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# *Sustainable Grassland Productivity*

*Edited by*

**J. Lloveras  
A. González-Rodríguez  
O. Vázquez-Yáñez  
J. Piñeiro  
O. Santamaría  
L. Olea  
M. J. Poblaciones**



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*Sustainable  
Grassland Productivity*



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Proceedings of the 21<sup>st</sup> General Meeting  
of the European Grassland Federation  
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## Foreword

Spain is a very large country with a great variety of grassland ecosystems ranging from the humid temperate North, where species such as Italian, perennial and hybrid ryegrasses, and red and white clovers are the main species sown to grasslands, being dairy and beef farming the main activities, to the very dry South East, where browsing of shrubs and grazing of cereal stubbles and fallows make the largest part of the fodder resources for livestock, mainly sheep and goats. In the middle is the *Dehesa*, which, together with the *Montado* of our Portuguese neighbours and very good friends, represents a unique and very special ecosystem, which is an excellent example of 'Grassland Sustainable Productivity', the subject of the 21<sup>st</sup> General Meeting of the European Grassland Federation.

As commented in the first opening paper, the *Dehesa* is the 'only possible form of rational, productive and sustainable land usage' in a place where arable farming would be unsustainable and unprofitable, and that, due to its diversification and efficiency, is 'also a very versatile system and has been able to successfully satisfy human requirements from the Middle Ages up to the twenty first Century'.

Taking into account the above mentioned features of the *Dehesa* system, located within an area of Mediterranean type of climate, the Spanish Society for the Study of Pastures decided to propose Extremadura as the most appropriate site within Spain to hold the 21<sup>st</sup> General Meeting because it represents a very different environment from those prevailing in most of the countries that organized the General Meetings in the past.

The theme is wide enough to be applicable at a Pan-European level and the Scientific Programme was designed to cover a wide variety of subjects, under the umbrella of 'Grassland Sustainable Productivity', namely 1) Overcoming seasonal constraints to forage production, 2) Role and potential of legumes, 3) Production and quality aspects of different animal feeds, 4) Changes in animal production systems to meet CAP reforms and 5) Grassland and climate change.

A total of 278 papers, from 38 countries, have been accepted to be presented as opening, plenary, oral or poster papers and will be an excellent basis for scientific discussions during the meeting.

The organizers would like to express their appreciation to the numerous people that helped in different aspects to make the meeting possible. Thanks are also specially given to 1) the Executive Committee that promoted a lively discussion among their members to define the theme and subjects of the different sessions, in order to meet EGF standards and traditions, 2) the Honorary Life Presidents who guarantee the scientific standards, being permanently ready to solve any question concerning a wide variety of subjects in a very short time, 3) the sponsors that made it possible to keep the individual fee at a reasonable level.

Very special thanks are given to the Scientific Committee members and to 133 external reviewers and language revisers who are a most important resource of the EGF family, in order to improve the language and scientific quality of the papers. It is a very difficult task because most of the contributions come from authors whose mother language is different from English.

We hope that all the participants enjoy the special atmosphere of the *Dehesa* and benefit from the scientific information and discussions of the 21<sup>st</sup> General Meeting of the European Federation.

Leopoldo Olea  
President of the Spanish Society  
for the Study of Pastures

Juan Piñeiro  
President of the  
European Grassland Federation



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# Opening Session

## Spanish Mediterranean pastoral systems



# The Spanish *dehesa*. A traditional Mediterranean silvopastoral system linking production and nature conservation

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## Abstract

The Spanish *dehesa* is a traditional, but also up-to-date, Mediterranean agrosilvopastoral system. It might be regarded as one of the most successful and efficient examples of how extensive agrosilvopastoral management is not only compatible with nature conservation and sustainable rural development within its environment, but also necessary for the achievement of both goals. Its area, of about 4 million hectares, is marked by two fundamental features: Mediterranean climate and low soil fertility.

The character, role, management and yield of its major components (tree layer, sward, crops, livestock and wildlife) are described. The current management of the Spanish *dehesas* is strongly influenced by the Common Agricultural Policy, showing a certain dissociation between the natural environment and its productivity, on the one hand, and agrosilvopastoral treatments, on the other. The main problem affecting the *dehesa* is the lack or shortage of regeneration of the tree layer.

Keywords: rangeland, Mediterranean, extensive livestock, Nature 2000 Network.

## Introduction

The term *dehesa* has many meanings. One of them reflects the word's etymology: *deffesa*, *defensa*, an early system of grazing land reserved for cattle used for land ploughing. Nowadays, the most widely accepted definition is that of an agrosilvopastoral (or pastoral-silvo-agricultural) system developed on poor or non-agricultural land and aimed at extensive livestock raising. Silviculture is not aimed at timber production but at increasing the crown cover per tree and at producing acorns, browse and fuelwood. The major goal of land cultivation is preventing the shrub invasion of grasslands and supplying fodder and grain for livestock, harvesting being a secondary goal (San Miguel, 1994, 2005; Montero *et al.*, 2000). According to Olea *et al.* (2005), the typical *dehesa* is located in the South Western part of the Iberian Peninsula, in Spain and Portugal, covering an area of about 3.5 – 4 million hectares. The greatest part of it is concentrated in Extremadura (1.25 M ha), Alentejo (800,000 ha) and Andalucía (700,000 ha).

The *dehesa* (*montado* in Portuguese) is an ancient system: the first written reference is from 924 (Olea *et al.*, 2005), though evidence of early *dehesas* is available from the Neolithic period (Stevenson and Harrison, 1992; Joffre *et al.*, 1999). Its expansion is closely linked with historical events: the reconquest of the Iberian Peninsula from the Moors and the subsequent re-distribution of that land, its re-population and the separation of heritages; the role of the Mesta, a powerful association of herdsmen and stockowners, and the sale of Church and nobility lands (Gómez-Gutiérrez, 1992; San Miguel, 1994; Joffre *et al.*, 1999).

The typical environment of the Spanish *dehesa* is marked by two fundamental features: the Mediterranean character of the climate (dry summers and somewhat cold winters) and the low fertility of the soil (particularly P and Ca), making arable farming unsustainable and unprofitable. Another important factor is topography, which is generally flat or hilly, but never rough. Within this difficult environment, the *dehesa* has arisen as the only possible form of rational, productive and sustainable land

usage. It does not try to maximize the output of any particular product. On the contrary, it tries to use a strategy of efficiency and diversification of structures with the aim of taking advantage of every natural resource (multiple, scarce and unevenly distributed in time and space) of its environment with a minimum input of energy and materials. Due to that diversification and efficiency, the *dehesa* is also a very versatile system and has been able to successfully satisfy human requirements from the Middle Ages up to the twenty-first century. That is the secret of its survival.

The link between the high structural and biological diversity of the *dehesa* and its efficiency and stability is the high diversity of relationships between its components. They are so closely entangled by that net of inter-relationships that the management of every single component necessarily affects each of the others. That is why the *dehesa* system should be described from a holistic point of view as a whole macro-organism; and why the *dehesa* is a paradigm of equilibrium and mutual dependence between production and nature conservation. Its high environmental value is a consequence of its extensive, integrated and efficient management. Therefore, that management should be considered as a powerful conservation tool (González and San Miguel, 2004).

### **Structure and management**

Due to its large area and its high economic, social and environmental importance, there is much available information on the *dehesa* system. However, most of it is written in Spanish and, what is even worse, deals exclusively with one or few of its components. Foresters deal almost exclusively with the tree layer but less with livestock or agriculture; agronomists, with crops but not with trees or wildlife; experts in animal production, with livestock but not with trees or wildlife; biologists, with flora, fauna or biodiversity but not with management, and so on. As a consequence, the aim of this paper will be to give a comprehensive view of the whole *dehesa* system, integrating the management of its different components and environmental aspects. To achieve that goal, we will present the essential information of every component as tables in which we describe the major role of each component and their essential features, regarding composition, production, management and improvement, as foresters do in silvopastoral management projects.

### **The tree layer**

The *dehesa* is a savannah-like open woodland (with summer drought instead of summer rainfall, as the in true savannah) where trees play a fundamental role of general stabilization providing the so-called services or indirect benefits. However, they contribute to the direct general production of the *dehesa* with acorns, browse, fuelwood, cork, edible fungus, pollen and some more resources. Its major features are summarized in Table 1. The tree layer is an essential component of the *dehesa* system and, as a consequence, sustainable management must be concerned not only with adult trees but also with their natural regeneration. This is the most important problem of the *dehesa* system, since natural regeneration is usually absent or scarce. The almost complete abandonment of transhumance, a partial substitution of sheep by cattle due to the shortage of shepherds, the increase of stocking rates and grazing periods allowed by socio-economic improvement and the Common Agricultural Policy are the most important reasons for that situation. In addition, it is getting worse as a consequence of the accelerated disappearance of adult trees due to the so called 'seca' (sudden dying-off caused finally by a fungal disease and promoted by climatic, edaphic and biological reasons).



Table 1. Major features of the *dehesa* tree layer and its management.

TREE LAYER	Major role	STABILITY: structure, landscape, climate (Joffre and Rambal, 1988, 1993), erosion, water and nutrient cycles, shelter, biodiversity, C fixation, cultural benefits, fodder,...). Perennial sclerophyllous species might be considered as permanent fodder reserves for livestock and wildlife
	Species	<i>Quercus ilex rotundifolia</i> (= <i>Q. ilex ballota</i> ), <i>Q. suber</i> (sclerophyllous and perennial), <i>Q. faginea</i> , <i>Q. pyrenaica</i> (semi-deciduous) and other less important species.
	Density	(15) 20 – 100 (200) adult trees ha <sup>-1</sup>
	Crown coverage	(5) 10 – 50 (70)%
	Basal area	2 – 10 (15) m <sup>2</sup> ha <sup>-1</sup>
	Products: Mean annual yield	Fuelwood: 800-5000 kg ha <sup>-1</sup> -rotation (DM)
		Browse (pruning or direct browsing): 400-1500 kg ha <sup>-1</sup> (DM)(pruning). Direct browsing is important in coppices (usually cold <i>dehesas</i> , with low acorn yield)
		Acorn: (100) 200 – 600 (800) kg ha <sup>-1</sup> , with inter-annual variations (Olea <i>et al.</i> , 2004; López-Carrasco <i>et al.</i> , 2005)
	Silvicultural rotations	Cork (only <i>Q. suber</i> ): 500-1500 (2000) kg ha <sup>-1</sup> -rotation
		Regeneration felling: tree senescence (150 years for <i>Q. suber</i> and 250-300 years for other species)
Pruning: 10-15 years Debarking: 9-12 years		
Threats	The lack or shortage of natural regeneration of trees in many <i>dehesas</i> is by far their most important threat. Besides, it is getting worse due to the sudden dying-off of many trees known as ‘seca’.	

### Natural pastures

The most important objective of the *dehesa* is extensive livestock rearing. Therefore, natural pastures, as the main source of fodder for livestock, are an essential component of the system. As a consequence of the Mediterranean climate, natural pastures are usually annual grasslands. However, perennials play a fundamental role in valley bottoms and particularly in dense swards created and maintained by intense and continuous grazing, known as majadales. Their major features are summarized in Table 2. The management of natural pastures is aimed at increasing their quality (legumes: protein, minerals), since quantity is much less important due to high variability (up to 200 %, according to Olea *et al.*, 1989) and the typical uneven seasonal distribution of their production (Figure 1). Therefore that management is based upon three fundamental topics: rational livestock grazing, legumes and phosphorus. A suitable management might result in a significant improvement of the quality of natural pastures (Table 3). However, seasonal periods of shortage of fresh fodder can not be avoided, so browse, fruits (particularly acorns), crops and supplementary food also contribute to a suitable nutrition of livestock in hunger periods: summer and winter. The shrub layer is typically absent or sparse.

Table 2. Major features of the *dehesa* natural pastures.

NATURAL PASTURE S	Major role	Providing fodder for livestock
	Communities	Usually annual grasslands: Helianthemetalia, Thero-Brometalia, Sisymbrietalia. Edapho-hygrophilous perennial grasslands ( <i>Agrostietalia</i> ) grow on valley beds and wither in mid-summer. The optimum grassland community is the 'majadal' ( <i>Poetalia bulbosae</i> ), a dense sward of annuals and perennials with a rather high representation of legumes (protein) created and maintained by intensive and continuous livestock grazing.
	Production	1000-2700 kg ha <sup>-1</sup> yr <sup>-1</sup> (DM). Majadal pastures usually around 3000 kg ha <sup>-1</sup> yr <sup>-1</sup> DM, with early growth start in autumn and late withering.
	Yearly distribution of the fresh fodder yield	Spring: 60-70% Summer: 0% Autumn: 15-25% Winter: 5-15% Highly variable due to a very high climatic variability
	Management goals	Legumes are essential due to their protein supply but also because, after withering, their nutritional quality is high enough to satisfy the maintenance requirements of livestock. Supplementary feeding could then be avoided or reduced (Olea <i>et al.</i> , 1989; Olea and Viguera, 1998).
	Improvement	Sustainable but intensive <u>grazing</u> aimed at increasing the pasture quality and at recycling limiting nutrients <u>P fertilization</u> (25 to 35 kg ha <sup>-1</sup> P <sub>2</sub> O <sub>5</sub> /ha during the first year and 18 - 25 thereafter) aimed at favouring legumes, whenever their abundance is high enough to ensure good results (Moreno <i>et al.</i> , 1993, 1994). The available P level should be high enough: 8-12 mg kg <sup>-1</sup> , Olsen method (Granda <i>et al.</i> , 1991). Superphosphate is the usual product, but natural phosphates (ecological products) are also showing good results (Olea <i>et al.</i> , 2005)

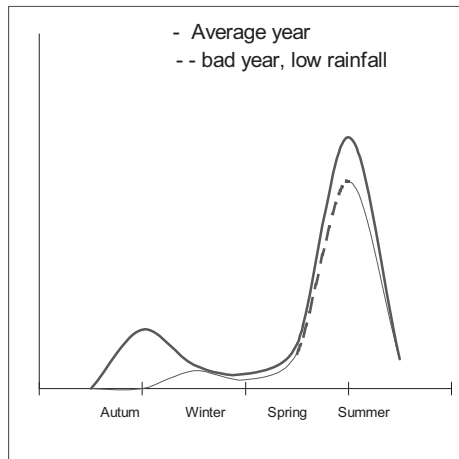


Figure 1. Annual production of natural pastures.

Table 3. Nutritional quality of the *dehesa* natural pastures.

Crude protein (g kg <sup>-1</sup> )			O.M.D.			% of Legumes		
Max	Min	Average	Max	Min	Average	Max	Min	Average
148	85	103	63.3	40.0	55.2	24.0	4.0	8.5

O.M.D.: Organic Matter Digestibility (%)

#### Crops, including sown pastures

Crops and sown pastures often play a fundamental role in livestock feeding, as a complement to natural pastures, both in seasonal distribution (summer and late winter) and in quality (Joffre *et al.*, 1988). In addition, cropping is usually carried out in cycles of several years (3-6) with the aim of keeping intolerant invading shrubs out of natural grasslands. Some *dehesa* owners allow other farmers to cultivate their *dehesas* for free when their natural pastures are being invaded by intolerant shrubs, usually *Cistus* sp. The major features of the *dehesa* crops and sown pastures are summarized in Table 4.

Table 4. Major features of the *dehesa* crops and sown pastures.

	Major role	Complementing the fodder yield of natural pastures, both in seasonal distribution and quality
CROPS	Types	<p><u>Cereal crops</u>: oat, barley, rye, wheat, triticale. They complement the fodder yield of natural pastures both in seasonal distribution (summer, late winter) and quality (energy). Grain is the most valuable product. It is usually collected, but it may also be harvested by direct summer grazing, since transhumance is no longer being carried out. Straw is also collected or grazed. Sometimes, there is a late winter grazing period of leafy biomass followed by a resting season until the summer grain harvest.</p> <p><u>Sown pastures</u>. Aimed at being used by grazing or cutting. In the first case, legumes are essential, so subterranean clover (<i>Trifolium subterraneum</i>) and other auto-reseeding legume species are the basis for permanent sown pastures (Olea <i>et al.</i>, 2005). They complement the fodder yield of natural pastures in quality (protein) and, to a lesser degree, in seasonal distribution (air dry biomass and seeds). In the second case, vetch-cereal (oat, triticale, barley), with a 3:1 weight rate and conservation as hay, is the usual choice. However <i>Lolium multiflorum</i> and winter cereals are also a choice. Hay is used as summer and winter fodder.</p>
	Production (average climatic year)	<p>Cereal crops: grain (1000-3000 kg ha<sup>-1</sup>), straw (2000-5000 kg ha<sup>-1</sup>)</p> <p>Sown pastures:            Legume rich permanent pastures: around 3000 kg ha<sup>-1</sup> (DM)            Vetch-cereal: 3000-6000 kg ha<sup>-1</sup> (DM). Hay making</p>
	Management	<p>Two-three tilling treatments before sowing (late winter, late spring, early autumn)            Early autumn sowing            Fertilization:            Cereal crops: N-P-K usually 200-300 kg ha<sup>-1</sup> (8-24-8 or 15-15-15)            Legume rich permanent pastures: P (at least 35-40 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> before sowing)            Vetch-cereal: N-P-K usually 200-300 kg ha<sup>-1</sup> of 8-24-8            Legume rich permanent pastures should be sown only when natural pastures show a very low abundance of legumes. In any other case, P fertilization becomes a better option.</p>

Table 5 compares the yield (quantity and quality) of natural pastures, P fertilized natural pastures and P fertilized sown pastures.

Table 5. Yield and quality of natural pastures, P-fertilized natural pastures and P-fertilized sown pastures at the *dehesa* system of Badajoz (Extremadura, Spain).

	Yield		Quality		
	kg DM ha <sup>-1</sup>	Average response	Crude protein (g kg <sup>-1</sup> )	O.M.D. (%)	Legumes (%)
Natural pastures	1440	-	103	52.0	8.5
P fertilized natural pastures	2238	55%	110	58.9	18.0
P fertilized sown pastures	2670	86%	136	62.5	30.0

O.M.D.: Organic Matter Digestibility

## Livestock

Table 6. Major features of the *dehesa* livestock

	Major role	The most important direct product
LIVESTOCK	Species (breeds)	<u>Cattle</u> : avileña-negra ibérica, morucha, retinta, lidia, blanca cacereña, berrenda en colorao, berrenda en negro, atigrada de Salamanca, ... <u>Sheep</u> : merino, Ille de France, Fleischschaff, Landschaff, ... <u>Swine</u> : Iberian pig (negro lampiño, negro entrepelado, colorado,...) <u>Goat</u> : verata, retinta, serrana,... <u>Horse</u> (español,...); <u>Donkey</u> (andaluz,...)
	Sustainable stocking rate	<u>Cattle</u> : 0.2–0.4 ha <sup>-1</sup> <u>Sheep</u> : 2–4 ha <sup>-1</sup> <u>Goat</u> : 2–3 ha <sup>-1</sup> <u>Iberian pig</u> : 0.4–0.6 ha <sup>-1</sup> The usual management is with several species, each one taking advantage of the optimal usage of specific natural resources (e.g. Iberian pig is preferred for fall and early winter acorn yield) An even distribution of livestock is desired with the aims of reducing damages to the tree layer, increasing the efficiency of grazing and reducing the prevalence of parasites and diseases
	Management	Periods of high nutritional requirements of livestock (late pregnancy and lactation) should coincide with seasons showing peaks of fresh fodder supply. <u>Cattle</u> : desired calving season from November until March, depending on winter cold. Lactation: 5-6 months <u>Sheep-goat</u> : two systems. One lambing season/year: spring or autumn (better prices). Three lambing seasons/ 2 years. Lactation: 45 days. <u>Iberian pig</u> : two farrowing seasons/year: spring and autumn (López-Bote, 1998). Piglets born in autumn are fed for one year (to reach 90-110 kg live weight) and then they are fed on acorns and grass from October until January, gaining around 0.7 kg day <sup>-1</sup> (to reach 140-160 kg live weight)

Extensive livestock is the most important direct product of the *dehesa*, but also a fundamental tool for creating and improving natural and sown pastures and for dispersing their seeds (Malo and Suárez, 1995; Malo *et al.*, 2000) and fertility (Gómez-Sal *et al.*, 1992). As a consequence, sustainable and extensive livestock management is an essential tool for the preservation of the *dehesa* system and its biodiversity. However, it should be compatible with the presence and regeneration of the tree layer, since trees are browsed and damaged by livestock with different intensities (trees up to 12-15 cm of diameter at breast height, or 20-40 years of age, might be shattered by cattle, especially if they are fed with concentrates including urea). Due to the high diversity of the *dehesa* system, different livestock species are required. The major features of the *dehesa* livestock and its management are summarized in Table 6.

## Hunting species

Hunting species have always been present in the *dehesa* system, but in low densities (with the exception of wild rabbit) since they were considered only as a source of complementary food. However, since 1960s the situation changed dramatically because hunting became a major economic activity and now is often the most important one in many *dehesas*. Wild ungulates, especially red deer (*Cervus elaphus hispanicus*) and wild boar (*Sus scrofa*), are now regarded as expensive renewable natural resources, so *dehesa* owners have usually fenced their properties. The result is a dramatic increase of wild ungulate densities (usually over 50 red deer individuals/km<sup>2</sup>). This has given rise to a new problem of sustainability (because of impacts on woody vegetation and fauna, prevalence of parasites and diseases which may affect livestock and even man, genetic loss,...) and new concepts of land use (Vargas *et al.*,

1995; San Miguel *et al.*, 1999). Wild rabbit densities have suffered a dramatic decrease because of myxomatosis, viral haemorrhagic disease and predators (wild boar included). This has become a major environmental problem (Villafuerte *et al.*, 1995; González and San Miguel, 2004), since rabbit is the basic prey of many predators (Iberian imperial eagle and Iberian lynx included) and necrophages (e.g. black vulture). Red legged partridge, another traditional hunting species, is also endangered by many problems including the common introduction of farm-raised individuals (with their parasites, diseases and sometimes different genetic heritage) and predators (wild boar also included). Finally, wood-pigeon densities have increased, even though they compete with livestock (especially Iberian pig) and wild ungulates for acorns. The major features of the *dehesa* hunting species and their management are summarized in Table 7.

Table 7. Major features of the *dehesa* hunting species.

	Major role	The most important direct product in many cases
	Species	Wild ungulates: Red deer ( <i>Cervus elaphus hispanicus</i> ), wild boar ( <i>Sus scrofa</i> ), roe deer ( <i>Capreolus capreolus</i> ), fallow deer ( <i>Dama dama</i> ), mouflon ( <i>Ovis ammon musimon</i> ) Wild rabbit ( <i>Oryctolagus cuniculus</i> ), hare ( <i>Lepus granatensis</i> ) Red legged partridge ( <i>Alectoris rufa</i> ), wood pigeon ( <i>Columba palumbus</i> ), turtle-dove ( <i>Streptopelia turtur</i> ) and some more
HUNTING SPECIES	Sustainable stocking rate	Ungulates: 10-20 ind km <sup>2</sup> . Problems of overstocking. Wild rabbit: traditionally over 10 ind/ha. Nowadays it has disappeared from many <i>dehesas</i> and their densities are much lower. Red legged partridge: densities vary with food and shelter supply. Wood-pigeon: high densities in autumn and winter there where acorn yields are high. Estate owners often scare them with the aim of reserving acorns for livestock or wild ungulates.
	Management	Wild ungulates: usually 'montería' (individuals are driven towards concealed hunters by dogs and dog handlers), but also, in a lesser extent, trophy-stalking. Culling: about 15-20% with the exception of wild boar (higher, up to 100% or even more). Lagomorph and bird species: stalking. Red legged partridge is also hunted by 'ojeo' (individuals are driven towards concealed hunters by people).

### Environmental quality

The *dehesa* is a system protected by the 92/43/EEC Habitat Directive, and included in the Nature 2000 network. In addition, it provides a wide variety of services, or environmental benefits: structural and biological diversity, environmental stability (erosion, climate, nutrient and water cycles, fire,...), landscape, leisure activities, tourism, cultural heritage and some more (Table 8). It is also the habitat of many protected animal and plant species and communities. As a consequence, in spite of the fact that it is usually a private property, the environmental quality of the *dehesa* system should be considered as a fundamental objective of its management and results in the so-called environmental rent (Campos *et al.*, 2001). However, as we stated above, that high environmental quality is a consequence of its extensive, integrated and efficient management and, therefore, that management should be considered as a powerful conservation tool. As an example of its importance agro-silvo-pastoral management is a basic activity of three LIFE Projects aimed at the conservation of Iberian lynx, Iberian imperial eagle, black vulture and black stork (González and San Miguel, 2004). The conclusion is that this kind of management, whose profitability is usually low, should be supported by European, Spanish and regional governments.

The tree crown coverage and distribution has shown to be a major factor in determining the diversity and population density of many animal groups in the *dehesa* system. It is widely known for livestock

species, ungulates, small mammals and birds, but has also been demonstrated for lizards (Martín and López, 2002), ants (Reyes *et al.*, 2003) and dung-feeding beetles (Galante *et al.*, 2001).

Table 8. Major aspects of the *dehesa* environmental quality.

ENVIRONMENTAL QUALITY	Major role	The most important service or indirect benefit of the <i>dehesa</i> system. Demanded by society and considered as a fundamental goal by every public policy (European Community, Spain, Autonomous Communities), even though most <i>dehesas</i> are private estates. The so-called environmental rent of the <i>dehesa</i> is very high and is still increasing (Campos <i>et al.</i> , 2001)
	Endangered fauna	Iberian Imperial Eagle ( <i>Aquila adalberti</i> ), <i>Hieraetus fasciatus</i> , <i>Elanus caeruleus</i> , Iberian lynx ( <i>Lynx pardinus</i> ), black vulture ( <i>Aegypius monachus</i> ), black stork ( <i>Ciconia nigra</i> ), crane ( <i>Grus grus</i> ), Cabrera's vole ( <i>Microtus cabrerae</i> ) and many others, invertebrates included
	Other environmental services	Structural and biological diversity: $\alpha$ , $\beta$ , $\gamma$ Environmental stability: erosion (Bernet, 1995; Olea <i>et al.</i> , 2005), climate (Joffre and Rambal, 1988, 1993), nutrient and water cycles (Gómez-Gutiérrez, 1992), fire,... Genetic biodiversity: traditional livestock breeds, traditional varieties of agricultural species, ecotypes of pasture species selected by grazing over centuries. Landscape Cultural heritage

The tree crown coverage, as well as the percentage of land covered by natural or sown pastures or shrubs, is also closely related with erosion in the *dehesa* system (Maldonado *et al.*, 2004). The arrival of autumn rainfall is the worst season from the point of view of erosion risk in the *dehesa* system, so suitable land use policies (Table 9) may significantly contribute to soil conservation.

Table 9. Land vegetation coverage (%).

Treatment	November	February	March	April
Fallow (recently ploughed land)	0	0	0	0
P fertilized sown pasture (1 <sup>st</sup> year)	18	76	83	99
Burnt pasture	25	68	79	79
P fertilized natural pasture	82	95	97	99
Natural pasture (unimproved)	70	81	84	90

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# Mediterranean dryland mixed sheep-cereal systems

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## Abstract

In dryland areas of Spain and in other similar areas of the Mediterranean Basin, mixed agro-pastoral systems where sheep rearing is associated with cereal-fallow crops are very common.

The present review analyses first the history and origin of the mixed sheep-cereal system; secondly describes the system in Spain and other Mediterranean countries; thirdly, compares it with other arable and livestock systems and finally, makes proposals to improve the sheep-cereal system in the context of sustainable rural development and nature conservation.

The historical analysis suggests that after several millennia of independent development in the Mediterranean Basin, sheep and cereal systems merged probably during the Middle Age, as a risk reduction strategy, because mixed sheep-cereal systems are more productive, diversified, reliable and better adapted to fluctuating climatic and social environments. Economic globalization and intensification of crop and livestock systems are threatening the viability of the Mediterranean sheep-cereal systems, but compensatory payments for public services such as protection of rural life, environment, biodiversity, landscapes, and traditional food production systems (organic farming), are some of the justifications to maintain and integrate extensive sheep rearing in association with low-intensity cereal crops in marginal drylands.

Key words: sheep-cereal systems, barley, stubbles, fallow weeds, steppe-birds, rural life.

## 1. History and origin of mixed sheep-cereal systems

Agriculture was a prerequisite for any high civilization, like those of Mesopotamia and Egypt, because city dwellers are consumers, not food producers. The bulk of the food produced and consumed came from four domesticates: wheat, barley, sheep, and goats (Harlan, 1975). However, goat and sheep domestication (pastoralism) started before cereal cultivation.

According to mitochondrial DNA analysis, there were at least three geographically and temporally separate captures of founder female bezoar goats (*Capra aegagrus*) during the formation of early domestic populations. Fossil records suggest that the three goat lineages (Asia, Fertile Crescent and Europe) diverged much earlier (200,000 BC) than its domestication (c. 13,000-9,000 BC) (MacHugh and Bradley, 2001; Luikart *et al.*, 2001). In the case of sheep, mtDNA indicates they derived from two different maternal lineages, and that some of the modern domestic sheep (*Ovis aries*) and European mouflon (Hiendleder *et al.*, 2001) derive from one of these two common ancestors. These recent genetic discoveries indicate that goats and sheep could have been domesticated in Europe and North Africa (di Lernia, 2001), independently from its domestication in the Near Eastern Centre, and under different forms of pastoralism.

According to archaeological records, goat domestication started in the Eastern Mediterranean ca. 7200 BC, but size reduction, an indicator of domestication, began later in sheep, ca. 6500 BC. Cereal cultivation appeared about a millennium after caprine husbandry. By 5500 BC, domestic goats and sheep are found at Neolithic sites throughout Southwest Asia, frequently associated with evidence of cereals. Therefore we can expect that agriculture and pastoralism became the dominant mode of subsistence across much of the region between the 7<sup>th</sup> and 5<sup>th</sup> millennium (Bar-Gal *et al.*, 2003).

Initially, herders and cultivators of lands surrounding villages coexisted, but as populations increased, and with it the demand of foodstuffs, both activities dissociated and finally shepherds became nomads

who only contacted sedentary village cultivators to trade animals and animal products (Abdi, 2003; Johnson, 1983). Mixed cereal-sheep husbandry is a relatively modern type of pastoralism, not identified as such among the early types of prehistoric pastoralism in the Near East.

The globalization process in the Roman period led to large scale cereal production in the so-called Rome's granaries, scattered around the Mediterranean (Syria, Turkey, Egypt, Cyrenaica, North Algeria, Tunisia, Morocco, Sicily, and the Iberian Peninsula). These granaries may have offered the optimal conditions for the origins of sheep-cereal mixed systems during the fall and disintegration of the Roman Empire, and with the social changes associated to the beginnings of the medieval period. Since then, the convergence of sheep pastoralism with cereal agriculture became a common practice around the Mediterranean, especially in its more marginal areas, as a strategy to reduce risk and diversify production.

During the Middle Ages, as populations increased, more forest was cleared and converted to farmland and pastures with livestock excreta being the main source of fertilizer. As livestock numbers increased and due to the seasonality of Mediterranean climate and pasture resources, livestock rearing demanded seasonal movement of animals along networks of drove roads connecting summer and winter pastures (Bignal and Cracken, 2000).

In Spain, this transhumant pastoral system was well developed by the 8<sup>th</sup> century, and by the year 1273 the king Alfonso X had established a national livestock association, the 'Honrado Concejo de la Mesta', which defended the rights of livestock raisers undertaking transhumance across Spain, until its abolition in 1836. The Mesta maintained large numbers of Merino flocks moving towards regions where natural conditions provided green pastures (highland and mountains in summer, plains and coastal areas in winter) or to cropping areas like winter cereals to graze stubbles and weedy fallows. During the last decades, old agrosilvopastoral sheep rearing systems have evolved towards more intensive and economically efficient agro-pastoral systems, where use of concentrates and agricultural by-products make up the largest part of the animals feed. However, in marginal environments, like those prevailing in semi-arid Mediterranean basin countries, agriculture and livestock are still associated, and sometimes integrated.

## **2. The sheep-cereal mixed farming system**

In Mediterranean countries, low-intensity arable systems are mainly confined to drylands (non-irrigated) which are particularly significant in the Southern European Union (Spain, Portugal, Italy and Greece), Middle East and North Africa (Syria, Tunisia, Morocco, etc). Very often, winter cereals use fallowing to maintain soil fertility, and associated with them, sheep rearing; animals graze stubbles in summer and weedy fallows in autumn-winter. The proportion of fallow (30-80%) and the importance of livestock increases as rainfall decreases. The number of fallow years also increases in poor soils (2-3 years). The components of the sheep-cereal/stubble/fallow systems are described below.

### **2.1 Cereal year**

In Spain, winter cereals occupy around 6 mill ha (35% of arable land), and in summer, the majority of cereal stubbles are grazed by livestock, as well as nearly 3 million hectares of fallow land in the autumn-winter. During the last 20 years, the area cultivated with wheat has been maintained around 2.2 mill. ha, while the area of barley has been reduced from 4.3 mill. ha in the period 1985/92, to 3.1 mill. ha in 1999/2004. EU subsidies to increase arable land under fallow in marginal areas are probably the main cause of the reduction of barley cultivation. Oats and rye occupy small areas (391.000 ha and 161.000 ha respectively). During the decade 1995-2004, mean barley production in Spain was 9 mill. tons of grain (ranging from 5 to 11 mill. tons) and average yields 2400 kg ha<sup>-1</sup>. Spanish barley production represents about 20% of EU barley production and 6% of world production.

The amount of straw produced by each unit of grain is 0.60 for wheat, 0.72 for barley, 0.78 for oats and 1.20 for rye (Kossila, 1984). In the barley straw, 35% corresponds to leaves, 58% to stems and 7% to awns and axis of spikes. Straw quality in Mediterranean semi-arid and marginal areas is generally better than in temperate zones (Rihani, 2001). Barley grain and straw are the most important livestock supplements during seasonal feed scarcity periods in dry Mediterranean areas (providing up to 50% of

animal nutritional requirements with 70% in the Middle East). When cereal yields are very low, as in dry years, grain is not harvested and animals graze cereals directly, maintaining their nutritional requirements during summer.

## 2.2 Stubble

After cereal harvesting, livestock enter the fields to graze the stubbles, which supply the remaining straw, weeds and the fallen grains. Sheep graze selectively; first the spikes and grains, secondly dry leaves, thirdly cut and fallen stems, and finally, standing stems. Summer weeds, such as *Polygonum*, *Cynodon*, *Amaranthus*, *Salsola*, *Chenopodium*, can be present in cereal stubbles, especially in areas where summer rains are relatively frequent. Some of these weeds are positively selected by sheep and contribute a fundamental part of their stubble diet. The biomass available in the stubbles ranges from 0.8 t ha<sup>-1</sup> in dry years, to 4.5 t ha<sup>-1</sup> in wet years. The quantity of fallen grain is highly variable, being around 200 kg ha<sup>-1</sup> (Robledo, 1991), and it is related to spike density, soil topography, and soil stone content. According to Caballero *et al.* (1992), cereal stubbles represent a regular and important source of nutrients during the summer season for the Spanish sheep population –a mean of 24 million ewes during the last 15 years-. The capacity of stubbles to maintain sheep is limited because grain is consumed soon and the other stubble fractions cannot satisfy the nutritional requirements of gestating ewes (Guessous *et al.*, 1989). In such cases, animals are supplemented with concentrates. Sometimes, summer weeds represent an important fraction of stubbles: in North Greece, 33% of stubble biomass was made of weeds contributing up to 78% of the total diet (Yiakoulaki & Papanastasis, 2003).

## 2.3 Fallow year

The cycle of the fallow year starts with the autumn rains, which induce the germination of fallen cereal grains and other spontaneous plants. The biomass produced is highly dependant on rainfall and winter temperatures. Species of the Gramineae are the dominant fallow 'weeds', barley being the most important, followed by *Lolium*, *Bromus*, *Avena*, Leguminosae (*Vicia*, *Lathyrus*, *Medicago*, *Trigonella*, *Melilotus*, *Coronilla*), Cruciferae (*Eruca*, *Biscutella*, *Sisymbrium*, *Diplotaxis*), Papaveraceae (*Papaver*, *Hypocoum*, *Roemeria*, *Fumaria*) and others (*Centaurea*, *Bupleurum*, *Orlaya*, *Bifora*, *Silene*, *Vaccaria*, *Cerastium*, *Anchusa*, *Galium*, *Linaria*, *Consolida*, *Muscari*, etc.). At the end of spring, summer species germinate (*Salsola*, *Chenopodium*, *Polygonum*, *Hypericum* and *Amaranthus*).

Surprisingly, very few data are available on the biomass produced by cereal fallows, despite the important area they cover and the feed they provide to livestock. In SE Spain, Robledo (1991) and Robledo *et al.* (1989) estimated a mean production of 500-900 kg DM ha<sup>-1</sup> during the period December-May; barley contributed 50% of the total biomass, followed by *Lolium rigidum* (19.3%), *Bromus diandrus* (11.3%), *Papaver rhoeas* (2.3%), *Vicia peregrina* (1.2%), *Trigonella polyceratia* (1.2 %) and others (77 species with a 14% contribution). It is so important that the yield contribution of Gramineae species during the fallow year could be consider as another cropping year, but one achieved without ploughing the soil and with the incorporation of manure from the grazing animals.

## 2.4 Sheep and herds

Sheep are the livestock species most related to cereal crops, with many races well adapted to different climatic and topographic conditions and able to convert cereal by-products into animal products. In 1995, a majority of Spanish livestock herds (60%) had less than 200 ewes, and very little economical future; 25% of total herds having 200-500 sheep made up one third of the census; however, herds with more than 500 sheep (13% of total) represented 50% of the national census and provide the best economic option for the future of the sheep-cereal system. Castilla-La Mancha and Aragón, two of the Spanish regions where sheep herds are larger (210-240 ewes/holding), are also regions where winter cereals are widely cultivated (Hoyos, 2003).

## 2.5 Climatic scenarios

The productivity of the system is very much related to rainfall; thus in Spain, during the last 20 years, mean barley yields oscillated from 1.4 t ha<sup>-1</sup> in a dry year, to 3.4 t ha<sup>-1</sup> in a wet year (mean yields 2.4 t ha<sup>-1</sup>). In a mean year, the sheep-cereal system is well balanced, but during dry years, its components must be adjusted to get a minimal economic return that assures its continuity. Global climatic change scenarios open up new questions, but the versatility of the sheep-cereal system provides opportunities for adaptation to future climatic changes.

During dry years, production is reduced to 50% of a normal year (90% in lower rainfall regions) and in these occasions, cereal crops are fully grazed at the end of the spring. Thus livestock are a fundamental tool to get an economic benefit from failed crops, a situation that can be prolonged during several years of drought, as occurs rather frequently in Mediterranean dryland regions. The price of cereal grains, straws and concentrates increases in dry years, what in turn increases the cost of animal feeding, because cereal products make up a large part of the animals diet.

During wet years, barley yields in Spain are 25% higher than in average years, and livestock feed resources are abundant; additionally, cereals are sometimes grazed during winter, with little reductions in grain yields. In wet years, weeds proliferate and compete with cereals, reducing the final grain yields, but in compensation, fallows are more productive.

## 2.6 Subsidy scenarios

EU subsidies to zones with environment or structural factors limiting development were initially used to increase sheep numbers and stocking rates in many regions of Mediterranean countries. In Spain, sheep numbers increased 52% during 1982-1993, and something similar happened to the area cultivated with cereals. With the new CAP starting in 1992, agriculture subsidies were given to producers instead of products. Since then, sheep numbers and cereal areas have stabilized or experienced a small reduction. In the case of livestock subsidies, they have not been used to improve forage production, infrastructure of farms, or to manage agrosilvopastoral territory, but have resulted in the intensification of farms and a movement of sheep from dryland to irrigated areas.

In Middle East and North African countries, governments offered subsidized grain to encourage the supplementation of grazing with cheap barley. This produced a rapid increase in the number of animals and the meat produced, but also meant more animals on less land, leading to degradation of rangelands to a generalized use of concentrate feeds and increased cost of meat production (feed costs represent more than 50% of livestock output; Nefzaoui and Ben Salem, 1999).

Fodder by-products from cereals represent the second largest resource in quantitative terms (after rangeland and natural pastures), but in terms of quality and seasonal availability, they represent the most reliable fodder resource for livestock during most of the year. Any future reduction of cereal area in economically marginal areas, due to reductions in subsidies, will diminish livestock feed resources and consequently, the stocking rate capacity of these areas.

In the near future, EU support to farmers must be linked to a double function: as producers of quality food, and as managers of a large territory with responsibilities for preservation of the environment. Future sustainable models for these territories should consider economic aspects, such as rural tourism, creation of second residences, winter sports, preservation and restoration of historical and cultural holdings, hunting activities, etc, as well as activities for the preservation and rehabilitation of the environment, such as reforestation, maintenance of natural parks, control of soil erosion, management of the territory, etc (Tió, 1996).

## 2.7 Maintenance of biodiversity

The cereal-fallow system maintains a pseudo-steppe landscape of great importance for nature conservation. The adventitious flora associated with cereal cropping represents about 20% of western Mediterranean flora, but also provides supplemental feeding and habitat for steppe wild birds such as partridges, quails, wood-pigeons, turtledoves, great bustard, etc, which are becoming under danger of extinction with the reduction of cereal-steppe habitats. Changes in the agricultural production systems in

recent years (intensification, low-tillage, general use of herbicide, pesticide and chemical fertilizers, etc), loss of landraces and old crop varieties and abandonment of traditional field uses, have reduced the quantity and number of weeds to critical levels, resulting in some bird species becoming close to extinction in many European countries.

### **3. Comparison of sheep-cereal systems with other systems**

Farming systems can be classified as extensive, semi-extensive or semi-intensive, and intensive, depending on the inputs, labour, capital and biodiversity involved.

#### **3.1 Extensive-low input systems**

According to Bignal and McCracken (2000), extensive systems can be classified as: livestock systems, arable systems, permanent crop systems and mixed systems, in which sheep-cereal and sheep cereal-legume systems could be included. Livestock systems are still present in upland and mountainous regions and in arid zones, but sheep numbers are decreasing, resulting in an increase of inflammable biomass and of forest areas affected by fires.

Arable systems, which are mainly confined to semiarid areas, are low yielding and use fallowing to maintain soil fertility, frequently in association with grazing. Permanent crop systems, such as olives, fruits and vines are an important component of Mediterranean lands, but inter-cropping with cereals and livestock grazing is practised in poorer areas.

Sheep-cereal systems are examples of mixed agro-pastoral systems, occupying an intermediate position between livestock and arable systems. Winter cereals represent the best alternative in terms of yields compared with the potential biomass produced by dryland pastures and rangelands. As an example, in semi-arid NW-Murcia (SE-Spain), where about 50% of the land is under cereal cultivation, the mean productivity of the biannual barley-fallow systems ( $2.7 \text{ t DM ha}^{-1} \text{ year}^{-1}$ ) is very high compared to that of native rangelands ( $1.8 \text{ t DM ha}^{-1} \text{ year}^{-1}$  in scrublands and steppes) and dry land pastures ( $1.2 \text{ t DM ha}^{-1} \text{ year}^{-1}$ ) (Ríos *et al.*, 1992).

Higher labour cost and declining prices have contributed to the reduced viability of farming in marginal areas where forestation, marginalisation or complete abandonment has occurred in some places. Thus, a loss of agricultural habitats associated with the drier, traditionally less intensive farming systems has been detected in southern Europe countries.

#### **3.2 Intensive-high input systems**

Intensive systems like continuous cropping, or animal feedlots, in general are economically more efficient, and can feed more people per unit area in terms of calories and protein, than extensive systems. In intensive cropping systems, the role of animals is taken over by resources from fossil reserves; tractors using diesel oil instead of animals, fertilizers instead of dung, and herbicides instead of grazing. In the case of feedlots, the role of grazing is taken over by cereals, with livestock now consuming more than a third of the entire world's grain as feed concentrates. Every kilo of meat produced uses 5-21 kg of animal feed, which has to be grown somewhere. Grain cereals are also used to produce ethanol by fermentation, a process that leaves a 50% residual (DDGS), rich in protein, which is used as animal feed.

#### **3.3 Intermediate systems**

A semi-extensive system which is becoming generally adopted in Spain and other semiarid Mediterranean countries is that of maintaining dry ewes on grazing resources from cereal crops and rangelands, but fattening lambs and supplementing animals with barley grain and other concentrate feeds during periods of high nutritional requirements. The energy requirement of a sheep in a shaded feedlot may be 70% lower than that of a sheep grazing on stubble (Landau *et al.*, 2000). There are also semi-intensive agro-pastoral system around irrigated areas and fertile valleys, where intensive agriculture generates large amounts of by-products, which together with concentrates, make up the

largest part of the animals feed, and nearby urban populations absorb the animal products (Correal and Sotomayor, 1998).

#### 4. Proposals to improve the sheep-cereal systems

The Mediterranean region is a complex mosaic of diversified landscapes. Much of the region is semi-arid and soils become saline, dry and unproductive in response to a combination of natural hazards (droughts, floods, forest fires) and human-controlled activities, notably over-tilling and overgrazing. Hence, proposals must be diverse and related to particular regions.

'Integration' by which farmers produce cereal and livestock to the mutual benefit of each enterprise is a must; however, in most cases owners of livestock do not have land, and cereal farmers do not have animals.

Long term security of land tenure and cooperative management of large territories will provide opportunities to improve livestock feed calendars with measures such as: controlling stocking rates on fallows and stubbles; practise deferred grazing in autumn to get more green feed in winter from weedy fallows; increase water supplies and fence part of cropland to make better use of feed resources; replace part of the fallow with forage legumes, etc.

##### 4.1 Review cereal breeding in relation to sheep utilization of stubble products

Land-race based cultivars with improved straw quality for feeding purposes have been released by ICARDA in Middle East and North African countries. In dry areas, drought stress is associated with a marked reduction of stem height and grain production in barley, but the nutritive value of the straw increases; stubbles also contains more protein in years of lower rainfall. In Syria, barley straw with shorter stems had more leaves (55 vs 36%) and appeared more extensively degraded in vitro (80.6 vs 68.3%) in research by Thomson and Ceccarelli (1991) which showed a relationship between leaf to stem ratio and straw degradability. Ohlde *et al.* (1992) also found leaves were more degradable than stem internodes.

The quality of stubble from early maturing cultivars is lower than from late maturing cultivars. For this reason, selection of tall and early maturing varieties, which can escape drought, is in conflict with the increase in straw quality required for arid areas, where straw represents an important feed resource (Susmel *et al.*, 1994).

Recently, breeders have engaged in 'reverse evolution', aimed at establishing barley as a permanent pasture crop: a) selecting for wild seed to enable self-regeneration of the pasture; b) replacing awns by hoods to increase palatability of barley hay (Hadjichristodoulou, 1997).

##### 4.2 Use of forage cereals and cereals as forage

When cereal yields are low, as in semiarid marginal areas, whole cereal crops such as barley can be used as forage for livestock, either for winter and spring grazing, or cut for hay at the end of the cycle (milky grain stage) for later use in periods of forage scarcity, such as winter. In Aragon, NE-Spain, several authors have evaluated the possibilities of cereals as forages. Joy and Delgado (1989) evaluating barley, oats and rye, measured winter yields between 200-400 kg DM ha<sup>-1</sup>, which reduced harvested grain and straw 20% and 30% respectively. When cut for hay in spring, forage yields were 2-3 t DM ha<sup>-1</sup>, rye being more productive in winter, and oats in spring.

Andueza *et al.* (2004) studied *in vivo* the nutritive value for sheep of hays made from barley, oats, rye, and triticale, and reported that daily intakes of oat and triticale hays (67.7 and 64.4 g DM BW<sup>-0.75</sup>) were higher than those of barley and rye (53.8 and 54.5 g DM BW<sup>-0.75</sup> respectively). Barley had the highest dry matter digestibility (0.69 DMD) and crude protein (8.02 CP), but gave lower intake values than oat and rye, which the authors suggest could be due to differences in palatability related to presence of awns.

Valiente (2003) investigated the use of whole barley crop as a sheep summer diet; animals consumed first the spikes (50% of the initial biomass), secondly the leaves and thirdly the straw. The barley crop maintained a stocking rate of 65 ewes/ha for 30 days, with liveweight gain of 100 g/animal/day, but

46% of the initial production was left as residual (80% straw and 20% leaves and spikes) when animals were withdrawn from paddocks.

#### 4.3 Organic farming

People living on marginal areas are confronted by several challenges: ecological sustainability (produce whilst preserving basic resources), feed survival (get enough food to feed the population living on it), and economical return (get a commercial benefit). Our modern, mechanized intensive agriculture is not a renewable resource because it consumes fossil energy and requires more work and energy per unit of food. Only a small part of land surface –deep fertile soils- is suitable for intensive agriculture; the rest of the land, which is better suited for range and forest and can be used by domestic ruminants that transform plant production that man cannot utilize (Harlan, 1975).

During recent decades, sheep rearing has gone through an intensification process that has given a negative selection of local breeds, as with ‘Segureña’ sheep, which lost adaptation to stress conditions and the capacity to transform feed resources of low nutritional value (Belmonte *et al.*, 1991). Thus a new selection process to recover robustness may be required before moving sheep flocks to extensive conditions, as proposed by the new EU-CAP.

Current EU policy on sustainable rural development promotes livestock systems adapted to local resources and environment, and oriented towards production of quality food; however, future predictions indicate that rural populations will continue their current decline and cereal production will evolve towards a competitive open market, as promoted by GATT agreements. With such a scenario, it seems logical that part of winter cereals, particularly barley, should be used for *in situ* consumption in extensive livestock systems.

Organic farming is an alternative to sustain sheep-cereal extensive systems in marginal Mediterranean drylands. In place of fertilizers and pesticides, organic farming relies on local biological resources: fertilizers vs. animal manure or legume cover crops; herbicides vs. animal grazing; confined animals vs. walking animals; medicine vs. plant’s medicinal effects. In summary, organic farming could offer consumers foods free of chemicals, environmentally friendly and better tasting. There is more hand labour in organic farming, but livestock are healthier and prices of animal products are usually higher; however, there is still a big gap in the technical-scientific knowledge of Mediterranean agro-ecosystems and its self regulating capacity (Fersino *et al.*, 2002) and hence, a lot of research is still needed (IFOAM-EU, 2004) before the economical, ecological and human sustainability goals of organic farming can be fully achieved.

#### 4.4 Introduction of woody forage species

To establish crop hedges and field margins in environmentally sensitive areas, could provide food and habitat for wild fauna and reduce soil erosion (Atkinson *et al.*, 2002). Similarly, introducing woody forage species as natural fences and as protein feed supplements in cereal cropping areas could improve feeding calendars, preserve biodiversity and protect soils.

Fodder shrub plantations (e.g., winter legumes *Medicago*, *Cytisus*, or summer ‘green’ C4 species *Atriplex*, etc.) can be used for several purposes: a) to create fodder banks for annual and inter-annual feed scarcity periods, b) as protein or mineral supplements to improve sheep intake of nutritionally deficient feeds (i.e., cereal straws, *Stipa* grasses, etc), c) to control soil erosion in cultivated areas with steep slopes, d) to provide refuge and feed to wild fauna, (Correal, 1993).

Perennial woody legumes, like tree medics (*M. arborea*, *M. citrina*) are a good option for winter-spring grazing. Cereal-*Atriplex* alley cropping with saltbushes planted in widely-spaced rows following contour lines provides an *in situ* protein supplement to straw/stubble and protects the soil against soil erosion during autumn heavy rains. In Morocco, Narjisse (2005) reported that a barley-*Atriplex* system gave 31% and 97% more grain and straw respectively than the barley-fallow system. In Spain, cereal farmers in Murcia (SE-Spain) planted a few thousand hectares of *Atriplex* (mostly *A. halimus* and *A. nummularia*) as feed banks or supplements for summer and winter periods, but in most cases, poor management reduced the life and success of the plantations, which were well grazed by sheep (in fact, were overgrazed). Sotomayor and Correal (2000) showed that dry sheep fed with a mixed diet of



*Atriplex* and cereal straw could be maintained during summer with significant increases in weight and body condition.

#### 4.5 Introduction of legumes in cereal fallows

Before the massive mechanization of agriculture, farmers had to cultivate forages of high feeding value as grain and hay legumes, and maintain cereal-legume fallows to produce enough feed for draft animals. Thus, in Seville (Spain), cropped land was rotated in a three year systems in which 1/3<sup>rd</sup> was cultivated with cereals, 1/3<sup>rd</sup> was fallow land, and 1/3<sup>rd</sup> was cropped with legumes (peas, beans or vetch); animals consumed cereal and legume stubbles, weedy fallows, the harvested straw and some of the harvested grain (Kayser, 1992).

With the advent of mechanization, most forage and pasture legumes were lost; however, fallow replacement with legumes for food, feed and pasture has been investigated by ICARDA (Jones, 1992). Grain legumes, such as lentils and chickpeas, had a certain degree of success in areas with good soils receiving more than 300 mm of rainfall, but annual pasture legumes in rotation with cereals were badly accepted by farmers. Approximately 350,000 ha of medic pastures were sown on North Africa and the Middle East during the 1970's and 1980's, mainly using imported seeds, but the results were far from being positive, and few of them are still functioning as the intended ley farming (Riveros *et al.*, 1989). In Morocco, the small farms size forced farmers to use high stocking rates, which reduced seed production and the regeneration of the self-seeding legumes (Jaritz, 1992). In fact, the majority of farmers in North Africa have a multifunctional concept of livestock rearing with the livestock being a source of revenue, nutrition, financial liquidity and providing status in society (Riveros *et al.*, 1989). Hence, they keep stocking rates far in excess of potential plant growth and any significant increase in animal feed is reflected in an increase in the animal population (Jaritz, 1992).

The ley farming system fits well to Australian conditions where properties and flocks are large, there are infrastructures to maintain sheep permanently grazing on the field (fences, watering points, etc), and flocks are stocked at reasonable rates, allowing high seed production and persistence of pasture legumes. However, such conditions do not exist in a majority of semiarid Mediterranean countries where attempts were made to introduce annual legumes, with the result that legumes are almost absent from cereal-fallow rotations.

#### 4.6 Annual forage calendars to match resources with sustainable stocking rates

The planning of annual feed calendars for livestock, combining all the possibilities offered by fodder resources such as cereal crops, fodder cereals, grain legumes, pasture legumes, and fodder shrubs can reduce grazing pressure on degraded rangelands and improve the efficiency of animals. Delgado *et al.* (2004) experimented in semiarid Zaragoza (NE-Spain) with a forage system based on the combined use of alfalfa, winter cereals and the forage shrub *Atriplex halimus*; paddocks were fenced, and forage resources rotationally grazed by a flock of local ewes. This continuous forage system maintained 2 ewes ha<sup>-1</sup>, and produced 1.2 lambs ewe<sup>-1</sup> year<sup>-1</sup> with one lambing year<sup>-1</sup>. Ewes lambed outdoors and lambs were kept permanently with their mothers until reaching a slaughter weight of 22-25 kg. Fálagan (1992) proposed a similar forage calendar using winter cereals, alfalfa and *Atriplex*.

The traditional system in Zaragoza is an alternate cereal-fallow producing an average of 1.8 t ha<sup>-1</sup> of barley; additionally, a stocking rate of 0.7 ewes ha<sup>-1</sup> is maintained with cereal by-products and other rangeland forage resources, but the system is in crisis because of scarcity of shepherds and the limited gross margins of farms. With a final product of 2.4 lambs ha<sup>-1</sup> year<sup>-1</sup>, the proposed forage system could economically compete with the traditional system and is also socially and environmentally more sustainable because of reduced needs for shepherds and labour, and because livestock are maintained in balance with forage resources and are less dependent on external inputs.

The feeding calendar for a majority of livestock-cereal mixed systems in North African and Middle East countries (Nefzaoui, 1999) is based on the following feed resources: rangelands, cereal stubbles, cereal fallows, standing barley (green or whole dry crop), cereal and legume straws, barley grain, wheat bran, crop residues, olive tree by-products (cake, leaves, twigs), and other supplements (cactus, *Atriplex*, etc.). The contribution of the resources mentioned is changing rapidly with the contribution from native

pasture and rangelands decreasing while the contribution from cereal grains, straw and crop residues is increasing.

## 5. Promotion of plant and animal diversity

Agricultural policy in Europe is changing from supporting production to encouraging environmental benefits in the context of sustainable rural development. Biodiversity may benefit from integrated farming techniques, such as sheep-cereal systems, but these need to incorporate environmental objectives explicitly, rather than as a fringe benefit. The loss of traditional crop rotations and the polarization of pastoral and arable farming has led to a marked reduction in mixed agriculture, and with it, a dramatic reduction in landscape diversity (Robinson and Sutherland, 2002). The intensification and modernization of cereal cropping has reduced the food supply for wild fauna because of: a) the removal of hedgerows and “rough patches”, b) the use of herbicides that eliminate weeds, c) suppression of fallow lands and d) earlier ploughing of stubbles (Newton, 2004).

Weeds are major constraints to crop production, yet they have a role within agro-ecosystems in supporting biodiversity, especially phytophagous insects and birds (Marshall *et al.*, 2003). Fields left fallow after harvest (i.e. stubble fields) support high wintering densities of many species of granivorous birds. In central England, seed abundance and area of bare earth were significantly greater on barley stubbles than on wheat stubbles (Moorcroft *et al.*, 2002). In S Portugal, studies on the diet of wild rabbits (*Oryctolagus cuniculus*) stress the relevance of cereals crops to increase the carrying capacity of ‘montados’ for rabbits (Martins *et al.*, 2002). In Spain, Verdú and Galante (2002) found positive relationships between density of rabbits, surface grazed by sheep and goats, and the dung beetle endemism index.

### 5.1 Arable weeds

Relations between arable field crops (cereals, legumes, etc.) and arable weeds are a very old process handled by men. Many weeds may be consumed as wild food and weeds are a source of medicinal products and potential sources of nutraceuticals (Rivera *et al.*, 2005) with some weeds providing animal self-medication (Engel, 2002).

Many countries of central Europe and the UK (Sprenger *et al.* 2002; Sutcliffe and Kay, 2000) have a red list of endangered plant, where arable weeds represent about 20% of the wild flora targeted for conservation. According to Waldhardt, *et al.* (2001), in Central Europe’s marginal cultivated landscapes, the seed bank of arable-land weeds is depleted after cultivation is abandoned and largely exhausted after only ca 20 years; on the other side, several aggressive weed species have increased markedly over the past 30 years, mostly because of the increased nitrogen input from intensive techniques.

### 5.2 Steppe birds

The conversion of forest lands to pastures and cereal crops produced a transformation of the territory and the appearance of spaces similar to secondary steppes and pseudo-steppes (Tucker and Dixon, 1997). About 60% of endangered birds in Europe live on these steppe habitats, and the areas where cereal-sheep mixed systems are present are the most important habitat for the preservation of steppe birds (Suárez *et al.* 2005). In Europe, the largest steppe area is in the Iberian Peninsula (about 17 mill. ha). Of all steppe birds, the great bustard (*Otis tarda*) and the little bustard (*Tetrax tetrax*) are the two species most threatened. Both species are classed as ‘endangered’ under current IUCN conservation criteria, with 50% of the world bustard population found in the Iberian Peninsula. The measurements suggested to protect steppe bustards are: to maintain fallows and their rich flora; preserve or create borders and living hedges; eliminate herbicides and pesticides, fertilize with organic manure; use native seeds; and maintain traditional cropping cycles (Alonso *et al.*, 2003).

### 5.3 Cereal land races and old cultivars

In Spain, Gadea (1954) made a cereal inventory through all the country, finding over 200 landraces of several wheat species. The provinces with greater diversity were Asturias, Murcia, Albacete, Cuenca and Eastern Andalusia, provinces where sheep-cereal mixed systems did exist; on the contrary, other Spanish provinces with better conditions for cereal production (e.g. Castilla-León), had lower levels of agro-diversity.

A recent ethnobotanical study in the province of Albacete catalogued 28 local races of wheat, but five less than in 1954 (Fajardo *et al.* 2000). Conservation of old cereal cultivars could be of great value for future breeding of cereals as forage for livestock.

### 5.4 Local animal breeds: sheep and goats

In the mountain areas of Italy, France Spain and Portugal almost half (47%) of the breed races present in Mediterranean countries are localized with the Alps-Pyrenean axis being like a hot spot for ovine diversity (Mason, 1967; MAPA, 1985). A particular example is the *Merino* ewe (origin in Spain and Portugal), whose diversity has been multiplied through all European countries, South America, Australia and New Zealand. Other important racial groups are the *Awassi* (with fat tail), from the Middle East, and the *Barbarine*, from North Africa. More than 200 ovine races and a smaller number of goat races are distributed in the Mediterranean area, occupying a large diversity of ecological niches. At least 80% of these races are bound to agriculture by-products for its feeding. Unfortunately, the mounting extinction rates among domestic breeds are diminishing the genetic diversity on which adaptability to marginal conditions and future breed improvement might depend (Luikart *et al.*, 2001).

## 6. Conclusions

The sheep-cereal farming is an original Mediterranean system which probably appeared in the Middle Age, during a critical economical situation, as a diversification response to reduce risk and optimize food and feed production.

Sheep-cereal systems survive better on marginal dry land, where cereal yields are low and animal production is economically more interesting; cereals are consumed *in situ* as animal feed in its different forms.

The winter cereal-stubble-fallow system maintains a cereal-steppe landscape where an important part of Mediterranean flora and fauna, especially steppe birds, depends on the habitat and feed resources generated by stubbles and fallows.

Mixed sheep-cereal systems are restricted by their low profitability and the poor integration between agriculture and livestock activities, which limit their economic, ecological and social efficiency.

The practise of sheep-cereal organic farming in marginal areas could not only help the preservation of local cereal races and local sheep and goat breeds, which are better adapted and more productive under difficult conditions than selected races and breeds, but also be justified by the originality and quality of their final products.

The preservation of extensive production systems adapted to the environment, and the quality products, biodiversity and landscape associated with them, are reasons that justify their maintenance in Mediterranean marginal areas. However the search for economic and ecological solutions for Rural Development in these territories, is most crucial to avoid human desertification, a possible scenario that would make unviable their preservation.

Seasonal fluctuation of animal nutritional requirements and forage and pasture resources must be matched in an optimal way. However, even if fodder gaps exist, there are possibilities to improve the system by approaches such as creating fodder banks or hedges with forage shrubs, which can also support biodiversity, or introducing infrastructures like fences, water points and animal shelters in part of the farmland, to maintain animals grazing permanently during long periods, and thus, reduce labour cost and shepherd's demand.

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# Session 1

## Overcoming seasonal constraints to forage production





# Overcoming seasonal constraints to production and utilisation of forage in Europe

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## Abstract

Climatic zones in Europe range from semi-arid in the Mediterranean basin to polar and so intensity of seasonality in forage production differs widely, influenced mainly by temperature and soil moisture. Management systems which overcome seasonal limitations vary widely. Forage may be conserved at times of excess supply, whether of grass or crops, for feeding stock in times of undersupply, principally as silage and hay. Seasonal forage growth curves can be manipulated by the use of fertilizer (mainly nitrogen), by irrigation, by selection of appropriate species and varieties, including development of secondary grasses and legumes, and by grazing management. Alternative forages or feeds such as maize, cereals, brassicas, a range of crop byproducts, and fodder trees and shrubs (in some regions) are valuable complements to grassland forage. Management systems including adjustment of stocking rates to reflect more closely grass growth and forage supply and their efficient integration, or changing stock reproduction cycles, contribute to overcoming seasonality. The additional constraint of minimizing adverse environmental impact in any strategy to overcome seasonality is acknowledged and some possible means of utilizing the feed resources to minimize nitrogen and phosphorus losses are considered. It is concluded that while decision support systems are useful in aiding farmers to make decisions to deal with seasonality problems, more information is required on the true costs of producing grassland forage and complementary feed alternatives to ensure options can be intelligently chosen.

Keywords: climate, growth curves, alternative feeds, conservation, grazing, environment.

## Introduction

Seasonality of production of grassland and forage in Europe is primarily influenced by temperature and soil moisture which limit the length and determine the intensity of the growing season. In most of Europe, temperature dictates the main seasonal trends in herbage growth but, in southern and eastern Europe in particular, summer trends are conditioned by the availability of soil moisture. Excluding the countries in the Mediterranean Basin, Topp and Doyle (2001) have divided Europe into eight agroclimatic zones, based on spring temperatures and summer rainfall/potential evapotranspiration, each zone having distinct seasonality. For example in the zone with the mildest winter, western Brittany has a mean average air temperature of about 6°C or above for all 12 months of the year in contrast to Rovaniemi in Finland, in the north east of Europe, where on average only 4 months have mean temperatures at 6°C and above, the remaining months averaging -4°C (<http://www.climate-zone.com/climate/>). The balance between precipitation and potential evapotranspiration during the growing season, an indication of the likelihood of drought adversely affecting growth, is almost 100 mm in deficit for Debrecen in Hungary compared to 200 mm in surplus at Dublin in Ireland.

In southern Europe, while all of the area is characterized by warm to hot summers mean monthly average temperatures for June to August typically approach 25°C in southern areas, and winter temperatures vary from mild to cool. For example in Italy around Milan the mean monthly temperature from November to February is about 4.5°C in contrast to Cagliari in Sardinia of 10.5°C for the same period. In these southern European countries annual rainfall varies between 400 and over 1000 mm with

potential evaporation in the most southerly areas exceeding 1200 mm, and so soil moisture deficit is severe in much of the area during summer.

### Seasonality in grass production

Examples of seasonal grass growth curves of timothy (*Phleum pratense* L.) grown under similar management at a range of sites throughout northern Europe are presented in Figure 1 (adapted from Corral, 1984). They highlight the long growing season in the south west of Ireland in contrast to the short season in Iceland and while the growing season in southern Finland is relatively short, growth rate in spring is rapid. The adverse impact of summer drought in continental Europe, in this case the Netherlands, is also highlighted. So, unless animal requirement mirrors herbage supply, herbage will be in surplus in some periods and deficit in others relative to immediate animal requirement.

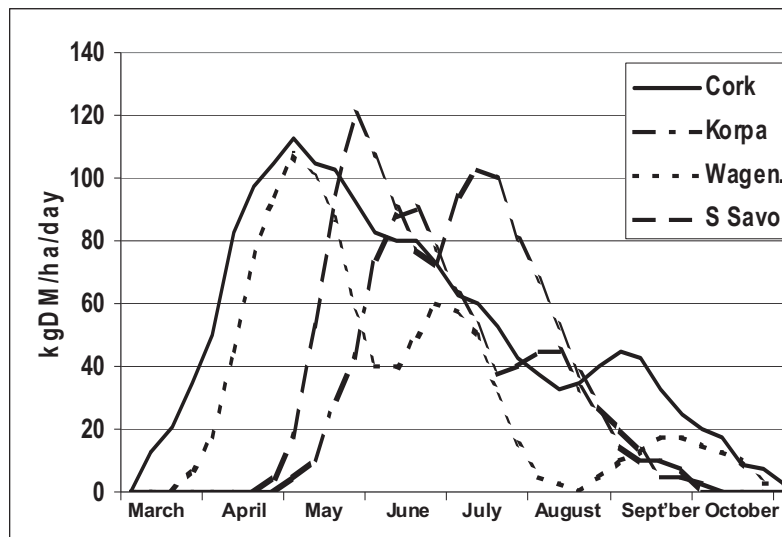


Figure 1. Daily growth rates for timothy cultivar Kämpe II (mean of 2 years) at Cork (Ireland), Korpa (Iceland), Wageningen (Netherlands) and South Savo (Finland) (From Corral, 1984).

A stylized growth curve of grassland production rate in semi-arid or warm Mediterranean areas shows the consequence of the excessively high evapotranspiration rate in summer during which there is no growth (Figure 2). This is followed by a small autumn peak as growth resumes with onset of rain. Low temperatures in early winter reduce growth but in late winter and spring rapid growth ensues until soil moisture deficit severely reduces growth (Papanastasis and Mansat, 1996).

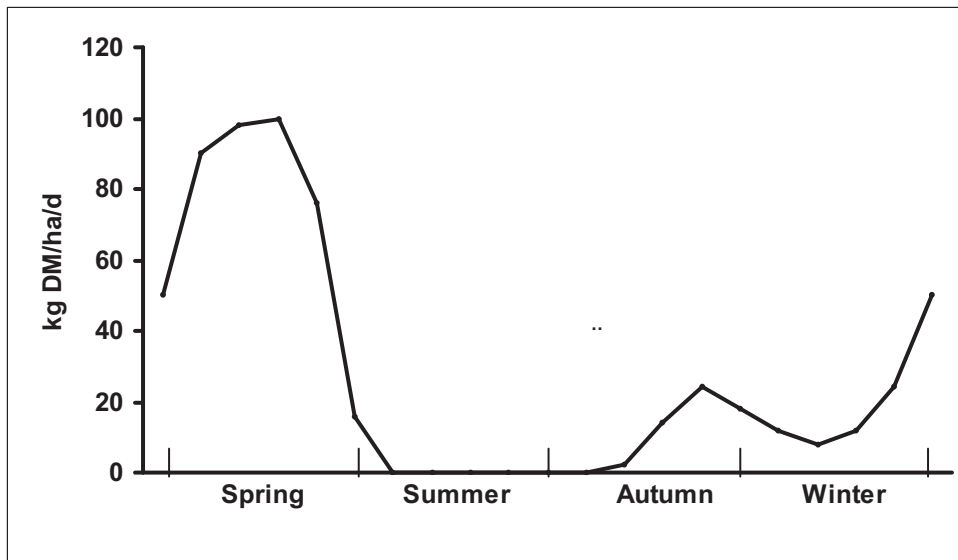


Figure 2. Diagrammatic growth curve of grass production in semi-arid or warm Mediterranean area (adapted from Papanastasis and Mansat, original from Olea *et al.*, 1991).

The requirement for feed for ruminants does not usually correspond to forage availability as outlined in the above growth curves. Therefore systems have had to be developed which 1. reduce demand for feed by adapting the animal production system to meeting the limitations in supply of fresh herbage from a given area, e.g. manipulation of the reproductive cycle of the livestock; transhumance is another more extreme example, or 2. require non-forages (such as grains or arable crop byproducts) to be fed or 3. require forages which grow during the gap in normal grass growth to be included or 4. retain excess herbage at times of oversupply to be fed during gaps (In this last case, herbage or forage can be retained either by conservation or by deferring its use for grazing (stockpiling)) or 5. manipulating the growth curve, e.g. by fertilizer nitrogen use to reduce imbalances in forage supply. Developments in the application of options 3–5 have been reviewed, particularly in production systems in the British Isles and Spain, by Frame *et al.* (in press). Often, more than one of these strategies is adopted within the one system to synchronise forage supply and demand. Of all of these strategies, conservation of herbage (either as surplus or to preserve a forage crop specifically grown) for silage or hay is probably the most widely adopted method to reduce seasonality throughout most of Europe.

### Conservation

Although the aim in silage making is to conserve herbage at as high a quality as grazed fresh grass, the quality of the grass at harvest and management from cutting until feeding, including all of the preserving/ensiling processes, are variables which prevent that aim being attained (Mayne and O’Kiely, 2005). The main method of storage is by sealed clamps but wrapped big bale silage has become popular for both large amounts, for which silos represent a significant capital cost, and for small amounts when there are temporary surpluses of grass growth. Systems based on big bale silage have proved particularly suitable for small holders in all parts of Europe, and the operation can be contracted out or the holding can belong to a cooperative machinery ring.

While grass and forages may be grown specifically for grazing or conservation, as discussed later, often good management dictates that the same sward be grazed and cut depending on demand for grazed herbage at specific times. So decisions need to be taken as to how much and when the sward in whole or

part should be closed off for conservation. Feed budgeting, which involves balancing animal requirements and grass supply on an annual, intermediate and short term (immediate) basis, provides a framework for taking decisions on the management of grassland (Mayne *et al.*, 2000).

During periods of low pasture growth decisions on how animals are to be fed have to be taken. While conserved herbage is an option, some systems rely heavily on bought-in feed (supplements) which may be calculated to be cheaper than home produced forage. In Northern Ireland, comparing a dairy production system which relies on high quality silage, high allowance of grazed grass and moderate amounts of concentrates with a system comprising tight grazing, medium quality silage and dependence on a high concentrate level, Ferris *et al.* (2003) found that overall animal performance was similar but stocking rate in the latter allowed one third more cows to be kept on the same area. While it may be inferred that the latter is the more cost effective system, when the economics of dairy production are considered in relation to the reliance on grass in the diet, Dillon *et al.* (2005) in a worldwide survey calculated that for every 10% increase in the diet comprising grass, the cost of producing 1 litre of milk declined by about €2.7. Some studies calculate grazed grass to be substantially cheaper (e.g. Wilkins *et al.*, 1999) or only slightly cheaper (e.g. Keady *et al.*, 2002) than high quality grass silage and alternative forages. Apparent lack of unanimity in methods of calculating relative costs of grazed and conserved grass demonstrates the complexity of whole systems management when dealing with seasonality and also points to the need for more detailed studies using standardized methodology.

For many years there has been little hay conservation research in Europe in spite of its important role in small farms in central and southern Europe. However, the tenets of good haymaking are well established (McCartney, 2005). Losses during harvesting, storage and feeding were the traditional causes for its replacement by silage, certainly in northern Europe. Harvesting in southern Europe where hay crops may or may not be grown under irrigation, is less of a problem because of long rain-free periods. Some countries e.g. Spain and Italy, produce lucerne pellets dehydrated by a combination of field and artificial drying; these pellets are then used for a protein rich feeding supplement when grass for grazing is inadequate. Silage from maize (*Zea mays* L.), grass or cereal/vetch (*Vicia* spp.), arable byproducts and hay are options for use as a 'buffer' feed to assure adequate forage intake and animal performance. The use of buffers can also prevent overgrazing and so avoid jeopardizing pasture persistence and vigour.

Current practices and developments in forage conservation, ensiling in particular, have been covered recently (Park and Stronge, 2005) and so will not be considered further here. However one development in plant breeding which has direct relevance to ensiling but also to animal production generally is the breeding of increased water soluble carbohydrate (WSC) content in perennial ryegrass which has potential to improve ensilability and nitrogen efficiency in the rumen, the latter has both financial and environmental implications. Difference in WSC content in trials between a high WSC cultivar and control were higher in Norway (12%) than in Ireland (7%) and differences between the cultivars were less marked at a high rate of N fertilization (O'Kiely *et al.*, 2005).

## **Manipulation of the seasonal herbage growth curve**

### **Use of fertilizers**

Producing herbage at a time when it is most likely to be needed is a major function of fertilizers, especially nitrogen, and also especially if an extended grazing strategy is to be adopted. In Ireland Hennessy (2005) has shown that it is possible to control herbage availability throughout the season by adjusting the distribution of nitrogenous fertilizer applied over the season. Applying only 20% of the annual total up to May (in contrast to the conventional 60%) and the remainder thereafter, herbage available in autumn was increased at the expense of spring-early summer herbage but total annual production was not affected. In Norway, in a two cut silage system, only at the higher annual rate of N application was there a slight advantage in favour of half or 60% being applied to produce the first cut (Lunnan and Nesheim, 2002). The growth curve of grass/white clover (*Trifolium repens* L.) swards can be altered by applying nitrogen fertilizer in spring i.e. when clover contribution to production is low. Grass growth is promoted without unduly adversely affecting clover contribution later in the season (Frame and Paterson, 1987). In semi-arid areas, water supply is a necessary adjunct to efficient use of fertilizers, N and supporting nutrients, in manipulating herbage supply and quality.

All aspects of the role and use of fertilizer N and other nutrients on grassland have been reviewed by Whitehead (2000). However, there are increasing limitations on N use in EU countries, due to CAP reforms, which have specific restrictions on N inputs to land. With current uncertainties over oil supplies and price which affect the energy cost required for N fertilizer manufacture, and therefore its cost to farmers, N-fixing forage legumes have undergone substantial reappraisal. As a result it is evident that they will play a more important role in the N economy and sustainability of European and indeed world grasslands (Frame, 2005; Frame and Laidlaw, 2005).

### Use of irrigation

Drought may depress grass growth during the growing season. Options to overcome this shortage of herbage include irrigating or feeding supplements e.g. silage. In northern Europe, soil moisture deficits (SMD) of 70 or 80 mm are not uncommon during mid-late summer and this can represent a reduction in potential growth of 50% or more. Herbage growth is usually reinstated quite soon after SMDs fall. In central and eastern parts of the region, e.g. the Netherlands, irrigation in intensively managed grassland farms is practised although economics of cost of irrigation and response of the grass or forage crop and also the environmental implications of large scale water usage need to be taken into account.

In semi-arid regions irrigation can increase forage yields 5- to 8-fold and for example production of 10-16 t DM ha<sup>-1</sup> has been achieved (Olea *et al.*, 1990). In trials in southern Spain tall fescue (*Festuca arundinacea* Schreb.) proved particularly suitable because of its productive persistence in mixture with white clover (*Trifolium repens* L.) or lucerne (*Medicago sativa* L.) though a lucerne monoculture was even more productive (Ratera *et al.*, 1977). Lucerne is the most popular species for irrigation on the more fertile land (MAPA, 2003) and is mainly hayed or dehydrated for onward sale. With irrigation, the growing season in north-east Spain can extend from early March to the end of October and achieve maximum growth rates of over 90 kg DM ha<sup>-1</sup> day<sup>-1</sup> in June and an annual yield of 11.5 t DM ha<sup>-1</sup> (Delgado *et al.*, 2004). In the Cantabrian Atlantic region, a 5-year annual increase of 37% was attained by summer irrigation (Martínez and Piñeiro, 1994).

### Species and varieties

In Europe, the number of bred varieties registered in the European Catalogue has increased in recent years, for example, from 857 in 1989 to 1594 in 1999 (European Commission, 1999 cited by Peeters, 2004). The most numerous were 565 for perennial ryegrass (*Lolium perenne* L.) and 237 for Italian ryegrass (*L. multiflorum* L.), together representing 50% of the total. Numbers of other species such as tall fescue, cocksfoot (*Dactylis glomerata*, L.), timothy, hybrid ryegrass (*Lolium x hybridum* Hausskn.) and meadow fescue (*Festuca pratensis* Huds.) ranged between 106 and 45. Several mainly secondary species were within the range 33 to 1 making up only 7% of the total. Clearly, this situation in Europe whereby selection by breeders has been concentrated on relatively few species is not ideal; conversely it represents breeding opportunity for the future. The two main ryegrasses above accounted for 83% of the amount of grass seed sold for forage production in the recent past while meadow fescue, cocksfoot, tall fescue and smooth meadowgrass (*Poa pratensis* L.) made up 17% (Kley, 1995), a situation which is not likely to have changed much currently.

Species differ in their annual growth pattern as do cultivars within species. The prevalence of grass species sown in intensive grassland varies throughout Europe. For example in western Europe perennial ryegrass is widely grown while in Nordic countries timothy and meadow fescue are more prevalent as they can withstand lower winter temperatures than perennial ryegrass. In Norway smooth meadowgrass is commonly sown, in mixture with other species, for the same reason. However the supremacy of these species is being challenged, especially as silage crops. In Finland tall fescue produces more dry matter in the cuts subsequent to the primary cut than meadow fescue, although its nutritive value under these conditions has still to be determined (Niemeläinen *et al.*, 2001). If nutrient levels applied to intensively managed grassland fall as predicted due to current implementation of the CAP reforms areas the current most appropriate species may need to be re-evaluated for lower input conditions and some species hitherto regarded as secondary need to be re-assessed (Peeters, 2004). He nominated three species for priority breeding: tall oat-grass (*Arrhenatherum elatius* L. Beauv. ex J. & C. Presl), red fescue (*Festuca*

*rubra* L.) and Yorkshire fog (*Holcus lanatus* L.). Compatibility with legumes in mixture will also assume greater importance.

Maturity class of varieties of grass species sown influences the seasonal growth curve. Perennial ryegrass varieties are classified according to their date of heading. Maximum growth rate is reached during the reproductive phase and so early heading varieties can reach their maximum 4 to 6 weeks earlier than the latest varieties in the late heading group and post-flowering depression is usually more pronounced than for late heading types (Laidlaw, 2005). In a dairy production trial, yield and digestibility in early summer were lower in swards containing intermediate than late heading cultivars (O'Donovan, 2001).

While components of mixtures in long term swards may behave unpredictably, depending on which component is favoured, in short term leys all of the components may make a predictable contribution. For example, mixing barley (*Hordeum vulgare* L.), Italian ryegrass and legumes for a short term organic ley receiving compost and rotationally grazed by dairy cows, the dominant species is barley for the first two grazings after which a balance is maintained between the ryegrass and legumes (Kuusela *et al.*, 2004).

Plant breeding has contributed to meeting the problem of low herbage growth in early spring. White clover tends not to grow as early in the season as perennial ryegrass or any of the other commonly sown temperate grasses. However, the white clover variety AberHerald has also been bred to grow earlier than others in its peer group and shown to do so in a range of northern European environments (Wachendorf *et al.*, 2001). Breeders also take account of overcoming environmental stress in breeding programmes which contribute to seasonality in forages. Freezing temperatures, snow cover, low light, heat, drought, anoxia (due to ice encasement), and flooding are all potentially stress-inducing factors which influence long term growth patterns of forages (Wilkins and Humphreys, 2003). Although attempts to produce more winter hardy ryegrasses by hybridizing perennial ryegrass with meadow fescue to produce festuloliums have not been successful when tested under Nordic conditions (Nesheim and Brønstad, 2000), hybrids between Italian ryegrass and tall fescue are showing more promise (L. Nesheim, unpublished data).

In addition to the obvious advantages in mineralizable soil nitrogen content which legumes confer, mixing grass and white clover can be advantageous in reducing the adverse impact of post flowering decline of perennial ryegrass in dry matter production and in digestibility. White clover's seasonal growth pattern reaches a maximum around mid-summer and so the seasonal growth curve of the mixture is likely to be more uniform for most of the summer than for the grass component on its own (Frame and Paterson, 1987). A database of the most common temperate forage legumes grown in northern Europe has been compiled from 330 trials carried out over 20 years. Analysis of the data confirms the higher yield potential of red clover (*Trifolium pratense* L.) and lucerne (*Medicago sativa* L.) than of white clover, birdsfoot trefoil (*Lotus corniculatus* L.) and galega (*Galega orientalis* Lam.) across northern Europe (Halling *et al.*, 2004). See also the review by Peeters *et al.* (2006) in these proceedings. Notable legumes used in southern Europe include lucerne, red clover (*T. pratense* L.), white clover and sulla (*Hedysarum coronarium* L.) and of course a range of annual legumes from *Trifolium* and *Medicago* spp. in particular. Annual species, including Italian ryegrass and cereals, assume increasing importance with increasing length and intensity of the summer drought. Conversely, with a less severe climate, the use of perennial species increases, whether in the natural pastures or in sown swards. Lucerne and Italian ryegrass are popular sown species for potential high production under irrigation. Tall fescue and cocksfoot of Mediterranean origin are two relatively drought resistant grass species, *inter alia* because of their ability to develop a deep rooting system and in the case of cocksfoot, dehydration tolerance in surviving tissues and the ability of roots to extract water at low soil water potentials (Volaire and Lelièvre, 2001). However, cocksfoot has become less popular in many parts of Europe because of the nutritive value and stock acceptability shortcomings (Peeters, 2004).

Some plant species which are weeds on cultivated land are also part of the grazing resource (Porquedda *et al.*, 2005). Garland (*Chrysanthemum coronarium* L.) is a spontaneous species in Mediterranean grassland acceptable to animals (but classed as a weed in grain cereal crops). Preliminary work suggests that it may also have potential for ensiling (Valente *et al.*, 2003).

The use of deep rooting herbs such as chicory (*Cichorium intybus* L.) is likely to increase. The re-introduction of potentially productive legume species from Australian breeding programmes based on

Mediterranean plant collections is well documented. However, there can be potential genetic erosion of existing adapted landraces in such situations (Rebuffo and Abadie, 2001) and imported cultivars do not always outperform local ecotypes (Falcinelli *et al.*, 1993).

### **Grazing management**

Growth curves of rotationally grazed or intermittently cut swards tend to reach a peak at flowering, followed by a trough or a plateau lower than the peak while the growth of set stocked swards is less extreme (Parsons and Chapman, 2000). Management at specific time of the year may also influence the growth and quality of the sward later. Grazing in early spring in Finland can have a beneficial impact on the growth and quality of swards later in spring and summer in rotationally grazed swards and swards are more easily managed later in the season (Virvajärvi *et al.*, 2003). Provided stocking rates are not extreme in spring, early turn out (March in Ireland) results in higher grass utilization and milk yield (about 1 cm lower post grazing height and 1.5 l cow<sup>-1</sup>, respectively) than turning out in mid April (O'Donovan and Delaby, 2004).

While in northern Europe growth during winter contributes little to herbage production, in some areas in Mediterranean regions, especially the most southerly, this period may account for most of annual herbage production. Although very little herbage is likely to be produced in winter grazing may extend beyond the growing season in western Europe in early winter and late winter-early spring where climate and ground conditions allow. This can be achieved by allowing herbage in autumn to accumulate and by deferring grazing until later, usually by extending the rotation by introducing swards which would otherwise have been cut in late summer/early autumn for silage (Laidlaw and Mayne, 2000). The amount of herbage which can be saved for out-of-season grazing is limited as rapid death of leaves and tillers in heavy stands reduces the amount of green herbage, although the limit rises with length of growing season (Hennessy, 2005). Guidelines introduced into Europe from New Zealand to minimize damage to swards in winter are summarized by Mayne and Laidlaw (1995).

Allowing cows access to grazing for 2-3 h d<sup>-1</sup> in early winter or turned out in early spring in Northern Ireland increased milk production (more than 2 l cow<sup>-1</sup>day<sup>-1</sup>) and reduced silage consumption (2.5-4 kg DM day<sup>-1</sup>) compared with those continuously housed (Mayne and Laidlaw, 1995; Sayers and Mayne, 2001). Turning out steers to grass in mid March for finishing before slaughter in summer produced a 7 kg advantage in carcass weight relative to those remaining indoors until early May on a grass silage diet before turnout (Steen, 2002). These examples demonstrate that in areas in which ground conditions will sustain grazing, benefits to animal performance can be accrued from utilizing grass by grazing, and manure accumulation during the winter housing period is reduced.

In regions with a Mediterranean or warm temperate climate, annual legume-based swards, e.g. subterranean clover (*Trifolium subterraneum* L.), which have a life cycle adapted to escaping summer drought, are an important resource. The swards are hard-grazed in winter but then leniently grazed in spring so as to permit seed production, the seed burrs being embedded or buried in the soil. The subsequent sward density and forage production depend on adequate seeding density which comes from current production together with seed reserves from the soil seed bank (see Porqueddu and Gonzalez, 2006, these proceedings for a review).

In mild winter Mediterranean conditions grazing lucerne with sheep has very little impact on spring production and improves nutritive value (Chocarro *et al.*, 2005). Lloveras *et al.* (1998) also noted that a late-autumn harvest of lucerne in Mediterranean conditions had little effect on the yield of the first spring harvest or on total annual yield. However, it is well documented that a late autumn rest period prior to dormancy is required in colder climates to ensure winter survival.

### **Alternative forages**

Some farming systems are unable to produce sufficient or consistently maintain high quality conserved grass. So alternatives are grown to contribute to meeting animal requirement. These can be used to provide a) fresh forage in autumn and winter, b) buffer grazing feed during the growing season when grass growth or quality are poor i.e. to bridge the summer gap or c) a conserved forage fed as a

substitute or, more commonly, as a supplement to grass and other constituents of a winter feeding programme (Leaver, 1990).

### Forage crops for grazing or feeding fresh

Brassica crops are the most common alternatives grown for grazing in many parts of northern Europe of which kale (*Brassica oleracea* L. ssp. *acephala*) is the highest yielding under English conditions producing about 9 t DM ha<sup>-1</sup> with a metabolisable energy content of about 12 MJ kg<sup>-1</sup>DM, followed in order of DM production by swedes (*Brassica napus* L. var. *rapifera*) (7 t DM ha<sup>-1</sup>, ME about 13.5 MJ kg<sup>-1</sup>), forage rape (*Brassica napus* L. ssp. *oleifera*) (t ha<sup>-1</sup>, ME about 10 MJ kg<sup>-1</sup>) and stubble turnips (*Brassica rapa* L. var. *oleifera*) (4 t ha<sup>-1</sup>, ME 12.5 MJ kg<sup>-1</sup>) (Ingram, 1990). So they compete favourably with grass silage as a source of ME. These are utilized at times when grass quantity or quality is low which in northern Europe is usually in autumn and winter. Of these, turnips and rape are usually grazed in autumn having been sown in early summer while kale and swedes are harvested and fed in autumn and early winter as are the two high yielding and high energy forages in the family Chenopodiaceae i.e. fodder beet and mangolds (both *Beta vulgaris* L.). Rye (*Secale cereale* L.) or triticale sown in autumn can produce 2 t ha<sup>-1</sup> of utilisable herbage in early spring. All of these crops, especially the brassicas and rye, have the capability to grow at relatively low temperatures and so are suited to the extremities of the growing season in cool temperate conditions. This also applies to early sown turnips offered to dairy cows during late summer in dry periods when grass is scarce. Utilisation of forage crops grazed, or even harvested, in late autumn and winter is often difficult especially on heavy soil and the high labour demand of root forages is a disincentive to their wide usage, despite their high energy content.

In southern Europe fast growing fodder crops are a valuable complement to grazed pastures. Cereals feature strongly and, for example, in Sardinia local landraces of oats (*Avena sativa* L.) and barley are winter grazed prior to allowing the crops to ripen for grain while forage brassicas e.g. stubble turnips, provide late summer/autumn feed (Porqueddu *et al.*, 2005). In some areas densely sown maize may be cut at the vegetative stage in summer and fed to stock. Byproducts of processing industries e.g. olive, grape, citrus, cottonseed, are available as feed in some localities (Seligman, 1998). The importance of successfully integrating available feed resources of pasture, cereal, byproducts and annual forage crops cannot be overemphasized (Talamucci and Pardini, 1999).

Performance of animals grazing brassicas, especially root crops, can be variable. This has been ascribed to variability in the efficiency of utilization of the standing crop, due to ground conditions, and variably high contents of glucosinilates which can have an adverse effect on intake and of S-methyl-cysteine sulphoxide (SCMO) which reduces the efficiency of utilization of ingested dry matter (Milne, 1990). Inclusion of brassicas, especially the leafy types, is limited when fed to cattle and dairy cows (usually a maximum of about 5 kg DM head<sup>-1</sup> day<sup>-1</sup>) to ensure that potential animal health problems are avoided. Grazing brassica crops in early winter may be environmentally disadvantageous as, in a study of nitrate losses in a dairy system in southern England, Allingham *et al.* (2002) recorded very high losses when the cows were grazing turnips in early winter.

### Fodder trees and shrubs

Several species of fodder trees and shrubs are a valuable source of animal fodder via grazing, cutting or browsing in the summers of semi-arid regions (Papanastasis *et al.*, 2006). Examples are the 'Dehesa' system in western Spain or the 'Montado' system in Portugal whereby oak trees (*Quercus suber* L. and *Q. ilex* ssp. *rotundifolia* (Lam) Schwarz ex Tab. Mor.) provide leaves and small branches for cattle feed and tagasaste (*Chamaecytisus palmensis* (Christ.) Hutch and *C. proliferus* Link.), which originated in the Canary Islands and whose use has spread elsewhere (Frame, 2005). Black locust (*Robinia pseudoacacia* L.), amorphia (*Amorpha fruticosa* L.), retama (*Retama sphaerocarpa* (L.) Boiss) and the tree medic (*Medicago arborea* L.) are valuable leguminous shrubs. Some species suggested for further investigation (Porqueddu *et al.*, 2005) are *Atriplex* spp., *Salsola vermiculata* L. and *Opuntia ficus-indica* (L.) Miller. Most authors indicate that substantially more research is needed concerning establishment, management, and nutritive value in particular, because of various anti-quality factors such as excessive



tannin or salt content. In more temperate areas there is scope for greater use of trimmings from poplars (*Populus* spp.) and willows (*Salix* spp.) (see New Zealand review by Charlton, 2003).

### **Forage crops for conservation**

The most prevalent role of forage crops is as an ensiled crop fed in conjunction with grass or grass/clover silage. Forage or ensilable arable crops which can grow and develop under temperate or cool temperate conditions include whole crop cereals, vetches (*Vicia* spp.), forage peas (*Pisum sativum* L.) or field beans (*Vicia faba* L.), cereal/bean or cereal/pea bicrop, and cereal/kale. Even as far north as central and eastern Norway, field beans, peas and vetch produce acceptable yields (Nesheim and Bakken, 2004). Crops for Mediterranean conditions include some of the above species such as cereal/vetch mixtures (e.g. Caballero *et al.*, 1995) and peas, but maize is of major importance. In the search for potential alternative crops, saffron (*Carthamus tinctorius* L.) for silage or hay is a promising example for adoption as a cattle feed (Landau *et al.*, 2004)

The highest yielding of the cereal crops for forage in the more southerly and western countries of Europe is winter wheat (*Triticum aestivum* L.) which is capable of producing about 15 t DM ha<sup>-1</sup> under British conditions (e.g. Weller *et al.*, 1993). It is ensiled if harvested at DM contents of up to 40% while more mature crops are preserved by treatment with urea. Other ensiled whole crops which offer possibilities to improve intake and animal performance include cereal/legume and barley/kale bicrops. Although the higher energy and starch content in whole crop cereal provides a potentially complement to grass silage and often increases intake, it does not always result in increased animal performance (Keady, 2005).

The benefit of forage maize exploited in southern Europe for many years has gradually extended north due to the development of early maturing hybrid maize varieties. While high yields can be sustained under conditions in southern Europe, covering the seed bed with a plastic mulch at sowing to promote early establishment usually results in earlier maturation, and higher yields (by 3-4 t ha<sup>-1</sup>) and starch content (by up to 50%) in more northerly environments (Easson and Fearnhaugh, 2003). Despite these advances risk of failure still limits the areas in northern Europe in which it can be exploited currently. While maize can be grown as far north as Norway, it is limited to very specific milder climatic areas of the country (Bakken *et al.*, 2005).

Feeding maize silage with grass silage generally increases DM intake, milk yield and milk protein content although benefits decline when harvested at a very mature stage, the optimum dry matter content being about 300 g kg<sup>-1</sup>DM. In a recent review of the value of maize silage for beef and dairy cattle Keady (2005) concludes that it has a concentrate sparing effect of up to 5 kg cow<sup>-1</sup>day<sup>-1</sup> and is as cheap to produce and use as grazed grass. However, these costs depend on the assumptions made and include Arable Aid which is less relevant under EU Single Farm Payment.

### **Environmental implications of overcoming seasonality**

#### **N and P losses during extended grazing**

Although in northern Europe, grazing is considered as the norm for utilization of grass in summer, it is a major source of nitrate leaching, especially when intensive (e.g. Cuttle and Jarvis, 2005). With regard to extended grazing, while Laidlaw *et al.* (2000) did not detect an effect on soil nitrate levels by dairy cows grazing for 2-3 h each day throughout November, Webb *et al.* (2005) calculated that extending the grazing season by 30 days on dairy farms receiving 250 kg N ha<sup>-1</sup> during the grazing season increased nitrate-N leaching by 3 to 10 kg ha<sup>-1</sup> meaned over total grazed and slurry-applied area. These calculations were based on models of N mass balances and data from five dairy farms in England, on a range of soil types, subject to different managements.

Similarly, phosphorus losses over winter increase progressively with delay in housing. McGechan (2003) has calculated that loss is approximately 0.2 kg inorganic P ha<sup>-1</sup> month<sup>-1</sup> delay in housing, depending on extent to which soil is at or close to field capacity. From an environmental aspect, animals, particularly dairy cows which are more likely to be farmed intensively, should have access to

pasture for out of season grazing for only limited periods each day to maximize intake rate and minimize soil damage and N and P loss.

Ammonia emission from grazing animals is likely to be about 5 times less than from housed animals due to infiltration of ammonia into the soil rather than be volatilized (Webb *et al.*, 2005). The nature of manure produced determines the amount of emission from storage and during spreading. Farmyard manure emits about 35% less ammonia and leaches about 65% less nitrate than a corresponding amount of slurry so the system of housing has an impact on the environment.

### **Integrating crops and grassland to reduce losses**

An inevitable consequence of housing animals is the accumulation of manure either as slurry or farmyard manure. Effective use of manure reduces the amount of inorganic fertilizer necessary to sustain a given level of forage production and so reduces N surplus leading to lower N loss (Cuttle and Jarvis, 2005). Due to the Nitrates Directive, all EU countries should have action plans to reduce N surpluses. In surveys of N balances, soil mineral N concentrations and efficiency of N utilization of systems involving grassland and forages, grass cut for conservation and lucerne crops are more nitrogen efficient than systems relying on annual forage crops (e.g. Grignani and Zavattaro, 2000). However, in some situations integration of grassland and forage crops offers opportunities to reduce N loss and increase N use efficiency, mainly on dairy farms. Introducing a forage crop with high energy content and efficient uptake of inorganic N into ploughed grassland reduces the amount of N fertilizer required to produce forage for ensiling compared with that required for grass for ensiling. In a long term experiment involving 3 year ley-3 year maize rotation in Belgium compared with all arable and permanent pasture (Nevens *et al.*, 2004) about 50 kg N ha<sup>-1</sup> annum<sup>-1</sup> applied to maize crops could be saved compared with current practice. The maize crop's requirement for N was met from N released by ploughing the ley in the first year and from slurry with very little N fertilizer in the two subsequent years. In northern Germany on free draining soils comparing the 3 component rotation (clover/grass, silage maize, triticale) at 3 intensities of fertilizer inputs (75, 150 and 225 kg ha<sup>-1</sup>), the high N use efficiency of maize and triticale, especially the former, resulted in potentially low N surpluses for the whole rotation (Wachendorf *et al.*, 2004). However, prevention of loss of nitrate N leached subsequent to ploughing cannot be assured and so this risk when ploughing grassland has to be taken into account.

### **Conclusions**

Due to the wide range in climatic conditions and levels of intensiveness throughout Europe there is not a fixed common formula to deal with seasonality of grass and forage production to ensure adequate profitability and satisfactorily meet the demands of agri-environment programmes. Information on which farmers can make informed decisions about, for example, whether their systems should be more or less dependent on forage relative to supplements, or grass silage relative to alternative forages or grass silage relative to grazing is not always clear. Also decision making is further complicated by the complexity of integration of grazing and conservation, animal requirement and level of supplementation. Environmental legislation and prescriptions are becoming progressively influential in ruminant production systems imposing steps to control emissions e.g. Nitrates Directive (imposing limits on production of organic N) and to improve perception of welfare of animals (e.g. obligatory grazing season in Nordic countries). In addition, climate change cannot be ignored which is a further factor influencing seasonality (Rounsevell *et al.*, 2005). Decision support systems are being developed to deal with some of the components of these systems e.g. forecasting growth in continuously stocked swards in Denmark (Søegaard *et al.*, 2005), predicting intake of grazing dairy cows (Delagarde *et al.*, 2004) or predicting outcomes from whole systems e.g. Dairy\_sim (Fitzgerald *et al.*, 2005). As more regulation and limits are imposed on farming, the requirement for such aids becomes increasingly pressing.

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# Irrigation rate and grazing heights influence on herbage mass and pasture composition, soil fertility and porosity

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## Abstract

The objective was to evaluate the carrying capacity, the evolution of botanical composition, soil fertility and porosity of an irrigated pasture of perennial ryegrass (*Lolium perenne* L.), tall fescue (*Festuca arundinacea* Schreb.), white clover (*Trifolium repens* L.) and lucerne (*Medicago sativa* L.), subjected to different irrigation rates and grazing heights. Pasture was divided and subjected to irrigation rates A, B and C during summer. The irrigation rates were 100% (plot A), 125% (plot B) and 150% (plot C) of potential evapotranspiration. Cuttings were done when lucerne was: 15 to 20cm (subplots 1), 25 to 30cm high (subplots 2) or full bloom (subplots 3), for a 114 day-period. Subplot A1 was grazed 5 times (22 grazing days), B1 and C1 6 times (32 grazing days) while A2, B2 and C2 were grazed 4 times – A2 and B2 were grazed 39 days while C2, 43 days. Irrigation rate increased the proportion of lucerne in the pasture although higher cutting frequency reduced lucerne and increased white clover. The proportions of ryegrass and fescue were low in every harvest. Soil parameters showed a statistically significant increase over time regardless of grazing height and irrigation rate. Neither irrigation rate nor grazing height influenced porosity, which decreased from 50% to 40%.

Keywords: irrigated pastures, carrying capacity, herbage composition, soil fertility and porosity.

## Introduction

Irrigated pastures in Mediterranean type climate areas constitute an important feed resource allowing to extend grazing throughout the year. The utilization of the pasture, controlling the species present, will be reflected on its nutritive value since the botanical composition and the proportion leaf/stem will be influenced. Knowing the evolution of the botanical composition, its nutritive and feeding value as well as the recycling potential of the nutrients in the soil, will provide a tool for the producers to decide their livestock feeding management and the production system sustainability.

## Materials and methods

An irrigated pasture with rye-grass, fescue, white clover and lucerne, sown on a clay-calcareous soil, was subjected to different irrigation rates and grazing heights during summer. The irrigation rates were 100% (plot A), 125% (plot B) and 150% (plot C) of potential evapotranspiration for the Santarém region. Grazing was performed whenever lucerne was 15 to 20cm (subplots 1) and 25 to 30 cm high (subplots 2) or as hay at full bloom (subplots 3). The experiment was a split plot in time design with three replicates. Subplots 1 and 2 were grazed individually by 50 kg dry-ewes, tied to the centre of the subplot allowing for a grazing area of 41.8m<sup>2</sup>, every time lucerne reached the planned heights, while subplot 3 was cut for hay. Sampling was done before grazing or haymaking. Herbage botanical composition was evaluated for all treatments. Nutritive value was only evaluated for grazing heights treatments. The dry matter (DM) content and chemical composition of the pasture species were evaluated by cutting the canopy inside quadrats clamped on the grazing plots. Pasture samples were analysed for DM, ash and nitrogen (AOAC, 1990), for fibre composition (Goering and Van Soest, 1970) and for *in vitro* organic matter digestibility (OMD) (Tilley and Terry, 1963). Determinations of minerals were made after dry-ashing of the samples, by atomic absorption spectrometry (Shimadzu Corporation, 1991) except phosphorus that was measured colorimetrically (AOAC, 1990). Soil was sampled at two



depths, 10 and 30cm for fertility, and 5 and 10cm for porosity analyses, at the beginning and at the end of the trial. Pasture was fertilized with phosphorus and potassium at the beginning of the trial according to previous soil analysis.

Whenever the F-test of ANOVA was significant the Tuckey's test was used to detect which means were different (Kaps and Lamberson, 2004).

## Results and discussion

During the 114 day-period of observation, the legume species dominated the pasture for all the grazing heights. The species evolution, evaluated on the clamped quadrats by weighing the cut canopy was: grazing height 1 - the proportion of ryegrass decreased from 24 to 13%, with a minimum of 9% by the end of August, fescue varied between 9 and 14%, lucerne decreased steadily from 36 to 8% and white clover dominated the pasture increasing from 31% to 65% by the end of the period; grazing height 2 – the proportion of the grass species was small, decreasing throughout the period, however, this grazing frequency allowed lucerne and white clover to maintain their proportions, respectively 50 and 36% of the green matter recorded inside the sampling quadrats. Results obtained from grazing height 3 are not shown. The lower grazing height decreased lucerne DM production. Values obtained for grazing heights 1 and 2 were, respectively, 32 and 74g m<sup>-2</sup>. Statistical differences were not observed for the other species between grazing heights. The highest irrigation rate increased significantly lucerne DM production with the following values: 36, 41 and 68g m<sup>-2</sup>, respectively for A, B and C treatments. All other species were not affected by irrigation rates.

Subplot A1 (irrigation rate A and grazing height 1) was grazed 5 times for a total of 22 grazing days, while B1 and C1 were grazed 6 times (27 and 32 grazing days, respectively). Grazing height 2 allowed subplots A2, B2 and C2 to be grazed 4 times – A2 and B2 were grazed during 39 days while C2 lasted for 43 days. Total dry matter produced for grazing height 1 was 572.3, 619.0 and 789.2 g m<sup>-2</sup> for irrigation rates A, B e C, while grazing height 2 produced 499.8, 630.3 and 709.3 g m<sup>-2</sup> for the same irrigation rates.

The species nutritive value did not vary significantly throughout the study period. However, significant differences were found for the OMD between grazing heights 1 and 2, with values of 738 and 669 g kg<sup>-1</sup> DM. The nutritive parameters evaluated did not differ between ryegrass and fescue or lucerne and white clover.

When species were grouped under grass or legume, significant differences were found for the following parameters – ash (131 vs. 110 g kg<sup>-1</sup>), crude protein (223 vs. 252 g kg<sup>-1</sup>), neutral detergent fiber (552 vs. 306 g kg<sup>-1</sup>), acid detergent fiber (309 vs. 255 g kg<sup>-1</sup>) and OMD (653 vs. 742 g kg<sup>-1</sup>). Mean mineral content was similar for all species, with the following values: phosphorous, 4.1 g kg<sup>-1</sup> DM; sodium, 3.4 g kg<sup>-1</sup> DM; potassium, 28.1 g kg<sup>-1</sup> DM; and magnesium, 2.3 g kg<sup>-1</sup> DM. On the other hand, calcium mean values were 7.7 g kg<sup>-1</sup> DM for grasses and 16.0 g kg<sup>-1</sup> DM for legumes.

In general, there was an increase in mineral content for iron, magnesium, manganese, sodium, zinc, nitrogen and organic matter while phosphorus and potassium were maintained at high levels due to the fertilization applied. Both irrigation rate and grazing heights did not influence the levels of minerals in the soil. Porosity was not significantly affected by irrigation rate or grazing heights. However, porosity under grazing decreased compared with the haymaking areas.

## Conclusions

The lower grazing height (15-20cm) increased the percentage of white clover in the pasture.

The maximum irrigation rate (150% PET) favoured lucerne. The availability of dry matter throughout the study period increased on the plots subjected to higher irrigation rates.

The different grazing heights did not influence the nutritive value of the species in the pasture but the OMD was greater for grazing height 1. Phosphorous and potassium levels in the soil were maintained throughout the study. All soil minerals determined increased significantly, regardless of irrigation rates or grazing heights.

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# Effects of grazing access time and silage supplementation on performance and feeding behaviour in grazing dairy cows

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## Abstract

The objective of this study was to evaluate the performance and feeding behaviour of dairy cows by restricting daily grazing access time and supplementing with maize (*Zea mays* L.) silage. The experiment was carried out during 8 weeks (May and June 2005) using 48 mid-lactation Holstein cows allocated to 4 treatments in a 2x2 latin square design on ryegrass pasture. The treatments were 4 and 8 h per day of access time available for grazing (A) and 5 and 10 kg DM of maize silage (S) per day (treatments: A4S5, A4S10, A8S5 and A8S10). Herbage allowance above 5 cm was 6.5 and 11.0 kg DM cow<sup>-1</sup>d<sup>-1</sup> for S10 and S5 treatments, respectively. Feeding behaviour on grazing was estimated by visual recording. The effect of grazing access time and maize silage are additive for milk production: 20.5, 21.9, 21.8, 22.7 kg for treatments A4S5, A8S5, A4S10, and A8S10 respectively. Grazing time shows an interaction between the two factorial treatments: 216, 353, 194, 297 min d<sup>-1</sup> for treatments A4S5, A8S5, A4S10 and A8S10 respectively.

Keywords: dairy cow, grazing, access time, maize silage.

## Introduction

The major problems encountered in dairy systems under grazing conditions are the unfavourable weather conditions and day length, especially at the end of winter and in autumn. This reduces growth and herbage availability, so animals are often supplemented to satisfy their nutritional requirements. Restricting daily access time to pasture can avoid the risks of sward degradations and can be used as a mean to improve grazing management. However, few experiments have been carried out on the performance of dairy cows when daily access time is restricted and dairy cows are supplemented with maize silage. The objective of this study was to evaluate the performance and feeding behaviour of dairy cows with short grazing access time at two levels of maize silage supplementation.

## Materials and methods

Four treatments were arranged in a 2x2 latin square design with 2 durations of daily access time for grazing, 4h (A4) and 8h (A8), associated with 5 (S5) or 10 (S10) kg DM cow<sup>-1</sup> d<sup>-1</sup> of a supplement based on maize silage and soyabean meal (ratio 83:17) (A4S5, A4S10, A8S5, A8S10). The experiment was carried out over 4 periods each lasting 14 days, from 29 April to 24 June 2005. Forty-eight mid-lactation Holstein cows were allocated to groups of 12 according to days of lactation (166 d), milk production (29.6 kg d<sup>-1</sup>) and composition, live weight (599 kg) and condition score. During the experiment, each morning, grazing session started at 09:00. Animals restricted to 4 h d<sup>-1</sup> grazing were removed from the pasture at 13:00 and housed in a concrete area. Those restricted to 8 h d<sup>-1</sup> were removed from the pasture at 17:00 and also confined. Cows were milked twice daily at 07:00 and 17:30. All cows were fed the supplement indoors in individual stalls once daily subsequent to the afternoon milking. The pasture area of 7.5 ha consisted of perennial ryegrass (*Lolium perenne* L. cv Ohio). Each paddock was sub-divided into 4 sub-paddocks, each allocated to one of the four treatments. These paddocks were fertilized after each grazing period at a level of 60 kg N ha<sup>-1</sup> of ammonium nitrate (33% N) and were strip-grazed with short rotation periods (18 d) in order to have relatively low pre-grazing sward heights. Daily herbage allowance was 7.5 and 12 kg cow<sup>-1</sup> d<sup>-1</sup> for S5 and S10 treatments, respectively. The difference between these two levels of herbage allowance was determined to get

similar post-grazing sward height whatever the maize silage level. Herbage mass and sward bulk density were estimated twice weekly by motor scythe cutting. Pre and post-grazing sward heights were determined daily using a plate meter.

Individual milk yields were recorded at each milking. Milk protein and fat concentrations were measured over 4 consecutive days at the end of each period. Grazing activity was recorded individually every 5 minutes by direct observation, 2 days during the second week of every period.

## Results

Pre-grazing sward height did not differ between treatments. Herbage mass above 5 cm was slightly higher ( $p < 0.05$ ) on A4 compared to A8 swards. Herbage CP content and OM digestibility averaged 225 g kg<sup>-1</sup> DM and 0.774, respectively. Herbage allowance was slightly lower than expected whatever the treatments. Residual sward height was higher on A4 than that on A8 (on average +1.25 cm,  $p < 0.01$ ) and lower on S10 than on S5 (-0.7 cm,  $p < 0.05$ ).

Table 1. Effects of grazing access time and supplementation level on sward and grazing characteristics, milk production and composition and on grazing activity in dairy cows.

Treatment	A4S5	A8S5	A4S10	A8S10	Syx	Significance		
						A	S	A*S
Sward height (cm)	11.1	10.6	11.2	10.3	0.65	NS	NS	NS
Herbage mass <sup>1</sup> (kg DM ha <sup>-1</sup> )	1468	1351	1478	1289	140.6	*	NS	NS
Herbage allowance <sup>1</sup> (kg DM d <sup>-1</sup> )	11.1	10.6	6.6	6.7	0.92	NS	***	NS
Residual sward height (cm)	7.2	5.7	6.3	5.3	0.54	**	*	NS
Milk production (kg d <sup>-1</sup> )	20.5	21.9	21.8	22.7	1.10	***	***	NS
Milk protein concentration (g kg <sup>-1</sup> )	29.3	29.8	29.9	30.4	0.65	***	***	NS
Milk fat concentration (g kg <sup>-1</sup> )	40.3	39.4	39.5	39.4	1.64	*	NS	NS
Grazing time (min d <sup>-1</sup> )	216	353	194	297	29.2	***	***	**
Proportion of time spent grazing	0.91	0.74	0.82	0.63	8.3	***	***	NS

A, grazing access time; S, supplementation level; Significant level \*,  $p < 0.05$ ; \*\*,  $p < 0.01$ ; \*\*\*,  $p < 0.001$ <sup>1</sup> above 5 cm.

The average intake of the supplement was 4.8 and 9.8 kg DM for S5 and S10, respectively. The effects of grazing access time and of supplementation level were additive for milk production and composition. Milk production was significantly lower in cows receiving 4 h than those receiving 8 h of grazing access time (21.1 and 22.1 kg for A4 and A8, respectively,  $p < 0.001$ ). Milk production was higher on S10 than on S5 (22.3 and 21.2 kg, respectively,  $p < 0.001$ ). Milk fat content was slightly higher on A4 compared to A8 (+0.5 g kg<sup>-1</sup>,  $p < 0.05$ ) and was not affected by supplementation level. Milk protein content was lower on A4 than on A8 (-0.5 g kg<sup>-1</sup>,  $p < 0.001$ ) and higher on S10 than on S5 (+0.6 g kg<sup>-1</sup>,  $p < 0.001$ ).

Grazing time was affected by grazing access time according to supplementation level (interaction:  $p < 0.01$ ). It was lower on A4 compared to A8 by 137 min with 5 kg of supplement and by 103 min with 10 kg of supplement. On average, grazing time was reduced by 39 min when supplementation level was increased from 5 to 10 kg ( $p < 0.001$ ).

The proportion of time spent grazing was higher on A4 than on A8 (0.86 vs. 0.68,  $p < 0.001$ ) and lower on S10 than on S5 (0.72 vs. 0.82,  $p < 0.001$ ).

## Discussion

The objective of this experiment was to test the ability of the grazing dairy cows to adapt their feeding behaviour when restricting the access time for grazing in order to maintain their performance. This study clearly showed that cows were unable to maintain milk production when access time is restricted to 4 h, even for a high supplementation level. Similar results were found by Mattiauda *et al.* (2003) when comparing 4 and 8 h of grazing access time. When comparing higher grazing access time (6 vs. 8 h or 8 vs. 16 h), Chilibroste *et al.* (2004) found no effect on milk production.

However, in our experiment, the reduction of milk production at low grazing access time (-1.1 kg, i.e. -5%) is much less pronounced than the relative variation of the time that cows spent to graze (-120 min, i.e. -37%). It is probable that cows showed a higher motivation to graze and a higher intake rate when grazing access time was restricted, as supported by the results of Gekara *et al.* (2005).

The decrease of milk protein content at low grazing access time probably reflects the lower energy intake, but was not observed by Mattiauda *et al.* (2003). The observed effect of grazing access time on milk fat content was opposite to the results of Mattiauda *et al.* (2003).

The low response in milk production is not easy to compare to the literature supplementation results because the herbage allowance has been reduced according to the supplementation levels and the residual sward height was lower at high supplementation level, contrary to the initial objective. It can be hypothesized that milk response to supplementation level would be higher at similar residual sward height.

## Conclusions

Restricting access time for grazing to 4 h daily only slightly decreases the milk production whatever the supplementation level. Short grazing access time can thus be used as a tool to improve the grazing management in seasons with adverse weather conditions.

## Acknowledgements

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# Investigating the contribution of hybrid ryegrass to early spring grazing systems

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## Abstract

The objective of this study was to compare hybrid and perennial ryegrass cultivars in a simulated grazing system with particular focus on the early spring grazing period. Five grass cultivars were used for this plot study – a late diploid and tetraploid perennial ryegrass and three hybrid ryegrass cultivars. Three different managements were imposed – management A (control) was defoliated in early February and mid-April, management B was defoliated in early February, early March and mid-April while management C was defoliated in early February, late March and mid-April. Two different defoliation heights (4cm-low and 7cm-high) were applied across each management. Silage was harvested on 8 June from all plots. Plots were defoliated with a motor Agria and DM yields were calculated, pre - and post - cutting heights were measured and growth rate was determined. Following all defoliations there was a significant effect ( $P < 0.001$ ) of defoliation height, spring management and grass variety on cumulative DM yields up to mid-April. A higher total DM yield was achieved by defoliating to 4cm (+1,109 kg DM ha<sup>-1</sup>, s.e. 27.7). The highest DM yields were harvested from management B (4,324 kg DM ha<sup>-1</sup>, s.e. 33.9). The hybrid cultivars had higher total DM yields than both the late heading diploid and tetraploid cultivars.

Keywords: hybrid ryegrass, spring grazing, DM yields.

## Introduction

Increasing the proportion of grazed grass in the diet of the dairy cow is seen as one of the main avenues to reduce production costs. The greatest potential to increase the proportion of grazed grass in the diet of the spring calving dairy cow exists during the peripheries of the grass-growing season (i.e. early spring and late autumn). The concept of early spring grazing has been well researched by Sayers and Mayne (2001), Dillon *et al.* (2002) and Kennedy *et al.* (2005). However, due to the deficit of grass growth during this early spring period grass silage and concentrate have previously constituted a large proportion of the spring calving cow's feed budget. In the overall context of the grazing system, extra dry matter (DM) produced during the early spring period can replace grass silage or concentrate or a combination of both in the animal's diet at this time. Therefore hybrid ryegrasses (Perennial × Italian ryegrass cultivars) may now have a role to play in an early grazing system. The objective of this study is to compare hybrid and perennial ryegrass cultivars in a grazing system with particular focus on the early spring grazing period.

## Materials and Methods

The study was carried out at Moorepark Research Centre, Fermoy, Co. Cork, Ireland (50°07'N; 8°16'W). The soil is a free-draining, brown earth soil with a sandy loam-to-loam texture. Ninety plots [1.5m x 6m (9m<sup>2</sup>)] were sown in September 2004 in a 5 x 3 x 2 factorial design. This incorporated 5 different ryegrass cultivars, 3 defoliation managements and 2 defoliation heights. The cultivars used were Navan (late heading tetraploid perennial ryegrass, heading date (HD) 6 June), Foxtrot (late heading diploid perennial ryegrass, HD 6 June), Foyle (hybrid tetraploid ryegrass, HD 22 May), Brutus (hybrid diploid ryegrass, HD 21 May) and Belleek (hybrid tetraploid ryegrass, HD 16 May). Navan was used as the control cultivar. Late heading date cultivars were used as the control varieties as they represent over

70% of seed sales in Ireland. All plots were harvested before closing on 22 October the previous year. Three different spring defoliation managements were imposed across all plots – management A (control) was defoliated on 2 February and 20 April, management B was defoliated on 2 February, 3 March and 20 April while management C was defoliated 2 February, 23 March and 20 April. Two different defoliation heights (4cm - low and 7cm - high) were applied across each management. All plots were closed and silage was harvested on 8 June.

Subsequent to sowing all plots were fertilised with 50 kg N ha<sup>-1</sup>. Forty kg N ha<sup>-1</sup> were applied to each plot after the initial defoliation in February. Following the final defoliation in April all plots had received a total of 140 kg N ha<sup>-1</sup>. Phosphorus (P) and Potassium (K) were applied at the rate of 15 kg ha<sup>-1</sup> and 40 kg ha<sup>-1</sup>, respectively divided into two applications during the year. The first application was applied in late January while the second was applied in late June.

Plots were harvested with a motor Agria (Etesia) scythe; all the mown herbage from each plot was collected and weighed. A subsample of approximately 0.5 kg was taken from each defoliated plot. Approximately 0.1 kg of the herbage subsample was dried for 48 h at 60 °C to determine the DM content. Samples were then milled through a 1mm sieve for chemical analysis. All pre and post defoliation heights were also recorded. DM yields and sward density were determined from the weight of herbage defoliated from the plots. Tiller density was also measured.

## Results and Discussion

The results of the study are displayed in Table 1. There was a significant ( $P < 0.001$ ) difference in DM yield between varieties when defoliated in early February. Navan (1,049 kg DM ha<sup>-1</sup>), the control variety had a higher DM yield than Foxtrot, Foyle, Brutus and Belleek (-341, -77, -44 and -27 kg DM ha<sup>-1</sup>, respectively). A significant ( $P < 0.001$ ) difference in DM yield at both defoliation heights also occurred, however this was because of the management imposed.

When the cumulative yields from February to April were analysed by management there was a significant effect ( $P < 0.001$ ) Management B had the highest cumulative DM yield (4,324 kg DM ha<sup>-1</sup>), managements A (-77 kg DM ha<sup>-1</sup>) and C (-454 kg DM ha<sup>-1</sup>) yielded less than management B. A significant ( $P < 0.001$ ) variety effect was recorded for the cumulative DM yield (Feb - April). Navan recorded a cumulative yield of 3,958 kg DM ha<sup>-1</sup>, Foxtrot yielded 478 kg DM ha<sup>-1</sup> less, while Foyle, Brutus and Belleek yielded +128, +742 and +548 kg DM ha<sup>-1</sup> more than Navan, respectively.

Cumulative DM yield was significantly ( $P < 0.001$ ) higher for the lower defoliation height, swards defoliated to 4cm yielded 1,109 kg DM ha<sup>-1</sup> more than those defoliated to 7cm (3,592 kg DM ha<sup>-1</sup>).

Table 1. A comparison of perennial and hybrid ryegrass cultivars under three managements and defoliated at two defoliation heights.

	Navan		Foxtrot		Foyle		Brutus		Belleek		SED	H	M	V	V * H
	High	Low	High	Low	High	Low	High	Low	High	Low					
DM Yield 2 Feb.	694	1403	416	1001	605	1339	678	1331	657	1389	25.2	**	N	**	N
												*	S	*	S
	Management A														
Cum yield Feb-Apr Silage DM yield	3411	4705	3052	4110	3684	4689	4307	5294	4007	5205	48.0	**	**	**	*
												*	*	*	*
	6611	6355	6613	6421	7054	5824	6760	6402	6043	6368	200.5	*	N	N	*
													S	S	
	Management B														
Cum yield Feb-Apr Silage DM yield	3667	4604	3122	4194	3615	4912	4328	5427	4115	5251	48.0	**	**	**	*
												*	*	*	*
	6809	6264	6959	6182	6388	6596	6925	6344	6268	6249	200.5	*	N	N	*
													S	S	
	Management														
Cum yield Feb-Apr Silage DM yield	3134	4228	2606	3802	3298	4320	3804	5043	3734	4725	48.0	**	**	**	*
												*	*	*	*
	6855	6628	6804	6746	6699	6694	6882	6795	7130	5796	200.5	*	N	N	*
													S	S	

NS=Non-significant, \*= $P \leq 0.05$ , \*\*= $P \leq 0.01$ , \*\*\*= $P \leq 0.001$ . <sup>abc</sup> values in the same row not sharing a common superscript are significantly different. Cumul = Cumulative (All cuts from 2 Feb to 20 April). V = Variety, H = Defoliation Height, M = Management.

There was a defoliation height  $\times$  management interaction ( $P < 0.05$ ) between the three managements; management A (+1,317 kg DM ha<sup>-1</sup>) had the largest difference in cumulative DM yield between defoliation heights. Brutus was deemed the more productive of varieties as defoliation height had least of an effect on DM yield.

Defoliation height had a significant effect ( $P < 0.05$ ) on silage DM production. There was also a significant interaction ( $P < 0.05$ ) between defoliation height  $\times$  variety.

## Conclusion

Hybrid ryegrass cultivars yielded a greater quantity of herbage from early February to late April than the two perennial ryegrass varieties. However, from the different defoliation dates used it is evident that the hybrids did not out perform the late tetraploid (Navan) in the early spring period (February /early March). Higher DM yields were achieved by all varieties when defoliated to a lower defoliation height. However, it should be noted that this is only one year's data collected from newly reseeded pastures. Thus the study needs to be repeated in order to account for between year variations.

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## Delimitation of agricultural and cattle districts in Bardenas Reales

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### Abstract

The Natural Park of Bardenas Reales is an important territorial base for a large number of cattle enterprises, along the banks of the River Ebro, and migrating flocks from the Pyrenees of Navarra. Management practices have led to the degradation of landscape and natural resources. In order to rationalise the use of the pasture resources according to profitable management systems, the park manager group have decided to divide the region into individual agricultural and cattle districts. To current project was developed to achieve this goal, using methodology based on the application of Geographical Information systems. (GIS) (Wilwy, 1999).

Keywords: GIS, land degradation.

### Introduction

The Natural Park of Bardenas Reales is a broad area of 41,800 ha located in the south-east of Navarra (Spain), in a semi-arid area. Cattle production is traditionally the main use of this area. The pasture resources of the region have widely different ecological and nutritional characteristics and support 100,000 animals throughout the year. The prevailing management systems have negatively affected not only the sustainability of the habitats but also aspects of enterprise profitability. The high density of cattle during specific times of the year, the absence of high quality resources and the absence of grazing control have led to a degradation in the vegetation cover, producing, in some cases, erosion problems. The present project aimed to optimise the allocation and use the forage resources in the natural park by dividing the area in individual agricultural and cattle districts. Its main objective was the classification of the Bardenas Reales area into agricultural and cattle districts, based on objective criteria and the environmental demands consequent to natural park status. In summary, it used GIS tools to facilitate the planning of natural resources. A cattle district is defined as the area which can support an UTH (*Man work unit*), consisting of around 600 sheep in extensive pasture.

### Materials and methods

The methodology consisted of the following three steps:

*1. Collection, integration and analysis of existing data over the studied area:* The project approach and methodology are made possible by the large quantity of territorial data available for Bardenas Reales area. The following cartographic information was used: Crops and land use digital maps of Navarra (1/25.000); Vegetation series digital map. (1/25.000); Soils digital map (1/25.000); Cadastral data base (1/25.000); Digital color orthophoto (1/5.000); Digital Terrain Model; Digital topographic map (1/5.000); Digital topographic map (1/25.000) (Servicio de Estructuras Agrarias, 1998) Additional sources of information included: Location of cattle infrastructures such as livestock and cattle pens; location of watering places(ponds); road networks (cattle tracks, roads and paths) and protected areas (natural parks and natural areas, "ZEPAS").

2. Pasture evaluation of the area: The first step was the pastoral evaluation of the area, cartographically represented as a Base Map in which every parcel was assigned a pastoral value in Forage Units (FU/ha/an). Natural feed resources were evaluated using the method designed by Daget and Poissonet (1972). The pastoral evaluation index and its transformation into energetic units were obtained by field work. Evaluation of agricultural resources, including yield, productivity and edaphological information, was achieved by two approaches: (1) taking the current situation (one year barley) and (2) taking the future situation (different crops rotated over an eight year period, including barley, cereal and legumes).

3. Data process and delimitation of the agricultural and cattle districts using GIS technology: To identify agricultural and cattle districts all the data were incorporated into a GIS project, according to the following procedure:

*3.1. Pasture base map generation:* Cropping and land use digital maps of Navarra. Scale 1/25.000 were used to generate the pasture and cartographic base provided by the methodology described above.

*3.2. Delimitation of areas that should be excluded from pasture evaluation:* According to the criteria of environmental or legal constraints on access, the following areas were excluded: a) Protected areas with pasture restriction, b) areas used for military exercises, c) steeply sloping areas and those at risk from erosion, and d) road networks including a three meter protection strip and cattle tracks.

*3.3. Definition of potential district limits:* It was decided that boundary lines should follow physical features of the area, be regular and easily located. These included: steeply sloping areas, roads, cattle tracks, main and secondary paths, ravines and field limits.

*3.4. Definition of cattle district limits:* Districts were allowed to cover different surface areas but had similar pasture contents with respect to meeting the nutritive requirements of an average sheep flock during a defined period of time, without degrading the pasture resources. These conditions provided objective criteria for delimiting districts. Further parameters prescribing the district pattern were:

Irregular and elongated topographical features should be avoided to minimise movements.

Boundaries should follow regular physical features of the area, including roads, paths and cattle tracks.

Districts should exclude certain areas for military reasons, proximity to main roads and erosion risk (e.g. cliffs).

To ensure accessibility, all districts should be reached by navigable paths.

The above criteria were accommodated in the interactive design of the districts. However, although this appeared relatively straightforward in theory, in practise it was very difficult.

## **Results**

The main results of this project are:

The pastoral evaluation of the area, cartographically represented as a Base Map in which every parcel is assigned a pastoral value. Furthermore every parcel has been linked to a comprehensive database containing information on land use, percentage of this use and the total pastoral value, etc.

The delimitation of agricultural and cattle districts under two different scenarios (Figure 3); the current situation consisting of one year barley, consisting of 66 districts, and a future situation consisting of different crops under an "eight year" rotation, consisting of 87 districts.

The generation of summary descriptions of the main features of every district, including surface areas, land-use and pasture values. The graphical information shows the general location in Bardenas Reales, topographical information and geographical limits over the orthophoto, together with the location of cattle infrastructures and watering places.

## **Conclusions**

The procedure followed in this project has proved to be an excellent tool for environmental resource planning. The use of GIS technology allows the analysis of different scenarios to be conducted without too much effort, and district areas identified according to different parameters, incorporating layers of new information and modifications in agricultural practices (i.e. crop rotations, new irrigation areas, etc.).

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## Content of nitrate in green forage and its effect on silage quality

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### Abstract

The content of nitrate in forage is important either due to poisoning when contents are high or due to its effects on silage fermentation. The aim of this work was to determine the nitrate content in green forage produced under different grassland management intensities and its subsequent effects on the silage fermentation process. Therefore 780 samples of green forage varying in the nitrogen fertilisation intensity and cutting frequency that had been imposed on grassland were investigated for nitrate content. Furthermore, silages made under laboratory conditions from green forage of low (N= 111), high (N= 178) and zero (N= 281) nitrate content were analysed for relationships with butyric acid, acetic acid and ammonia. The results show that the nitrate content is independent of N fertilisation:  $< 4.4 \text{ g nitrate kg}^{-1} \text{ DM}$  in nearly 90 % of all investigated samples of green forage made under practical conditions. Depending on nitrate content of green forage, metabolism and pattern of fermentation products in silages were significantly different during comparable fermentation stages. The problems in practice with evaluation of fermentation quality have to be traced back to differences in material being ensiled.

Keywords: Silage, nitrate, fermentation quality, fermentation process, butyric acid.

### Introduction

The nitrate content in green forage depends on factors like the level of N fertilisation and the intensity of grassland management. For the present situation only, the results of surveys indicate that most green forages are nitrate-free or contained  $< 4.4 \text{ g NO}_3 \text{ kg}^{-1} \text{ DM}$ .

The knowledge of ensilage applies currently to forage with nitrate contents between 4.4 to 13.3 g NO<sub>3</sub> kg<sup>-1</sup> DM (Kaiser, 1981). At the beginning of the fermentation process nitrate acts as a natural inhibitor of clostridia. Furthermore, nitrate has also substantial effects on the metabolic pathways in the fermentation process (Hein, 1970; Kaiser, 1981), mainly in lactate degradation. Nitrate acts as a hydrogen acceptor in this process in which acetic acid (AA) is formed. The fermentation processes when ensiling materials that are low in nitrate or nitrate-free are substantially different from those in green forage containing nitrate (Weiß, 2000).

The aim of this study was to determine the nitrate content when the level of nitrogen fertilisation and cutting frequency of grassland were varied. In the second part of this study the relationships between the parameters of undesirable metabolism in silages were investigated, depending on low, high or absent nitrate content in herbage at ensiling.

### Materials and methods

For the determination of nitrate content, green forage was harvested within the project “performance and N balance of grassland on sandy soils” (Trott *et al.*, 2004). The experiment was carried out at the experimental farm Karkendamm in northern Germany in a split-plot design with 4 replicates (plot size: 18 m<sup>2</sup>) including four mineral nitrogen (N) application rates and two slurry levels. Mineral N fertilizer levels were 0, 100, 200, 300 kg N ha<sup>-1</sup> yr<sup>-1</sup> as calcium ammonium nitrate. Cow slurry (0 and 20 m<sup>3</sup> ha<sup>-1</sup>) with an average total N content of 3.5 kg m<sup>-3</sup> was applied in March (see also Trott *et al.*, 2004). Additionally cutting frequency was varied using a simulated grazing system (SG) with on average six cuts per year and a cutting only system (CO) with four cuts per year. Two sward types were compared: pure grass swards and grass-white clover mixtures. At the end of the season, the swards were dominated by perennial ryegrass in swards receiving high amounts of nitrogen and by a mixture of perennial

ryegrass and white clover in swards receiving no nitrogen or moderate levels of nitrogen. Samples were taken during the years 2000 and 2001.

For the determination of silage quality depending on the nitrate content in herbage at ensiling the relationships with BA, AA and ammonia (NH<sub>3</sub>) in silages were investigated. Therefore under laboratory conditions 570 silages were produced from different green forages of known chemical composition: 281 silages from green forage that was nitrate-free (< 1 g NO<sub>3</sub> kg<sup>-1</sup> DM), 111 silages from forage low in nitrate (< 4.4 NO<sub>3</sub> kg<sup>-1</sup> DM) and 178 from forage containing nitrate > 4.4 NO<sub>3</sub> kg<sup>-1</sup> DM. The ensiling material was analysed for dry matter (DM), watersoluble carbohydrates (WSC), buffering capacity (BC) and nitrate. The silages were analysed for pH, volatile fatty acids (GC) and ammonia (Conway Method). Data were submitted to analysis of variance. In all variants the normal distribution was not reached, therefore the medians were compared with Kruskal-Wallis-test and test of Nemenyi.

## Results and discussion

Independent of the cutting frequency and N-fertilisation level the median nitrate content was very low in green forage (shown in Table 1). The range of nitrate contents was high in the variants. Higher nitrate contents occurred in clover containing swards produced under the conditions of SG. As mineral N-fertilisation increased the nitrate content also increased. The single highest value for nitrate was 11.23 g NO<sub>3</sub> kg<sup>-1</sup> DM. In 89.7 % of all analysed samples the nitrate content was < 4.4 g NO<sub>3</sub> kg<sup>-1</sup> DM.

Table 1. Nitrate content in grass and clover depending on utilisation intensity (exemplary).

N kg ha <sup>-1</sup>	CO Pure grass swards								SG White clover-grass swards							
	Without cow slurry				Cow slurry				Without Cow slurry				Cow slurry			
	2000		2001		2000		2001		2000		2001		2000		2001	
	Median of nitrate content (g NO <sub>3</sub> kg <sup>-1</sup> DM)															
	N	x	N	x	N	x	N	x	N	x	N	x	N	x	N	x
0	12	0.05 a	8	0.05	10	0.07 a	8	0.07 a	23	0.22 a	11	1.70 ab	24	0.19 a	12	1.41 a
100	14	0.08 ab	8	0.07	14	0.10 a	7	0.09 ab	27	1.48 ab	12	0.60 a	25	0.69 a	12	1.48 a
200	31	0.31 bc	15	0.07	30	0.20 a	16	0.11 ab	28	2.64 b	14	1.95 ab	29	3.05 b	14	2.52 ab
300	17	1.14 c	7	0.09	16	1.49 b	8	0.37 b	27	4.86 c	16	3.83 b	26	4.38 b	16	4.92 b

Significant at P<0.05. N, Number ; x, median.

The investigations with green forage as the ensiling material (EM) varying in nitrate content showed that the relationships between the parameters of undesirable metabolism differ depending on the nitrate content of EM. In EM with high nitrate levels AA is formed in silages during lactate degradation, as long as nitrate is available. In such BA-free silages with increasing AA-content, ammonia is formed (see Figure 1, a) and thereby the pH is increased. After complete nitrate reduction BA is metabolised and the ammonia content increases considerably due to protein degradation (Figure 1,b). There is no strong correlation between Ammonia and AA levels. In silages from nitrate-free EM the AA is not formed during lactate decomposition irrespective of BA formation (Figure 2) and the content of AA is very low, under 30 g kg<sup>-1</sup> DM. The content of ammonia is also negligible. Ammonia increased significantly due to protein degradation with BA formation in the later stage of lactate degradation. The change of pH depends also on the occurrence of nitrate in EM. During the lactate degradation in EM with nitrate the pH increased more than in nitrate-free EM.

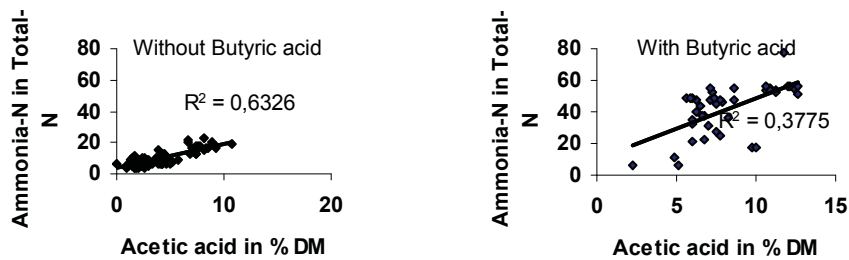


Figure 1. Relationships between BA, AA and ammonia in silages a) without BA (N = 135) and b) with BA (N = 43) from green forage with nitrate ( $> 4.4 \text{ g NO}_3 \text{ kg}^{-1} \text{ DM}$ )

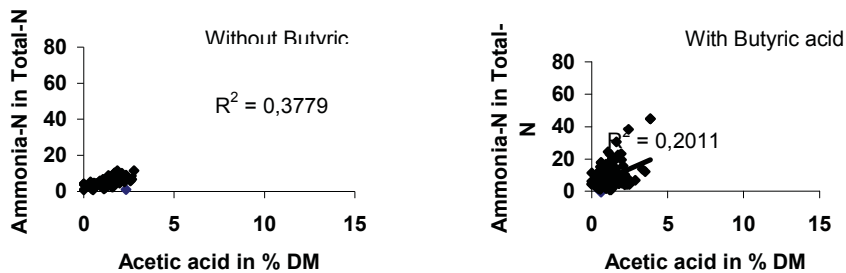


Figure 2. Relationships between BA, AA and ammonia in silages without BA (N = 83) and with BA (N = 198) from green forage without nitrate ( $< 1 \text{ g NO}_3 \text{ kg}^{-1} \text{ DM}$ ).

## Conclusions

Current intensities of grassland management in Germany are obviously linked with low levels of nitrate in green forage. Depending on the nitrate content there are differences both in the fermentation process and the resulting pattern of fermentation products. The content of ammonia and the pH are directly related to the nitrate content in green forage. Knowledge about fermentation process, pattern of fermentation products, assessment of ferment ability of herbage and silage quality are basically different by ensiling of green forage with nitrate and green forage with low or free in nitrate.

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## Maize silage production in a kura clover living mulch

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### Abstract

Cropping systems that improve soil conservation and reduce nitrogen fertilizer inputs for mixed crop and livestock enterprises are needed to improve environmental and economic sustainability. Our objectives were to determine whether established kura clover (*Trifolium ambiguum* M. Bieb.) could serve as a living mulch and nitrogen source for no-till maize (*Zea mays* L.) silage production. Maize was no-till sown into kura clover that had been either killed (control) or suppressed with herbicides in two Wisconsin (USA) locations over 3 years. Maize grown in kura clover living mulch was fertilized with from 0 to 84 kg ha<sup>-1</sup> nitrogen and the control treatment with 84 kg ha<sup>-1</sup>. Maize silage yield (dry matter basis) ranged from 9.8 to 20.5 t ha<sup>-1</sup> over six environments, and adequate suppression of the living mulch resulted in no yield loss in the mulch compared to the control system. Silage yield in kura clover living mulch did not respond to nitrogen fertilizer application over six environments, indicating that maize silage nitrogen requirements were fully met by the suppressed kura clover. In all environments, kura clover recovered to full production by June the year following maize production.

Keywords: kura clover, living mulch, maize silage.

### Introduction

Alfalfa (*Medicago sativa* L.) and maize silage, grown in rotation, have long been the primary high quality forages harvested to support the dairy industry in the North-Central USA. However, removal of essentially all plant residue with maize silage production results in excessive erosive soil loss (Gallagher *et al.*, 1996), prompting the need for alternative soil conserving systems. Furthermore, the ever-increasing cost of nitrogen fertilizer encourages the search for cropping systems that rely on biologically fixed nitrogen. Legume living mulches have been tested in the northern USA as a means to meet nitrogen requirements of maize (Eberlein *et al.*, 1992; Hartwig and Ammon, 2002), but perennial legumes evaluated reduced maize yields or failed to recover after maize harvest. Kura clover seems to be ideally suited to serve as living mulch. It is extremely persistent through frigid winters and produces rhizomes that allow it to fill in gaps that may otherwise be invaded by weeds (Albrecht and Kim, 1998). Our preliminary research (Zemenchik *et al.*, 2000; Affeldt *et al.*, 2004) has demonstrated that with adequate suppression, kura clover can be managed to provide minimal competition to maize and that this system results in reduced soil erosion compared to conventional maize silage production systems (Eleki, 2003). Our objectives were to test the system under a wide range of environmental conditions and to determine if kura clover living mulch could fully meet nitrogen requirements of maize silage.

### Materials and Methods

Field studies were conducted near Arlington (43°18'N, 89°21'W) and Lancaster (42°50'N, 90°47'W), Wisconsin, USA, in 2001, 2002 and 2003. Glyphosate resistant maize (hybrid DKC50-73 RR46) was no-till sown into 3 to 6 year old fields of 'Endura' kura clover that had been suppressed with glyphosate (1.66 kg a.e. ha<sup>-1</sup>) and dicamba (0.14 kg a.e. ha<sup>-1</sup>) (living mulch) or killed with clopyralid (0.10 kg a.e. ha<sup>-1</sup>) (control plots) the previous day. After sowing, a 20-cm band of clopyralid (0.10 kg a.e. ha<sup>-1</sup>) was applied over the maize seed rows in the living mulch plots. A second application of glyphosate (0.83 kg a.e. ha<sup>-1</sup>) was made at the V3 maize growth stage to control annual weeds and provide additional kura clover suppression. Nitrogen fertilizer treatments of 0, 28, 56 or 84 kg ha<sup>-1</sup> were applied to the living mulch treatments and 84 kg ha<sup>-1</sup> to the control plots at the maize V3 stage. Whole plant maize (as for silage) was harvested when grain was at the 50% kernel milk line stage. Maize was chopped, weighed,

and a 1-kg subsample was taken to calculate dry matter yields. The experiment was arranged as a randomized complete block design with five treatments replicated four times. Analysis of variance was conducted with SAS, and means were compared with Fisher's protected LSD ( $P=0.05$ ). Single degree of freedom contrasts were used to compare treatment groups or specific treatments.

## Results and Discussion

Abnormally low precipitation at both locations in 2001, and at Lancaster in 2003 resulted in relatively low whole plant maize DM yields, and a cool wet spring resulted in delayed maize development and reduced yield at Arlington in 2002 (Table 1). Yields approaching 20 t DM ha<sup>-1</sup> were produced at Lancaster in 2002 and Arlington in 2003 when growing conditions were excellent.

Table 1. Yield of whole-plant maize grown in kura clover living mulch or killed kura clover near Arlington and Lancaster, WI, USA over 3 years.

Nitrogen Treatment (kg N ha <sup>-1</sup> )	Arlington			Lancaster		
	2001	2002	2003	2001	2002	2003
	t DM ha <sup>-1</sup>			t DM ha <sup>-1</sup>		
0	11.9a†	14.0b	18.8a	9.8a	20.2a	13.8a
28	12.1a	14.5ab	18.3a	9.8a	18.5b	13.7a
56	11.3a	16.3a	19.3a	9.9a	18.8ab	13.1a
84	13.6a	13.3b	18.8a	10.8a	19.0ab	14.4a
84 (killed kura control)	13.7a	14.4ab	20.5a	10.3a	18.9ab	14.4a
CV, %	14.2	11.0	14.1	9.3	5.5	7.1
Contrasts	Significance					
Control vs. rest	NS	NS	NS	NS	NS	NS
84 N vs. control	NS	NS	NS	NS	NS	NS
Linear	NS	NS	NS	NS	NS	NS

† Within columns, means followed by the same letter are not significantly different at  $P = 0.05$  according to Fisher's protected LSD. NS is non-significant at  $P = 0.05$ .

Over all six environments, maize silage yields in suppressed kura clover living mulch were equal to the control treatment where maize was grown in a killed kura clover stand (Table 1). Clearly the herbicide suppression that was applied was effective in controlling competition from kura clover over a wide range of environmental conditions. But because well-established kura clover has a massive rhizome system, glyphosate was not effective at killing it, and the clover recovered to full production by June in the season after maize production (data not shown). The lack of whole-plant yield response among nitrogen fertilizer treatments suggests that suppressed kura clover living mulch fully satisfied the nitrogen requirements of the maize crop (Table 1). Earlier research (Zemenchik *et al.*, 2000) revealed borderline deficient soil nitrate concentrations under maize-kura clover living mulch, but no apparent loss of yield, suggesting that nitrogen was mineralized from the decaying clover tissues at rates rapid enough to meet the maize requirements.

## Conclusions

Maize silage can routinely be produced in a kura clover living mulch with no maize yield loss if kura clover is adequately suppressed. Furthermore, all of the nitrogen required for maize silage production appears to be available through the suppressed clover, and kura clover recovers to full production by



June the following season. We used glyphosate resistant maize (GMO) and glyphosate herbicide in these experiments, but it is likely that other herbicides could be used to control both kura clover and weeds in this living mulch system.

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## Influence of supplemental irrigation on annual ryegrass

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### Abstract

The objective of this research was to evaluate the effect of supplemental irrigation on increasing and stabilizing yield and quality of forage produced by annual ryegrass (*Lolium multiflorum* Lam.) in southern Portugal. The following four irrigation treatments were tested: rainfed (control), and irrigation up to 25%, 50% and 100% of soil water holding capacity. The results showed that water irrigation doubled the number of harvests, four instead of two compared to the rainfed treatment, and also doubled the yields of dry matter, crude protein and dry matter digestibility which shows the importance of supplemental irrigation in stabilizing forage availability along the year, even at the lowest level of irrigation used. The differences between the control and the irrigation treatments were more noticeable with respect to crude protein yield.

Keywords: productivity, nutritive value, annual ryegrass, irrigation.

### Introduction

Annual ryegrass (*Lolium multiflorum* Lam.) yield in southern Portugal is strongly affected by soil water availability in early autumn and especially late spring. This species is usually cultivated under rainfed conditions allowing usually two harvests. Lourenço and Palma (2001) reported total dry matter yield values ranging from 5,274 to 6,790 kg ha<sup>-1</sup>. However, values lower than 3,500 kg ha<sup>-1</sup> have been reported by Lourenço and Palma (2005). This shows the great variability of forage production of the region depending mostly on total amount and rainfall distribution along the year. Supplemental irrigation, can increase and stabilize yields, but since water is becoming an expensive and scarce resource, it is important to investigate the response of this species to irrigation.

### Material and Methods

The experiment was conducted in 2003/2004 that, in spite of October being very rainy, was a dry year since the amount of rainfall (433 mm) was lower than the normal (634 mm). On the other hand, the temperatures were higher than the normal except in October. The field trial was set up in a Luvisol of the Experimental Center of Currais, located near Évora (14 km), with 82 mg kg<sup>-1</sup> of P<sub>2</sub>O<sub>5</sub>, 62 mg kg<sup>-1</sup> of K<sub>2</sub>O, and pH (H<sub>2</sub>O) of 5.78. The following four irrigation treatments were tested: rainfed (control), and irrigation up to 25%, 50% and 100% of soil water holding capacity. The seeded area of 8,100 m<sup>2</sup> was divided into four areas, one for each treatment. A sprinkler irrigation system was used with two lines for each of the three irrigation treatments. A profile probe PR1/4 was used to monitor soil water content at different depths (10, 20, 30, and 40 cm). The values measured, usually twice a week, were used to adjust the irrigation requirements, estimated by the Cropwat model (FAO, 1992) using meteorological data and the Penman-Monteith equation (Allen *et al.*, 1994), in order to maintain the soil water content at each desired irrigation treatment level. Planting date was on October 8, 2003, using the Pollanum variety, and seeding at the rate of 750 live seeds m<sup>-2</sup> (38 kg ha<sup>-1</sup>). Harvest dates were the following: March 8, May 11, June 15, and July 13. Nine randomised samples, of 1 m<sup>2</sup> each, were harvested in each irrigation treatment to evaluate forage production. After harvests, 50 kg ha<sup>-1</sup> of nitrogen was applied.

Dry matter yield was determined after oven drying at 65°C during 48 to 72 hours. Crude protein was analyzed by the Kjeldhal standard procedure (AOAC, 1975), and *in vitro* dry matter digestibility by the Tilley and Terry technique (Tilley and Terry, 1963).

## Results and Discussion

Dry matter yield values are presented in Figure 1. Irrigation allowed to double the number of harvests and to increase dry matter yield even at the lowest irrigation treatment, which shows the importance of irrigation in regularizing the curve of dry matter production in the Mediterranean environment.

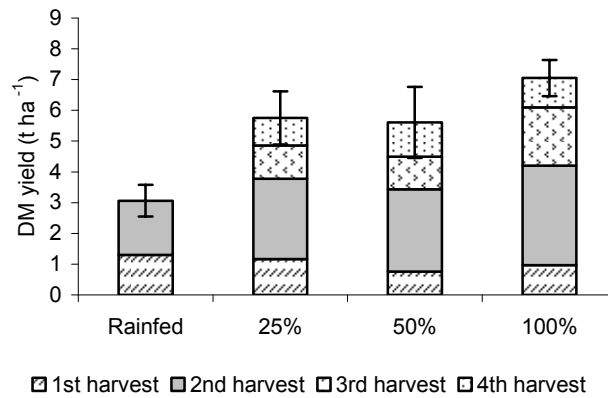


Figure 1. Seasonal dry-matter yield for annual ryegrass subjected to three irrigation treatments in relation to the rainfed control.

With respect to protein yield the advantage of irrigation seemed to be even bigger since the values (Figure 2) were more than 50% higher, compared to the results for rainfed conditions.

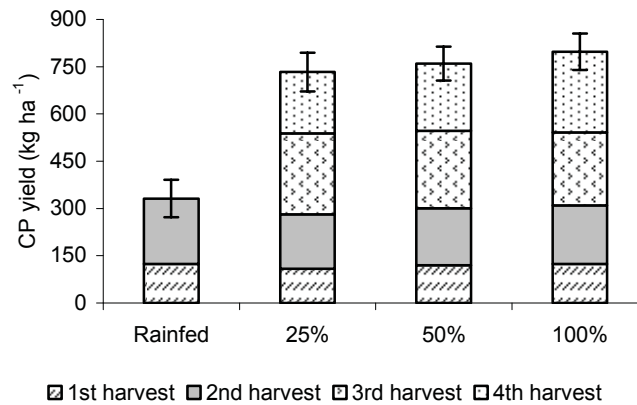


Figure 2. Seasonal crude protein yield for annual ryegrass subjected to three irrigation treatments in relation to the rainfed control.

Results for dry matter digestibility yields (Figure 3) were not so consistent compared to protein yield but, once again, even the lowest irrigation treatment produced more than the rainfed one.

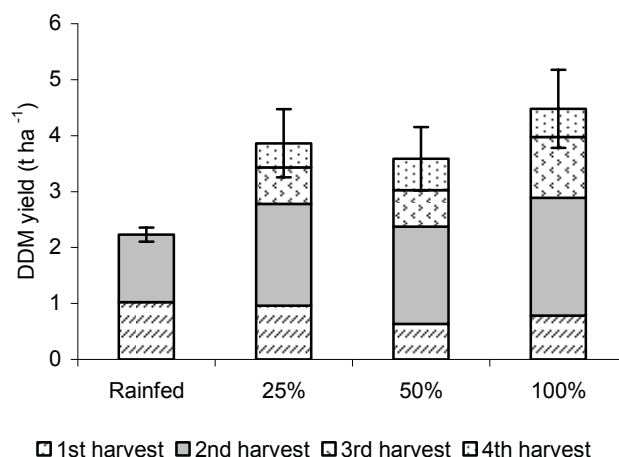


Figure 3. Seasonal digestible dry matter yield for annual ryegrass subjected to three irrigation treatments in relation to the rainfed control.

### Conclusions

As a general conclusion, it can be stated that in years drier than average, such as 2003/2004, even a small amount of water can make a big difference in stabilizing the curve of forage production along the year, doubling the number of possible harvests. With respect to crude protein and digestible dry matter yields, the differences between the control and the irrigation treatments were more noticeable for crude protein.

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## Irrigation of grass/clover under grazing and cutting

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### Abstract

With the aim to examine the drought sensitivity and to assess evapotranspiration in grazed grass/clover an irrigation experiment with cutting and continuous grazing with heifers was carried out on coarse sandy soil in 2004-2005. There were three irrigation treatments; full irrigation at 25 mm soil water deficit, half irrigation with irrigation every second time of full irrigation and no irrigation. Irrigation was managed by decision support system Vandregnskab. The treatments greatly affected the seasonal growth and proportion of white clover (*Trifolium repens* L.). For the whole season the utilization of the irrigation water (kg dry matter mm<sup>-1</sup>) was the same under cutting and grazing. However, measurements of soil water content showed that the Vandregnskab DSS model overestimated evapotranspiration under grazing, which then had caused an overestimation of the irrigation needs. In the second year there were nearly no yield increase due to irrigation, which indicates the need for more varied tools taking into account precipitation forecasts and grazing parameters such as residual effects on growth rates and needs for continuous growth.

Keywords: irrigation, grazing, cutting, white clover.

### Introduction

On sandy soils an important constraint for grass/clover production is the availability of soil water. Under continuous grazing, especially with dairy cows, there is a need for constant herbage production, whereas under cutting a period with depressed growth is less critical. Under grazing it has been indicated that the root depth is less than under cutting (Deinum, 1985), which decreases the amount of available water. Further white clover is often reported more drought sensitive than grass. These circumstances mean that grass/clover pastures on sandy soils are irrigated frequently with high amounts of water. In Denmark a great part of intensive managed grass/clover is placed on coarse sandy soil with low soil water availability. Currently used irrigation management tools like Vandregnskab and Markvand (Plauborg and Olesen, 1991, Plauborg *et al.*, 1996) are based on data from irrigation experiments under cutting. However, the soil water balance is affected by continuous grazing, which maintain a more constant and often lower leaf area index than under cutting.

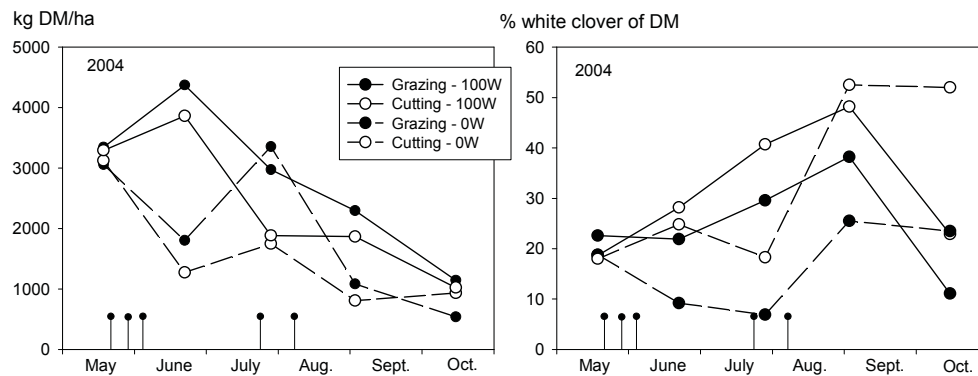
### Materials and methods

In 2003 a perennial ryegrass/white clover sward (*Lolium perenne* L. and *Trifolium repens* L.) was established and an experiment with irrigation was carried out during 2004 and 2005 with the same treatment in the plots each year. Results up to September 2005 are included in this paper. The swards were either cut (60 m<sup>2</sup> plots) or grazed continuously (600-1000 m<sup>2</sup> plots) by second year heifers. The plots were (100-W) irrigated with 25 mm at a soil water deficit of 25 mm in the internet DSS Vandregnskab ([www.planteinfo.dk](http://www.planteinfo.dk)), (50-W) irrigated every second time 100-W was irrigated after start of a new drought period and (0-W) unirrigated. The plots were irrigated by a spray boom with an intensity of 36 mm hour<sup>-1</sup>. A buffer area for grazing was used to keep the compressed sward height at 8 cm in the plots in the beginning of the season decreasing to 6 cm later in the season. The grazed sward was topped once per season about July 1. There were three replicates in a split plot design with irrigation as main plots. Herbage production was measured by a Haldrup plot harvester five times during the season both in the cut and grazed plots. In the grazed plots an area was cut by the plot harvester to obtain the same stubble height as in the cutting plots and the area was fenced off in the same period as a regrowth period in the cutting plots. In that way the production was measured in an area, which until

fencing off had been grazed. Compressed sward height was measured weekly by 50 measurements with a plate raising meter. Soil water content was measured weekly by the TDR-method. The soil type was coarse sandy with c. 90 % coarse and fine sand and 2-3 % humus and with a field capacity for plant available water of c. 60 mm.

## Results and discussion

In 2004 there was a drought period in the 1<sup>st</sup> regrowth and 100-W was irrigated three times (Figure 1) and 50-W was irrigated once. Later in the season there were only two smaller drought periods that released one irrigation in 100-W and none in 50-W. A similar picture was found in 2005 with the only difference that the strong drought period occurred one month later. The seasonal growth is shown in figure 1 for the first experimental year. Because of no drought in spring and no residual effects of cutting/grazing there was no differences in 1<sup>st</sup> cut. In the rest of the season, dry matter (DM) yield was higher in the grazed plots than in the cut plots and the clover content was lower. This can be due to the N-excretion from the heifers and the effect of grazing on the plant morphology. The drought in 1<sup>st</sup> regrowth greatly depressed the yield in 2<sup>nd</sup> cut, but as the drought in the 2<sup>nd</sup> regrowth was limited, the DM yield in 0-W was the same as in 100-W, which could be due to compensatory growth in 0-W after a drought period. The proportion of white clover in the herbage was lower in 0-W plots than 100-W plots in most of the season, which also was expected due to the normally found greater drought sensitivity in white clover than perennial ryegrass. However, at the last cut it was opposite, and in 2005 especially in 100-W cut plots the clover content was still low (Table 1).



W) at grazing and cutting respectively. | : 100-W irrigated with 25 mm.

The hypothesis was that the grazed sward should be more drought sensitive than the cut sward. The utilization of the irrigation water ( $\text{kg DM mm}^{-1}$  irrigation) was, however, not higher under cutting than under grazing (Table 1). In the second harvest year, 2005, there was surprisingly nearly no effect of irrigation. This could be due to a later drought than in 2004 and residual effects from 2004.

Table 1. Dry matter yield (t DM ha<sup>-1</sup>), amount of irrigation (Irr) (mm), yield increase per mm irrigation (kg DM mm<sup>-1</sup>) and proportion of weighted white clover (% clover of herbage DM).

		2004				2005			
		Clover (%)	Yield (t ha <sup>-1</sup> )	Irr (mm)	kg (DM/mm)	Clover (%)	Yield (t ha <sup>-1</sup> )	Irr (mm)	kg (DM/mm)
0-W	Grazing	14.7	9.8	0		21.7	11.0	0	
	Cutting	27.6	7.9	0		35.1	8.3	0	
50-W	Grazing	19.1	9.8	25	0	19.9	10.9	25	-2
	Cutting	22.4	8.1	25	9	28.8	8.4	25	4
100-W	Grazing	26.0	14.1	125	34	22.0	11.2	100	3
	Cutting	31.4	11.9	125	32	16.2	8.5	100	2
Cutting/grazing		**	***			NS	***		
Irrigation		*	***			NS	NS		
Irrigat.*cut/graz.		NS	NS			NS	NS		

\*\*\*: P<0.001, \*\*: P<0.01 and NS: non significant.

The soil water balances of the treatments were simulated by the Vandregnskab DSS model for grass under cutting. This model uses temperature sums to predict the leaf area index (LAI), which assumes a value of 0.5 during winter and after cutting and then raises to a maximum LAI of 5, corresponding to full potential evapotranspiration from the grass. From simulations of the 2004 TDR-measured soil water content data, it soon became clear, that the model overestimated evapotranspiration and soil water deficit in the grazed treatments. The maximum LAI of the model was then calibrated to the 2004 data to give a better fit. While a maximum LAI of 5 was ok under cutting, it was shown that a maximum LAI of about 1.5 gave the best prediction of seasonal data of soil water deficits under grazing. This is in accordance with LAI measurements under continuous grazing showing an average of 1.1 (Søegaard, 2002). The new model for grazing was tested on the 2005 soil water deficit data, and fitted the data fairly well.

Accordingly, since the original model (with a max. LAI of 5) was used for irrigation management in all of the experiment, irrigation needs were overestimated in grazing treatments. Therefore higher yield responses to irrigation could probably have been obtained by better scheduling taking into account that evapotranspiration under grazing does not reach full potential level for much of the season. For 2005 the model triggered an irrigation of 25 mm on 31<sup>st</sup> of May, which however was followed by 49 mm of precipitation the following days. This may have contributed to leach recently applied nitrogen, decrease yield compared to unirrigated; and it underlines the need to take into account precipitation forecasts in irrigation scheduling.

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## Drought resistance evaluation of perennial grasses

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### Abstract

In semi-arid Mediterranean regions, perennial forage species could play a primary role in rain-fed agricultural systems by complementing the commonly used annual species in order to widen the forage utilization by animals. To identify cultivars characterized by high drought resistance and water use efficiency a greenhouse study was carried out on 3 cultivars of *Dactylis glomerata* L., 1 cultivar of *Festuca arundinacea* Schreb and 1 cultivar of *Phalaris aquatica* L. Net photosynthesis, transpiration rate, stomatal conductance, Relative Water Content and Specific Leaf Area were monitored during the water stress period. *D. glomerata* 'Ottava' showed the highest photosynthetic activity while *P. aquatica* 'Partenope' and *F. arundinacea* 'Tanit' showed the lowest transpiration and photosynthesis rates. Under drought conditions, stomatal conductance and transpiration rate were less variable than net photosynthesis. *D. glomerata* seemed more adapted to severe water deficit conditions since it maintained longer a high photosynthetic rate even at the most severe drought treatment.

Keywords: perennial forage grasses, drought resistance, net photosynthesis.

### Introduction

Forage species have a strategic importance in semi-arid and arid regions. In Mediterranean areas, dry matter production of pastures and its seasonal distribution are strongly related to the meteorological pattern. The choice of suitable forage species and varieties, taking into account their growth behaviour and their adaptation to drought conditions, represents one of the main strategies to improve forage production in semi-arid areas. Drought resistance is generally defined as the maintenance of plant production during moderate or severe water deficit. Several studies have been carried out on drought resistance in annual forage species while less experimental results are available on perennial forage grasses (Sanderson *et al.*, 1997). To identify the main ecophysiological characteristics of herbaceous perennial grasses conferring drought survival, as the ability to survive at low soil moisture under Mediterranean summer drought and optimal water use efficiency (WUE), an experiment on 5 perennial grasses was carried out.

### Materials and Methods

On December 2003, seeds were sowed into containers and at the beginning of March transplanted at the 5<sup>th</sup> leaf stage in 45 l pots arranged in a factorial complete randomised design with five replicates. From the end of March, three water regimes were applied: 1) control, soil humidity was kept at the field capacity, 2) W1, water losses from pots were reintegrated at 75% 3) W2, water losses from pots were reintegrated at 50%. Three genotypes of *Dactylis glomerata* L. ('Ottava', 'Jana' and 'Gusana'), 1 cultivar of *Festuca arundinacea* Schreb ('Tanit') and 1 cultivar of *Phalaris aquatica* L. ('Partenope') and 3 water regimes were compared in a pot experiment trial under greenhouse from December 2003. At the beginning of March 2004, after seed germination in containers, 2 plants per pot at the 5<sup>th</sup> leaf stage were transplanted in 45 litres of 50:50 sand:nursery substrate mix and well irrigated until the drought treatment started. Two times per week, pots were weighed to determine water losses and water volumes for irrigation. Net photosynthesis, transpiration rate and stomatal conductance by a gas analyser (CIRAS-2), Relative Water Content (RWC) and Specific Leaf Area (SLA) were monitored during the water stress period.



## Results

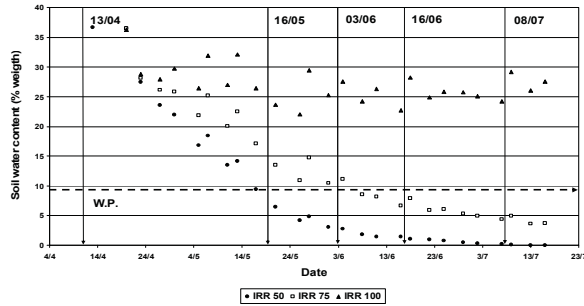


Figure 1 - Evolution of soil water content in three water regimes. While ‘Tanit’ tall fescue had a RWC around 65%. SLA was not significantly influenced by substrate water content, whereas different SLA were measured between accessions: ‘Tanit’ and ‘Jana’ showed the lowest values, on average 20 m<sup>2</sup> kg<sup>-1</sup> (Table 2).

Table 1 - Relative Water Content (RWC, %) in relation to ecotypes and water regimes.

Genotype	Water regimes			Mean
	Control	W1	W2	
Ottava	83.2	81.4	57.0	73.9b
Gusana	84.2	82.5	80.0	82.2a
Jana	86.6	81.7	41.8	70.0c
Tanit	82.0	72.4	41.1	65.2d
Partenope	81.5	72.2	61.9	71.9bc
Mean	83.5a	78.1b	56.3c	

Means with the same letter are not different for P≤0.05;

Table 2 - Specific Leaf Area (SLA, m<sup>2</sup> kg<sup>-1</sup>) in relation to ecotypes and water regimes.

Genotype	Water regimes			Mean
	Control	W1	W2	
Ottava	25.7	31.4	24.9	27.30ab
Gusana	32.8	36.6	30.4	33.27a
Jana	19.8	23.2	19.0	20.67b
Tanit	22.1	18.0	17.2	19.11b
Partenope	28.2	29.9	32.0	30.01a
Mean	25.70a	27.83a	24.69a	

Means with the same letter are not different for P≤0.05;

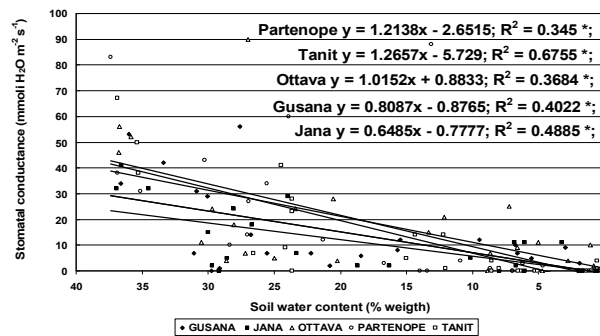


Figure 2 - Relationship between stomatal conductance vs soil humidity in 5 ecotypes.

Regression analysis between stomatal conductance and soil water content (Figure 2) and net photosynthesis and soil water content (Figure 3) evidenced the influence of available soil water reduction on physiological behaviour. Considering the relation between stomatal conductance and soil water content, ‘Gusana’ and ‘Jana’ showed lower regression coefficients, 0.8087 and 0.6486 respectively, seeming to respond less rapidly to drought. ‘Partenope’ and ‘Tanit’ had the highest regression coefficients for the

photosynthesis rate vs soil water content. ‘Partenope’ showed a lower photosynthesis activity than *D. glomerata* accessions, probably due to an earlier phenological development as a response to progressive water deficit.

## Conclusion

The experiment allowed a complete control of the drought imposition through water content monitoring in pots and simulated a progressive soil water deficit in late spring. At the beginning of the drought, plants maintained a high photosynthetic activity, in order to accumulate carbohydrates to be used for the autumn vegetative re-growth (Voilare and Lelièvre, 1997). As a response to water stress, all the accessions tended to reduce the length of the phenological cycle anticipating the summer senescence induction. *D. glomerata* accessions, and in particular ‘Ottava’, were characterized by the highest photosynthesis rate. *D. glomerata* seemed more adapted to severe water deficit conditions since it maintained longer a high photosynthetic rate even at the most severe drought treatment. Among *D. glomerata* accessions, the high photosynthesis activity of ‘Gusana’ could be explained by a lateness of the reproductive stage, probably due to an inefficient fulfilment of cold requirements for material collected at high altitude and low winter temperature environment (Ledda *et al.*, 2002; Ledda and Seddaiu, 2003). *Phalaris* ‘Partenope’ responded to severe drought drastically reducing the stomatal conductance, the transpiration and photosynthesis rate, and closing earlier the growth cycle. *Festuca* ‘Tanit’ showed a similar pattern of the monitored physiological variables to *Phalaris* ‘Partenope’, even though the reproductive stage was reached later. *Phalaris* ‘Partenope’ seemed to react to drought stress closing early the growth cycle, which could be considered a *drought escaping* mechanism, while the behaviour of *Dactylis* accessions could be mainly explained as *drought tolerance* (Turner, 1979).

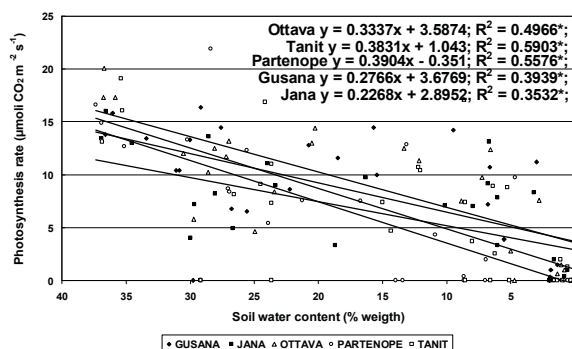


Figure 3 - Relationship between photosynthesis vs soil humidity in 5 ecotypes.

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## Water Use Efficiency in three cultivars of *Dactylis glomerata* under different soil water contents

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### Abstract

Water use efficiency (WUE) of forage crops is becoming a major character under Mediterranean conditions. Thus, WUE will be a main trait in the selection of new cultivars for these areas. The need of accurate and simple variables to estimate WUE in large selection field experiments will also be of great interest. In this sense, the objective of the present work was to evaluate WUE in three cultivars of *Dactylis glomerata* L. by gas exchange parameters and gravimetric methods. Potted plants of cultivars Porto, Jana and Kasbah were subjected to three water regimes namely 50%, 70% and 100% field capacity. Soil water content and water consumption were measured by gravimetric methods every day along a four-week period. Leaf gas exchange was measured one and three weeks after the beginning of the treatments to determine intrinsic WUE at leaf level (A/g). At the end of the experiment, plant biomass parameters were determined and WUE at plant level was calculated as leaf biomass production per water consumed. Large differences were observed among cultivars and treatments, with cultivar Porto showing the highest biomass accumulation and WUE at plant level. The relationship between WUE at plant level and A/g was not always clear.

Keywords: biomass production, *Dactylis glomerata*, drought, photosynthesis, stomatal conductance, water use efficiency.

### Introduction

The greater needs of water and food resources as a consequence of the world population increase lead to a future scenario where agricultural production should increase without an extra income of irrigation water. In such a situation, water use efficiency (WUE) of forage crops will be a main trait in the selection of new cultivars for Mediterranean areas where, in addition to the increase of water requirements, the foresighted climate change predicts an increase of aridity (McCarthy *et al.*, 2001). In this sense, several attempts have been made to establish accurate and simple variables to estimate WUE in large selection field experiments. Moreover, the relationship between leaf-level WUE, crop WUE and plant production has been found to be different depending on species and conditions (Condon *et al.*, 2004 and references therein). Under Mediterranean conditions, where the rainfall pattern is highly variable and heterogeneous within and among years, the use of perennial forage species, usually less productive than annuals, but with the ability to take profit of late summer precipitation, and thus of a longer growing season, could be interesting in order to extend the grazing season. In this sense, the objective of the present work was to evaluate WUE in three cultivars of *Dactylis glomerata* by gas exchange parameters and gravimetric methods.

### Materials and methods

The experiment was carried out in the experimental field of the Balearic Islands University in Mallorca, West Mediterranean Basin, during late-spring and early-summer 2005. The climate is typically Mediterranean with warm and dry summers and cool wet winters. Seeds of three contrasting cultivars of *Dactylis glomerata*: Kasbah (summer dormant), Porto (non dormant) and Jana ('mild' dormant) were sown in seed benches with horticultural substrate in January. At late March, seedlings were transplanted to 5 L pots (four seedlings per pot) and randomised disposed outdoors under a rainout shelter. The

substrate was a mixture of 50% horticultural substrate and 50% clay calcareous soil. All plants (24 pots per cultivar) were kept under optimal soil moisture conditions until the beginning of the experiment, when they were cut at 5 cm height. At this point, the total biomass of six pots per cultivar was measured (initial biomass). After cutting, the remaining plants (18 pots per cultivar) were kept under saturating irrigation during one week and then three different water regimes were imposed: field capacity (control), 70% field capacity (mild drought) and 50% field capacity (severe drought). The soil water content capacity was previously calculated by gravimetric methods on four substrate samples. The pot weight at field capacity and its water content were then calculated. Soil water regime was controlled by weighing each pot daily and restoring the soil water content specific to each treatment. Three weeks after treatments were imposed, leaf, stem and root biomass were determined in six pots per treatment and cultivar. The leaf area was measured in a sub-sample (of total leaf biomass) by using a portable AM-100 leaf area meter (ADC, Herts, UK) in order to calculate the specific leaf area and the total pot leaf area. In addition, green and senescent aerial biomass were considered separately. The leaf biomass production ( $\Delta B$ ) was calculated by:  $\Delta B = B_2 - B_1$ , where:  $B_2$  is the mean (six replicates) pot leaf biomass at the end of the experiment and  $B_1$  is the mean (six replicates) pot leaf biomass at the initial point of the experiment. Gas exchange measurements (six replicates per cultivar and treatment) were taken one and three weeks after the beginning of the treatment by using an infrared gas analyzer (Li-Cor 6400, Li-Cor Inc., Nebraska, USA). The cuvette conditions were fixed at 1500  $\mu\text{mol photons m}^{-2}\text{s}^{-1}$  and 370 ppm of  $\text{CO}_2$ . The measurements were done at mid-morning of sunny days on healthy sunny exposed leaves. An ANOVA was performed, by using SPSS 11.0 statistical package (SPSS, Chicago, IL), to study the influence of cultivars, treatments and its interaction on water use efficiency at plant level, measured as the leaf biomass produced per water consumed.

## Results and discussion

The climatic conditions during the experiment were those typical of Mediterranean summer (data not shown). Biomass accumulation differed among cultivars (Figure 1). Porto showed the highest biomass accumulation and Kasbah the lowest. Jana accumulated less biomass than Porto when irrigated at field capacity, but the opposite was observed at 50% field capacity. Differences among treatments in biomass accumulation were only clearly observed in Porto plants. In Jana, root biomass was lower in field capacity plants than in 70% and 50% field capacity, as a consequence total biomass accumulation was very similar in the three treatments. In a similar way, water use efficiency at plant level (WUE, considered as leaf biomass production per water consumed) was significantly higher in Porto than in Jana (except in 50% field capacity), while Kasbah plants showed a significantly lower WUE than Porto and Jana in the three treatments (Table 1). In all cultivars, WUE was significantly higher in well watered plants than in 50% field capacity.

Porto was the most productive and water use efficient cultivar when plants were subjected to a good water regime or to a mild drought. Nevertheless, when a severe drought was imposed, Jana showed a slightly higher biomass accumulation and WUE than the other two cultivars. Moreover, the low biomass accumulation and WUE showed by Kasbah plants, suggest that they were, at least, partially dormant even under optimal soil water conditions.

A negative relationship between WUE at plant level, measured as the leaf biomass production per water consumed, and WUE at leaf level measured, after three weeks of treatment, by gas exchange techniques (intrinsic WUE,  $A/g$ ) was observed in cultivars Jana and Porto (Figure 2). By contrast, Kasbah was clearly out of this relationship. In fact, Condon *et al.* (2004) suggested that WUE at leaf level does not always reflect WUE at plant level.

Table 1. WUE measured as leaf biomass produced per consumed water ( $\text{g L}^{-1}$ ). Values are means of six replicates  $\pm$  S.E. Different letters indicate significant differences at  $p < 0.05$ .

	Jana	Kasbah	Porto
100% Field Capacity	0.59 $\pm$ 0.06ef	0.15 $\pm$ 0.062b	0.75 $\pm$ 0.04g
70% Field capacity	0.49 $\pm$ 0.04de	0.12 $\pm$ 0.031ab	0.67 $\pm$ 0.07fg
50% Field capacity	0.39 $\pm$ 0.05cd	0.0003 $\pm$ 0.0423a	0.34 $\pm$ 0.02c

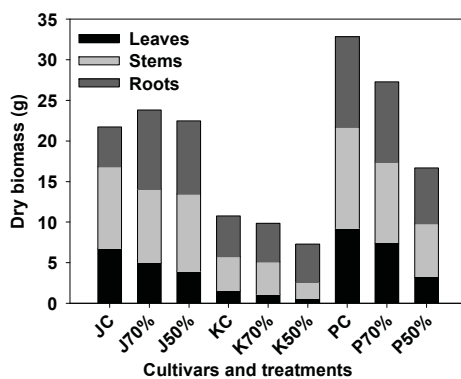


Figure 1. Dry biomass of leaves, stems and roots of each cultivar (Jana, Kasbah and Porto) and treatment (field capacity (C); 70% field capacity (70%) and 50% field capacity (50%)). Values represent mean of six replicates.

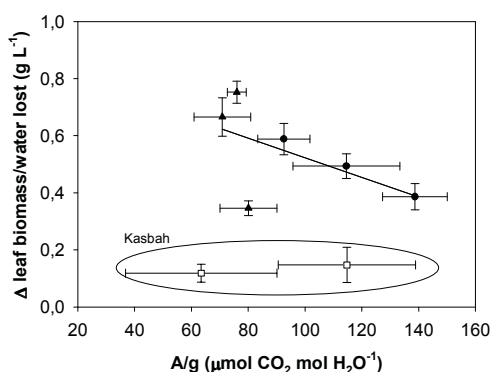


Figure 2. Relationship between WUE plant level, measured as the leaf biomass production per water consumed, and intrinsic WUE at leaf level (A/g) in Porto (closed triangles), Jana (closed circles) and Kasbah (open squares) plants. Each point represent mean of six replicates

### Acknowledgments

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# **Necromass and soil water availability in Mediterranean grasslands: simulation under two extreme experimental conditions**

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## **Abstract**

The relationship among plant spatial distribution and water availability of soil and necromass was studied in Mediterranean grasslands. Regional and local variability have been considered: five sites along an altitudinal gradient of 1100 m were sampled in Central Spain, each one comprising plots on the upper and the lower part of a slope. Spatial distribution of herbaceous vegetation has been analysed for both plant species and morpho-functional traits (MFTs), revealing an ecological variation trend associated simultaneously with altitude and geomorphological position. This trend was related to water availability, assessed as a function of evaporation under two simulated extreme temperature conditions. Results depended on temperature setting and allowed examining hypotheses on the consequences of various scenarios of climatic change on Mediterranean grasslands.

Keywords: altitudinal gradient, geomorphology, water stress, temperature influence, ecosystem function.

## **Introduction**

In Mediterranean grasslands, water availability varies at regional (changes in temperature and rainfall with altitude) and local (geomorphological processes in a slope system; Solntsev, 1974) scale. This variability determines well-known patterns in species composition and associated morpho-functional traits (MFTs; Montalvo *et al.*, 1991). Our aim is to investigate the relationship between grassland vegetation and water evaporation in soil and necromass, considering different altitudes and geomorphological positions. The process of evaporation has two phases (Mellouli *et al.*, 2000); at the beginning, water loss occurs at a constant rate (lineal phase) and at a certain point, evaporation decreases progressively (exponential phase), since water is retained harder. In this study Water evaporation was assessed experimentally under two extreme temperatures and was characterized by different parameters and correlated to plant variability (floristic composition and MFTs).

## **Materials and Methods**

The study was carried out in five sites situated along an altitudinal gradient of 1100 m in Central Spain Mountain Range, from El Pardo area (642 m.a.s.l.), representing the driest conditions, to Puerto de La Morcuera (1719 m.a.s.l.), representing the moistest and coldest conditions. In each site, two topographical situations were studied: upper and lower zones of a southerly oriented slope. In each zone, plant species were sampled in five 20 cm<sup>2</sup> plots randomly distributed. Relative abundance of 53 qualitative MFTs was evaluated as the percentage of species that presented a given trait. Thus, vegetation was characterized with a Detrended Correspondence Analysis (DCA) of the relative abundance of species and MFTs. Besides, necromass and surface soil were sampled in each plot. The samples, previously dried, were watered until saturation and weighted every hour until they reached a constant weight. The evaporation process was evaluated for two temperature conditions (hot, 30°C, and cold, 12°C) and described by six evaporation parameters. Finally, relationship among water availability and floristic composition was investigated using stepwise multiple regressions between evaporation parameters (considering separately soil and necromass) and, as the dependent variable, dimension 1 of DCA analyses.

## Results and Discussion

In the two DCA for species and MFTs, locations distributed along dimension 1 depending on their altitude. Independently, dimension 2 differentiates upper and lower zones, absorbing less than 8% of total variability in both cases. However, in the same location lower zones are comparatively displaced towards coordinates related to higher altitudes. Concisely, dimension 1 revealed a pattern of variation associated simultaneously to elevation and geomorphology (Figure 1). These trends were identified as an ecological persistence gradient (Montalvo *et al.*, 1991): species and MFTs resembling strategies type *r* were more abundant in lower altitudes (annual life cycle, wind and animal seed dispersion and large plant size, represented by species such as *Biserrula pelecinus* and *Galium parisiense*), while strategies type *K* prevailed in higher altitudes (perennial life cycle, bulb, tuber or root tuber and high palatability with *Festuca iberica* and *Carex muricata* subsp. *lamprocarpa* as the dominant species).

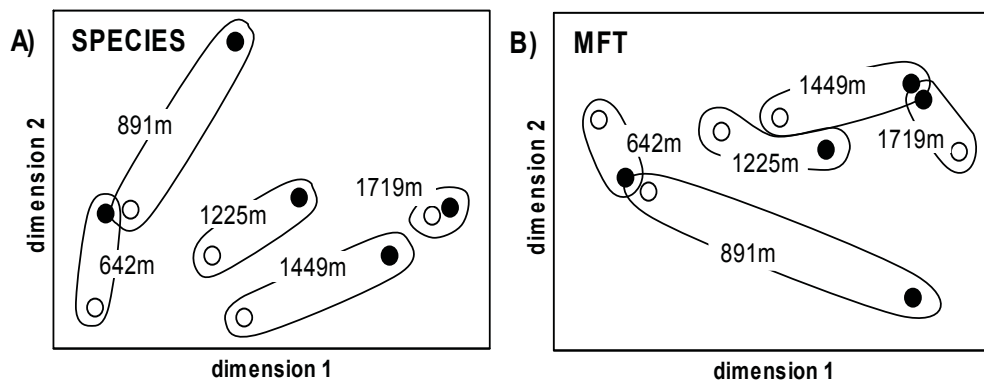


Figure 1. Vegetation gradient defined by an ordination analysis (DCA) of 50 observations of plant species (A) and MFT (B) abundance. The evolvents comprise the two geomorphological locations (upper part: close circles, lower part: open circles) in each of the five altitudes. Dimension 1 of both analyses simultaneously correspond to the *ecological persistence gradient* defined in the text.

Temperature determined important differences in the evaporation parameters of soils where these plants communities exist (Sánchez-Jardón *et al.*, 2004). The water content at the end of the lineal phase ( $W_c$ ) and total evaporation length ( $T_t$ ) parameters showed linear relationships with the ecological persistence gradient, in both soil and necromass (Figure 2). However, these relationships depended on the temperature considered: when temperature was high the trend in vegetation showed a significant relation with  $W_c$  (Fig. 2.A,C), while at a low temperature,  $T_t$  was the important parameter (Fig. 2.B,D). In soil, both  $W_c$  and  $T_t$  increase with altitude leading to *K* strategies (Fig. 2.A,B). On the contrary, in necromass, these parameters tend to decrease as moving from *r* to *K* strategies in the ecological persistence gradient (Fig. 2.C,D).

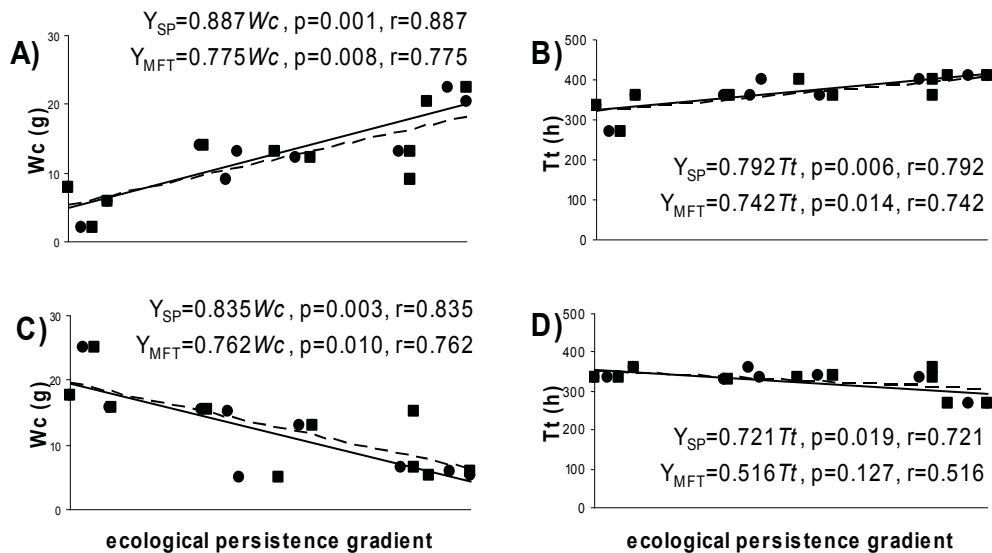


Figure 2. Relationship among evaporation parameters of soil (A,B) and necromass (C,D) and the *ecological persistence gradient* (composed by the two first dimensions of DCA analyses for both species (circles) and MFTs (squares); see text and Fig.1).

## Conclusions

Results allow inferring possible consequences of climate change on Mediterranean grasslands in relation to water availability for the herbaceous plants. If global temperatures increase, soil from high altitudes will need higher water content in soil to maintain their characteristic vegetation. On the contrary, if global temperatures eventually decrease, plants associated to higher altitudes will get favourable conditions also in lower altitudes, which would result in uniformed vegetation along the altitudinal gradient.

Additionally, we have found that necromass in lower altitude grasslands retains a higher amount of water (has a greater water holding capacity) than necromass from higher altitudes. Necromass buffers evaporation from soil and, subsequently, prevents soil desiccation, which diminishes water stress for plants.

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## Effects of the grazing period on nitrate leaching

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### Abstract

On experimental dairy farm De Marke, which is located on a leaching sensitive soil, actual nitrate content of the upper groundwater is 54 mg L<sup>-1</sup>, although a specific fertilization and grazing strategy has been applied to restrict nitrate leaching. The grazing period on De Marke starts at the end of May, after a first cut for silage, and ends mid September. By modeling, possibilities were explored to reduce leaching by starting and ending the grazing period earlier. Results suggest that the nitrate content can be reduced to 47 mg L<sup>-1</sup> by starting grazing in spring as soon as possible.

Keywords: grazing, nitrate, excretion.

### Introduction

Grazing can be attractive to dairy farmers as it reduces costs and stimulates animal health. Besides, it is appreciated by society for its contribution to an attractive rural landscape. However, excretion during grazing will contribute to nitrate leaching. On experimental farm De Marke, a prototype dairy farming system is practiced that should reduce nitrate content of the upper ground water to a level below 50 mg L<sup>-1</sup>. Grazing should be maintained, if possible. A specific grazing strategy has been developed to restrict nitrate leaching. The N-excretion during grazing is restricted by allowing cows to graze only 5.5 hours a day and by feeding low protein products indoors at night. In order to distribute excreta evenly over the fields, rotationally grazing is practiced (Aarts *et al.*, 1992). The grazing period starts at the end of May, after a first cut for high quality silage, and ends mid September, what is one month earlier compared to commercial farms. In spite of this strategy, the nitrate concentration below pastures stabilized at 54 mg L<sup>-1</sup>, level above the desired one (50 mg L<sup>-1</sup>). An analysis that was carried out earlier pointed out that grazing contributes to more than 50% of leaching (30 mg L<sup>-1</sup>). As the target is not realized, a further reduction of nitrate leaching is required. The objective of this paper is to present the results of model calculations on the effects of starting and ending the grazing season earlier.

### Materials and methods

Experimental farm 'De Marke' is situated on light sandy soil. The rooting zone of 30 cm, with an average organic matter content of 4.8 %, overlays a layer of coarse sand without organic matter. Groundwater depth is 1 to 3 meter below soil surface. As a result, the soil is very susceptible to leaching of nitrate. Of the total farm area of 55 ha, 11 ha of permanent grassland and 13 ha of temporary grass, in rotation with maize, are available for grazing by 77 cows and associated young stock. Grassland is fertilized with 250 kg N ha<sup>-1</sup> from slurry. No mineral fertilizer is used. Input by clover is about 21 kg N ha<sup>-1</sup>. The average amount of N excreted during grazing is 94 kg N ha<sup>-1</sup>. The characteristics of pasture management are summarized in Table 1.

Effects of shifting the grazing period on nitrate leaching were calculated with the model NURP (Vellinga *et al.*, 2001), calibrated with data of 1997-2004.

### Results and discussion

Results of model calculations indicate that, grass is available for grazing from the end of April onwards. Therefore, on De Marke the grazing period can start more than one month earlier. Calculations suggest

that nitrate leaching can be reduced to a level below 50 mg L<sup>-1</sup> by starting grazing at that time. Therefore, a further reduction of grazing intensity (by a shorter grazing time or grazing period) is not necessary to realize the target regarding nitrate leaching. The beneficial effects of starting grazing period earlier can be explained by the longer period in which excreted N can be taken up by the crop. At the end of the season crop growth decreases, mainly as a result of low light density. At higher grazing intensities, as practiced by most farmers, the effect of shifting the grazing period would be even stronger. The effects on De Marke, are relatively small, due to the fact that grazing is already restricted and the protein level of the diet low. The calculations with NURP suggest that grazing contributes to 26 mg L<sup>-1</sup> which is comparable with the value of 30 mg L<sup>-1</sup> mentioned earlier.

In early spring energy content of grass is higher than in August and September. To realize a high silage quality spring grass is cut and not grazed. On De Marke 225,324 kg silage is harvested in total, annually. The uptake of grass by grazing cows is 5 kg DM d<sup>-1</sup>, complying with 11,935 kg dry matter in total, in 31 days. When grazing starts 31 days earlier 11,935 kg grass dry matter is needed for grazing in that period, and therefore can not be used for silage making. Because grazing period ends also 31 days earlier, a comparable amount of herbage comes additional available for silage making in August and September. The amount of silage involved with the shift of the grazing period is 5% of total silage yield of the farm. On average, silage of the first cut of grass contains 1.04 MJ NEL kg DM<sup>-1</sup> and silage from grass cut in August and September contains 0.96 MJ NEL kg DM<sup>-1</sup>. The average energy content of silage on De Marke is 0.99 MJ NEL kg DM<sup>-1</sup>. Therefore, the deviation in spring and autumn of the energy content is less than 10% of the average level. The effect of shifting grazing season on the average energy level of the silage of the farm is less than 1%.

Table 1. Main characteristics of grazing on De Marke.

Method	Rotational grazing
Number of cows ha <sup>-1</sup>	3.2
Grazing period	End of May – Mid Sept
Grazing hours per day	5.5

Table 2. Nitrate content of the upper groundwater below pastures on De Marke in the actual situation, model calculated after shifting the grazing period (starting and ending one month earlier) and at full time housing (zero grazing).

Scenario	Grazing period	Nitrate in upper groundwater (mg L <sup>-1</sup> )
Actual situation	End of May – Mid Sept	54
Early grazing	End of April – Mid August	47
Zero grazing	-	28

## Conclusion

The grazing period can start earlier by abandoning the strategy to devote high quality spring grown grass to silage making. In a farming system that has already been strongly adapted to restrict nitrate leaching, shifting grazing season by starting early in spring will reduce the nitrate content of the upper ground water from 54 mg L<sup>-1</sup> to 47 mg L<sup>-1</sup>. Because of the low grazing intensity, early grazing does not lead to a significant effect on average silage quality.

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## Grain sorghum silages as an alternative to irrigated maize silage

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### Abstract

Silage maize production for feeding dairy cows has to be secured by irrigation in areas affected by summer drought. The objective was to determine how best to combine the maintenance of milk production and the conservation of water resources. We evaluated in 2004-2005 the benefits and limits of two sorghum silages (*Sorghum bicolor* L.) (grain sorghum silage GS and sweet grain sorghum silage SS) compared to an irrigated maize silage (MA). Irrigation was provided only to the maize crop. Each of the three diets were given to a group of eight dairy cows for a 15 week winter experiment. Forage yield and composition, cropping costs, intake and animal performance were recorded. The sorghum crops yielded 14.3 and 17.8 t DM ha<sup>-1</sup> for SG and SS respectively, compared to 20.1 t for irrigated MA. Sorghum input costs were lower. Their quality was also good (less starch but more protein than MA diet). Cows fed SG had a significantly higher intake than those offered MA and gave the same milk production. Cows fed SS had an intake comparable to MA but yielded less milk. Non-irrigated sorghum could become a good alternative to irrigated maize if its agro-environmental and economic benefits are confirmed in various conditions.

Keywords: grain sorghum, water resource, dairy cows, maize, silage, costs.

### Introduction

Under European conditions, whole plant maize silage is the main ingredient in the diet of dairy cows, especially during the winter because of its yield potential and feeding value. Nevertheless maize production could be limited in some areas because summer rainfall is insufficient. So it has to be supplemented by irrigation and then generates an important agricultural water consumption. Grain sorghum hybrids and especially sweet sorghum types should be of great interest to avoid this additional water consumption (Lemaire *et al.*, 1996, Legarto, 2000). After a preliminary trial (Nascimento *et al.*, 2005) we evaluated in 2004 the benefits and limits of two grain sorghum silages (*Sorghum bicolor* L. Moench) for dairy milk production, compared to an irrigated maize silage. We paid special attention to forage quality and yield, cropping costs and animal performance.

### Materials and methods

The two grain sorghum hybrids were a traditional grain type, cv. Solarius, and a sweet type, cv. Topsilo, the latter with a higher biomass potential yield but a later flowering date. Those hybrids and the maize hybrid, cv. Argenteo were grown in Lusignan (0°08'35''E, 46°40'39''N, Poitou-Charentes, France) on two ha plots each. Irrigation to prevent summer drought stress was provided only to the maize crop. Sowing densities were 100 000, 220 000 and 370 000 grains ha<sup>-1</sup>, respectively for MA, GS and SS. The row spacing was 0.75 m in order to allow a silage harvest with the same material. Mineral fertilization was calculated using the balance method taking into account the soil furniture. Weeds and pest control (*Ostrinia nubilalis* H., European corn borer) were conducted using standard practices. Farm direct costs, including pesticides, fertilizers, seeds and energy power for irrigation were recorded. Mechanisation, labour costs and fixed costs were not taking into account. Harvest, storage and distribution conditions of both silages were strictly comparable. Twenty four Holstein-Friesian dairy cows (33.2 kg milk d<sup>-1</sup>, 640 kg body weight, 54 days in milk in average) were paired and assigned for 15 weeks to one of the three experimental diets : maize silage diet (MA), grain sorghum silage (GS) or sweet sorghum silage diet (SS). Cows were individually fed the experimental diets *ad libitum* each morning, using Calan type feeding doors, targeting 10% refusals. The three diets of cows were balanced with the same amount and

nature of concentrates (2.2 kg of N rich concentrate and 3 - 5 kg of energy rich concentrate, according to the expected milk yield) except urea which was only given to the MA cows (0.05 kg). Dry matter yield was evaluated at the date of harvest with 5 samples (two meter long on a single row). Fresh forage was sampled in the silos to evaluate the water soluble carbohydrates content (WSC). Offered forages were daily sampled and monthly bulked. Fiber (Neutral Detergent Fiber, NDF), crude protein and starch contents such as *in vitro* dry matter digestibility (IVDMD) were measured throughout the experiment. Forage and concentrate intakes and milk production were daily recorded. Milk composition (fat and protein contents) and body weight variations were weekly observed. Diets and animal effects were tested by analysis of variance. Diet square means were compared by a Student Newman Keuls test.

## Results

The mean characteristics of the experiment are given in Table 1 (crops and forages), Table 2 (costs) and Table 3 (animal performances).

Table 1. Crops and forages characteristics.

Treatments	Rainfall + Irrigation mm	DM yield (t ha <sup>-1</sup> )	Crude protein (g kg <sup>-1</sup> )	NDF (g kg <sup>-1</sup> )	Starch (g kg <sup>-1</sup> )	WSC (g kg <sup>-1</sup> )
MA Maize	191 + 152	20.1	82	390	30.4	41
GS Sorghum	174 + 0	14.3	104	400	27.7	46
SS Sorghum	190 + 0	17.8	95	383	29.6	46

DM: Dry matter; NDF: Neutral detergent fiber; WSC: Water soluble carbohydrates.

Table 2. Cropping costs.

Treatments	Fertilizers €	Seeds €	Pesticides €	Electric power <sup>(a)</sup> €
MA Maize	97	158	177	76
GS Sorghum	59	99	129	0
SS Sorghum	59	51	129	0

<sup>(a)</sup> Electric power, in euros, for irrigation.

Total rainfall was 174 to 190 mm between sowing and harvest, allowing a good growth of the sorghum crops. Maize was given 152 mm more in five irrigation times from early July to mid August. The costs of the sorghum crops were lower than the maize (minus 269 and 221 euros per ha for SS and GS respectively) because of the nature of the weeds control strategy, the lack of European corn borer biological control and the lack of irrigation (electric power supply). As expected the irrigated maize silage provided a high forage yield with a good quality (20.1 t DM ha<sup>-1</sup> and digestibility of 72.7 g kg<sup>-1</sup>). In comparison with the maize, the grain sorghum had a lower yield (- 29 %) with a good quality (less starch but more protein). The sweet sorghum reached a high yield (only - 11 %) and an intermediate position for starch and protein contents. Despite its characteristics it did not present any difference in water soluble carbohydrates content (WSC). The two sorghums were of lower digestibility according to the IVDMD and fibre content evaluations.

Table 3. Animal performances.

Treatments	Intake kg DM	Dry matter (g kg <sup>-1</sup> )	IVDMD (g kg <sup>-1</sup> )	Milk (kg d <sup>-1</sup> )	Fat (g kg <sup>-1</sup> )	Protein (g kg <sup>-1</sup> )	BW gain (kg)
MA Maize	17,0 b	347	727	29.9 a	40.1 a	32.1 a	+ 20 a
GS Sorghum	19,9 a	332	715	30.3 a	42.6 a	32.1 a	+ 29 a
SS Sorghum	15.8 b	311	710	25.5 b	41.3 a	31.7 a	+ 15 a

IVDMD: *in vitro* dry matter digestibility; BW: body weight

a-d: different superscripts mean significant differences between the values (P<0.05), SNK test.

Cows fed the GS diet had a significantly higher intake than cows fed the MA diet (+17 %). Despite its lower feeding value, this higher intake led to similar milk production and protein content throughout the experiment and to weak tendencies to an improved fat content and a higher body weight gain. Cows fed the SS diet had a non significantly lower intake (- 7 %) but a significantly lower daily milk production (- 15 %).

### Conclusions

The results of the present study confirm that non-irrigated sorghum is able to provide quite a high biomass yield at a low cost. It also has a good efficiency for dairy cow milk production. It could clearly permit water conservation or improved water management during summer drought in areas involved in rearing ruminants. This type of sorghum could then become a good alternative to maize. Its agro-environmental and economic benefits have to be confirmed in a range of conditions. Further experiments with special focus on environmental impacts are also planned. This study pointed to great differences in yield potential and quality between the two sorghum types. The evaluation of such an intraspecific variability will probably allow farmers to choose improved hybrids for feeding dairy cows with low environmental impacts.

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# Productivity and sward composition of semi-natural pasture under different N fertilizing regimes

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## Abstract

The mountain pastures in Croatia often remain unfertilized or poorly fertilized and produce low quantities of poor quality forage. The objective of this study was to evaluate changes in the botanical composition and productivity of semi-natural mountain pasture, rotationally grazed by sheep, under different rates of N application (35, 100 and 150 kg N ha<sup>-1</sup> yr<sup>-1</sup>). Significant differences between years occurred in all the investigated parameters. The dry matter (DM) yield in the second year (4,805.7 kg ha<sup>-1</sup>) was significantly lower compared with the DM yield in the first and the third year (8,721.2 and 9,210.6 kg ha<sup>-1</sup>, respectively). In the second year, the annual content of grass, legumes and dead plant material in total DM yield decreased ( $P < 0.01$ ), while the content of broadleaf plants increased more than 65 % in comparison with the first year. The N fertilizer did not cause significant differences in DM yield and botanical composition among treatments within each of the three years. This indicates that low input management does not necessarily result in sward productivity losses.

Keywords: nitrogen, yield, sward composition.

## Introduction

Current upland pasture management in Croatia includes fertilizing with low amounts or even no N fertilizers applied per year. The productivity of grazed pastures is frequently limited by the supply of N from soil organic matter (Steele and Vallis, 1988). The effect of high rates of nitrogen application on white clover (*Trifolium repens* L.) performance in mixed swards is well described (Frame *et al.*, 1997). However, if N application is too high, the clover portion of the sward becomes too low to provide sufficient N to the sward later in the growing season (Caradus *et al.*, 1993; Thomas, 1992). Grass-clover relationship is intensified under grazing especially on semi natural swards containing large number of species. Grazing, fertilizing and cutting regime as well as climatic factors was recognized as factors influencing species composition. The objective of this study was to evaluate the response in productivity of an investigated pasture to N fertilizing at low and moderate rates, under grazing conditions.

## Material and Methods

The experiment was conducted in north-western Croatia, during three years (2002-2004), at Faculty of Agriculture experimental field Medvednica (650 m altitude, mountain region, 1230 mm average annual precipitation, 6.6°C mean annual temperature), on semi natural grassland-association *Arrhenatheretum medioeuropaeum* (*Br-BI-19*), that have been grazed rotationally by steers. Prior to beginning of the experiment (March 2002), the sward was composed of 67.70 % grass, 16.08 % white clover, and 16.22 % forbs in dry matter yield. In March each year of the experiment, 500 kg ha<sup>-1</sup> N-P-K (7:20:30) was applied as compound fertilizer. The three fertilizer N levels applied annually (kg ha<sup>-1</sup>) were 35 (N<sub>35</sub>), 100 (N<sub>100</sub>) and 150 (N<sub>150</sub>). The treatment N<sub>35</sub> received all N in the spring application of N-P-K fertilizer, and treatments N<sub>100</sub> and N<sub>150</sub> additionally received 65 and 115 kg ha<sup>-1</sup> N as 27 % ammonium nitrate (NH<sub>4</sub>NO<sub>3</sub>), in three dressings, after each grazing. A randomized-block design was used with four replicates of three treatments giving a total of 12 plots (plot size 27.5 m<sup>2</sup>). Each replicate was rotationally grazed by Charolais sheep. The sward was stocked when herbage height ranged from 13 to 17 cm. The end of each grazing period was at 5 cm residual stubble height or maximum 24 h per replication. At the beginning of each grazing period, three samples from each plot were taken for

measuring pasture productivity using a 1.0 × 0.3 m quadrat and hand scissors. A 0.1 kg subsample was taken for separation into constituent floristic components, oven dried for 48 h at 60 °C and weighed to ascertain DM yield. Data were analyzed using mixed model procedures (SAS Inst., 1998).

## Results and discussion

The annual DM production was not increased significantly ( $P>0.05$ ) by increasing fertilizer N rate (Table 1). Nonsignificant fertilizer N rate × year interaction ( $P>0.05$ ) indicates that DM production responded similarly under various N fertilizing rates in each year. The growing season significantly affected annual DM production ( $P<0.01$ ). These results demonstrate a great influence of climatic factors on DM production for the same pasture type. Low DM yield occurred in 2003 and was caused by low precipitation and high temperatures through vegetative period (204.6 mm less than average precipitation and 1.6 °C higher temperature).

Table 1. Dry matter yield over growing seasons and N fertilizing rates.

	DM (kg ha <sup>-1</sup> )			mean
	2002	2003	2004	
Fertilizer N (kg ha <sup>-1</sup> annually)				
35	8,097.8	4,541.2	8,174.9	6,937.9
100	8,864.2	4,880.3	9,123.1	7,622.5
150	9,201.6	4,995.8	10,334.0	8,177.0
Significance		NS		NS
Mean	8,721.2	4,805.7	9,210.6	
LSD (0.01)§		953.2		

NS not significant

§ LSD value for comparing mean annual DM yields.

Averaged over years, N fertilization did not affect annual content of legumes, grasses and forbs in DM yield ( $P>0.05$ ). Fertilizing with 35 kg N ha<sup>-1</sup> significantly ( $P<0.05$ ) increased annual content of dead plant material in DM yield in comparison with treatments N<sub>100</sub> and N<sub>150</sub>. The annual content of legumes, grasses, forbs and dead plant material in DM was significantly different ( $P<0.01$ ) between growing seasons (Figure 1). The proportion of legumes decreased from 118.75 g kg<sup>-1</sup> DM in 2002 to 17.7 and 15.64 g kg<sup>-1</sup> DM in 2003 and 2004, respectively. Similar trend occurred with grasses, while the proportion of forbs significantly increased from 289.65 g kg<sup>-1</sup> DM in 2002 to 488.19 and 420.26 g kg<sup>-1</sup> DM in 2003 and 2004, respectively.



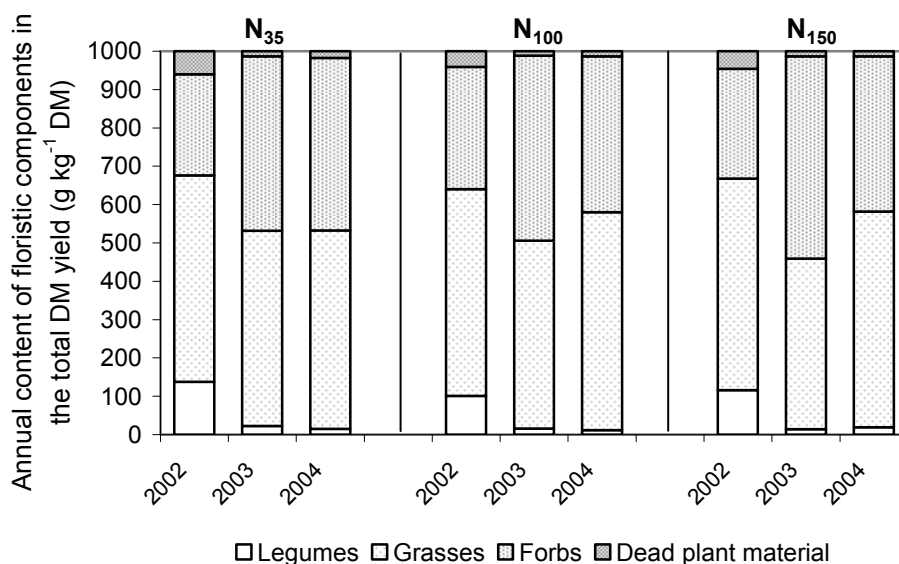


Figure 1. Annual content of floristic components in the total DM yield (g kg<sup>-1</sup> DM).

This type of pastures has been associated with unstable herbage production over growing seasons and consequently with changes in botanical composition. Higher nitrogen levels than usual did not improve yield stability and sward productivity. Similarly, Rodwell (1992) shows that large numbers of grassland communities are not dependent upon management for the maintenance of their species composition, being maintained solely by extreme climatic and/or edaphic factors. In temperate pastures there is a need to use N as efficiently as possible for economic and environmental reasons. Reducing nitrogen fertilizer application does not necessarily result in reducing sward productivity.

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## **Influence of fertilisation and management on degraded pastures**

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### **Abstract**

During the period 2003-2005, the influence of organic and mineral fertilizers on the productivity, biodiversity and quality of a pasture with *Festuca valesiaca* Schleich. ex Gaudin was analyzed. By fertilization and changing its way of being used, from heavy grazing to the use by mowing (in the optimum period) one could notice a significant increase of the yield, an improvement of the floristic composition and of the forage quality. The highest yields were obtained in the variants fertilized with 20-40 t ha<sup>-1</sup> manure on a soil background of N<sub>60</sub>P<sub>30</sub>, with increases of 69-82% compared non-fertilized control. It was also noticed an improvement of the floristic composition by increasing the number and the percentage of those species with good feeding value, such as: *Poa pratensis* L., *Festuca valesiaca* Schleich. ex Gaud, *Medicago lupulina* L.

Keywords: fertilization, usage management, biodiversity, productivity, quality.

### **Introduction**

The permanent pasturelands from Romania stretch for a surface of 4.9 million ha, from with 340 thousand ha are situated in steppe and forest steppe zones.

Generally speaking, these pasturelands are situated on degraded, poorly productive lands, having an inadequate floristic composition, and the yields obtained are low and of poor quality. From the basic measures for rehabilitation of these pasturelands, an important place occupies the balanced fertilization and the rational use (Jeangros and Bertola, 2002; Peeters and Kopec, 1996; Vintu *et al.*, 2003). The organic and mineral fertilization and the rational use lead to substantial increases of the production, biodiversity and the fodder quality improvement (Carlen *et al.*, 1998; Scehovic, 1999; Peeters *et al.*, 2004).

### **Materials and Methods**

The experiment was carried on during the years 2003 – 2005, on a pasture with *Festuca valesiaca* Schleich. ex Gaudin, from the forest steppe in the north-east of Romania, characterized by average annual rainfall of 520 mm. The soil on which the experiment was made was of cambic chernozem type, not strongly lavigated. This experiment aimed at finding the influence of the fertilization with manure which is applied annually, every two or three years, in doses of 10-20-40 t ha<sup>-1</sup>, combined with N<sub>60</sub>P<sub>30</sub>, on a permanent pasture used by mowing or grazing (mowing when the predominant grass are of 15 - 18 cm height).

### **Results and discussion**

The average yields obtained in the years 2003 – 2005, on the permanent pasture used by mowing or grazing, on which were applied different doses and combinations of organic and mineral fertilizers are presented in Table 1.

Table 1. The influence of fertilization on the production of dry matter.

No.	Type of fertilization	Grazing		Mowing	
		t ha <sup>-1</sup>	%	t ha <sup>-1</sup>	%
1	Unfertilized control	2.74	100	2.94	100
2	10 t ha <sup>-1</sup> annual manure + N <sub>60</sub> P <sub>30</sub>	4.03**	147	4.83***	164
3	20 t ha <sup>-1</sup> annual manure + N <sub>60</sub> P <sub>30</sub>	4.63***	169	4.96***	169
4	20 t ha <sup>-1</sup> manure at 2 years + N <sub>60</sub> P <sub>30</sub>	4.21***	154	5.15***	175
5	30 t ha <sup>-1</sup> manure at 3 years + N <sub>60</sub> P <sub>30</sub>	4.26***	155	5.13***	174
6	40 t ha <sup>-1</sup> manure at 3 years + N <sub>60</sub> P <sub>30</sub>	4.54***	166	5.36***	182
Average		4.07**	149	4.73**	161
		DL 5% = 0.78 t ha <sup>-1</sup>		1.0 t ha <sup>-1</sup>	
		DL 1% = 1.06 t ha <sup>-1</sup>		1.37 t ha <sup>-1</sup>	
		DL 0.1% = 1.41 t ha <sup>-1</sup>		1.88 t ha <sup>-1</sup>	

The data in Table 1 show that the yields obtained when the pasture is exploited by mowing are bigger, in all the fertilizing variants, than the ones obtained by grazing.

Table 2 presents data regarding the structure of the vegetal carpet in 2005. There can be noticed that the average weight of the grass is bigger when exploiting by mowing, while

The average weight of the legumes is bigger when exploiting by grazing.

Table 2. The influence of fertilization on the structure of vegetal cover (%), 2005.

No.	Type of fertilization	Grazing			Mowing		
		Grass	Legumes	Others	Grass	Legumes	Others
1	Unfertilized control	45	36	19	46	31	23
2	10 t ha <sup>-1</sup> annual manure + N <sub>60</sub> P <sub>30</sub>	53	37	10	69	19	12
3	20 t ha <sup>-1</sup> annual manure + N <sub>60</sub> P <sub>30</sub>	62	17	21	53	31	16
4	20 t ha <sup>-1</sup> manure at 2 years + N <sub>60</sub> P <sub>30</sub>	55	33	12	79	12	9
5	30 t ha <sup>-1</sup> manure at 3 years + N <sub>60</sub> P <sub>30</sub>	54	25	21	75	6	19
6	40 t ha <sup>-1</sup> manure at 3 years + N <sub>60</sub> P <sub>30</sub>	62	24	14	57	27	16
Average		55	29	16	63	21	16

Watching the evolution of the fodder quality under the influence of the experimental factors (Table 3) we can notice that the average percentage of crude protein (CP) is bigger when exploiting by grazing, being also correlated with a lower average percentage of crude fibre.

Table 3. The influence of experimental factors on the quality of fodder (g kg<sup>-1</sup> DM).

No.	Type of fertilization	Grazing				Mowing			
		CP	CF	ASH	FAT	CP	CF	ASH	FAT
1	Unfertilized control	15.3	19.9	6.3	1.64	14.8	19.3	6.2	1.46
2	10 t ha <sup>-1</sup> annual manure + N <sub>60</sub> P <sub>30</sub>	16.8	20.3	5.1	1.73	15.3	20.4	5.2	1.80
3	20 t ha <sup>-1</sup> annual manure + N <sub>60</sub> P <sub>30</sub>	15.8	22.5	6.3	1.71	15.4	21.9	5.8	1.45
4	20 t ha <sup>-1</sup> manure at 2 years + N <sub>60</sub> P <sub>30</sub>	16.0	20.6	7.5	1.87	14.8	20.8	5.4	1.59
5	30 t ha <sup>-1</sup> manure at 3 years + N <sub>60</sub> P <sub>30</sub>	16.3	21.4	6.9	1.45	14.5	20.7	7.3	1.30
6	40 t ha <sup>-1</sup> manure at 3 years + N <sub>60</sub> P <sub>30</sub>	16.1	20.9	7.0	1.08	14.4	21.6	7.2	1.50
Average		16.1	20.9	6.5	1.58	14.9	20.8	6.2	1.52

CP – crude protein; CF – crude fibre; ASH – total ash; FAT – crude fat.

In Figure 1 are presented data about the botanical composition of the vegetal cover differentiated according to the level of the anthropic intervention. Generally we can see an increase of the species-rich at those levels of fertilization in which bigger doses of organic and mineral fertilizers were applied.

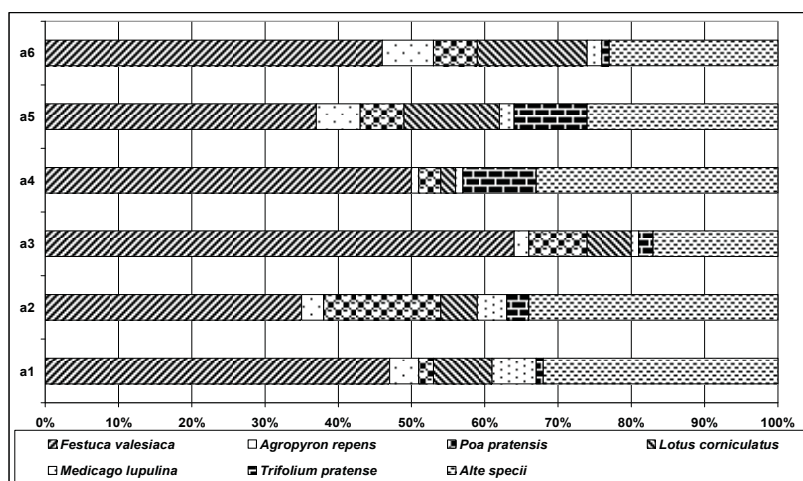


Figure 1. The influence of antropic intervention on the botanical composition.

### Conclusions

The conclusions of this experiment show that the use of organic and mineral fertilizers determined yield increases ensured statistically by both ways.

The yield increases compared to non-fertilized control were between 47 - 69% when grazing was used and between 64 - 82% when mowing was used.

After the use of organic and mineral fertilizers in different doses and combinations we can notice a better quality of the fodder obtained when the pasture was exploited by grazing.

The anthropic intervention determined an increase of the weight of species-rich when the doses of fertilizers also increased.

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# Effects of cattle grazing on selected soil chemical and soil physical properties

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## Abstract

At present, in Austria there is an attempt to propagate intensive cattle grazing. Therefore, the effects of trampling and cattle grazing on selected soil chemical and physical properties at the scale of a representative paddock in a mountainous region in Austria were investigated. Excessive trampling and cattle grazing lead to a distinct soil compaction especially in the 5-10 cm soil layer, resulting in stagnant water conditions, and to an accumulation of nutrients in topsoil (mainly potassium, phosphorus, boron). Furthermore, there is a permanent transfer of soil nutrients (mainly potassium and nitrogen) and organic matter by grazing cattle within a paddock. Fertilizer recommendations for a sustainable pasture management will be given.

Keywords: soil chemical properties, soil physical properties.

## Introduction

Grazing management practices need to be both ecologically and economically sustainable. Therefore, an efficient utilization of fertilizers and a grazing intensity adapted to the site are necessary. The aim of this study was to examine the effects of trampling and cattle grazing on selected soil chemical and physical properties at the scale of a representative paddock in a mountainous region in Austria in order (1) to maintain or increase soil quality, (2) to decrease nutrient losses from pasture soils, and (3) to formulate fertilizer recommendations for a sustainable pasture management.

## Materials and methods

This investigation was conducted in the Styrian Enns valley (Austria) at an altitude of 675 m. The climate is relatively cool and humid, with a mean annual air temperature of 6.7 °C and annual precipitation of 1023 mm, of which 60 % falls during the growing season (April-September). The mean monthly temperature varies from -4.5 °C in January to 17.0 °C in July. The soil is a deep, base-rich Cambisol with a loamy sand texture. The paddock investigated has a total area of about 2 ha and had been grazed by dairy cattle (Brown Swiss) five times per grazing season for more than 10 years. The stocking density was approximately 4 cows ha<sup>-1</sup> during 180 days from early May to the end of October with an average grazing time of about 8 hours per day. The strip grazed permanent pasture was fertilized regularly with farmyard manure, mostly cattle slurry. Within the paddock investigated, three different vegetation types, representing different levels of disturbance were identified. The *Matricario-Polygonetum arenastri* has been developed near the entry to the paddock where trampling intensity is greatest. This vegetation type is characterized by the 'trampled plot' (3x8 m). The 'grazed plots' (5x10 m, three replicates) were distributed in the middle of the paddock and their vegetation belongs to the *Alchemillo monticolae-Cynosuretum cristati*. The 'boundary plot' (0.5x20 m) was located under the fence and the plant species composition can only be referred to the order *Arrhenatheretalia*. The untrampled plot below the fence was grazed during the five grazing periods and had not received farmyard manure or excreta from grazing cattle. During the grazing season soil samples from the 0-10 cm soil layer (A horizon) for chemical analyses were collected before each grazing period (5 analyses of composite samples per plot). At the beginning and the end of the grazing season also soil samples for physical analyses were taken from topsoil. Soil analyses were carried out according to the ÖNORM

methods (Austrian Standards Institute). Yield and mineral element content in the harvestable above-ground plant biomass were determined by using standard methods. For each plot, the arithmetic mean and coefficient of variability were calculated.

## Results and discussion

Intensive trampling and cattle grazing lead to a soil compaction mainly in the 5-10 cm soil layer (Table 1) and to a degradation of soil structure. The porous crumb structure in the freely drained topsoil of the 'boundary plot' was replaced by a compact platy structure in the imperfectly drained topsoils of the 'grazed plots' and 'trampled plot'. Plant species such as *Ranunculus repens* L., *Agrostis stolonifera* L., and *Poa annua* L. are bioindicators of such compacted, periodically wet topsoils. When comparing the three different plots, the topsoil of the 'trampled plot' had a lower  $C_{org}$  content and consequently also a lower  $N_{tot}$  content than the topsoils of the 'grazed plots' and the previous two had a higher  $C_{org}$  and  $N_{tot}$  content than the topsoil of the 'boundary plot' (Table 2). The comparatively lower  $C_{org}$  content in topsoil of the 'boundary plot' despite a 5-fold to 8-fold higher below-ground phytomass (data not shown) can be explained by a permanent removal of organic matter due to cattle grazing without a compensatory return by farmyard manure and cattle excreta. The lower annual input of carbon from below-ground phytomass (data not shown) and a higher mean soil temperature seem to be the main reasons for the lower  $C_{org}$  content in topsoil of the 'trampled plot' compared to those of the 'grazed plots'. Primarily the topsoil of the 'trampled plot' was enriched with nutrients due to enhanced external inputs with farmyard manure and cattle excreta (Table 2, 3). Especially potassium, phosphorus, and boron are accumulating in topsoil. In the untrampled and unfertilized but grazed 'boundary plot' the nutritional status of the topsoil was very low due to the continuous removal of nutrients by grazing cattle. The long-term effect of this redistribution of nutrients within a paddock is that small areas below the fence become depleted of nutrients, while areas especially near the entry to the paddock accumulate high amounts of nutrients in topsoil, primarily those which are returned in a high degree with cattle excreta (e.g. potassium, phosphorus, boron). On the other side, potassium and nitrogen are depleted from topsoil more easily than phosphorus and sulphur due to a comparatively higher transfer from soil to the harvestable plant biomass (Table 3).

Table 1. Selected soil physical properties (means of 8 analyses and coefficient of variability).

cm Soil depth	Bulk density (g cm <sup>-3</sup> )			Pore space (%)		
	Trampled plot	Grazed plot	Boundary plot	Trampled plot	Grazed plot	Boundary plot
0-5	1.21 (9)	1.06 (14)	0.99 (19)	52 (7)	59 (9)	62 (11)
5-10	1.52 (5)	1.17 (10)	0.95 (4)	43 (5)	55 (8)	64 (2)
10-15	1.56 (2)	1.37 (0.1)	1.08 (16)	42 (4)	48 (0.1)	59 (11)

Table 2. Selected soil chemical properties (A horizon, 0-10 cm).

	n	g kg <sup>-1</sup>						mg kg <sup>-1</sup> 7d <sup>-1</sup>	meq 100 g <sup>-1</sup>	%
		$C_{org}$	$N_{tot}$	$S_{tot}$	$C_{org}/N_{tot}$	$C_{org}/S_{tot}$	$N_{tot}/S_{tot}$			
Trampled plot	5	39.8	4.2	1.0	9.5	40	4	246*	26.5	7.3
Grazed plot	15	45.0	4.7	1.0	9.6	45	5	253*	19.7	2.1*
Boundary plot	5	32.3	3.1	1.0	10.4	32	3	120*	16.7	1.1*

n = number of analyses; p.m.N. = potentially mineralizable nitrogen;  $CEC_{eff}$  = effective cation exchange capacity (BaCl<sub>2</sub>-extract); K % = percentage potassium saturation (BaCl<sub>2</sub>-extract); \* = coefficient of variability > 30 %.

Table 3. Selected soil chemical properties (A horizon, 0-10 cm) and nutrient transfer from topsoil (0-10 cm) to harvestable plant biomass.

	n	CaCl <sub>2</sub>	μS cm <sup>-1</sup>	mg kg <sup>-1</sup>				% Plant removal			
		pH	EC	P <sub>CAL</sub>	K <sub>CAL</sub>	B	C	N	P	S	K
Trampled plot	5	7.2	214	235	761	2.9	3.3	2.9	0.5	0.8	4.0
Grazed plot	15	6.4	97	110	165*	1.3	6.7	6.1	1.4	1.3	15.2
Boundary plot	5	6.4	84	15*	45*	0.6*	1.8	1.0	0.2	0.3	1.3

n = number of analyses; EC = electrical conductivity; P<sub>CAL</sub>, K<sub>CAL</sub> = lactate-soluble phosphorus and potassium content; B = acetate-soluble boron content according to BARON; % plant removal = element content in harvestable annual plant biomass in % of soil storage (C<sub>org</sub>, N<sub>tot</sub>, S<sub>tot</sub>, aqua regia extractable P and K); \* = coefficient of variability > 30 %.

### Conclusions

Although the soil chemical and physical properties prior to cattle grazing are not known, it seems reasonable to assume that the observed differences are attributable to long-term excessive grazing and trampling. The different grazing and trampling intensity as well as the uneven return of cattle excreta in grazed pastures not only creates a mosaic of vegetation structures (Mendarte *et al.*, 2005), but also results in a spatial heterogeneity in soil nutrient content and soil compaction. A sustainable pasture management takes into consideration this large-scale heterogeneity in pasture soils. Both from an ecological and economical point of view, there is a need to control trampling and grazing intensity at certain parts of a permanent pasture such as areas near the entry to the paddock, because there is a risk of overgrazing and poaching, leading to a distinct soil compaction, nutrient accumulation in topsoil (mainly potassium, phosphorus, boron), bare patches, and weed infestation. Furthermore, these intensively trampled areas need no or only small amounts of fertilizer (especially K-rich fertilizer such as cattle slurry). Therefore, within intensively grazed paddocks differential fertilizer practices and variations in grazing management are necessary.

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# Cartography and evaluation of the Red Natura 2000 rangelands in Navarra

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## Abstract

The 92/43/CEE Directive is a Community instrument that establishes a common framework for the conservation of wild animal and plant species and natural habitats of Community importance. It provides for the creation of a network of special areas of conservation called "Natura 2000". The application of the Directive in the Navarra county (north Spain) has led to the declaration of 42 Common Areas of Interest (*LICs*) which occupy 255 141 hectares (25% of the Navarra county surface), with a relevant portion of rangelands. Since Red Natura 2000 sites need Integrated Management Plans. A basic and previous step is the characterization and evaluation of their rangelands in order to establish the best grazing recommendations for the protected pastured areas. In this context, the Department of Agriculture of the Navarra Government carries out a project whose objective is identify, characterize, evaluate and perform the cartography of grazing resources present at the *LICs*. As a result of this project, a detailed digitalized cartography (scale 1:25,000) is obtained, where the spatial distribution and grazing value of the different rangelands, as well as existing cattle facilities, are included. For the maintenance and management of the alphanumeric and graphical data, a specific GIS computer program has been conceived.

Keywords: *LICs*, rangeland characterization, grazing value, cartography, GIS.

## Introduction

In 1992, the European Community promulgated the Directive 92/43/CEE. The main aim of this Directive is the preservation of the biodiversity and the implementation of models of sustainable development that warrant the preservation of habitats and species of communitarian European interest. The application of the Habitats

Directive in the Navarra county (Figure 1) has led to the declaration of 42 Common Areas of Interest (*LICs*) (Figure 2) which will involve, when applying article 6, the designation of these sites as Special Areas for Conservation (*SACs*).

The implementation of an Integrated Management Planning for each site will assure the establishment of actual and preventive measures that enable their preservation. A detailed data base that incorporates complete information on the habitats developed at each site is the first necessary step for future planning. For that reason, the Area of *Evaluación de Recursos Agrarios Departamento de Agricultura de Navarra* conducts a project which objectives are:

To identify and characterize the grazing resources present at the *LICs*.

To evaluate these grazing resources, taking into consideration their forage production, quality and seasonality.

To perform the cartography of rangelands (typology, grazing value and existing cattle facilities) at 1:25,000 scale.



Figure 1- Location of Navarra





Figure 2- Common Areas of Interest (LICs) in Navarra

**Materials and methods**

The methodological process is as follows:

Demarcation of the vegetation units using different cartographic documents available such as Digital map of Navarra Land Uses and Crops (1:25,000), Digital map of Vegetation series (1:25,000), Digital aerial restored photographs (1:5,000) and Digital Topographic map (1:5,000).

Characterization of the vegetation considering different issues such as physiography, plant community shape and structure, floristic composition and degree of preservation. Detailed field samplings are done in order to analyze community structure and floristic composition. The method used is the linear transect procedure (Welch and Scott, 1995). Nomenclature used follows the *Nomenclator de la Sociedad Española para el Estudio de los Pastos* (Ferrer *et al.*, 2001).

Estimation of the grazing value using the methodological approach proposed by Daget and Poissonet (1972) and implemented in Spain by Amella and Ferrer (1979) and Ascaso *et al.* (1996), among others. The estimate, called *Valor Pastoral* (Pastoral Value, VP), is transformed in Energy Units using the following empirical formula:

$$UF \text{ ha}^{-1} \text{ yr}^{-1} = k \times VP$$

where UF is the energy expressed in forage units, and k is a transformation coefficient that varies in function of the biogeographic environment.

To verify the relationship between the estimate VP and the UF, production and quality forage controls are carried out (cut of herbaceous biomass in exclusion plots). Once the mean grazing value of the different vegetation types is determined, they are classified in pre-established intervals of grazing value.

Cartography: the types of vegetation differentiated are drew on the aerial restored photographs and digitalized. Because of the work scale, 1/25,000, the minimum cartographic area represented in the map is 2.5 ha. Also, cattle facilities and field sampling areas are included in the map.

Information management: for the maintenance, search and use of the alphanumerical and graphical data a specific computer program has been implemented based on ArcMap, ESRI (Figure 3).



Figure 3- GIS based computer program

## Results

From each study site we obtain the following results:

Digitalized map at 1:25,000: on a topographic map the different vegetation units are represented, as well as their grazing values and the existing cattle facilities (Figure 4). Using different matrix and colours, the spatial distribution of the vegetation units and the areas with different grazing values are indicated. The legend includes information about the vegetation units belonging to each class of grazing value, their surface and their mean forage units (UF ha<sup>-1</sup>yr<sup>-1</sup>), as well as the total forage value that corresponds to each unit and to the site as a whole (in UF yr<sup>-1</sup>). Eventually, it comprises the area occupied by each grazing value class.



Figure 4- Map of the different vegetation units and their grazing values as well as the existing cattle facilities

Final document of synthesis: this document uses a simple format, similar to a filing card, where technical data related to each vegetation type is included: plant community description, floristic composition, cattle use, degradation symptoms, grazing value and impact, community dynamics and recommendations for grazing management. In addition, basic data on the area and its actual farming use are included.

Rangelands catalogue: using all the obtained information, a Navarra rangelands catalogue is being implemented. This catalogue includes information about all the rangeland units differentiated, their grazing value and the recommendations for their grazing management.

## Conclusions

The accomplishment of this project allows the compilation of relevant, biological and land-use data. This information (rangelands typology, value and degree of preservation, actual grazing pressure and optimal stocking rates, etc), is extremely useful to elaborate the Integrating Management Plans of the Natura 2000 sites. The computer program specifically designed, allows a helpful and practical access to the information related to each site.

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# Influence of the phosphoric fertilization in grasses of “dehesas” of degraded areas

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## Abstract

Two field experiments were conducted on dehesa soils, having a very low pH, aluminum in toxic levels, low amounts of calcium, magnesium and assimilable phosphorus. The objective of the experiments was to study the effect of applications of plaster, transformation residuals of the sugar industry (SIR) and phosphoric rock (possible alternative to calcium superphosphate) on natural grasses for three years. The amount of crude fiber in the grasses increased with time, and in the fertilized treatments there were significantly higher levels than the unfertilized treatment. With regard to crude protein there were significant differences between treatments in the first year, but not in the following years. In 2002 and 2003, greater protein concentrations were reached but this effect was not consistent. The application of phosphoric rock resulted in very similar production levels to those obtained with calcium superphosphate (18%). Production increased over time. Phosphoric rock could be an ecological alternative to calcium superphosphate, and SIR can give acceptable improvements in production.

Keywords: acid soils, sugar industry, phosphoric rock, calcium superfosfate.

## Introduction

The "Siberia extremeña" district is located in eastern Extremadura. This geographical zone is poor, with technological limitations and low yields from its agrarian activities. Livestock production in this zone is generally of sheep, normally for meat, but also producing milk for traditional, non-industrial, cheese-making. By far most of the estates are used for game (big and small, and many of them are close to being categorized as dehesas according to the concept defined in Ferrer *et al.* (2002). The use of the characteristic soils of this zone is made very difficult by the presence of aluminium at levels which are toxic for the growth of crops (Espejo, 1993), very low calcium, magnesium, and potassium content, and extremely low availability of phosphorus. Previous studies have shown the beneficial effect of phosphates (Olea *et al.*, 1989) and gypsum (Vizcayno *et al.*, 2001) on the biomass production of these soils. Natural phosphorites (phosphate rock) increase the soil's phosphorus and—as also does gypsum—calcium content. Furthermore, surface-applied gypsum has been shown to be an effective corrector of the aluminium toxicity in the subsurface horizons (Espejo *et al.*, 1993). These are aspects of great interest for improving the nutritional quality and persistence of legumes in these pastures (increased seed production). The goal of the present work was to study the effects of applying different sources of phosphorus in combination with the use of gypsum as a pH corrector on the nutritional quality and natural biomass production in these zones, and to examine the behaviour of phosphate rock as an alternative to the use of 18% calcium superphosphate.

## Materials and methods

A field trial was conducted in the eastern zone of Extremadura (Extremeñan Siberia) on a dehesa in Navalvillar de Pela (Badajoz), located in areas of soil with a highly acidic pH and levels of aluminium toxicity that are representative of the zone. The experimental design was of random blocks, with four replicates, and unit plots of 15 m<sup>2</sup>, with a total of 256 samples in each study year. Three fertilizer sources were studied: phosphate rock (micronized and pelleted), 18% calcium superphosphate (used traditionally), and vinasse (sugar industry residue, SIR, with a high content of phosphorus, calcium, etc.), all in combination with the application of gypsum (3,500 kg ha<sup>-1</sup>) in the first year of study. The

phosphate fertilizer was applied on the surface after the first autumnal rains, at an annual dose of 36 U.F.  $P_2O_5$   $ha^{-1}year^{-1}$ . Management was continuous-deferred sheep grazing (Olea and Paredes, 1997), the same as in the rest of the enclosure in which the trial was performed. In each year, we determined the fibre and total protein content of the biomass which was sampled by randomly throwing four  $0.25m \times 0.25m$  quadrats in each plot, as well as the biomass production corresponding to the different treatments. The parameters of the study were subjected to an analysis of variance, comparing the means with the Duncan test at a 95% confidence level. The program used was SPSS 11.5.

## Results and discussion

Total protein and total fibre: Figures 1 and 2, respectively, show the results of the ANOVA.

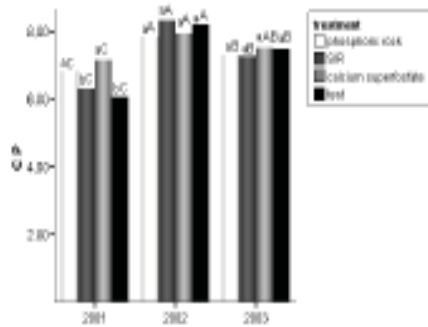


Figure 1. Evolution of crude protein content.

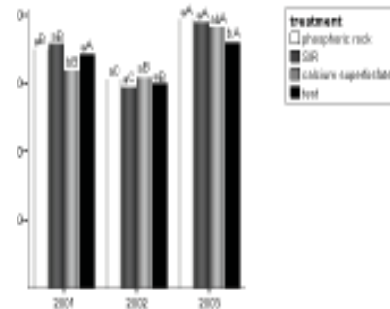


Figure 2: Evolution of crude fiber content.

Lower case letters represent the comparison between treatments. Upper case letters represent the comparison between years for each treatment. Different letters represent the existence of a minimal significant difference, with  $P < 0.05$ .

In 2001, there were significant differences between the treatments with sources of phosphorus and the vinasse and control. In the following two years, however, there were no significant differences between treatments. With respect to the comparison between years, 2002 was the year of significantly greatest content in all the treatments except for that with superphosphate which also stood out significantly in 2003. For the fibre content, in 2001 the vinasse-treated samples had the significantly lowest levels, and in 2003 the lowest contents were in the treatment with vinasse and in the control. There were no significant differences between treatments in 2002. With respect to the comparison between years, 2003 was the year of significantly greatest levels in all the treatments except with the control in 2001. The values were significantly less in the treatments with phosphate rock and vinasse in 2002.

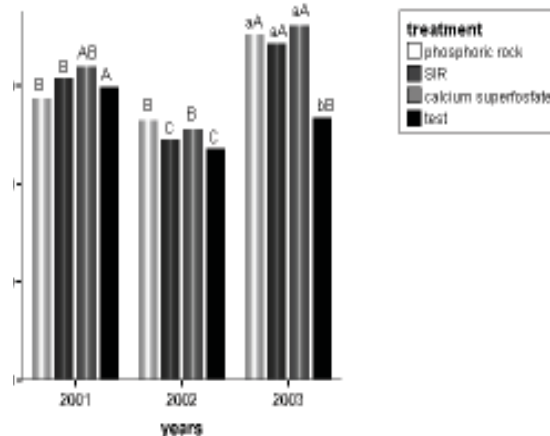


Figure 3. Evolution of biomass production.

There were no significant differences between treatments in biomass production in the first two years. In the last year of the study, however, the treatments presented significantly higher levels than the control. In the comparison between years, 2003 stood out in all the situations studied, with the highest levels of production in the treatments with fertilizer. The vinasse treatment was that of the lowest levels in 2002, a year in which there was a generalized reduction in production.

#### Conclusions

There was a significant increase in biomass production starting from the third year of fertilizer treatment. Phosphate rock gave levels of improvement that were similar to those of calcium superphosphate. Its use could therefore be considered as an ecologically friendly alternative. No major differences were observed in the quality of the biomass following the treatments.

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## Evolution of the chemical composition of the “dehesa” grasses

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### Abstract

*Trifolium subterraneum* L. and *Ornithopus compressus* L. species, in addition to graminaceous species are considered the most important pasture plants of “dehesa” of Extremadura. In the present paper, 6 “dehesa” stands representative of the south-west of the province of Badajoz (200,000 ha) were studied over a period of two agricultural years (2002-2003 and 2003-2004). Soils and plants were analyzed approximately every fortnight, from autumn until the end of spring. Samples of plants from different plots were taken to check the proportions of neutral detergent fiber (NDF) and crude protein. Climatologically two different weather conditions were studied. It was observed that NDF during the vegetative cycle of the plant increased with maturity and there were significant differences between samples taken at the end of spring and those taken previously. There was a greater amount of crude protein in plants in winter, it being significantly different from spring.

Keywords: crude protein, fiber, mediterranean pastures.

### Introduction

The semi-arid southwest of the Iberian Peninsula is characterized by the presence of vegetation consisting of herbaceous formations with an open and scattered tree canopy, known as “dehesas”. The origin of these formations lies in the limitations of the physical medium—both the poverty of the soil and the harshness of the climate have given rise to the transformation of the original ecosystems into systems of extensive, mixed-use exploitations.

Mediterranean systems are characterized by markedly seasonal production, and by meteorologically driven interannual variability in both production and quality (Ferrer and Broca, 1999).

The present work is a study of the quality of Mediterranean dehesa pastures in the SW of the province of Badajoz, estimated as the neutral detergent fibre (NDF) and total protein content in legumes (*Ornithopus compressus* and *Trifolium subterraneum*), grasses, and a representative biodiverse fraction of the ration available for grazing ruminants. The evolution of the species and groups of species was studied over the course of 2 years.

### Materials and methods

The study was conducted in 6 representative dehesas. Samples were collected in each experimental field in winter, and at the beginning and end of spring, in the years 2002-03 and 2003-04. Sampling in each zone was at random, with 4 replicates, collected by reaping the herbaceous material included within a 0.25 m square quadrat.

The assays made for each sample were of total protein (by the Kjeldahl method) and NDF using the methods described by Goering and Van Soest (1970).

The results were subjected to an analysis of variance and means were compared using the Duncan test at a 95% confidence level. The program used was SPSS 11.5.

### Results and discussion

It is shown in Figure 1 that the total protein available for ingestion declines as the plant matures (Viguera and Olea, 1999), with the content being significantly greater in winter than in spring. The ANOVA showed no significant differences ( $p < 0.05$ ) between the beginning and end of spring in

*Ornithopus compressus* or the grasses in either study year. There were significant differences, however, for *Trifolium subterraneum* and the biodiverse fraction. The fraction available is appropriate for the needs of grazing ruminants (A.R.C. 1968).

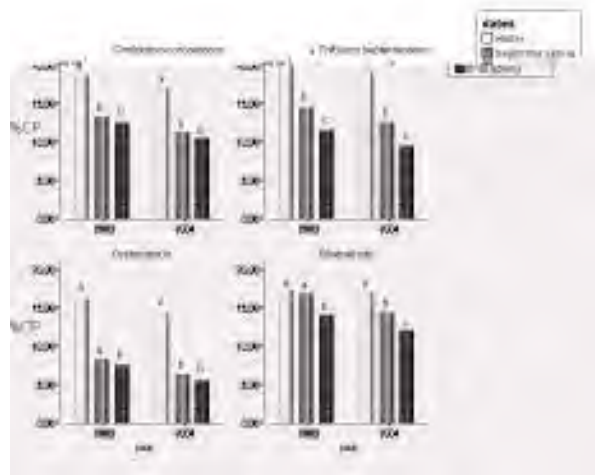


Figure 1. Seasonal crude protein evolution. Different letters represent the existence of a minimal significant difference at  $P < 0.05$ .

As the plant matures, the NDF content increases. One observes in Figure 2 that the NDF levels were greater in the grasses than in the legumes in both years of the study. This is consistent with the findings of Pérez-Corona *et al.* (1995) and Vázquez de Aldana *et al.* (2000) that the hemicellulose content of grasses is around twice that of legumes and other families. Significant differences ( $p < 0.05$ ) were found between the beginning and the end of spring in both years of the study except for *Trifolium subterraneum* in 2003. Whereas in 2004 in no case was there any significant difference between winter and the beginning of spring, in 2003 there were significant differences in all cases. This indicates that the year of sampling has a significant influence.

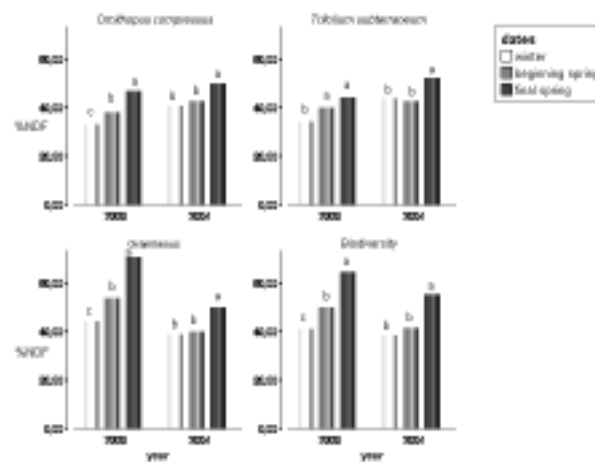


Figure 2. Seasonal NDF evolution. Different letters represent the existence of a minimal significant difference at  $P < 0.05$ .

## Conclusions

The quality of the pasture in the dehesas of the SW of the province of Badajoz displays a significant temporal variation which affects the total protein and neutral detergent fibre content.

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## Does the response of natural pastures to improvement techniques depend on soil fertility level?

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### Abstract

The need for increasing the productivity of rain-fed natural pastures is the subject of concern for farmers in the Portuguese Mediterranean regions. Their concerns surround the stocking rates and how to ensure the recovery of old and eroded arable lands. Basically, two techniques might be applied: fertilisation and sowing plus fertilisation. The response to these procedures might be dependent on soil fertility level, since it limits natural pasture productivity. To test this hypothesis we developed an 'on-farm' experiment, at 3 sites: Elvas (1.8% soil organic matter, 42 mg kg<sup>-1</sup> P), Caria (1.5% soil organic matter, 120 mg kg<sup>-1</sup> P) and Coruche (0.7% soil organic matter, 10 mg kg<sup>-1</sup> P). The annual dry matter yield of natural pasture, natural fertilized pasture and sown fertilised pasture, was recorded under grazing conditions. At the Coruche site, annual yield was lower, compared to the other sites. However, Coruche was the only site with a positive response to fertilization (97%) for natural pasture. Higher autumn and spring growth rates explained this response. The results suggested that under low soil fertility the potential for increasing pasture yield through fertilisation is higher than in high soil fertility conditions.

Keywords: rain-fed annual type pastures, fertilization, natural pasture.

### Introduction

The evolution of Portuguese agricultural systems induced high levels of soil erosion and a reduction of natural pastures biodiversity. These two facts led to a reduction of the potential yields in natural pastures. Several studies showed that yield increase and soil protection could be achieved by sowing bio diverse mixtures of annual legumes and grasses together with rational level of fertilisation (Crespo, 1982; Olea *et al.*, 1986). However, in situations where pasture biodiversity could be preserved, the fertilisation alone induced a significant response of dry matter annual production (Almeida, 1989). On the other hand, sowing bio diverse mixtures were not always efficient, and the positive response could be dependent on several growing conditions (Almeida, 2002). If soil fertility level induces strong limitations on pasture growth, then we expect, beside species richness of pastures, that the fertilisation *per se* could induce a positive response on yield. To test this hypothesis, we developed an on-farm experiment under grazing, at three different soil fertility conditions.

### Materials and methods

The present work was developed at 3 sites: Elvas, clay loamy soil, 1.8% soil organic matter, pH 7,2 and 42 mg kg<sup>-1</sup> P (High fertility level); Caria, loamy sandy soil, 1.5% soil organic matter, pH 5,1 and 120 mg kg<sup>-1</sup> P (High-medium fertility level); Coruche, sandy soil, 0.7% soil organic matter, pH 5,5 and 10 mg kg<sup>-1</sup> P (Low soil fertility level). In each site, three treatments were established under grazing conditions: natural pasture (control, NP), natural fertilized pasture (FP) and sown (mixture of annual legumes and grasses) fertilized pasture (FSP). The experiment was established in a randomised complete block design, 3 sites x 3 treatments, with 6 replicates. Grazing 'exclusion' cages (1m x 1m)

were used to evaluate aboveground biomass, in 'open-closed cut method', per plot. Sub-samples were used to estimate botanical composition by manual separation. The rest of samples were dried (65° C during 48 hours) and then analysed for N, P, Mn and B. The cutting dates were: November, February and April. Rainfall during growing season was 562,8 mm at Elvas, 725,8 mm at Caria and 428,5 mm at Coruche. The SPSS 12 software was used for statistical GLM procedure of univariate analysis of variance. Means were compared by LSD test ( $p < 0,05$ ).

## Results and discussion

Table 1. Means of total annual aboveground biomass (DW kg ha<sup>-1</sup>), % of legumes and biomass mineral content of natural pasture (NP), improved with fertilisation (FP) and with fertilisation plus sowing (FSP), in low (Coruche), High-medium (Caria) and High (Elvas) soil fertility level.

Site	Treatments	DW (kg ha <sup>-1</sup> )	Legumes (%)	N (%)	P (%)	B (mg kg <sup>-1</sup> W)	Mn (mg kg <sup>-1</sup> DW)
Coruche	NP	800	0.3	1.42	0.21	10.13	233,5
	FP	1577	8	1.51	0.25	8.8	293
	FSP	2087	25.6	19.3	0.32	11.12	268,2
Caria	NP	1681	8.1	1.54	0.26	13.98	252,4
	FP	1756	8.2	1.65	0.28	15.01	212,7
	FSP	4340	31.4	2.39	0.34	15.9	198,4
Elvas	NP	4100	41.8	2.86	0.36	23.49	75,7
	FP	4300	34.4	2.62	0.37	23.11	84,6
	FSP	7259	46.1	2.74	0.31	21.46	80,4
Standard Errors		197	0.11	4.1	0.02	16.8	1.27

Total aboveground biomass from different sites (Table 1) corresponded to the defined soil fertility levels. N concentration of biomass (Table 1) seems to be positively correlated to % legumes in pasture. In contrast, P and B content of biomass (Table 1) seem to be dependent on the soil fertility levels. In particular B concentrations are likely to reveal limitations to plant growth, strongly at Coruche and more weakly at Caria as considering critical levels suggested by Marschner (1995). Therefore, at Coruche growth was apparently B-limited. Furthermore, biomass Mn concentration (Table 1) was significantly higher at Coruche ( $P > 0.001$ ), showing a possible toxicity effect (Marco *et al.*, 1995). Significantly ( $P < 0.001$ ), the yield response of FP and FSP relative to natural pasture (NP) in each site (Figure 1) shows the stronger response at Coruche. However, the significant ( $P < 0.001$ ) interaction (Site x Treatment) showed at Caria and at Elvas no response of FP treatments.

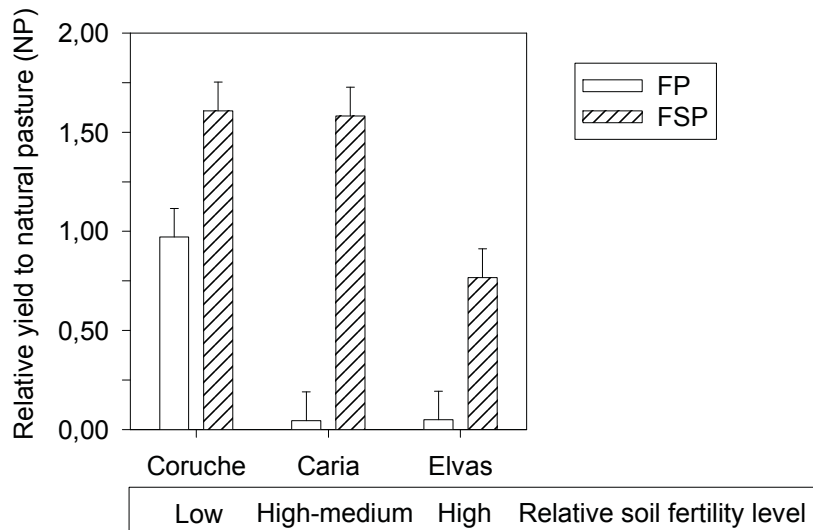


Figure 1. Yield response of fertilisation (FP) and sowing plus fertilization (FSP) relative to annual total above ground biomass of natural pasture, in 3 different sites, with different soil fertility levels. Values are means of 6 replicates and one standard error is displayed.

Therefore we suggest that under low soil fertility conditions, the alleviation of nutrient limitation, by the use of correct fertilizers might be enough to ensure a significant increase in aboveground biomass production. In contrast, in situations where soil conditions do not present limitations to plant growth, then the increase in biomass might be dependent on sowing well-adapted pasture species.

#### Acknowledgements

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## Wastewater and phosphorus effects on the growth of berseem clover

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### Abstract

The aim of this paper is to study the influence of irrigation with treated wastewater plus fertilization with phosphorus on growth pattern and internal water status of berseem clover (*Trifolium alexandrinum* L.). The field experiment was carried out in northern Greece where the climate is semi-arid. Two irrigation regimes were applied: the first with treated wastewater and the second with tap water. In each water regime two fertilizer treatments were applied, first one with phosphorus and the second one without fertilization. Experimental design consisted on four replications of each treatment and subplot size was 20 m<sup>2</sup>. The water salinity was not high enough to cause any effect on plant water potential. Wastewater and fertilizer application increased significantly plant leaf area. Plant growth expressed as aboveground dry biomass followed the same pattern as the leaf area.

Key words: wastewater, *Trifolium alexandrinum*, fertilization, leaf area, plant water potential.

### Introduction

The use of treated domestic wastewater for crop irrigation is an inevitable, common and important practice in many countries in the world as well as in the Mediterranean region (Angelakis *et al.*, 1999). Problems such as wastewater disposal and lack of water availability in arid zones could be controlled using treated wastewater for irrigation. Additionally, the treated wastewater has a high nutritive value that may improve plant growth (Shahalam *et al.*, 1998). However, using wastewater for irrigation is associated with hazards, one of which is the increase of soil salinity (Poole *et al.*, 2004). It is well documented that salinity affects plant water potential and therefore its growth (Dirksen, 1985). The aim of this paper is to study the influence of irrigation with treated wastewater plus fertilization with phosphorus on growth pattern and internal water status of berseem clover (*Trifolium alexandrinum*).

### Materials and Methods

Berseem clover (*Trifolium alexandrinum* var. *alex*) was sown at April 15 2005, in a field nearby the local Wastewater Treatment plant of Drama city in Eastern Macedonia, Greece. The soil texture is silty clay and pH 8.03. The climate of the area is semi-arid with mean annual temperature of 15.5°C and mean annual precipitation of 589 mm. The dry period is from June to September. Treatments consisted of source of irrigation water (treated domestic wastewater and tap water) and fertilization practice (with and without fertilization). The experimental design was a split-plot with four replications. Main plot treatments were source of irrigation water and subplot treatments the fertilization practice. Thus, four treatments were used: wastewater irrigation plus fertilization (WF), wastewater without fertilization (WwF), tap water plus fertilization (TF) and tap water without fertilization (used as a control) (TwF). The total wastewater or tap water supply for each plot was 87mm, in five irrigations the dates of which are presented in Figure 2. Fertilizer that contained 19.8 % phosphorus was applied at loading rates of 30g m<sup>-2</sup> twice: a. after sowing (22nd of May) and b. after the first cutting (6th of July). Main plot size was 9 x 23 m and subplot size was 4 x 5 m. The plots were separated by a 3m wide pathway and the subplots divided by 1m pathway.

Table 1. Mean values of several chemical features present in wastewater and tap water

	Conductivity at 20°C ( $\mu\text{S cm}^{-1}$ )	Nitrates ( $\text{mg L}^{-1}$ )	Ammonium ( $\text{mg L}^{-1}$ )	Phosphorus pentoxide ( $\text{mg L}^{-1}$ )	Potassium ( $\text{mg L}^{-1}$ )	Magnesium ( $\text{mg L}^{-1}$ )	Iron ( $\text{mg L}^{-1}$ )
Wastewater	760.3	27.13	0.13	2.57	6.73	21.66	0.01
Tap water	420.3	14.00	0.01	0.00	0.80	16.59	0.01

Two cuttings were done (by scissor) in order to measure the yield at the early flower stage of development. The first was at 28th of June 2005 and the second at 26th of July 2005. In order to measure the plant growth pattern after the first cut, four measurements of above ground dry biomass have been done, at weekly intervals. At every sampling three plants from each subplot were chosen randomly to be pulled up by the roots. Then, leaves and stems were separated in the lab. Fresh leaves surface was scanned to measure their leaf area using the Program *Image Tool for Windows 98 version 3.00*. The plant parts were oven dried at 75°C for 48 hours and weighed to estimate their above ground dry biomass.

Predawn plant water potential was measured, using a pressure chamber (Arimad-2, Kfar Charuv), every week in four stems of each subplot, that is, sixteen for each treatment.

Data were subjected to analysis of variance. LSD values were calculated and used to compare treatment means ( $p \leq 0.05$ ).

### Results and discussion

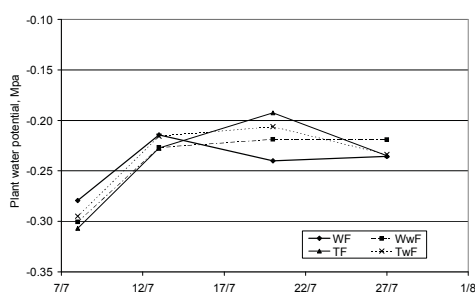


Figure 1. Changes of plant water potential along the study period

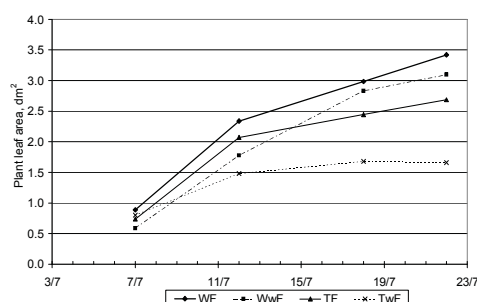


Figure 2. Seasonal changes of plant leaf area along the study period

The mean values of several chemical parameters related to potential fertilizer value of wastewater and tap water during the measurement period are presented in Table 1. According to these parameters, wastewater had higher values than tap water, except for Fe. The conductivity, which is considered to be an indicator of salinity, was higher in wastewater than in tap water. It is likely that this result will affect the salinity of the soil, therefore the plant water potential, with a consequent reduction in plant yield (Poole *et al.*, 2004). However, the plant water potential values during the measurement period did not show any differences between the irrigation treatments (figure 1), although this might be attributed to low additions of wastewater in this experiment. Further irrigation with wastewater might increase soil salinity (Ramirez-Fuentes *et al.*, 2002), and for that reason studies comprising longer periods will be needed to draw more definitive conclusions.

Leaf area of plants irrigated with wastewater and/or fertilized was significantly higher than that for plants at the treatment irrigated with tap water without fertilization, although only after the second measurement (Figure 2). The changes in plant leaf area could partly explain the plant growth pattern, as a strong relationship exists between leaf area and plant productivity.

The plant growth, measured as aboveground biomass, is illustrated on figure 3. Plants grow up without differences among treatments until 12th of July (fifteen days after the regrowth of plants begun). After this date the plants grown under tap water irrigation and without fertilization (TwF) decreased their growth rate and therefore do not increase their aboveground biomass. In the final measurement at 27th of July, the results of analysis of variance indicated significant differences only between the TwF and the rest of the treatments, while there were not significant differences between the WF, WwF, TF treatments. However, the dry weight obtained by WF tend to be greater compared to WwF and this greater to TF. In these results there is a tendency showing a positive effect of wastewater and fertilizer use. However, we consider that the results maybe affected by the rainy weather conditions and the consequent low wastewater irrigation supply. Similar findings were reported at other studies (Shahalam *et al.*, 1998).

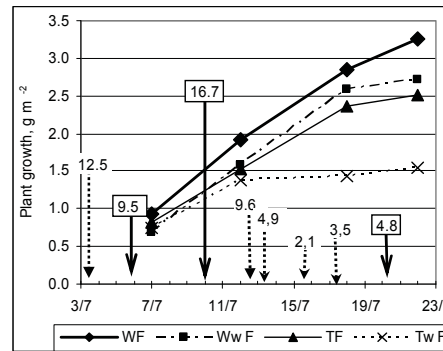


Figure 3. The plant growth rate after the first cutting. The date and amount of irrigation are given by continuous line arrows (irrigation) and dotted line arrows (rain).

### Acknowledgements

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# The influence of different foliar fertilisation on the yield and botanical composition of a meadow sward

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## Abstract

During the years 2000-2002 a study was carried out on a 3-cut meadow on mineral soil to compare the effects of different foliar fertilisation treatments on dry matter (DM) yield and botanical composition. Experimental treatments were allocated in a randomized block design with four replications of plots of 10 m<sup>2</sup> area established. These comprised 8 fertilisation treatments: 1. control (0), 2. NPK, 3. N + ½PK, 4. N + ½PK + foliar Ekolist PK, 5. NPK + foliar Mikrosol U, 6. NPK + complex foliar (Ekolist PK, Mikrosol U and Ekolist Mg); 7. NPK + foliar complex (Ekolist PK, Mikrosol U, Ekolist Mg) + Mikrosol Oz after the last regrowth; 8. N + ½PK + foliar Plonvit P. The highest DM yields were obtained from NPK fertilisation and after supplementary complex foliar fertilisation and complex and Mikrosol Oz. Comparable effects were observed in the case of N + ½PK and Ekolist PK foliar fertilisation. The changes of botanical composition were low: there was only a tendency of a positive influence of complex foliar fertilisation and Mikrosol Oz on persistence of favourable grasses, and of Ekolist PK and Plonvit on the persistence of legumes, mainly *Trifolium repens* L.

Keywords: meadow, mineral soil, foliar fertilisation, yield of DM, botanical composition.

## Introduction

There is increased interest in Poland in using foliar fertilisation, as a supplement to soil fertilisation, not only in intensive agriculture but also ecologically sensitive situations (Ciepiela *et al.*, 1999). It can have great importance in extreme conditions and in plant-stressed situations, i.e. in periods of drought, excessive moistening, inadequate pH of soils, and also in situations where nutrients are yield-limiting foliar feeding can be supplementary to soil mineral fertilisation (Nurzyński, 1996). The aim of these investigations was to compare the effects of foliar fertilisation and top-dressing fertilisation on dry matter (DM) yield and to evaluate resulting changes in botanical composition.

## Material and methods

The study was carried out during 2000-2002 on a 3-cut meadow on a mineral soil (soil type was podsollic and pseudo podsollic, classified as valuation class IV b - characterised by a considerable mosaic, either periodically too dry or locally periodically too wet). Before establishment of the experiment (1999), the soil was pH 5.0 with low content of phosphorus (P) and high in potassium (K) and magnesium (Mg). The vegetation period typically lasts from 1 April to 1 November, the average annual temperature is 6.9 C, and precipitants 638.4 mm. Experimental treatments were allocated in a randomized block design with four replications of plots of 10 m<sup>2</sup> area established. These comprised 8 fertilisation treatments: 1. without fertilisations (control), 2. NPK (N - 180 kg ha<sup>-1</sup>, P<sub>2</sub>O<sub>5</sub> - 70 kg ha<sup>-1</sup>, K<sub>2</sub>O - 160 kg ha<sup>-1</sup> (80 + 40 + 40)), 3. N+1/2PK, 4. N + 1/2 PK + foliar Ekolist PK (10 litre ha<sup>-1</sup>), 5. NPK + foliar Mikrosol U (2 litre ha<sup>-1</sup>), 6. NPK + complex foliar (Ekolist PK with urea 0.5% - in spring and after every regrowth - after the last one with urea 0.2%; Mikrosol U - 2 l ha<sup>-1</sup> + Ekolist Mg (5 litre ha<sup>-1</sup>) - after 5-7 days of Ekolist PK spraying), 7. NPK + complex foliar (as in treatment 6.) + before winter - Mikrosol Oz (2 l ha<sup>-1</sup>), 8. N + 1/2 PK + foliar Plonvit P (10 litre ha<sup>-1</sup>). Every year the sward was cut three times and the DM yields were measured, the botanical composition of sward was evaluated (valuation method) and plant samples were taken for analysis of the chosen chemical compounds. Phenological observations and measurements of precipitants were conducted (Table 1). Composition of foliar fertilisers is shown in Table 2. They were applied in spring and after every harvest on plants with high above 10 cm. Doses of mineral fertilisers were: N -180 kg ha<sup>-1</sup> (3 x 60), P<sub>2</sub>O<sub>5</sub> -70 kg ha<sup>-1</sup>, K<sub>2</sub>O -160 kg ha<sup>-1</sup> (80 + 40 + 40).

Table 1. Annual precipitation (mm) in years 2000-2002 and precipitation in vegetation season recognized as optimal for grasslands.

Months	2001	2002	Optim.
I	40	40	
II	20	33	
III	63	44	
IV	135	12	65
V	36	124	120
VI	111	112	115
VII	180	54	100
VIII	81	63	80
IX	35	35	70
X	11	14	
XI	38	43	
XII	50	57	

Table 2. Composition of fertilisers (g l<sup>-1</sup>).

Component	Foliar fertiliser				
	Ekolist PK	Mikrosol U	Mikrosol Oz	Ekolist Mg	Plonvit P
P	50.0	0.0	0.0	0.0	0.0
K	200.0	0.0	20.0	0.0	0.0
N	0.0	50.0	30.0	0.0	8
Mg	0.0	35.0	35.0	60	5
S	0.0	32.0	30.0	0.0	0.0
Fe	0.0	8.4	12.0	0.0	0.50
Mn	0.0	11.9	7.0	0.0	0.55
Zn	0.0	7.0	9.0	0.0	0.50
Cu	0.0	8.4	6.0	0.0	0.30
B	0.0	7.0	2.0	0.0	0.15
Mo	0.0	0.05	0.25	0.0	0.005
Na	0.0	0.0	7.0	0.0	1.5
Ti	0.0	0.0	0.0	0.0	0.01
J	0.0	0	0.0	0.0	0.003

## Results and discussion

The highest DM yields from the period of study (Table 3) were obtained after soil NPK fertilisation (treatment 2. - 9.41 t ha<sup>-1</sup>), and then after soil NPK fertilisation supplemented with complex foliar fertilisation (treatment 6 - 9.21 t ha<sup>-1</sup>) and complex with Mikrosol Oz before winter (treatment 7 - 9.26 t ha<sup>-1</sup>).

Table 3. Yields of DM ( t ha<sup>-1</sup>). Years 2000-2002.

Fertilisation treatments	2000	2001	2002	Total	Mean
1. without fertilisation	4.90	8.44	5.15	18.49	6.16
2. NPK	7.36	13.21	7.67	28.24	9.41
3. N + 1/2 PK	7.02	11.14	6.89	25.05	8.35
4. N + 1/2 PK + Ekolist PK	7.31	12.89	7.20	27.40	9.13
5. NPK + foliar Mikrosol U	7.27	12.42	7.22	26.91	8.97
6. NPK + foliar complex*	7.65	12.73	7.25	27.63	9.21
7. NPK + foliar complex*+ Mikrosol Oz	7.74	12.78	7.27	27.79	9.26
8. N + 1/2 PK + Plonvit P	5.58	11.50	6.46	23.54	7.85
Standard deviation	1.04	0.90	0.79	3.09	1.03

\*Ekolist PK (on spring and after every cut with urea 0.5%, after the last cut with urea 0.2%), Mikrosol U + Ekolist Mg - after 5-7 days from spraying with Ekolist PK.



But in year with large deficiency in rainfall, (2001), especially in spring, effects of complex foliar fertilisation were annually about by 4-5% higher than effects of soil NPK fertilisation. Higher yields of *Lolium perenne* were reported from foliar fertilisation with urea, compared with soil fertilisation (Jankowski *et al.*, 1994). Comparable DM yields (9.13 t ha<sup>-1</sup>) to the NPK treatment were obtained from treatment 4 (N + 1/2 PK + Ekolist PK) that is after replacement of part of soil fertilisation (PK) with foliar fertilisation. The increase of DM yield in relation to N + 1/2 PK fertilisation (treatment 3. - without Ekolist PK) was over 9%. The soil NPK fertilisation and foliar application of Mikrosol gave worse results, compared with both the NPK-only and the NPK supplemented by complex foliar fertilisation. The changes in botanical composition of sward were not large (Table 4). The contribution of grasses decreased slightly, most of all on the control (by 25%, mostly in favour of herbs and weeds), and in least range (by 2-3%) after supplementary foliar fertilisation, especially with Mikrosol U and Mikrosol Oz before winter. The share of legumes decreased also by over 50%, with the exception of the treatment with foliar fertilisation by Ekolist PK and Plonvit P, on which the decrease was 40%, and in case of *Trifolium repens* only by 20%.

Table 4. Botanical composition of meadow sward before establishment of experiment (1999) and in 2002.

Species/group of plant	Before establishment of experiment	Fertilisation treatments							
		control	NPK	N + 1/2 PK	N + 1/2 PK + Ekolist PK	NPK+ Mikrosol U	NPK + foliar complex.	NPK + foliar complex + Mikrosol Oz	N + 1/2 PK + Plonvit P
<i>Lolium perenne</i> L.	20	8	19	17	18	18	17	19	18
<i>Lolium multiflorum</i> L.	10	4	6	7	8	4	6	7	6
<i>Festuca pratensis</i> Huds.	25	8	20	15	18	15	18	18	20
<i>Phleum pratense</i> L.	20	12	20	19	21	22	21	19	19
<i>Poa pratensis</i> L.	15	7	11	13	10	18	12	15	13
Other grasses*	0	29	11	14	12	13	12	10	11
Grasses total	90	68	87	85	87	90	86	88	87
<i>Trifolium pratense</i>	5	1	1	2	2	1	1	2	2
<i>Trifolium repens</i> L.	5	3	4	3	4	2	3	3	4
Legumes total	10	4	5	5	6	3	4	5	6
Herbs and weeds **	0	28	8	10	7	7	10	7	7

\*Other grasses: *Holcus lanatus*, *Poa annua*, *Agrostis vulgaris*. \*\* Weeds and herbs: *Taraxacum officinale*, *Plantago lanceolata*, *Matricaria chamomilla*, *Ranunculus repens*, *Rumex acetosella*, *Cardamine pratensis*, *Capsella bursa pastoris*, *Geranium pratense*, *Stelaria graminea*.

## Conclusions

The highest yields of DM were obtained after soil NPK fertilisation, and among treatments with foliar fertilisation – after the complex foliar and complex with Mikrosol Oz before winter. These exceeded the DM yields after NPK fertilisation in one year with large deficiencies of precipitation. The replacement of half of the soil dose of PK by foliar Ekolist PK also gave comparable yields to full NPK soil fertilisation. Changes in botanical composition were small. There were tendencies for positive influence of the foliar complex fertilisation and Mikrosol Oz on the persistence of valuable grasses, and for Ekolist PK and Plonvit on the persistence of legumes, mostly *Trifolium repens*.

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# The influence of organic fertilisers on the biodiversity of a *Festuca rubra* meadow

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## Abstract

In this paper we present the results obtained through the fertilization with farmyard manure (FYM) of a pasture of *Festuca rubra* L. on two types of soil: *terra rossa* and *brown eu-mezobazic rendzinic*. The yield of pasture dry matter increases proportionally with the increase of the quantities of FYM applied, while biodiversity decreases.

Keywords: fertilization, manure, biodiversity.

## Introduction

The fertilization with organic fertilizers causes, according to the quantity applied, substantial alterations of pasture yield levels, floristic composition and quality of the fodder obtained thereof (Rotar *et al.*, 2003). Manure fertilization is still being used on a large scale on the pastures of Apuseni Mountains (Păcurar, 2005; Reif *et al.*, 2005).

## Materials and methods

The experiment was placed in 2001 on two types of soil, one being *terra rossa* and the other *brown eu-meobazic rendzinic*. The setting of the experiment was done according to the rules of experimental techniques. The experimental treatments were as follows: V1 – control, V2 – 10 t ha<sup>-1</sup> manure, V3 – 20 t ha<sup>-1</sup> manure, V4 – 30 t ha<sup>-1</sup> manure. The size of the elemental plot was 10 m<sup>2</sup>. The harvesting was done at the flowering of the Poaceae, that is, at the beginning of July. The changes in the canopy cover were measured with a quadrat, as well as the biodiversity, and interpreted through the Shannon index.

## Results and discussion

Yield increases on both types of soil are proportional to the increase in the amount of FYM applied (Table 1).

Table 1. The influence of organic fertilizers on the yield of dry matter DM(2004).

Type of soil	Treatment	Yield/(t ha <sup>-1</sup> )	(%)	Difference	Significance.
Terra rossa	control	0.64	100.0	0.00	
	10 t FYM ha <sup>-1</sup>	1.00	155,5	0.36	-
	20 t FYM ha <sup>-1</sup>	2.23	348.8	1.59	***
	30 t FYM ha <sup>-1</sup>	2.80	437,1	2,16	***
DL(p5%)- 0.59		DL(p1%)- 0.86		DL(p0,1%)- 1.26	
Brown eu-mezobazic rendzinic	control	1,47	100,0	0,00	
	10 t FYM ha <sup>-1</sup>	1,91	129,7	0,44	-
	20 t FYM ha <sup>-1</sup>	2,08	141,1	0,61	*
	30 t FYM ha <sup>-1</sup>	2,18	148,2	0,71	*
DL(p5%) - 0.60		DL(p1%) - 0.87		DL(p0,1%) - 1.28	

On the *terra rossa* soil following the application of FYM it was observed a decrease of the surface cover of Poaceae as compared to the control, an increase of the surface cover of Phabaceae, a decrease of Cyperaceae and Juncaceae down to their disappearance and a slight increase of the cover of plants from other families. On the *brown eu-mezobazic rendzinic* soil the response of the canopy cover to the organic fertilization was slightly different. The Poaceae increased their cover as compared to the control, up to 40.6%, the Phabaceae reduced their cover proportionally with the increase in FYM application down to 10.9%, while the cover of plants from other families dropped down to 34.5%. On both types of soil the number of species was reduced following the application of treatments. On the *terra rossa* soil, when 10 t ha<sup>-1</sup> FYM were applied 8 species disappeared (*Lathyrus pratensis*, *Carex pallescens*, *Arabis hirsuta*, *Leontodon autumnale*, *Scabiosa columbaria*, etc.), when treated with 20 t FYM ha<sup>-1</sup> 10 species disappeared (*Campanula patula*, *Polygala vulgaris*, etc.), while at the 30 t FYM ha<sup>-1</sup> treatment 16 species disappeared (*Luzula campestris*, *Cerastium glomerata*, *Carum carvi*, *Trollius europaeus*, *Viola declinata*, etc). In the phytocenosis on the *brown eu-mezobazic rendzinic* soil at the treatment of 10 t FYM ha<sup>-1</sup> 3 species disappeared (*Anthyllis vulneraria*, *Medicago lupulina*, *Leontodon hispidus*), at the application of 20 t FYM ha<sup>-1</sup> 5 species disappeared (*Campanula patula*, *Hypericum maculatum*, etc.) while at the treatment of 30 t FYM ha<sup>-1</sup> 9 species disappeared (*Carex pallescens*, *Luzula campestris*, *Cerastium glomeratum*, *Lathyrus pratensis*). On both types of soil through the application of treatments some nitrogen-loving species get into the pasture (*Festuca pratensis*, *Poa trivialis* etc.). The decrease of floristic diversity as a result of treatment application is outlined by the evolution of the Shannon index, which dropped considerably according to the intensification of the fertilization system.

Table 2. Influence of organic fertilization on floristic composition.

	Terra rossa				Brown eu-mezobazic rendzinic			
	Control	10 t ha <sup>-1</sup>	20 t ha <sup>-1</sup>	30 t ha <sup>-1</sup>	Control	10 t ha <sup>-1</sup>	20 t ha <sup>-1</sup>	30 t ha <sup>-1</sup>
Poaceae %	42.9	30.2	21.3	30.7	24.2	26.2	40.6	40.6
Phabaceae %	9.4	29.2	35.2	28.5	19.7	18.1	18.7	10.9
OF %	44.4	53.9	51.8	45.0	50.1	48.3	43.0	34.5
Cyperaceae %	0.6	0.3	0.1	0.0	0.1	0.2	0.8	0.0
Total	42	35	34	30	38	37	35	32
Species that go out		8	10	16		3	5	9
Species that enter		1	2	4		2	2	2
Shannon index	2.845	2.788	2.680	2.473	3.302	2.932	3.119	2.630

## Conclusions

The application of stable manure on *Festuca rubra* meadows caused a significant increase of pasture yield as compared to the control, on both types of soils, from the rate of 20 t ha<sup>-1</sup> up. The phytocenosis of the *brown eu-mezobazic rendzinic* soil showed a more pronounced stability as compared to that of *terra rossa* soil. The floristic diversity decreases proportionally with the intensification of the system on both types of soil.

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## Effects of fertiliser phosphorus on soils initially low in phosphorus

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### Abstract

The resources of rock phosphates are limited and their common use as P fertiliser has to be considered, both from environmental and economical perspectives. Applied superphosphate in many former plant nutrient investigations ruled out the possibility of consistent discussions of the separate S and P effects. The objective of the presented field experiments was to test how plant growth in grasslands relates to proposed critical values of herbage P/N ratio. Three field trials were established on soils low in P. Five levels of P fertilisation (0, 5, 10, 20 and 50 kg P ha<sup>-1</sup> yr<sup>-1</sup>) and two levels of N (normal and 50 % of normal) were applied. The trials were harvested in two subsequent years. Plant P uptake on unfertilised plots was higher than expected, and uptake of applied P was lower. When 20 kg P ha<sup>-1</sup> was applied only 14 % of the P applied was found in the harvested material. Thus, the relation between relative dry matter yield and P/N ratio in plants was not examined such in detail as planned; however, the yield decrease following low P/N was in accordance to our hypothesis.

Keywords: critical value, grass, P/N, response, superphosphate, DM yield.

### Introduction

Common use of the limited rock phosphates resources as phosphorus (P) fertiliser has to be carefully considered, both from environmental and economic perspectives. It is important to know how plant growth relates to the concentration of P in the plants. Superphosphate has been applied in many former plant nutrient experiments, and thus, experimental data confounded with sulphur (S) effects have reduced the possibilities for interpretation of P effects. Fystro (unpublished data) has accomplished pot trials with perennial ryegrass (*Lolium perenne* L.), timothy (*Phleum pratense* L.) and red clover (*Trifolium pratense* L.) in order to investigate critical values of P/N in the plants in relation to dry matter yield (Figure 1). The objective of the presented field trials was to test the proposed relation between plant growth and P/N in harvested herbage.

### Materials and methods

Three field experiments, situated at Hegra (63°30'N), Begnadalen (61°7'N) and Naerbo (58°47'N) were established on loamy soils in 1-2 year old swards dominated by timothy and meadow fescue (*Festuca pratensis* Huds.). The intention was to select fields with a P-AL content lower than 2.6 mg 100 g<sup>-1</sup> soil (Loes and Falk Ogaard, 2001), however, actual values in the upper 10 cm layer at time of establishment were 4.8 (Hegra), 2.2 (Begnadalen) and 3.4 (Naerbo) mg P 100 g<sup>-1</sup> soil. Treatments consisted in yearly applications of two levels of N (normal and 50 % of normal) and five levels of P (0, 5, 10, 20 and 50 kg ha<sup>-1</sup>) in a factorial design and were replicated three times at each field. The P was applied as Na<sub>2</sub>HPO<sub>4</sub> × 2 H<sub>2</sub>O. All trials were harvested twice in two subsequent years. Statistical analysis of variance is carried out by use of GLM procedure in the program package SAS.

### Results and discussion

No significant dry matter yield response was found after added P except when combined with the highest N level at Begnadalen in 2003 (Table 1).

Table 1. Proportional (%) N and P treatment effects on total dry matter (DM) yields. Means of highest N level and means of no P added = 100, respectively.

N and P fertilisation kg ha <sup>-1</sup> yr <sup>-1</sup>	Hegra		Begnadalen		Naerbo	
	2002	2003	2002	2003	2002	2003
N: 100-140	100	98	94	93	98	95
200-280 (= t DM ha <sup>-1</sup> )	100 (13.4)	100 (9.8)	100 (8.4)	100 (10.7)	100 (5.8)	100 (5.9)
P: 0 (= t DM ha <sup>-1</sup> )	100 (13.1)	100 (9.5)	100 (8.2)	100 (10.0)	100 (5.4)	100 (5.5)
5	98	92	95	101	97	108
10	96	97	98	104	98	105
20	93	104	98	101	102	110
50	92	104	104	109	108	111
S.E.: N	2.1	2.1	1.2	1.5	2.5	1.7
P	3.1	3.4	1.9	2.5	4.0	3.0
P-value: N	0.95	0.71	0.47	0.32	0.86	0.04
P	0.39	0.08	0.10	0.12	0.33	0.13
N*P	0.49	0.71	0.15	0.01	0.54	0.47

On average of all fields the P yields increased significantly by increasing P fertilisation (Table 2). The uptake of P on unfertilised plots was 18 kg ha<sup>-1</sup> in year 2002 and 15 kg ha<sup>-1</sup> in year 2003. Only 14 % of P added at the rate of 20 kg P ha<sup>-1</sup> was found in harvested material. The corresponding figure for 50 kg P ha<sup>-1</sup> was 10 %. The concentration of P in the plants was generally lower than that found by Johansen *et al.* (2003).

Table 2. Proportional (%) N and P treatment effects on total phosphorous yields. Means of highest N level and means of no P added = 100, respectively.

N and P fertilisation kg ha <sup>-1</sup> yr <sup>-1</sup>	Hegra		Begnadalen		Naerbo		All fields	
	2002	2003	2002	2003	2002	2003	2002	2003
N: 100-140	100	85	94	104	98	78	101	87
200-280 (= kg P ha <sup>-1</sup> )	100 (25)	100 (17)	100 (20)	100 (19)	100 (13)	100 (14)	100 (19)	100 (17)
P: 0 (= kg P ha <sup>-1</sup> )	100 (23)	100 (15)	100 (18)	100 (18)	100 (11)	100 (11)	100 (18)	100 (15)
5	94	100	97	97	92	131	94	109
10	103	102	104	103	97	130	101	112
20	105	116	108	112	119	147	111	125
50	113	127	123	128	123	178	120	144
S.E.: N							1.9	2.7
P							3.2	5.3
P-value: N							0.08	0.28
P							<0.001	<0.001
N*P							0.33	0.34

The relation between dry matter growth and the ratio P/N in harvested plant material is plotted in Figure 1 together with a curve fitted to data collected from a pot experiment with perennial rye grass (unpublished). Although the field experiment data in the present study did not create as low P/N figures

as expected, these results are well in correspondence with the dry matter responses found in the independent pot experiment data. Therefore, the lack of dry matter yield responses expected after added P is in accordance to the unexpected high P/N ratios observed when no P was added. Dry matter yield responses in relation to the P and N status have been presented earlier (Duru and Th  lier-Huche, 1997), and the use of such relations in P diagnosis discussed (Calvi  re and Duru, 1999).

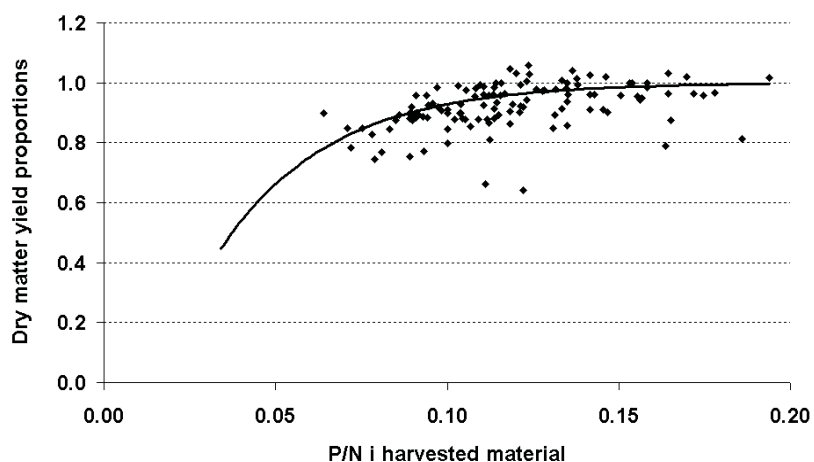


Figure 1. Dry matter yield as related to the phosphorous and nitrogen ratio (P/N) in harvested timothy and meadow fescue dominated herbage. Observations from first and second cut in the 2-year field trial with different P and N treatments are plotted and compared to a curve fitted to data collected from a pot experiment with perennial rye grass (unpublished).

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# Influence of fertilization rates on *Lolium perenne* sward photosynthetic characteristics and seed yield

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## Abstract

Perennial ryegrass *Lolium perenne* L. is a popular forage species in Latvia and have several important performance characteristics: high seed and herbage yield, long growing season, tolerance to a wide range of environmental conditions and grazing practices, rapid seedling establishment, weed suppression, excellent persistence under close grazing, compatibility with white clover, high forage quality and palatability. The research objective was to study the influence of N, P and K fertilizers on perennial ryegrass Leaf Area Index (LAI), Net Photosynthesis Productivity (NPP) as well as to examine the effect on seed yield. Perennial ryegrass cultivar 'Spidola' was sown in experimental field of the Research Institute of Agriculture on sod podzolic sandy loam soil (Luvic Phaeozem) in 1999 – 2002. Randomize complete block design with four replications was used. The following mineral fertilizer rates were applied: N and P<sub>2</sub>O<sub>5</sub>, each 0, 30, 60, 90, 120 and K<sub>2</sub>O – 0, 40, 80, 120, 160 kg ha<sup>-1</sup>. The highest seed yield was obtained 557, 861 and 839 kg ha<sup>-1</sup> in 2000, 2001 and 2002, respectively. Average net photosynthesis productivity was 0.6 – 2.1 and 1.1 – 1.9 g m<sup>-2</sup> d<sup>-1</sup>, but leaf area index – 9.0 – 15.8 and 7.1 – 18.5 in 2002 and 2003, respectively.

Keywords: *Lolium perenne* L., fertilization, photosynthesis, seed yield.

## Introduction

Perennial ryegrass (*Lolium perenne* L) is a dominant forage species of Europe and other humid and semi-arid parts of the world. Photosynthesis is one of the basic processes in the nature because 90 – 95% of the plant organic mass is created through photosynthesis. The most significant indices of photosynthetic activity are crop index, utilisation of photosynthetically active radiation and net photosynthesis productivity (Adamovich, 2001). When plant tissue is combusted the residual ash represents those mineral elements taken up from the soil via the roots. In grasses they generally make up about 6 to 9 % of the plant dry weight. Leaf photosynthesis is influenced by the current environment, by the leaf age and by the environment the leaf experienced while its photosynthetic apparatus was being laid down (Robson *et al.*, 1998).

The objective of this work was to determine ryegrass *Lolium perenne* cultivar 'Spidola' photosynthetic ability (leaf area and net photosynthesis productivity) as well as seed yield as function of applied N, P, K amounts in conditions of Latvia.

## Materials and methods

Field experiments were carried out on sod-podzolic sandy soil (Luvic Phaeozem, WRB, 1998), pH<sub>KCl</sub> 6.5, plant available P<sub>2</sub>O<sub>5</sub> – 110 and K<sub>2</sub>O – 204 mg kg<sup>-1</sup> (Egner-Riehm), soil organic carbon – 12.2 g kg<sup>-1</sup> (Tyurin's method). Randomized complete block design with four replicates was used. The plot size was 17.5 m<sup>2</sup>. Five fertilizer rates were applied in both years. Perennial ryegrass (12 kg ha<sup>-1</sup>) was planted using Nordsten seed drill in May 1999, 2000, 2001 and 2002 after field preparation. The following mineral fertilizer rates were used: N and P<sub>2</sub>O<sub>5</sub>, each of them 0, 30, 60, 90 and 120 kg ha<sup>-1</sup>, K<sub>2</sub>O – 0, 40, 80, 120, and 160 kg ha<sup>-1</sup>. Weed control was performed using MCPA 1 l ha<sup>-1</sup> in mixture with 8 – 10 g ha<sup>-1</sup> of Granstar. Seed yield was recorded from the first year 2000, 2001 and 2002 sward use. Analysis of yield components and other parameters were also recorded. During vegetation period in 2002 and 2003 ryegrass 'Spidola' samples from 1 m<sup>2</sup> were cut and collected and determined dry matter (DM) and



leaf area surface (LAI). Dynamics of plant leaf area (LAI) expansion, net photosynthesis productivity (which characterises the increase of plant DM production per leaf area unit per unit of time, expressed in  $\text{g m}^{-2} \text{day}^{-1}$ ), were determined for cuts 1. Sampling of plants for determination of these indices was carried out for each treatment in 5 to 12 day intervals. These indices in a sward were determined according to previously approved method (Coombs *et al.*, 1989).

Only the effects of nitrogen fertilizer are shown in Table 1. The influences of the potassium and phosphorus fertilizers were insignificant at all used levels.

Analysis of variance (ANOVA) was conducted using the GLM procedure of SAS (SAS Inst., 1990) at  $P=0.05$  to test the effects of year, location, N treatment, and all interactions.

## Results and discussion

Our control data show that without added nitrogen fertilizer leaf area was only  $7.1 - 8.0 \text{ m}^2 \text{ m}^{-2}$ . If added nitrogen was higher than  $\text{N}_{30}$  leaf surface area was in the range  $10.5 - 18.5 \text{ m}^2 \text{ m}^{-2}$  (Table 1).

Table 1. Indices of photosynthesis activity in perennial ryegrass ‘Spidola’ swards (2002 and 2003 growing seasons).

Nitrogen level, $\text{kg ha}^{-1}$	Leaf area, $\text{m}^2 \text{ m}^{-2}$		Net photosynthesis productivity, $\text{g m}^{-2} \text{ d}^{-1}$	
	2002	2003	2002	2003
0	8.0	7.1	1.0	1.1
30	14.3	10.5	1.9	1.7
60	14.1	14.4	2.1	1.9
90	13.7	18.1	1.9	1.8
120	15.8	18.5	0.6	1.7
LSD <sub>0.05</sub>	2.8	1.6	0.6	0.9

In grass swards important characteristic is net photosynthesis productivity which characterizes organic matter increase per leaf area unit. NPP for ‘Spidola’ control plot swards in 2002 and 2003 was  $0.1 - 2.3 \text{ g m}^{-2} \text{ d}^{-1}$ , but 2003 –  $0.6 - 1.4 \text{ g m}^{-2} \text{ d}^{-1}$ . In treatments receiving NPK fertilizers net photosynthesis productivity was  $0.6 - 3.5$  and  $0.6 - 3.3 \text{ g m}^{-2} \text{ d}^{-1}$  (Table 1).

Obtained data show that in optimal photosynthetic and moisture conditions perennial ryegrass ‘Spidola’ can achieve good growth and photosynthetic productivity. In non optimal conditions additional fertilizer can help to improve the photosynthesis. Seed yields (Figure 1) differed considerably between years and between control ( $\text{N}_0\text{P}_0\text{K}_0$ ) and fertilized plots. The lowest seed yield was obtained in the 2000 growing season,  $284 \text{ kg ha}^{-1}$  for control ( $\text{N}_0\text{P}_0\text{K}_0$ ) and in the range of  $344 - 557 \text{ kg ha}^{-1}$  for fertilized plots. The highest seed yields were obtained in 2001 growing season,  $225 - 344 \text{ kg ha}^{-1}$  for control ( $\text{N}_0$ ) and  $560 - 861 \text{ kg ha}^{-1}$  for plots fertilized with additional nitrogen. In the 2002 growing season seed yield between control and fertilized plots were more pronounced.

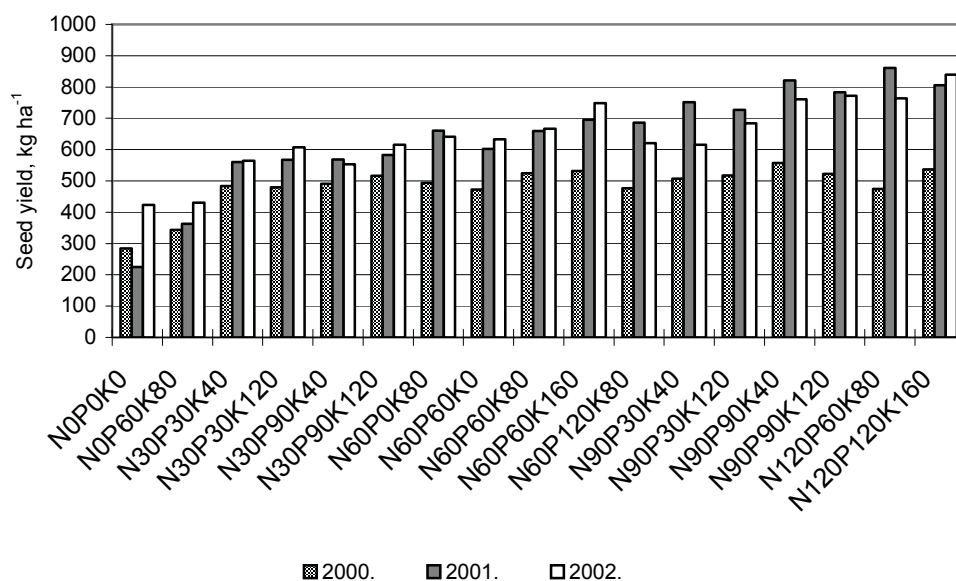


Fig. 1. Perennial ryegrass 'Spidola' first year seed yield as a function of the NPK fertilizer, kg ha<sup>-1</sup>.

### Conclusion

Additional nitrogen fertilizer stimulated perennial ryegrass cultivar 'Spidola' plant development and growth increasing leaf area index, a number of shoots per square unit, dry matter yield and seed yield in conditions of Latvia.

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# Predicting response of perennial ryegrass/white clover swards to strategic spring application of nitrogenous fertilizer

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## Abstract

This study was carried out to validate a grass growth model modified to take account of white clover (*Trifolium repens* L.) and to test its ability to predict the response of perennial ryegrass (*Lolium perenne* L.)/white clover swards to nitrogenous fertilizer applied in spring under western European conditions. The model, which is briefly described, requires daily meteorological data (air temperatures, rainfall and radiation), an estimate of initial clover stolon growing point density and an approximate profile of soil mineral N during the growing season (to estimate grass growth in the mixture). When validated against data sets of grass and clover yields from cut plot experiments managed under simulated grazing and with no fertilizer N application, the model predicts clover yield and content reasonably well but is less successful in predicting the impact of N fertilizer on annual production of grass and clover. Improvements to the model and implications of the requirements of the model for development of a decision support system for grass/clover swards are discussed.

Keywords: white clover, model, nitrogenous fertilizer.

## Introduction

Limits on production of organic nitrogen within the EU imposed by the Nitrates Directive restrict stocking rate and hence output per unit area, therefore increasing the importance in grassland of effective use of nitrogenous fertilizer (a significant variable cost) and white clover. Grass/white clover swards grow slowly in early spring compared with grass receiving N fertilizer but this may be overcome by strategic application of N fertilizer to the grass/clover swards. However, the effect on clover and on the sward generally is variable. This study describes the testing of a grass growth model adapted to include white clover and to validate its ability to predict clover growth and content in swards subjected to simulated grazing management with and without N fertilizer in early spring.

## Materials and methods

The grass growth model of Johnson and Thornley (1985) which had been adapted for field conditions has been further modified to include white clover. The model is driven by daily meteorological inputs (maximum and minimum air temperatures, rainfall and radiation) which, in conjunction with initial leaf area index (LAI), determine the rate of photosynthesis and respiration of both grass and white clover, and fertilizer N application, clover growing point number at the beginning of the growing season in mid March and coefficients to control the rate of soil N mineralization during each regrowth. Briefly, the features of the modified model include: an inverse relationship between grass growth and white clover growing point density (GPD) to early May, linear increase in density with time thereafter to September, LAI of clover at the beginning of each regrowth period calculated from GPD at that time and a predetermined leaf area per growing point, photosynthesis of the grass and clover components determined by the relative contribution of each to the canopy, extinction coefficients, photosynthetic efficiencies and positions within the canopy, respiratory losses due to growth, maintenance and, for clover, nitrogen fixation and estimates of senescence.

The model was validated against DM and clover yields at each harvest in trials comparing grass/white clover receiving no N fertilizer (0N) and 75 – 90 kg N ha<sup>-1</sup> in spring (SN) (depending on study). These

are reported in Laidlaw (1980; 1984) for each of 4 years after the first full harvest year in an experiment carried out at Belfast, Northern Ireland, and Frame (1987) and Frame and Paterson (1987) for the means of each of two trials carried out in Ayr, Scotland. Inputs are: daily meteorological conditions, as described above, and initial GPD (estimated from clover yield at the first harvest, as growing points were not measured in these trials). Nitrogen mineralization coefficients are also inputs, and are varied so that grass yield at each harvest in the model is similar to that actually harvested at each cut. This takes account in the validation of the variation in grass growth in mixed swards.

## Results and discussion

The validation data set ranges from swards with annual DM yields of 5.74 to 8.45 t ha<sup>-1</sup> and annual clover yields of 1.82 to 5.75 t ha<sup>-1</sup> at 0N. Corresponding yields for the SN range were 7.95 to 9.60 t ha<sup>-1</sup> for total yields and 0.79 to 4.85 t ha<sup>-1</sup> for white clover yields.

Linear regression of predicted on actual annual clover yield and content in 0N swards shows, in general, that the model predicts these quite well with regression line slopes close to 1 and reasonably high R<sup>2</sup> values, though slightly over predicting the annual amount of harvested clover (Table 1). The effectiveness of the model in predicting the effect of N is less clear as the relationships for annual yields and content of clover are very poor (negative R<sup>2</sup> values). However, when individual harvests up to early July are considered, i.e. when N effects are expected to be most marked, the relationship improves with high R<sup>2</sup> values particularly for clover content for both N treatments, although the slope indicates that the model under predicts contents in SN swards by 20%.

Table 1. Regression equations relating predicted (y) against actual (x) stated yields or clover content.

Character	No N in spring		N in spring	
	Equation	R <sup>2</sup>	Equation	R <sup>2</sup>
Annual clover yield	y = 1.12x	0.89	y = 1.00x	-0.91
Annual clover content	y = 1.05x	0.69	y = 1.02x	-0.84
Total annual DM yield	y = 1.06x	0.20	y = 1.12x	0.48
Clover yield at each harvest	y = 0.99x	0.60	y = 1.02x	0.58
Clover yield at each harvest to early July	y = 0.94x	0.73	y = 0.84x	0.53
Clover content at each harvest to early July	y = 1.03x	0.84	y = 0.83x	0.82
Annual DM response to N			y = 0.86x	0.80
Annual grass response			y = 0.86x	-2.67
Annual clover response			y = 0.96x	-1.98

The model slightly under predicts the response of total annual dry matter to spring applied N fertilizer but still accounts for 80% of variance. The response of the components, however, is poorly predicted.

From this analysis some changes to the model are required. When not constraining the regression line relating actual and predicted clover yields in SN to pass through the origin, the best-fit line has a large intercept due to the model greatly over predicting clover yield in swards with low clover yields. This suggests that the adverse effect of N on white clover is underestimated in swards of low clover content mainly in autumn as the relationship is reasonably strong in SN swards for harvests until early July.

## Conclusions

A more detailed analysis of the impact that N applied in spring exerts on grass/white clover swards is required, and this may reveal that a more flexible relationship between grass growth and the pattern of stolon GPD is required than the simple phases of net stolon production used in this model.

However, in the absence of spring applied N fertilizer, the grass/clover model can predict clover yield and content of swards where the growth pattern of the grass can be anticipated, meteorological conditions can be intelligently forecast and the clover status of the sward at the beginning of the season

can be judged. Of these, the availability of N particularly during the first half of the growing season is likely to be the most difficult to estimate. Nevertheless, as it plays a major role in the subsequent development of clover, some means of readily estimating this variable needs to be devised before a reliable decision support system to predict clover yield and content can be developed.

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# Effect of liming and fertilization on the productivity of a long-term pasture

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## Abstract

Acid soils account for about 30% of agricultural land in Lithuania. White clover (*Trifolium repens* L.) persists and spreads well in limed ( $\text{pH}_{\text{KCl}}$  5.6-7.0) legume/grass pastures. The objective of this study was to study the effect of soil pH and fertilizer addition on herbage yield and clover abundance on the sward. Thus, prior to pasture establishment soil was limed and fertilizer treatments applied every year through the 12 year study period. A large variation in herbage dry matter (DM) yield, resulting from weather conditions, liming and fertilization was recorded. The highest herbage DM yield on the  $\text{P}_{60}\text{K}_{60}$  (PK) treatment control was obtained at a soil  $\text{pH}_{\text{KCl}}$  level of 5.6-6.0. Nitrogen application,  $\text{N}_{120}\text{P}_{60}\text{K}_{60}$  (NPK), increased herbage DM yield by  $1.59 \text{ t ha}^{-1}$ . Pasture yield was also affected by the amount of rainfall falling during the growing season. A medium-strong curvilinear correlation ( $r=0.557$ ) was obtained between herbage DM yield and amount of rainfall during the growing season on the PK control treatment at  $\text{pH}_{\text{KCl}}$  6.6-7.0. The lowest herbage DM yield variation occurred at soil  $\text{pH}_{\text{KCl}}$  level of 5.1-5.5 and 5.6-6.0 for the two fertilization levels, and the highest yield variation on PK background at  $\text{pH}_{\text{KCl}}$  6.6-7.0

Keywords: permanent grassland, yield,  $\text{pH}_{\text{KCl}}$ , fertilization.

## Introduction

Long-term pastures in Lithuania play an important role in the development of animal husbandry. Acid soils account for about 30 % of agricultural land in Lithuania. White clover/grass pastures, with a  $\text{pH}_{\text{KCl}}$  level of 5.6 – 6.0 and fertilized with  $\text{P}_{60}\text{K}_{60}$ , produce on average  $5.1 - 5.8 \text{ t ha}^{-1}$  dry matter yield. One kg of nitrogen produces an extra 9.6 kg DM (Daugėlienė, 2002). However, annual yields in many European countries are over 7-8 t DM  $\text{ha}^{-1}$ . The nitrogen application efficiency in terms of increment in DM yield was always higher than the economic standard limit (10 kg DM  $\text{kg}^{-1}$  N) (Scotton *et al.*, 2003). In this context, the present research aims to analyze the effects of different soil  $\text{pH}_{\text{KCl}}$  and PK and NPK fertilization on herbage yield in a long-term pasture.

## Materials and methods

The soil at the experimental site was a sod podzolic *Hapli-Endohypogleyic Luvisol* (IDg4-p) light loam on medium loam with topsoil  $\text{pH}_{\text{KCl}}$  5.2, available  $\text{P}_2\text{O}_5$  108  $\text{mg kg}^{-1}$  and  $\text{K}_2\text{O}$  142  $\text{mg kg}^{-1}$ . Liming was applied before pasture sowing. Limestone rate was calculated according to the titration curves neutralizing the soil with a 0.033 N  $\text{CaCl}_2$  solution. A seed mixture containing 35% *Trifolium repens* L., 40% *Phleum pratense* L. and 25% *Poa pratensis* L. was sown. The sward was fertilized annually in spring with 60  $\text{kg ha}^{-1}$  of both  $\text{P}_2\text{O}_5$  and  $\text{K}_2\text{O}$ . Nitrogen ( $\text{N}_{120}$ ) fertilizer was split in two equal parts after the 1<sup>st</sup> and 2<sup>nd</sup> grazing. Treatments were replicated four times. Pasture was grazed four times a year with a herd of dairy cows. Dry matter yield was determined on the basis of total DM amount per plot and calculated as  $\text{t DM yield ha}^{-1}$ . The data of DM yields were statistically analyzed by the ANOVA according to Tarakanovas (1999).

## Results and discussion

Average data suggest that in those plots in which  $PK_{60}$  fertilization was applied to the pasture the highest herbage DM yield was obtained at 5.6-6.0  $pH_{KCl}$  level. When NPK fertilization was applied soil reaction did not have any appreciable effect on DM yield (Table 1). Dry matter yield was determined by weather conditions during the summer period. The lowest DM yield, 1.99-2.40  $t\ ha^{-1}$ , was obtained in 1994, when there was no rainfall in July. On  $P_{60}K_{60}$  control treatment clover constituted 23-25 % of the herbage (Figure 1). Water deficit influences the basic processes connected with grass productivity (Jones, 1998). When the amount of rainfall in May (1996) exceeded long-term mean by 3.3 times, the yield on  $P_{60}K_{60}$  control treatment amounted to 4.26-4.69  $t\ DM\ ha^{-1}$ , and on the NPK treatment was 5.43-5.87  $t\ DM\ ha^{-1}$ . White clover constituted 42-48 % and 5-9% of herbage, respectively. The amount of rainfall falling in June had the greatest impact on DM yield. In 2001, when long-term mean rainfall was exceeded by 2.4 times, and white clover constituted 30-35 % of the pasture, on the  $P_{60}K_{60}$  control the herbage yield was 0.36-1.19  $t\ DM\ ha^{-1}$  higher than in 1996, when May was rainy. Dry matter yield variations due to rainfall differences were more marked at a higher  $pH_{KCl}$  level. On the  $P_{60}K_{60}$  control ( $pH_{KCl}$  6.6-7.0) a medium-strong curvilinear correlation ( $r=0.557$ ) was obtained between herbage DM yield and amount of rainfall during the growing season. On both fertilization treatments the highest minimal DM yield was recorded at a soil pH level of 5.6-6.0. The highest herbage DM yield was obtained on the  $P_{60}K_{60}$  control treatment at soil  $pH_{KCl}$  of 6.6-7.0, and on the NPK treatment at  $pH_{KCl}$  of 6.1-6.5 (Table 1).

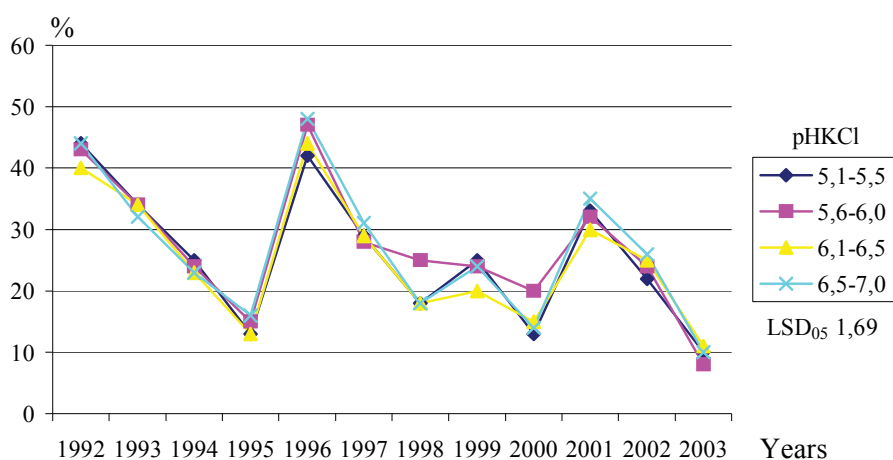


Figure 1. The contribution of white clover in pasture sward depending on soil  $pH_{KCl}$  ( $P_{60}K_{60}$ ) through the study period.

Analysis of variance of the data from the bi-factorial experiment suggests that irrespective of PK and NPK fertilization, the effect of soil reaction on herbage DM yield was inappreciable ( $F_{\text{fakt.}} < F_{\text{teor.}}$ ) (Table 2). At a soil  $pH_{KCl}$  level of 5.6-6.0 the herbage DM yield exceeded the average yield by 0.11  $t\ ha^{-1}$ . Liming did not increase the total DM yield, however, some effect was observed, as a 10 % yield increase was noted in the treatment limed with 8  $t\ limestone\ ha^{-1}$ , compared with the treatment without liming (Stypinski *et al.*, 2003). Nitrogen fertilization significantly increased herbage DM yield ( $F_{\text{fakt.}} > F_{\text{teor.}}$ ). On the NPK treatment herbage DM yield was 1.59  $t\ ha^{-1}$  higher than in the PK treatment. Herbage yield ( $t\ DM\ ha^{-1}$ ) was significantly higher if N fertilization was used (Alibegovic-Grbic and Civic, 2003).

Table 1. Herbage dry matter yield in relation to soil pH and fertilization.

Soil pH <sub>KCl</sub>	Mean (t ha <sup>-1</sup> )	Error of mean	Minimum DM yield (t ha <sup>-1</sup> )	Maximum DM yield (t ha <sup>-1</sup> )	Coefficient of variation V %
P <sub>60</sub> K <sub>60</sub>					
5.1-5.5	3.02	0.21	2.04	4.39	23
5.6-6.0	3.37	0.25	2.40	5.12	25
6.1-6.5	3.18	0.28	2.05	5.05	29
6.6-7.0	3.15	0.29	1.99	5.41	31
N <sub>120</sub> P <sub>60</sub> K <sub>60</sub>					
5.1-5.5	4.82	0.35	3.31	6.52	24
5.6-6.0	4.81	0.33	3.48	6.44	23
6.1-6.5	4.69	0.36	3.36	6.62	26
6.6-7.0	4.76	0.34	3.21	6.38	24

Table 2. Analysis of two factors (pH<sub>KCl</sub> and fertilization) of dry matter yield data.

Soil pH <sub>KCl</sub> Factor A	DM yield (t ha <sup>-1</sup> )	Fertilization Factor B	DM yield (t ha <sup>-1</sup> )	
5.1-5.5	3.92	P <sub>60</sub> K <sub>60</sub>	3.18	Difference
5.6-6.0	4.09	N <sub>120</sub> P <sub>60</sub> K <sub>60</sub>	4.77	1.59
6.1-6.5	3.94	R <sub>05</sub>	0.099	0.198
6.6-7.0	3.96			
R <sub>05</sub>	0.171			
<sup>1</sup> F <sub>fakt.</sub> 0.61; <sup>2</sup> F <sub>teor.</sub> 2.74		F <sub>fakt.</sub> 257** ; F <sub>teor.</sub> 3.98		
Interaction of factors AB LSD <sub>05</sub> 0.261		F <sub>fakt.</sub> 0.6 ; F <sub>teor.</sub> 2.74		
Mean of the experiment 3.98; LSD <sub>05</sub> 0.524;		Sx % 3.52; F <sub>fakt.</sub> 37** ; F <sub>teor.</sub> 2.14		

<sup>1</sup>F<sub>fakt.</sub> – actual yield; <sup>2</sup>F<sub>teor.</sub> – theoretical yield; \*\*R<sub>01</sub>.

## Conclusions

Liming did not give any marked increase in the yield of white clover/grass, which resulted from white clover better tolerance of lower pH values. White clover grows and spreads well at a soil pH<sub>KCl</sub> of 5.6-7.0. The highest DM yield on the P<sub>60</sub>K<sub>60</sub> background was recorded at 5.6-6.0 pH<sub>KCl</sub>, with 40 % of white clover in the sward. Nitrogen (N<sub>120</sub>P<sub>60</sub>K<sub>60</sub>) increased herbage DM yield by 1.59 t ha<sup>-1</sup> compared to the P<sub>60</sub>K<sub>60</sub> treatment.

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## Soil and pasture potassium content after sewage sludge application

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### Abstract

The use of sewage sludge as fertilizer is promoted by the European Union due to its content of N, P and K. The objective of this experiment was to test the effect of different sewage sludge doses, (with or without added potassium), on soil and pasture K concentration. The experiment was located in Lugo (NW Spain) on agriculturally abandoned land which was afforested with *Pinus radiata* D. Don. in 1997 at a density of 1667 trees ha<sup>-1</sup>. Before planting in autumn 1997, pasture was sown at 25 kg ha<sup>-1</sup> of *Lolium perenne* L., 10 kg ha<sup>-1</sup> of *Dactylis glomerata* L. and 4 kg ha<sup>-1</sup> of *Trifolium repens* L.. Fertilizer was applied during 1998, 1999 and 2000. In June 1998, pasture K content was reduced when sewage sludge dose was increased, but soil potassium was significantly lower following the low sewage sludge dose compared with no fertilizer. In the other seven harvests, there were no differences between treatments, probably due to climatic conditions and pasture composition.

Keywords: silvopastoral systems, organic fertilizer, orchard grass, perennial ryegrass, white clover.

### Introduction

Recently, sewage sludge retention has been increased in EU countries due to the implementation of EU directives. Inadequate disposal of this residue can create important environmental problems. Sewage sludge contains appreciable amounts of N, P and K, which makes it suitable for using as fertilizer (Smith, 1996). In pastures growing on acid soils, P and K fertilization is usually applied before sowing to reach target levels of pasture growth and establishment. These elements are also necessary for the development of legumes. Potassium is an essential element for plant and animal development. It is easily translocated from soil to plants and these can utilise higher quantities than needed, especially legumes. Mineral nitrogen is applied to pasture to encourage the growth of sown species of grass (Whitehead, 1995). Anaerobic sewage sludge K concentration is usually low and, therefore, a supply of this element as inorganic fertiliser can be needed to increase pasture growth and enhance the percentage of clover in the pasture. Sludge with higher heavy metal content than is found in the soil is less convenient to use as fertilizer. This problem is even more important in acid soils due to the higher availability of heavy metals in acid soils. The objective of this experiment was to test the effect of different sewage sludge doses, with or without potassium, on the potassium concentration in soil and pasture.

### Materials and methods

The experiment was carried out on agricultural land at Lugo, in north-west Spain, at an altitude of 450 m and with a mean annual precipitation of 1350 mm. Pasture was sown (25 kg ha<sup>-1</sup> *Lolium perenne* cv 'Brigantia', 10 kg ha<sup>-1</sup> *Dactylis glomerata* cv 'Artabro', 4 kg ha<sup>-1</sup> *Trifolium repens* cv 'Huia') in Autumn 1997 on a sandy soil with a pH of 6.3, 31.5 g kg<sup>-1</sup> of N, a high available phosphorus status (0.028 g kg<sup>-1</sup>) and a medium available potassium status (K<sub>2</sub>O: 0.087 g kg<sup>-1</sup>). In January 1998, a plantation of *Pinus radiata* was established at a density of 1667 trees ha<sup>-1</sup>. Eight treatments were applied in a randomised block design with three replicates and plot size of 24m<sup>2</sup>. Treatments consisted of three annual sewage sludge doses (L1: 160 kg N total ha<sup>-1</sup>; L2: 320 kg N total ha<sup>-1</sup>; L3: 480 kg N total ha<sup>-1</sup>) with or without potassium (200 kg K<sub>2</sub>O ha<sup>-1</sup> per year). Two control treatments were established: no fertilizer (NF) and inorganic fertilizer usually used in the region (MIN: 80 kg N ha<sup>-1</sup>, 120 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> and 200 kg K<sub>2</sub>O ha<sup>-1</sup>). These treatments were applied in spring 1998, 1999 and 2000. Available herbage

was clipped 8 times in July and December of 1998 and May, July and November of 1999 and 2000. Four samples per plot (0.09 m<sup>2</sup>) were taken using hand clippers. At the same time, soil samples per plot were taken at a depth of 25 cm. K content in soil and plant was estimated after drying and microkjeldahl digestion, as described Castro *et al.* (1990). Plant and soil K concentration was determined using a VARIAN 220FS spectrophotometer by atomic emission (Varian, 1989). The data were analysed using ANOVA and means were separated by the Duncan test.

## Results and discussion

The lowest soil K concentration (0.16 mg kg<sup>-1</sup>) in June 1998 was obtained when 160 kg total N ha<sup>-1</sup> was applied (Fig 1). This was probably because of (a) the high proportion of *Trifolium repens* in pasture and this species absorbs K in great quantity, and (b) the high forage production of L1, compared with L2 and L3 (Rigueiro *et al.*, 2000). L2 and L3 were similar to the non-fertilizer treatment, due to the higher K application with sewage sludge and the lower productivity of the NF treatment. López-Díaz *et al.* (2005) observed a similar response in exchangeable K in soil when similar doses of sewage sludge were applied in a more acid soil (forestland), even if the K levels in soil were lower than in the present experiment.

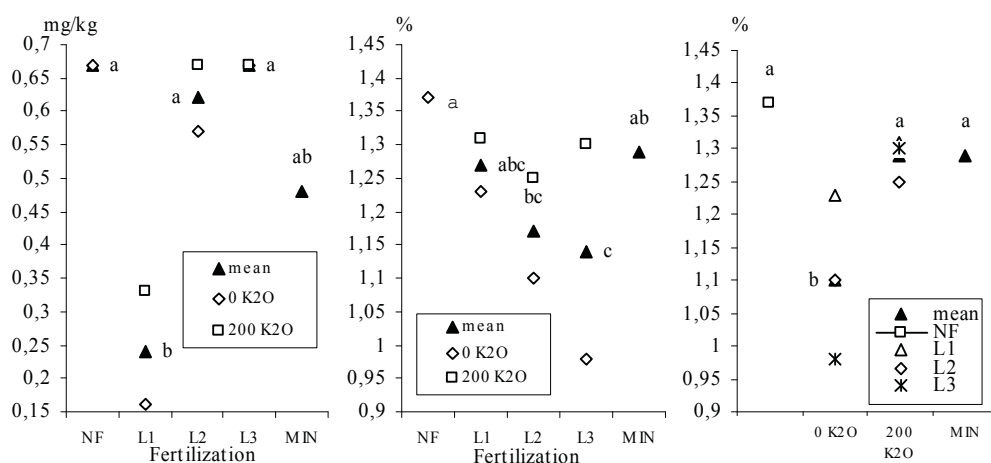


Figure 1. Potassium content (mg kg<sup>-1</sup>) in soil (left graph) and plant (two right graphs) in June 1998 with different fertilizer treatments in June 1998. 0 K<sub>2</sub>O: 0 kg K<sub>2</sub>O ha<sup>-1</sup>; 200 K<sub>2</sub>O: 200 kg K<sub>2</sub>O ha<sup>-1</sup>; NF: no fertilizer; L1: low sewage sludge doses (160 kg total N ha<sup>-1</sup>); L2: medium sewage sludge doses (320 kg total N ha<sup>-1</sup>); L3: high sewage sludge doses (480 kg total N ha<sup>-1</sup>). Different letters indicate significant differences between fertilization treatments (▲).

K levels (0.97-1.37 %) in pasture were similar to those summarised by Mosquera *et al.* (1999) and were reduced when sewage sludge dose was increased. This could be explained by a reduction in *Trifolium repens* and an increase in *Dactylis glomerata* in higher sewage sludge doses, as white clover has higher K values (1.6-3%) compared to cocksfoot (0.5-1%) (Whitehead, 1995). Secondly, higher sewage sludge doses increase pasture production (Rigueiro *et al.*, 2000), which could have caused a dilution effect in the K concentration. Nitrogen application at a low rate increased herbage K levels which has the potential to enhance root growth in clover (Whitehead, 1995). However, when there was no clover in the pasture, López-Díaz *et al.* (2005) found in other experiments carried out in more acid soils, there was an increment of pasture K with higher sewage sludge doses. This could be attributed to the absence of clover and the lower proportion of potassium in soil, compared with the present experiment.

Concentration of pasture K was higher when 200 kg K<sub>2</sub>O ha<sup>-1</sup> was applied, than when 100 K<sub>2</sub>O ha<sup>-1</sup> was applied. There was, however, no significant differences between K levels in soil, probably due to the higher extraction by plants and the mobility of this element from soil to plant.

Inorganic fertilizer had no effect on K levels in soil or plants. However, in more acid soils López-Díaz *et al.* (2005) obtained an increment in K concentration in pasture when mineral fertilization was applied due to K application with mineral treatment and the lower initial K content in soil. Over the three years, all treatments produced forage with enough potassium for equine (0.4%) (NRC, 1989), goat (0.5%), ovine (0.5%) (NRC, 1985) and bovine (0.6%) (NRC, 2000) nutrition.

### Conclusions

K content in soil and plant was significantly affected by different treatments where there were higher levels of clover in the swards. K in plants was reduced when sewage sludge dose was increased, but soil K concentration decreased significantly with the lower sewage sludge dose compared with no fertilization.

### Acknowledgements

We are grateful to CICYT and XUNTA DE GALICIA for financial assistance; to Divina Vázquez Varela, Teresa Piñeiro López and José Javier Santiago-Freijanes for help with laboratory and field work.

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# Influence of post – mushroom compost on the yielding and botanical composition of meadow sward destroyed by fire

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## Abstract

The aim of this research was to estimate the potential of post – mushroom compost utilization as organic manure for the recultivation of a burnt meadow. The following combinations were applied: control (without fertilization); mineral fertilization (NPK); mineral fertilization (NPK + liming), mineral fertilization (NPK) + post- mushroom compost. All the fertilizer combinations were applied on the burnt meadow as well as unburnt one. The best renovation method, on the burnt meadow, was the fertilization with the NPK together with post - mushroom compost. The best change in the botanical composition was observed on the meadow fertilized with NPK separately and together with post - mushroom compost. A positive influence of the post - mushroom compost on the renovated meadow showed the possibilities of utilization of this kind of organic waste for meadow fertilization.

Keywords: post - mushroom compost, burnt meadow, botanical composition.

## Introduction

Grasslands are important elements of agricultural production under many management systems. The biodiversity of meadows changes under the influence of different factors, e.g. soil, moisture, climate and human activities. These factors may cause the occurrence of some plant species and disappearance of others (Grzyb and Prończuk, 1995).

The meadows can be destroyed by human activity through burning. One method of renovation of such meadows can be fertilization with minerals or with organic matter (Jankowski, 1997). Many organic substances are not utilized at present (Jankowski *et al.*, 2005), and are regarded as organical wastes e.g. post - mushroom compost. The aim of this research was to estimate the potential of using post - mushroom compost as organic manure for the recultivation of a burnt meadow.

## Material and methods

The experiment was carried out in 1999 – 2001 on a permanent meadow located on a mineral soil. The experiment was set up in four replicates in 5 x 2 m plots. The following combinations were applied: control (without fertilization); mineral fertilization (NPK); mineral fertilization (NPK + liming), mineral fertilization (NPK) + post- mushroom compost.

The treatments were applied to the burnt and unburnt meadow in the following dose: 180 kg N ha<sup>-1</sup> in three doses each year; 110 kg P ha<sup>-1</sup> once in spring each year; 150 kg K ha<sup>-1</sup> in three doses each year; liming 1t ha<sup>-1</sup> once in spring 1999; post - mushroom compost 20 t ha<sup>-1</sup> of fresh matter once in spring 1999. During the vegetation season three harvestes were done. From the first mowing samples of green matter (about 1kg) from each plot were taken for the estimation of botanical composition. The results were analysed using the ANOVA/ MANOVA and the means were compared using the LSD test.

## Results

Large differences between harvests, years and treatments were found in this study (Table1).

The highest dry matter yields were on the meadow fertilized with NPK together with post- mushroom compost, where the yield on the unburnt meadow 9,5 t ha<sup>-1</sup> was. With regard to the kind of meadow destroyed (burnt or unburnt) the yield of the unburnt meadow was higher than the burned one.

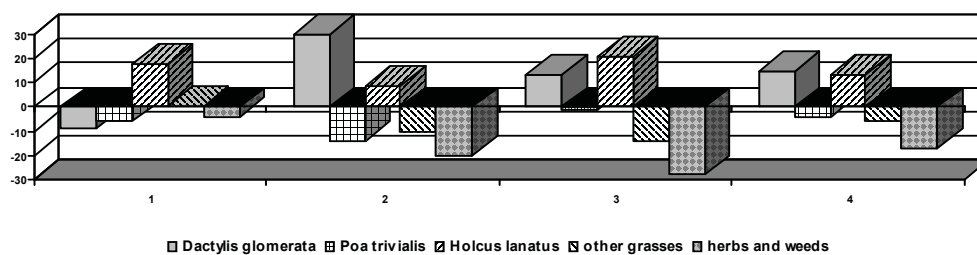
In successive years regardless of the renovation methods used, an increase in *Dactylis glomerata* L. was observed. The highest increase (about 36%) was on the burnt meadow fertilized with NPK. One of the consequences of the applied renovation methods was a significant decrease in the amount of weeds and herbs. The highest decrease (about 28%) was found on the meadow fertilized with NPK together with liming (Figures 1 and 2).

Table 1. Yield of meadow hay (t ha<sup>-1</sup>) in successive years for the analyzed combinations.

Combination		Yield of hay			
		1999	2000	2001	Mean
Control	A	2,6	2,5	2,5	2,5 a
	B	2,8	2,8	2,6	2,7 a
NPK fertilization	A	7,1	6,9	7,0	7,0 b
	B	7,7	7,9	7,8	7,8 c
NPK + liming	A	9,4	8,6	6,3	8,1 c
	B	10,4	10,0	7,0	9,1 d
NPK + post-mushroom compost	A	9,5	9,9	8,0	9,1 d
	B	10,0	10,4	8,2	9,5 e

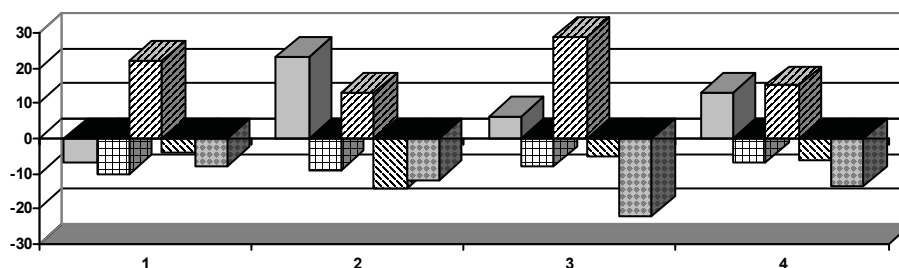
A – Burnt meadow, B – Unburnt meadow

Values followed by different letters (a, b, c, d, e) are significantly different at the level of significance  $\alpha < 0,05$ .



1.- control; 2- NPK ; 3 – NPK + liming; 4 – NPK + post-mushroom compost

Figure 1. Changes in botanical composition in successive years for burnt meadow.



■ *Dactylis glomerata* ▨ *Poa trivialis* ▩ *Holcus lanatus* ▪ other grasses ▫ herbs and weeds

1.- control; 2- NPK ; 3 – NPK + liming; 4 – NPK + post–mushroom compost

Figure 2. Changes in botanical composition in successive years for unburnt meadow.

### Conclusion

With regard to yield the best renovation method on the burnt meadow was fertilization with NPK together with post - mushroom compost.

The botanical composition of meadow sward was significantly dependent on the kind of a renovation method. The best change in the botanical composition was observed on the meadow fertilized with NPK separately and together with post - mushroom compost.

The positive influence of post - mushroom compost on the renovated meadow showed the possibilities of utilization of this kind of organic waste for meadow fertilization.

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## Agromorphological diversity in North Spanish *Agrostis capillaris*

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### Abstract

Fourteen *Agrostis capillaris* L. accessions collected from northern Spain were characterised in a low-fertilisation regime at Carreño (43° 35' N, 5° 47' W, 80 m.a.s.l.) in Asturias during two years (2004 and 2005) in a randomised complete block design with two replicates of 15 plants per accession. One commercial cultivar (*Agrostis capillaris* cv 'Golfin') was also included in this study. The entries included accessions and cultivar were evaluated for agronomical and turfgrass quality characteristics each year. The collected data was reduced to four principle components that cumulatively explained 82.6% of the total variance. Cluster analysis was useful in identifying four groups of accessions describing 57% of the phenotypic variation among accessions. Cluster 1 consisted of seven accessions with an intermediate growth habit, late heading and with the narrowest flag leaf and shortest upper internodes. Cluster 2 contained three accessions with the latest heading, the most erect growth habit, darkest green colour as accessions of cluster 3. Cluster 3 contained the two earliest heading accessions and the widest flag leaf and longest upper internodes. Cluster 4 consisted of two accessions and the cultivar 'Golfin' lightest green colour and intermediate characters among the other clusters.

Keywords: bentgrass, characterisation, multivariate analysis, turf.

### Introduction

Browntop in Europe or Colonial bentgrass in United States (*Agrostis capillaris* L. 2n=4x=28), native to Europe and temperate Asia, is commonly used for tennis courts, high-grade lawns, fairways and erosion control (Hubbard, 1984).

To conserve and study the existing genetic resources of colonial bentgrass Northern Spanish accessions for use in breeding was investigated using amplified fragment length polymorphism (AFLP) markers (Zhao *et al.*, 2006).

Objective of this research was to characterise the variability of northern Spanish *Agrostis capillaris* accessions, on the basis of morphological characteristics. Knowledge of this variability should provide useful information concerning the potential value of these accessions to Spanish and North American breeding programs.

### Materials and methods

Originally, seed of 14 colonial bentgrass accessions was collected from grasslands in northern Spain. The morphological study was established at the University of Oviedo in a farm of the community of Carreño (43° 35' N, 5° 47' W, 90 m.a.s.l., on an inceptisol soil type). The trial was arranged in a randomized complete block design with two replications of 15 plants per accession. Plants were transplanted to the field in March 2003, 50 cm apart. One commercial cultivar 'Golfin' (*Agrostis capillaris*) was also included as control.

The site received the same amount of fertilizer throughout the two years of study, a total of 10 g N m<sup>2</sup> per year. The plants were maintained at mowing height of 5 cm with a rotary mower.

A total of eleven morphological traits including (1) leaf width (1=narrow to 9=broad), (2) growth habit (1=erect to 9=prostrate), (3) colour (1=light green to 9=dark green), (4) autumn growth (1=very little to 9=very green), (5) winter growth (1=very little to 9=very green), (6) flag leaf length in centimetres, (7) flag leaf width in millimetres, (8) length of longest stem, included inflorescence, in centimetres, (9)

Length inflorescence, in centimetres, (10) length upper internode, in centimetres, (11) heading date as the number of days after January the first. These traits were evaluated during a 2-year period.

Analysis of variance of the morphological data was performed first per year and to observe errors homogeneity to combine the statistical analysis for the two years.

Multivariate relationships among entries were revealed with a principal component analysis (PCA) using a correlation matrix derived from the significant characters. The original variables were reduced to four independent linear combinations, principle components (PC) of the variables, with eigenvalues greater than 1. These PCs were used as the input for an agglomerative hierarchical clustering analysis to detect groups of similar agromorphological types. The squared Euclidean distance was the measure of distance, and the Ward's clustering algorithm, was the method for combining entries into clusters. With the purpose to examine differences between the clusters obtained, a table of means, standard deviations and the results of a one-way ANOVA (F tests) are also presented. Statistical analyses were computed using SPSS 11.5 (SPSS, 2002).

## Results and discussion

The data set was reduced to four principle components that cumulatively explained 82.6% of the total variance.

Hierarchical clustering analysis performed on the first four principal components leads to a dendrogram that was cut at the four cluster level describing 57% of the phenotypic variation among entries.

Significant statistical differences were detected between clusters for growth habit, colour, flag leaf width, length of upper internodes and heading date (Table 1).

Table 1. Between-Groups differences for leaf width (Lw), growth habit (Gh), colour (Co), autumn growth (Ag), winter growth (Wg), flag leaf length (Fl), flag leaf width (Flw), length of longest stem (Lls), length inflorescence (Lin), length upper internodes (Lui) and heading date (Hd) traits. M = mean, SD = Standard deviation. \*\*\*  $p < 0.001$ , \*\*  $p < 0.01$ , \*  $p < 0.05$ , NS =  $p > 0.05$ .

Trait	Group 1 (n = 7)		Group 2 (n = 3)		Group 3 (n = 2)		Group 4 (n = 3)		Anova F ratios
	M	SD	M	SD	M	SD	M	SD	F (3,11)
Lw	6.19	0.66	6.57	0.40	5.95	0.05	6.00	0.73	0.5NS
Gh	4.13	0.24	3.10	0.08	3.85	0.05	3.27	0.05	24.4***
Co	6.49	0.45	6.87	0.05	6.90	0.00	5.33	0.47	8.0**
Ag	6.36	0.54	6.43	0.31	5.40	1.20	5.27	0.90	1.8NS
Wg	6.49	0.56	6.90	0.71	5.90	1.20	6.13	0.87	0.6NS
Fl	7.66	1.95	8.37	1.45	10.80	1.20	7.13	1.77	1.5NS
Flw	3.39	0.61	3.40	0.41	5.10	0.40	4.73	0.54	7.0**
Lls	63.54	5.36	68.23	5.43	74.70	2.90	59.60	3.50	3.4NS
Lin	11.70	2.12	12.50	1.72	16.55	3.35	10.43	2.17	2.3NS
Lui	9.86	1.19	11.87	0.79	14.35	0.85	10.23	0.49	9.3**
Hd	158.29	1.90	159.67	0.47	147.50	0.50	155.00	2.94	15.4***



Cluster 1 consisted of seven accessions with an intermediate growth habit, late heading and with the narrowest flag leaf and shortest upper internodes. Cluster 2 contained three accessions with the latest heading, the most erect growth habit, darkest green colour as accessions of cluster 3. Cluster 3 contained the two earliest heading accessions and the widest flag leaf and longest upper internodes. Cluster 4 consisted of two accessions and the cultivar lightest green colour and intermediate characters among the other clusters.

Certain colonial bentgrass accessions are widely recognized as having good resistance to snow mold (Vergara and Bughrara, 2005) and dollar spot (Belanguer *et al.*, 2004). For this reason we are now evaluating the susceptibility to two important turf diseases (snow mold and dollar spot) on these accessions.

### **Conclusions**

The assessment of the morphological diversity among North Spanish colonial bentgrass accessions suggested the potential value of this germplasm in turfgrass cultivar improvement.

### **Acknowledgements**

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# Effect of mulching frequency on botanical composition of grassland

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## Abstract

Effects of different mulching frequency treatments on the botanical composition of permanent grassland were studied in upland areas of the Jizerské Mountains in the Czech Republic from 2000 to 2005. Treatments investigated were unmanaged grassland, and mulching once, twice and three-times per year. Dominant plant species before the experiment were *Dactylis glomerata* L., *Festuca pratensis* Huds., *Elytrigia repens* L., *Aegopodium podagraria* L. and *Veronica chamaedrys* L., and legume cover was about 8%. During the experiment the legume cover increased in both of the multiply mulched treatments. Abundance of *Trifolium repens* L. increased from 1 to 11% under three-times mulched and from 1 to 8% under the twice- mulched treatment. In the 2003 and 2004 growing seasons the cover of *T. repens* was the highest and reached over 20% and 15%, respectively, in the three-times mulched treatment. In unmanaged or once-mulched plots legumes disappeared and tall forbs such as *Cirsium arvense* L., *Urtica dioica* L. and *Galium album* L. increased.

Keywords: meadow, mulching frequency, legumes, number of species, management.

## Introduction

Reductions in livestock grazers has led to decreased importance of semi-natural grasslands for forage production in marginal areas of the Czech Republic during the last decade of 20th century. Large areas of meadows and pastures were abandoned. Tall grasses and dicotyledonous species with high competitive ability for light and nutrients have spread on unmanaged grasslands. Some are undesirable perennial weeds, e.g. *Elytrigia repens*, *Rumex obtusifolius* L., *Cirsium arvense*, *Urtica dioica*. The absence of defoliation has lead in many cases to succession by shrubs and trees (e.g. alder, birch or aspen). Mulching is an alternative grassland management without forage utilization. The aim of our study was to compare the biodiversity on grassland receiving different mulching frequencies with unmanaged grassland.

## Material and methods

The experiment was carried in the Jizerské Mountains in the northern part of the Czech Republic from 2000 to 2005. Altitude is 420 m above sea level, average annual temperature is 7.2°C and annual precipitation is 803 mm. *Dactylis glomerata*, *Elytrigia repens* and *Festuca pratensis* were the dominant species. Treatments were arranged in four complete randomized blocks in 5 x 10 m plots. Investigated treatments were: (1) unmanaged grassland (U); (2) 1x mulching in July (1M); (3) 2x mulching in June and August (2M); (4) 3x mulching in May, July and September (3M). Mulching was done by the machine Uni Maher UM 19 which crushes and breaks down biomass to an even layer on the surface of the sward. Percentage cover of all vascular plant species was recorded at the end of May each year. Nomenclature: Kubát *et al.* (2002). ANOVA was used for analysis of collected data.

## Results and discussion

In the frequently mulched treatments (2M, 3M) the prostrate dicotyledonous species (e.g. *Trifolium repens*, *Taraxacum* spp.) spread during the experiment. There was a significant increase ( $P < 0.001$ ) of legumes (Table 1). Similarly, Pavlů *et al.* (2003) recorded an increase of white clover under frequent defoliation on pasture.

Table 1. Proportional contributions (%) of agrobotanical groups for years and treatments.

Treatment	U					1M					2M					3M								
	00	01	02	03	04	00	01	02	03	04	00	01	02	03	04	00	01	02	03	04				
Grasses	62	50	46	44	41	42	55	48	57	41	41	30	54	47	56	48	48	39	56	45	50	39	31	26
Legumes	7	6	0	1	2	2	9	12	4	9	8	6	7	20	10	12	12	11	9	23	23	30	21	17
Forbs	22	29	33	36	48	51	25	28	32	44	40	58	30	23	26	35	35	38	27	21	23	25	31	36
Empty pl.	9	16	22	19	10	6	11	13	7	7	11	7	9	11	7	6	6	13	8	11	5	6	17	21

*Trifolium repens* increased from 1 to 11% under the 3M treatment and from 1 to 8% under the 2M treatment. The cover of *T. repens* was the highest in the 2003 and 2004 vegetation seasons and reached over 20% and 15%, respectively, on the 3M treatment (Figure 1).

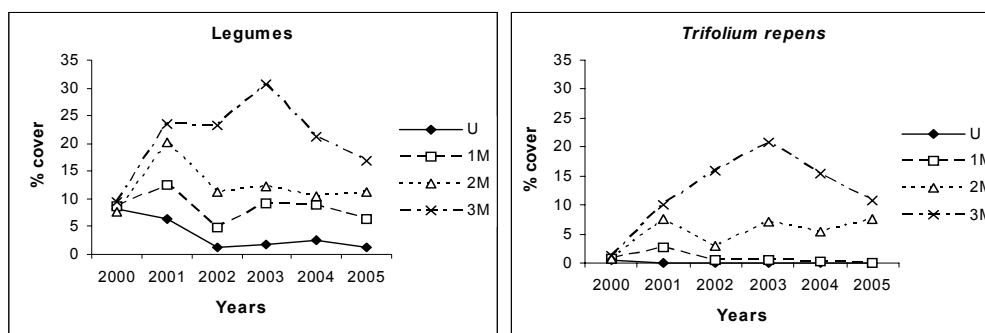


Figure 1. Mean cover of legumes and *Trifolium repens* in the sward (years 2000-2005).

In the frequently mulched treatments the cover of prostrate herbs increased, while in the unmanaged and once-mulched plots some tall dicotyledonous species and weeds such as *Cirsium arvense*, *Urtica dioica* and *Galium album* became widespread (Figure 2).

Cover of *Taraxacum* spp. increased from 2 to 7% in the 2M and from 2 to 9% in the 3M treatments, respectively. Abundance of *Cirsium arvense* increased from 2 to 6% in U and 1M treatments; however, it decreased in the 2M and 3M treatments. The cover of *Urtica dioica* increased from 1 to 7% and that of *Galium album* increased from 3 to 10% on unmanaged grassland during the experiment. The number of plant species recorded was slightly increased in the mulched treatments, but it decreased in the unmanaged grassland (Figure 3). This trend is in accordance findings reported elsewhere ( Bakker, 1989, Ryser *et al.*,1995) that the appropriate frequency of disturbance is one of the key factors for coexistence of a high number of plant species.

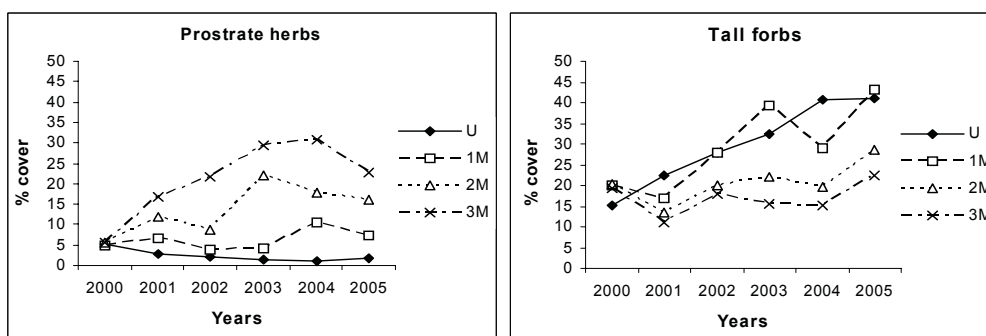


Figure 2. Mean cover of prostrate herbs and tall forbs in the sward (years 2000-2005).

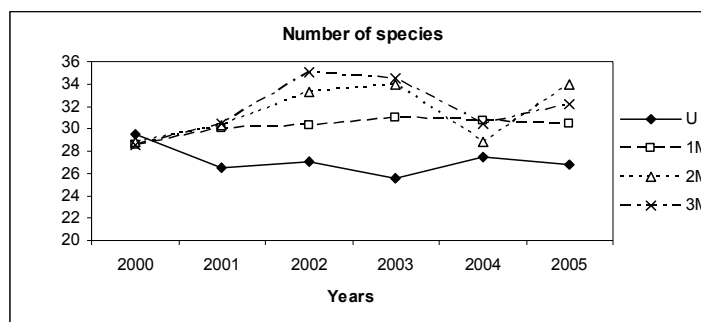


Figure 3. Mean number of plant species (years 2000-2005).

## Conclusions

The increase of legume cover in the sward was promoted by frequent mulching. Tall forbs (e.g. *Urtica dioica*, *Cirsium arvense* and *Galium album*) spread in unmanaged grassland and in plots mulched once per year, in July. Frequent mulching resulted in a small increase in the number of plant species recorded. To restrict the spread of weeds and the decrease of species diversity, alternative managements must be practised in areas where the grasslands are not utilised for fodder production in the Czech Republic.

## Acknowledgements

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# Influence of the seeder type and the initial sward composition on the success of overseeding

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## Abstract

The botanical composition of agriculturally poor permanent grassland can be improved by overseeding, but this process often needs to be repeated in order to have success. We used four kinds of seeders (seed broadcaster with roller, seed broadcaster with harrow, drill seeder, and seeder with rotary band cultivator) at six locations to assess the influence of the seeder type and the initial sward composition on the success of overseeding. The proportion of the sown grass species was successfully improved at only three of the six sites. The seeder with rotary band cultivator gave slightly better results at two of these three successful sites but the resulting difference between the types of seeders was small. At one of the unsuccessful sites, *Agrostis stolonifera* L. and *Poa trivialis* L. had a yield proportion of 75% in the initial sward, and independently of the seeder used these stoloniferous species immediately out-competed the seedlings. At the other two unsuccessful locations, rainfall was probably insufficient to sustain the growth of the seedlings, independent of the seeder treatments. The results show that the composition of the initial sward and the moisture conditions have a greater influence on the rate of success of overseeding than the type of seeders used.

Keywords: overseeding, seeder type, botanical composition.

## Introduction

Overseeding commonly used to improve the botanical composition of unsatisfying permanent grassland. The rate of success of this method is nevertheless low. Therefore the operation often needs to be repeated before an increase in the proportion of the sown species can be achieved. Beside the competition for water and light exerted by the established plants on the seedlings, another probable cause of failure is the unsatisfying contact between the seeds and the soil. Different types of seeders and combinations of machines are in use to try alleviating this problem. We tested four kinds of seeders at six sites to assess the influence of the seeder type and the initial sward composition on the success of overseeding.

## Materials and Methods

The four kinds of seeders tested were: 1) seed broadcaster with roller, 2) seed broadcaster with harrow, 3) drill seeder, and 4) seeder with rotary band cultivator. Six experiments were carried out at the following locations: Ebenrein, Böckten, Dürrenroth, Gossau, Reckenholz and Reichenburg. Each experiment consisted of a randomized complete block design with 4 replicates. No overseeding was performed in the control plots. The overseeding took place in the middle of May 2000, after the first utilization of the meadows. The seed mixture used was 12 kg ha<sup>-1</sup> *Lolium perenne* L., 6 kg ha<sup>-1</sup> *Poa pratensis* L. and 2 kg ha<sup>-1</sup> *Trifolium repens* L., except at Gossau where a mixture of 3 kg ha<sup>-1</sup> *Lolium perenne* L., 7 kg ha<sup>-1</sup> *Poa pratensis* L., 8 kg ha<sup>-1</sup> *Alopecurus pratense* L. and 2 kg ha<sup>-1</sup> *Trifolium repens* L. was used. The botanical composition of the swards, in terms of yield proportions, was estimated according to Dietl (1995), modified to 12 yield proportion classes, during springtime of each experimental year.

## Results and discussion

The overseeding successfully increased the yield proportion of the sown grass species on three (Gossau, Reckenholz and Reichenburg) of the six locations (Figure 1). At the other locations, no significant difference in botanical composition was found between the oversown plots and the control, until 3 years after overseeding. The yield proportion of *Trifolium repens* was not increased by overseeding at all locations. Combining the locations where overseeding was successful with the ones where it was not, in the analysis of variance, resulted, as expected, in a significant seeder by location interaction (data not shown). On the other hand, combined analysis of variance for the three locations that had significant differences with the control (Gossau, Reckenholz, Reichenburg) showed no significant seeder by location interaction (Table 1).

Table 1. Combined analysis of variance of the log(x + 1) transformed yield proportion data of sown grass species, for the locations Gossau, Reckenholz and Reichenburg.

Source of Variation	d.f.	2002		2003	
		MS	Computed F	MS	Computed F
Treatment (T)	4	0.5751	49.29**	0.5511	22.93**
E <sub>1</sub> : Control vs. Seeders	(1)	2.0551	176.16**	2.1071	87.66**
E <sub>2</sub> : Between Seeders	(3)	0.0817	7.01**	0.0324	1.35 <sup>n.s.</sup>
Location (L)	2	2.0619	34.37**	0.1599	3.85 <sup>n.s.</sup>
Block in L	9	0.0411		0.0573	
T X L	8	0.0600	5.14**	0.0415	1.73 <sup>n.s.</sup>
E <sub>1</sub> X L	(2)	0.1762	15.10**	0.1255	5.22*
E <sub>2</sub> X L	(6)	0.0213	1.82 <sup>n.s.</sup>	0.0135	0.56 <sup>n.s.</sup>
Pooled error	36	0.0117		0.0240	

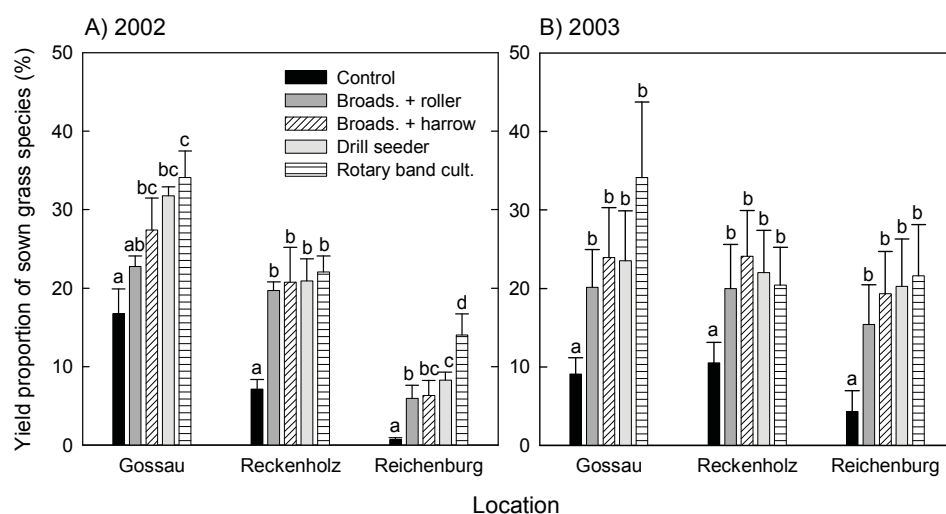


Figure 1. Yield proportion of the oversown grass species at Gossau, Reckenholz and Reichenburg in A) 2002, and B) 2003. Data are means + SE of 4 replicates. At a same location, means labelled with a common letter are not significantly different at 5 % level by LSD test.

In 2002 in Gossau and Reichenburg, the ranking of the seeders for the success of the overseeding, in terms of the increase in the proportion of the sown grass species, was as follows: seed broadcaster with roller < seed broadcaster with harrow < drill seeder < seeder with rotary band cultivator. However, significant differences at both sites were found only between the seed broadcaster with roller and the seeder with rotary band cultivator. No differences between the types of seeders were found at Reckenholz. In 2003, the differences found between the seeders were not statistically significant at any of these three sites.

The high proportion of the stoloniferous grass species *Agrostis stolonifera* L. and *Poa trivialis* L. in the initial sward probably caused the failure of the overseeding treatments at Dürrenroth: in 2000, the combined yield proportion of these two species was around 75%, and their competition was presumably too strong. We observed that these two grass species even closed the vegetation-free bands opened in the sward by the rotary band cultivator within a few weeks after overseeding. At all other locations, the yield proportion of *Agrostis stolonifera* and *Poa trivialis* in the initial sward was less than 30%.

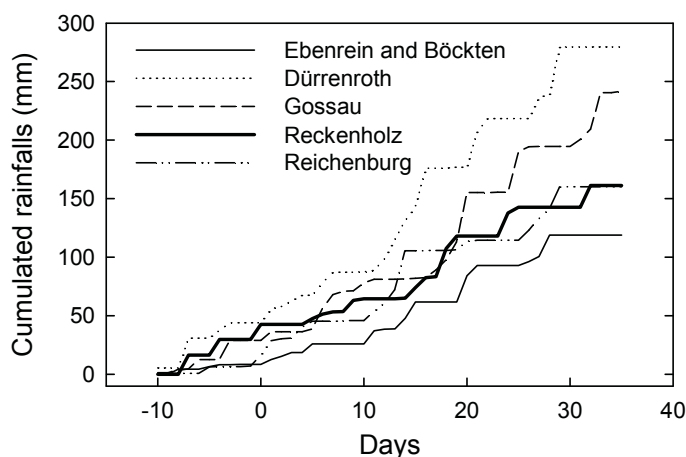


Figure 2. Cumulated amounts of rain falling in the area of the experimental sites starting 10 days before overseeding (day 0 = day of overseeding). Data are measurements of the nearest available meteorological station (largest distance from the experimental site = 15 km).

The failure of the overseeding treatments at Ebenrein and Böckten might have been due to insufficient water availability to the seedlings. At these locations, rainfall before and after overseeding was markedly less abundant than at the other locations (Figure 2).

### Conclusions

At the locations where overseeding was successful, the four types of seeders were able to improve the botanical composition of the swards in similar proportions, and there were no statistical differences between the types of seeders three years after overseeding. At the locations where the weather conditions were dry, or large proportions of *Agrostis stolonifera* and *Poa trivialis* in the initial sward were competing with the seedlings, none of the types of seeders tested was successful in establishing the sown species. We conclude that the composition of the initial sward and the moisture conditions have a greater influence on the rate of success of overseeding than the type of seeder used.

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## Effect of different grazing intensities on some simple plant traits

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### Abstract

The effect of different grazing management imposed on abandoned grassland was studied in an upland area in the northern part of the Czech Republic over seven years (1998-2004). We established completely randomized blocks experiment with the following treatments: unmanaged control (U), intensive (IG) and extensive (EG) continuous grazing, 1<sup>st</sup> cut followed by intensive (ICG) and extensive (ECG) continuous grazing for the rest of the season. All plant species within the study area were *a priori* categorized according to their simple vegetation traits. Cover of the short grasses positively responded to all managed treatments and was the highest in IG treatment. Abundance of tall grasses as well as tall forbs reflected intensity of management and was in order U>EG, ECG>IG, ICG. On the other hand prostrate forbs increased their cover with the intensity of management (ICG>IG>ECG>EG). This result indicates a replacing of tall dominants by short grasses and prostrate forbs.

Keywords: cattle grazing, intensity, functional group, grassland.

### Introduction

During the last two decades considerable changes in the utilization of grassland in Europe has occurred. Reduction of number of cattle led to the extensification of grassland utilization and resulted also in abandonment of the low productive sites. These changes in agricultural land use were stronger in the eastern and central European countries, which had to build up a market economy. Reintroduction of management on formerly abandoned grassland is necessary to prevent natural reforestation. Suitable management and its intensity depend on present vegetation, local possibilities and our target goal.

Although species response gives very useful information, a functional analysis of vegetation may help to understand and predict the impact of management in more general way (Louault *et al.*, 2005). The aim of this study was to compare different grazing managements, focusing on simple vegetation traits.

### Materials and methods

The experiment was undertaken in the Jizerské Mountains in the northern part of the Czech Republic. The altitude of the investigated grassland is 420 m, the average annual precipitation is 803 mm, and the mean annual temperature is 7.2 °C. The dominant species in 1998 were *Agrostis capillaris* L., *Alopecurus pratensis* L., *Festuca rubra* L., *Aegopodium podagraria* L. and *Galium album* Mill. Experiment was arranged in two completely randomized blocks. Applied treatments were: intensive grazing (IG), 1<sup>st</sup> cut followed by intensive grazing (ICG), extensive grazing (EG), 1<sup>st</sup> cut followed by extensive grazing (ECG), and unmanaged grassland (U) as the control. All treatments were grazed continuously by young heifers.

Samples were taken in permanent 1 x 1 m plots using four replications in each paddock. All plant species within the study area were *a priori* categorized according to their main vegetation traits: short grasses, tall grasses, prostrate forbs, tall forbs and others. The redundancy analysis (Lepš and Šmilauer, 2003) was used to evaluate trends in plant species composition.

### Results and discussion

Remarkable successional developments independent of the all treatments as well as significant differences among study treatments were detected. (Table 1).



Table 1 Results of RDA analyses of functional groups data. PlotID = plot identifier; % explained variability = species variability explained by axis 1 (all ordination axes) - measure of explanatory power of the explanatory variables; F-ratio = F statistics for the test of particular analysis (all axes); P-value = corresponding probability value obtained by the Monte Carlo permutation test (999 permutations), i.e. Type I error probability in testing the hypothesis that the effect of one (all) explanatory variables is zero.

Analysis	Explanatory variables	Covariables	% expl. 1 <sup>st</sup> (all) axis	F ratio 1 <sup>st</sup> (all) axis	P 1 <sup>st</sup> (all) axis
A1: There are no directional changes in time in the species composition, that are common to all the treatments or specific for particular treatments. <b>Yes</b>	Year, Year*IG, Year*EG, Year*ICG, Year*ECG, Year*U	Plot ID	29.0 (36.6)	95.85 (27.17)	0.001 (0.001)
A2: The temporal trend in the species composition is independent of the all treatments. <b>Yes</b>	Year*IG, Year*EG, Year*ICG, Year*ECG, Year*U	Year, Plot ID	9.1 (14.6)	23.63 (10.08)	0.001 (0.001)

Tall grasses (with dominant *A. pratensis*) were generally replaced by short grasses (with dominant *A. capillaris*) similarly in all managed treatments (Figure 1). The prostrate forbs represented by two dominants, *T. repens* and *Taraxacum* spp., increased in cover in all managed treatments. *T. repens* increased cover as a function of intensity management (ICG>IG>ECG>EG), whereas *Taraxacum* spp. was strongly promoted by first cut followed both grazing intensities (ICG, ECG). In this study both tall dominant forbs (*A. podagraria* and *G. album*) e decreased by grazing on abandoned grassland. Total abundance of tall grasses as well as tall forbs reflected intensity of management and was in order U>EG, ECG>IG, ICG. These results indicate a replacing of tall dominants by short grasses and prostrate forbs.

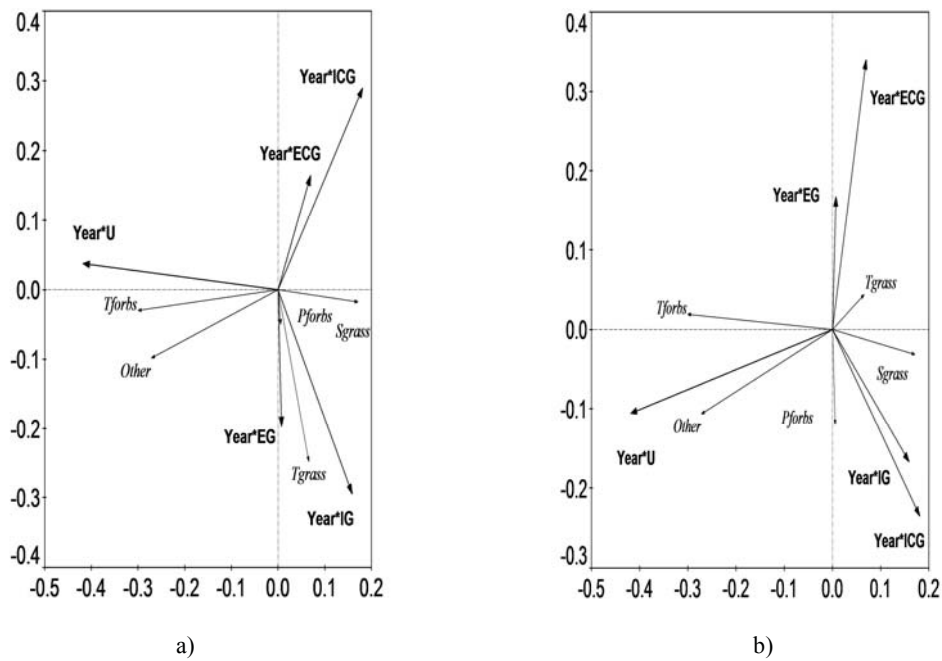


Figure 1. Ordination diagram showing the result of RDA analysis a) first and second axis b) first and third axis, Abbreviation: \*-indicates interaction of environmental variables, Tforbs-Tall forbs, Tgrass-Tall grass, Sgrass-Short grass, Pforbs-Prostrate forbs, Other-Other species.

### Conclusions

Simple vegetation traits can be used to predict response of different grazing management, however it is strongly dependent on several dominants sometimes resulting in miscellaneous responses.

### Acknowledgements

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## Methods of seed germination in four leguminous forage shrubs

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### Abstract

The germination of *Chamaecytisus palmensis* Hutch., *Teline canariensis* L., *Teline osyrioides sericea* Kuntze. and *Teline osyrioides osyrioides* Svent. seeds was studied in relation to the following treatments, taken individually or in various combinations: a) aril removal; b) no aril removal; c) scarification in water at 80° C and 100° C; d) scarification in concentrated sulphuric acid. The method which gave the best results for all the species was aril removal plus scarification with acid during 30-40 min.

Keywords: *Chamaecytisus*, *Teline*, seed germination.

### Introduction

There are numerous species of endemic shrubs of the family *Fabaceae* in the Canary Islands. Many of them are potentially useful in agroforestry systems for arid and semi-arid regions. Only *Chamaecytisus palmensis* (tagasaste) has been established in forage systems, proving itself to be one of the most interesting species in this respect (Lefroy, 2002). In our opinion, however, there are many other species which may also be useful for these agrobiosystems, such as those of the genus *Teline*. In fact that they have not been studied from this perspective, despite being a potential forage resource. This study proposes a simple, efficient and useful pre-germination method for four shrub-like leguminous plants endemic to the Canary Islands.

### Materials and Methods

The seeds were gathered in ripe pods during the summer of 1998, from four populations located in their natural habitat. They were washed with detergent and those that floated were eliminated.

The germination of seeds with and without aril removal was studied in the following conditions: a) not treated (control), b) scarification in water at 80° C and 100° C and c) scarification in concentrated sulphuric acid of a specific density of 1.8 for 30, 35 and 40 minutes. Aril removal was carried out individually using a scalpel, with efforts being made not to damage the seed coating. Scarification with water at 80° C was carried out by placing the seeds in the water at this temperature until the water cooled. Seeds with and without aril removal were scarified separately. In the scarification with water at 100° C, the seeds with and without aril removal were submerged for 50 seconds at this temperature and then washed in normal water. The treated and control seeds were put to germinate in plastic boxes measuring 17x12x3.5 cm. The substrate was picón (ashes of a basaltic composition) sterilised at 150° C for 12 hours. The seeds were placed on the surface to make it easier to follow their germination. Seeds were considered to have germinated when the radicle reached a length of 2 mm. The trays were watered in order to maintain constant humidity close to field capacity with a solution containing 1 mL L<sup>-1</sup> of fungicide (active principle: Hymexazol). Each treatment was repeated in four trays. After sowing, the trays were placed in a germination chamber at a constant temperature of 16° C with 16 hours of daylight.

A statistical analysis (single-factor Anova) was carried out and Duncan's test was used for comparing germination percentages.

## Results and Discussion.

Table 1. Percentages of germination obtained on *Chamaecytisus palmensis* for different scarification treatments applied before or after aril removal.

	With aril			Without aril		
Check	7	(8.3)	a	37	(9.6)	b
Water 80° C	32	(1.2)	b	54	(18.6)	c
40 min acid	28	(3.5)	b	60	(8.5)	c
Mean	22			50		

Values are test means. The standard deviations appear in parenthesis.

Values followed by the same letter are not significantly different (Duncan's test,  $P < 0.05$ ).

Tagasaste shows significantly different results (Table 1), among treatments with a maximum of 60% germination (with aril removal plus 40 minutes in acid) and a minimum of 54% germination (with aril removal plus water at 80° C). The statistical analysis shows no significant difference between 60 and 54% germination. These values are lower than those obtained for the same species without aril removal by Olea *et al.* (1993), who achieved 75% by boiling the seeds in water for one minute. Pérez de Paz *et al.* (1986) demonstrated that immersion in boiling water for a few minutes gave much higher germination percentages.

Table 2. Percentages of germination obtained on *Teline canariensis* for different scarification treatments applied before or after aril removal.

	With aril			Without aril		
Check	13	(9.0)	a	18	(3.5)	a
Water 80° C	20	(14.4)	a	37	(13.4)	b
60 sec water 100 °C	13	(10.5)	a	30	(7.8)	ab
35 min acid	17	(14.8)	a	43	(6.8)	b
Mean	16			32		

Values are test means. The standard deviations appear in parenthesis.

Values followed by the same letter are not significantly different (Duncan's test,  $P < 0.05$ ).

Table 3. Percentages of germination obtained on *Teline osyrioides sericea* for different scarification treatments applied before or after aril removal.

	With aril			Without aril		
Check	11	(9.6)	a	35	(4.6)	de
Water 80° C	20	(4.2)	abc	45	(7.0)	e
50 sec water 100 °C	16	(12.2)	ab	28	(6.6)	cd
60 sec water 100 °C	25	(2.7)	bcd	31	(4.4)	cd
30 min acid	33	(5.6)	d	79	(2.3)	f
35 min acid	25	(6.0)	bcd	76	(11.9)	f
Mean	22			49		

Values are test means. The standard deviations appear in parenthesis.

Values followed by the same letter are not significantly different (Duncan's test,  $P < 0.05$ ).

On the other hand, *T. canariensis* shows significant differences among treatments (Table 2), with the highest germination values reaching 37% and 43% or over (with aril removal plus water at 80° C and with aril removal plus 35 minutes in acid respectively). These percentages are lower than those obtained for the same species without aril removal by Peréz de Paz *et al.* (1986), who achieved germination rates of 89% with boiling water and manual scarification.

The results obtained with *T. o. sericea* form three significantly different groups (Table 3). The group with the highest rate of germination corresponds to seeds with aril removal scarified for 30 minutes in acid (79%) and seeds with aril removal scarified for 35 minutes in acid. These percentages are higher than those obtained for the same species without aril removal by Barquín and Chinae (1995), using submersion for one hour in acid (31%).

Table 4. Percentages of germination obtained on *Teline osyrioides osyrioides* for different scarification treatments applied before or after aril removal.

	With aril			Without aril		
Check	27	(10.1)	a	42	(6.0)	bc
Water 80° C	45	(5.7)	cd	48	(1.8)	cde
50 sec water 100 °C	34	(6.4)	ab	45	(2.9)	c
30 min acid	55	(6.1)	def	61	(5.3)	f
35 min acid	47	(7.9)	cd	50	(4.1)	cde
40 min acid	62	(8.9)	f	58	(7.9)	ef
Mean	45			51		

Values are test means. The standard deviations appear in parenthesis.

Values followed by the same letter are not significantly different (Duncan's test,  $P < 0.05$ ).

*T. o. osyrioides* shows also three clearly defined groups (Table 4). Those with a rate of 61% or over correspond to treatments without aril removal plus 40 minutes in acid (62%), and treatments with aril removal plus 30 minutes in acid (61%). These values are lower than those obtained by Lucía Sauquillo *et al.* (1994), using scarification with sandpaper (74 %).

The presence of the aril interferes with the germination process, since when this element is eliminated germination levels increase in all species, both in untreated and treated seeds with the exception of *T. o. osyrioides*. From these trials we can conclude that the removal of the aril followed by scarification with concentrated sulphuric acid for 30-40 minutes at a constant germination temperature of 16° C provides optimum results. This germination system is suitable for both research studies and for the large-scale propagation of this group of species, (tagasaste, *T. o. sericea*, *T. o. osyrioides*), with an average germination percentage of 60%. On the another hand, the results for *T. canariensis*, which we considered valid for our purposes with values of 43%, could be improved by making some modifications to the method.

#### Acknowledgements

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## Effect of fertigation on *Chamaecytisus proliferus*

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### Abstract

Four nutrient solutions with different P, K, Ca and Mg concentrations were studied, in relation to their effect on one endemic leguminous forage shrub for arid regions (Canary Islands). The substrate used showed a high cation fixation capacity and a retention of phosphate anions, mainly due to its volcanic composition. A direct relationship was observed between assimilable P, K, Ca and Mg concentrations present in the substrate and in the leaves, showing that *Chamaecytisus proliferus* Link subsp. *angustifolius* (Kuntze) G. Kunkel (escobón) has a good capacity for assimilating nutrients.

Keywords: fertigation, *Chamaecytisus*.

### Introduction

*Chamaecytisus proliferus* Link subsp. *angustifolius* (Kuntze) G. Kunkel, is a shrubby leguminous plant native to the Canary Islands. Despite its lower forage value in comparison with tagasaste (*Chamaecytisus palmensis* Hutch., it is still routinely used as a forage plant, particularly in the municipalities located in the south of Tenerife (Pérez de Paz *et al.*, 1986). The escobón is held to be more resistant to drought and more severe arid conditions, hence its value for agroforestry uses and the recovery and reforestation of soil. It can be introduced successfully in areas in which tagasaste would not thrive due to unfavourable ecological conditions. The current neglect of agricultural soil, combined with the improper use of fertilisers and the high salinity levels resulting from the use of poor-quality water, makes it necessary to develop adequate fertigation techniques. In order to take advantage of all the potential of fertigation, it is necessary to apply a nutritive solution with the most adequate concentrations of different nutrients, as well as to distribute the irrigation points in accordance with the specific needs of the crop in question. In this way, we can optimise the quality while achieving at the same time a maximum production.

In this fertigation study, different concentrations of P, K, Ca and Mg are used to establish the specific fertiliser requirements of different types of soil, in order to obtain a fodder of a high quality and high production rate.

### Material and Methods

A total of 240 two-month-old plants were selected. Those with a healthy appearance and a mean growth respect to the total group were chosen. Individual forest containers were used with a substrate composed by a mix (4:3:2) of peat, "picón" (ashes of a basaltic composition) and soil. The trial lasted five months and was carried out in a greenhouse. The mean climatic conditions were: temperature 23° C and 73% RH. Drip irrigation was used, almost up to field capacity. A total of 34 irrigation sessions were carried out.

The interaction between the substrate and the nutrient solution was studied to optimise the solutions. For this purpose, pastes saturated with the solutions were prepared and the concentrations of nutrients available for the plant in the extract after 24 hours were analysed. Four nutrient solutions were used with four replicates (Table 1). At the end of the trial the plants were cut flush with the ground for subsequent mineralisation and analysis. The substrate was dried and sifted at 2 mm. P, K, Ca and Mg levels were determined in both the plant and the substrate, using AOAC methods (1990). The results were used to calculate the regression lines and their R<sup>2</sup> correlation coefficients.

## Results and discussion

The solutions (Table 1) showed a pH level of between 6.4 and 8.5 for the treatments; the E.C. oscillated between 0.41 and 5.21 dS m<sup>-1</sup>. In the substrate-solution interaction, the substrate showed a notable retention of P and K and a release of Ca and Mg, thereby increasing the level of these nutrients in the solution. Fixation and release processes were found between the nutrient solution and the substrate that are characteristic of the volcanic materials used in the substrate (Blesa and Luque 1976; Cid Ballarín *et al.* 1996; Luque 1981).

Table 1. Composition of the nutrient solutions and the substrate-nutrient solution balance.

	T-0	T-1	Ti-1	T-2	Ti-2	T-3	Ti-3
P (meq L <sup>-1</sup> )	0	0.82	0.01	1.63	0.01	2.45	0.02
K (meq L <sup>-1</sup> )	2.69	14.7	1.96	30.89	6.41	45.07	11.18
Ca (meq L <sup>-1</sup> )	0.03	0.12	4.18	0.12	9.55	0.12	12.24
Mg (meq L <sup>-1</sup> )	0.72	2.1	3.13	2.1	5.76	2.1	7.74
pH	8.5	6.41		6.48		6.69	
E.C.(dS m <sup>-1</sup> )	0.41	1.97		3.73		5.21	

Note: T= treatment; Ti = solution-substrate interaction.

The phosphorous content in the plant (Table 2) was lower in the T-0 treatment, being 0.1% when this nutrient was not added and rising as the phosphorous levels in the solution increased, with the levels being 0.16% (T-1), 0.19 % (T-2) and 0.28% (T-3). The phosphorous content in the plant was higher than the 0.07% obtained by Pérez de Paz *et al.* (1986) in escobón. It was observed that the increases in P (Olsen) available in the substrate corresponded to increases in the phosphorous levels in leaves, with a correlation of R<sup>2</sup>= 0.675.

Table 2. List of nutrients in the substrate, the plant and their correlation at the end of the cultivation period.

T	P <sub>s</sub> (mg kg <sup>-1</sup> )	P <sub>f</sub> (g kg <sup>-1</sup> )	K <sub>s</sub> (meq 100g <sup>-1</sup> )	K <sub>f</sub> (g kg <sup>-1</sup> )	Ca <sub>s</sub> (meq 100g <sup>-1</sup> )	Ca <sub>f</sub> (g kg <sup>-1</sup> )	Mg <sub>s</sub> (meq 100g <sup>-1</sup> )	Mg <sub>f</sub> (g kg <sup>-1</sup> )
T-0	22.89	1	1.26	15	10.68	5	4.78	2
T-1	29.35	1.6	8.35	23	9.51	4	4.67	2
T-2	43.82	1.9	17.71	34	9.04	2	4.21	2
T-3	38.10	2.8	23.60	42	8.72	1	3.80	1
R <sup>2</sup>	0.675		0.999		0.963		0.973	

Note: T = Treatment; s = substrate; f= plant.

For K (Table 2), an excellent correlation was found between the concentration available in the substrate and that found in the plant (R<sup>2</sup>= 0.999). Increased levels in the substrate were reflected in increased levels in the plant, with treatments T-0 (1.5%) and T-1 (2.3%) being lower than the 2.4% found in natural tagasaste plants by China *et al.* (2002). Treatments T-2 and T-3, with a higher potassium fertilisation, showed increased levels of this nutrient in the plant, reaching values of 3.4% and 4.2%, both higher than for tagasaste (China *et al.*, 2002).

The decrease in assimilable Ca and Mg in the substrate corresponded to a drop in these nutrients in the plant (Table 2). This result showed a very good correlation (R<sup>2</sup>= 0.963 for calcium and R<sup>2</sup>=0.973 for magnesium). The calcium levels in the plant oscillated between 0.5 and 0.1%, levels lower than the 0.7% found in escobón by Pérez de Paz *et al.* (1986). Mg content in the plant oscillated between 0.1 and 0.2%. Treatments T-0, T-1 and T-2 exceeded the 0.16% found in tagasaste by China *et al.* (2002).

## Conclusions

The escobón demonstrated a good capacity for assimilating the nutrients P, K, Ca and Mg, with a direct relationship being found between the concentrations present in the substrate and the plant. These nutrients play an important role in improving the forage quality of the plant, and treatments T-1 and T-2 proved to be the most suitable for this crop and this type of substrate.

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## RAL-based measurement of turf grass colour by image analysis

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### Abstract

Colour is one of the major criteria used to evaluate the quality of turf and lawn. In Germany assessment of turf grass colour is usually estimated along with other attributes in a scale system with grades 1 to 9 (Bundessortenamt, 1999). The rating results, however, are influenced by individual estimates and are difficult to reproduce. In the present paper, camera and image analysis technology is applied to measure turf grass colour quantitatively by its reflectance in the Hue-Lightness Chroma (HLC Systems). The CCD camera used has been calibrated by means of the so-called RAL-colour system which includes 1688 colour charts in the HLC frame. To provide a range of colour and structural variation, turf grass plots have been established with 2 different turf grass species. It is demonstrated that image analysis is a suitable tool to assess turf grass colour in a reproducible and calibrated manner, over a wide span of structural and colour attributes of turf grass.

Keywords: turf grass, colour, image processing, RAL.

### Introduction

Colour is an important criterion of turf grass quality. Colour of turf grass is influenced by species and variety composition and the treatment of the established sod. Normally, turf grass colour is estimated visually (Bundessortenamt, 1999) and this is a major source of inaccuracies. In the present study, images of turf grass plots have been taken with a high resolution RGB-digital camera and these images classified according to the German RAL system. RAL is a standard of colour which enables normalized measures of hue (H), lightness/intensity (L) and chroma (C) (RAL, 1999).

### Materials and methods

Two species of turf grass (supina bluegrass *Poa supina* L. and red fescue *Festuca rubra* L., were established in plots from which images were taken with a RGB digital camera, CANON Powershot, at 5Mpixels resolution, with calibration of the camera using RAL desing colour system. RAL is a physical standard consisting of 1688 colour chips arranged in the HLC (Hue, Lightness, Chroma) system. It is based on a cylindrical coordinate system, which enables the arrangement of colour in a numerically equidistant manner and related to visual perceptions (CILELAB, 1976). For the camera calibration, a halogen light source was applied at different intensities while taking images of the RAL chips together with a white standard at 5 intensity levels of illumination. The readings were stored in a data base system. Figure 1 shows RGB reflection values of a specific RAL chip as related to the white standard, as a polynomial function. The parameters given in Fig.1 depend on the individual colour chip and the type of camera used. All 1688 RAL colours were calibrated in the same way and the respective data were stored in a data base. Turf grass images were taken in the RAW-data format, i.e. not compressed while camera-specific functions being switched off. During application, the grass sod and small spots of the white standard within were imaged simultaneously; the white standard spots serving as an illumination measure enabling the correction of illumination variance within the image frame. Numerical determination of turf grass colour consisted of three steps: (1) establishment of RGB values of the white standard, (2) calculation of RGB values of all the stored 1688 RAL colours taking into account the white standards into account, and (3) attributing RGB values of the turf grass plots to the best fitting RGB values of the RAL chips. During the validation procedure it became evident that images of all RAL chips taken together with the white standard at different illumination intensities and

spectral compositions of the light source could be identified with the same HLC-values. In other words the Hue, Lightness and Chroma values of all RAL-chips could be re-established irrespectively of the illumination intensity and spectral composition of light applied. Area portion of the RAL colours and their HLC values were determined for each turf grass plot. For example, an RAL reading of 1202010 represents Hue=120, Lightness=20, Chroma=10. (theoretical range: Hue:0...360, Lightness 0..100, Chroma 0..100).

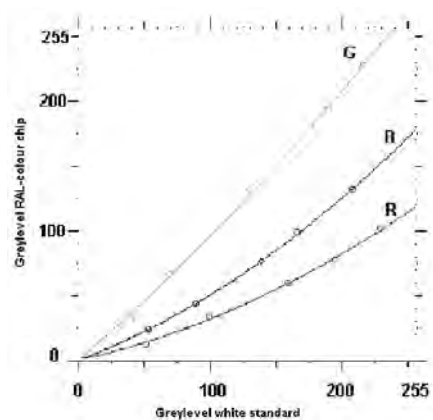


Figure 1. Relationship between RGB-values of a specific RAL-chip and the white standard.

### Results and discussion

The  $2^{24}$  colour grades of the turf grass images were transformed into 6 RAL grades. Fig.2 shows the true colour image of supina blue grass, whilst Figs. 3 to 5 show the RAL-colours found in the image. Dark areas in the image (Figure 3) are caused by shadowing within the grass sod.

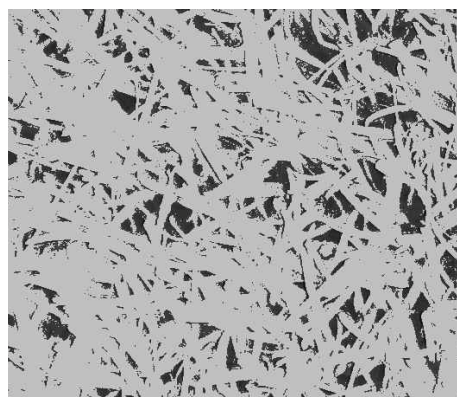
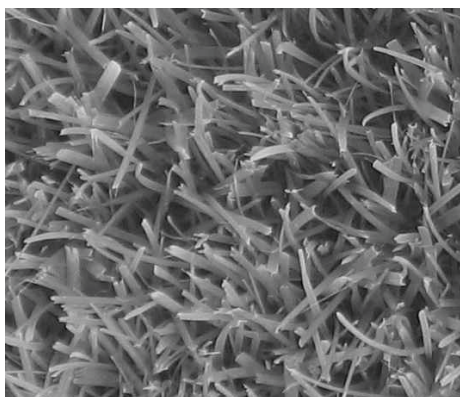


Figure.3 Supina bluegrass RAL 1202010, 17% area proportion.

Grass plots with higher proportional areas of dark shadow appear darker; this area is close to the ground where light intensity is at its minimum. The same turf grass image contains two further colour classes representing the most illuminated leaf portions in Fig. 4 and 5. The RAL figures, 1203020 and 120402, respectively contribute 22% (Figure 4) and 26% (Figure 5) of the image frame area. Colour differences between leaves are represented by their RAL-colour classes. The fifth RAL-colour class arises from the bright tips of the cut leaves, observed in both grass species tested (Fig.5 and Table 1 last column).The

RAL colour values and their proportional areas measure the observed colour in an objective way. Moreover, the effect on visual colour perception can be analysed numerically as the number of turf grass structures conferring high shadow in the sod will appear as a fixed proportion of darker areas within the image area frame.

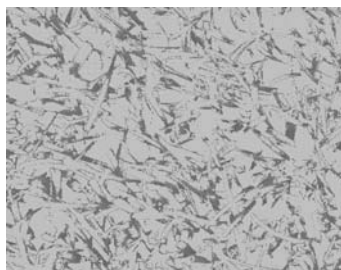


Figure 4. Supina bluegrass RAL1204020, 22% area proportion.



Figure 5. Supina bluegrass RAL 1205030, 26% area proportion.

For example, the fine structured turf grass sod of red fescue causes a greater number of shadow elements and reduces lightness values more than supine blue grass. However, turf grass colour is a very complicated phenomenon and in reality the observed colour will depend on the 3D structure generated by the size of leaf blades, tiller density etc. and the number of different colours. The numerical classification of turf grass colour by means of RAL provides a method for describing not only differences of colours caused by plant pigments but also structural influences, and provides a more objective measurement of colour compared to visual estimates under standardized imaging geometry.

Table.1 Area portion of RAL –colour components for two analysed turf grass species.

	RAL	120 20 10	120 30 20	110 40 20	120 40 20	120 50 30	120 60 40
	Portion	%	%	%	%	%	%
Plot 1	Supina bluegrass	17	32		22	26	3
	Red fescue	23	38	17		17	5
Plot 2	Supina bluegrass	15	31		24	27	3
	Red fescue	21	31	18		22	8

## Conclusion

The colour of a turf grass canopy is influenced by the actual colour of the component leaves and the individual 3-dimensional leaf structure. Digital image processing enables analysis of the multicoloured structure of turf grass. Combined with a physical standard like RAL, it provides an objective and reliable method for obtaining numerical values instead of visual estimates.

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# Analysis of the land use and grazing management in dairy sheep farms using a GIS methodology

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## Abstract

Aim of this paper was to test a GIS methodology in order to describe the relationship among land use and grazing in dairy sheep farms located in a hilly area of the North-West Tuscany (Livorno, Italy). Three dairy sheep farms were selected as a sample of three different levels of management intensification. A structured questionnaire was designed to collect information on: farm structure, dairy sheep breed, proportion of pasture land and forage crops, grazing management, feeding and reproduction techniques. Farm maps were also acquired in order to correctly set the forage crops and pasture lots. Each lot was coupled with a descriptive function that included the kind of crop or pasture and its use. Lots with the same function were together assembled and rearranged in order to obtain a thematic farm map. The adopted methodology resulted able to put in evidence the relationship between land use and management system in the observed dairy sheep farms, and may be a useful tool in order to control the land evolution due to changes in the dairy sheep management, with special focus on the level of grazing intensification, the proportion of forage crops and pasture, the level of annual stocking rate and grazing pressure on vegetation.

Keywords: sheep, land analysis, GIS, farm modelling.

## Introduction

In the Livorno Province (North-Western Tuscany), dairy sheep farms include 75% of the total ewes. Massese breed is the largest population (more than 50% of total ewes), followed by the Sarda breed (37%). Although these two breeds significantly differ in the lambing rhythm (three lambing in two years and one lambing/year for Massese and Sarda, respectively) and in the milk production level (Sarda sheep is more productive), the farming system is quite similar. Dairy flocks are managed on pasture all year round, with a little concentrate integration in milking parlour. As general rule, farmers crop for pasture production, while native forages represent a small percentage of total forage resources. Aim of this paper was to test a GIS methodology in order to describe the relationship among land use and grazing in dairy sheep farms, and to put in evidence the role of farming system in the rural landscape characterization.

## Materials and methods

Three dairy ewe farms located in the Livorno province were selected as representative of local farming system, according to the results of a previous study (Secchiari *et al.*, 2000). In order to understand how farmers organise the farm area for feeding dairy ewes, each farm was visited and a structured questionnaire was designed to collect information on: farm structure, dairy sheep breed, proportion of native pasture land and forage crops, grazing management, feeding and reproduction techniques, period of pasture use, where and when forage resources are grazed or grazed and cut for hay. Moreover, geographical information system was applied in order to map the area of each farm, according to land use and pasture management. Geographic datasets were managed, analysed and displayed by using ArcGIS 9.0 (ESRI, USA). In this way, different feeding functions were associated to each lot of the farm area and the organisation of farm was differentiated by assembling lots with the same function in a homogeneous block, in order to obtain a thematic map of each farm.

## Results and discussion

The characteristics of the three sample farms agreed with those of the other dairy ewe farms located in the Livorno province: small size, elevated stocking rate, forage crops as the main forage resource, native forages were practically absent, moderate use of feeding supplies (Secchiari *et al.*, 2000). As expected, dairy farm with Sarda ewes was larger, but also with a higher number of animals (Table 1).

Table 1. Characteristics of the three sample farms.

	Farm A	Farm B	Farm C
Farm area	65 ha	40 ha	19 ha
Number of ewes	550	130	160
Ewe breed	Sarda	Massese and Garfagnina	Massese
Forage crops	<i>Trifolium alexandrinum</i> + <i>Lolium perenne</i>	<i>Trifolium alexandrinum</i> + <i>Lolium perenne</i>	<i>Trifolium alexandrinum</i> + <i>Trifolium incarnatum</i> + <i>Lolium perenne</i>
Other crops	<i>Avena sativa</i> <i>Trifolium incarnatum</i> + <i>Lolium perenne</i>	Lucerne <i>Hedysarum coronarium</i>	Durum wheat Common wheat Maize
Percentage of forage crops	100	65	100
N° of blocks	3	2	1

These farm characteristics lead farmers to substitute native forage with forage crops in order to maximize the forage production and to avoid deficit of feed resources during definite periods. This practice allows better managing the feed supply of the flocks, but at the same time preventing from qualifying milk for cheese of “terroir” (Dubeuf, 2002). In the present study, the application of a GIS methodology coupled with data collected by the questionnaire allowed to create thematic farm maps according to the functions associated to the land use. In this way, three blocks in the farm A (Figure 1), two in the farm B and only one in the farm C were identified. A similar study performed in the Corsica region highlighted that farmers perceived the farm space according to the value that they assigned to it: the relationship between the distance of the lot to the sheepfold and the degree of the investment were explained by a decreasing gradient (Santucci *et al.*, 1997). Actually, only the organization of the farm A partially agreed with this scenario. The lots of the first block, in fact, are close to the sheepfold and belong to the farmer, which intend this area to grazing dairy ewes and young animals, for nine months per year. During the remaining three months the lots are cut for hay and dairy ewes move to the lots of the second block (Figure 1). The farmer uses this area in consequence of a lease contract. The feeding function of these lots does not differ to that of first block, but the farmer considers this area only as a complement of the first block, as a consequence of both the larger distance to the sheepfold and the different possessory title. The feeding function of the third block is only for hay provision. This area belongs to the farm by means a verbal agreement and the farmer considers this block additional to the farm.

The farms B and C are smaller than farm A and their organization is, therefore, more simplified. Farmer B chose to crop with cereal a minor part of the farm land, in order to be independent to commercial supplies. So the Farm B resulted in two blocks: one with the double function grazing/hay and the other with the function cereal crops for feeding supplementation.. On the contrary, farmer C, with the smallest farm, cropped the whole land with the same forage mixture for grazing and hay, and bought concentrate supplementation.

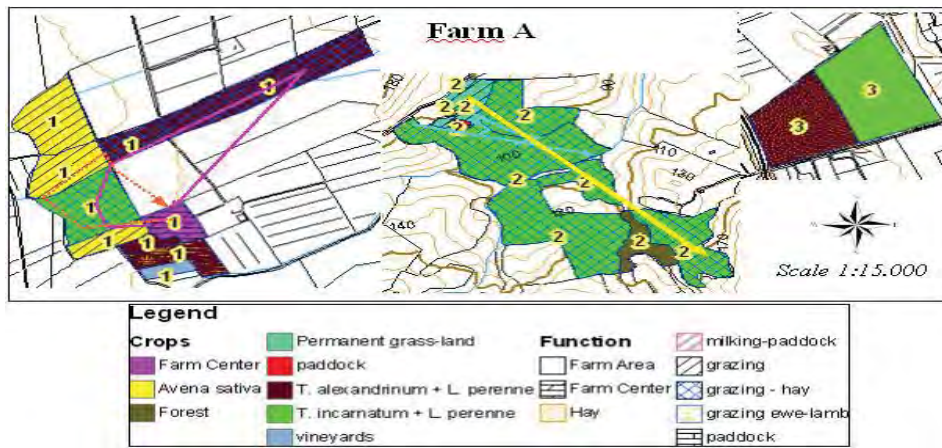


Figure 1. Map of land use in the Farm A. Different numbers inside polygon indicate different functions.

### Conclusions

The application of GIS methodologies allowed to create thematic farm maps according to the strategies that farmers applied in order to feeding dairy ewes. The subdivision of farm land in blocks according to the feeding function allowed to put in evidence the logic that farmers adopt in order to optimize the land use. The analysis of the farm maps confirmed that the small size of farms and the high stocking rate led farmers to exclusively crop with forage mixture, that completely substituted native forage resource. This fact does not make easy a milk qualification for cheese “terroir” and may interfere with the development of dairy ewe sector in the Livorno province.

### Acknowledgements

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## Productivity aspects of *Festulolium* and *Lolium x boucheanum* cultivars

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### Abstract

The objective of this research was to investigate crop yield and photosynthesis activity of '*Festulolium* and *Lolium x boucheanum*' ryegrass hybrids under agro-ecological conditions of Latvia. Field trials were established on the sod-podzolic soil and fertilized with N 120<sub>(60+60)</sub>, N180<sub>(60+60+60)</sub>, P 78 and K 90 kg ha<sup>-1</sup>. Forages were harvested three times during the growing season. The productivity of grasslands and pastures mostly depends on cultivated grass cultivars, other traits were also affected by genetic characteristics of particular cultivars.

Keywords: *Festulolium*, *Lolium x boucheanum*, productivity, photosynthesis.

### Introduction

Sustainability is a measure of our ability to produce food with the maximum efficiency combined with the minimum damage to the environment. Each region needs cultivars combining specialized combinations of stress tolerance, and productivity for local climate conditions, combining attributes of Italian and perennial ryegrass to produce an efficient hybrid of ryegrass. In Baltic climate conditions it is not widely used for the reason of unsatisfactory response to cold conditions (Gutmane and Adamovics, 2004). Greater sward productivity may be obtained through use of hybrid combinations of contrasting grass species. '*Festulolium*' hybrids are among the most persistent and productive grasses of the grasses used in many Europe countries, especially in adverse environments (Nesheim and Bronstad 2000; Kohoutek *et al.*, 2004). Important requirement for '*Festulolium*' is combining such characters of ryegrass as productivity, growth potential and feeding quality, and of fescues-stress tolerance such as cold and drought during the growth period (Casler *et al.*, 2002).

### Materials and methods

Field trials were conducted in Latvia on sod-podzolic soils pH was 7.1 and phosphorus and potassium level were 253 and 198 mg kg<sup>-1</sup> respectively, organic matter content was 31 g kg<sup>-1</sup>. Swards were composed of: perennial ryegrass 'Spidola' (control); festulolium 'Perun' (*L. multiflorum x F. pratensis*), 'Punia' (*L. multiflorum x F. pratensis*), 'Saikava' (*L. perenne x F. pratensis*), 'Lofa' (*L. multiflorum x F. arundinacea*), 'Hykor' (*L. multiflorum x F. arundinacea*); hybrid ryegrass – 'Tapirus' (*L. multiflorum x L. perenne*). The total seeding rate was 1000 germinating seeds per m<sup>2</sup>. The plots were fertilized as follows: P 78 and K 90 kg ha<sup>-1</sup> and two N fertilizer treatments-N 120<sub>(40+40+40)</sub> and N 180<sub>(60+60+60)</sub>. Swards were cut three times per season. Dynamics of plant leaf area expansion, net photosynthesis productivity (which characterizes the increase of plant DM production per leaf area unit of time, expressed in g m<sup>-2</sup> day<sup>-1</sup>), were determined for first cut. Sampling of plants was carried out in 7-day intervals after spring regrowth.

### Results and discussion

Unfavorable weather conditions occurring in 2002 – 2003 didn't cause winterkilling of the studied cultivars except for early heading hybrid ryegrass cv. 'Ligunda'. The average DM yield distribution during the years showed that maximum yield was obtained in the 1st year of sward use in both N treatments the average DM yields were 22% higher compared to 2nd year of used. There were no significant differences in annual sward yield use between the cultivars. For the 1<sup>st</sup> and 2<sup>nd</sup> year of sward use, the highest DM yields were provided by DLF-trifolium '*Festulolium*' cultivar 'Hykor' at the

N 180 treatment 18.9 and 17.4 t ha<sup>-1</sup>, respectively. The average DM yields of ‘*Festulolium*’ cultivars were by 4.78 t ha<sup>-1</sup> or 45%, but those of hybrid ryegrass by 2.67 t ha<sup>-1</sup> or 25% higher compared to perennial ryegrass (Table 1).

Table 1. Average winter hardiness and DM yield for two years of sward use (t ha<sup>-1</sup>).

Nitrogen rate (kg ha <sup>-1</sup> )	Varieties	Spidola	Lofa	Felina	Hykor	Perun	Tapirus	Punia
N120	DM, t ha <sup>-1</sup>	9.86	13.17	14.30	16.84	14.33	12.24	14.31
	Sx	0.19	0.31	0.26	0.14	0.11	0.23	0.08
	Winterhardiness	7.8	7.3	7.8	7.8	7.5	7.5	8.0
	Sx	0.3	0.5	0.3	0.3	0.3	0.6	0.4
N180	DM, t ha <sup>-1</sup>	11.48	14.59	15.87	18.13	16.50	14.44	16.47
	Sx	0.28	0.12	0.28	0.35	0.20	0.17	0.12
	Winterhardiness	8.0	7.5	8.0	7.8	7.5	7.5	8.0
	Sx	0.4	0.5	0.0	0.3	0.3	0.3	0.0

Determination of ‘*Festulolium*’ and hybrid ryegrass leaf area dynamics showed that the development of the maximum leaf area index (LAI) was achieved before ear emergence stage. The LAI and net photosynthesis productivity for ‘*Festulolium*’, hybrid ryegrass and perennial ryegrass were different. The weather conditions affect leaf development and their photosynthetic capacity. Late and cool spring in 2004 and 2005 leads to slowed formation of leaf area. There was a negative correlation between the leaf area and net photosynthesis productivity ( $r = -0.31$ ) for ‘*Festulolium*’ and hybrid ryegrass swards. As leaves age, their photosynthetic capacity declines (Figure 1).

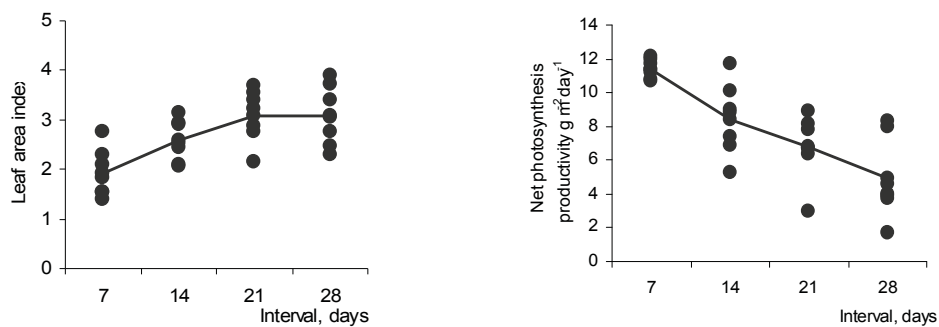


Figure 1. Indices of photosynthesis activity for *Festulolium* and hybrid ryegrass swards (on average for 2004-2005).

The maximum values of LAI were achieved by ‘*Festulolium*’ cv. Hykor’ and ‘Punia’ respectively 3.89 and 3.71. No significant differences were found for LAI when compared on a cultivar basis.

The highest average net photosynthesis productivity was showed by ‘*Festulolium*’ cv. ‘Saikava’ 9.08 g m<sup>-2</sup> day<sup>-1</sup>. All ‘*Festulolium*’ and hybrid ryegrass cultivars exceeded perennial ryegrass cv. ‘Spidola’ – 6.58 g m<sup>-2</sup> day<sup>-1</sup>. The average net photosynthesis productivity of ‘*Festulolium*’ cultivars was 20% higher compared to perennial ryegrass. A significant DM yield depending on leaf area index was observed in the year of 2004. It is characterized by equation of linear regression, with P-value = 0.0121 < 0.05 (Figure 2).



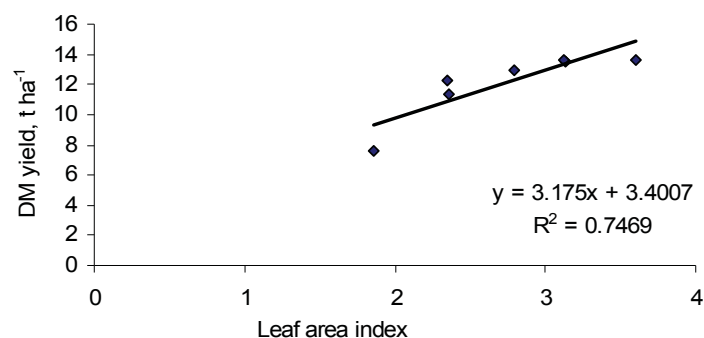


Figure 2. Equation of linear regression between net LAI and plant DM yield, t ha<sup>-1</sup>, (2004).

### Conclusions

The productivity of photosynthesis and biomass was dependent on the cultivar to be used. Cultivars of '*Festulolium*' and '*Lolium x boucheanum*' are promising species to be used as fodder grasses in the climatic zone of Latvia. Early heading hybrid ryegrass cultivars are less appropriate for Baltic climate conditions than the latest ones.

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# Ecologo-genetical estimation of perennial ryegrass (*Lolium perenne*) European cultivars and local populations in Bulgaria

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## Abstract

Perennial ryegrass (*Lolium perenne* L.) cultivars were studied in the Institute of Forage Crops-Pleven during the period 2002-2004 in four different environments. Cultivars were distinguished by maturity group, ploidy level (diploid vs. tetraploid) and origin. Different harvesting time was applied in the different environments. The influence of directionally created limiting environmental conditions – water deficiency / optimum irrigation and different densities on the perennial ryegrass productivity was studied as described by Dragavtsev (2005). The genotype and cultivars behaviour were estimated similar to Eberhart and Russell (1966). A rank analysis was performed by productivity and coefficient of linear regression. Some cultivars were most productive over all environments and years and are useful for direct use in pasture and for breeding program to improve the species in Bulgaria. The different environments had differentiating ability for identification of genotype by phenotype without changing the generations.

Keywords: perennial ryegrass, linear regression, ecologo-genetical estimation, genotype.

## Introduction

The yield value and stability are determined by different genetic bases. This is a prerequisite for successful breeding aimed at their combining (Katova *et al.*, 2003; Katova, 2005). When environmental limits are changed it results in regular redetermination of gene spectra and number of loci, determining a mean value and genetic variability of a quantitative character (Christov *et al.*, 2002, Dragavtsev, 2005). Any character of plant productivity in terms of ecological model is not only a product of action of genes or chromosomes, but rather a result of interaction of environmental limiting factors and the systems of gene complex.

The aim of the study is to select high productivity and more stable genotypes in diverse environmental condition.

## Materials and methods

Twenty cultivars and populations of perennial ryegrass were studied in the Institute of Forage Crops-Pleven during the period 2002-2004 in four different environments (with and without irrigation, and two levels of plant density: 120,500 plants ha<sup>-1</sup> and 62,500 plants ha<sup>-1</sup>). The soil type is leached chernozem and area is typical of continental climate. The warmest months for the Pleven region are June, July and August. Temperature maximums over 40°C have been recorded. The coldest months are December, January and February. The temperatures often reach to - 20°C and lower - 28°C. Genetically varieties and populations were distinguished by maturity group - early, ploidy level (diploid vs. tetraploid) and origin. Different number of cuts were applied in the second (2003) and third (2004) years of production in the 8 different environments. Totally 80 individual plants per cultivar were measured. The influence of directionally created limiting environmental conditions - water deficiency / optimum irrigation and different densities on the perennial ryegrass productivity was studied described by Dragavtsev (2005). The genotype and variety behaviour was estimated similar to Eberhart and Russell (1966). A rank analysis was performed by productivity and coefficient of linear regression.

## Results and discussion

The study of the quantitative trait 'biomass productivity' for perennial ryegrass as a several cuts and perennial species is accomplished under strong genotype-environment interaction. The selection of elite genotypes is performed on the background of this interaction. The yield value and stability are determined by different genetic bases. The ANOVA analysis showed significant interaction between genotypes and environments (Table 1) for early maturity group. We obtain the information about polymorphism and individual varietal effects of genes of adaptivity (on the basis of the polymorphism of genes conferring resistance to drought and cold), tolerance to density and length of the periods in ontogenesis.

Table 1. Two-way ANOVA analysis for the perennial ryegrass cultivars and genotype-environment interactions for early maturity cultivars.

Source of Variation	SS	df	MS	F	P-value	F-criteria
Genotypes (early type)	43,368	19	2,282.5	5.1551	4E-12	1.5937
Environments	531,142	7	75,877	171.37	2E-184	2.0159
Interaction	244,044	133	1,834.9	4.1442	7E-42	1.2223
Within	637,586	1,440	442.77			
Total	1E+06	1,599				

Table 2. Estimation of ecological stability of the perennial ryegrass cultivars for dry matter productivity (2003-2004).

\* T- tetraploid.

Varieties and populations	Origin	Total dry matter productivity g plant <sup>-1</sup>	Average dry matter productivity (xi) g plant <sup>-1</sup>	Coefficient of linear regression bi	Ecological stability Si <sup>2</sup>	Significance F-criteria
Svichtov	Bulgaria	400.58	50.07	1.88	49.67	1.12
Syn IV	Bulgaria	355.62	44.45	1.00	1,183.90	26.74
Marta – T*	Romania	354.05	44.26	0.78	2,915.06	65.84
Abereclair – T*	United Kingdom	336.20	42.02	1.10	196.48	4.44
Devnya	Bulgaria	331.24	41.40	1.40	41.62	0.94
Belene	Bulgaria	329.42	41.18	1.49	38.99	0.88
Varna	Bulgaria	328.45	41.06	1.53	122.50	2.77
Sredeec	Bulgaria	326.73	40.84	1.15	51.39	1.16
Vabel	Bulgaria	322.83	40.35	1.05	73.34	1.66
Balgarene	Bulgaria	321.00	40.13	1.03	99.26	2.24
Syn III – T*	Bulgaria	298.88	37.36	0.92	125.76	2.84
Tetramax – T*	Denmark	297.81	37.23	0.60	656.04	14.82
Anaconda – T*	Netherlands	295.23	36.90	1.02	345.43	7.80
Rebecca	Belgium	288.61	36.08	0.94	2,211.43	49.95
Targovichte - St	Bulgaria	286.10	35.76	0.77	2,058.47	46.49
Meretti – T*	Belgium	264.37	33.05	0.81	519.35	11.73
Abergold	United Kingdom	261.10	32.64	0.83	187.68	4.24
Moy	United Kingdom	255.83	31.98	0.62	1,576.87	35.61
Targovichte -St	Bulgaria	248.94	31.12	0.48	286.94	6.48
Labrador – T*	Denmark	222.42	27.80	0.60	1,478.62	33.40

Significant differences in forage DM productivity and in the stability parameters over environments were found (Table 2). Unstable genotypes were those with  $S_i^2$  (sum of square deviation) significantly deviating from regression line and  $b_i$  (stability coefficient) higher than 1. The genotype which has  $b_i = 1$  and  $S_i^2 = 0$  was assumed to be 'ideal'. If values were lower than 1, the genotype may be stable in unfavourable conditions. Breeding is directed to selection of genotypes which combine high productivity with lower (or equal)  $b_i$  value than 1, between 0.7 and 1 (Christov *et al.*, 2002). This research displayed a great variation for the two parameters.

### Conclusions

The application of directionally created limited environmental conditions gives ability for identification of genotype by phenotype without changing the generations.

The yield value and stability are determined by different genetic bases and breeding aimed at their combining could be successful. The varieties with the highest ecological stability can spread in a wider ecological area.

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# Influence of partial Total Mixed Rations amount on the grass voluntary intake by dairy cows

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## Abstract

The aim of this work was to determine if the voluntary fresh grass intake of dairy cows during grazing is optimised according to the amount and type of Total Mixed Ration (TMR) ingested as supplementation. Twelve Holstein-Friesian cows, between the third and fifth month of lactation, were housed in metabolic houses. They were fed a TMR that included either Italian ryegrass (*Lolium multiflorum* Lam.) or fava-bean (*Vicia faba* L.) silages that were offered at two levels of feeding (15 and 25 g DM kg<sup>-1</sup> LW) in a change-over design with four treatments. The TMR was offered to the animals once a day (8:00 h) and freshly cut grass was offered at 16:00 h, with the cows allowed *ad libitum* intake. Refusals were removed and weighed daily. Cows were milked twice daily at 7:00 and 15:00 h. The voluntary grass intakes and the milk yield and composition were recorded. The results showed that the higher level of offered TMR decreased the voluntary intake of grass, increased total voluntary intake, with the production and quality of milk unaffected.

Keywords: voluntary intake, italian ryegrass, fava-bean, silage, total mixed ration, dairy cows.

## Introduction

Different production systems are available to optimise milk production. Many dairy producers avoid grassland management systems because milk production per cow is often lower than with a confinement or housing system based on feeding mixed rations (White *et al.*, 2002). When pasture is the only forage and no supplement is fed, this leads to low milk production per cow and reduced milk fat and protein concentrations in the milk. The digestive and metabolic utilization of concentrates, like supplements of forages, increases with the number of daily meals (Church, 1975), so the usual supplementation to dairy cows of two meals daily after milking is not efficient. It is possible to achieve a more consistent intake if the supplementation is offered with other forages in the form of TMR that is offered to cows during an indoor feeding period and between the twice-daily milking times. This system is called partial TMR because the pasture grazed by the cows is not physically part of the TMR. Bargo *et al.*, (2002) under North American conditions found that this practice is less effective than the feeding with TMR during the whole day, but in Northwest Spain the feeding system is interest because many dairy farmers have both grasslands for grazing and also equipment for TMR feeding systems. The aim of this work was to determine the grass voluntary intake in dairy cows according both the amount of Total Mixed Ration (TMR) ingested and the type of silage included in the TMR, as well as measuring the effect on milk production and quality.

## Materials and methods

A plot of 4.2 ha was divided in half, with one half sown with 40 kg seed ha<sup>-1</sup> of Italian ryegrass (*Lolium multiflorum* Lam.) and the other half with 150 kg seed ha<sup>-1</sup> of fava bean (*Vicia faba* L.). Prior to ensiling the crops were chopped using a conventional forage harvester at the phenological stage of ear emergence (Italian ryegrass) and pods with grain (Fava beans). After harvest, and 24 hours of wilting of the ryegrass due its low DM content, three clamps of silage of both species were made. Twelve Holstein-Friesian cows in the third to fifth months of lactation were housed in a tie-stall barn. At the start of the trial the daily milk production of the cows was 27.7 kg of milk per day, 26.5 g fat kg<sup>-1</sup> and 32.4 g protein kg<sup>-1</sup> from partial TMR and rotational grazing. The cows were fed with two types of

isoenergetic and isoproteic TMR, containing either Italian ryegrass or fava-beans silages. The TMR was offered at two levels of feeding (15 and 25 g DM kg<sup>-1</sup> LW) in a change-over design with four treatments in two periods (trials 1 and 2). Each experimental period lasted 22 days, including 15 days of adaptation followed by 7 days of experimental measurements to determine the voluntary feed intake and production and quality of milk. Daily TMR was offered at 8:00 h and sufficient freshly-cut grass (*Lolium perenne* L. and *Trifolium repens* L.) was offered at 16:00 h to allow each cow *ad libitum* intake. Refusals were removed and weighed daily. Cows were milked at 7:00 and 15:00 h daily. Dry matter (DM) of samples was determined by drying in an air-forced oven at 60 (TMR) or 102°C (forage) for 24 h. Their chemical composition was determined by near reflectance spectroscopy (NIRS). Milk was analyzed by MilkoScan FT6000. The data was analysed using SAS (1999).

## Results and discussion

As expected, the composition of the TMR did not differ between both types; only the ash content in the TMR containing fava bean silage was lower. As can be seen in Table 1, there are more important differences between the trials in grass quality. In the second period grass had a lower proportion of protein and a higher fibre content, due to the slower rate of grass growth leading to increased maturity and lower quality of the grass.

Table 1. Nutritive value of TMR with different silages and grass in each experimental period.

	Trial 1			Trial 2			s.e.		Significance		
	FB	RG	grass	FB	RG	grass	to TMR	to grass	TMR	Trial to TMR	Trial to grass
DM (g kg <sup>-1</sup> DM)	495	503	152	491	501	160	3.4	2.5	NS	NS	NS
Ash (g kg <sup>-1</sup> DM)	76	80	115	73	77	96	0.7	1.8	**	*	***
CP (g kg <sup>-1</sup> DM)	143	147	210	143	145	169	1.2	2.2	NS	NS	***
NDF (g kg <sup>-1</sup> DM)	n.d.	n.d.	487	n.d.	n.d.	523	-	4.9	-	-	***
CF (g kg <sup>-1</sup> DM)	182	181	n.d.	192	193	n.d.	2.7	-	NS	*	-
ME (MJ kg <sup>-1</sup> DM)	10.8	10.7	10.5	10.8	10.7	10.1	0.34	0.28	NS	NS	***

FB: fava bean silage; RG: ryegrass silage. DM: dry matter; CP: crude protein; NDF: neutral detergent fiber; CF: crude fiber; ME: estimated metabolizable energy. n.d.: no determined.

\*, \*\* and \*\*\* Significant at 0.05, 0.01 and 0.001 % levels respectively. NS p>0.05.

The results of voluntary grass intake, production and quality of milk according to the level of feeding and type of silage included in the TMR, are shown in Table 2. The level of TMR offered significantly affected the voluntary intake of grass DM (6.8 vs 3.8 kg DM per day for the low and high level respectively, p<0.001) and total DM intake (17.1 vs 18.2 kg DM per day respectively, p<0.01). The interaction between the level of feeding and type of silage could be explained only at the highest level. In this case, the total intake had increased (p<0.01), however these effects only happened when silage was made with ryegrass (1.85 kg d<sup>-1</sup> higher DM intake). The studied parameters did not affect the milk production and its quality, except for higher lactose (p<0.05) and lower urea (p=0.07) proportions when the cows were fed with TMR that included fava bean silage. This fact could be attributed to the higher starch concentration in this feed (277 vs 236 g starch kg<sup>-1</sup>DM to FB and RG respectively, p<0.001). The higher rumen degradation kinetic of the leguminous cellular wall can stimulate the voluntary intake (Illius and Gordon, 1991). However, this increase was not shown in the current study. This could be due to palatability problems or changes in the structure of the cellular walls as a result of the silage process (Jaurena and Pichard, 2001).

Table 2. Voluntary intake of grass and milk production and quality according to TMR level.

	15		25		S.e.	Silage	Significance	
	FB	RG	FB	RG			Level	Silage*Level
Grass intake (kg DM d <sup>-1</sup> )	6.8	6.8	3.8	3.8	0.04	NS	***	NS
Total intake (kg DM d <sup>-1</sup> )	17.7	16.6	17.9	18.5	0.10	NS	**	*
▲LW (kg d <sup>-1</sup> )	-0.33	-0.71	-0.85	-0.45	0.337	NS	NS	NS
Milk production								
kg d <sup>-1</sup>	24.5	24.9	24.5	25.5	0.25	NS	NS	NS
Fat (g L <sup>-1</sup> )	27.2	27.5	25.7	28.2	0.42	NS	NS	NS
Protein (g L <sup>-1</sup> )	31.1	30.8	31.0	31.0	0.10	NS	NS	NS
Lactose (g L <sup>-1</sup> )	49.3	49.1	50.5	49.4	0.10	*	*	NS
No fat solids (g L <sup>-1</sup> )	87.3	86.7	88.0	87.2	0.14	NS	NS	NS
Urea (mg L <sup>-1</sup> )	275	306	259	289	5.3	NS	NS	NS

▲LW: live weight variation. \*, \*\* and \*\*\* Significant at 0.05, 0.01 and 0.001 % levels respectively. NS p>0.05.

### Conclusions

The results of this work show that milk yield and fat, protein and urea contents of the milk were not affected by the level of TMR fed. In addition, the substitution of Italian ryegrass silage by fava bean in the TMR for feeding dairy cows, offered at two DM levels of 15 and 25 g DM per kg of live weight, reduced the protein required in other ingredients in the diet without affecting voluntary intake.

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# The impact of natural factors on mountain grassland under fallow

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## Abstract

The paper presents an analysis of grassland fallowing which considers natural factors such as: slope, exposure and soil-agricultural complexes in a commune representative of the Sudeten. The analysis was based on field inventory of fallow grasslands and on digital database of natural factors. The greatest percent of fallow grasslands was found between 450 and 500 m a.s.l. on slopes of 12-15° inclination and northern exposure and in a poor (3z) soil complex. Significant positive relationship was found in the two highest zones (550-600 m a.s.l. and 600-650 m a.s.l.) inclined more than 20° and exposed to the north.

Keywords: Sudetes, mountainous grassland, grassland fallowing, natural factors.

## Introduction

The Sudeten (4800 km<sup>2</sup> within the Polish borders) are medium mountains in Europe, the highest peak is elevated 1602 m a.s.l. Agriculture in the region is oriented to breeding ruminants based on fodder from grasslands. The grasslands area comprises c. 50% of croplands and it is at altitudes up to 700 m a.s.l. The problem which has intensified since the beginning of the nineties is the process of grassland fallowing. The main reason for this trend was unprofitability of animal production and difficult conditions which largely depend on natural factors. In 54 Sudeten communes 39 thousand ha of meadows and pastures (31% of the total) are not used (Agricultural census, 2002). Fallowing is most widespread in areas of natural conditions which are favourable for agricultural production. Therefore, economically and naturally valuable grasslands undergo degradation. The impact of basic natural factors like elevation a.s.l., slope, exposure and soils on fallowing grasslands has been analysed in the paper. The analysis was carried out in Kłodzko, the commune representative of the Sudeten, and based on field studies and digital database on natural factors. According to the agricultural census of 2002, 59% of grasslands were excluded from management.

## Materials and methods

The Kłodzko commune is situated in the Middle of Sudeten and is the second largest commune in the region. It occupies the central part of the Kłodzko Valley surrounded by the mountains. The commune is of definite agricultural character. Cropland areas amount to 20 189 ha or 73% of the total area. The grassland area is 5 849 ha which constitutes 21% of all croplands (Fatyga and Górecki, 2001). The altitude of 650 m a.s.l. is the upper boundary for grasslands in the commune and 97% of them are situated below 500 m a.s.l. and on slopes inclined no more than 15°. Northern exposure of grasslands dominates (in 28%) in the study area. Grasslands of average quality prevail in the commune (76%) as it is common in the whole Sudeten. Field inventory of fallow grasslands was carried out in May 2004 in the Kłodzko commune. Areas of fallow grasslands were charted on maps on a scale of 1:1000 and 1:5000 and then edited in the polygon form in the digital database of natural factors based on topographic maps on a scale of 1:10 000. This approach allowed for estimating the areas of fallow grasslands and their percentage share in the altitude ranges (every 50 m), slope classes (0-3°, 3-6°, 6-9°, 9-12°, 12-15°, 15-20°, >20°), exposures (N, S, E, W and flat areas) and soil-agricultural complexes of grasslands. Three types of such complexes were distinguished in Poland: 1z - very good (>4 t DM of yield ha<sup>-1</sup>), 2z - average (2-4 t DM ha<sup>-1</sup>) i 3z - poor (<2 t DM ha<sup>-1</sup>). Classification was based on soil



quality classes and general soil characteristics. A relationship between percentage share of fallow grasslands and analysed factors was tested by linear correlation.

## Results

The field inventory demonstrated that 27% of meadows and pastures were not used in the commune. This result is by more than half lower than that recorded in the agricultural census of 2002 (Table 1).

Table 1 Percentage share of fallow grasslands in the altitude ranges and slopes.

Altitude (m) a.s.l.	Slope (°)							Total	Significance
	0-3	3-6	6-9	9-12	12-15	15-20	>20		
< 300	20	12	35	15	22	0	23	19	NS
300-350	14	16	24	24	34	9	15	16	NS
350-400	25	24	30	41	47	35	6	27	NS
400-450	40	45	42	42	39	31	19	42	-0,929*
450-500	50	48	40	51	45	18	32	45	-0,779*
500-550	12	31	25	24	44	43	18	30	NS
550-600	29	0	24	17	13	37	50	22	0,884*
600-650	0	0	0	22	5	15	100	12	0,770*
Total	21	26	33	39	40	27	21	27	
Significance	NS	NS	NS	NS	NS	NS	0,736*		

\*significant at  $\alpha=0,05$ ,  $r=0,632$  (altitude),  $r=0,666$  (slope), NS no significant.

As seen in table 1 fallowing increased up to the elevation of 500 m a.s.l. and slope of 15° achieving the highest values of 45 and 40%, respectively, in the range 450-500 m a.s.l. and at a slope of 12-15°. Further increases in slope were accompanied by a greater percentage of fallow grasslands at higher elevations. On slopes inclined more than 20° and in the zone of 600-650 m a.s.l. grasslands were not used at all. Significant positive correlation between the intensity of fallowing and elevation was found only for the inclination class >20° and for other inclination classes in the two highest altitude groups. Negative significant correlation was found in the zones of 400-450 and 450-500 m a.s.l.

Table 2. Percentage share of fallow grasslands in the altitude zones according to exposure.

Altitude (m) a.s.l.	Exposure				Flat area
	N	S	E	W	
< 300	29	17	10	13	35
300-350	17	17	15	17	14
350-400	32	21	25	31	19
400-450	47	37	52	38	23
450-500	57	39	47	36	51
500-550	46	36	18	25	15
550-600	46	2	11	23	
600-650	100	0	0	0	
Total	32	25	25	27	22
significance	0,812*	NS	NS	NS	NS

\*significant at  $\alpha=0,05$ ,  $r=0,632$  (N,S,E,W),  $r=0,707$  (flat area), NS no significant.

The greatest percentage of unused meadows and pastures was found on slopes with the northern exposure (32%) and the smallest - on southern and eastern slopes (25%) (Table 2).

Percentage share of fallow in particular exposures increased with elevation but a significant correlation was found only for the northern slopes. Data from Table 3 demonstrate that most unused grasslands (42%) were found in poor soil complexes, with 26% in very good soil complexes and 23% in medium complexes.

Table 3. Percentage share of fallow grasslands in the altitude zones according to soil complexes of grasslands.

Altitude (m) a.s.l.	Grassland		
	1z - very good	2z - average	3z - poor
<300	34	15	15
300-350	0	16	26
350-400	0	25	38
400-450		35	55
450-500		41	48
500-550		34	28
550-600		56	15
600-650		0	18
Total	26	23	42
Significance	NS	NS	NS

NS no significant,  $\alpha=0,05$ ,  $r=0,878$  (very good)  $r=0,632$  (average and poor).

The study did not show significant relationships between the proportion unused and altitude in particular soil complexes.

### Conclusion

Inventory of fallow grasslands in the Kłodzko commune did not confirm the data from agricultural census of 2002. Decreased proportions of fallow land were possibly influenced by direct subsidies or other forms of financial support (for cultivated meadows and pastures) following Polish accession to the EU in 2004.

Analysis of the data demonstrated that:

The highest percentage of unused grasslands occurred in the zone between 450 and 500 m a.s.l. at the inclination of 12-15° on slopes of northern exposure and in the poor soil complex 3z.

The intensity of fallowing increased with elevation but the only significant relationship was found in slopes above 20° inclination and on slopes of northern exposure.

Percentage share of unused meadows and pastures increased with the inclination of slopes at higher altitudes of 550-600 m and 600-650 m a.s.l. In zones situated between 400-450 and 450-500 m a.s.l. the relationship was negative.

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# Photosynthesis, growth and productivity of tall fescue and their intergeneric hybrids

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## Abstract

Net assimilation rates of tall fescue (*Festuca arundinacea* Schreb.) and their intergeneric hybrids were investigated in two field experiments. Significant differences of net assimilation rate (NAR) were identified between individual re-growth periods between cuts. In the first experiment, NAR decreased to 45.5% and 36.7% in the second and third cuts compared with the first cut. A similar trend was confirmed in the second experiment, when NAR decreased to 65.1% and 51.8% in the second and third cut, respectively. As NAR correlated very well with DM production ( $r = 0.87^{++}$ ), it is suggested that decreasing NAR during the summer and autumn seasons (2<sup>nd</sup> and 3<sup>rd</sup> cut) could be responsible for summer depression syndrome in grassland. Results from the 2<sup>nd</sup> experiment confirmed that highly productive cultivars of tall fescue (13.33 t ha<sup>-1</sup> of dry matter (DM), cv. Kora, Felina, Hykor) attained higher values of NAR (1.85 g m<sup>-2</sup> d<sup>-1</sup>) in comparison to groups of medium and low productivity cultivars. DM production was 11.26 t ha<sup>-1</sup> and 6.75 t ha<sup>-1</sup>, and NAR reached 1.81 and 1.36 g m<sup>-2</sup> d<sup>-1</sup>, respectively. It was also confirmed that low productivity cultivars (cv. Lofa, Bečva) allocated substantially more assimilates to the root system compared with highly and medium productive cultivars.

Keywords: grasses, tall fescue, IGH, NAR, summer depression.

## Introduction

In the former Czechoslovakia (1991) an assortment of forage grasses was enriched with a new cultivar of tall fescue (*Festuca arundinacea*) cv. Lekora. Later, intergeneric hybrids of annual ryegrass (*Lolium multiflorum* Lam.) x tall fescue were introduced. C<sub>3</sub> forage grasses exhibit summer depression in productivity, caused by various genetic and environmental factors (Gáborčík, 2005) including leaf and sward photosynthesis. Generally, photosynthesis is measured gasometrically (Alberda, 1977, Woledge and Leafy, 1976), but this method does not take into account retention of assimilates (dry matter) in the above-ground part of sward. The main aim of this study was to determine net assimilation rate (NAR) of forage grasses, including tall fescue and their intergeneric hybrids using a growth analysis approach.

## Materials and methods

The field trial was established in upland region of east Slovakia in 1997 (Matejovce, elevation 700 m). The following grasses were studied: perennial ryegrass (*Lolium multiflorum* L. – cv. Mustang), tall fescue (*Festuca arundinacea* Schreb. - cv. Lekora), Italian ryegrass (*Lolium multiflorum* Lam. – cv. Kroto and Jiskra) and intergeneric hybrids (IGH) of tall fescue and Italian ryegrass (cv. Jiskra and Becva) and hybrid of meadow fescue (*Festuca pratensis* Huds.) and Italian ryegrass (cv. Perun). The fertilisation rate was 3 x 30 kg N ha<sup>-1</sup> (applied after each cut), 30 kg P ha<sup>-1</sup> and 60 kg K ha<sup>-1</sup> applied in spring. The mean daily temperature and rainfall amount over growing season are given in Table 1. The second trial was established (1999) at Banská Bystrica (altitude 460 m). Mean daily temperature and amount of rainfall in 2002 is given in Tab.1. Two tall fescue cultivars (cv. Lekora and Kora) and six hybrids were studied (cv. Felina, Lofa, Becva, Fortuna, Korina and Hykor). N fertilizer was applied in 2002 at a rate of 120 kg ha<sup>-1</sup> (3 x 40 kg ha<sup>-1</sup>). P and K were applied in spring at 30 and 60 kg ha<sup>-1</sup>,

respectively. The second and the third nitrogen rates were applied after the 1<sup>st</sup> and the 3<sup>rd</sup> cut. The plots (10 m<sup>2</sup>, 4 replications) were cut three times during the growing period: the first cut at heading date for the grasses, and the 2<sup>nd</sup> and 3<sup>rd</sup> cuts, respectively, 6 and 8 weeks after the first cut. Net assimilation rate (NAR) was determined by a method of growth analysis. Shoot samples (250 x 250 mm) were taken at the start ( $t_0, W_0$  and  $A_0$ ) and at the end ( $t_1, W_1$  and  $A_1$ ) of individual re-growth periods (three cuts) and NAR was calculated according to Gaborcik *et al.* (2004). The results were subjected to ANOVA.

## Results and discussion

Net assimilation rates (NAR) of the studied grass species and intergeneric hybrids (IGH) varied considerably (Table 2) between 1.44 (cv. Lofa) and 2.33 g m<sup>-2</sup> d<sup>-1</sup> (cv. Lekora and cv. Perun). The mean NAR for grasses during the growing period was 2.00 ± 0.36 g m<sup>-2</sup> d<sup>-1</sup>. The NAR values of individual grass species and IGHs decreased in the following order: tall fescue > annual ryegrass > IGH > perennial ryegrass (2.33 > 2.15 > 1.86 > 1.72 g m<sup>-2</sup> d<sup>-1</sup>). In spite of the great difference between maximum and minimum values of NAR (35.5 %) the differences are not statistically significant (P > 0.05). On the other hand, there was a downward trend in NAR over the growing season. NAR was highest during the recovery period after the first cut (3.25 ± 1.05 g m<sup>-2</sup> d<sup>-1</sup>) and was lower during the periods following the second (55%) and third cuts (68%). The difference in NAR between 1st and 2nd cuts was highly significant, but it was for the difference between 2<sup>nd</sup> and 3<sup>rd</sup> cuts.

The NAR values of tall fescue cultivars and IGHs (Tab.3) varied between 1.22 (cv. Lofa) and 1.97 g m<sup>-2</sup> d<sup>-1</sup> (cv. Kora). The mean value of NAR for tall fescue cultivars was 1.71 g m<sup>-2</sup> d<sup>-1</sup> and was reduced by 11.2% for IGHs. Values of NAR differed between individual recovery periods following cutting. These differences were significant (P < 0.05). Compared with the NAR after the first cut (2.10 g m<sup>-2</sup> d<sup>-1</sup>) the values after the 2nd and 3<sup>rd</sup> cuts were, respectively, only 85% and 56%. The results from this experiment confirmed the observations made in the first one (Table 1). A close relationship between NAR value and DM production in the 2nd experiment was confirmed (r = 0.87<sup>++</sup>).

It was also confirmed that dry matter production in the various grasses declined between the first and third cuts, corresponding with the changes of NAR over the growing period. Mean DM production for the first, second and third cuts was, respectively, 4.806, 2.722 and 3.008 t ha<sup>-1</sup>. The cultivars were classified into three groups based on dry matter production. The first group (cv. Kora, Felina and Hykor) was characterised by the highest DM production (12.329 t ha<sup>-1</sup>). The DM production of the second group (cv. Fortuna, Lekora and Korina) was only a little smaller – 11.264 t ha<sup>-1</sup>, but for the third group (cv. Lofa and Becva) it was much lower – 6.758 t ha<sup>-1</sup>. The relative differences of DM production between groups was 9.0 % and 45.0 % corresponding with the average NAR for individual groups of cultivars. The highest NAR was typical for the first and second group (1.85 and 1.81 g m<sup>-2</sup> d<sup>-1</sup>) and the lowest for third group (1.36 g m<sup>-2</sup> d<sup>-1</sup>). The relative differences in values for the second and third groups compared with the first group were, respectively, 2.2 % and 26.5 %. The less productive cultivars (cv. Lofa and Becva) were characterised not only by their low NAR values but also by the accumulation of higher amounts of assimilates (dry matter) in the root system (Gaborcik and Zibritova, in press).

Table 1. Mean daily temperature ( $T_d$ , °C) and sum of rainfall (R, mm) in individual growing period at both experimental sites.

Trait	Matejovce / cut			Mean/Σ	Banska Bystrica / cut			Mean/Σ
	1 <sup>st</sup>	2nd	3rd		1st	2nd	3rd	
$T_d$	11.6	17.9	11.7	12.7	12.3	17.9	16.1	15.4
R	166	98	144	408	92.5	187.2	241.3	562.2

The results of the two field experiments using a method of growth analyses showed a rapid decline in the net assimilation rate (NAR) of the species and IGHs studied, in agreement with the previous findings of Alberda (1977) and Woledge and Leaf (1976) using a gasometric method of photosynthesis determination. In the current experiments we confirmed that not only photosynthesis but also assimilate

retention in shoot (NAR) is decreased over summer period of growth. This feature may be responsible, together with a decline of leaf area index (LAI), for summer depression of DM production of grasses (Gaborcik *et al.* 2004).

Table 2. Net assimilation rate (NAR, g m<sup>-2</sup> d<sup>-1</sup>) of studied grasses.

Species	Cultivar	C u t			Mean ± s.e.
		1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	
<i>L.perenne</i>	Mustang	2.81	0.95	1.40	1.72 ± 0.97
<i>L.multiflorum</i>	Kroto	3.38	1.27	1.38	2.01 ± 1.19
<i>L.multiflorum</i>	Jiskra	3.67	1.95	0.91	2.27 ± 1.39
<i>F.arundinacea</i>	Lekora	3.55	2.04	1.39	2.33 ± 1.11
IGH	Bečva	2.81	1.25	1.28	1.78 ± 0.89
IGH	Lofa	1.76	1.56	1.16	1.44 ± 0.31
Mean ± s.d.		3.25 ± 1.05	1.48 ± 0.39	1.20 ± 0.22	2.32 ± 1.17

Table 3. Net assimilation rate (NAR) of tall fescue and intergeneric hybrids assortment.

Cultivar	NAR (g m <sup>-2</sup> d <sup>-1</sup> )			Xmean	DM yield (t ha <sup>-1</sup> )	SPAD
	1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>			
Lekora	2.85	1.75	1.15	1.92	11.484	40.1
Kora	2.69	1.65	1.56	1.97	12.408	40.8
Felina (IGH)	2.18	2.02	1.28	1.83	11.823	35.4
Lofa (IGH)	1.52	1.29	0.86	1.22	6.421	35.0
Bečva (IGH)	1.56	1.83	1.10	1.50	7.094	39.9
Fortuna (IGH)	2.38	1.93	1.65	1.99	11.474	36.9
Korina (IGH)	1.76	1.63	1.18	1.52	10.833	45.4
Hykor (IGH)	1.84	2.15	1.24	1.74	12.757	42.3
Mean	2.10	1.78	1.25	1.71	10.537	34.5

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# Characterisation of Cantabrian (Northwest Spain) tall fescue wild populations

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## Abstract

Nine tall fescue (*Festuca arundinacea* Schreb.) accessions collected on the Cantabrian mountains were characterised at the Centro de Investigaciones Agrarias de Mabegondo (43° 14' 50'' N, 8° 15' 45'' W) near the coast (100 masl) on a silt loam soil over a three-year period (2003-2005) in a randomised complete block design with three replicates of 10 plants per accession. Two commercial cultivars (Fawn and Tima) were also included in this study. The entries included accessions and cultivars were evaluated for nine agromorphological characteristics each year and at the end of the third year for endophyte presence. Tall fescue accessions showed higher agromorphological values than Fawn in 88% of the cases and than Tima in 55% of the cases. Eight (89%) of the nine accessions contained endophyte with a range of 8 to 30%.

Keywords: endophytes, *Festuca arundinacea*, genetic resources.

## Introduction

Tall fescue (*Festuca arundinacea* Schreb,  $2n = 6x = 42$ ) is a temperate outcrossing, native perennial grass that is usually found in the North of Spain (Lopez and Oliveira, 2000). While it is productive and persistent in this area, its use has often been limited by weak seedling vigour and lower palatability than perennial ryegrass (Piñeiro and Perez, 1981). In North Spain, tall fescue populations are commonly infected by endophytes. Oliveira and Castro (1997) found that seventeen accessions (90%) of nineteen accessions contained endophyte fungus with a range of 7 to 100%. This endophyte/plant symbiosis has been shown to enhance insect, disease, and drought resistance in tall fescue (Funk and White, 1997).

Since 2003, nine wild populations collected on the Cantabrian mountains are being evaluated together with other grass species (Costal *et al.*, 2004). The objective of this research was to characterise the variability among nine tall fescue accessions of the Cantabrian Mountains, on the basis of agromorphological characteristics and endophyte presence. Knowledge of this variability should provide useful information concerning the potential value of these accessions to Spanish breeding programs especially to improve seasonal growths, seed production and stem rust tolerance.

## Materials and Methods

Originally, seed of nine tall fescue accessions was collected from natural grasslands in the Cantabrian Mountains (northern Spain). The agromorphological study was established at the Centro de Investigaciones Agrarias de Mabegondo (43° 14' 50'' N, 8° 15' 45'' W) near the coast (100 masl) on a silt loam soil over a three-year period (2003-2005) in a randomised complete block design with three replicates of 10 plants per accession. Plants were transplanted to the field in February 2003, 50 cm apart. Two commercial cultivars (Fawn and Tima) were also included in this study. The site received the same amount of fertilizer throughout the three years of study, a total of 3.2 g N m<sup>-2</sup>, 1.7 g P m<sup>-2</sup> and 3.3 g K m<sup>-2</sup> per year.

The characters studied are showed in Table 1. For the ordinal-scale characters (visual estimates from 1 through 5), the non-parametric Kruskal-Wallis test was used to test the existence of statistically significant differences between entries. For the quantitative heading date, analysis of variance was

performed first per year and to observe error homogeneity furthermore to combine the statistical analysis for the three years.

The level of endophyte in each accession was checked by collecting at least 150 seeds per accession at the end of the third year in the nursery and using the method described by Latch *et al.*, 1987. Statistical analyses were computed using SPSS version 11.5 (SPSS, 2002).

## Results and Discussion

For the ordinal-scale, non-parametric Kruskal-Wallis test showed significant differences ( $P < 0.05$ ) for all the characteristics. For the only quantitative trait studied (heading date), there were significant differences ( $P < 0.05$ ) among accessions. Tall fescue accessions from the Cantabrian region showed higher seasonal growths, inflorescence abundance and autumn tolerance to stem rust than Fawn in 88% of the cases and than Tima in 55% of the cases. These two cultivars are standard cultivars well known for its productivity in Spain. It is noteworthy the good seasonal growths and autumn stem rust tolerances of the accession 1294 collected at Cardeo (Mieres). Of the nine accessions evaluated, none had high levels of endophyte infection (greater than 75% infected seeds), eight (89%) had seeds infected with endophyte, one was moderately infected (25% to 75%), seven had low levels of infection (less than 25%), and another entry had no infected seeds. The proportion of Cantabrian tall fescue accessions examined that was endophyte infected (89%) was similar as that reported by Oliveira and Castro (1997) in tall fescue populations from North Spain (90%) but the percent infected seeds was lower (8-30%) than in that study (7-100%).

Table 1. Means, standard deviations (SD) of the traits studied on nine tall fescue populations collected in the Cantabrian mountains and the controls 'Fawn' and 'Tima'.

NUMBA	CRO	CRI	CRP	HAB	AH	FES	ABIN	CRESP	ENF	ENDO
1223	2.6	2.2	2.9	3.0	3.9	45.0d	2.9	3.0	3.2	15
1224	2.9	2.7	3.1	3.2	2.8	40.7e	3.0	2.9	3.2	30
1249	3.1	2.9	3.6	3.2	3.4	44.8d	3.3	3.6	3.5	13
1250	3.2	2.9	3.5	2.9	2.8	49.3bc	3.1	3.6	3.5	14
1251	3.4	3.1	3.8	2.9	2.9	48.2bc	3.4	3.9	3.6	0
1294	3.7	3.3	4.1	3.1	3.1	47.3cd	3.8	3.7	3.6	8
1295	3.0	2.7	3.5	3.0	3.5	44.6d	2.8	3.3	3.4	12
1296	2.9	2.8	3.1	2.7	3.1	50.5b	3.0	3.2	3.4	13
1297	2.0	1.6	2.2	3.1	1.9	57.9a	2.8	2.2	3.6	18
Mean	2.9	2.7	3.3	3.0	3.1	47.6	3.1	3.3	3.5	
SD	0.4	0.5	0.5	0.1	0.5	4.6	0.3	0.5	0.2	
Fawn	2.2	2.1	3.1	2.9	3.4	43.0b	2.8	2.6	3.0	
Tima	2.8	2.5	3.4	3.0	2.9	47.4a	3.3	3.4	3.6	
Mean	2.5	2.2	3.2	2.9	3.1	45.2	3.0	3.0	3.2	
SD	0.3	0.2	0.1	0.1	0.2	2.2	0.2	0.4	0.2	

NUMBA1 is the accession code. The traits used are: CRO, CRI, CRP, growths in autumn, winter and spring (1 = very low to 5 = very high), HAB, growth habit (1 = prostrate to 5 = erect), AH, leaf width (1 very narrow to 5 = very wide), FES, heading date as the number of day after March first, ABIN, inflorescence abundance (1 = very low to 5 = very high), CRESP, growth at heading date (1 = very low to 5 = very high), ENF, autumn susceptibility to stem rust (1 = very high to 5 = very low), ENDO, infection percentage. Means of the quantitative character heading date were compared using the Duncan test. Means followed by different letters were significantly different at 5%.

## Conclusions

Accession 1294 from Cardeo (Mieres) showed the best seasonal growths and autumn stem rust tolerances. The high proportion of Cantabrian tall fescue accessions endophyte infected suggest that in this area grass breeders may be more likely to select endophyte infected plants, but for obtaining the benefits of endophytes, it is probably necessary to have higher percent infected seeds in the accessions

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## Forage production and quality of grass species for extended grazing season

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### Abstract

In the last decade suckler cow breeding has developed progressively in the Czech Republic (CR). Effective breeding also requires decreasing running costs. A possibility is to extend the grazing season in autumn (In the CR cattle graze from late April until the middle of October, depending on altitude). In mild winters grazing could extend into the winter months. Therefore in 1997 and 1999 small plot trials with selected grass species (*Dactylis glomerata* L., *Lolium perenne* L., *Festuca arundinacea* Schreb., *Festuca pratensis* Huds., genus hybrids and *Bromus marginatus* Nees.) were established at Jevíčko in the Czech Republic (335 m above sea level, average annual temperature 7.5 °C, average annual precipitation 629 mm) which were intended for harvest during the extended grazing season (from October to April of the following year). Regrowth after the second harvest in three time series (2001-2003) in the middle of July and August was assessed. Grasses were fertilized with 120 kg ha<sup>-1</sup> N (60 kg ha<sup>-1</sup> N in spring, 60 kg ha<sup>-1</sup> N after second harvest + P<sub>35</sub>K<sub>100</sub> in spring) in the form of ammonium nitrate with lime. Dry matter and concentrations of NEV, NEL, CP and CF were evaluated by infrared spectrometry.

Keywords: extended grazing season, grasses, forage quality, NIR spectrometry.

### Introduction

In the Czech Republic there has been a progressive development of suckler cow production in the last decade. In 1990 there were almost no suckler cows but by 2004 there were about 140,000. Effective management requires among other factors low running costs. This is possible by year-round grazing. Preparation for grazing vegetation grazing in winter requires the last summer cut to be taken June, July, or possibly in August (Opitz von Boberfeld, 1997).

### Material and methods

In 1997 and 1999 small plot trials with eight selected grasses species and varieties - *Dactylis glomerata* (cv. Niva), *Lolium perenne* (cv. Sport and Mustang), *Festuca arundinacea* (cv. Kora), *Festuca pratensis* (cv. Rožnovská), genus hybrids (cv. Hykor and Bečva) and *Bromus marginatus* (cv. Tacit) were established at the site Jevíčko in the Czech Republic (335 m above sea level, average annual temperature 7.5 °C, average annual precipitation 629 mm) for herbage during the extended grazing season (from October until April the following year). Yield of the third cut, taken during the extended season, regrown after the harvest in the middle of July or August in three time series was assessed. Grasses were fertilized

(120 kg ha<sup>-1</sup> N - 60 kg ha<sup>-1</sup> N in spring, 60 kg ha<sup>-1</sup> N after second cut) in the form of ammonium nitrate with lime + P, K applied in spring (35 kg ha<sup>-1</sup> P in the form of superphosphate and 100 kg ha<sup>-1</sup> K in the form of potash salt). Dry matter production was measured and forage quality parameters of NEV (net energy of fattening), NEL (net energy of lactation), crude protein (CP) and crude fibre (CF), were predicted by NIRSystems 6500. Differences between means were statistically evaluated by analysis of variance.

## Results and discussion

Mean dry matter production (Fig. 1) of eight varieties for three harvest years was influenced by the date of the summer regrowth from mid-July was more productive, with an average of six samplings of 1.54 t ha<sup>-1</sup> DM, compared to mid-August growth with a significantly ( $P < 0.01$ ) lower average production 0.93 t ha<sup>-1</sup> DM.

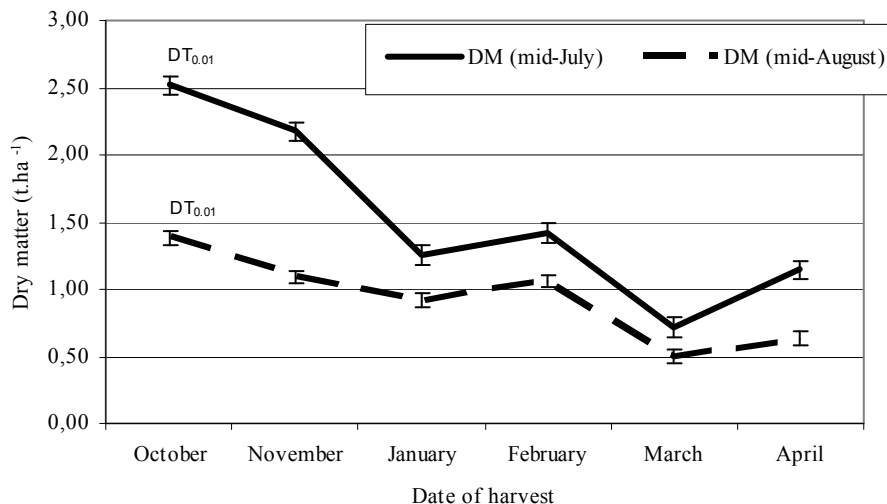


Figure 1. Dry matter production (t ha<sup>-1</sup>) during extended grazing season (average of sowing years, regrowth and varieties).

The highest dry matter production was reached in October and November sampling with yields from 2.52 to 2.17 t ha<sup>-1</sup> DM grown from July growth, and 1.38 to 1.09 t ha<sup>-1</sup> DM grown from August (Fig. 1). During the winter dry matter production fell as a result of decay of part of the organic matter, especially easily digestible fractions. *Dactylis glomerata* (cv. Niva), produced the highest dry matter, with an average of 2.80 t ha<sup>-1</sup> DM from six out-of-season samples of growth from mid July, followed by hybrid (cv. Hykor) at 1.94 t ha<sup>-1</sup> DM and *Festuca arundinacea* (cv. Kora) at 1.86 t ha<sup>-1</sup> DM, resp. from the growth from mid August cv. Niva 1.17 t ha<sup>-1</sup> DM, cv. Hykor 1.04 t ha<sup>-1</sup> DM and cv. Kora 0.89 t ha<sup>-1</sup> DM. These varieties had significantly higher DM production compared to other varieties ( $P \leq 0.01$ ). Concentration of NEV and NEL (Fig. 2) unlike dry matter production was higher from August than July regrowths, because the vegetation was younger. NEV and NEL concentrations had maximum values from samplings until the beginning of winter i.e. 5.8 – 6.3 MJ kg<sup>-1</sup> DM, while during winter season until the beginning of March they fell sharply to 3 – 4 MJ kg<sup>-1</sup> DM, starting to rise with initiation of growth in the beginning of spring. CP concentration followed a similar

pattern, i.e. 133 g kg<sup>-1</sup> DM falling to 75 g kg<sup>-1</sup> DM and rising again with the beginning of growth in spring.

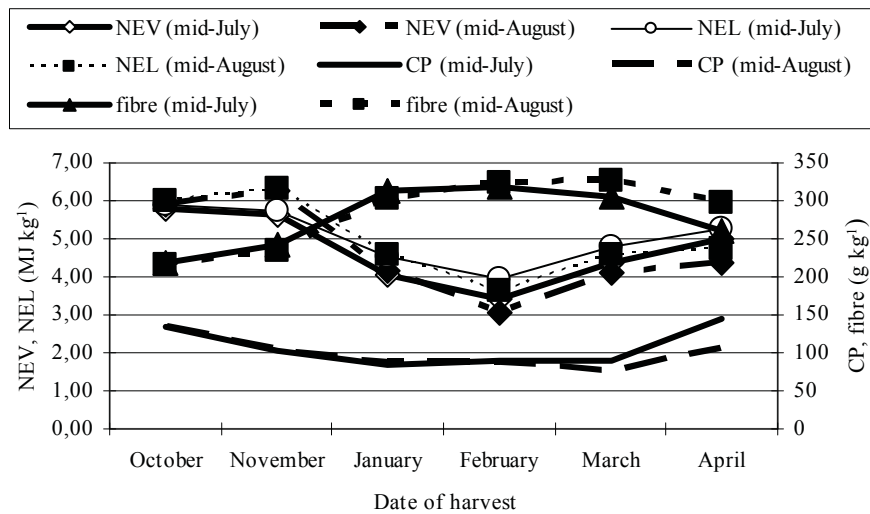


Figure 2. Quality of grass forage in extended grazing season from two regrowth periods (average of sowing and harvest years and varieties).

Fibre concentration was at its lowest in October (216.1 – 217.3 g kg<sup>-1</sup> DM, rising to over 300 g kg<sup>-1</sup> DM in winter. As growth commenced in spring its concentration began to decrease. These results agree with the findings of Skládanka (2005), Opitz von Boberfeld and Wöhler (2002) and others.

### Conclusion

In the Czech Republic it is practicable to extend the autumn grazing season for suckler cows by 2 – 3 weeks, and even longer on lower land without snow cover. The forage quality is still high in this period; however it decreases during winter due to decay of organic matter. *Dactylis glomerata*, *Festuca arundinacea* and genus hybrid are suitable grass species. To achieve sufficient vegetation it is necessary to allow regrowth from July or August at the latest.

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## Diet and trophic relationships of domestic herbivores in Central Spain

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### Abstract

This paper analyzes the diet and trophic relationships of domestic herbivores in summer, on the north side Sierra de Gredos (Central Spain). Cattle (*Bos Taurus*), horse (*Equus caballus*) and domestic goat (*Capra hircus*) diets were evaluated on the basis of fecal analysis. Cattle and horse consumed *Nardus stricta* L. (67.2 % and 50 % respectively) and *Carex* species. *Festuca elegans* Boiss. and *Erica arborea* L. were the most heavily consumed species by domestic goat. Overlap between the diets of these herbivores was low, with the exception of cattle and horse. Cattle and horses proved to be strict grazers. The diet composition of domestic goat was more diversified. Domestic goat increased consumption of shrubs and followed both grazing and browsing behavior patterns, particularly the former.

Keywords: cattle, horse, domestic goat, feeding habits.

### Introduction

In natural areas in the Iberian Peninsula cohabit domestic herbivores of social and economic importance, due to their role in traditional extensive grazing systems. One such area is the middle and high-altitude belt of Sierra de Gredos (central northwest Spain), which is occupied from mid-June to September by domestic herbivores: cattle, the most abundant domestic livestock, a small numbers of horses and domestic goats. The plant-herbivore relation is based on a series of variables and parameters (food availability and quality, selection diet, trophic relationships and possible competence among herbivores, etc), which would be convenient to know and analyze in order to accomplish an adequate and sustainable management of the different ecosystems. The competitive exclusion theory (Hardin, 1960) proposes that two species cannot coexist using the same resource if it is limited. Under such conditions, diets are divergent as a result of the effects of potential competition for resources and/or different feeding habits and the strategies adopted by ungulates for survival and maintenance. The present study compare the diet of these herbivores, define their overlap and analyze the feeding habits of each species in a deforested mountain area with abundant grasslands and patches of scrub.

### Material and methods

The study area is located on the north side of the Sierra de Gredos, 40°20'N 40°15'S and 5°10'E 5°20'W at an altitude of 1500-2400 metres a.s.l. Cattle, horse and goat diets were studied using microhistological analysis of fecal samples. Fecal samples from each animal group were collected in July. Cattle diet was estimated from 100 samples (1 sample per individual) collected in different grassland zones. There were few horses in the area (40-50 individuals), and 25 samples were used in the study. Domestic goat diet was estimated from samples collected from 50 individuals in two flocks (25 per flock). The Cavender and Hansen (1970) technique was used with slight modifications in chemical and physical treatments (Martínez, 2002). Two pellets were taken from each goat fecal sample and two extracts were taken from each collected cow and horse dung pat. The degree of overlap between diets was estimated using the Kulczynski's Similarity Index. Spearman's rank correlation ( $r_s$ ) was used also for a comparison of diets. Diet diversity was calculated using the Shannon-Weaver index ( $H = - \sum p_i \log_{10} p_i$ ).

## Results and discussion

In cattle, horse and domestic goat diet, 25, 20 and 30 species of plants respectively were identified. Table 1 shows the most consumed species by the different animals. One common feature of all 3 herbivores was the heavy consumption of grasses.

Table 1. Species of plants (5) most consumed\* (biomass percentage) by cow, horse and domestic goat in the north side Sierra de Gredos (Central Spain).

	<i>Nardus stricta</i>	<i>Carex nigra</i>	<i>Festuca iberica</i>	<i>Festuca elegans</i>	<i>Carex. Binervis</i>	<i>Agrostis castellana</i>	<i>Erica arborea</i>	<i>Quercus pyrenaica</i>	<i>Cytisus scoparius</i>
Cow	67.2*	8.5*	5.4*	4.3*	4.0*	1.0	-	-	-
Horse	50*	17.5*	6.0*	2.5	6.0*	5.3*	-	-	-
Domestic goat	1.3	-	-	40.3*	0.7	4.6*	9.4*	5.1*	4.6*

Cattle were strictly grazers and consumed grasses almost exclusively. This feeding pattern has also been detected elsewhere (McInnis and Vavra, 1987). As with cattle, horses had purely herbaceous feeding habits and consumed large amounts of grasses, also found elsewhere to be an important component of horse diet (McInnis and Vavra, 1987). The fact that grasses together with certain sedges and rushes are almost 100 % of cattle and horse diets is related to the abundance of grasslands in the study area and the fact that these groups comprise the majority of the herbaceous biomass in the environment (Martínez, 2001). The relatively abundant sedges in the area, were specially valued by horses, with major consumption near watercourses due to their greater abundance and quality (continuous regrowth). Although domestic goat consumed considerable amounts of herbaceous vegetation (especially grasses), it was the ungulate which made most use of woody vegetation (Table 2).

Table 2. Diet diversity and group of plants consumed (biomass percentage) by cow, horse and domestic goat in the north side Sierra de Gredos.

	Herbaceous plants	Woody plants	Diet diversity
Cow	100	-	0.62
Horse	100	-	0.70
Domestic goat	72.1	27.9	0.85

The importance of herbaceous resources in domestic goat diet is linked to the abundance of grasses in the zone and the clearly preferred group for goats (Morand-Fehr *et al.*, 1983; Bullock, 1985; Martínez, 2001). On the other hand, the existing scrubland is limited to a small number of species that form the heaths and broomfields. This suggests a concordance with Campbell *et al.* (1962), who found that domestic goat behavior, its preferences and its effects on plants all depend on the relative palatability of trees and shrubs, the available herbaceous vegetation and the number of goats per area unit.

Cattle diet correlated with that of horse diet ( $r_s = 0.76$ ,  $n=25$ ,  $P<0.001$ ). In contrast, domestic goat diet did not correlate with any of the other two. The diet diversity was highest for domestic goat, followed by horse and cattle. The highest overlap was between cattle and horse diet (74.9%). Overlaps between domestic goat and cattle or horse diets were low (25.2% and 23.4% respectively). The low overlap degree suggest that there is little evidence of competition for forage between cattle/horse and the domestic goat. According to the competitive exclusion principle (Hardin, 1960), food resources should be distributed among the different species that feed in different plant communities. Domestic goat yielded the greatest difference in diet from the other two herbivores, given that caprine species, particularly domestic goat, tend to be browsers (Malechek and Provenza, 1983, Aldezabal, 2001). In the present study area, both species occupied the same grassland communities, but the number of horses

was very small and resource availability was considered to be acceptable (Martínez, 2001), suggesting that there was, in fact, no competition for forage between them.

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# Cooperative livestock systems in Europe: Pastoral organisations to overcome seasonal constraints

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## Abstract

Cooperative livestock systems are present all over Europe. Inherited from the past centuries or recently established, these systems are mostly located in marginal areas. The relief, the latitude and the climate hampered the process of agricultural intensification. Cooperative livestock systems can be regarded as a form of adaptation to the seasonal constraints in order to overcome the problems of forage scarcity. Ideally, they enable a fair distribution of the resources between the farmers and improve the viability of small farms. The paper presents three cooperative livestock systems within Europe: reindeer husbandry in Northern Fennoscandia, sheep grazing in Central Spain and cattle grazing in the German Alps. The results depict the utilisation intensity of the grazing resource in each system. Experts and farmers interviews reveal that the utilisation of the pastures is not well-balanced. Abandonment or overgrazing seems to endanger these systems. Factors like technical innovation, support measures, price policy, conflict interests and user rights influence severely the economic and ecological sustainability of these systems.

Keywords: cooperative pasture, low intensity, grazing, Europe.

## Introduction

Regions with restrictive seasonal availability of pastoral forage are particularly pronounced in areas with marked relief (e.g. mountainous areas) or extreme climate conditions (for instance in Mediterranean areas with warm summer and low rainfall or in Boreal regions where low temperature year-long persists).

Joint utilisation of pastoral resources allows the exploitation of these hard areas whose utilisation would otherwise not be economically feasible for a single farmer and therefore it offers an alternative to overcome seasonal constraints. These organisations are based on a consensus between several land owners and land users. Scale effects induced from the collective action allow the exploitation of larger areas and the saving of production (materials, labour...) and transaction costs.

In the course of the LACOPE project, cooperative livestock systems in seven European countries are being studied. The aim of the project is to focus on their importance for the agricultural activities, the rural territories and the landscape conservation. In the following sections three examples of cooperative systems in three different regions of Europe are presented. The current trend of the utilisation of the pastoral resource is analysed and considerations about the current stakes are discussed.

## Description of the cooperative livestock systems

A detailed description of the cooperative livestock systems, presented below, is available in Gueydon *et al.* (2004).

### *Sámi reindeer herding in Northern Fennoscandia*

The area of reindeer management is located in the northernmost parts of Norway, Sweden and Finland. This area traditionally inhabited by the Sámi covers more than a third of the total area of the respective countries and supports about 560,000 reindeers. The harsh climatic conditions of this region impede most kinds of agriculture but reindeer husbandry. The Sámi people herded traditionally reindeer for centuries in these vast areas.

*Sedentary sheep grazing in Castilla – La Mancha, Central Spain*

In Castilla – La Mancha (CLM) roughly 80% of the Total Agricultural Land of the region is grazed by sheep. Most of the land is occupied by non-irrigated cereal farming so that the sheep are grazing on the stubble after harvest, as well as on fallow. Sheep farmers own on average only a few hectares. However, they have acquired since centuries user rights on the land of this region. Agricultural land of different holdings of each municipality are 'merged' together into several polygons, called *poligonos parcelarios*. Each polygon has a size that can theoretically maintain one sizeable sheep flock.

*Cattle grazing in the Upper Bavarian pre-Alps and Alps, Germany*

The agricultural land in the pre-alpine and alpine areas of Upper Bavaria is almost exclusively composed of grassland (BayStMELF, 2002). The *Allmende*, the old designation for the joint organisation, are inherited since the 5<sup>th</sup>-6<sup>th</sup> centuries (BStMELF, 1972). Most of them are located in the higher altitudes of the Alps and are usually grazed by heifers from the dairy farms of the valleys.

*Current stakes*

In the three regions interviews were carried out with representatives of the cooperative livestock systems. Among others they were asked to give an appraisal of the intensity of utilisation of the pastures ranking from overgrazing, well-balanced or undergrazing. With overgrazing it is meant that the current utilisation endangers the natural re-growth of the pastures. The second category means that the pastures are not endangered through current utilisation. The last category intends that the current utilisation support shrub and forest growth. The appraisals enable us to realise an overall appreciation for each investigated regions. Additional inquiries with the farmers using the cooperative pastures set straight the socio-economic reasons of the current resource exploitation (Table 1).

Table 1. Appreciation of the utilisation intensity of the cooperative pastures in the different regions.

	Utilization intensity	Socio-economic reasons
Northern Fennoscandia	Overgrazing/ Undergrazing	1) Increasing competition for grazing areas between farmers 2) Hampering of seasonal migration through infrastructure, tourism
CLM	Undergrazing	1) Reducing pastoral activities 2) Decreasing number of animals (Year round indoor farming)
Upper Bavaria	Undergrazing	1) Declining interest to use cooperative systems 2) Reducing labour (work off-farm)

The results reveal that the utilisation intensity of the cooperative pastures in each region is unbalanced. In Northern Fennoscandia, competition for land occurs in a twofold matter. On the one hand farmers are motivated to increase their herd size for enhancing the profits. This creates a scarcity of land. On the other side, the state is competing for the land for developing infrastructure and tourism activities. These effects create a gradient of land use. Overgrazing occurs especially on the pastures in the vicinity of the settlement where competition is high. Undergrazing comes out on land too far from the households or difficult to reach. In CLM and Upper Bavaria, a main problem is the currently economic unattractiveness of grazing systems in comparison with the intensive production systems. In CLM the farmers tend to cease grazing activities and shift towards year round indoor farming in order to reduce the workload. In Upper Bavaria the farmers tend to work off-farm in order to improve their livelihood. In these two regions the pastures tend to be underused.



## Discussion and conclusion

The previous section depicts the relative inappropriate utilisation of the cooperative pastoral systems. It seems that they could be on the long run threatened. On the one hand, for economic reasons the interest of the farmers for using these systems is declining. On the other hand competition for land impedes their sustainability. These trends are mostly influenced by external factors like market and price policy (competitiveness), technical innovation (productivity, work load, loss of grazing areas), agricultural and support measures (increase of revenues), improved standards of living (work load, indebtedness) and changes in the mind and behaviour (conflict interest, free-riding).

However, considering their historical development, these cooperative systems survived for centuries. The cooperative grazing systems never existed in a closed and stable environment of their own but were always influenced by various changes in the rural environment. Since some of the menacing factors are mainly influenced by politics the cooperative systems could also be foster with changed political measures. In France for instance a specific regulation for the establishment and support of cooperative livestock systems was settled down in the 70's. Since there, around 900 cooperative systems have been created on the French territory (Chambres d'Agriculture, 2005). Apart from this, it seems also necessary that the systems evolve and so far adapt their intern structure to external changes.

All in all, the matter of establishing cooperative pastoral systems shall remain a priority. All over Europe vast areas of agricultural land are of low value. In view of the fact that they give the farmers low potential for economic valuation, they are often set aside. Jointly exploitation would offer a good option to optimise the utilisation of these areas. Moreover this meets the commitments of the multifunctionality of the agriculture. Indeed cooperative livestock system account for an interesting option to maintain and sustain rural territory especially of marginal regions.

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# Quantification of the initiation date, heading date and critical day length of ryegrass cultivars

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## Abstract

The objective of this study was to determine the minimum day-length requirement for reproductive initiation of eight diploid perennial ryegrasses (*Lolium perenne* L.). The hypothesis tested was that the minimum day-length required to initiate reproductive growth in spring might define an equivalent minimum day length in summer (post-solstice), after which further reproductive initiation ceases. Using forty spaced plants per variety, tillers were examined for reproductive budding, to give an average critical day length for each variety. Mean date of ear emergence was also determined. There was a large variation between varieties with initiation date varying from the 31<sup>st</sup> March (s.d. 2.2 days) (AberDart) to 2<sup>nd</sup> May (s.d. 6.9 days) (Twystar) and heading from 30<sup>th</sup> May (s.d. 11.8 days) (Fennema) to 17<sup>th</sup> June (s.d. 4.2 days) (Twystar). The expected relationship between dates of ear initiation (EI) and ear emergence (EE) was observed with an  $R^2 = 0.935$  between variety means. As rate of EE is energy dependent, meteorological data were used to refine the EI to EE relationship for each variety. The potential of these observations for providing an understanding of the control of secondary seed-head development in mid-summer swards is considered.

Keywords: Tillers, initiation date, heading, critical day length.

## Introduction

In spite of good grassland management, inflorescent development in mid-summer tillers has become a recognised problem, as it results in below optimum production, reduced grass DMI and reduced milk protein which subsequently causes a decline in the quality of milk for processing. Terry and Tilley (1964) have shown how the digestibility of ryegrass is affected by ear emergence. The dry matter digestibility of perennial ryegrass fell slowly prior to ear emergence at a rate of 0.2% per day but declined more rapidly at 0.5% per day after ear emergence and up to 0.8% per day with stem fractions. The day-length dependent control mechanisms that trigger the switch of vegetative tillers to reproductive tillers in spring are known. It is unknown, however, how climatic, management and genotypic factors act or interact to initiate new secondary and even tertiary reproductive tillers after defoliation of these primary reproductive tillers. The objective of this study was to define the relationship between EI and EE for a group of perennial ryegrass varieties of differing maturities, examine the meteorological requirements for these physiological stages and to determine the possible 'sensitive period' for each variety during the growing season.

## Materials and methods

Forty spaced plants of eight test varieties were grown at a site at Crossnacreevy in Northern Ireland (latitude 54°32'N). The eight diploid perennial ryegrass cultivars used were either intermediate (I) or late (L) heading. The varieties were AberDart (I), Fennema (I), Corbet (I), AberAvon (L), Mezquita (L), Foxtrot (L), Melle (L) and Twystar (L). These plants were vernalised over the winter of 2004. From previous data collected, the expected EI date for each variety was estimated. Examination of individual tillers began on April 1<sup>st</sup> 2005. One tiller was removed from each plant on alternate days and examined under the microscope for reproductive budding as described by Sweet *et al.* (1991). All mature and developing leaves were removed, until the apex was visible. Under close examination, the presence of a double ridge on the apex indicated that the tiller had initiated or turned reproductive. The mean EI for each variety was determined. The mean date of EE for each variety was also recorded as

the date when three seed heads had visibly emerged on a spaced plant. From these results, the critical day length for initiation was then determined for each variety as the minimum length of daylight required to trigger the growth of the reproductive apex of a tiller. Day length in hours was read from a table compiled by Shing Lam (personal communication) who calculated the year-round hours of daylight at five degree latitude intervals based on a formula published by Meeus (1991). From meteorological data collected, the total accumulated temperature receipt above 0°C from EI to EE was determined and expressed in degree-days and degree hours. Photosynthetically active radiation (PAR) is the amount of useable light energy received by a plant and is dependent on light intensity and day-length. The total PAR<sub>(EI-EE)</sub> was tabulated from EI to EE for individual tillers.

## Results and discussion

Table 1 shows the estimated and actual EI and EE dates of the eight test varieties. The overall mean EI date for all varieties was up to eight days earlier than that estimated. As EI is day-length dependent, this difference should not be affected by growing conditions, beyond the energy needed to develop the minute 'double-ridge' structure in the embryo. Virtually complete cessation of growth would be necessary to move initiation dates by an average of eight days. The estimates were extrapolated from work done in the 1970's by Camlin (personal communication), who examined three varieties, of early, intermediate and late maturity. Extremely poor growing conditions were not experienced in this earlier study. It is, therefore, likely that the discrepancy resides with the inaccuracy associated with the extrapolation rather than with the current work. Mezquita was the only variety to initiate on the 'average' initiation date. Overall, the average critical day length for the eight varieties ranged from 12.7 to 15.0 hours (Table 1). The actual and estimated EE dates of the varieties were also not the same. The four earlier varieties headed 2-4 days earlier than expected, three of the four later varieties headed 0-2 days later and Twystar, the latest variety, headed two days prior to the expected date. These estimates were taken from a database constructed from a ten-year average EE date for the varieties growing at Crossnacreevy. Ear emergence is a physiological process requiring considerable energy to develop and elongate stem and seed-head structures. It is therefore highly dependent on growing conditions and clearly 2005 was not a perfect 'average' year, climatically. The observed difference indicates the level of variation in EE that growing conditions can affect and how the discrepancy can widen and contract as ambient conditions change. A direct consequence of this was that the time from EI to EE ranged from 46 to 61 days across the varieties. The accumulated air temperature recorded above 0°C from EI to EE ranged from 11,635 hours (485 days) to 12,804 hours (534 days) for all varieties (Table 1). The accumulated air temperature was higher for intermediate heading varieties but there was significant variation within each of the maturity pairs (intermediate and late heading varieties). On average there were six degree days more with intermediate than late heading varieties from EI to EE. Due to variable cloud cover, the degree of solar radiation varied from day to day, which subsequently affected the PAR<sub>(EI-EE)</sub> accumulation. The mean PAR<sub>(EI-EE)</sub> of the eight test varieties ranged from 346 to 409 MJ m<sup>-2</sup> during the period of EI to EE. PAR<sub>(EI-EE)</sub> did not decrease with later heading varieties but the mean light energy requirements for late heading varieties was 34.6 MJm<sup>-2</sup> less than intermediate heading varieties. However there were differences in the total accumulated temperature and PAR<sub>(EI-EE)</sub> between the four varieties that initiated before the April 20th, which were AberDart, Fennema, Corbet and AberAvon and the remaining four varieties that initiated after April 20<sup>th</sup>. The four varieties that initiated prior to April 20<sup>th</sup>, had on average 433 degree hours and 19 degree days more accumulated temperature than those that initiated later and also 45.1MJ m<sup>-2</sup> added PAR<sub>(EI-EE)</sub>. AberAvon has a critical day length more comparable to the intermediate heading varieties than the late heading varieties, this results in a high total accumulated temperature and PAR<sub>(EI-EE)</sub> requirement.

Table 1. Initiation (EI) dates, heading (EE) dates, critical day length, total accumulated temperature and PAR of eight test varieties.

Variety	Ear Initiation (EI)		Ear Emergence (EE)		Critical Day Length (hrs)	Total Accumulated Temperature Degree Hours	Degree Days	PAR EI-EE MJm <sup>-2</sup>
	Estimated	Actual (s.d.)	Estimated	Actual (s.d.)				
AberDart	08 Apr	31 Mar (2.2)	27 May	31 May (8.5)	12.7	12590	525	409.7
Fennema	10 Apr	02 Apr (2.1)	28 May	30 May (11.8)	12.8	11971	499	393.4
Corbet	17 Apr	13 Apr (6.0)	03 Jun	7 Jun (6.7)	13.6	12312	513	387.0
AberAvon	19 Apr	14 Apr (5.7)	05 Jun	9 Jun (5.7)	13.6	12804	534	400.5
Foxtrot	22 Apr	21 Apr (3.6)	07 Jun	8 Jun (4.0)	14.2	11635	485	355.4
Mezquita	23 Apr	23 Apr (5.0)	08 Jun	10 Jun (4.9)	14.3	11981	499	358.6
Melle	04 May	01 May (6.1)	17 Jun	17 Jun (5.3)	14.9	12295	512	346.3
Twystar	04 May	02 May (6.9)	19 Jun	17 Jun (4.2)	15.0	12034	501	349.5
Mean	-	-	-	-	13.9	12203	508	375.0
CV (%)	-	-	-	-	6.3	3.1	3.1	6.7

s.d. = standard deviation (days) CV = Coefficient of Variance.

### Conclusion

From this investigation, it can be concluded that the eight test varieties differ in their requirements for reproductive initiation. Primary reproductive initiation in spring is dependent on day length but ear emergence and possibly further replacement reproductive tillering depends on environmental factors, such as light intensity and temperature. Varieties that initiated after April 20<sup>th</sup> tended to have lower degree hour requirements. This date generally represents the beginning of the second grazing rotation of a typical Irish dairy farm. Cultivars that initiate at this stage in the growing season will have a shorter time interval for the production of reproductive material throughout the growing season than cultivars that initiated earlier in the season, and this may impact on sward quality mid-season.

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## Seed production and germination of different ecotypes of *Bromus erectus* Hudson

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### Abstract

A field trial was established in the lowlands to compare different ecotypes of *Bromus erectus* collected in several mountain grasslands in terms of their potential seed yield (PSY) and germinability. PSY was tested comparing the broadcast and drill sowing methods. Three germination treatments, control (no treatment before germination test), prechilling and prechilling+KNO<sub>3</sub> were compared. PSY was around 2300 and 1000 kg ha<sup>-1</sup> respectively in broadcast and drill sowing. Germination % in control and prechilling treatments was on average 77% and in the prechilling+KNO<sub>3</sub> was 70%. The higher PSY was not associated to the higher germination energy of the ecotypes.

Key words: seed production, germination, mean germination time, ecotypes, *Bromus erectus*.

### Introduction

According to Giannangeli *et al.* (2005) the use of local ecotypes of *Bromus erectus* for restoration of disturbed sites in their origin areas, could be a suitable alternative to less adapted commercial species or varieties due to their adaptation to the ecological conditions in these areas. Taking into account both advantages and problems connected with the propagation of species outside their natural area a field trial was established in lowlands in order to compare different ecotypes of *Bromus erectus* collected in mountain grasslands in terms of potential seed yield and germinability.

### Materials and methods

A field trial was established at the experimental farm of the Faculty of Agriculture of Università Politecnica delle Marche. Mean temperature and precipitation from November to June 2004 were 10.5°C and 589 mm (for details see Giannangeli *et al.*, 2005). Seeds of 8 *B. erectus* ecotypes were collected during summer 2003 in 8 grasslands sites (Giannangeli *et al.*, 2005). The trial was established in 2 m<sup>2</sup> plots (1 x 2 m) arranged in a two-factorial completely randomised design with 3 replicates: ecotype x sowing method. Sowing by drilling (DS: 5 rows per plot with 20 cm row width) was used for all ecotypes and broadcast sowing (BS) only for 4 ecotypes. Sowing was performed on 25/11/2003 with a seed rate of 5 g m<sup>-2</sup>. Potential seed yield (PSY) was estimated during the 2<sup>nd</sup> year of cultivation since no seed production occurred in the 1<sup>st</sup> one. Tiller density was counted within 0.2 m quadrats in 3 pre-fixed points along plot diagonals in the BS plots and in 5 pre-fixed 0.2 m rows along plot diagonals in DS. At maturity the number and weight of seeds were determined on 20 random tillers. PSY was calculated considering nr. of inflorescences m<sup>-2</sup>, nr. of seeds per tiller and thousand seed weight (TSW). Before germination tests, seeds were stored at room temperature for 30 days. Germination tests were performed only on the pure seed fraction produced in the drill sowing plots. Three germination treatments, tested in 4 replications of 50 seeds each, were applied: (i) control, no treatment before germination test; (ii) prechilling at 4-6°C in the dark for 7 days; (iii) prechilling + 0.2% KNO<sub>3</sub> solution. Seeds were submitted for 21 days to 20°C with 8 hrs of light. Weekly, germination % was monitored according to ISTA (1996). Data were submitted to ANOVA analysis and Least Significant Difference (LSD) test was used for mean comparison. Percentage data was arcsine transformed prior to be analysed.

## Results and Discussion

In all ecotypes, PSY was significantly higher in the BS (2303 kg ha<sup>-1</sup>) compared to the DS method (1002 kg ha<sup>-1</sup>) (Table 1). This result was highly related to the nr. of tillers m<sup>-2</sup> ( $r = 0.86^{***}$ ;  $n=24$ ) and to the nr. of seeds per tiller ( $r = 0.65^{***}$ ;  $n=24$ ), although in the latter trait no significant difference was found between ecotypes or sowing methods. Consequently, it appears that the higher tiller density recorded for BS led to a higher PSY. DS adversely affected tiller density (428.3 and 854.7 in DS and BS respectively) due to more intense intraspecific competition.

Table 1. Potential seed yield per unit area, number of tillers per unit area and number of seeds per tiller in relation to ecotype and sowing method (BS: broadcast; DS: drill).

Ecotype	Potential seed yield (kg ha <sup>-1</sup> )			Number of tillers m <sup>-2</sup>			Number of seeds per tiller		
	Sowing method			Sowing method			Sowing method		
	BS	DS	Mean	BS	DS	Mean	BS	DS	Mean
1	2156	943 <sup>cd</sup>	1549 <sup>ab</sup>	805.3	468.3 <sup>bc</sup>	636.8 <sup>ab</sup>	52.6	38.6	45.6
2	2910	1130 <sup>cd</sup>	2020 <sup>a</sup>	1024.0	386.7 <sup>c</sup>	705.3 <sup>ab</sup>	49.7	51.6	50.6
3	1423	729 <sup>d</sup>	1076 <sup>b</sup>	624.0	381.7 <sup>c</sup>	502.8 <sup>b</sup>	44.9	38.7	41.8
4	2721	1206 <sup>bcd</sup>	1964 <sup>a</sup>	965.3	476.7 <sup>bc</sup>	721.0 <sup>a</sup>	57.4	51.7	54.6
5	.	1969 <sup>a</sup>	.	.	708.3 <sup>a</sup>	.	.	51.0	.
6	.	1096 <sup>cd</sup>	.	.	402.0 <sup>c</sup>	.	.	52.6	.
7	.	1378 <sup>abc</sup>	.	.	543.3 <sup>abc</sup>	.	.	40.6	.
8	.	1760 <sup>ab</sup>	.	.	670.0 <sup>ab</sup>	.	.	47.6	.

In columns, means with no common letters differ significantly at  $P \leq 0.05$ .

LSD<sub>0.05</sub> to compare the sowing methods = 497.0 kg ha<sup>-1</sup>, 111.0 tillers m<sup>-2</sup>, 12.1 seeds per tiller.

With regard to DS, the different soil and environmental conditions of the collection sites probably contributed to select genotypes characterized by different productivity. For instance, ecotypes 5, 7 and 8, collected on calcareous marls and marly limestone substratum had higher PSY, while ecotype 3, gathered on marls and sandstones substratum, showed a lower PSY (729 Kg ha<sup>-1</sup>). Even though ecotypes 1, 2, 3, 5 and 6 belong to the same *Centaureo bracteatae-Brometum erecti* plant association, the higher PSY of ecotype 5 could be related to a higher abundance of some calcicolous species such as *Briza media*, *Ononis spinosa*, *Leucanthemum vulgare*, *Rhinanthus alectorolophus*, *Poa pratensis* and *Poa compressa* (Grime *et al.*, 1988). Concerning the BS treatment, no significant difference was found among the 4 ecotypes, although the lowest PSY of ecotype 3 was confirmed (Table 1).

The germination % after 7 days of some ecotypes was higher in the control treatment (2, 3, 5 and 6) and with prechilling (5 and 6). In the first seedling count ecotype 3 (30%) and 6 (21%) showed the highest germination % compared to the others in the control and in prechilling treatments respectively (data not shown). No significant interaction ecotype x treatment was found for FGP (Table 2). In all ecotypes, control and prechilling treatments recorded the highest FGP. Comparing ecotypes, 1 and 7 had the lowest FGP. FGP and MGT were not significantly correlated ( $r = -0.66^{n.s.}$ ;  $n=8$ ), although there was a tendency for lower FGP to be associated with increased MGT. Within treatments, ecotypes 3 and 6 had lower MGT than the others. Prechilling and prechilling+KNO<sub>3</sub> did not reduce MGT. Differences among treatments were not consistent across ecotypes.

Table 2. Final germination percentage (FGP) and mean germination time (MGT) in relation to ecotype and sowing method.

Eco-type	-----Final germination percentage (%)-----				-----Mean germination time (days)-----			
	Control	Prechilling	Prechilling + KNO <sub>3</sub>	Mean	Control	Prechilling	Prechilling + KNO <sub>3</sub>	Mean
1	64.5	60.5	59.5	61.5 <sup>b</sup>	17.7 <sup>d</sup>	18.4 <sup>b</sup>	18.8 <sup>c</sup>	18.3
2	84.5	79.0	77.0	80.2 <sup>a</sup>	15.2 <sup>b</sup>	18.0 <sup>b</sup>	16.8 <sup>b</sup>	16.6
3	83.0	83.0	75.5	80.5 <sup>a</sup>	13.0 <sup>a</sup>	15.1 <sup>a</sup>	14.8 <sup>a</sup>	14.3
4	84.0	82.5	68.5	78.3 <sup>a</sup>	17.9 <sup>d</sup>	17.6 <sup>b</sup>	18.5 <sup>c</sup>	18.0
5	82.0	80.5	73.5	78.7 <sup>a</sup>	17.9 <sup>d</sup>	17.1 <sup>b</sup>	16.9 <sup>b</sup>	17.3
6	87.0	88.5	82.0	85.8 <sup>a</sup>	15.9 <sup>bc</sup>	14.6 <sup>a</sup>	16.0 <sup>ab</sup>	15.5
7	59.0	50.5	45.0	51.5 <sup>c</sup>	17.8 <sup>d</sup>	18.4 <sup>b</sup>	18.6 <sup>c</sup>	18.3
8	79.5	82.0	78.5	80.0 <sup>a</sup>	16.8 <sup>cd</sup>	17.4 <sup>b</sup>	16.9 <sup>b</sup>	17.0
Mean	77.9 <sup>a</sup>	75.8 <sup>a</sup>	69.9 <sup>b</sup>	74.6	16.5	17.1	17.2	16.9

In rows (only for FGP) and columns, means with no common letters differ significantly at  $P \leq 0.05$ ; FGP:  $LSD_{0.05}$  among treatments and ecotypes = 5.1% and 8.3%; MGT:  $LSD_{0.05}$  among treatments within ecotypes = 1.5 days.

## Conclusions

Taking into account the different seed rates used in similar studies, the mean PSY (about 1600 kg ha<sup>-1</sup>) found in this experiment was higher than those observed in other *B. erectus* cultivations (200-500 kg ha<sup>-1</sup> with 1.2-1.5 g m<sup>-2</sup> seed rate) (Krautzer *et al.*, 2004). These results also highlight higher yields compared to similar productive species such as *Festuca nigrescens* (up to about 1000 kg ha<sup>-1</sup> with 0.5 g m<sup>-2</sup>) (Peratoner and Spatz, 2004) and *Festuca arundinacea* (more than 1000 kg ha<sup>-1</sup> with 4 g m<sup>-2</sup>) (Santilocchi *et al.*, 1989). The high seed rate (5 g m<sup>-2</sup>) used in this experiment may be justified by the high resulting PSY and easier weed control. Moreover, TSW (on average 5.47 g) was among the highest reported values for *B. erectus* (Krautzer *et al.*, 2004) probably contributing to the high PSY. Based on the results, PSY obtained with the broadcast method was more than double that obtained with drill sowing, suggesting that the former could be adopted for large-scale cultivation.

Overall, germination % in the control and prechilling treatments was on average 77% and in the prechilling+KNO<sub>3</sub> treatment was 70%, indicating that good germination could be achieved even without the application of the treatments tested in this experiment. The study showed that higher PSY was not associated with higher FGP. In fact, although ecotypes 5 and 3 showed a high FGP (around 80%), ecotype 5 produced 1240 kg ha<sup>-1</sup> more than ecotype 3. Despite being less productive, ecotype 3 germinated more rapidly than most of the ecotypes, therefore it could be used for restoration purposes when rapid seed germination and establishment are needed.

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# The effect of supplementary crude protein concentration on the performance of grazing dairy cows

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## Abstract:

Sixty autumn-calving cows were randomized on days in milk (140, s.d = 24.0), lactation number (3.1, s.d = 2.1) and pre-experimental milk yield (28.5 kg day<sup>-1</sup>, s.d = 5.4) into 5 treatments. Two treatments consisted of grass only – 20 kg dry matter (DM) d<sup>-1</sup> >4cm (HG) and 15 kg DM d<sup>-1</sup> (LG). The remaining three treatments consisted of a basal herbage allowance of 15 kg DM d<sup>-1</sup> and were supplemented with either 4 kg DM d<sup>-1</sup> maize (*Zea Mays* L.) silage (M), a high protein ration (180 g kg<sup>-1</sup> - HC), or citrus pulp (70 g kg<sup>-1</sup> of CP - LC). Milk yield was measured daily while milk composition, body weight and body condition score were measured weekly. The milk yield of the LG treatment (20.7 kg day<sup>-1</sup>) was lower (P<0.01) than that of the supplement treatments but not the HG treatment. Milk protein content was greater (P<0.05) for HG (34.8 g kg<sup>-1</sup>) than for LG and HC. Milk fat content was lower (P<0.05) for HC (35.3 g kg<sup>-1</sup>) than for HG, M and LC. The response to offering 4 kg DM d<sup>-1</sup> of maize, concentrate and citrus pulp was 0.75, 1.00 and 0.78 kg milk kg DM<sup>-1</sup> respectively.

Keywords: Dairy cows, supplementation, protein, maize, concentrate.

## Introduction

Grazed grass is the cheapest feed available to lactating dairy cows. It has relatively high intake characteristics and a high energy content which makes it an obvious feed choice. Grass growth in Ireland is seasonal and therefore grass shortages are evident at the beginning and end of the grazing season. To combat this, farmers are forced to offer their herd a suitable supplement to increase the total dry matter intake (DMI). Supplementation allows cows to achieve a high level of nutrition while maintaining a high level of pasture utilization (Stockdale, 1995). Concentrates are the obvious choice of supplementation but can be expensive especially when feeding high crude protein (CP) concentrates. The trend in recent years has been to feed a concentrate of high CP content in an effort to stimulate or maintain a high level of milk production. When diets based on fresh pasture are formulated for grazing dairy cows, rumen degradable protein is supplied in excess. It is likely that supplements with lower protein levels would suffice for grazing cows in many situations. The objective of this experiment was to investigate the effects of feeding different levels of CP concentrations in forage and concentrate to dairy cows in early spring on milk production and total DM intake.

## Materials and Methods

Sixty autumn calving dairy cows were balanced on the basis of days in milk (140, s.d = 24.0), lactation number (3.1, s.d = 2.1) and pre-experimental milk yield (28.5 kg DM d<sup>-1</sup>, s.d = 5.4) into five groups and assigned randomly from within groups to the following treatments; (i) 20 kg DM d<sup>-1</sup> herbage allowance >4cm (HG), (ii) 15 kg DM d<sup>-1</sup> herbage allowance (LG), (iii) LG + 4 kg DM d<sup>-1</sup> maize silage (M), (iv) LG + 4 kg DM d<sup>-1</sup> of 18% CP concentrate (HC), (v) LG + 4 kg DM d<sup>-1</sup> of 7% CP concentrate (LC). The study began on 28<sup>th</sup> February and ended 1st May 2005 (9 weeks). The animals grazed as three separate herds. Both the HG and LG groups grazed separately while the three supplemented treatments grazed together as a herd of 36 cows. Maize silage was offered in forage bins mounted on electronic load cells (Griffith Elder); this weighed feed removals and thereby determined DM intake. The concentrate offered to the HC treatment was offered in the parlour while the concentrate offered to the LC treatment was offered in out-of-parlour feeders. Supplements were offered after morning milking. Fresh grass was



allocated on a daily basis after morning milking. Herbage intake was measured once during week 7 using the n-alkane technique. Herbage mass (>4 cm) was determined in each grazing paddock by cutting four strips (1.2 × 10m) with an Etesia motor scythe. Ten grass height measurements were recorded from each cut strip (pre and post harvesting) to determine the sampled height and calculate the bulk density (kg DM cm<sup>-1</sup> ha<sup>-1</sup>). Average paddock sward heights were measured, this sward height multiplied by the mean bulk density from the Etesia cuts was used to calculate paddock herbage mass. Thirty pre and post grazing sward heights were measured daily for each treatment using a rising plate meter. Milk yield was recorded daily while concentrations of milk fat, protein and lactose were determined by one successive morning and evening milk sample per week. Body weight and body condition score were measured once weekly. Data were analysed using PROC MIXED (SAS, 2005) whereby the effects of treatment, experimental week, block and treatment × experimental week were included in the model; cow was included as a repeated effect.

## Results and Discussion

The results of the experiment are shown in Table 1. Mean herbage mass was 1564 kg DM ha<sup>-1</sup> (s.d. 314). Mean pre-grazing sward heights were 11.1 (s.d. 1.62), 11.0 (s.d. 1.42) and 10.8 cm (s.d. 1.22), post grazing heights were 5.1 (s.d. 0.72), 4.2 (s.d. 0.58) and 4.4 cm (s.d. 0.63) for HG, LG and supplemented herds, respectively. Herbage disappearance was 16.4 (s.d. 2.71), 14.0 (s.d. 2.21) and 13.5 (s.d. 2.03) kg DM d<sup>-1</sup> for HG, LG and supplemented herds, respectively. Herbage DMI was higher for the HG group (17.1 kg DM d<sup>-1</sup>) than the supplemented treatments but not LG. The maize supplemented group recorded the highest herbage intake of the supplemented groups (13.8 kg DM d<sup>-1</sup>). The higher herbage intake of the animals supplemented with maize is reflected in the supplement intake which was lower than the HC and LC treatments. The highest numerical milk yield over the experimental period was recorded from the HC treatment. Treatment LG had a significantly lower milk yield than the three supplement treatments but not HG. Milk fat, protein and lactose yield was significantly lower for the LG treatment than the other four groups. The HG treatment recorded the highest milk protein concentration and was significantly higher than the LG and HC treatments. Milk fat concentration was lower for HC than HG, M and LC. There was no significant difference in lactose concentration, bodyweight, bodyweight change, body condition score and body condition score change between treatments.

Table 1. Effect of supplementation of autumn calving cows in spring time.

	HG	LG	M	HC	LC	s.e.m	Sig.
Herbage intake (kg DM d <sup>-1</sup> )	17.1 <sup>a</sup>	15.2 <sup>ab</sup>	13.8 <sup>b</sup>	13.4 <sup>b</sup>	13.1 <sup>b</sup>	0.57	***
Supplement intake (kg DM d <sup>-1</sup> )	-	-	3.2 <sup>a</sup>	4.0 <sup>b</sup>	3.9 <sup>b</sup>	0.13	***
Total DMI (kg DM d <sup>-1</sup> )	17.1 <sup>ab</sup>	15.2 <sup>a</sup>	17.0 <sup>ab</sup>	17.4 <sup>b</sup>	17.0 <sup>a</sup>	0.77 <sup>ab</sup>	*
Milk yield (kg d <sup>-1</sup> )	23.2 <sup>ab</sup>	20.7 <sup>a</sup>	23.7 <sup>b</sup>	24.7 <sup>b</sup>	23.8 <sup>b</sup>	0.76	**
Milk fat yield (kg d <sup>-1</sup> )	0.88 <sup>a</sup>	0.77 <sup>b</sup>	0.92 <sup>a</sup>	0.86 <sup>a</sup>	0.96 <sup>a</sup>	0.023	***
Milk fat content (g kg <sup>-1</sup> )	38.9 <sup>a</sup>	37.8 <sup>ab</sup>	39.4 <sup>a</sup>	35.3 <sup>b</sup>	40.4 <sup>a</sup>	0.125	*
Milk protein yield (kg d <sup>-1</sup> )	0.80 <sup>a</sup>	0.66 <sup>b</sup>	0.79 <sup>a</sup>	0.81 <sup>a</sup>	0.79 <sup>a</sup>	0.021	***
Milk protein content (g kg <sup>-1</sup> )	34.8 <sup>a</sup>	32.0 <sup>b</sup>	33.6 <sup>ab</sup>	32.6 <sup>b</sup>	33.3 <sup>ab</sup>	0.066	*
Milk lactose yield (kg d <sup>-1</sup> )	1.06 <sup>a</sup>	0.90 <sup>b</sup>	1.07 <sup>a</sup>	1.09 <sup>a</sup>	1.11 <sup>a</sup>	0.039	**
Milk lactose content (g kg <sup>-1</sup> )	46.0	43.9	45.1	43.9	46.5	0.100	n.s
Milk Fat + Protein yield (kg d <sup>-1</sup> )	1.68 <sup>a</sup>	1.43 <sup>b</sup>	1.71 <sup>a</sup>	1.65 <sup>a</sup>	1.75 <sup>a</sup>	0.040	***
Bodyweight (kg)	575	562	570	562	570	12.0	n.s
Bodyweight change/day (kg)	0.43	0.28	0.25	0.40	0.27	0.102	n.s
Body Condition Score	2.85	2.68	2.86	2.86	2.83	0.093	n.s
Body Condition Score change	0.05	-0.11	0.05	0.14	-0.09	0.074	n.s

Means within rows having different superscripts differ significantly (P<0.05).

\* = P<0.05, \*\* = P<0.01, \*\*\* = P<0.001, n.s. = not significant.

Herbage DM intake was measured from one intake period (Week 7) while supplement intake is the average intake over the experimental period.

## **Conclusion**

There is a large milk production benefit to supplementing grazing cows, on a restricted grass allowance in early spring. Extra herbage offered gave similar solids yield response to supplementation. Extra herbage was more beneficial in terms of improved milk protein concentration. Supplementing with a low CP concentrate or maize silage did not restrict production compared to a high CP concentrate.

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# Seasonal changes in biomass and composition of legume based swards under moderate and extensive grazing

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## Abstract

Grazing frequency affects the persistence of species, botanical composition and productivity of swards. Different swards consisting of *Trifolium repens* L., *Medicago sativa* L., *Lolium perenne* L., *Poa pratensis* L. were investigated under moderate and more extensive grazing management on a loamy Cambisol. Sward composition and grazing frequency had a significant effect on sward botanical composition and productivity. The yield of legumes accounted for nearly half of the total yield, and in the swards with lucerne even more than half. Legume yield declined annually in both grazing frequencies until the fourth year of swards use but to a greater extent under moderate grazing. From the fifth year the sward yield increased again. Dry matter yield was more stable under the more extensive grazing treatment, but more equal distribution of yield within the grazing season was observed under the moderate grazing management. The experiment demonstrated a positive effect of legumes on the yield and distribution over the grazing season.

Keywords: grazing frequency, botanical composition, lucerne, white clover, yield.

## Introduction

Seasonality is an important limit to herbage yield and animal production, and total annual grassland production can be less meaningful than its distribution throughout the year (Porqueddu *et al.*, 2005). Frequency of grazing can have a major influence on the pattern of pasture growth. More intense or more frequent defoliations interrupt the productive development, reducing seasonal variation by lowering the spring or summer peak, but also lowering total production (Korte and Harris, 1987; Tonkunas and Kadziulis, 1977). The choice of proper combinations of grass and legumes and defoliation management is important to exploit the complementary growth and balance of species. There is some evidence that plant species diversity can also be maintained by moderate and more extensive grazing (Isselstein *et al.*, 2005). The aims were to investigate the balance between species during the growing season in response to different grazing regimes and to assess effects of different swards on the annual yield and its distribution over grazing season.

## Materials and methods

During 1998 – 2004 a bi-factorial field trial of pasture utilization was carried out on a loamy Endocalcari-Epihypogleyic Cambisol near Dotnuva, Lithuania (55°24'N, 23°50' E, average annual rainfall 555 mm, average annual temperature 6° C, average length of growing season 194 days). The pastures were re-established in the spring of 1998 with an oat/vetch cover crop for green forage. Soil pH varied between 6.5 to 7.0, humus content was 2.5-3.2 %, C content 14.5 g kg<sup>-1</sup>, available P 50-80 mg and K 100-150 mg kg<sup>-1</sup>. The treatments of the A factor involved different swards consisting of white clover (*Trifolium repens* L.), lucerne (*Medicago sativa* L.), perennial ryegrass (*Lolium perenne* L.), and meadow grass (*Poa pratensis* L.) Lithuanian varieties. Legume/grass proportion in the seeding mixtures was 40:60 or 60:40. The treatments of the B factor involved moderate (M) and more extensive (E) grazing with 6 and 5 grazings per season, respectively. In 2004 the swards in all treatments of both factors were grazed 4 times. Swards were grazed with dairy cows. The grazing season lasted from the beginning of May until the middle of October. Grazing intensity was 2-2.5 cows ha<sup>-1</sup> yr<sup>-1</sup>, grazing season duration 150 days. Before grazing the dry matter (DM) yield was estimated in four replicates. The yield data were statistically processed using analysis of variance.

## Results and discussion

The total DM yield was affected by sward composition and grazing frequency. Moderate grazing caused significant annual DM losses from the first to the fourth year of use (Table 1). DM yield in the fourth year accounted for only 19-35% of the first year DM yield level, depending on sward composition. DM yield decreased from the first to the fourth year under more extensive grazing, too. However, the yield of legume/grass swards in the fourth year in more extensive grazing declined more slowly than in moderate grazing. Nevertheless, from the fifth year the sward yield started to increase and in the sixth year it was nearly the same as in the first year. The lucerne/grass and white clover/lucerne/grass swards gave the best yield under both grazing frequencies in all four years of use, and in five years under more extensive grazing.

Table 1. Total annual dry matter (DM) yield of different swards under moderate (M) and extensive (E) grazing.

Swards	DM (t ha <sup>-1</sup> )											
	1999		2000		2001		2002		2003		2004	
	M	E	M	E	M	E	M	E	M	E	M	E
<i>T. repens/L. perenne</i>	5.61	6.12	3.47	5.49	3.05	5.02	1.42	2.58	1.98	2.69	5.53	5.26
<i>T.r./L.p./Poa prat.</i>	5.62	6.56	3.39	5.36	3.21	5.16	1.08	2.47	1.64	2.20	5.22	4.73
<i>M. sativa/L.p./P.p.</i>	6.51	7.55	6.32	8.87	4.44	7.19	2.30	3.04	3.97	5.59	7.21	8.63
<i>T.r./M. s./L.p.</i>	5.96	6.96	4.91	8.11	3.99	6.56	1.62	3.02	3.14	4.76	6.66	7.04
LSD <sub>0.05</sub> (A/B factor)	0.303/0.124		0.300/0.122		0.243/0.099		0.320/0.130		0.257/0.105		0.327/0.133	

Table 2. Annual share of legumes in the dry matter DM yield of swards under moderate (M) and extensive (E) grazing.

Swards	Legume (%)									
	1999		2000		2001		2002		2003	
	M	E	M	E	M	E	M	E	M	E
<i>T. repens/L. perenne</i>	52.8	43.1	20.6	15.5	31.7	22.8	26.0	22.5	13.7	6.9
<i>T.r./L. p./Poa prat.</i>	55.2	48.8	19.4	20.6	31.2	24.0	26.6	29.6	17.4	8.2
<i>M. sativa/L.p./P.p.</i>	68.5	69.8	72.7	68.2	39.3	52.7	57.2	52.2	68.1	72.5
<i>T.r./M. s./L.p.</i>	61.2	58.1	47.3	34.6	36.6	35.5	49.7	53.9	55.4	66.8

The share of legumes varied between sward type and grazing frequency (Table 2). A higher legume share was observed for the swards of lucerne/grass and white clover/lucerne/grass. Even under moderate grazing the share of legumes in these swards was higher than in swards of white clover/grass. The greatest share of legumes was obtained in the first year. However, by the second year the yield of legumes had decreased, except for the lucerne/grass sward. The content of white clover decreased more rapidly than the content of lucerne. The more extensive grazing treatment had a negative effect on white clover, but positive on lucerne content in the swards. In the fourth year of sward use lucerne competed well even under moderate grazing. This can be explained by the dry climatic conditions, which were more adverse to white clover. Some researchers remarked that complex forage mixtures were more productive than simple grass-legume mixtures during drought and also reduced weed pressure (Sanderson *et al.*, 2004).

The share of legumes in botanical composition was the greatest in second half of the grazing season, therefore legumes had a positive impact on yield distribution over whole season. Distribution of the

annual DM yield over grazing season in average was more even in the swards containing lucerne or lucerne and white clover, less equal in white clover/grass swards (Table 3). In this aspect moderate grazing was noticeably superior over more extensive. The climatic conditions of season had affected considerably the distribution of annual yield over the whole grazing period. The superiority of swards with lucerne in such worse season was especially significant.

Table 3. Distribution of sward annual yield over the grazing season under moderate (M) and more extensive (E) grazing, average data, 1999-2003.

Swards	Annual DM yield share in grazings (%)										Yield dispersion		
	1 <sup>st</sup>		2 <sup>nd</sup>		3 <sup>rd</sup>		4 <sup>th</sup>		5 <sup>th</sup>		6 <sup>th</sup>		s <sup>2</sup>
	M	E	M	E	M	E	M	E	M	E	M	E	
<i>T. repens/L. perenne</i>	26	32	21	26	19	17	13	16	13	9	8	43	82
<i>T.r./L. p./Poa prat.</i>	26	36	19	25	18	16	13	17	15	11	9	34	70
<i>M. sativa/L.p./P.p.</i>	26	27	14	22	17	24	24	20	13	7	6	55	56
<i>T.r./M. s./L.p.</i>	24	26	16	24	18	23	20	20	14	7	8	30	58

### Conclusions

A positive effect of legumes on the distribution of yield over the whole grazing season was obtained. Swards containing lucerne exhibited the highest sustainability over five years under both grazing frequencies. Moderate grazing was favourable for more even distribution of yield over season, however, higher and more stable annual DM yields were obtained under more extensive grazing.

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## Concentrate supplementation effects on dairy cows grazing Galician pastures during spring.

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### Abstract

Concentrate supplementation of grazing dairy cows is a strategy for optimal management of pastures and milk production in Galicia. Ninety-one dairy cows grazed a grass-clover pasture area of 17.2 ha from April to June. The cows were assigned to three treatment groups and offered 0, 4 or 8 kg dry matter  $\text{d}^{-1}$  of concentrate. Milk production and quality, live weight, pasture intake and herbage production and composition were measured during the experimental period. Milk yields were 26.0, 30.2 and 32.5 kg milk  $\text{d}^{-1}$  for 0, 4 and 8 kg  $\text{d}^{-1}$  dry matter of concentrate supplemented, respectively from April 15 to May 15. Corresponding values for the period May 16 to June 30 were 20.4, 24.6 and 28.1 kg milk  $\text{d}^{-1}$ . Pasture intake was lower for the highest concentrate group. Seasonal variations in milk production and composition were associated with changes in pasture production and composition. The results show that Galician pastures can maintain high milk production during spring but changes in pasture allowance and quality through the season influence the milk response to supplementation.

Keywords: grazing, dairy cows, supplementation

### Introduction

Galicia produces 40% of Spanish milk and milk production is the main economic activity for 18000 farmers. Although pastures represents most of the arable area of the dairy farm, recent studies (López Garrido and Barbeyto Nistal, 2005) show that concentrate utilisation is over 0.4 kg per kg of milk produced, which indicates a poor forage use.

Concentrate supplementation allows high genetic merit cows to reach higher rates of milk production and compensate for deficiencies in seasonal pasture production and quality. The marginal milk response per kg of concentrate consumed is an indicator of the optimal level of concentrate supplementation.

The objective of this study was to determine the effects of offering supplementary concentrate to dairy cows grazing Galician pastures during spring on milk response and pasture dry matter intake.

### Material and methods

Ninety-one primiparous and multiparous dairy cows (mean 173 days in milk) at pasture were randomly assigned to three treatment groups. The treatments were 0, 4 and 8 kg supplementary concentrate dry matter (DM)  $\text{d}^{-1}$ , respectively. During the pre-experimental period (March 15 to March 31) all cows grazed and were supplemented with concentrate, grass silage and corn silage after milking and then grazed between supplementation periods. The experimental period was from April 15 to June 30. This period was divided in two based on the length of the grazing rotations. Period 1 was from April 15 to May 15 and period 2 from May 16 to June 30. There was only one rotation in each period and the paddocks grazed were the same for both rotations. The concentrate supplied during pre-experimental and experimental periods was composed of 247 g  $\text{kg}^{-1}$  DM of crude protein and 1.145 UFL  $\text{kg}^{-1}$  DM. Milk production was recorded daily. Milk samples were collected every 15 days to determine milk components (fat, protein, somatic cells account and urea).

The cows grazed a pasture area of 17.2 ha divided on 27 paddocks. Each paddock was grazed to a pasture height target of 5-6 cm. Pregrazing and postgrazing herbage mass (HM) was measured by cutting 3 quadrants before and after the cows grazed each paddock. Paddock size varied between 0.39 and 1.16 ha and average residence time was 4.0, 4.1 and 3.0 days for 0, 4 and 8 kg DM  $\text{d}^{-1}$  concentrate groups respectively. Cutting height was about 4 cm above ground level. Pasture DM intake

was measured as the difference between pregrazing and postgrazing HM and pasture allowance was measured as the pregrazing HM times the paddock area divided by the number of cows grazing and the residence time. Herbage samples were used to estimate DM, *in vitro* organic matter digestibility, crude fibre, neutral detergent fibre and acid detergent fibre.

Performance data were analysed using a repeated measures design including concentrate level, Cows within Concentrate level, Period, Period x Concentrate level and residual. The effect of Concentrate level was tested using Cows within Concentrate level and the Period and Interaction effects were tested using the residual. The ANOVA procedure of SAS (1999) was used for the analysis.

## Results and Discussion

There were significant ( $P < 0.05$ ) differences between groups in mean pasture DM intakes. The difference between the 0 and 4 kg d<sup>-1</sup> supplemented groups was not significant but these had higher values than the highest supplemented group (Table 1). No substitution of concentrate for pasture was observed when concentrate supplementation was 4.0 kg DM d<sup>-1</sup>. The substitution rate for 8 kg DM d<sup>-1</sup> of concentrate was 0.38 and 0.27 for period 1 and 2, respectively.

Pasture allowance was lower for the highest supplemented group (Table 1) and this could account for a lower pasture intake but this difference was not significant ( $P < 0.05$ ). Pregrazing pasture height and HM ranges were more extreme for this group too (average pasture heights were 15.4 and 27.3 cm for period 1 and 2 respectively). No significant differences were found for postgrazing HM. Pasture quality was the same for all the supplementation groups but *in vitro* DM digestibility of the pasture was lower during period 2.

Table 2 shows the milk production and quality variables. There were significant differences ( $P < 0.05$ ) between treatment groups for milk production. Daily milk production was 26.0, 30.2 and 32.5 kg for groups supplemented with 0, 4 and 8 kg d<sup>-1</sup> DM of concentrate respectively during period 1, and 20.4, 24.6 and 28.1 kg during period 2. Milk response to concentrate supplementation was 0.95 kg milk per kg of concentrate for the 4 kg DM supplemented group in both grazing periods. In contrast, the highest supplemented group had a lower milk response but it increased from period 1 to period 2 (0.73 to 0.87 kg milk per kg of concentrate, respectively). These values were close to those reported by Bargo *et al.* (2002) of 0.96 and 1.36 kg milk kg<sup>-1</sup> of concentrate for low and high pasture allowances, respectively.

No significant differences were found between groups for milk fat or SCC but significant differences were found for milk protein and milk urea. Milk protein and milk urea were higher for the supplemented groups. This could be attributed to the high protein content of the concentrate which was formulated for the pre-experimental period where forages had lower crude protein content.

Body weight was significantly higher for the highest supplemented group (Table 2). This indicates that portion of the concentrate supplement was diverted to the recovery of body reserves.

Table 1. Pasture dry matter intake (PDMI), pasture allowance, height, crude protein (CP) and *in vitro* organic matter digestibility (IVOMD) and pregrazing and postgrazing herbage mass (HM) means for the treatment groups. Grazing period 1 lasted from April 15 to Mai 15 and period 2 from Mai 16 to June 30.

Concentrate kg DM d <sup>-1</sup>	0		4		8		SEM	sig.		
	1	2	1	2	1	2		Group	Period	Group*Period
PDMI Kg DM d <sup>-1</sup>	16.4	17.7	16.8	17.7	13.0	15.3	3.83	0.04	0.10	0.64
Pregrazing, HM t DM ha <sup>-1</sup>	2.91	3.81	2.77	3.85	2.48	4.39	0.58	0.66	<0.01	<0.01
Postgrazing, HM t DM ha <sup>-1</sup>	0.76	1.02	0.59	1.03	0.70	1.26	0.32	0.23	<0.01	0.09
Pasture allowance, Kg DM/d/cow	22.9	24.2	21.4	23.1	18.6	21.5	11.2	0.20	0.12	0.79
Pasture height, cm	16.4	20.8	16.9	23.4	15.4	27.3	5.4	0.26	<0.01	0.04
Pasture CP, g kg <sup>-1</sup>	159	136	158	133	153	115	34	0.53	0.16	0.60
Pasture IVOMD, g kg <sup>-1</sup>	809	741	810	733	819	711	48	0.67	<0.01	0.95

Table 2. Milk production and composition and animal body weight of the treatment groups.

Concentrate, kg DM d <sup>-1</sup>	0		4		8		SEM	sig.		
	1	2	1	2	1	2		Group	Period	Group*Period
Milk, kg d <sup>-1</sup>	26.0	20.4	30.2	24.6	32.5	28.1	5.4	<0.01	<0.01	0.26
Milk fat, g kg <sup>-1</sup>	35.9	36.8	35.7	36.7	34.6	35.4	5.6	0.37	0.08	0.98
Milk protein, g kg <sup>-1</sup>	30.2	30.3	32.0	32.0	31.7	31.2	1.9	0.09	0.19	0.28
Somatic cells account	124	146	124	180	158	125	243	0.90	0.36	0.14
Milk urea, mg l <sup>-1</sup>	172	253	252	311	308	363	80	<0.01	<0.01	<0.01
BW, kg	594	607	595	610	607	637	20	0.31	<0.01	<0.01

## Conclusions

The results show that Galician pastures can maintain high milk production during spring and there is a decreasing response to increasing levels of concentrate supplementation. During late spring the quality of the pasture decreased and the milk response to concentrate supplementation increased. This result could be influenced by the pasture management because herbage regrowth interval was larger and pasture was more mature.

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# Alternatives to conventional feeding systems for beef production in Ireland

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## Abstract

The liveweight gain (LWG) of bulls grazing different pasture allowances in winter was compared with those accommodated on out-wintering pads at Teagasc's Grange Beef Research Centre, Ireland. Fifty-six yearling bulls grazed pasture at allowances of 3, 5, or 7 kg dry matter (DM) head<sup>-1</sup> d<sup>-1</sup> (Low, Medium, or High), or were offered silage *ad libitum* on out-wintering pads (Pads) for 124 days in winter. There were two replicates of the four treatment groups. All animals were supplemented with 2 kg DM head<sup>-1</sup> d<sup>-1</sup> of barley-based concentrate. LWG averaged 0.87 kg d<sup>-1</sup> for bulls on Pads and the High pasture allowance, compared with 0.74 kg d<sup>-1</sup> and 0.63 kg d<sup>-1</sup> for bulls on the Medium and Low pasture allowances respectively. In the following spring/summer all bulls grazed similar pasture allowances for 139 days until slaughter. LWG of bulls that were wintered on Pads and the Low pasture allowance averaged 1.22 kg d<sup>-1</sup> compared with 1.02 kg d<sup>-1</sup> for the Medium and High pasture allowance groups. Final live weights (~597 kg) and carcass weights (~310 kg) were similar. These results show that bulls maintained on restricted pasture allowances in winter can achieve similar animal performance overall compared with those maintained on out-wintering pads.

Keywords: bull beef, liveweight gain, out-wintering pads, pasture allowance, winter grazing.

## Introduction

The profitability of beef production in Ireland is greatly influenced by the cost of feeding over winter. The conventional system of over wintering cattle is to feed silage and concentrates to housed animals. Recently, over wintering cattle on 'out-wintering pads' has been shown to provide positive animal performance while minimising costs (French and Hickey, 2005). Furthermore, systems that maximise the contribution of grazed grass in the diet of growing cattle are likely to be more competitive. Potentially, an alternative to housing cattle and feeding silage is to maintain cattle outdoors to graze pasture over the winter, which has accumulated since the autumn. A wide body of information exists on the management of grass swards in the autumn to produce herbage for winter utilisation (Hennessy *et al.*, 2004). However, much less information is available on the management of animals grazing in the winter to optimise animal production while minimising damage to the sward. Thus, the objective of this study was to compare the performance of yearling bulls either grazing different pasture allowances in winter or accommodated on out-wintering pads.

## Materials and Methods

The experiment was conducted at Teagasc's Grange Beef Research Centre, County Meath, Republic of Ireland. Fifty-six Charolais × Limousin yearling bulls with a mean initial live weight of 342 kg (± s.e. of 44.2 kg) were blocked on live weight and randomly assigned to eight groups, giving seven animals per group. Six groups of bulls grazed at pasture allowances of either 3, 5, or 7 kg DM head<sup>-1</sup> d<sup>-1</sup> (Low, Medium, or High), and two groups were offered silage *ad libitum* on out-wintering corrals (Pads). There were two replicates of the four treatments, which were imposed in winter from 1 December 2004 to 6 April 2005 (124 days).

The three pasture allowances were based on pre-grazing pasture mass above a cutting height of 4 cm, and were achieved by adjusting the area of pasture offered each day, using temporary electric fences. In addition all animals received 2 kg DM head<sup>-1</sup> d<sup>-1</sup> of barley-based concentrate (87% barley, 6.75%

toasted soya bean, 4.75% sugar cane molasses, calcium carbonate and sodium chloride). Portable troughs were used to feed the concentrate and water. The total area grazed in winter was 2.2, 2.7 and 3.3 ha for the Low, Medium and High pasture allowances respectively. Fresh grass silage and water were offered each day to the bulls accommodated on the out-wintering pads. The pads comprised of compacted clay soil covered with a wood chip bedding which was renewed four times in winter, and liquid effluent drained into septic tanks.

After winter, all animals were rotationally stocked in spring/summer from 6 April to 22 August 2005 (139 days). Similar pasture allowances were achieved by shifting each group to a new area of pasture every 3 – 5 days when the grazed pasture height had reached 6 – 7 cm. The bulls were supplemented with ~3 kg DM head<sup>-1</sup> d<sup>-1</sup> of barley-based concentrate for the final 76 days until slaughter. The pastures grazed in spring and summer were not grazed in winter.

Liveweight gain was measured every 3 – 4 weeks. Carcass weight and conformation (EUROP scale 1 – 5) were measured when animals were slaughtered on 23 August 2005. Pre- and post- grazing pasture masses were measured each week in winter and every 2 – 3 weeks in spring and summer, by cutting three 0.55 m × 5 m strips per group to a stubble height of 4 cm. The freshly cut herbage was weighed and sub-samples dried to determine DM percent.

## Results and Discussion

In winter, the LWG of bulls on the High pasture allowance averaged 0.89 kg d<sup>-1</sup> compared with ( $P < 0.05$ ) 0.64 kg d<sup>-1</sup> for bulls on the Low allowance (Table 1). This resulted in an extra 35 kg of live weight produced in winter although differences in live weight were not significant. The average growth rates of bulls accommodated on Pads and on the Medium pasture allowance were intermediate at 0.85 and 0.74 kg d<sup>-1</sup> respectively. In comparison, French and Hickey (2005) reported that steers offered silage plus concentrates on out-wintering pads had average growth rates of about 1.4 kg d<sup>-1</sup> compared with 1.2 kg d<sup>-1</sup> when housed on slatted floors.

Closing paddocks from late September onwards provided an average pre-grazing pasture mass of 1870 kg DM ha<sup>-1</sup> in winter (Table 2). Accumulating pasture before this time can lead to a reduction in total herbage mass due to decreases in leaf production rate and number of tillers, and an increased leaf senescence rate in grasses, which all result from an increase in canopy accumulation in winter (Laidlaw and Mayne, 2000). Post-grazing pasture mass averaged 400 kg DM ha<sup>-1</sup> and 370 kg DM ha<sup>-1</sup> for the High and Medium allowances respectively compared with ( $P < 0.01$ ) 260 kg DM ha<sup>-1</sup> for the Low allowance (Table 2). The bulls on the Low allowance were observed to have spent more time grazing and treading each day than the bulls on the Medium and High allowances. Thus, grazing at high pasture allowances in winter is likely to increase individual animal performance and reduce damage to the subsequent growth of the sward.

In spring and summer, growth rates of the bulls ranged from 1.4 to 1.7 kg d<sup>-1</sup> for the first 35 days, and from 0.8 to 1.1 kg d<sup>-1</sup> for the next 109 days (Table 1), when all animals were given similar pasture masses (Table 2). Although the bulls that were wintered on Pads and the Low pasture allowance subsequently grew 0.2 – 0.3 kg d<sup>-1</sup> faster ( $P < 0.01$ ) than those on the Medium and High allowances, all animals achieved acceptable liveweight gains in spring and summer irrespective of their previous winter feeding regime. As a consequence, final live weight (~597 kg), carcass weight (~310 kg) and carcass conformation (~2.9) were similar across all treatments when the animals were slaughtered. Further research is required on the environmental impacts of winter grazing systems.

Table 1. Mean live weight (LW), liveweight gain (LWG), carcass weight (CW) and carcass conformation of yearling bulls in winter and spring/summer.

Treatment	----- Winter (1/12 – 6/4) -----			----- Spring/summer (6/4 – 23/8) -----				
	LW 1/12 (kg)	LWG 1/12–6/4 (kg d <sup>-1</sup> )	LW 6/4 (kg)	LWG 6/4–10/5 (kg d <sup>-1</sup> )	LWG 10/5–22/8 (kg d <sup>-1</sup> )	LW 22/8 (kg)	CW 23/8 (kg)	Conf. (1-5)
Low	344	0.63 <sub>b</sub>	425	1.70	1.00 <sub>ab</sub>	594	303	2.9
Medium	337	0.74 <sub>a</sub>	431	1.45	0.82 <sub>b</sub>	574	302	2.8
High	343	0.89 <sub>a</sub>	460	1.41	0.82 <sub>b</sub>	605	319	3.1
Pads	345	0.85 <sub>a</sub>	443	1.61	1.06 <sub>a</sub>	616	317	2.9
<i>P</i>	NS	*	NS	NS	**	NS	NS	NS
s.e.d.	16.7	0.087	24.1	0.141	0.080	27.3	17.6	0.19

NS = not significant, \* $P < 0.05$ , \*\* $P < 0.01$ .

Within columns, means with the same or no letter subscript are not significantly different ( $\alpha = 0.05$ ).

Table 2. Mean pre- and post-grazing pasture masses (PM) in winter and spring/summer.

	----- Winter (1/12 – 6/4) -----		----- Spring/summer (6/4 – 23/8) -----	
	Pre-grazing PM (kg DM ha <sup>-1</sup> )	Post-grazing PM (kg DM ha <sup>-1</sup> )	Pre-grazing PM (kg DM ha <sup>-1</sup> )	Post-grazing PM (kg DM ha <sup>-1</sup> )
Low	1,870	260 <sub>b</sub>	2,190	550
Medium	1,870	370 <sub>a</sub>	2,240	630
High	1,870	400 <sub>a</sub>	2,230	600
Pads	-	-	1,890	700
<i>P</i>	NS	**	NS	NS
s.e.d.	68	7	56	45

NS = not significant, \*\* $P < 0.01$ .

Within columns, means with the same or no letter subscript are not significantly different ( $\alpha = 0.05$ ).

## Conclusion

This study has shown that feeding systems which involve all-year grazing of cattle or accommodating cattle on out-wintering pads have potential for integration into beef production systems in Ireland. Bulls maintained on restricted pasture allowances in winter can achieve a similar animal performance overall compared with bulls offered silage on out-wintering pads.

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# Species composition of the soil seed bank in comparison with the floristic composition of meadow sward

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## Abstract

The objective of this study was evaluation of the soil seed bank in the surface layer of the soil, seed distribution at four depths and a comparison of the species composition of seeds in the soil with the floristic composition of the meadow sward. Soil samples were taken from two stands: a permanent meadow and a meadow subject to renovation (after previous rotary tillage). Research has shown that the species structure of the soil seed bank does not reflect the floristic composition of the sward, due to the fact that the sward is dominated by species whose survival strategy is related to vegetative reproduction. Although changes in sward management occurred seeds of the species characteristic of the previous managements remained in the soil. The most numerous of these were the seeds of dicotyledonous plants, whereas mainly the *Chenopodium album* L. seeds determined the size of the soil seed bank. The greatest reserves of seeds were in the surface layer of the soil (0-10 cm). The application of rotary tillage resulted in the replacement of seeds, allowing for development of seedlings from seeds lingering in the deeper soil layers.

Keywords: meadow, soil seed bank, rotary tillage, vertical distribution of seeds.

## Introduction

Plants developing from seeds in the top layer of soil, usually dicotyledonous weeds, represent strong competition to the seedlings of grasses, which develop more slowly. They decrease the renovation effect of grasslands (Janicka, 2005). This fact encouraged the study of the reserve of seeds in the soil. The composition of the soil seed bank depends on the plant communities appearing in a particular area both at the present moment and also in the past, as well as on the biological properties of plants. Changes in land-use and management practices influence the distribution of seeds in the soil and the established vegetation (Bekker *et al.*, 1998; Reine *et al.*, 2004). The objective of this study was the evaluation of the seed reserve in the top layer of the soil and the distribution of seeds at four depths. A further aim was comparison of the composition of seed species in the soil with the floristic composition of the vegetation.

## Materials and methods

The study was carried out in central Poland, at a moderately dry meadow site (water table below 150 cm), situated on a mineral soil (degraded black earth). Soil samples were collected in April, before the fresh seed rain. Samples were taken at random, to a depth of 20 cm, from two stands: 1) a permanent meadow, extensively managed and fertilised, 2) a meadow subject to renovation at that time (after rotary tillage). A steel cylinder of 110 mm diameter and 100 mm height was used for sampling. Samples of 10 cm of soil were collected twice; then they were divided into 4 levels: 0-5 cm, 5-10 cm, 10-15 cm and 15-20 cm. In total, 10 samples were collected from each combination. Subsequently, they were subjected to laboratory analysis on the basis of a seed separation method. The samples were washed on sieves (mesh width 0.25 mm); then the material was drenched with potassium iodide solution (density 1.60 – 1.66 g cm<sup>-3</sup>). At that point the mineral parts started to deposit at the bottom and the seeds and other organic components stayed on the surface of the solution. They were transferred onto filters and dried at a temperature of 60°C. Live seeds were identified and taken out under a binocular microscope,

and they were analysed with respect to the species classification and number. During the growing season the species composition of meadow sward was analysed.

## Results and discussion

In the permanent meadow the number of seeds in the 0-20 cm layer in an area of 1m<sup>2</sup> amounted to 17 850; these seeds represented 23 species, including 15 meadow species and 8 segetal. In the soil seed bank the most numerous were dicotyledonous species (Table 1). More than 80% of the seed bank consisted of only three species: *Chenopodium album*, *Taraxacum officinale* Weber. and *Polygonum convolvulus* L. The next four most abundant species amounted to 1-5%, and the remaining 16 species – contributed less than 1%.

Table 1. The number of species and percentage of plant groups in a permanent meadow sward and soil seed bank.

Group of plant species	Vegetation		Soil seed bank	
	Number of species	Percentage in annual yield	Number of species	Percentage according to number of seeds
Grasses	15	83.6	8	3.9
Papilionaceous	8	2.2	4	1.1
Other dicotyledonous:	15	14.2	11	95.0
Annual	3	0.1	9	87.7
Perennial	12	14.1	2	7.3
Total	38	100.0	23	100.0

These results confirmed that the greatest seed reserves are in the top layer of the soil. The application of rotary tillage resulted in the replacement of some of the seeds from the surface layers of the soil to the layer located lower (Table 2). Moreover, this treatment encouraged the development of seedlings from seeds, which were previously located deeper. As a result the vegetation in the areas subject to the application of rotary tillage comprised 8 species which did not occur in the permanent meadow sward but whose seeds were present in the soil, i.e. *Capsella bursa pastoris* L., *Chenopodium album*, *Fumaria officinalis* L., *Lamium amplexicaule* L., *Polygonum convolvulus*, *P. persicaria* L., *P. nodosum* Pers. and *Viola arvensis* Murray. These are annual species, associated with arable land, which do not play any important role in the regeneration of the species structure, but appear in large quantities in the initial period after renovation.

The mass and the shape of seeds are the two main determinants of their vertical distribution in the soil (Bekker *et al.*, 1998). Fruits with pappus, seeds with a large surface area/volume ratio and which have awns have less chance of passive movement down cracks in the soil, as well as translocation by soil animals. These results showed that 80.6% of grass seeds were located in the surface layer of the soil (0-5 cm). The seeds of *Taraxacum officinale* were distributed in a similar manner, whereas the small round seeds, e.g. *Chenopodium album*, were mostly located deeper (Table 2). In the seed bank and the vegetation of the permanent meadow there were 46 species in total, 8 of which were present only in the seed bank and 23 only in the vegetation, whereas 15 species were common. A great number of meadow species are characterised by high vegetative reproduction; cutting prevents the flowering and fruiting of many species and therefore limits their contribution to reserves in the soil. Moreover, species present in the vegetation and which do not occur in the seed bank are usually characterised by a transient or short-term persistent seed bank (e.g. grasses), as opposed to the species found only in the seed bank, which create a persistent seed bank (annual weeds). As a consequence, a more varied species list of vegetation is not reflected in the soil seed bank (Table 1). It has to be stressed that the studied meadow was established in the 1950's. Previously this area was arable land, and most of the crops were root crops and vegetables. Along with the change in the management, seeds of such species as *Chenopodium album*, or species of the *Polygonum* genus, remained in the soil bank in large numbers. A crucial

biological feature of these species, which determines their position in the soil seed bank, is the longevity of their seeds. Seeds whose mass is below 3 mg and which are small and round are persistent (Thompson *et al.*, 1993), e.g. the seeds of *Chenopodium album*. They are able to survive in the soil for several years, which results in the fact that they often determine the volume of the seed bank.

Table 2. Vertical distribution of total number of viable seeds per m<sup>2</sup> (N°) and percentage of all species, grasses, *Chenopodium album* and *Taraxacum officinale* seeds in the four layers of the soil of permanent meadow (A) and soil after rotary tillage (B).

Layer (cm)	N° of seeds of all species		All species %		Grasses			<i>Chenopodium album</i>			<i>Taraxacum officinale</i>		
					N°	%		N°			%		
	A	B	A	B	A	A	B	A	A	B	A	A	B
0-5	5148 a*	4243 b	30.8	22.3	874 a	80.6	17.5	1969 b	17.0	20.6	1126 a	74.8	10.2
5-10	4527 ab	6264 a	27.1	33.0	105 b	9.7	50.0	3579 a	31.0	32.6	211 b	14.0	56.4
10-15	3401 b	3643 b	20.4	19.2	92 b	8.4	18.5	2948 ab	25.5	19.3	137 b	9.1	30.4
15-20	3632 ab	4843 ab	21.7	25.5	14 b	1.3	14.0	3053 ab	26.5	27.5	32 b	2.1	3.0
NIR <sub>0.05</sub>	1590.6	1590.6			177.4			1388.1			450.7		

\*in the columns figures indicated by the same letters are not significantly different.

## Conclusion

There were 17 850 seeds in the 0-20 cm layer of soil within 1 m<sup>2</sup>. The most numerous group were the seeds of dicotyledonous plants. Seeds of *Chenopodium album* largely determined the size of the soil seed bank, amongst the 23 identified species. The greatest seed reserves were in the surface layer of the soil (0-5 cm). Rotary tillage resulted in a replacement of seeds, allowing for the development of seedlings from seeds which were previously located in the deeper layers of the soil. The species structure of the soil seed bank did not reflect the floristic composition of the meadow sward, because: 1) the sward was dominated by species whose survival strategy is related to vegetative reproduction; 2) along with the change of management the seeds of species characteristic for the previous type of management remained in the soil, creating a persistent seed bank.

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## **Session 2**

### **Role and potential of legumes**





## Temperate legumes: key-species for sustainable temperate mixtures

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### Abstract

The paper summarises the current knowledge on temperate legumes used in Europe, lucerne excluded except for comparison with other species, and discusses the possibilities for new development of these species in the farming sector as well as some research priorities. The yield potential of lucerne, red clover and white clover is high but, despite undeniable breeding progress, persistence remains a problem especially for red clover, probably mainly related with disease attacks by *Sclerotinia trifoliorum* but by other fungi species like *Fusarium* spp. as well. For white clover, new aggressive cultivars are much more persistent than old cultivars, and management conditions for balanced and persistent clover/grass mixtures are now much better understood. For *Lotus* spp. and Caucasian clover a lot of research is still needed to define their potential and optional management requirements. Superior nutritive values, intake characteristics and animal performances have been demonstrated for legumes compared to grasses. Utilization of protein remains though a problem that can lead to losses to the environment, bad conservation of silage and necessary high-energy consumption for the synthesis of urea. More research programmes are needed on ways to reduce proteolysis. Grazing legume/grass mixtures can increase the polyunsaturated fatty acid content of milk and can thus have a positive impact on consumers' health. Nitrogen fixation is very important in productive legume species but its variability still needs to be studied to understand better the factors affecting fixation in the field. Nitrate leaching in grazed white clover/grass swards and after the ploughing of clover-based leys has been amply studied but fewer data are available for other legume species and on nitrogen amounts available for the subsequent crops in ley/crop rotations. Breeding efforts should concentrate on developing more persistent cultivars especially in red clover and *Lotus* spp., on species more resistant to environmental and biological stresses and on the introduction of genes of condensed tannins (CT) synthesis in clover species. Nitrogen-fertilised swards imply a high consumption of fossil energy for the synthesis of inorganic N fertilizers; the use of legumes can thus reduce CO<sub>2</sub> emissions in the atmosphere.

Keywords: production, N fixation, animal performance, utilization, environmental issues.

### Introduction

After a long period of decline, there is a renewed interest in forage legumes in the temperate area of Europe for several reasons: farmers have to adapt their management to changing economic and political conditions and other stakeholders want to improve the impact of agriculture on the environment. In a context of a decrease of agricultural prices and premiums, and of a limitation to production, farmers must decrease their production costs. Legumes are seen as a way to reduce the use of inputs, mainly nitrogen fertilizers but also, to some extent, concentrates. Since 1992, a significant proportion of producers have converted to organic farming mainly because of attractive financial supports and/or specific higher prices. Forage legumes are an essential part of these organic systems where symbiotic N fixation must replace inorganic N fertilizers. Farmers are also under the pressure of environmental regulations, notably the European Nitrate directive and its national transpositions. Rochon *et al.* (2004) estimated that the Common Agricultural Policy (CAP) could now shift the balance of economic advantage towards legumes and away from high usage of inorganic N fertilizers on grass swards. They estimated the benefit to be 137 € ha<sup>-1</sup> for farmers using legume or legume/grass silages instead of grass silages. According to Doyle and Topp (2002), the benefit of replacing a tenth of the current forage (grass) area within the EU by legume/grass mixtures for silage making would gain as much as 1,300 million € for the European livestock farming sector. The use of forage legumes is also considered as a

way to protect the environment since symbiotic N fixation is an alternative to industrial N fertilizer synthesis, which consumes high levels of fossil energy. In optimal conditions, legume swards can decrease nitrate pollution of water tables compared with highly N-fertilised grass swards. Legumes also undoubtedly have a better impact than grasses on the landscape and on some wildlife species.

Two important research programmes financed by the European Commission have recently focused on legumes: LEGSIL and LEGGRAZE. LEGSIL (FAIR CT96-1832) 'Legume Silages for Animal Production' was carried out between 1997 and 2001 (Wilkins and Paul, 2002). It associated 6 teams from 4 North European countries. LEGGRAZE (QLK5-CT-2001-02328) 'Low input animal production based on forage legumes for grazing systems' developed its activities between 2001 and 2005 (<http://www.univ-perp.fr/leggraze/>). It associated 5 teams from 4 countries. A research network is also financed by the European Commission, namely the project COST action 852 'Quality legume-based forage systems for contrasting environments'. It started in 2001 and will end in 2006. It includes 26 teams from 20 countries ([http://www.iger.bbsrc.ac.uk/COST\\_852/COST852Homepage.html](http://www.iger.bbsrc.ac.uk/COST_852/COST852Homepage.html)).

White clover (*Trifolium repens*) is the most important legume species in temperate Europe, followed by lucerne (*Medicago sativa*) and red clover (*T. pratense*). Some other secondary legumes can have a local importance: sainfoin (*Onobrychis viciifolia*), birdsfoot trefoils (*Lotus* spp.), alsike clover (*T. hybridum*) and galega (*Galega orientalis*). Caucasian clover (*T. ambiguum*) is a novel species for forage production in Europe. All these species have very different characteristics and are adapted to contrasting managements and environments. Lucerne is not tackled in this paper except for comparison with other species; it is the subject of the paper of Veronesi *et al.* (in this book). Reviews on these legume species have been published for white clover (Frame and Newbould, 1986; Baker and Williams, 1987), red clover (Frame, 1990; Taylor and Quesenberry, 1996) and temperate legumes in general (Taylor, 1985; Frame *et al.*, 1998; Frame, 2005).

### Legume-grass mixtures

In cool climates, legumes have to face strong competition from grasses especially in spring. Companion grasses must thus be chosen with care in order to ensure a persistent and balanced mixture. For instance, white clover, which has a prostrate habit, can be mixed with small-size grasses like *Lolium perenne*, *Poa pratensis* or *Festuca rubra*. The mixture of white clover with tall-size grasses is much more problematic. Within species, an adequate choice of cultivars can improve the viability of the mixture. In recent years, much information has been accumulated on the compatibility between the different types of *L. perenne* and white clover cultivars. Large-leaved clover cultivars should be associated with late diploid *L. perenne* cultivars, medium-size-leaved clover cultivars with late tetraploid *L. perenne* cultivars and small-leaved clover cultivars with very late tetraploid *L. perenne* cultivars (Le Gall and Guernion, 2004). Red clover that is very competitive in the early production years has to be associated with competitive grasses like *L. perenne* and *L. multiflorum*; it is also traditionally mixed with *Phleum pratense* and *F. pratensis*. Alsike clover is notably used with *P. pratense* and *F. pratensis*. The compatibility of Caucasian clover with grasses has still to be studied in Europe but non-aggressive grasses seem to be the best solution. *Lotus corniculatus* is best mixed with small-size grasses like *P. pratensis* and *F. rubra* or with taller but low-tillering grasses like *P. pratense* and *Bromus inermis*. *Lotus uliginosus* is mainly mixed with *P. pratense* and *F. pratensis*; in the Azores, in a very humid climate, it thrives spontaneously in mixture with *Holcus lanatus*. Galega is compatible with a wide range of grasses but its persistence is reduced when mixed with aggressive species. Overseeding has proved to be successful with several legume species if basic rules are respected (Tiley and Frame, 1991; Frame, 2005). The proportion of clover in harvested forages can be precisely evaluated by NIR spectroscopy (Wachendorf *et al.*, 1999; Deprez *et al.*, 2005).

### Fertilization – soils

Nitrogen fertilization is not recommended on legume/grass mixtures because it reduces N fixation and decreases the proportion of legumes but tactically at about 50 kg N ha<sup>-1</sup> it can be used to compensate for a slow growth of the legume at the beginning and the end of the growing season. With cold weather in spring, white clover growth is particularly slow. When red clover disappears in mixtures in the second

or the third year of production, N fertilization can be applied to compensate for the lack of fixed N for the grass component. The maintenance of a balanced proportion of legumes in mixtures with grasses requires sufficient S, P and K availability. Legumes are more susceptible than grasses to low pH. Adequate pH-H<sub>2</sub>O values (above 5.5 - 6.0) are necessary for a good S, P, K, Ca and micro-nutrients (i.a. Mo and Co) nutrition of legumes. Superphosphate that provides P and S is thus an excellent fertilizer of legume/grass mixtures. Liming must be applied on the basis of regular soil analysis.

## Production

White clover annual production in pure stand and in favourable growing conditions is about 6-9 t DM ha<sup>-1</sup> (Castle *et al.*, 1983; Frame and Newbould, 1984) but the species is almost always used in mixture with grasses and the contribution of white clover to total yield is highly variable. Annual production of white clover/grass mixtures in good conditions in the west of Europe are 7-11 t DM ha<sup>-1</sup> (Frame and Newbould, 1984) and theoretically up to 20 t DM ha<sup>-1</sup>. In the north of Europe (Scandinavia, Baltic States), typical annual production values of mixtures are about 6-8 t DM ha<sup>-1</sup>. The peak yield of white clover/grass mixtures occurs later in spring compared with N-fertilized grass swards. Since white clover is a very plastic species, differing defoliation frequencies can have diverse effects on its production influenced by the differing density and height of the companion species.

Red clover is higher yielding than white clover, at least under cutting. In most cases, red clover/grass mixtures are more productive than pure red clover swards cultivated on the same sites (Arnaud and Niqueux, 1982; Halling *et al.*, 2000; Kravale *et al.*, 2002; Sebastia *et al.*, 2004). On the basis of a synthesis of several authors for experiments in many areas of Europe (Charles and Lehmann, 1973; Frame *et al.*, 1973; Frame, 1975; Arnaud and Niqueux, 1982; Frame and Harkess, 1983; Aldrich, 1984; Sheldrick *et al.*, 1986; Charles *et al.*, 1988; Gielen *et al.*, 1990; Fisher *et al.*, 1996; Nykänen *et al.*, 2000; Skuodiene, 2000; Halling *et al.*, 2002; Kravale *et al.*, 2002; Lugic *et al.*, 2002; Cupina *et al.*, 2004; Eric *et al.*, 2004; Nesic *et al.*, 2004), typical annual yields of pure red clover swards are summarized in Table 1.

Table 1. Typical annual yields (t DM ha<sup>-1</sup>) for different areas of Europe.

	A1	A2	A3
<b>Pure red clover swards</b>			
West of Europe	10-14	7-10	3-4
South of Europe	13-21	6-13	-
North of Europe	7-8	7-8	-
<b>Red clover/grass mixtures</b>			
West of Europe	11-17	8-15	-
North of Europe	6-9	7-9	5

A1: first production year; A2: second production year; A3: third production year.

In Belgium (Deprez *et al.*, 2004b), very high annual yields were recorded. At sea level, average yields (t DM ha<sup>-1</sup>) were 18.6 in A1 and 16.4 in A2. For mixtures in less favourable conditions, average annual yields (t DM ha<sup>-1</sup>) of mixtures reached 16.4 in A1, 16.4 in A2 and 16.3 in A3 at 120 m above sea level and 14.1 in A1 and 9.0 in A2 at 500 m above sea level. High yields persisting over 3 years of production were achieved with Mattenkleer cultivars. Most authors recorded a yield decrease in the second year of production and very low or nil yields in the third year (Halling *et al.*, 2000; Hadjigeorgious and Thanopoulos, 2004; Frame and Harkess, 1983). This decrease may be due to the presence of a fungi or a complex of fungi species. It can be concluded from several studies (Fisher *et al.*, 1996; Halling *et al.*, 2000; Hadjigeorgious and Thanopoulos, 2004; Deprez *et al.*, 2004b) that the present cultivars of red clover have similar yields to lucerne cultivars, which was probably not the case in the past.

Legumes or legume/grass mixtures have higher summer production than that of pure *Lolium perenne* swards; they reduce the summer forage production deficit and have thus more regular yields throughout the growing season than pure grass stands.

Alsike clover has a yield potential intermediate between red and white clovers and yields decrease with

time. The potential of Caucasian clover has still to be studied in Europe. Birdsfoot trefoil seems to be not very productive. A recent study (Fychan *et al.*, 2003) recorded annual yields of about 5-7 t DM ha<sup>-1</sup> for monocultures during two years, the second year being less productive than the first one. Sainfoin is less productive than lucerne and red clover (about 7-15 t DM ha<sup>-1</sup>) and difficult to establish, while a low competitiveness against weeds and a low N fixation can limit its production. In the Baltic States and in Finland, galega gives typical annual yields in pure stand or in mixture with grasses of about 8-10 t DM ha<sup>-1</sup>.

### **Nutritive value, voluntary food intake, animal performance**

Legumes have usually higher digestibility, crude protein (CP), pectin, lignin, ash, Ca and Mg contents than grasses. They are poorer in total cell wall or neutral detergent fibre (NDF), hemi-cellulose and water-soluble carbohydrate (WSC) (especially when compared with *Lolium perenne* for this last constituent). Lignin decreases digestibility of cell walls and some other constituents, e.g. proteins, though the total digestibility of the organic matter is better in legumes. A lower WSC content is a real disadvantage in conservation for silage. These chemical characteristics cannot entirely explain their nutritional superiority and higher intake characteristics compared with grasses. Other parameters seem to be involved (Beever and Thorp, 1996): a higher rate of particle breakdown, an enhanced rate of digestion in the rumen, a higher amount of non-ammonium nitrogen (NAN) (more microbial proteins) to the small intestine, a higher efficiency of energy utilization. Faster rates of particle breakdown and digestion of legume material in the rumen have a critical importance compared with the high fibre concentration and bulkiness of grass material for enabling a higher voluntary intake. The morphology of white clover leaves also contribute to a better intake by grazing animals compared with grass. High protein content in legumes can also be a problem. In grazing and silage, inefficient use of protein in the rumen can lead to high levels of N-based pollution. A reduced proteolysis can be a way to solve the problem. Sainfoin and birdsfoot trefoils contain significant concentrations of CT (soluble polyphenols) and these reduce the degradation of the main leaf protein (Rubisco) and to a lesser extent its solubilisation in the rumen (Min *et al.*, 2000). More non-ammonium nitrogen is thus supplied to the small intestine. When tannin contents are at 20-40 g kg<sup>-1</sup>, which are typical values for these species, they induce a better efficiency in N utilization, prevent bloat (Barry and McNabb, 1999) and reduce the negative effects of internal parasites in sheep (Cosgrove and Niezen, 2000); however, above 60 g kg<sup>-1</sup> DM, they decrease digestibility, intake and animal performance.

White clover differs from most other legume and grass species since the stems are usually not harvested; only leaflets, petioles, flowers and peduncles are grazed or cut. Leaf population is regularly regenerated. Petioles are highly digestible, while flowers and peduncles are less so but are produced mainly in summer. These characteristics result in a high and relatively stable digestibility. White clover is highly acceptable to livestock and in mixed swards or in trials where white clover and ryegrass are separated in different plots, livestock show a preference for clover. Compared with grass, grazing white clover requires less time per bite (higher biting rate) and intake per bite is often higher. Grazing time can be less than for a pure grass sward (Penning *et al.*, 1998). Animal production is usually higher in mixed swards compared with pure grass swards, and with increasing proportions of clover in the sward (Thomson *et al.*, 1985; Wilkins *et al.*, 1995; Ribeiro-Filho *et al.*, 2003). Animal performance per ha on white clover/grass swards is about 80% of those recorded on pure grass swards fertilized with 250-400 kg N ha<sup>-1</sup> (Bax and Schils, 1993; Davies and Hopkins, 1996). It is usually recognized that the target white clover proportion in the swards must average about 30% in the sward DM (40-50% in summer) (Le Gall and Guernion, 2004). Higher proportions of clover induce excessive protein contents in the forage, which requires high-energy consumption for the synthesis of urea by the animals and causes increased N excretion. Since the quality of white clover is longer lasting than grasses (Frame and Newbould, 1986), its management is more flexible (Vertès and Simon, 1992).

In silage, for an equivalent forage quality, legumes have nutritional advantages over grasses. For equal yields, red clover/grass mixtures have similar energy contents and higher crude protein contents than N-fertilized grass swards. Energy utilization is better and legume silages allow better energy gain than grass silage (Tyrrell *et al.*, 1992). Red clover/grass silage is consumed by dairy cows more than grass silage of similar digestibility (Heikkilä *et al.*, 1992). In comparison with grass silage, red clover silage

results in better animal performance in beef production (Thomas *et al.*, 1981; Vale *et al.*, 2000) and in dairy cows (Thomas *et al.*, 1985). However, in conservation, a part of the nutritional advantage of legumes can be reduced by leaf losses during the wilting and harvesting processes. In grazing, red clover gives higher dairy cow performance than grasses (Pedraza *et al.*, 1988).

#### **Anti-quality factors and other secondary metabolites**

Bloat in cattle and to a lesser extent in sheep is the main hazard associated with grazing white clover (Rumbaugh, 1984). Bloat does not occur with species with high levels of CT like sainfoin and birdsfoot trefoils. With other legume species (*Trifolium* spp., *Medicago* spp.), its occurrence is low. In New Zealand where dairy cows graze mainly white clover/grass mixtures, mortality is usually lower than 0.8% per year (Carruthers *et al.*, 1987). Several strategies can be developed to prevent it (by decreasing order of importance): a slow transition between silage feeding or pure grass sward grazing and legume-rich sward grazing, the use of legume/grass mixtures rather than pure legume swards, the distribution of a fibrous complement (hay or straw) during grazing, the use of a rumen anti-foaming agent (e.g. non-ionic poloxalene surfactant) incorporated in the feeding ration, the use of mineral oils sprayed on the sward, mixed with drinking water or in feed supplements. Since marked differences exist among animals in susceptibility to the disease, selection and disposal of chronically bloating animals in the herd is a longer-term measure. The inclusion of the genes of CT synthesis of *Lotus* spp. in clover by biotechnology would be extremely useful but consumers would first have to accept the use of genetic modification (GMO) in agriculture.

Cyanogenic glucosides (lotaustralin and linamarin) and the enzyme linomerase can be present in some cultivars of white clover and *Lotus* spp.. In cyanogenic plants, when leaves are damaged, the enzyme hydrolyses the cyanogenic glucoside and produces hydrocyanic acid (HCN), which induces asphyxiation in grazing animals, although levels of this compound and their effect are generally low. There is evidence that slugs, snails and other pests discriminate against cyanogenic varieties.

Significant infertility problems can occur in sheep grazing red clover before or during mating periods because of phyto-oestrogens (Collins and Cox, 1985), the main molecule being the phyto-isoflavone, formononetin. Symptoms are delayed rebreeding, reduced ovulation rates and reduced twinning percentage. Cattle are less susceptible than sheep. The contents of the hormone vary with cultivars and are lower in summer. Cultivars with a low content are increasingly available. When the forage is conserved as hay the risk is reduced by about 70%. Silage does not reduce the risk and could even increase it.

Legumes can contain other secondary plant metabolites, such as flavonoids. These molecules as well as fatty acids can affect the chemical and sensorial properties of sheep (Cabiddu *et al.*, 2001) and cow (Bertilsson *et al.*, 2002) milk. It seems that some forage legumes could increase the milk polyunsaturated fatty acid concentration and affect in a pleasant way the flavour of milk and cheese. However, oxidation products of polyunsaturated acids, such as *n*-aldehydes and peroxides, can produce bad flavour in dairy products (Rochon *et al.*, 2004).

#### **Nitrogen fixation**

The ability of legumes to fix N is based on a symbiosis with bacteria developing in plant galls (nodules) produced by the plants on root hairs. These bacteria are relatively specific to host legume genera: *Medicago* spp. and *Melilotus* spp. are associated with *Sinorhizobium meliloti*, *Trifolium* spp. with *Rhizobium leguminosarum* biovar *trifolii* and *Lotus* spp. with *Mesorhizobium loti* (Amarger, 2001). Atmospheric di-nitrogen (N<sub>2</sub>) is reduced by bacteria into ammonia (NH<sub>4</sub><sup>+</sup>) by action of their nitrogenase enzyme system. Ammonia is then transformed in organic products that are partially transferred to the host plant. Nitrogen is also released by senescent nodules into the soil and then reabsorbed by legume roots. Defoliation induces a decline in nitrogenase activity; complete defoliation leads to a decrease of 5 to 20% (Hartwig, 1998). Nitrogen fixation requires sufficient levels of Fe and Mo, which are constituents of the nitrogenase enzymes; it is also very sensitive to K and P availability. Low soil pH can adversely affect N fixation by heavy metal toxicity (Al notably) or by reducing P, Ca, Mg and Mo availability. High soil pH (> 7.5) on calcareous soils has similar effects by decreasing P and Fe

availability. Low and excessive temperatures reduce fixation but large genetic variability exists for the tolerance to this factor. Drought reduces the number of nodules and N fixation activity but probably confers a comparative growth advantage to legumes compared to grasses because drought reduces more soil N mineralization than N fixation. Elevated CO<sub>2</sub> concentrations in the atmosphere stimulate N fixation and increase the legume proportion in legume/grass mixtures or in complex mixed swards; these two phenomena being reinforced when P availability is improved (Hartwig and Soussana, 2001). The transfer of N from legume to grass can occur in three ways: (i) exudation of low molecular weight organic-N compounds, (ii) degradation of senescent legume organs (nodules, roots, leaves and stems), and (iii) excrements of grazing livestock, especially urine. The second and the third route are the most important in grasslands. The underground transfer is 25-50% of the total (Ledgard, 1991). The proportion of N fixed transferred to grasses seems to be highly variable; values of 13 to 34% have been cited for instance (Heichel and Henjum, 1991). Theoretically, N fixation could be improved by selection of more effective strains of *Rhizobium* or strains better adapted to specific legume cultivars; Graham and Vance (2000) have discussed potential future approaches.

Several types of methods have been developed for N fixation assessment:

i) Nitrogen yield difference (NYD). The fixing plants can be legumes in pure stand or a legume/grass mixture. The non-fixing plant can be a non-legume (usually a grass species) or a non-nodulating legume of the same species or cultivar. Both fixing and non-fixing plants are not fertilized with N. The method is based on the assumption that both fixing and non-fixing plants have the same yield potential and take up the same N amount from the soil. This may not be totally correct since the grass and the legume species have different seasonal growth patterns, total yield potential and root morphology. Moreover, the calculation of N fixation is affected by soil N mineralization; when soil N availability is high, the calculated fixation is lower compared with a soil with low N availability because the yield of the non-fertilised grass is higher in the first case but that does not necessarily mean that fixation is reduced. In most experiments, fixation is calculated on the basis of harvested yield, taking not into account the nitrogen present in roots, stubble, stolons and soil. The method tends thus to underestimate the amount fixed (Jorgensen and Ledgard, 1997). For all these reasons, the results only provide an apparent value of N fixation.

ii) Nitrogen Fertilizer Replacement Value (NFRV). The N yield of a legume is compared with the N yield of a non-fixing plant fertilized with increasing rates of N. A regression equation between N fertilization rates (X-axis) and N yields of the non-fixing plant (Y-axis) is then calculated. Since legume N yield (Y-axis) is recorded it is possible to evaluate the equivalent N fertilization rate (X-axis) for reaching this yield with a N-fertilised non-fixing plant. The same remarks as in the previous methods can be made. Moreover, high N fertilization rates can affect N mineralization from the soil.

iii) Acetylene reduction. The method is based on the fact that the nitrogenase enzyme that transforms N<sub>2</sub> into NH<sub>4</sub><sup>+</sup> in the nodules can also reduce acetylene (C<sub>2</sub>H<sub>2</sub>) to ethylene (C<sub>2</sub>H<sub>4</sub>). The technique can only measure short periods of fixation and suffers from other limitations.

iv) N<sup>15</sup> isotope-based methods. Among these methods, one technique supplies an inorganic fertiliser enriched in N<sup>15</sup> to a fixing and a non-fixing plant. The fertiliser and the atmosphere differ in N isotope abundance. The fixing and the non-fixing plants are harvested, sorted out and analysed separately by a mass spectrometer. The N fixed can then be deduced.

All the above methods have disadvantages and none is totally reliable in estimating the absolute amount of N fixed. However, some of the isotope-based techniques may be more precise.

N fixation by legume species has been estimated by a number of authors and some typical and maximum values are given in Table 2. N fixation by sainfoin can be very low. This can be due to plant characteristics (Witty *et al.*, 1983) but also to inefficient *Rhizobium* strains.

Table 2. Some examples of amounts of N fixed by temperate legumes.

Country	Species	Amount (kg N ha <sup>-1</sup> year <sup>-1</sup> )	Reference
New Zealand	White clover	Typical values: 100-350	Hoglund <i>et al.</i> , 1979; Caradus, 1990; Ledgard <i>et al.</i> , 1990.
	Birdsfoot trefoil	Maximum: 680 Maximum: 140	Sears <i>et al.</i> , 1965 Laidlaw and Teuber, 2001
Denmark	White + red clover	128-305	Hogh-Jensen, 1996
Switzerland	White clover	270-370	Boller and Nosberger, 1987
Belgium	Red clover	Typical values: 300-400 Maximum: 545	Deprez <i>et al.</i> , 2004a
Lithuania	White clover	12-113	Kadziulene, 2004
	Red clover	21-231	
United Kingdom	White clover	Typical values: 100-200 Maximum: 445	Whitehead, 1995
North America	Red clover	Typical values: 100-250	Smith <i>et al.</i> , 1985

The amount fixed is proportionate to the legume content of the sward. Vinther and Jensen (2000) estimated the amount of N (kg) fixed per ton of DM production at about 30-46 for white clover and 24-36 for red clover. Apart from water availability, flux of photosynthetically active radiation (PAR) reaching the canopy, and adequate amounts of micronutrients like Fe and Mo, fixation is affected mainly by the frequency of defoliation and N availability. Defoliation reduces fixation especially if severe and frequent. Høgh-Jensen and Kristensen (1995) showed that fixation varies according to the cutting regime. Fixation is reduced in grazing compared with cutting. External N has a depleting effect whether it is provided by soil mineralization, by organic or inorganic fertilizers or by grazing animal excrements (urine mainly). Fixation can also be reduced by specific organisms, which weaken the legume host plant.

After ploughing one- to four-year red clover/ryegrass leys cut for silage, Deprez *et al.* (2005) found an average residual effect of 63 kg N ha<sup>-1</sup> on the subsequent wheat crop but no influence of the sward age of the leys on N availability for the following crop. Clotuche (1998) recorded high values of available N after land set-asides of one year. The biomass was cut and chopped and left on the ground during the set-aside period, making 80-160 kg N ha<sup>-1</sup> available for the following crop after legume/grass mixtures and 160-260 kg N ha<sup>-1</sup> after pure legume swards.

#### Pests and diseases

Legumes are more susceptible than grasses to pest attacks. However, most of the time treatments are uneconomical and integrated pest management (IPM) is preferred including the use of pest resistant cultivars or species. White clover can be attacked by several diseases that contribute to decreased persistence, yield and quality (Clements, 1994; Clements and Cook, 1998). The ascomycete fungus *Sclerotinia trifoliorum* is often considered as the main agent responsible for the lack of persistence of red clover. *S. trifoliorum* is a necrotic fungus that attacks leaves and crowns weakened by cold winter temperatures. The swards sown in spring are generally not affected the first year, but are attacked the following years. The fungus persists as sclerotia in the soil. Clover rot caused by *S. trifoliorum* can be destructive in red clover swards throughout the world (Scott, 1984). The deterioration of red clover roots may also be caused by *Fusarium* root rot through the action of complexes of pathogenic root-rotting fungi, such as species of *Fusarium*, *Cylindrocarpon* and *Phoma* (Skipp *et al.*, 1986).

## Breeding

In the last decades, breeding efforts have produced significant advances, notably in white clover and lucerne. Many new cultivars, more productive, more compatible with grasses in grazing and more persistent have been released. Again, Mattenkleee Swiss cultivars of red clover are undoubtedly more persistent than the usual Ackerkleee cultivars. Breeding objectives for legumes in general should focus more on qualitative rather than on quantitative characters, for instance on persistence, pest and disease resistance, better early spring growth, adaptation to low and high temperatures and reduction of anti-quality factors. Sufficient seed yield will remain an important condition for marketing reasons. From an agronomical point of view, breeding effort should be intensified on red clover and previously neglected secondary species.

## Utilization

White clover in mixture with grasses is adapted to grazing. The balance between grasses and white clover and to a certain extent other legume species can be manipulated by (i) N fertilization, (ii) type of grazing animal, (iii) combination of grazing and cutting, (iv) defoliation interval and (v) grazing system. Sowing density of the legume species has little effect on the final composition of the mixture at least for white clover and for sowing rates ranging from 2 to 8 kg seeds ha<sup>-1</sup>. N fertilisation decreases legume proportion in the sward, especially when spread in spring and early summer. It has a smaller negative effect in late summer and autumn. Organic fertilizers generally favour legume species in mixtures, particularly white clover, in comparison with inorganic-N fertilizers (Nesheim *et al.*, 1990; Jeangros and Thoni, 1994). Cattle allow more white clover to develop than sheep. Reduction of clover proportion can be rapid with sheep grazing, especially in continuous grazing (Orr *et al.*, 1990). Compared with cattle grazing, the branching of stolons is low, stolons have thin short internodes, leaf size is small and the WSC content of the stolons is reduced (Jones and Davies, 1988). However, sheep grazing in autumn and early spring can have beneficial effects by reducing grass growth at those periods of slow clover growth (Laidlaw *et al.*, 1992). Goats consume proportionately less clover than sheep in similar mixed swards and so exert a lower pressure on the legume (del Pozo *et al.*, 1996; Penning *et al.*, 1997). Defoliation interval has more importance than the type of management (cutting or grazing); frequent defoliation prevents grasses from dominating white clover and this is better achieved by grazing than by cutting. Cutting late for hay is very detrimental to white clover. In a tall sward, cut or leniently grazed, the far red/red light ratio is high, and so stolon branching and number of growing points are reduced (Thompson, 1995). Trampling by animal hooves can harm the stolons. However, while grazing is better than cutting for silage, an interposed silage cut may allow restoration of stolons, for instance in June just before the main growing period of the legume (Gooding *et al.*, 1996). Continuous grazing is less favourable than rotational grazing (Curl and Wilkins, 1983) but continuous grazing in spring followed by rotational grazing in summer has proved to be an efficient system to maintain white clover in swards. Small-leaved cultivars are better adapted to continuous grazing by sheep (Swift *et al.*, 1992). Continuous grazing with cattle at low stocking rate is detrimental to white clover because the clover has to spend too much energy to extend the petioles to the top of the tall canopy (Teuber and Laidlaw, 1995).

Red clover is adapted to infrequent defoliation and is thus mainly suitable for conservation. In conservation, leaf losses increase with increased wilting; and so are higher for hay than for silage. Dry matter losses at harvest can range between 14% and 45% (Rebischung, 1963; Ciotti and Cavallero, 1979; Stilmant *et al.*, 2004; Van Bol *et al.*, 1993). Leaf loss can be minimized by reducing the number of times hay is handled in the field, by handling hay at high humidity, by using hay conditioners and by using clover/grass mixtures. Despite its low WSC, high CP contents and high buffering capacity, red clover can be successfully ensiled if wilted to a sufficient DM content. The use of an additive can also help. Mixing red clover with grasses reduces the disadvantages of the pure legume. Wheel tracking during wilting and harvesting operations or during fertilizer spreading can deplete red clover density and growth vigour (Frame, 1987), and so mechanical operations must be as limited as possible. Red clover can be occasionally grazed for short periods and preferably in rotational grazing. Late cultivars are more tolerant to grazing than early cultivars. Red clover/*Lolium multiflorum* mixtures, mainly managed for



silage production, can be grazed in early spring and thus can extend the grazing season since the mixture starts growing faster than *L. perenne* and permanent grassland swards.

Sainfoin is best used on calcareous, shallow soils and in dry climates. It is mainly adapted to cutting and for silage it has the advantage of a low buffering capacity. It can be grazed but infrequent intervals, and low stocking rates are necessary. Alsike clover can be used in short-term leys (3-4 years) in mixture with other legume and grass species, especially in wet conditions or when there is fluctuation of the soil water supply.

More research is needed to understand the potential and the requirements of *Lotus* spp. and Caucasian clover for grazing (Hopkins *et al.*, 1993; Rochon *et al.*, 2004). *Lotus* species are often not very persistent with grazing at least in some site conditions. *Lotus corniculatus* is mainly adapted to dry conditions and low-fertility soils. In contrast, Caucasian clover can be very persistent under grazing (Taylor and Smith, 1998); lamb liveweight gain was comparable to *L. corniculatus* (Sheaffer *et al.*, 1992). The integration of these species into grassland systems has still to be explored (Seguin *et al.*, 2002; Rochon *et al.*, 2004).

### **Environmental issues in legume-based systems**

Legume-based systems have genuine advantages for the efficiency of N use compared with those based on inorganic N fertilizers. Excess of N supply is avoided and the ammonium form is more abundant in the available soil N pool (Jarvis and Barraclough, 1991; Scholefield and Titchen, 1995); thus the grass component of the mixture can absorb high amounts of available N released by the system. In farm conditions, nitrate leaching losses from grazed white clover/grass mixtures are generally much smaller than those from highly N-fertilized grass swards but stocking rates are usually lower in legume-based systems. Despite these theoretical advantages and encouraging survey results, several experiments have suggested that the two sward types release similar amounts of nitrate at comparable levels of animal production per hectare (Cuttle *et al.*, 1992; Tyson *et al.*, 1997; Hooda *et al.*, 1998) but other experiments (Ledgaard *et al.*, 1999) found that leaching in a clover/grass system relative to a 200N grass system varied from 50% to less than 30%. Nitrate leaching after ploughing of clover leys can be high and incompatible with the protection of water quality if specific measures for limiting losses are not taken (Djurhuus and Olsen, 1997). Spring ploughing instead of autumn ploughing is one of the most efficient of these measures.

The main environmental advantage of legume-based systems is probably the reduction of fossil energy that is necessary to synthesize inorganic N fertilizers. Pimentel *et al.* (2005) compared a conventional cash-grain system (5-year crop rotation) with a typical livestock system in which grain crops were grown for cattle feed and legume leys used for animal feeding and as a source of nitrogen. Fossil energy inputs were about 30% lower in the second system and this decreased the emissions of CO<sub>2</sub> to the atmosphere.

Legumes are undoubtedly a better source of pollen and nectar for some insect species than grasses and legume flowers are more attractive for the landscape. However, many legume-based swards are too dense for wildlife, notably birds that cannot use them as a breeding and a feeding cover. Lucerne is a notable exception being permeable to bird circulation and providing arthropods and nutritious leaves, a shelter against predators and a good nesting place if cut late (Peeters and Decamps, 1998).

### **Conclusions and prospects**

Two main attributes of legumes should be better exploited in future agriculture: their capacity for N fixation and their high nutritive value and intake potential. These characteristics can reduce production costs and thus increase farmers' income (Fau, 1993; Frame *et al.*, 1998; Pecetti and Piano, 2000; Rochon *et al.*, 2004). In particular, legumes can play an essential role in extensive systems in order to reduce N fertilization while maintaining sward yields at an acceptable level. They are also one of the pillars of organic systems. They have however a main shortcoming, a lack of persistence. For grazing, the occurrence of bloat should be minimized by the introduction of the genes of CT synthesis within the genome of clovers. For conservation, there is still a need for the development of techniques that can

minimize leaf losses and control buffering capacity, ammonia content and development of undesirable micro-organisms.

The main positive effect of legumes for the environment is the reduction of fossil energy use and consequently of the emission of CO<sub>2</sub> to the atmosphere. In certain conditions, legumes (especially lucerne) can be attractive for wildlife and improve the landscape while N emissions from legume-based systems have to be well controlled by management. Future research should focus on N fixation and utilization efficiency for legumes other than white clover (Rochon *et al.*, 2004) and the impact of legume-based systems on the environment compared with other systems should be monitored by a set of reliable indicators.

Sustainable systems of animal production based on white clover/grass mixtures have been developed for beef cattle, dairy cows and sheep, but for livestock producers replacing grass by legume/grass swards, is a question of trade-offs between performance per head and performance per hectare. Changes in the European CAP could encourage a greater adoption of legume-based grazing systems. However, this would be a fundamental modification of forage-based systems of livestock production and in many cases would imply a complete reversal of past practices: greater extensification, an extension of the grazing season, reduction in the conservation of fodder resources, a decrease of concentrate use per litre of milk and the introduction of new management practices. In dairy production, the quest for increasingly high production per cow would not be compatible with these new goals. Sustainable systems relying on persistent swards need to be developed and evaluated. Sufficient income should be achieved by the decrease of production costs through reducing: (i) manpower, mainly by extending the grazing season, (ii) inorganic N fertilizer use on grasslands and in grassland/crop rotations, (iii) the housing period and the proportion of conserved feeding in the total diet, (iv) concentrate per kg output thanks to a better intake of legumes compared with grasses, and through improving (i) conservation of legumes, (ii) persistence (disease resistance, competitiveness), (iii) overall herbage quality, which leads to increased intake. Getting higher prices for animal products thanks to organic or other sustainable farming labels can also increase income. Research needs therefore to evaluate the benefits for the environment and for consumers of food produced from low-input legume-based livestock systems (Rochon *et al.*, 2004).

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# Role and potential of annual pasture legumes in Mediterranean farming systems

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## Abstract

This paper reviews the current use of self-regenerating pasture legumes in the farming systems of southern Europe. Annual legumes are mainly the base for the improvement of low quality native pastures in extensive farming systems. Traditional pasture legumes such as subterranean clovers and annual medics are the base plants of Mediterranean pastures, and are essential for pasture improvement of semiarid zones. The role of a recent second generation of annual pasture legumes is discussed. Some key agronomic aspects and the level of forage production and forage quality are considered. The constraints related to the low persistence of introduced materials and sward management are examined. The potential of using pasture legumes in simple and complex legume-legume or grass-legume mixtures is analysed. The implication of legume-rhizobia symbiosis is also briefly touched upon. Finally the alternative uses of annual self-reseeding legumes are listed.

Keywords: legume pastures, pasture legume biodiversity, grass-legume mixtures, pasture legume-based management, alternative uses.

## Introduction

In the Mediterranean pastures of semiarid areas, annual self-regenerating legumes play a special role as pasture species for their forage quality and aptitude to fix atmospheric nitrogen in symbiosis with their root-nodule bacteria, increasing soil fertility and assisting the nutritional needs of other plants (Crespo, 1997). Spontaneous annual legumes are an important component of Mediterranean pastures and their survival is strictly linked to their self-reseeding ability. We will refer to annual pasture self-regenerating legumes that show the adaptive advantage of having an annual cycle combined with a “seed escape” habit. To cope with the high environmental variability, both climatic and pedologic, under the ancient tradition of grazing utilisation, Mediterranean self-reseeding legumes have developed specific strategies to ensure adaptation and reproduction. Complex biological and ecological mechanisms involving seed yield and soil seed bank dynamics allow long-term regeneration of perennial-like stands. Such reasons make annual legumes from the Mediterranean basin a valuable source of germplasm. These species were introduced to other Mediterranean-type areas, and their naturalisation and diffusion had a remarkable impact on the new environments, particularly in Australia where annual legumes have effectively contributed to sustaining and increasing cereal and animal production (Cocks, 1999). Moreover, the Mediterranean seed market for annual pasture legumes has been heavily reliant in recent decades on the Australian selections from germplasm often originating from the Mediterranean basin.

## Annual self-reseeding legumes and farming systems

Because of the large number of species and the relevant diversity of their adaptive characteristics, annual self-regenerating legumes have the important potential role to be utilised in the widest range of environmental conditions and relevant farming systems of the Mediterranean areas (Piano and Talamucci, 1996). In response to the high environmental variability and local rural traditions there is a wide range of farming systems typologies in the Mediterranean regions of Europe (Porqueddu and Sulas, 1998). Extensive livestock production systems with integral open-air grazing during much of the year are prevalent under rainfed conditions. Annual legumes are mainly the base for the improvement of low quality native pastures in agro-silvopastoral (e.g. Dehesa in Spain) and agropastoral systems. When

a natural seed bank of pasture legumes is present, fertilisation without overseeding may be sufficient to obtain satisfactory agronomic results, particularly when repeated over several years (Bullitta *et al.*, 1989). It is likely that the seed bank of annual pasture legumes in a natural unfertilised pasture is not quantitatively sufficient to guarantee a prompt response to P soil enrichment, but “domestication” of structured wild populations under less oligotrophic conditions is often agronomically more reliable than the introduction of selected uniform varieties (Yañez *et al.*, 1991; Roggero and Porqueddu, 1999).

The annual self-reseeding pasture legumes are the key component of the ley farming and ‘phase farming’ system supporting wheat/wool industry on millions of hectares in southern Australia. The traditional ley farming is based on an annual cereal/pasture legume rotation while the second one relies on longer phases of cropping between pasture periods. Phase farming differs from ley farming in the requirement to re-sow the pasture after each cropping phase while pastures in ley farming are only rarely re-sown. In the last thirty years the annual self-reseeding legumes have also been increasingly utilised in Mediterranean Europe. However, commercial varieties of annual self-reseeding legumes imported from Australia sometimes proved unsuitable for pasture improvement in southern Europe mainly due to the different climatic conditions and management systems (Olea and Viguera, 1999). In many cases the agronomic performance of the introduced legumes under both field trials and farm conditions were satisfactory the first few years after sowing, but subsequently, the contribution of sown varieties to the forage yield often decreased as native species became more competitive. Fara *et al.* (1997) found that native ecotypes were, on average, more persistent and better adapted than commercial varieties. These reasons have stimulated different European research institutes to carry out eco-geography studies and selection programmes aimed at the valorisation of local germplasm (Piano and Francis, 1993, Loi *et al.*, 1995). In Spain and Italy seven and five varieties of *Trifolium subterraneum* L. *sensu lato* (subterranean clover) respectively were selected in late '80 and '90 (González, 1994; Piano *et al.*, 1997). New varieties of *Medicago polymorpha* L. (burr medic) were also selected in Italy (Porqueddu *et al.*, 1999) and France (Prosperi *et al.*, 1999). Unfortunately, despite these new varieties proving to be superior than Australian ones, the lack of a European seed multiplication has not allowed their diffusion except for a few Italian varieties of subclovers multiplied in Australia.

### Traditional pasture legumes

There is a marked association between distribution in nature and performance in agriculture of legume species and soil properties, related to the nature of parent rock, soil texture, soil chemical characteristics and associated differences in morpho-physiology of the species (Piano and Francis, 1993).

Subclovers (*T. subterraneum* L., *T. brachycalycinum* Katzn. and Morley, *T. yanninicum* Katzn. and Morley) are the base plants of Mediterranean pastures, essential in any pasture improvement of semiarid zones with acid, neutral or lightly alkaline soils (Frame *et al.*, 1997). Their importance relies on their good winter growth, high reseeding capacity, perfect adaptation to grazing due to their prostrate habit, ability to bury the seed heads in the soil, and excellent persistence. They grow well in moderate continental and semicontinental climates, either warm or temperate. The most favourable levels of rainfall are between 400 and 1000 mm. In terms of soil adaptation, *T. brachycalycinum* prefers clay, weakly acid or lightly alkaline soils whereas *T. subterraneum* prefers sandy acid soils. *T. yanninicum* is especially adapted to hydromorphic or flooded soils. There are around 40 varieties available in the seed market with a wide range of growing season length and hardseededness.

Annual medics (*Medicago spp.*) are the principal component of pastures in alkaline or lightly acid soils in areas of low and medium rainfall (250-600 mm). Medics show a high potential for seed and forage production, as well as producing a high percentage of hard seeds. These species are better adapted than subclovers to hard and clay soils, since they do not have to bury their seed heads. The dominant species is *M. polymorpha*, due to its adaptation to a wide range of edaphic conditions, from acid to alkaline and from sandy-loam to clay soils (Loi *et al.*, 1995). *M. murex* Willd. (murex medic), is tolerant to acid soils (from pH 4.5 up to alkaline soils) while *M. truncatula* Gaertn. (barrel medic) and *M. scutellata* Mill. (snail medic) grow well in heavy soils, neutral to alkaline. Other less minor commercial species are: *M. rugosa* Desr. (gama medic), *M. littoralis* Rhode (strand medic), and *M. tornata* Mill. (disc medic). A total of about 30 annual medic varieties is commercialised.

Yellow serradella (*Ornithopus compressus* L.) is another species widely distributed in the Mediterranean regions and recognised to be successful on acid and sandy soils, especially in granitic soils where subclovers and medics are not productive. It grows best in well drained soils but does not tolerate waterlogged conditions.

### **A second generation of pasture legumes**

Despite the unequivocal success of the southern Australian ley-farming systems, several contemporary factors have challenged their sustainability (Howieson *et al.*, 2000a). These are mainly associated with the excessive dependence on herbicides, incomplete use of water and nutrients by shallow-rooted annual species, and the emergence of new pests and diseases. In addition, the increased sensitivity to the costs, both economic and environmental, associated with suction harvesting of medics and subterranean clovers for seed production has had an impact on their popularity. These limitations led to a serious re-examination of the pasture legume components required for contemporary ley farming, and this resulted in the recognition that alternative legume species with different traits were probably required (Loi *et al.*, 2005a). Traits sought in the new species are deeper root systems, improved persistence from higher production of hard seed, acid tolerant rhizobial symbioses, tolerance to pests and diseases and ease of harvesting with conventional cereal harvesters. Several cultivars of a second generation of annual pasture legumes are now commercially available, e.g. biserrula (*Biserrula pelecinus* L.), French serradella (*Ornithopus sativus* L.), gland clover (*T. glanduliferum* Boiss) and improved varieties of arrowleaf clover (*T. vesiculosum* Savi.), balansa clover (*T. michelianum* Savi.), Persian clover (*T. resupinatum* L.) and yellow serradella, and have been rapidly adopted (Loi *et al.* 2000). Some other species are in an advanced phase of evaluation and are close to being released as commercial cultivars, e.g. *T. formosum* d'Urv. (eastern star clover), *T. spumosum* L. (bladder clover), *T. hirtum* All. (rose clover), *Melilotus albus* Medik (*white sweet-clover*), *Trigonella balansae* Boiss. and Reuter (trigonella) and *Lotus ornithopodioides* L. (annual birdsfoot trefoil). The availability of a wider range of species for Mediterranean agricultural systems in Australia are of benefit to farmers there, who can thus choose from a wider range of species with different characteristics. The question posed by pasture specialists is whether or not these alternative pasture legumes are really of interest to European farmers. Some of the traits considered important for ley farming systems do not seem so relevant for establishment of permanent pasture in southern Europe, e.g. insect tolerance, high hardseededness. Preliminary results on the evaluation of these alternative pasture species in the Mediterranean basin are contrasting (Campiglia *et al.*, 2005; C. Porqueddu, unpublished data; A. Franca, pers. comm.). Pure swards of several new Australian varieties have shown difficulties in establishment and re-establishment and lower forage production compared to subterranean clovers that produce higher levels of soft seed and consequently are able to compete against the native flora, usually richer than the Australian ones.

### **Key agronomic aspects and productivity**

The adoption of legume-based grazing systems depends on farmers feeling confident about growing and utilising pasture legumes and this depends on overcoming the difficulties related to sward establishment, maintenance and management under grazing.

#### ***Establishment***

The success of pasture establishment strictly depends on a good sowing method. Seed must be sown superficially, never deeper than 1 cm, and the soil consolidated by rolling, which is the key to provide a protection against ants and birds. Sowing should be carried out in late summer or at the beginning of autumn, and if possible also under dry soil conditions before the first rains or immediately after the season break, when warm temperatures allow good germination and fast establishment of legumes. It is very important to reach at least 200 plants m<sup>-2</sup> to assure a good establishment of the improved pasture.

### **Fertilisation**

Mediterranean semiarid pastures are mainly located on soils which are deficient in phosphorus (P) and nitrogen (N), even with shortcomings of potassium (K) in some areas. The needs of N are covered by the legume-rhizobia symbiosis, although a low rate of N (less than 30 kg ha<sup>-1</sup>) could be beneficial in the first stages of seedling development. Therefore, the needs for P are the most important, its addition to the soil being an essential requisite for the correct establishment of annual legume-based pastures. The first fertiliser should be applied during seed-bed preparation before sowing depending on soil P content while, in successive years, P fertilisation should be applied on the soil surface, after the first autumn rains, at around 30-40 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>.

### **Forage production**

The most productive and essential species in any pasture improvement scheme are subclovers. Yields depend upon the specific growing conditions, but especially rainfall during the growing season, Bolger *et al.* (1993) found a linear relationship between water use and dry matter (DM) yield up to 440 mm of growing-season rainfall with yields ranging from 3 to 12 t ha<sup>-1</sup>. Most evaluations are made under a mowing regime that underestimates the potential yield of pasture legumes, particularly for species with a prostrate habit, such as subclovers. Pasture improvement does not only mean an absolute increase of DM, but also implies an increase of pasture production in critical periods (autumn, winter and late spring), which leads to a reduced need for complementary feedstuffs. Gloag *et al.*, (2004) found that *T. vesiculosum* extended the growing season by 3-4 weeks, had higher digestibility than *T. diffusum* Ehrh. (diffuse clover) and *T. subterraneum* in late spring, and could increase lamb live weights in late summer by more than 10%.

### **Persistence**

In the presence of low persistence all other characteristics of success, including productivity, are meaningless. In order to obtain sward persistence it is fundamental to take into account certain considerations, because annual legumes will not remain in the pasture if the following situations occur: i) incorrect election of species and varieties ii) nutritional problems (mainly P deficiency) iii) incorrect sward utilisation (e.g. overgrazing). Apart from these main causes, there can be other factors which account for the lack of persistence, such as mature seeds being damaged by a plague of insects (Fara *et al.*, 1997). An important buffer mechanism against adverse abiotic factors or a flexible sward spring management is a long duration of the flowering phase and the ability to keep flowers and ripe pods at the same time. Many factors are responsible for the persistence but a high seed bank is fundamental for a successful use and long term persistence of annual dominant Mediterranean grassland types. Although winter grazing is advantageous to seed production, avoiding excessive predation of seed by livestock grazing stubble is also a critical aspect in legume persistence. The persistence is not only related to the seed yield but also to the pod and seed characteristics of each pasture legume e.g. seed size, hardseededness, softening pattern, seed dispersal capacity and seed burial ability. Seed size influences the persistence of pasture legumes in several ways. Larger seeds emerge from greater depths than smaller ones but are less likely to survive ingestion by sheep. Moreover small-seeded species guarantee a dense population of prostrate, small seedlings having higher survival rates and maintaining an adequate photosynthetic area than a sparser stand of large seedlings. The softening of hard seeds differs between and within species, and in many situations the level of hardseededness may be less important than the softening pattern (Porqueddu *et al.*, 1996).

### **Qualitative aspects**

Annual pasture legumes have a high feeding quality, determined by high crude protein (CP), mineral and vitamin contents, low proportion of cell walls and high animal intake levels. Forage quality in general was not included in most of the Australian legume breeding targets. However an emphasis was put in to selecting varieties with low oestrogen levels. The nutritive value of annual pasture legumes can

vary between species, cultivars and plant organs according to the stage of development. Late maturing cultivars maintain higher DM digestibility and contain more essential nutrients than early maturing cultivars during late spring. Moreover, the differences in nutrient concentrations among cultivars within the same species are sometimes of the same magnitude of the differences among species. The main criteria of selection to improve feeding value in annual medics were suggested by Porqueddu (2001).

Relevant differences among annual legumes in terms of palatability have been identified. Specific indexes (SI, score from 1 to 5) have been attributed to define the “palatability” of a single pasture species in different environments and an inventory of SI has been made available by Roggero *et al.* (2002). Nevertheless high relative palatability may lead to an excessive selective grazing pressure for the species and its subsequent demise. The selection and use of varieties with limited and/or temporary-limited palatability suggested by some authors (Kellaway *et al.*, 1993) is a useful strategy for the varieties’ survival in a mixed sward or it may be used as a tool for controlling undesirable weeds under heavy grazing with high stocking rates, e.g. use of *T. formosum* (Loi *et al.*, 2005b).

The nutritive value of the whole plant is related to the quality of the individual plant parts and their proportions in the sward. Little attention has been given to stem thickness, which is also associated to variable plant habit. Prostrate and erect plants differ in the physical distribution of structural components (e.g. lignin) so that prostrate stems are less rigid and have higher digestibility. Differences in stem digestibility are usually associated with variations in numbers of vascular bundles. The stem anatomy of six annual legumes (*M. murex* cv Zodiac, *T. subterraneum* L. cv Junee, *T. michelianum* Savi cv Paradana and *T. resupinatum* L. cv Kyambro, Maral and SA12240) was examined. It was found that *M. murex* had a very high vascular bundles density mm<sup>-2</sup> of stem tissue, which also resulted in a poor performance under grazing (Kellaway *et al.*, 1993). Therefore, species with stems of higher nutritive value are of major interest as this is the part of the plant most available to livestock in late spring and summer.

Regarding anti nutritional factors, coumestans, which induce temporary infertility in ewes, are especially present in annual medics. In contrast to oestrogenic activity of formononetin in subterranean clover, which ceases as the plant matures and dries, cumestrol in medics increases with maturity and reaches a high level in dry pastures. Intraspecific genetic variations in the content of oestrogenic substances enabled cultivars to be selected for reduced estrogenic activity or for its absence. It has also been observed that plants under stress, induced by deficiencies in nutrients or biotic diseases, increase estrogenic activity. If grazing sheep have a varied feeding regime, the infertility problems may be avoided.

Annual pasture legumes are an important feed resource not only as green forage during the growing season but also as standing hay and pods in summer and early autumn (Fois *et al.*, 2000). Pods of annual medics are an important component of the diet of sheep during the summer dry period. Medics with an appropriate spring management can produce over 1 t ha<sup>-1</sup> of seed (Lelievre and Porqueddu, 1994) or 3 t ha<sup>-1</sup> of pods with high CP content (Sitzia and Fois, 1999). However because of easy consumption of medics pods by sheep, grazing management control is needed during summer to avoid impoverishment of the seed bank.

### **Sward management**

Grazing is a fundamental tool for enhancing sward persistence and productivity, but, as pointed out in a recent review by Rochon *et al.* (2004), there is still limited scientific information regarding grazing management of legumes in Mediterranean basin areas. Lightly grazed pastures are likely to become grass dominant, while heavy grazing increases the proportion of legumes in the sward, especially of species with very prostrate habit (e.g. subclover, murex medic). In autumn it would be advisable to initiate grazing at least one month after the first rains, so that seedlings can well establish or re-establish and grow before low temperatures occur. During winter, until the beginning of the flowering period, intensive grazing is useful to favour legume growth. Contrastingly, in spring, during flowering and seed development, grazing should be limited to stocking rates below sward potential, thus allowing a suitable production of new seeds to rebuild the seed bank. During summer, grazing allows animals to eat the excess of pasture and also favours pod burying, increased seed softening, and autumn seedling emergence. Regarding grazing methods, Sitzia *et al.* (1997) found higher forage availability under

rotational grazing than continuous stocking in a mixed sown pasture of *Lolium rigidum*, *T. subterraneum* and *M. polymorpha*, but ewes' body weight and condition scores, as well as total milk yields, were similar between the two grazing techniques.

### Use of legumes in mixtures

A productive and balanced pasture sward, able to persist under marginal conditions, can be obtained using subclover mixed with other species and cultivars well adapted to the local conditions. But the difficulties found in the establishment and persistence of these pastures during adverse climatic years have led to the conclusion that the more diversified the seed mixture, the greater likelihood of getting a productive, balanced and persistent pasture (Crespo, 1997). Likewise, Tilman and Downing (1994) already affirmed that the more diverse the botanical composition, the stabler the pasture during drought conditions. This is the main reason why other annual legumes (e.g. *O. compressus*, *B. pelecinus*) present in natural pastures (Moreno and Gallardo, 1983), are utilised for sward improvements in the SW of the Iberian Peninsula, together with other less abundant species (e.g. *T. michelianum*, *T. resupinatum*). Traditionally, pasture seed mixtures available in southern Europe consisted of a small number of legume species or 4-5 subclover varieties mainly differing in earliness, and sometimes including low rates of annual grasses such as *L. rigidum* Gaudin (annual ryegrass), *L. multiflorum* Lam. (Italian ryegrass), *Avena sativa* L. (common oat). Nowadays, it is common to find complex mixtures including up to 20 annual self-regenerating legumes. In some cases e.g. pasture improvement in areas with higher rainfall, perennial grasses, such as *Dactylis glomerata* L. (cocksfoot), *Phalaris aquatica* L. (bulbous canarygrass), *Festuca arundinacea* Schreber (tall fescue) are utilised in grass-legume mixtures. The compatibility of perennial grasses and annual legumes when grown in mixtures has been reviewed by Dear and Roggero (2003), who discussed particularly the effects of perennial grasses on emergence, survival and seed set by annual legumes. Encouraging results have been obtained by Porqueddu and Maltoni *et al.* (2005) evaluating 4-species mixtures belonging to different functional types as grass/legume and fast-annual/slow-perennial establishing species (*L. rigidum*, *D. glomerata*, *M. polymorpha* and *M. sativa*) in different proportions, within the common activity of the COST Action 852 (2002). Compared with pure stands, grass-legume mixtures provided higher yields with better seasonal distribution and decreased unsown species presence. Moreover, the constraints related to management of perennial grass-annual legume mixtures under grazing at a farm scale are being studied in the PERMED (2005) project.

It is very important to determine the compatibility of the species to be utilised in the mixtures. According to several authors subclover used in mixture with annual medics and *T. michelianum* tends to dominate from the second year of establishment (Taylor and Rossiter, 1974; Dear *et al.*, 1999; Porqueddu *et al.*, 2004). Some studies are being carried out at the SIDT station of Extremadura, Spain, using the same number of seeds for each species (i.e. *T. subterraneum*; *T. cherleri*; *T. glomeratum*; *T. striatum* L. (knotted clover); *M. polymorpha*; *O. compressus* and *B. pelecinus*, up to a whole of 20 kg ha<sup>-1</sup>). The results show the dominance of *M. polymorpha* and *T. subterraneum*, suggesting that it is necessary to modify seed proportions taking into account seed vigour, and controlling the most vigorous species in the first stages by means of grazing (González, pers. comm.). Seed proportion in a species mixture is fundamental to get a balanced mixture. Advantages may be achieved combining seeds of different size (small and large seeds), hardseedness levels and softening patterns with the aim to guarantee long term pasture regeneration at satisfactory productive levels and to reduce inter- and intra-annual fluctuations. Moreover, every species colonises a different ecological niche so that the inclusion and ideal combination in mixture of new pasture legumes with traditional species, could be used to cope better with unpredictable climatic fluctuations and the extremely high heterogeneity of environmental and farming system situations (from large pasture areas to small single field scale).

### Legume-rhizobia symbiosis

N-fixation in nodulated pasture legumes is fundamental for the economic and environmental sustainability of Mediterranean farming systems. In the Mediterranean basin soils where rhizobia are usually widespread their importance has been overlooked, while contrastingly, in-depth studies and

selection programmes have been carried out in new Mediterranean environments such as southern Australia, where the lack of adequate native rhizobia strains makes inoculation strictly necessary. Inoculation with suitable rhizobia is recommended, since there can be rhizobia shortcomings in soil, e.g. introduction or re-introduction of sown pastures into cereal crop fields, high acid soils. Nevertheless, in the Mediterranean basin there is a strong body of evidence that despite co-evolution of legume and rhizobia, the relationship is not always optimal (Howieson *et al.*, 2000b). Moreover, the commercial seed imported from Australian producers is often inoculated by the local seed companies or they suggest and provide inoculants also produced in Australia. However, there is no practical evidence on the effectiveness of such new inoculant strains and there is poor scientific knowledge on the interactions with natural populations of rhizobia and the fate of the introduced strains (Sulas, 2005).

The quantity of N fixed by Mediterranean legumes differ widely between species and environments (Unkovich and Pate, 2000). There is the need to determine the amount of N-fixation by pasture legumes at a local level for the development of appropriate agronomic techniques under both conventional and organic farm conditions. For example, in Sardinia, Sulas and Sitzia (2005), using the  $^{15}\text{N}$  dilution method, estimated fixed N of 52 and 154 kg ha<sup>-1</sup> for grazed mixtures and a harvested pure stand of burr medic, respectively.

#### **Alternative uses of annual self-reseeding legumes**

Annual self-regenerating legumes tend to be more and more used in non-conventional systems of southern Europe for sustainable environmental improvement. During the last decade they have been increasingly used as cover crops in vineyards, olive groves and orchards allowing low impact soil management alternatives to traditional tillage and herbicide application (Porqueddu *et al.*, 2000). Self-regenerating cover crops cope with many functions: reduce erosion risks and summer evaporation throughout the dead mulch, avoid the costs associated with tillage, improve soil fertility, increase soil carrying capacity for agricultural machinery and can be also used to reduce the vigour of vines. In many situations sward mowing can be substituted by sheep grazing making management easier and more economical. Subclovers and annual medics have been tested successfully in several Mediterranean regions (Masson and Gintzburger, 2000). Although they compete quite effectively with weeds it seems necessary to re-sow the cover crop in 3 to 4 years to ensure legume dominance or alternatively setting up more appropriate management techniques (e.g. minimum tillage in autumn) and using legume or grass-legume mixtures (Nieddu *et al.*, 2000). Subclover has been also proposed for use in cereal crops as living mulch, then subsequently used as cover crop and green manure (Campiglia *et al.*, 2000). Moreover, several varieties of subclovers proved suitable for understorey oversowing to improve forage quality and quantity in agroforestry and silvopastoral systems (Papanastasis, 1998). Pasture legumes have been also used to develop management systems of fire-break lines with grazing for fire prevention in some woody areas (Etienne, 1996; Caredda *et al.*, 2002).

Some annual legumes can be utilised as pioneer species for land rehabilitation and to restore biological activity in degraded sites (e.g. quarries, mine sites, roadsides). Preliminary results comparing native and commercial accessions are promising (Maltoni *et al.*, 2005) e.g. *O. sativus*, *T. campestre* Schreb. (hop clover) and *M. indica* (L.) All. (yellow sweet-clover) performed well on acid sandy soils.

#### **Prospects for annual pasture legumes**

The use of annual pasture legumes in the Mediterranean agro-pastoral and cereal-based farming systems increased in recent decades due to the rapid conversion of many farms to organic management, despite the general decrease of forage legumes across Europe. However, many farmers recently decided to go back to conventional farming as a result of new European Union Directives, more restrictive in terms of subsidies for organic farming. At the same time seed demand for pasture legumes increased in Australia pushing up the seed price, and consequently seed demand for pasture legumes in some Mediterranean regions of Europe fell in the last two years. Moreover, the real prices for animal products are declining (e.g. milk ewe in Sardinia), reducing the product:inorganic-N fertiliser price ratio. Decoupling payments from production-related activities to agri-environment opportunities will reduce the attraction of growing arable crops such as cereals. This will encourage Mediterranean farmers to seek ways of

reducing costs, associated with the use of inorganic-N fertilisers, soil tillages, purchased feed, and to increase their reliance on low-cost and low-input grassland systems with an associated important potential role of legume-based natural or sown permanent pastures. Because farmers in low-input systems cannot afford expensive seed, the easiness of seed harvesting is an essential trait for the development of new pasture legume species. Moreover, the ease of harvesting may play a major role in their adoption because, as in Australia, seed production can be achieved locally at the farm scale with conventional cereal harvesters. This may require the valorisation of new native pasture legumes with different traits to those possessed by the currently commercial materials, e.g. legumes with erect habit that set heads on the top of the plants such as *T. nigrescens* Viv. (ball clover). But at the same time a successful development of a European seed industry for Mediterranean pasture species is needed to make effective the selection activities carried out by several public research institutions. On the other hand, more efforts in on-farm experimentation and knowledge transfer to farmers about the correct incorporation and management of annual legumes is necessary for their full exploitation.

There is a great potential for the use of mixtures (i.e. only different annual legumes, annual grass-legumes, perennial grass-annual legumes) in forage and non-conventional systems. However, further studies should be aimed at investigating the adaptation mechanisms of annual legumes to associated companion grasses and the effects of management factors on the maintenance of a balanced sward botanical composition over time.

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# Lucerne breeding in Europe: results and research strategies for future developments

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## Abstract

Lucerne breeding efforts in Europe are analysed from 1995 to the present considering three main topics: (i) germplasm collection, conservation and evaluation; (ii) genetics, breeding and genetic transformation; (iii) forage quality. The importance of European germplasm resources has been clearly evidenced with special interest for material which could be useful to add new traits such as grazing tolerance and resistance to stresses to cultivated lucerne. The results of genetic research and breeding activities are more relevant in the area of modern biotechnologies and basic research than in the area of variety development, the most recent varieties being characterized by a forage production which is, on average, only 5% higher than old varieties and adapted ecotypes. The reasons for this slow progress are discussed. Transgenic technology is giving interesting results but, at the moment, the negative attitude of European public opinion toward genetically modified plants and the possibility of gene flow from transgenic lucerne to cultivated or wild populations make it difficult to hypothesize the release of genetically modified lucerne in the near future. An interesting evolution of lucerne breeding has been evident in the last few years from the efforts aimed at creating more digestible varieties with high agronomic performances.

Keywords: germplasm, genetics, breeding, genetic transformation, selected traits.

## Introduction

Cultivated lucerne (*Medicago sativa* L. subsp. *sativa*,  $2n=4x=32$ ) is a species native to the Middle East and the most common forage species in the world. In a large part of Europe, it is the most important forage legume, and is grown for hay, dehydrated forage, pellets and silage. Lucerne is also especially important for restoring soil fertility: with no cost for nitrogen due to N-fixation by *Rhizobium*, the residues increase soil organic matter, the root system mobilizes nutrients deep within the soil profile and the soil structure, permeability to water and water retention are improved. Furthermore, lucerne only requires low inputs of herbicides and pesticides and no N fertilisers. This agrees with EU policies and public concern about the environmental impact of agricultural activities. Lucerne hay fields were also shown to be highly favourable to biodiversity enrichment. Its high protein content meets the requirements of the feed market, particularly after the problems caused by the use of concentrates of animal origin, presently forbidden in the EU (Torricelli *et al.*, 2003).

In approaching the general theme of the review, papers published since 1995 have been divided into three main topics: A. germplasm collection, conservation and evaluation; B. genetics, breeding and genetic transformation; C. forage quality. Even if several papers could be included into more than one topic, we collected 26 papers in A, 119 in B and 23 in C. We subdivided the large B group into: B1. genetics and cytogenetics (36 papers), B2. breeding, variety development, identification and evaluation (65 papers) and B3. genetic transformation (18 papers). In the following pages, we summarize what was obtained.

## **A. Germplasm collection, conservation and evaluation**

With regard to this topic, the experiences of French and Spanish researchers working on natural Spanish populations of lucerne named Mielgas deserve particular interest. Stock rearing is a traditional component of Mediterranean agriculture systems and nowadays it also represents the best way to valorise population-drained marginal areas for agriculture, landscape maintenance and spare-time activities (Delgado Enguita, 1989; Ben Chaâbane, 1997; Prosperi *et al.*, 1999). As a consequence, plant breeding activities in these areas must be targeted to obtain lucerne varieties adapted to grazing and resistant to environmental constraints. A prerequisite to reach the target is the collection and evaluation of local germplasm. Since the second half of the eighties, more than 100 natural populations of Mielgas have been collected from Spanish pastures and evaluated under both pasture and cutting managements. Most populations were characterized by a prostrate habit and a great ability of soil colonisation. They showed a wide variability for growth rhythm (for example, the first cut Dry Matter Yield, DMY, ranged from 39% to 75% of the total DMY, depending on the accession) and, on the whole, better persistence with respect to the control varieties, fast spring growth and interesting seed yield.

The different behaviour of the Spanish natural populations with respect to the maintenance of their own distinctiveness when grown in close proximity to lucerne crops established with seed belonging to cultivated ecotypes or bred varieties makes the issue of gene flow from cultivated to wild plants of critical importance. The effects of gene flow have been studied with the use of quantitative traits, allozymes and molecular markers (Jenczewski *et al.*, 1999). Some Mielgas populations appeared to be intermediate between wild populations and cultivated ecotypes; as a consequence, gene flow from cultivated to wild populations is clearly present, resulting in the formation of hybrid populations. Some other populations seem to be able to maintain themselves completely separated from the neighbouring cultivated ecotypes as a probable consequence of time, spatial and reproductive isolation. Differences among natural and cultivated populations are more evident for agronomic traits than for neutral markers. This can be the consequence of a natural selection pressure that, at least in some locations, is acting against the gene flow which tends to transfer agronomic traits typical of cultivated material to natural populations. On the whole, Mielgas germplasm is very interesting for the presence of different positive traits such as prostrate growth habit, persistence, tolerance to water stress, a good level of tolerance to stem nematode and interesting seed production, but needs to be selected at least for total DMY and for vegetative growth in autumn. This work evidenced the possibility of gene flow from cultivated to wild material, possibility which deserves great attention if we consider the feasibility to introduce transgenic varieties of lucerne in open fields.

Evaluation of lucerne germplasm for agronomical and physiological traits has also been conducted in Spain for the Tierra de Campos ecotype (Fombellida, 2001), Greece (Vaitsis, 1999), Czech and Slovak Republics (Drobna *et al.*, 1999), Italy (Piano *et al.*, 1996; Torricelli *et al.*, 2003), France (Crochemore *et al.*, 1998) and Bulgaria (Keretikova *et al.*, 2003). An overview of the current state of lucerne genetic resources in Europe has been recently presented by Meglic *et al.* (2003). Last but not least, since the second half of the nineties more and more interest has been devoted to the characterization of lucerne genetic resources by means of molecular markers (Veronesi *et al.*, 1997).

## **B. Genetics, breeding and transformation**

### ***B1. Genetics and cytogenetics***

A basic research area implemented in the last decade has been the one devoted to the analysis of reproductive mutants with possible interest for lucerne breeding (Barcaccia *et al.*, 1996; 2000; Tavoletti *et al.*, 2000). On the whole, the widespread occurrence of  $2n$  gametes (i.e. gametes with the somatic chromosome number) in the *Medicago sativa-coerulea-falcata* complex supports the concept that gene flow from diploid to tetraploid species occurs continuously in nature and plays a key role in lucerne evolution. Cytological investigation provided insights into the types of meiotic abnormalities responsible for the production of  $2n$  gametes. Alterations were defined as genetically equivalent to first (FDR) or second division restitution (SDR) mechanisms.

For breeding purposes, data have proven that  $2n$  gametes of the FDR type are more advantageous than those obtained by SDR for transferring parental heterozygosity and retaining epistatic interactions. The use of diploid meiotic mutants that produce  $2n$  gametes is now recognized as one of the most effective methods available for exploiting heterosis and introgressing wild germplasm traits into cultivated tetraploid lucerne via unilateral (USP) and bilateral sexual polyploidization (BSP) schemes. Furthermore, apomixis has the potential of cloning plants through seed and thus provides a unique opportunity for developing superior tetraploid varieties with permanent fixed heterosis and epistatic effects. A possible main goal in lucerne breeding could be the introduction of functional apomixis (i.e. apomeiosis and parthenogenesis) in cultivated lucerne stock. All this area has been recently reviewed by Barcaccia *et al.* (2003).

Molecular Markers (MMs) and mapping methods have been applied to lucerne by European researchers. After the pioneering paper of Kiss *et al.* (1993), a Restriction Fragment Length Polymorphism (RFLP) linkage map of a diploid lucerne meiotic mutant based on a  $F_1$  population has been obtained by Tavoletti *et al.* (1996a) who also performed a half tetrad analysis using RFLP to estimate the relative frequencies of  $2n$  gametes produced by mechanisms genetically equivalent to FDR or SDR (Tavoletti *et al.*, 1996b). At diploid level, Amplified Fragment Length Polymorphism (AFLP) fingerprinting in *Medicago* spp. has been developed and applied in linkage mapping by Barcaccia *et al.* (1999) while, more recently, the Sequence Specific Amplification Polymorphism (S-SAP) method, derived by the AFLP technique, has been utilized within the genus *Medicago* (with particular interest for the *M. sativa-falcata-coerulea* complex) to produce amplified fragments containing a retrotransposon LTR sequence at one end and a host restriction site at the other (Porceddu *et al.*, 2002). The technique appeared suitable for studying genetic diversity within, and relatedness between, lucerne species.

Considering that the cultivated forms of lucerne are autotetraploid ( $2n=4x=32$ ), population structure parameters commonly used for diploid species were re-examined and a specific population genetic software prepared (Ronfort *et al.*, 1999). MMs and mapping methods have been applied to  $4x$  materials using both AFLP dominant markers and microsatellite codominant markers (Simple Sequence Repeats, SSRs) (Julier *et al.*, 2003a). The genetic structure of lucerne populations and varieties makes the construction of such maps difficult. To reach the objective and to be able to detect Quantitative Trait Loci (QTLs) of breeding interest, specific mapping procedures for autotetraploids were applied using a  $F_1$  population produced from the cross of two heterozygous parental plants. The results were consistent with the hypothesis of a tetrasomic inheritance in lucerne. Furthermore, data coming from SSRs studies permitted to define that for most of the SSR loci Double Reduction (DR) was not significant. A development in the area of estimation of DR coefficient (an essential feature of tetrasomic inheritance) is still underway. SSR markers have been also used to analyse the structure of genetic diversity among and within lucerne varieties (Flajoulot *et al.*, 2005).

In order to clarify the domestication history of lucerne, Muller and co-authors (Muller *et al.*, 2001) applied a phylogeographical approach based on a characterization for mitochondrial DNA variation through RFLP of samples of natural and cultivated populations. In the wild pool from the presumed centre of origin (Near East to central Asia), diversity was high but the absence of geographic differentiation hinders a more precise location for domestication. Within cultivated lucerne, genetic differentiation associated with a geographic structure strongly suggests the existence of at least two independent routes for lucerne dissemination (East and West) from its centre of origin. The same authors studied DNA sequence diversity of a nuclear gene in wild and cultivated *M. sativa-falcata-coerulea* accessions concluding that a population bottleneck could be associated to lucerne domestication (Muller *et al.*, 2002). Cytological studies of the nucleolus organizing regions in the *M. sativa-coerulea-falcata* complex showed that the number of active Nucleolus Organizing Regions (NORs) in *M. sativa* is twice the number found in *M. coerulea* and in *M. falcata*. Consequently, if *M. sativa* originated from sexual hybridisation of  $2n$  gametes involving one or both diploid species, no major reorganization or loss of structural or functional rDNA loci occurred (Calderini *et al.*, 1996).

## **B2. Breeding, variety development, evaluation and identification**

The theoretical problems of lucerne breeding related to autotetraploidy have been carefully analysed in Europe a long time ago by Demarly, Gallais and Rotili (Demarly 1963; Gallais, 1977; Rotili 1976, 1979); nothing really new appeared in this area in the last decade. Mass, phenotypic, recurrent selection and synthetic variety development schemes are still the basis of variety development while the first so-called “chance hybrids” or “free hybrids” are entering the commercial seed market.

Considering new targets of breeding efforts for lucerne, the most important one, at least for Southern European Countries, appears to be grazing tolerance. Traditional lucerne varieties generally present lower persistence under grazing management relative to mowing while, in the last two decades, lucerne grazing has aroused new interest due to the need for extensive livestock systems, either to reduce the environmental risks related to intensive animal husbandry or to decrease the cost of production as a way to maintain competitiveness of livestock enterprises. On the basis of these considerations, Spanish, French and Italian research teams started selection programs for grazing tolerant lucerne (Delgado Enguita, 1989, Charrier *et al.*, 1993; Piano *et al.*, 1996). Some cultivars with a better survival under grazing were released such as Coussouls and Luzelle in France. Research is still in progress (Pecetti and Piano, 2005) and there is a hope that, in the near future, European lucerne varieties specifically adapted to grazing will enter the seed market.

Other European selection targets appear to be improved seed yield (Svirskis, 1997; Huyghe *et al.*, 1999), DMY (Torricelli *et al.*, 2004), resistance to pea aphid (Bournoville *et al.*, 2002), persistence under frequent cutting regimes (Nagy, 2003), increased root size (Chloupek *et al.*, 1999), salt tolerance (Hefny and Doliski, 1999) and tolerance to soil acidity and aluminium and aluminium toxicity (Hauptvogel, 1999).

DMY still remains the most important breeding target for lucerne and therefore it deserves a particular attention; if we look at data coming from Europe, in the last few years it is evident that DMY increases with respect to old varieties and local ecotypes have been very limited, being not more than 5% (Lloveras *et al.*, 1998; Kerticova and Scotti, 1999; Babinec *et al.*, 2003; Delgado *et al.*, 2003; C. Huyghe and F. Veronesi, unpublished data). In our opinion, the slow genetic gains are largely due to: (i) the very good adaptation of local populations; (ii) the wide genetic basis of the varieties; (iii) the small number of European research teams devoted to applied plant breeding; (iv) the inherent difficulties connected to the tetrasomic inheritance of the species. The presence of an important environmental influence on the varietal performances makes the investigation of variety x location interaction of special interest for lucerne, as indicated by the research work of Annichiarico and Piano (2005) based on Additive Main effects and Multiplicative Interaction (AMMI) analysis. The identification of morphological traits associated with general and specific adaptation patterns and possibly coupled, in the long run, with that on the relationship between adaptive traits and the genome level as revealed by MMs can improve the understanding of the variety x location interaction effects as well as assist plant breeding aimed at definite adaptation targets (Annichiarico, 1999).

Methodology of lucerne breeding with special interest to progress in molecular biology and to the use of MMs has been recently reviewed by Julier *et al.* (2003b) and by Veronesi *et al.* (2003). Thanks to the proximity to the model species *Medicago truncatula*, genomic databases, knowledge and tools have been expanded and theoretical developments made for the obtainment of linkage maps for an autotetraploid species such as lucerne. As a result, QTLs are located for an increasing number of traits and candidate genes will be shortly identified. In particular, the use of MMs in lucerne breeding can be characterized under four categories: (i) germplasm characterization and management, (ii) genetic linkage mapping, (iii) gene targeting and marker assisted selection, (iv) heterosis and inbreeding. MMs associated to useful traits have been already found (Scotti *et al.*, 2000; Julier *et al.*, 2002) even if, in spite of a quite large number of studies devoted to this area, no lucerne variety developed using MMs technology has been released to date. However, MMs and DNA sequencing technologies evolve rapidly and we hope that these technologies will become available in the near future to lucerne breeders.

According to the opinion of some researchers, MMs could also be of interest for variety identification (Smolikova and Nedelnik, 1999; Labombarda *et al.*, 2000), even if the wide genetic variability typical of lucerne makes quite difficult to identify private bands useful for a real control of the commercial seed market.

### **B3. Genetic transformation**

In the last few years, research involving lucerne genetic transformation has been carried out by different European public research teams, predominantly in Belgium, France and Italy. According to Veronesi and Rosellini (2000), transformation efforts in Europe can be summarized as follows: (i) improved protein quality through the expression of seed storage proteins rich in sulfur amino acids such as  $\gamma$ -zein (Bellucci *et al.*, 2002); (ii) improved digestibility through the reduction of lignin content obtained with the inhibition of specific enzymes in the lignin biosynthesis pathway (Baucher *et al.*, 1999); (iii) male sterility obtained at Plant Genetic Systems, Gent, Belgium by transformation with a construct containing *Barnase* gene under the control of a tobacco anther tapetum specific promoter. The resulting transgenic male sterility has been characterized by Rosellini *et al.* (2001) who indicated that it should be possible to obtain good male sterile plants by backcrossing this trait into different genetic backgrounds; (iv) in an attempt to produce plants tolerant to soil acidity, lucerne has been transformed with a bacterial citrate synthase gene. Some transgenic plants showed a reduced aluminium absorption in root tips, a better root growth and a total DMY higher than controls in a greenhouse trial conducted with Al-toxic soil (Rosellini *et al.*, 2003); (v) basic research, where lucerne genetic transformation is used as a tool for investigating the mode of action of genes involved in the plant-*Rhizobium* symbiotic interactions (Bauchrowitz *et al.*, 1994; Bauer *et al.*, 1996; Jimenez-Zurdo *et al.*, 2000); (vi) plastid transformation is presently attempted in several labs due to the potential of the plastome to produce high transgene expression levels in green tissue (D. Rosellini, personal communication) and to other advantages of plastome transformation over genetic engineering of the nucleus such as site specific integration.

### **C. Forage quality**

Lucerne contains proteins which are rapidly degraded in the rumen; inducing a poor dietary efficiency, risk of bloat, and nitrogen loss detrimental to the environment. For these reasons, Julier and Colleagues have been active in the investigation of variation in ruminal protein degradability in lucerne (Julier *et al.*, 2003c). As a matter of fact, up to now the range of genetic variation within cultivated lucerne for *in situ* crude protein (CP) degradation appears to be narrow. Similar results have been obtained in a practical breeding program by Torricelli *et al.* (2001) who did not find a large variability for CP among lucerne plants. Even if these authors evidenced the absence of a negative correlation of statistical significance between DMY and CP, they concluded that, from a practical point of view, too many single plants required to be analysed to apply selection differentials for CP within lucerne populations and to start a specific breeding program. It seems worthwhile paying more attention to the variability for CP among lucerne populations. The presence of an interesting genetic variation for quality components within the lucerne complex has been evidenced by Pecetti and Colleagues in their research project aimed at developing grazing type lucerne varieties (Pecetti *et al.*, 2001).

Feeding value is an important trait for lucerne, which is characterized by high protein content but moderate energy value. Several studies have been performed to analyse the possibilities of genetic progress. In addition to laboratory measurements (enzymatic digestibility and fibre content, neutral detergent fibre, NDF, acid detergent fibre, ADF, acid detergent lignin, ADL), tools have been developed using near infrared spectroscopy (NIRS), making it possible to measure the feeding value for breeding purposes (Andueza *et al.*, 2001; Odoardi *et al.*, 2001). Large genetic variation was evidenced among varieties and among individual plants, and the inheritance of feeding value proved to be mainly additive (Julier *et al.*, 2003d).

On the basis of the above reported findings, new selection criteria have been added in lucerne breeding programmes trying to create more digestible varieties with high agronomic performances. The target is not a simple one, due to the negative genetic correlation evidenced between digestibility and forage yield (Julier *et al.*, 2003d). Molecular mapping is underway with the objective of identifying MMs associated with growth and digestibility traits to obtain a better understanding of the relationships among these traits and to produce tool kits useful for lucerne breeders.

Data coming from the dehydration industry seems to indicate that, at least for the moment, the choice of varieties is important for DMY while small differences in quality among varieties lead to the conclusion that agronomic technique, harvest frequency and weed control are the major factors influencing the



quality of the dehydrated product (Corsi *et al.*, 2001). As a consequence, it should be clarified if breeding approaches for quality could be sustained by private breeders in the absence of a clear economic return in terms of higher market prices.

## Conclusions

The European experience in lucerne germplasm collection, conservation and evaluation shows the importance of this topic for the development of varieties adapted to grazing and resistant to environmental constraints. The use of MMs increases the possibility of studying the effects of gene flow from cultivated to wild materials. MMs and mapping methods have been applied to lucerne by European researchers. Thanks to the proximity to the model species *Medicago truncatula*, genomic databases, knowledge and tools have been recently expanded and theoretical developments made for the obtainment of linkage maps for an autotetraploid such as lucerne. As a result, QTLs have been located for an increasing number of traits and candidate genes identified. Reproductive mutants may have impact on lucerne breeding; a main goal could be the introduction of functional apomixis (i.e. apomeiosis and parthenogenesis) in cultivated lucerne stocks.

Considering new targets for lucerne breeding, one of the most important appears to be grazing tolerance. Some cultivars with a better survival under grazing have been already released and there is a hope than, in the near future, new European lucerne varieties specifically adapted to grazing will enter the seed market. Other European selection targets appear to be improved seed yield, insect resistance, persistence under frequent cutting regimes, increased root size, salt tolerance and tolerance to soil acidity and aluminium toxicity. DMY still remains the most important breeding target for lucerne but it is evident that, in the last decade, increases with respect to old varieties and local ecotypes have been very limited, being on average 5%. Out of the several reasons which can be adducted for these slow genetic gains, the low number of research teams actually active in lucerne breeding in Europe should be mentioned.

In the last few years, research involving genetic transformation has been carried out by different teams with interesting results but, at the moment, the situation in Europe is very unfavourable to the release of GM crops. Lucerne has high risks of transgene dissemination due to its perenniality, outcrossing behaviour and long distance of pollen dispersal. There is also the presence of compatible materials such as local landraces and wild populations, some of them with a strategic importance for the maintenance of lucerne germplasm.

A new selection criterion, digestibility, has been added in the last few years to lucerne breeding programmes trying to create more digestible varieties with high agronomic performances. The target is not a simple one, due to the negative genetic correlation between digestibility and forage yield but molecular mapping is under study with the objective of identifying MMs associated to growth and digestibility traits useful for practical breeding.

On the whole, it looks like lucerne research in Europe reached a good scientific standard in the last decade but is carried out by few public research teams and is prevalently devoted to the analysis of basic problems; as a consequence, a bigger economic effort and a coordination between basic research and applied plant breeding are required priorities.

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## Red clover for silage: management impacts on yield and digestibility during the season after sowing

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### Abstract

Successive harvests were used to quantify the yield and digestibility of monoculture and binary plots of red clover (*Trifolium pratense* L.) and perennial ryegrass (*Lolium perenne* L.). Twenty-four treatments examined the effects of cultivar, companion grass, harvest schedule and N fertiliser in plots containing red clover, and benchmarked these effects against ryegrass receiving different rates of N fertiliser. In general, red clover had a higher yield and lower digestibility than ryegrass grown without N fertiliser. Inclusion of ryegrass with red clover increased annual yield and digestibility compared to monocultures of red clover. Swards with Ruttinova had a higher annual yield than with Merviot, but had a similar digestibility. The early first-cut harvest schedule improved mean annual digestibility without altering overall yield. Applying inorganic N during March to treatments containing red clover influenced neither annual yield nor digestibility. Monocultures of red clover and the earlier first-cut harvest schedule resulted in a higher ground cover by red clover at the end of the first growing season than binary mixtures or the late first-cut harvest schedule, respectively.

Keywords: red clover, perennial ryegrass, management, yield, digestibility.

### Introduction

Red clover is considered a short-lived perennial herbage legume that can be highly productive for two and sometimes three years, and whose upright growth habit makes it particularly suited for hay and silage making (Frame *et al.*, 1998). In countries where permanent grassland dominates farmland it is important to define aspects of the management of red clover that will improve its persistence and its contribution to feed supply. This experiment quantified the impacts of cultivar, companion grass, harvest schedule and nitrogen fertiliser on crop yield and digestibility in its first year after reseeding. The effects of these factors will be assessed in subsequent years to fully monitor their impacts on persistence.

### Materials and methods

Within a randomised complete block (n=4) design, field plots (24 per block, each 10m x 2m) were used to evaluate a 2 (cultivars) x 2 (alone or with companion grass) x 2 (harvest schedule) x 2 (application of N fertiliser in spring) combination of factors relating to red clover, and a 2 (harvest schedule) x 4 (application of N fertiliser in spring) combination of factors relating to a monoculture of perennial ryegrass. Two cultivars of red clover (Merviot and Ruttinova) were each sown in monoculture or with perennial ryegrass (*cv.* Greengold) in August, 2001. They received 0 or 50 kg inorganic N fertiliser ha<sup>-1</sup> in mid-March 2002 and had a first-cut harvest date of 2 June or 19 June. Sequential harvests following 2 June were taken after 50, 44 and 97 days, with the corresponding durations after 19 June being 44, 42 and 88 days. Monoculture plots of perennial ryegrass (*cv.* Greengold) received 0, 50, 100 or 150 kg inorganic N ha<sup>-1</sup> in mid-March and immediately after the first three harvests, and had similar harvest dates to the red clover. All plots were harvested to a 5 cm stubble height and received 22 kg P and 95 kg K ha<sup>-1</sup> after the first, second and third harvests, and double those rates after the fourth harvest. Immediately before the final harvest, a visual assessment was made of the proportion of ground cover contributed by red clover. Dry matter (DM) content was determined by drying in a forced-air oven at 98°C for 16h. Samples dried at 40°C for 48h were milled through a 1mm screen before being assayed for DM digestibility (DMD) (Tilley and Terry, 1963). Clover data were analysed using a General Linear

Model that accounted for each of the four factors and all two-, three- and four-way interactions. Linear and quadratic equations were fitted to the data from the ryegrass treated with different rates of N fertiliser.

## Results and discussion

Table 1. Herbage yield (kg DM ha<sup>-1</sup>) and digestibility (DMD) (g kg<sup>-1</sup>) for red clover treatments at each harvest in the year after sowing.

Cult. <sup>1</sup>	Grass <sup>2</sup>	N <sup>3</sup>	Date <sup>4</sup>	Cut 1		Cut 2		Cut 3		Cut 4	
				Yield	DMD	Yield	DMD	Yield	DMD	Yield	DMD
Merv. <sup>5</sup>	No	No	Early	4,196	719	3,564	707	3,442	735	255	700
	No	No	Late	5,734	662	3,503	715	2,730	709	400	744
	No	Yes	Early	5,063	717	3,737	709	3,437	719	260	700
	No	Yes	Late	6,083	643	2,833	721	2,383	741	477	731
	Yes	No	Early	6,027	754	3,415	741	3,498	743	1,090	718
	Yes	No	Late	6,743	682	2,166	698	2,687	755	1,045	779
	Yes	Yes	Early	6,545	764	2,807	754	3,449	744	1,129	727
	Yes	Yes	Late	7,466	701	1,139	737	2,009	762	1,026	777
Rutt. <sup>6</sup>	No	No	Early	4,890	721	3,998	711	3,387	735	470	723
	No	No	Late	6,166	656	4,023	707	2,520	732	231	737
	No	Yes	Early	5,184	731	3,972	699	3,015	723	361	718
	No	Yes	Late	6,774	662	3,937	688	2,754	714	302	731
	Yes	No	Early	6,624	756	3,050	739	3,434	731	1,293	725
	Yes	No	Late	7,009	692	2,845	729	2,106	750	910	775
	Yes	Yes	Early	6,481	762	3,004	746	3,084	738	1,374	750
	Yes	Yes	Late	8,103	675	3,018	756	2,434	736	1,043	774
s.e.m.											
1 <sup>7</sup>				175.1	3.4	166.0	4.9	90.1	3.7	52.1	3.3
2 <sup>8</sup>				247.6	4.9	234.8	6.9	127.4	5.2	73.7	4.7
3 <sup>9</sup>				350.2	6.9	332.1	9.7	180.2	7.4	104.2	6.6
4 <sup>10</sup>				495.2	9.7	469.6	13.8	254.8	10.5	147.4	9.4

<sup>1</sup>Clover cultivar; <sup>2</sup>Presence of companion grass; <sup>3</sup>Application of inorganic N in spring; <sup>4</sup>Early or late first-cut harvest schedule; <sup>5</sup>Merviot; <sup>6</sup>Ruttinova; <sup>7</sup>Single factor; <sup>8</sup>2 factors; <sup>9</sup>3 factors; <sup>10</sup>4 factors.

Inclusion of ryegrass with red clover influenced herbage yield and quality. Binary mixtures had a higher ( $p < 0.01$ ) annual yield (13,506 vs. 12,510 kg ha<sup>-1</sup>; s.e.m. 259.9) and average DMD (730 vs. 702 g kg<sup>-1</sup>; s.e.m. 2.5) than red clover monocultures. This agrees with Crowley (1978) and Frame *et al.* (1998). Herbage yield from swards with red clover was higher than reported by Connolly (1970) and Keane (1982) for binary mixtures but lower than reported by Crowley (1978) for a monoculture or binary mixture in the first year after sowing. Red clover cultivar influenced herbage yield. Swards with Merviot had a lower ( $p < 0.05$ ) annual DM yield than those with Ruttinova (12,542 vs. 13,474 kg ha<sup>-1</sup>), but did not differ ( $p > 0.05$ ) in weighted annual DMD (716 vs. 715 g kg<sup>-1</sup>). The yield advantage to Ruttinova was clearcut at the first and particularly the second harvest. Schedules with earlier first-cut harvesting of swards containing red clover had improved annual digestibility (733 vs. 699 g kg<sup>-1</sup>;  $p < 0.001$ ), although annual yield did not change ( $p > 0.05$ ). Application of inorganic N to red clover-based swards in spring influenced neither annual yield nor DMD ( $p > 0.05$ ). The only interaction related to annual output was a larger ( $p < 0.05$ ) increase in DM yield in response to the inclusion of ryegrass with



red clover for the early compared to the late first-cut harvest schedule. Treatment effects within individual harvests are summarised in Table 1, and most of the possible interactions (84/88) were not significant ( $p>0.05$ ).

Table 2. Linear and quadratic relationships between input of inorganic N fertiliser ( $\text{kg ha}^{-1}$ ) and ryegrass dry matter yield (DMY)( $\text{kg ha}^{-1}$ ) or DM digestibility (DMD)( $\text{g kg}^{-1}$ ).

Cut	Date	y <sup>#</sup>	a <sup>#</sup>	S.E.	b <sup>#</sup>	S.E.	Sig.	c <sup>#</sup>	S.E.	Sig.	R <sup>2</sup>
1	Early	DMY	5,893	414.2	21.2	13.30	0.135	-0.13	0.085	0.162	0.17
1	Early	DMD	772	10.1	-0.1	0.11	0.310				0.07
1	Late	DMY	6,511	383.6	30.0	12.32	0.030	-0.17	0.079	0.048	0.32
1	Late	DMD	690	15.4	-0.1	0.50	0.823	0.00	0.003	0.705	0.03
2	Early	DMY	1,514	428.3	15.8	4.58	0.004				
2	Early	DMD	806	8.1	-0.2	0.09	0.054				
2	Late	DMY	1,028	265.7	28.1	8.53	0.006	-0.15	0.055	0.018	0.50
2	Late	DMD	788	11.5	-0.5	0.37	0.226	0.00	0.002	0.316	0.13
3	Early	DMY	1,243	136.1	46.5	4.37	0.000	-0.22	0.028	0.000	0.93
3	Early	DMD	818	9.9	-0.6	0.32	0.103	0.00	0.002	0.257	0.32
3	Late	DMY	1,102	224.7	27.6	7.22	0.002	-0.08	0.046	0.095	0.81
3	Late	DMD	789	7.4	0.2	0.24	0.310	0.00	0.002	0.152	0.25
4	Early	DMY	1,038	209.8	27.8	6.74	0.001	-0.15	0.043	0.004	0.60
4	Early	DMD	796	13.2	-1.4	0.42	0.007	0.01	0.003	0.030	0.54
4	Late	DMY	886	170.3	25.2	5.47	0.000	-0.12	0.035	0.005	0.73
4	Late	DMD	785	10.7	0.1	0.34	0.743	0.00	0.002	0.334	0.31

\*Early or late first-cut harvest schedule; <sup>#</sup> $y=a+bx+cx^2$ .

Monocultures of ryegrass receiving no inorganic N had a mean annual DM yield of 9,311 and 9,594  $\text{kg ha}^{-1}$  for the early and late first-cut harvest schedules, respectively, with corresponding average weighted DMD's of 788 and 720  $\text{g kg}^{-1}$ . First and fourth cuts of red clover monocultures (Table 1) yielded less than ryegrass (Table 2), whereas the binary mixture had a similar yield to ryegrass that had up to 40  $\text{kg N ha}^{-1}$  applied. In contrast, the yield of red clover at the second harvest was similar or better than ryegrass plus 80 – 150  $\text{kg N ha}^{-1}$ , and was similar to ryegrass plus 40 – 80  $\text{kg N ha}^{-1}$  at the third harvest. Red clover generally had a lower DMD than ryegrass, although for the first and fourth harvests the binary mixture had a comparable DMD to a pure sward of ryegrass. There were some early indications of persistency towards the end of the first full season. The proportion of ground cover accounted for by red clover was higher ( $p<0.001$ ) with monocultures of red clover than with binary mixtures (0.79 vs. 0.57; s.e.m.0.031). The early first-cut system had a larger ( $p<0.01$ ) red clover ground cover than the late system (0.75 vs. 0.61). In contrast, neither spring application of N fertiliser (0.71 vs. 0.65 for 0 and 50  $\text{kg N ha}^{-1}$ , respectively) nor red clover cultivar (0.68 vs. 0.69 for Merviot and Ruttinova, respectively) altered ( $p>0.05$ ) the proportion of red clover. There were no interactions ( $p>0.05$ ) between these factors. Overall, the inclusion of grass with red clover gave a clear improvement in herbage yield and digestibility. Comparable improvements in yield and digestibility were mediated by red clover cultivar and harvest schedule, respectively. In contrast, the application of inorganic N in spring resulted in no benefit.

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## Regulation of symbiotic nitrogen fixation in grass-clover mixtures

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### Abstract

In the first evaluation of the data from the running multi-location experiment of COST 852, grass-clover mixtures generally yielded more than pure grass swards when fertilised with the same amount of nitrogen (Sebastian *et al.*, 2004). Symbiotic N<sub>2</sub> fixation of clover is expected to be an important advantage to the yield of grass-clover mixtures. To profit from legumes in productive but environmentally friendly forage systems, knowledge about the regulation of symbiotic N<sub>2</sub> fixation at the plant level is fundamental. We studied the effects of nitrogen fertilisation and the proportion of grass in the sward on the proportion of N derived from symbiotic fixation in red (*Trifolium pratense* L.) and white clover (*Trifolium repens* L.) (%N<sub>sym</sub>) during the 2003 growing season. We observed differences between red and white clover and between the harvests. The large differences of %N<sub>sym</sub> within the same harvest can mainly be explained by the proportion of grass in the swards and the N fertilisation as main, quadratic and interaction effects. This indicates that the legume plants efficiently regulate their N<sub>2</sub> fixing activity according to the availability of mineral N in their rhizosphere.

Keywords: fertilisation, simplex design, *Trifolium pratense*, *Trifolium repens*.

### Introduction

The European multi-location experiment of COST 852 is currently assessing the influence of the proportions of grass/clover on the yield of grass-clover mixtures. First results showed a yield advantage of grass-clover mixtures over pure grass swards when fertilised with the same level of nitrogen (N) (Sebastian *et al.*, 2004). This is thought to be due partly to the symbiotic fixation of atmospheric N by the clover species. It is therefore suggested that legumes are an important component of environmentally friendly forage production systems because they allow high yields with low inputs of N fertilisers. However, high rates of symbiotic N<sub>2</sub> fixation could also represent a risk of unwanted N losses to the environment. Thus, quantitative information about symbiotic N<sub>2</sub> fixation and its regulation is of primary importance in optimising the use of legumes. We studied the effects that N fertilisation and the proportion of grass in the sward had on the proportion of N derived from symbiotic fixation at the legume plant level.

### Material and methods

Perennial ryegrass (*Lolium perenne* L.), cocksfoot (*Dactylis glomerata* L.), red clover (*Trifolium pratense*) and white clover (*Trifolium repens*) were sown in pure swards and in four species mixtures according to the simplex design (Cornell, 2002). The advantage of this experimental design is that a large range of grass proportions in the sward, from 0 to 100%, is available at each N level. The plots were established in August 2002 in Zurich, Switzerland. In spring 2003, the first regrowth of all plots was fertilised with 30 kg N ha<sup>-1</sup>. Three levels of N fertilisation were established after the first harvest (low N = 10, moderate N = 30 and high N = 90 kg N ha<sup>-1</sup> regrowth<sup>-1</sup>). During the 2003 growing season, we applied the <sup>15</sup>N dilution technique to estimate the proportion of N derived from symbiotic fixation in the legume plants (thereafter referred to as %N<sub>sym</sub>). We analysed the first (H1), the third (H3) and the fifth harvest (H5). The factors affecting %N<sub>sym</sub> in both red and white clover were analysed for each harvest by multiple linear regression methods using the explanatory covariates 'proportion of grasses' (centred) at each harvest and the factor 'N fertilisation level' as main, quadratic and interaction effects.

## Results and Discussion

The results of the multiple linear regression analysis are presented in Table 1 and illustrated in Figure 1 for the fifth harvest. %N<sub>sym</sub> varied strongly during the growing season and slightly among clover species as shown by the intercepts in Table 1. In the moderate N treatment and averaged over all mixtures, red clover acquired 94% of its N by symbiotic fixation at the first, 79 % at the third and 61 % at the fifth harvest. For white clover, %N<sub>sym</sub> was on average 90 % at H1, 67 % at H3 and 60 % at H5. The high levels of %N<sub>sym</sub> in red and white clover demonstrate that symbiotic N<sub>2</sub> input was important in the different clover-grass mixed swards of our field experiment during the 2003 growing season. We suggest that at our site this is a main factor in explaining the high yield advantage of clover-grass mixtures compared with grass monocultures (Sebastia *et al.*, 2004). The proportion of grass in the swards affected %N<sub>sym</sub> within individual harvests (Table 1, Figure 1). We therefore found large differences in %N<sub>sym</sub> between the mixtures within individual harvests and clover species. An increasing proportion of grasses generally lead to an increase in %N<sub>sym</sub>. This increase might be due to the withdrawal of mineral N from the soil solution by the competing grass species. At H3 an additional significant quadratic effect of the proportion of grass occurred (%Grass<sup>2</sup> in Table 1). In the high N fertilisation treatment, %N<sub>sym</sub> was less influenced by the proportion of grasses than in the moderate and the low N treatment. The level of N fertilisation also affected %N<sub>sym</sub> within individual harvests (Table 1, Figure 1).

Table 1. Variables of the multiple linear regression models affecting the proportion of N from symbiosis (%N<sub>sym</sub>) in red and white clover, analysed for the first (H1), third (H3) and fifth harvest (H5) in 2003.

	Red clover			White clover		
	Estimate (%N <sub>sym</sub> )	sd		Estimate (%N <sub>sym</sub> )	sd	
H1						
Intercept	93.7	0.9	***	85.9	1.8	***
%Grass	5.8	2.1	**	3.8	8.0	ns
(%Grass <sup>2</sup> )	-6.4	7.6	ns	41.2	23.0	.
H3						
Intercept	89.7	1.3	***	80.8	2.2	***
Low N	8.2	4.1	.	8.6	6.8	ns
High N	-25.5	2.7	***	-51.2	4.4	***
%Grass	3.3	7.3	ns	21.1	11.1	.
(%Grass <sup>2</sup> )	-101.9	18.0	***	-87.9	28.6	**
%Grass x low N	9.7	14.3	ns	-20.7	23.7	ns
%Grass x high N	25.0	9.6	*	-29.0	15.6	.
H5						
Intercept	64.2	1.9	***	62.9	1.7	***
Low N	20.7	3.9	***	19.0	3.4	***
High N	-33.6	3.8	***	-42.2	3.2	***
%Grass	54.4	7.1	***	42.2	5.5	***
(%Grass <sup>2</sup> )	-38.6	21.0	.	-4.7	16.2	ns
%Grass x low N	-28.4	16.7	.	-11.7	13.8	ns
%Grass x high N	-44.9	12.9	***	-44.3	10.5	***

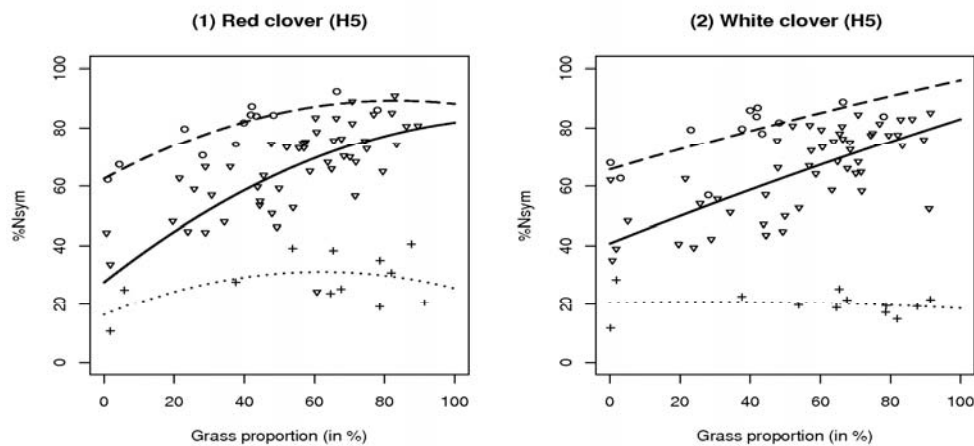


Figure 1. Effect of the proportion of grass in the swards on %  $N_{sym}$  in (1) red and (2) white clover as measured (points) and predicted (lines) for the fifth harvest 2003 and the three levels of N fertilisation, low N (circles, ---), moderate N (triangles, —) and high N (crosses, .....).

Compared with the moderate N fertilisation level, % $N_{sym}$  of red clover was increased on average by 2.2 % (H3) and 15.2 % (H5) in the low and decreased by 18.3 % (H3) and 36.3 % (H5) in the high N fertilisation level. The decrease in % $N_{sym}$  at high N was larger in white clover, with 50.8 % and 45.1 % less % $N_{sym}$  at H3 and H5 respectively. The strong effects of the proportion of grass and the N fertilisation on % $N_{sym}$  suggest that the legume plants efficiently regulated their  $N_2$  fixing activity according to the availability of mineral N in their rhizosphere, as discussed by Hartwig (1998). This is an advantage of legumes for environmentally friendly production systems since it enables the system to observe a certain self-regulation. At low N availability, N input from symbiotic fixation increases, while at high N availability, N input decreases.

### Conclusions

The high levels of % $N_{sym}$  in red and white clover demonstrate that symbiotic  $N_2$  input was important in the different grass-clover mixed swards of our field experiment during the 2003 growing season. Symbiotic nitrogen fixation in grass-clover mixtures was regulated by both the proportion of grasses in the swards and the level of N fertilisation. Legumes therefore seem advantageous to environmentally friendly production systems since they enable the system to observe a certain self-regulation of the symbiotic N input according to the availability of N from other sources.

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## Developing the role of *Lotus* species in UK grasslands

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### Abstract

Experiments have been carried out with the aims of quantifying: (a) the response of a cultivar of *Lotus corniculatus* L. to two contrasting cutting treatments, in comparison with other temperate forage legume species; and (b) variation within *Lotus* spp. for traits of agronomic interest, in particular productivity, morphology and tannin content. The results are discussed in terms of the needs of livestock systems in the UK and the future direction of the *Lotus* breeding programme at IGER is outlined.

Keywords: *Lotus*, yield, persistence, morphology, condensed tannins.

### Introduction

Until recently the putative advantages of including perennial *Lotus* spp. (*L. corniculatus* – birdsfoot trefoil, or *L. pedunculatus* Cav. – greater lotus) in UK pastures derived from, primarily, their capacity to grow on soils considered unsuitable for other forage legumes. Thus, in UK agriculture they have always had a marginal role, being mainly regarded as a potentially useful protein source for grazing animals under very low-input conditions in which the use of white clover (the major forage legume species in UK pastures) would not be economically sustainable. However, interest in *Lotus* spp. has increased in the UK in response to changing agricultural scenarios subsequent to CAP reform and it is envisaged that they could also make a valuable contribution to animal nutrition in medium-input pastures. The major nutritional advantage of *Lotus* spp., in addition to the high digestibility commonly found in forage legumes, is the relatively high concentration of condensed tannins (CT) in the leaves, stems and flowers (Barry and McNabb, 1999). These are chemicals that protect against bloat (retention of fermentation gases within the rumen) and improve the utilisation of protein by ruminant animals. CT are not found in useful quantities in white clover. An additional attribute of *Lotus* herbage is that it has anthelmintic properties, with ensuing animal health benefits. Thus, the inclusion of *Lotus* spp. in pastures offers potential advantages. Nevertheless, significant obstacles must be overcome before they can be included with confidence in sward mixtures. The most significant of these is that currently available *Lotus* cultivars lack persistence and competitive ability under UK conditions. There is clearly a need for plant breeders to produce robust, locally-adapted *Lotus* cultivars and the Legume Breeding and Genetics Team at IGER is currently evaluating a number of cultivars and selection lines as part of a strategy for developing germplasm that is suitable for UK conditions and farm practices, and that will play a significant role in sustainable pastures.

This paper describes (a) a field experiment in which the yield and persistence of four legume species, including *L. corniculatus*, were compared under two contrasting cutting treatments: a conservation-type management and a simulated grazing management, and (b) the main elements of the current *Lotus* germplasm screening programme at IGER.

### Material and methods

(a) The effects of cutting frequency on the yield and persistence of four legume species were evaluated in a field experiment carried out for two years. The legume species were white clover (cv. Klondyke), red clover (cv. Pirat), lucerne (cv. Ameristand) and birdsfoot trefoil (cv. Rocco). They were grown in binary mixtures with perennial ryegrass (cv. Fennema). Plots sown with lucerne and birdsfoot trefoil were irrigated with a solution containing the appropriate *Rhizobium* spp. The plots were 12 m<sup>2</sup> and each mixture was randomised within a block and replicated three times. A grass monoculture treatment was

also included. The plots were divided into two sections and subjected to two cutting frequencies: 'simulated grazing' (SG - interval between cuts was 30 days) and 'infrequent cutting' (IC - interval between cuts was 50 days). All plots were cut to a height of 5 cm. No fertiliser N, P or K was supplied to any of the treatments. At each harvest the cut herbage was weighed and subsampled for botanical analysis. Results (DM yield of legume and grass components) were analysed by ANOVA using a split-plot model, with cutting frequencies as the main plots and grass/legume mixtures as sub-plots. Cumulative DM yields for legume and grass components were derived for each year. (b) Variation within *L. corniculatus* and *L. pedunculatus* for yield, plant morphology and tannin content has been quantified. Genotypes from 24 *Lotus* populations (cultivars and selection lines) were grown in a spaced plant nursery and their DM production was measured after cutting in June and September. Genotypes from the same populations were grown in pots in a glasshouse and their morphology was evaluated by measuring plant height and stem number. In a sub-group of 9 of these populations a high throughput assay was used to quantify leaf and stem CT content.

## Results and discussion

Results from experiment (a) are presented in Table 1.

Table 1. Cumulative DM yields ( $\text{kg ha}^{-1}$ ) of the legume and grass components of binary mixtures under two cutting regimes in successive harvest years. SG = simulated grazing (interval 30 days); IC = infrequent cutting (interval 50 days).

Year 1

Legume						
Treatment	Species	White clover	Red clover	Lucerne	Birdsfoot trefoil	Perennial ryegrass
SG		4990	5262	484	226	-
IC		6125	8768	1497	1195	-
Grass						
Treatment		1843	1850	1599	1496	1701
SG		1843	1850	1599	1496	1701
IC		2393	2425	2436	2019	2504

Legume yield: Treatment x Species  $P=0.001$ ; lsd at 5% 847.8. Grass yield: Treatment  $P=0.011$ ; lsd at 5% 293.7; Species = ns; Treatment x Species = ns.

Year 2

Legume						
Treatment	Species	White clover	Red clover	Lucerne	Birdsfoot trefoil	Perennial ryegrass
SG		7228	7069	1273	203	-
IC		6115	7372	5058	803	-
Grass						
Treatment		2603	3399	3310	3854	3089
SG		2603	3399	3310	3854	3089
IC		2850	2407	3214	2668	3035

Legume yield: Treatment x Species  $P=0.04$ ; lsd at 5% 2308.6. Grass yield: Treatment = ns; Species = ns; Treatment x Species = ns.

In Year 1 the cumulative yield of all four legume species was adversely affected by the SG treatment, whereas that of perennial ryegrass was not. However, the legume species differed in the extent to which their yield was reduced by this treatment. The greatest reduction was found in birdsfoot trefoil, in which

yield under SG was less than 20% of that in the IC management; the least affected species was white clover, in which yield under SG was 81% of that in IC. In Year 2, white and red clover again responded differently to lucerne and birdsfoot trefoil in terms of their yield under the two treatments. Yield in the former two species was not significantly affected by cutting treatment, whereas large yield reductions were found in lucerne and birdsfoot trefoil in the SG management. As in Year 1, perennial ryegrass yield was not affected by cutting treatment in any legume/grass mixture. In both years the yield of birdsfoot trefoil was significantly lower than that of white and red clover, even in the IC treatment. In Year 1 and Year 2, total (grass + legume) yield in mixtures containing birdsfoot trefoil was not significantly different from grass monoculture yields in either of the cutting treatments, indicating that the legume was not present in sufficient quantities to contribute enough fixed N to benefit sward productivity. This experiment was intended to simulate a low-input system (*i.e.* no applications of N, P or K) with contrasting cutting treatments that approximated to conservation and lenient grazing managements. The birdsfoot trefoil cultivar used in this experiment, Rocco, is of an upright morphological type and clearly performed particularly poorly under the SG treatment, suggesting that this cultivar would be unsuitable for grazing systems. It was also relatively unproductive in the IC treatment, suggesting that herbage cuts should be carried out on a very infrequent basis when using germplasm of this morpho-type.

(b) Significant variation in DM productivity was found between the populations grown as spaced plants, both in terms of total productivity and seasonal growth pattern. Glasshouse measurements also revealed large differences within and between populations in terms of plant morphology, with plant morphotypes ranging from tall and erect to prostrate and spreading. One particular selection line contained plants with rhizomes as well as above-ground stems. The incorporation of the rhizomatous trait into more productive lines may be a route towards improved persistence of *Lotus* under grazing. There was significant variation in CT content between leaf and stem tissue, and also within and between populations. Quantifying the CT content is important, as tannins must be present in herbage in sufficient quantities to have positive impacts on animal health, whilst not exceeding levels of 45 g CT kg<sup>-1</sup> DM, which may reduce intake. Some genotypes screened contained CT concentrations of up to 70 g kg<sup>-1</sup> DM. The high level of genetic variation for DM production, plant morphology and CT content present within the range of *Lotus* germplasm investigated was encouraging, and suggests that there is much scope for selection for agronomically desirable traits.

#### **Acknowledgements**

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## Effect of preservation method on the nutritive value of sulla (*Hedysarum coronarium*)

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### Abstract

This study was conducted to investigate the effect of the preservation method of sulla (*Hedysarum coronarium* L.). Sulla was cut at full bloom stage and preserved by freezing, sun-drying (hay) and was ensiled after wilting to 30% dry matter (DM). Apparent digestibility, ruminal microbial protein synthesis and N balance were studied in four rams fed at maintenance level in a completely randomized block experiment. Three rumen-cannulated rams were used to determine *in situ* degradability. Chemical composition of sulla was 15.6% crude protein (CP), 14.8% total sugar, 45.6% neutral detergent fibre (NDF) and 38.1% acid detergent fibre (ADF). The "in vivo" apparent digestibility of organic matter (OM), CP, NDF and ADF were 62.5%, 68.3%, 35.3% and 34.7%, respectively. Conservation leads to a significant decrease in sugar and in CP digestibility. The effective degradability (ED) of DM, OM, CP, NDF and ADF in fresh feed were calculated considering a rumen passage rate of 2% hour<sup>-1</sup> and the following values were found: 71.7, 70.0, 81.1, 46.8 and 51.6, respectively. A decrease of ED with conservation was observed. No significant differences were observed for microbial protein synthesis. The conservation as hay or haylage decreased the nutritive value of sulla, particularly the protein fraction.

Keywords: *Hedysarum coronarium*, chemical composition, digestibility, degradability.

### Introduction

Sulla (*Hedysarum coronarium* L.) is a legume well grazed by animals and is resistant to grazing and trampling (Watson, 1982). Cutting for hay or silage is the best way to use the plant, achieving maximum productivity (Perez, 1993-94). The present study was conducted to investigate the effect of sulla preservation as hay and haylage on the chemical composition and nutritive value.

### Materials and methods

This study was carried out in center of Portugal, using sulla cultivar Grimaldi. Biomass was collected at full bloom stage and preserved by freezing, sun-drying (hay) and was ensiled after wilted in the field to 30% dry matter (DM). Sulla samples (fresh, hay and haylage) were analysed for dry matter (DM), nitrogen (N), neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL). Total sugar and starch, gross energy (GE) and calcium (Ca) and phosphorus (P) were determined. The fermentative characteristics of silage were analysed: pH, NH<sub>3</sub>-N, soluble N, volatile fatty acids and lactic acid. Apparent digestibility, ruminal microbial protein synthesis and N balance were studied in four rams, fed at maintenance level (45 g DM kg<sup>-1</sup> w<sup>0.75</sup>) in a completely randomized block experiment. Ruminal microbial protein synthesis was estimated by the Chen and Gomes (1992) method. Three rumen-cannulated rams were used to determine *in situ* degradability of organic matter (OM), crude protein (CP), NDF and ADF. Feed samples were incubated in the rumen during 4, 8, 16, 24, 48, 72 and 96 hours. The disappearance values were fitted to the equation,  $p = a + b(1 - e^{-ct})$ . To estimate effective degradability (ED) the equation  $ED = a + b(c/c + k)$  was used, where k is the outflow rate from the rumen assumed to be 0.02 h<sup>-1</sup>. Data were analysed with the GLM procedures of SAS (2004). When ANOVA's F test was significant, least significant test (LSD) was used to multiple means comparison.



## Results and discussion

Fresh sulla had low DM content and high levels of CP (Table1) .The preservation methods did not affect the CP content ( $P>0.05$ ), but decreased the soluble sugar content ( $P<0.01$ ), more evident in haylage than in hay, and increased the fibre fraction content (NDF and ADF).

Table 1. Chemical composition of fresh sulla (*Hedysarum coronarium*), hay and haylage.

Parameters	Fresh	Hay	Haylage	RSD	Significance
Dry matter (%)	13.4 <sup>a</sup>	85.5 <sup>c</sup>	36.8 <sup>b</sup>	4.66	**
Organic matter (%DM)	89.9	90.5	89.2	1.26	NS
Crude protein (%DM)	15.6	12.5	12.9	2.28	NS
Sugar (%DM)	14.8 <sup>c</sup>	9.1 <sup>b</sup>	2.8 <sup>a</sup>	1.21	**
Starch (%DM)	2.4	2.5	2.3	0.57	NS
Ca (%DM)	1.46	1.04	1.10	0.20	NS
P (%DM)	0.33	0.29	0.30	0.02	NS
NDF (%DM)	45.6 <sup>a</sup>	53.8 <sup>b</sup>	54.8 <sup>b</sup>	2.69	**
ADF (%DM)	38.1 <sup>a</sup>	44.2 <sup>ab</sup>	47.1 <sup>b</sup>	3.26	*
ADL(%DM)	7.3	8.2	8.7	1.21	NS
Gross energy (MJ) DM)	18.0	17.5	17.4	0.66	NS

<sup>a,b,c</sup> - Mean values within a row with unlike superscript letters were significantly different ( $P<0.05$ ).

RSD- Residual standard deviation, \*  $P < 0.05$ , \*\*  $P < 0.01$ , NS- Non-significant ( $P>0.05$ ).

Silage was well preserved. Lactic acid was the major organic fermentation product in a concentration of 48.8 g kg<sup>-1</sup> DM. This level allowed a pH decrease to 4.28 which was enough to ensure an adequate conservation. Other organic fermentation products (expressed g kg<sup>-1</sup> DM) were low, averaging 15.5 for acetic, 0.3 for propionic and 2.8 for butyric acid. The levels of NH<sub>3</sub>-N and soluble-N, expressed in % total N, were also low, averaging 6.43 and 21.8, respectively. These values indicate stable and good quality silage.

Table 2. *In vivo* digestibility, retained N, microbial protein, metabolizable energy of sulla.

	Fresh	Hay	Haylage	RSD	Significance
<i>In vivo</i> digestibility (%)					
Dry matter	61.0	57.3	57.7	3.4	NS
Organic matter	62.5	58.1	59.6	3.5	NS
Crude protein	68.3 <sup>c</sup>	57.3 <sup>b</sup>	48.6 <sup>a</sup>	3.4	**
NDF	35.3	40.8	42.7	5.9	NS
ADF	34.7	39.4	42.8	6.4	NS
GE	61.4	55.8	56.9	3.9	NS
Retained N(% N intake)	37.3 <sup>c</sup>	7.2 <sup>b</sup>	-13.2 <sup>a</sup>	4.5	***
Microbial protein (g kg <sup>-1</sup> FOM)	21.7	19.6	24.6	5.3	NS
Metabolizable energy (Mj kg <sup>-1</sup> DM)	9.30	8.09	8.07		

RSD-Residual standard deviation, \*  $P<0.05$ , \*\*  $P< 0.001$ , NS=Non-significant.

With the conservation process, CP digestibility and retained N decreased ( $P<0.01$ ) (Table 2). This decrease was greater in haylage than in hay. N retention was negative for animals fed with haylage, probably achieved by a higher proportion of ingested N excreted in the urine (60.9% in haylage vs

30.4% in fresh and 45% in hay). In silage, the CP soluble or rapidly degraded fraction (a) was greater than in fresh or hay forages (Table 3). This high fraction originates a rapid and high N-NH<sub>3</sub> level in the rumen (data not shown). If N-NH<sub>3</sub> is not completely used by the microbial population, it is absorbed through the rumen walls and excreted in the urine as urea. There were no significant differences among efficiency of microbial protein synthesis. Metabolizable energy was estimated by the equation ME=GE-(GEfeces+GEurine+GEmethane) where GEmethane was calculated according to Blaxter and Clapperton (1965). The value obtained for fresh sulla (1.3 MJ kg<sup>-1</sup> DM) was greater than for hay and haylage.

Table 3. In situ degradations parameters (g g<sup>-1</sup>) and effective degradability (ED) of dry matter (DM), Crude protein (CP), NDF and ADF.

		Fresh	Hay	Haylage	RSD	Significance
DM	a	0.42 <sup>c</sup>	0.31 <sup>a</sup>	0.37 <sup>b</sup>	0.0060	***
	b	0.35 <sup>a</sup>	0.38 <sup>b</sup>	0.35 <sup>a</sup>	0.0080	*
	c (h <sup>-1</sup> )	0.12	0.11	0.10	0.0088	NS
	ED	0.72	0.63 <sup>a</sup>	0.66 <sup>b</sup>	0.0098	**
CP	a	0.44 <sup>b</sup>	0.21 <sup>a</sup>	0.58 <sup>c</sup>	0.0112	***
	b	0.43 <sup>b</sup>	0.54 <sup>c</sup>	0.27 <sup>a</sup>	0.0214	***
	c (h <sup>-1</sup> )	0.14	0.12	0.09	0.0252	NS
NDF	ED	0.81 <sup>b</sup>	0.67 <sup>a</sup>	0.80 <sup>b</sup>	0.0179	**
	a	0.05	0.05	0.06	0.0062	NS
	b	0.51 <sup>b</sup>	0.45 <sup>a</sup>	0.45 <sup>a</sup>	0.0085	**
	c (h <sup>-1</sup> )	0.10	0.09	0.09	0.0130	NS
ADF	ED	0.47 <sup>b</sup>	0.42 <sup>a</sup>	0.43 <sup>a</sup>	0.0070	**
	a	0.13 <sup>b</sup>	0.11 <sup>a</sup>	0.10 <sup>a</sup>	0.0032	**
	b	0.47 <sup>b</sup>	0.42 <sup>a</sup>	0.44 <sup>b</sup>	0.0085	*
	c (h <sup>-1</sup> )	0.10	0.08	0.08	0.0124	NS
	ED	0.52 <sup>b</sup>	0.45 <sup>a</sup>	0.46 <sup>a</sup>	0.0070	*

RSD- Residual standard deviation, \*P < 0.05, \*\* P < 0.01, \*\*\* P<0.001, NS- Non-significant (P>0.05).

## Conclusions

Fresh sulla is a forage with good nutritive value. CP has a high apparent digestibility and was extensively degraded in the rumen. Conservation as hay or haylage led to a decrease in the nutritive value. Haymaking and ensilage reduced total sugar, CP digestibility, N-balance and ME. The N balance in haylage, was negative. The ED of CP was similar for fresh and haylage and is lower for hay. The efficiency of microbial protein synthesis was similar for feedstuffs.

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## Yield and forage yield components in winter vetch cultivars

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### Abstract

A small-plot trial was established at the Rimski Šančevi experiment field between autumn 2002 and summer 2005 to compare growth and yield of several vetch species. Seven vetches, including common vetch (*Vicia sativa* L. cvs. NS Sirmium, Neoplanta, L-386 and L-387), Hungarian vetch (*Vicia pannonica* Crantz cv. NS Panonika), hairy vetch (*Vicia villosa* Roth cv. NS Violeta) and bitter vetch (*Vicia ervilia* (L.) Willd. cv. Rodopi) were compared. Common, Hungarian and hairy vetch were from Serbia and Montenegro and the bitter vetch was from Bulgaria. All cultivars were sown in early October, at a crop density of 180 viable seeds per m<sup>2</sup>, and were cut when first pods began to appear (late May for bitter vetch, early June for common and Hungarian vetches and late June for hairy vetch). Number of plants per m<sup>2</sup> varied from 51 in NS Violeta to 165 in Rodopi. NS Violeta had the greatest plant height (158 cm) and number of internodes (32.7) and the largest plant mass (77.52 g). The greatest number of stems per plant was in NS Panonika (6.0). L-386 had the highest yield of fresh forage (32.5 t ha<sup>-1</sup>), while the highest yield of hay was in Neoplanta (9.2 t ha<sup>-1</sup>).

Keywords: vetches, winter cultivars, yield, yield components, fresh weight, hay.

### Introduction

Of the numerous *Vicia* species, it is common vetch (*V. sativa* L.), Hungarian vetch (*V. pannonica* Crantz) and hairy vetch (*V. villosa* Roth) are the most important in Serbia and Montenegro (Miladinović, 2001). Grown in an area of about 7,000 ha, vetches are used as winter and spring crops and in the form of fresh forage, hay, silage, haylage and green manure. Common vetch is the most widely distributed of the vetches due to its high and stable yields and reliable seed production. Hungarian vetch play an important role in the Eastern Mediterranean region (Orak, 2000), whereas hairy vetch is appreciated for its positive effects upon succeeding crop, soil structure and weed control (Hartwig and Ammon, 2002). Bitter vetch (*Vicia ervilia* (L.) Willd.) is used as hay in many countries of the Old World and is unknown in Serbia and Montenegro. Our study was aimed at determining forage yield and its components in winter cultivars of both traditional and introduced species such as common, Hungarian, hairy and bitter vetch.

### Materials and methods

A small-plot trial was conducted at the Rimski Šančevi Experiment Field between autumn 2002 and summer 2005. The trial included four cultivars of common vetch (NS Sirmium, Neoplanta, L-386 and L-387) and one cultivar each of Hungarian vetch (NS Panonika), hairy vetch (NS Violeta) and bitter vetch (Rodopi). The bitter vetch was from Bulgaria and all other vetches were from Serbia and Montenegro. All cultivars were sown in early October at a crop density of 180 viable seeds m<sup>-2</sup>, that was about 110 kg ha<sup>-1</sup> in common vetches, 90 kg ha<sup>-1</sup> in Hungarian and bitter vetches and 70 kg ha<sup>-1</sup> in hairy vetch. Each cultivar was sown in three replicate plots of 5 m<sup>2</sup> in size. The prevailing weather conditions during the growing period of these four vetch species are given in Table 1. Lodging was not prominent in any of the nine cultivars and did not affect forage yields. The vetches were cut when first pods began to appear (Kertikov, 2003). This occurred in late May for bitter vetch, early June for common and Hungarian vetches and late June for hairy vetch. The number of plants m<sup>-2</sup> before cutting, plant height

(cm), number of stems per plant, number of internodes, plant mass (g), yield of fresh forage and hay ( $t\ ha^{-1}$ ) were measured. The results were processed by the analysis of variance with the *LSD* test applied.

Table 1. Mean values of average monthly temperatures ( $^{\circ}C$ ) and monthly precipitation sums (mm) during the growing period of winter vetches between October 2002 and June 2005 at Rimski Šančevi as compared to the long-term mean values.

	Months	X	XI	XII	I	II	III	IV	V	VI	X-VI mean
Average monthly temperature	2002-05 mean	13.8	8.5	2.0	-0.9	-2.1	5.5	11.8	17.6	20.9	8.6
	Long-term mean	11.6	5.8	1.6	-0.5	1.8	6.2	11.3	16.7	19.7	8.2
Monthly precipitation sum	2002-05 mean	107.7	63.3	28.0	44.3	35.0	22.3	50.3	53.0	90.3	494.3
	Long-term mean	43.3	49.7	48.2	37.3	32.3	38.1	46.7	58.7	84.5	438.8

## Results and discussion

Bitter vetch proved the most winter hardy of the three vetch species with 165 plants  $m^{-2}$  that survived until cutting. The plant density of common vetch ranged from 138 in L-387 to 157 plants  $m^{-2}$  in Neoplanta, which confirms that winter genotypes of common vetch are well adapted to the harsh winter conditions of Serbia and Montenegro (Mihailović *et al.*, 2005a). Hungarian vetch had an intermediate value of 102 plants  $m^{-2}$ . There were more than 51 plants  $m^{-2}$  of hairy vetch after the winter, but that number was subsequently reduced due to non uniform growth and development within the stand.

Table 2. Average values of the forage yield components and forage yields of winter vetches cultivars at Rimski Šančevi for the period between October 2002 and June 2005.

Species	Cultivar name	No. of plants per $m^2$	Plant height (cm)	No. of stems per plant	No. of internodes per stem	Plant mass (g)	Yield of fresh forage ( $t\ ha^{-1}$ )	Yield of hay ( $t\ ha^{-1}$ )
Common vetch	NS Sirmium	147	112	3.3	23.8	24.08	29.7	8.3
	Neoplanta	157	92	2.9	22.6	22.87	31.9	9.2
	L-386	155	108	2.5	24.8	24.00	32.5	6.5
	L-387	138	113	2.3	27.3	27.01	30.5	8.3
Hairy vetch	NS Violeta	51	158	3.8	32.7	77.52	31.2	5.7
Hungarian vetch	NS Panonika	102	78	6.0	22.6	29.89	24.2	5.3
Bitter vetch	Rodopi	165	60	2.6	18.1	18.64	30.7	6.9
<i>LSD</i>	$P < 0.05$	12	10	2.6	4.3	7.18	2.8	0.7
	$P < 0.01$	17	14	3.3	5.8	11.03	4.3	1.1

Plant height was the greatest in hairy vetch (158 cm) and smallest in bitter vetch 60 cm. The greatest number of stems was in Hungarian vetch, which was more than the species average (Radenović, 2000). The smallest number of stems was in common vetch L-387 (2.3). The number of internodes per stem ranged between 18.1 in bitter vetch to 32.7 in hairy vetch.

The largest plant mass was produced by hairy vetch (77.52 g) because of excessive growth (Mihailović *et al.*, 2005b). Bitter vetch had the smallest plant mass (18.64 g). Yields of fresh forage in winter common vetch ranged from 29.7  $t\ ha^{-1}$  in NS Sirmium to 32.5  $t\ ha^{-1}$  in L-386 (Table 2). These yields were higher or at the same level as in spring cultivars (Mihailović *et al.*, 2005c). Yields of hay ranged from 6.5  $t\ ha^{-1}$  to 9.2  $t\ ha^{-1}$  and were similar to those obtained by others under conditions of Spain (Lloveras *et al.*, 2004). With a yield of 5.7  $t\ ha^{-1}$ , hairy vetch produced relatively high yields of hay

confirming its productivity in areas with a long growing season (Guldan and Martin, 2003). Hungarian vetch yielded 5.3 t ha<sup>-1</sup> of hay, which is considered satisfactory for dry regions with a continental climate (Uzun *et al.*, 2004). Bitter vetch yielded 30.7 t ha<sup>-1</sup> of fresh forage and 6.9 t ha<sup>-1</sup> of hay.

### Conclusions

Common vetch proved to be the most yielding vetch species in the prevailing continental conditions of Serbia and Montenegro. Hairy vetch has a great potential for high forage yields, but requires a certain improvement in terms of stand uniformity and determinate growth. There is a need to improve winter hardiness in Hungarian vetch, as well as to continue testing upon utilisation of bitter vetch as forage.

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## Lupins in sustainable agriculture: leaching losses following grain harvest

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### Abstract

Two varieties of blue flowering lupin (*Lupinus angustifolius*, L.var. Bora and Prima) and one of yellow (*L. luteus*, L.var. Wodjil) were compared with peas (*Pisum sativum*, L.var. Ventura) in the uptake of major nutrients. After the grain harvest, leaching losses were measured under a following winter cereal. Losses of N and P (but not K) were highest from previously bare fallow ground, and lowest after unfertilized grass. There were no differences in losses between the peas and the lupins. Prima was the most effective of the lupins at fixing N and the most suitable to precede a winter cereal.

Keywords: leaching, lupins, fallow, nitrate, phosphorus, potassium.

### Introduction

In both arable and livestock sectors, leaching and runoff of nitrogen (N) and phosphorus (P) into water courses is a major environmental issue reflected in the implementation of nitrate vulnerable zones (NVZ) and the EU Water Framework Directive. Alongside the need for environmentally sustainable farming there remains a requirement for economic sustainability, particularly in the livestock sector for efficient, high protein content crop production. The need for traceable, non-genetically modified sources of protein is essential for organic farmers, where N fixing grain legumes have the potential to satisfy this requirement, providing a source of oil and energy. This work examines the role of three spring sown lupins, as both sources of N (N fixation) and as sinks for other nutrients, in terms of the environmental impact of nutrient leaching under a following winter cereal.

### Material and methods

Two varieties of blue flowering lupin (*Lupinus angustifolius*, var. Bora and Prima) and one yellow (*L. luteus*, var. Wodjil) were compared with peas (*Pisum sativum*, var. Ventura) grown in field plots (15 x 15 m) on a freely draining soil (sandy loam, FAO dystric or eutric cambisol), pH 5.5 with 36 and 240 mg l<sup>-1</sup> available P and potassium (K); no N was applied. There were four replicate plots of each plant type which were randomized in four blocks. Each plot was split into two sub-plots, so that half received a seed bed application of triple super phosphate (40 kg P ha<sup>-1</sup>). Seeds were sown in May 2004 at rates adjusted to achieve planting densities (m<sup>-2</sup>) of 70 (Bora and Wodjil), 80 (Ventura) and 100 (Prima). An inoculum of *Bradyrhizobium* was applied to the lupin plots to encourage nodulation. Eight unfertilized control plots (7.5 x 15 m) were included in the experimental layout: half were kept fallow (i.e. no weeds) by hoeing throughout the growing season and the remainder sown (30 kg seed ha<sup>-1</sup>) with a diploid perennial ryegrass (*Lolium perenne* L., var. Glen). Crops were harvested using a 71 x 71 cm<sup>2</sup>, placed randomly in the sub-plots and cut to 2.5 cm above ground level. Seed pods were removed by hand and air-dried for 72 h before shelling. Peas were harvested after 128 d from sowing, but the harvest of lupins was staggered to accommodate variations in maturation: first was Prima (133 d) and then Bora, Wodjil and grass plots after 147 d. All plots were cleared using a small plot harvester and the residues ploughed in, power harrowed and sown on 26 October 2004 with winter wheat (*Triticum aestivum*, L. Var. Claire). Before emergence, five macrorhizon leaching tubes (Meijboom and van Noordwijk, 1996) were installed in each sub-plot. The sampling tubes were run to a central collection point between each pair of sub-plots to minimise damage to the new cereal crop when leachates were obtained. The macrorhizons were put under vacuum by applying a suction from a 60 ml syringe with the

plunger held fully open by a peg for 24 h. The frequency of sampling was dictated by the amount of precipitation and initiated whenever cumulative rainfall reached ca. 50 mm. Sampling continued from December 2004 to April 2005 (when drainage ceased): all samples were frozen shortly after collection and subsequently analysed for nitrate-N and P (soluble-reactive) by automated analyses and K by flame photometry.

## Results and discussion

No significant differences were found between the  $\pm$  P treatments, so yield and nutrient data have been combined in the Tables (8 sub-plots each) to show overall means for each species.

Table 1. Grain yield (at 85% DM) and nutrient offtake in the grain ( $\text{kg ha}^{-1}$ ) from peas and three lupin varieties.

	Peas	Bora	Prima	Wodjil
Grain yield	4820 <sub>a</sub>	1318 <sub>c</sub>	2455 <sub>b</sub>	1346 <sub>c</sub>
N	151 <sub>a</sub>	52 <sub>c</sub>	97 <sub>b</sub>	61 <sub>c</sub>
P	8.2 <sub>a</sub>	3.0 <sub>c</sub>	5.3 <sub>b</sub>	6.5 <sub>b</sub>
K	54 <sub>a</sub>	22 <sub>b</sub>	30 <sub>b</sub>	20 <sub>b</sub>

For comparisons between legumes, values that have different letters are sig. diff.  $P < 0.05$ .

Peas (Ventura) out-yielded all three lupin varieties (Table 1); the first of the lupins to mature was Prima, which out-performed Bora and Wodjil (which did not differ significantly) in grain yield. Nitrogen contents of grain followed the same trends in grain yield, but contents of P and K were similar in all the lupins (except for Bora which was significantly lower in P).

Table 2. Plant yield (without grain) and nutrient offtake ( $\text{kg ha}^{-1}$ ) from peas and three lupin varieties.

Plant offtakes	Peas	Bora	Prima	Wodjil
DM yield	3205 <sub>a</sub>	2414 <sub>bc</sub>	2005 <sub>bc</sub>	2898 <sub>ab</sub>
N	51 <sub>a</sub>	34 <sub>bc</sub>	26 <sub>c</sub>	40 <sub>ab</sub>
P	1.6 <sub>b</sub>	2.9 <sub>ab</sub>	1.5 <sub>b</sub>	4.0 <sub>a</sub>
K	19 <sub>c</sub>	46 <sub>ab</sub>	23 <sub>bc</sub>	55 <sub>a</sub>

For comparisons between legumes, values that have different letters are sig. diff.  $P < 0.05$ .

Plant DM yield and N content (Table 2) were highest in peas, but lowest in Prima, reflecting the greater investment in grain at harvesting than the two slower maturing varieties (Wodjil and Bora). Wodjil seeds were still not fully ripened at harvest, but sowing winter cereal could not be delayed further due to deteriorating soil conditions. Consequently, more P and K were removed in plant material with Wodjil than with Prima or peas, but amounts were not significantly different from Bora. An estimate of N fixation was obtained from the total N offtake in plant biomass, minus the offtake from the unfertilized grass, amounting to 184, 67, 104 and 82  $\text{kg N ha}^{-1}$  in peas, Bora, Prima and Wodjil, respectively. Before ploughing in the autumn, soil mineral N in plots which had previously grown peas or lupins was  $> 20 \text{ kg N}$  (inorganic) in the 0-60 cm soil profile, with the minor component in the lower (30-60 cm) layer. In contrast, plots previously with grass had  $< 20 \text{ kg N}$  and the fallowed plots had 55  $\text{kg N}$  (0-60 cm profile). Also, the distribution of inorganic N in the fallowed plots was more evenly spread between the upper and lower soil layers, showing a greater migration of inorganic N to depth when no plants had been allowed to grow before planting winter wheat.

Table 3. Nutrients leached ( $\text{kg ha}^{-1}$ ) from plots under winter wheat grown where previous treatments had been peas, lupins, grass or maintained fallow.

Nutrients	Peas	Lupin (Bora)	Lupin (Prima)	Lupin (Wodjil)	Grass	Fallow
N	15.3 <sub>ab</sub>	11.1 <sub>bc</sub>	17.0 <sub>ab</sub>	11.4 <sub>bc</sub>	5.5 <sub>c</sub>	19.7 <sub>a</sub>
P	0.05	0.06	0.05	0.06	0.03	0.07
K	2.8	2.9	2.7	2.8	2.6	2.1

For comparisons between previous plot treatments, values that have different letters are sig. diff.  $P < 0.05$ .

Table 3 shows nutrient leaching under winter wheat, following the harvest of lupins, peas and grass. Overall losses of nitrate-N ( $\text{kg ha}^{-1}$ ) were highest on soil that was previously kept fallow, but there was high variability in leachate samples and no significant differences were found between nutrient losses following lupins or peas; the lowest losses for N and P, however, were recorded after unfertilized grass. Concentrations of nitrate in leachates remained below the EU limit ( $11.3 \text{ mg NO}_3^- \text{N l}^{-1}$ ) for drinking water throughout (data not shown); the exception was the first sampling (December 2004) in previously fallowed plots (ca.  $16 \text{ mg NO}_3^- \text{N l}^{-1}$ ). The lack of response we found to added P, in either biomass or grain production was also observed by Watt and Evans (2003). Added P did not improve the soil index for available P and no differences were found in leached P (or K) under winter cereal. The vulnerability of bare fallow soil to winter leaching was clearly demonstrated and was lowest where the unfertilized grass cover provided an effective sink for available soil N. Anderson *et al.* (1998) also found less leaching under pasture than lupin crops and fallow soil (with weeds) was shown to leach more than soil growing lupins (McLenaghan *et al.*, 1996).

### Conclusions

The highest values for leached nutrients (N and P, but not K) were from bare fallow ground (preceding a winter cereal) with no significant differences in leaching following any of the legume crops. Unfertilized grass provided the most effective sink for available N and P with significantly lower losses of nitrate-N under the winter cereal. Of the three lupin varieties grown, Prima would appear to be best adapted to shorter growing seasons, if a winter crop is anticipated and was the most effective (except for peas) at fixing N.

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## Alfalfa production and quality in Northeast Spain

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### Abstract

The paper summarizes the data from published research and surveys on alfalfa (*Medicago sativa* L.) dry matter (DM) yields and crude protein contents in Northeast Spain. The average DM yields varied from 10.1 to 22.5 t DM ha<sup>-1</sup>, in irrigated conditions, and from 1.3 to 6.3 t DM ha<sup>-1</sup> in rain fed conditions. The crude protein contents of the crop ranged from 110 to 251 g kg<sup>-1</sup> DM under irrigation and averaged 205 g kg<sup>-1</sup> DM in non irrigated areas. The numbers of harvests per season was normally between 5 and 7. It is concluded that the Ebro valley is a suitable area for alfalfa production.

Keywords: harvest management, winter grazing, nutritive value.

### Introduction

Spain is the second largest alfalfa producer in Europe (FAO, 2004) with 260,000 ha, 77% of which are irrigated. Of this area, 58% is located in NE Spain, in the Ebro valley area (Aragón and Catalunya, with 107,000 ha and 42,500 ha, respectively) where alfalfa is an important part of the cropping systems based on the alfalfa-corn-wheat rotation. The forage production of the Ebro valley area is about 8,080,000 t of dry forage that represents 63% of the Spanish production. In this area alfalfa is used mainly for dehydration and exported to other areas and countries (MAPA, 2004). In the last 15 years, the economic importance of alfalfa in the area has motivated several research projects on the management of the crop, funded by the Spanish Ministry of Agriculture and by private firms. The aim of this paper is to summarize the published results on alfalfa dry matter (DM) yields and quality, related to different management techniques, in the Ebro valley.

### Materials and Methods

The data used in this review, presented in Table 1, is drawn from 10-15 years of published research trials conducted in CITA, Aragón (41° 43' N; 0° 52' W, 41° 39' N; 0° 23' W), in the University of Lleida (UdL)-IRTA research Center and in Mas Badia-IRTA Experimental Station in Girona (42° 03' N; 3° 04' W). The climate of the area is classified as Continental Mediterranean with an annual average temperature of 14°C, and a rainfall between 300 and 400 mm in the first two locations, and little higher in Gerona, near the Mediterranean coast. In all the trials, the forage harvested was oven-dried at 70°C for 48 hours and the crude protein (CP) was analyzed by Near-Infra Red Spectroscopy (NIRS) using a InfraAnalyzer 2000.

### Results and discussion

The number of harvests per season were two to three in rainfed areas and six to seven under irrigation and the results of field trials under different management studies (irrigation vs no irrigation, cutting and grazing, etc) show a range of dry matter yields from 10 to 22 t DM ha<sup>-1</sup> in irrigated conditions (Lloveras *et al.*, 1998; Delgado, 1988) depending on the amount of water available and the soil type.

Table 1. Dry Matter production (DM, t ha<sup>-1</sup> yr<sup>-1</sup>) and crude protein content (CP, g kg<sup>-1</sup>) in NE Spain for different locations and managements.

Loc	Altitude m	Coordinates	Sampling year	Soil	Tm °C	Rainfall (mm)	Irrigation m <sup>3</sup> ha <sup>-1</sup>	Cutting /grazing	Cuts yr <sup>-1</sup>	DM t ha <sup>-1</sup> yr <sup>-1</sup>	CP g kg <sup>-1</sup>
Palau Anglesola	180	41°39'N, 0°51'E	1990-93	Gypsic Xerocept	13.4	396	yes	Cut	6	19.4	-
Palau Anglesola	180	41°39'N, 0°51'E	1992-94	Gypsic Xerocept	13.6	418	Yes	Cut	6-7	21.6	212
Palau Anglesola	180	41°39'N, 0°51'E	1993-97	Oxyaquic Xerofluent	11.1	433	Yes	Cut	6	22.5	192
Alcolea Cinca	200	41°43'N, 0°07'E	2001-02	Typic Xerofluent	14.0	325	Yes	Cut	6	13.7	251
Villanueva Sigena	250	41°43'N, 0°08'E	2001-02	Calcixerolic Xerocept	14.0	325	Yes	Cut	6	10.4	223
Gimenells	258	41°39'N, 0°23'E	2001-02	Calcixercept Petrocalcic	13.6	409	Yes	Cut	6-7	21.7	203
Gimenells	258	41°39'N, 0°23'E	2001-02	Calcixercept Petrocalcic	13.6	409	Yes	Cut	6-7	13.7	201
Gimenells	258	41°39'N, 0°23'E	2002-04	Calcixercept Petrocalcic	15.8	407	Yes	Cut	6-7	18.2	110
Gimenells	258	41°39'N, 0°23'E	1998-2000	Calcixercept Petrocalcic	14.3	370	Yes	Cut	4	10.1	228
Gimenells	258	41°39'N, 0°23'E	1998-2000	Calcixercept Petrocalcic	14.3	370	Yes	Grazed Cut	4	8.28	246
Zaragoza	225	41°43'N, 0°52'W	1985-87	Fluvisol	14.3	315	Yes	Cut	6	19.33	211
Zaragoza	225	41°43'N, 0°52'W	1985-87	Fluvisol	14.3	315	Yes	Grazed	7	18.93	221
Zaragoza	225	41°43'N, 0°52'W	1987-91	Fluvisol	15.1	344	Yes	Cut	6	17.95	-
Zaragoza	225	41°43'N, 0°52'W	1987-91	Fluvisol	15.1	344	Yes	Grazed	7	16.91	-
Zaragoza	225	41°43'N, 0°52'W	1999-00	Fluvisol	15	412	Yes	Cut	6	16.36	203
Zaragoza	225	41°43'N, 0°52'W	1999-02	Fluvisol	14.9	323	Yes	Cut	6	14.34	199
Zaragoza	225	41°43'N, 0°52'W	1999-02	Fluvisol	14.9	323	Yes	Grazed	7	14.48	200
Zaragoza	225	41°43'N, 0°52'W	1999-02	Fluvisol	14.9	363	Yes	Cut	6	16.37	217
Marracos (Z)	450	42°07'N, 0°42'W	1979-81	Renzina	13.4	494	No	Cut	3	6.32	-
Pancrudo (Te)	1200	40°47'N, 0°42'E	1979-81	Brown soils	8.7	426	No	Cut	3	5.71	-
Peñaflo (Z)	250	41°46'N, 0°45'W	1986-87	Renzina	14.4	338	No	Cut	2	1.37	-
Marracos (Z)	450	42°07'N, 0°42'W	1986-87	Renzina	13.7	473	No	Cut	3	4.48	-
San Blas (Te)	900	40°20'N, 1°06'E	1986-90	Brown soils	11.3	485	No	Cut	4	4.52	-
Alcolea Cinca (Hu)	186	41°42'N, 0°6.98'E	2001	Xerofluent Typic	14.6	266	Yes	Cut	5	12.6	230
Villanueva Sigena (Hu)	231	41°42'N, 0°0.16'E	2001	Xerocept Calcixerolic	14.6	266	Yes	Cut	5	8.0	191
Tallada Empordà (Gi)	20	42°03'N, 3°04'E	2002	Xerofluent oxiaquico	14.9	634	No	Cut	3	9.1	205
Tallada Empordà (Gi)	20	42°03'N, 3°04'E	2002	Xerofluent oxiaquico	14.9	634	Yes	Cut	5	14.9	195
Tallada Empordà (Gi)	20	42°03'N, 3°04'E	2002	Xerofluent oxiaquico	14.9	634	No	Cut	3	9.9	202
Tallada Empordà (Gi)	20	42°03'N, 3°04'E	2002	Xerofluent oxiaquico	14.9	634	Yes	Cut	5	17.4	196

On the other hand, surveys of the alfalfa producers show that about 77% of the areas with alfalfa, in the Ebro valley, have average yields of between 14.5 and 17.5 t DM ha<sup>-1</sup>. In non irrigated conditions the forage DM yields are much lower, 1.3-6.3 t ha<sup>-1</sup>, depending mainly on the rainfall (Alvaro and Lloveras, 2003; Delgado, 1984; 1988). These yields are similar to those of many areas of Europe (FAO, 2004), showing the potential of the crop and its adaptation to the studied area. Winter grazing is common in the area, and studies show that this practice reduces very little the DM yields of the subsequent season, from 17.2 to 16.8 t ha<sup>-1</sup>, suggesting that this management can be a good way to extend crop use during the year (Chocarro *et al.*, 2005; Delgado *et al.*, 1992). In the conditions of the studies surveyed, slurry manure and K fertilization increased DM yields in soils with low fertility, whereas in high fertility soils, the use of slurry manure does not decrease forage yields (Lloveras *et al.*, 2001; Lloveras *et al.*, 2004). On the other hand, cutting stage trials showed that increasing from six to seven harvest per year (harvesting before flowering, compared with cutting after flowering) decreased DM yields by 20% from 25.5 t ha<sup>-1</sup> to 21.6 t ha<sup>-1</sup> (Lloveras *et al.*, 1988). As far as forage quality, in irrigated conditions, the average CP content ranged from 110 g kg<sup>-1</sup> DM to 251 g kg<sup>-1</sup> DM, depending on the harvest stage. In rainfed trials the CP contents ranged between 202-205 g kg<sup>-1</sup> DM. These values can be considered high

according to the European standards (Journet, 1993) and excellent according to USA standards (Undersander *et al.*, 1993). In general, in irrigated conditions, winter-grazing increased CP content of the first spring harvest from 205 g kg<sup>-1</sup>DM to 210 g kg<sup>-1</sup>.

## Conclusions

This study shows a good performance of the alfalfa crop in the irrigated conditions of North East Spain, producing high forage yields of good quality. However, the raise of the cost of irrigation water or its scarcity can increase the costs of production and possibly reduce the area under alfalfa in the future.

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## The influence of lucerne stand structure on fibre content in the forage

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### Abstract

The aim of this work was to investigate the influence of stand structure on fibre content in the aboveground biomass of lucerne (*Medicago sativa* L.). In 2004, the measurement and sampling were repeatedly realized from late vegetative to late flowering stage plants in the first cut. Compressed height (CH) of stand was measured by rising-plate meter and the number of plants recorded at each sampling point. The number of most mature stems, total number of stems, neutral detergent fibre (NDF), acid detergent fibre (ADF), and crude fibre (CF) content was assessed for each sample. Significant positive relationships were observed for NDF ( $P < 0.0000$ ), ADF ( $P < 0.0000$ ) and CF ( $P < 0.0000$ ) with CH, and negative significant correlations for NDF ( $P = 0.0145$ ), ADF ( $P = 0.0145$ ) and CF ( $P = 0.0200$ ) with the number of most mature stems. For CH, the  $R^2$  values obtained from the linear model were 88.80, 83.20 and 83.24 for NDF, ADF, and CF, respectively. For number of most mature stems, the corresponding  $R^2$  values were 19.51, 19.54 and 17.86, respectively. CH is simply measured and explained over 88% of fibre content variability evaluated by RDA analysis and therefore seems a good predictor of fibre content in the forage of lucerne in the first cut.

Keywords: lucerne, rising-plate meter, stands structure, fibre.

### Introduction

Morphological characteristics of lucerne are significantly correlated with yield and quality of dry matter (Katić *et al.*, 2003). In practice, a single plant of lucerne has no interest, because the exploitation concerns not a plant but the stand. Indeed, an optimal structure of stand is necessary but not sufficient to secure a high forage quality (Rotili *et al.*, 1999). The relationship between length of lucerne stems and development stages has been shown to be connected with forage quality by Sulc *et al.* (1999). They described relationships among developmental stages, height of lucerne stands, and neutral detergent fibre content (NDF) and obtained an  $R^2$  value of 0.89 for the linear regression of NDF content and length of tallest stem. It can be difficult to determine the length of tallest stem and furthermore the fibre content in lucerne forage can be influenced by stand density (Šantrůček, 1989). The aim of this work was to investigate the influence of compressed height (measured by rising-plate meter) and stand structure (density and number of stems) on CF, ADF, and NDF content in the aboveground biomass of lucerne (*Medicago sativa* L.).

### Materials and Methods

The experimental plants consisted of two lucerne cultivars and variety Jarka grown in two replications in completely randomised blocks. In 2004, the measurement and sampling were repeatedly realized from the late vegetative to the late flowering stage in the first cut. The compressed height (CH) of stand was measured by rising-plate meter (Castle, 1976) and number of plants was assessed per each sampling point. The number of most mature stems (stems in declared stage), total number of stems, neutral detergent fiber (NDF), acid detergent fiber (ADF), and crude fiber (CF) content was assessed for each sample. The data analyses were performed by simple linear regression in Statistica 6.0. For separating evaluated variable effects variation partitioning was made by RDA analysis in Canoco (Leps and Smilauer, 2003).

## Results and discussion

According to widely accepted knowledge (Dukić and Erić, 1995; Sulc *et al.*, 1999) about developmental stages on fibre content we used variation partitioning to separate the effects of developmental stages and measured parameters. These results are shown in Table 1. The candivars and variety Jarka explained only 0.6% of fibre content variability and were not included to other analyses.

Table 1 Results of RDA analysis. (explanatory variables of all canonical axes, unrestricted permutations, number of permutations 4999).

Variables	Covariables	% of explained variability	F-test	P-value
Measured characteristic and developmental stages	-	89.8	33.82	0.0002
Developmental stages	-	81.1	119.84	0.0002
Measured characteristic	-	89.5	199.03	0.0002
Developmental stages	measured characteristic	0.3	0.66	0.4822
Measured characteristic	developmental stages	8.8	3.96	0.0002

Generally, the measured parameters were closely related with developmental stages. The ratio of explained variability which can not be separated for measured characteristic or developmental stages is over 80% (89.5 – 8.8 or 81.1 – 0.3). In the test of measured characteristics without covariables the first canonical axe explained 99.7% of all and was closely positive correlated with CH and negatively with the number of most mature stems so we can suppose that CH and the number of most mature stems were the most important measured stand characteristics with relation to fibre content. After separating developmental stage effects CH explained an additional 8% or 8.8% of variability when taken together with the number of most mature stems. CH without other effect explained 88.4% of fibre content variability.

Our results of simple linear regressions show significant positive relationship for all forms of fiber: NDF ( $P < 0.0000$ ), ADF ( $P < 0.0000$ ) and CF ( $P < 0.0000$ ) with CH and negative significant correlations for NDF ( $P = 0.0145$ ), ADF ( $P = 0.0145$ ) and CF ( $P = 0.0200$ ) with number of most mature stems. For CH, the  $R^2$  values accounted for by the linear model were 88.80, 83.20 and 83.24 for NDF, ADF and CF, respectively. These results are in accordance with Sulc *et al.* (1999) regarding the relationship between length of most mature stems and NDF content ( $R^2 = 0.89$ ). For number of most mature stems, we obtained the corresponding  $R^2$  values 19.51, 19.54 and 17.86, respectively. Other evaluated parameters: number of plants per  $m^{-2}$ , total number of stems per  $m^{-2}$ , and number of stems per plant did not affect the quality of lucerne forage. Although stand density can influence the fibre content (Šantrůček, 1989) we included only close and complete stands in the second year of vegetation in this experiment. The number of plants and number of stems per  $m^{-2}$  ranged approximately from 200 to 400 and from 750 to 1600, respectively.

## Conclusions

The structure of stand was not significantly correlated with fibre content in close stands; however the number of most mature stems is moderately negatively correlated with NDF, ADF, and CF content. Compressed height measured by rising-plate meter was closely correlated with fibre, mainly for NDF. This parameter is simply measured and explained over 88% of fibre content variability evaluated by RDA analysis so it seems as a good predictor for fibre content. The experiment is in progress so these results should not be considered definitive.

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## Effect of harvest frequency on yield and quality of alfalfa forage

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### Abstract

The yield and quality of alfalfa depend on plant maturity at the time of harvest. If harvested earlier in the season, alfalfa produces a larger number of cuttings per year, a lower yield per cutting but higher forage quality. The objective of this study was to assess the yield and quality of alfalfa forage as affected by the frequency of cutting. In the year of establishment (2004), the study compared three cuts taken at the early flowering stage or two cuts taken at full flowering stage and in the second year of production, four cuts were taken at the early flowering stage and three cuts at the full flowering stage. In 2004, we obtained 18.48 t ha<sup>-1</sup> of green forage on average per cut (equivalent to 5.65 t ha<sup>-1</sup> dry matter, DM) in the two-cut harvest schedule and 17.16 t ha<sup>-1</sup> of forage (equivalent to 4.46 t ha<sup>-1</sup> DM) in the three-cut schedule. However, the leaf to stem ratio was significantly higher in the more intensive than in the less intensive schedule, 490 g kg<sup>-1</sup> and 410 g kg<sup>-1</sup>, respectively. The average plant heights in the three-cut and two-cut schedules were 57 cm and 78 cm, respectively.

Keywords: alfalfa, cutting schedule, quality, yield.

### Introduction

If harvested at the appropriate development stage, alfalfa can provide high yields of quality forage for 3 to 5 years. Alfalfa harvested at later stages (full flower) produces increased yields of green forage and DM whilst prolonging the productive life of the alfalfa field (Lloveras *et al.*, 1998). However, harvesting at earlier development stages (early flower) produces hay and DM with improved quality, i.e., more crude protein and less crude cellulose (Katić *et al.*, 2003). Alfalfa quality degradation with age is primarily a consequence of reduced leaf proportion and increased content of crude cellulose in the stem (Buxton *et al.*, 1985; Onstad *et al.*, 1983). More recently, alfalfa harvested at the early flower stage has been favored, as a compromise between yield quantity and quality (Sheaffer, 1988). Whilst cuttings taken earlier prevent the replenishment of carbohydrate reserves, significantly decreasing the productive life and yield of alfalfa, harvests taken at the early flower stage provide sufficient time for carbohydrate reserves to be replenished (Gabrielson *et al.*, 1985).

The objective of this study was to assess the yield and quality of alfalfa harvested at the early flower or full flower stages.

### Materials and Methods

Field trials were conducted in 2004 and 2005 in an experimental field at the Institute of Field and Vegetable Crops in Novi Sad (45° northern latitude, 19° eastern longitude, 80m altitude). The soil in the field was a chernozem with good physical properties (pH 7.25 in KCL) containing 2.36% CaCO<sub>3</sub>, 0.154 % N, 21.81 mg P<sub>2</sub>O<sub>5</sub> 100g<sup>-1</sup> of soil and 31.2 mg K<sub>2</sub>O 100g<sup>-1</sup> of soil in the 0-30 cm layer.

Thirty six alfalfa varieties were planted in three replications and two treatments: Block I -harvested at the early flower stage (EF) (10 % of plants in flower) and Block II - harvested at the full flower stage (FF) (> 50 % of plants in flower). Herbicides were applied to control weeds. In the year of establishment (2004), three cuts were taken at the EF stage compared with two cuts taken at FF. In the second year of production (2005), four cuts were taken at the EF stage compared with three cuts taken at FF. Green forage yield was measured in the field. Samples of 500 g were taken for determinations of DM content, leaf to stem ratio and plant height. Significance of differences between the means of the treatments was determined by the t test (df 35).

## Results and Discussion

Alfalfa harvested at the FF stage produced significantly higher ( $p < 0.05$ ) yields of green forage and DM on average per cut than the harvests taken at the EF stage (Tables 1 and 2).

In the first year (2004), the harvest at the EF stage brought higher yields of forage and hay. In the second year (2005), higher yields were obtained with the harvest at the FF stage. However, Zeidan *et al.*, (1988) did not obtain higher yields with harvests at the FF stage. Alfalfa plants harvested at the FF stage were 20 cm taller than those harvested at the EF stage (Tables 1, 2).

Table 1. Effect of harvest frequency on yields of green forage and dry matter, plant height and proportion of leaves in the year of establishment (2004).

Stage	Green forage yield (t ha <sup>-1</sup> )		Dry matter yield (t ha <sup>-1</sup> )		Proportion of leaves (g kg <sup>-1</sup> )	Plant height (cm)
	Mean per cut	Per Year	Per cut	Per Year		
Early flower	17.61 <sup>NS</sup>	52.8 <sup>**</sup>	4.46	13.4 <sup>**</sup>	490 <sup>**</sup>	57
Full flower	18.48 <sup>NS</sup>	36.9	5.65 <sup>*</sup>	11.3	410	78 <sup>**</sup>

NS- non significant; \* significant  $p < 0.05$ ; \*\* significant  $p < 0.01$ .

Katić (2001) obtained highly significant correlations between yield and plant height. However, the harvest at the EF stage produced a 7% higher proportion of leaves (Tables 1, 2).

Table 2. Effect of harvest frequency on yields of green forage and dry matter, plant height and proportion of leaves in the second year (2005).

Stage	Green forage yield (t ha <sup>-1</sup> )		Dry matter yield (t ha <sup>-1</sup> )		Proportion of leaves (g kg <sup>-1</sup> )	Plant height (cm)
	Per cut	Per Year	Per cut	Per Year		
Early flower	21.58	86.3 <sup>NS</sup>	3.88	15.5	470 <sup>**</sup>	61
Full flower	27.39 <sup>*</sup>	82.2 <sup>NS</sup>	5.38 <sup>**</sup>	16.1 <sup>**</sup>	400	83 <sup>**</sup>

NS- non significant; \* significant  $p < 0.05$ ; \*\* significant  $p < 0.01$ .

Proportion of leaves in DM was shown to reduce from the EF stage to the FF stage (Katić *et al.*, 2003).

At the EF stage, leaves and stems contained significantly higher amounts of crude protein, (29.01 % and 9.18 %, respectively), and significantly lower amounts of crude cellulose (11.51 % and 32.49 %, respectively). Michaud *et al.*, (2001) found that two thirds of the total crude protein was located in alfalfa leaves. Rotili *et al.*, (2001) suggested that the proportion of leaves in the forage might be considered as an indirect indicator of alfalfa quality.

Alfalfa harvest at the EF stage provides more net energy for milk production (Zeidan *et al.*, 1988), which seems to justify the use of the early harvest method. Also, alfalfa harvest at the EF stage brings the same or higher total yield as well as a significantly higher proportion of leaves, i.e., higher quality of forage or hay, than the harvest at the FF stage. Furthermore, the early harvest ensures a good combination of yield (green forage and DM) and quality (crude protein content).

## Conclusions

Alfalfa harvested at the FF stage produces higher yields of green forage and dry matter than harvesting at the EF stage.

A higher proportion of leaves, i.e., better quality of green forage and DM, are obtained when harvesting alfalfa at the EF stage. Alfalfa plants are taller if harvested at the FF stage than at the EF stage.



Alfalfa harvested at the EF stage provides the best combination of yield (green forage and DM) and quality (crude protein content).

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# Autumn harvest management of lucerne in seeding year on root carbohydrates content before overwintering

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## Abstract

Root reserves in lucerne, which are mainly represented by carbohydrates, influence the overwintering of plants. The effect of autumn harvest management of two cultivars of lucerne (*Medicago sativa* L.) – Jarka (Czech) and Europe (French) in the seeding year (early spring seeding) on root carbohydrates before overwintering was studied in 2000 and 2001. The autumn cut in seeding year was done in the three different dates of growing degree days from summer cut (at 630 GDD; 860 GDD and 980 GDD). Samples of roots (10 cm of taproot) were taken in the end of vegetation, were dried at 60°C and contents of starch, fructose and saccharose were analyzed. Content of these root reserves was recalculated on one squared meter ( $\text{g m}^{-2}$ ). The pools of root reserves in tap-root biomass of lucerne were significantly influenced by dates of last cut (defined by GDD) in the seeding year. The maximum of root reserves content was at 860 GDD with significant effect of year. Significant differences between cultivar only existed for saccharose content. There was a significant interaction between year and GDD by saccharose and fructose content.

Keywords: lucerne, autumn harvest management, root carbohydrates, growing degree day.

## Introduction

Root reserves in lucerne, which are mainly represented by carbohydrates, influence the overwintering of plants. The fall harvest does not consistently affected starch or total nonstructural carbohydrate concentration in lucerne roots (Dhont *et al.*, 2002). However, pools of root carbohydrate reserves are significantly reduced by fall harvest, and they are even more closely related to lucerne regrowth potential than their concentrations (Dhont *et al.*, 2004). Generally, the carbohydrate content of lucerne root reserves is affected by length of interval between the last summer and autumn cut. According to Gramshaw *et al.* (1993) root reserves were highest for 7 - and 8 - weekly cutting intervals. These results correspond with Edmisten *et al.* (1988) that the minimum interval should be of seven weeks long. Bélanger *et al.* (1992) conclude that the autumn-harvesting management of lucerne should be based on the duration of the growth period between the last two harvests, instead of autumn rest period based on calendar dates. In Atlantic Canada, a minimum interval of 500 GDD between the two last harvests to maintain dry matter yield across several years was required (Bélanger *et al.*, 1999). These principles of optimal autumn harvest management were not still fully investigated in the seeding year, and remain to be determined, therefore our objective was to characterize root organic reserve accumulation response to a fall harvest management based on GDD in the seeding year.

## Materials and methods

The plot experiment was established in the field of the Research station of Faculty of Agrobiological Sciences, Food and Natural Resource (CUAP) in Červený Újezd on 10<sup>th</sup> April 2000 (Experiment I) and 15<sup>th</sup> April 2001 (Experiment II). These experiments were established in split plot design with four replications and the harvest area of 10  $\text{m}^2$  per plots. Used cultivars were Jarka and Europe (quantity of seed sown 15  $\text{kg ha}^{-1}$ ). The site characteristics are: 405 m above sea level (latitude: 50°04' N, altitude: 14°10' E). Prevailing soil type is clay loam orthic luvisol, kind of soil is medium with the neutral or slightly alkaline soil reaction. According to the agro meteorological characteristics this place belongs from moderate to warm and mostly dry climatic area. There were two cuts in the seeding year. The second cut (autumn)

was taken in three different terms. The interval between summer and autumn harvest was based on cumulative growing degree-days (GDD). This parameter was calculated for each day using the maximum daily temperature ( $T_{max}$ ), the minimum daily temperature ( $T_{min}$ ), and the base temperature ( $T_{base}$ ) as:  $GDD = (T_{max} + T_{min}) / 2 - T_{base}$ . The base temperature ( $T_{base}$ ) is the temperature below which development is zero, for lucerne is used  $T_{base} 5^{\circ}C$  (Bélanger *et al.*, 1992; Dhont *et al.*, 2004). Samples of roots (10 cm of taproot) were taken in the end of vegetation, were dried at  $60^{\circ}C$  and contents of starch, fructose and saccharose were analyzed. The content of root reserves was calculated from determined concentrations and weight of roots of each sample. Results were statistically evaluated by multiple analyses of covariance with interactions (weight of roots was a covariate).

## Results and discussion

There was a significant influence of the last harvest date and their interactions on root reserves content. There were significant differences between years at starch and nonstructural carbohydrates. The effect of root weight (covariate) was highly significant for all variables (Table 1).

Table 1. The content of tap-root carbohydrates.

Cultivar	a.h.t *	Pools of carbohydrates (g m <sup>-2</sup> )					
		Starch		Fructose		Saccharose	
		2000	2001	2000	2001	2000	2001
Jarka	630 GDD	41.12	9.49	2.06	1.42	35.33	26.72
	860 GDD	50.72	8.10	1.92	1.08	39.55	21.67
	980 GDD	56.73	16.86	2.57	2.14	48.92	42.76
Europe	630 GDD	43.56	15.99	1.65	2.13	34.42	47.43
	860 GDD	61.45	14.60	2.20	2.18	44.67	43.22
	980 GDD	49.14	30.13	2.56	2.32	45.91	51.61
Significance probability							
Root weight (covariate)		0.0000		0.0000		0.0000	
a.h.t		0.0062		0.0001		0.0000	
cv.		ns		ns		0.0000	
Year		0.0000		0.0000		0.0000	
Interactions							
cv. x a.h.t		ns		0.0080		0.0012	
cv. x year		ns		0.0027		0.0000	
a.h.t x year		ns		0.0000		0.0000	
range test							
630 GDD x 860 GDD		630 < 860***		630 < 860***		630 < 860***	
630 GDD x 980 GDD		ns		630 < 980***		630 < 980***	
860 GDD x 980 GDD		860 > 980**		ns		ns	
Jarka x Europe		ns		ns		Jarka < Europe***	
2000 x 2001		2000 > 2001***		2000 > 2001***		2000 > 2001***	

\* a.h.t - autumn harvest treatments; 630, 860 or 980 GDD after the summer harvest, on 13<sup>th</sup> Sept., 11<sup>th</sup> Oct., 30<sup>th</sup> Nov. 2000, 11<sup>th</sup> Sept., 17<sup>th</sup> Oct., 30<sup>th</sup> Nov. 2001.

ns, not significant at  $P < 0.05$  (Tukey); \* significant at  $P 0.05$ , \*\* significant at  $P 0.01$ , \*\*\* significant at  $P 0.001$ .

Cunningham and Volenec (1998) described that starch concentrations increased from September to October, then declined in a linear fashion until March. In our experiment, the average highest content of starch before over wintering was obtained at 860 GDD harvested in October. We did not observe any significant differences between cultivars but cultivars provided different starch content dependence on year and date of autumn cut (table 1). The significant effect of the year without interactions indicates an influence of year only on the basic level of starch. The average fructose content was significantly higher at 860 and 980 GDD in comparison with 630 GDD. There was a significant interaction of GDD versus year. At 860 GDD fructose content was the same in both years, but in 2000 fructose content was lowest at 860 GDD, and not significantly different from 630 GDD whilst fructose content at 860 GDD in 2001 was significantly the highest value. The average saccharose content was significantly higher at 860 GDD and 980 GDD. Cultivar Europe accumulated significantly more saccharose than cv. Jarka, but only in 2001. Dhont *et al.* (2002) reported different levels of accumulation of saccharose between cultivars in various date of last cut of production year. The interactions GDD versus cv. showed significantly higher saccharose accumulation in Europe at 630 and 860 GDD. The last date 980 GDD was not significant. In 2000 the linear increase of saccharose content was observed from 630 to 980 GDD. In contrast to 2000, the significantly highest saccharose accumulation was at 860 GDD in 2001. It seems, that year in interaction with autumn harvest management strongly affected the nonstructural carbohydrates content in the seeding year.

### Conclusions

The pools of root reserves in tap-root biomass of lucerne were significantly influenced by dates of last cut (defined by GDD) in the seeding year. Generally, the maximum of root reserves content was at 860 GDD with significant effect of year and depended on total weight of roots. The year in interaction with autumn harvest management strongly affected the nonstructural carbohydrates content. From the point of view of root reserve content an optimal date of autumn cut at 860 GDD in seeding year seems the most suitable recommendation.

### Acknowledgements

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# Yield and botanical composition of pure alfalfa and alfalfa-orchardgrass mixtures at different levels of nitrogen

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## Abstract

A field trial was conducted near Belgrade to compare yields of alfalfa (*Medicago sativa* L.) and alfalfa-orchardgrass (*Dactylis glomerata* L.) mixtures fertilized with from 0 to 210 kg ha<sup>-1</sup> N fertilizer in 2003-2004 and to investigate how N fertilizers affect the botanical composition of the mixture. Yield and botanical composition of pure alfalfa and its mixture with orchard grass varied throughout the years. Pure alfalfa had higher dry matter yields (DM) than mixture in both years. In the first year the highest DM yield of pure alfalfa was obtained with 70 kg N ha<sup>-1</sup> (7.48 t ha<sup>-1</sup>) and in the second year with 140 kg N ha<sup>-1</sup> (16.96 t ha<sup>-1</sup>). In the first year the mixture of alfalfa and orchard grass had the highest yield without nitrogen (5.86 t ha<sup>-1</sup>) and in the second year with the highest amount of nitrogen (14.17 t ha<sup>-1</sup>). High amount of nitrogen had a depressive effect on proportion of alfalfa although it enlarged the proportion of grass in the mixture.

Keywords: alfalfa, alfalfa-orchard grass mixture, yield, botanical composition.

## Introduction

Alfalfa is forage plant most often used for livestock nutrition in Serbia, especially in Pannonian lowland region due to high yields and very good quality of forage. Growing alfalfa in mixture with grasses has many advantages such as: potential use through grazing since it reduces bloat incidence (Ocokoljic, 1984), makes making of hay easier as well as battle against weeds (Vuckovic, 1999) provides equal yields during the year (Casler and Walgenbach, 1990), reduces the need for application of N mineral fertilizers due to nitrogen fixation abilities of legumes (Mallarino *et al.*, 1990) and gives more sugar in forage necessary for successful ensiling (Djordjevic and Dinic, 2005). Disadvantage of mixtures in relation to alfalfa is lower yield and poorer quality of forage. Objective of this research was to provide higher yield of alfalfa mixture through application of N mineral fertilizers in order to promote its introduction into production and in this way to use valuable advantages of this plant.

## Material and methods

Trial was set on experimental field of the Institute for Animal Husbandry, Belgrade-Zemun over period of two years (2003-2004) on soil type low carbonate chernozem in moderate continental climate zone with annual precipitation amount of 645.2 mm and mean annual air temperature of 12.1°C. The effect of four doses of nitrogen mineral fertilizer (0, 70, 140 and 210 kg N ha<sup>-1</sup>) on dry matter yield and floristic composition of pure alfalfa crop and alfalfa/ orchard grass mixture was investigated. Fertilization was carried out twice, in the first year subsequent to sowing ½ N and after first cut the second half was applied. In the second year, ½ of N was applied at the beginning of vegetation and the second half after the first cut. Sowing was carried out in the spring of 2003. Trial was set according to method of random block system in four repetitions and size of main parcel of 10 m<sup>2</sup>. We are used 20 kg ha<sup>-1</sup> of seed for alfalfa monoculture and for mixture 10 kg ha<sup>-1</sup> of alfalfa seed and 20 kg ha<sup>-1</sup> orchardgrass. Previous crop was wheat fertilized with 350 kg ha<sup>-1</sup> of NPK. Yield of DM was determined by sampling of green mass of 1 kg, drying, measuring and calculating in relation to 1 ha, and botanical composition by sampling from 1m<sup>2</sup> in four repetitions, determining of individual species, measuring and calculating their participation in percentage. Statistical processing of data was carried out by variance analysis according to random block system, and significance of differences between mean values by LSD test.

## Results and Discussion

In Table 1 data on yield of dry matter in both investigation years is presented.

Table 1. Forage yield of sward (t DM ha<sup>-1</sup>), average for period 2003/2004.

Sward A <sub>n</sub>	kgNha <sup>-1</sup> B <sub>n</sub>	Cut 2003			Σ	Cut 2004				Σ
		I	II	III		I	II	III	IV	
Alfalfa A <sub>1</sub>	0 B <sub>1</sub>	2.39	2.88	1.64	6.92	4.98	4.91	3.30	1.48	14.68
	70 B <sub>2</sub>	2.10	3.41	1.97	7.48	5.30	5.56	3.19	1.61	15.65
	140B <sub>3</sub>	2.28	3.46	1.69	7.44	5.60	6.07	3.63	1.65	16.96
	210B <sub>4</sub>	1.45	2.28	1.67	5.39	5.96	5.02	3.37	1.65	16.33
Average A <sub>1</sub>		2.05	3.01	1.74	6.81	5.46	5.39	3.37	1.68	15.91
Alfalfa + Orchard grassA <sub>2</sub>	0 B <sub>1</sub>	2.30	2.32	1.25	5.86	5.18	4.45	2.92	1.45	13.75
	70 B <sub>2</sub>	1.33	2.10	1.52	4.94	4.79	4.83	2.72	1.57	13.66
	140B <sub>3</sub>	2.01	2.23	1.04	5.29	5.21	4.63	2.14	1.75	13.74
	210B <sub>4</sub>	1.50	2.21	1.05	4.77	5.96	4.43	2.22	1.56	14.17
Average A <sub>2</sub>		1.78	2.21	1.22	5.22	5.29	4.58	2.50	1.58	13.83
Lsd 0.05	A	0.2075*	0.9014	0.4386*	1.4587*	0.4031	0.2288**	0.2637**	0.2044	0.8338**
	B	0.6539**	0.7377**	0.3347**	1.3289**	0.5434**	0.7918**	0.5443**	0.3707	1.2936**
	AB	0.8300*	1.3014*	0.6125*	2.2263*	0.7881	0.9992**	0.7219**	0.5022	1.8096**
Lsd 0.01	A	0.3809	1.6547	0.8051*	2.6776*	0.7399	0.4200**	0.4841**	0.3753	1.5306**
	B	0.8957**	1.0105**	0.4585**	1.8203**	0.7443**	1.0847**	0.7456**	0.5078	1.7720**
	AB	1.1611	2.0837	0.9906	3.5091	1.1774	1.3931**	1.0340**	0.7270	2.6603**

Based on data presented in Table 1 it is obvious that in both years alfalfa gave statistically considerably higher yields of dry matter than mixture. In the first year alfalfa gave higher yield than the mixture by 1.59 t ha<sup>-1</sup> or 30.45%, and in the second year by 2.08 t ha<sup>-1</sup> or 15.04%. Yields obtained in our research are similar to results of Chakarov and Vassilev (1992) except in their research mixture gave better results than alfalfa. Same as in investigation of Alibegovic- Grbic *et al.* (2004) fertilization had statistically very significant effect on total yield of dry matter in both years and it can be noticed that in the first year pure alfalfa crops fertilized with 70 kg N ha<sup>-1</sup> gave maximal yield of 7.48 t ha<sup>-1</sup>, whereas in case of mixture fertilization decreased yield and the highest yield was registered in mixture that wasn't fertilized, 5.86 t ha<sup>-1</sup>. In the second year N fertilization increased yield of pure alfalfa crop, from 14.68 t ha<sup>-1</sup> without fertilization to 16.96 t ha<sup>-1</sup> with 140 kg ha<sup>-1</sup> which is by 2.28 t ha<sup>-1</sup> or 15.53% higher, whereas in case of mixtures, the highest dose of N gave the highest yields 14.17 t ha<sup>-1</sup> and fertilization with the lowest dose of N the lowest yield of 13.66 t ha<sup>-1</sup> or by 3.73%. Analysis of botanical composition showed that proportion of alfalfa in total yield of mixture was higher than proportion of orchard grass in the first year, and in the second year it decreased and in certain variants it was even lower than proportion of orchard grass which is in accordance with investigation of Vassilev and Chakarov (1999). On figure 1 we can observe that fertilization had the effect on proportion of alfalfa and orchard grass in mixture in the way that it decreased the share of alfalfa and increased the proportion of orchard grass in both investigation years, in other words it influenced the competitive ability of orchard grass.

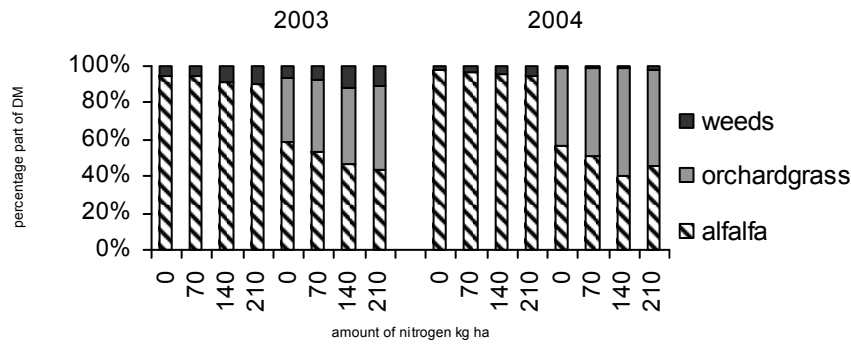


Figure 1. Percentage of species in total dry matter yield of swards.

In regard to presence of weeds it can be observed that it is higher in the first year than in the second year, however in the second year their presence in mixture was considerably reduced compared to pure alfalfa crop. Fertilization affected the increase of proportion of weeds in total yield.

### Conclusion

Fertilization of crops alfalfa and mixture of alfalfa + orchard grass had the effect on very significant increase of yield. Although that the yield was increased by fertilization, yield of mixture was lower than yield of pure alfalfa in both year. So, according to yield, alfalfa remains the best alternative in production of livestock feed in our ecological conditions of cultivation and exploitation.

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## Qualitative changes of lucerne and mixed lucerne-timothy swards during the time of usage

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### Abstract

The aim of the research (2002-2005) was to compare the impact of three different cutting schedules on the productivity, forage quality and swards' composition of three lucerne (*Medicago* sp.) varieties and three lucerne-timothy (*Phleum* sp.) mixed swards. Harvest management was: traditional for Latvia three cut schedule (providing stand longevity), four cut schedule using fixed time intervals, and intensive sward utilization (grazing simulation). All the treatments gave high dry matter yield in the first two years of swards' usage, and on average yield of mixtures was higher compared with pure lucerne stands. Composition of sward depends on lucerne variety – 2 varieties are very fast growing and suppress the growth of timothy. The 3<sup>rd</sup> variety is slower in growth, making good composition with timothy. Pure lucerne swards provided better quality during the same cut, but quality was strongly dependant on cutting schedule. Despite sowing mixtures of equal proportion, the yield proportion of mixed sward components was variable and dependant on lucerne variety, cutting time in season and harvest year. Drastic changes in sward density and composition occurred in 2005 thus affecting DM yield and its quality due to problematical wintering.

Keywords: lucerne, timothy, pure and mixed swards, quality.

### Introduction

Mixtures of lucerne-grass may or may not yield more than pure stands of either species (Chamblee and Collins, 1988). Companion grass can provide more stand stability if compared with pure lucerne stands especially in fields with changing soil quality. At the same time pure lucerne swards can provide higher forage quality. It is reported in many research works that lucerne is a superior pasture legume, and its role in pastures has grown significantly with the development of spreading type (rhizomatous or creeping rooted) varieties. Some researchers reported 2-3 years stand longevity under rotational grazing, some – more than 8 years (Van Keuren and Matches, 1988). The aim of our research was to compare the impact of three different cutting schedules including grazing simulation on the productivity, forage quality and sward composition of three lucerne (*Medicago* sp.) varieties and three lucerne-timothy (*Phleum pratense* L.) mixed swards.

### Materials and Methods

Field experiments were carried out in Research and Study farm 'Vecauce' of Latvia University of Agriculture (latitude: N 56° 28', longitude: E 22° 53') from 2003 to 2005 (lucerne was sown in 2002). Soil at the site was sod podzolic clay loam with pH<sub>KCl</sub> 6.7, containing P 226 mg kg<sup>-1</sup>, K 211 mg kg<sup>-1</sup> that was available for plants and an organic carbon content of 23 g kg<sup>-1</sup> of soil. Before the sowing (2002) mineral fertilizers were applied: 17.5 kg ha<sup>-1</sup> P and 33.2 kg ha<sup>-1</sup> K, and also in the spring of each usage year (2003 – 2005) – 34.9 kg ha<sup>-1</sup> P and 99.6 kg ha<sup>-1</sup> K. Three lucerne varieties (Karlu, WL-324 and Diane) were used in pure stands and mixed with timothy. Total seeding rate was 16 kg ha<sup>-1</sup> for pure stands and 12 kg ha<sup>-1</sup> lucerne + 4 kg ha<sup>-1</sup> timothy for mixed stands. The trial was arranged into 4 times replicated randomised blocks and plot size was 5 m<sup>2</sup>. Harvest management was as follows: traditional three cut schedule (1<sup>st</sup> cut – bud stage; 2<sup>nd</sup> cut – early to full bloom stage; 3<sup>rd</sup> cut – after October 1)-treatment A; four cut schedule using fixed time intervals (1<sup>st</sup> cut – May 25- June 1; 2<sup>nd</sup> cut – July 10; 3<sup>rd</sup> cut – August 20; 4<sup>th</sup> cut – October 10) – treatment B; intensive sward utilization (grazing simulation



when sward reaches 20-25 cm height) after the 1<sup>st</sup> cut in lucerne budding stage - treatment C. Meteorological conditions were different both during vegetation seasons and wintering periods; these influenced sward longevity radically. Particularly winter 2004/2005 characterized by sharply fluctuating temperatures was cause for a sharp decrease of lucerne in the swards followed by changes of composition, quality and DM yield. ANOVA procedures were used for processing the obtained data.

## Results and discussion

On average the total DM yield obtained per trial was high (18.04 t ha<sup>-1</sup> in 2003, 16.67 t ha<sup>-1</sup> in 2004, and 7.77 t ha<sup>-1</sup> from the 1<sup>st</sup> two cuts in 2005). Although the percentage of timothy was low (Fig.1) in mixed swards mixtures provided higher DM yield if compared with pure lucerne stands. Treatment A provided the highest ( $p<0.05$ ) DM yields per ha per season (on average 16.46 – 22.64 t ha<sup>-1</sup> in 2003 and 16.27 – 21.04 t ha<sup>-1</sup> in 2004). Treatment B gave also high, but substantially lower ( $p<0.05$ ) average DM yield (13.06 – 20.09 t ha<sup>-1</sup> in 2003 and 13.00 – 19.25 t ha<sup>-1</sup> in 2004). It was expected that treatment C would provide the lowest DM yield because 3 from 4 cuts were used for grazing. Despite this the first two years of research gave very optimistic results: average DM yield for treatment C was 13.86-18.34 t ha<sup>-1</sup> in 2003 and 13.57- 15.29 t ha<sup>-1</sup> in 2004. All the swards were dense in 2004 (Fig. 3), and only slightly diminished when compared with density in the sowing year, i.e. stand density compared to that in the fall of sowing year was 92 % for A, 95 % for B and 93 % for C. Only a very few herbs were observed in pure lucerne swards or in mixtures with timothy (Fig. 1).

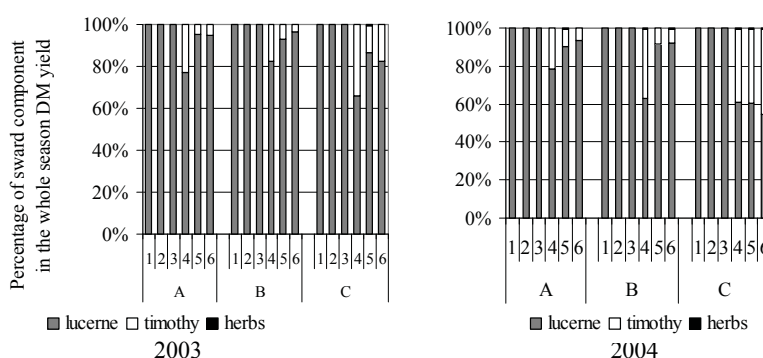


Figure 1. Percentage of the sward components in the whole season DM yield depending on cutting regime (1-Karlu; 2- WL-324; 3-Diane; 4-Karlu+tim.; 5-WL-324+tim.; 6-Diane+tim.).

Quality of obtained yield was high in first two years of usage. Although the main component in mixtures was lucerne (Fig. 1), the pure lucerne stands showed higher quality if it was measured by protein concentration (Fig. 2.). After the 3<sup>rd</sup> winter stand density decreased on average per trial by 26 % if compared with 2004 (Fig. 3) and major changes were observed in sward composition (Fig. 4) which were due to the appearance of large numbers of herbs in both types of swards (mainly *Taraxacum officinale*).

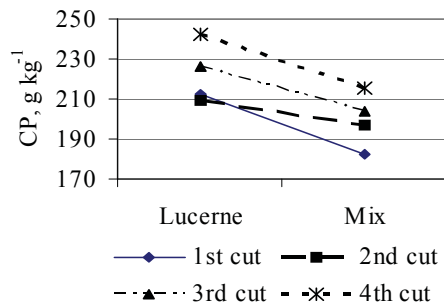


Figure 2. Average quality of lucerne and mixed swards (1<sup>st</sup> and 2<sup>nd</sup> cut 2003-2005; 3<sup>rd</sup>, 4<sup>th</sup> cut – 2003-2004).

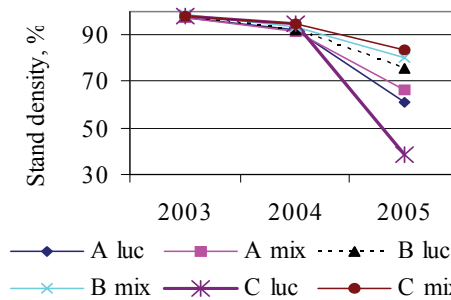


Figure 3. Stand density changes in research years 2003-2005 from that in the sowing year fall (2002 = 100 %).

The 3<sup>rd</sup> year of usage showed that only a spreading-type variety ‘Karlu’ in our experiment could be successfully used in intensive regime (i.e. according to treatment C) for long periods of time. Intensive varieties WL-324 and Diane showed excellent results for hay or silage production in the 3-4 cut schedules but did not persist under the grazing managements despite being first cut at the budding stage. In addition –lucerne rich swards are traditionally infrequently fertilised with nitrogen. N fertiliser was therefore not used in our experiment. However, we observed that in treatment C the amount of lucerne often decreased below 20 % of DM yield, the number of herbs in the sward increased greatly (Fig. 4) and protein concentration decreased to an average of only 100 g kg<sup>-1</sup> in the 1<sup>st</sup> cut. In the 2<sup>nd</sup> cut of 2005 protein concentration was on average 184.9 g kg<sup>-1</sup> that is of 65.0-86.7 g kg<sup>-1</sup> less than that in the 2<sup>nd</sup> cuts of the previous years.

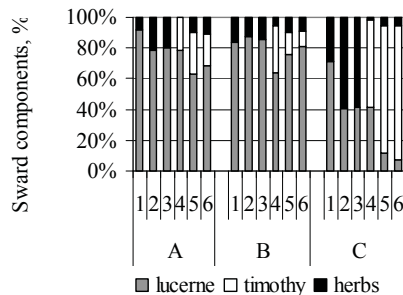


Figure 4. Percentage of the sward components in the total 1<sup>st</sup> and 2<sup>nd</sup> cut DM yield depending on cutting regime in 2005.

## Conclusions

Use of lucerne in mixture with timothy can provide higher total DM yield per season, but with lower crude protein concentration. If sward is used for hay or silage more intensive varieties could be used, but if sward is intended for grazing – special type varieties (e.g. Karlu) will ensure longer persistence and quality of sward. Intensive varieties (e.g. WL-324, Diane) have shown very high competitive capacity thus suppressing development of timothy in sward, but when conditions become improper – timothy can fill in the growth space. In such cases changing the fertilising scheme should be considered.

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## **Economic efficiency of some non-chemical methods of weed control in lucerne**

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### **Abstract**

In spite of its high efficiency, the chemical method of weed control in lucerne (*Medicago sativa* L.) stands is ambivalent. This necessitates the search for non-chemical environmentally friendly methods and the objective of this study was to determine their efficiency.

The study showed that in pure lucerne stands the biological method of weed control (lucerne under oat cover) was the most efficient and its profitability increased by 14 % as compared to control. The establishment of mixed stands of lucerne and perennial grasses was another non-chemical means, the herbaceous association of lucerne + wheatgrass being the most efficient because of cost reduction (increased profitability by 2.6 % to the control). The combined application of the two methods (legume grass mixture under oat cover) also increased the profitability by 23.56 %. The conclusion is the tested non-chemical methods are an efficient alternative in the development of a system of good plant protection practices meeting the European standards.

Keywords: efficiency, non-chemical methods, weeds, lucerne.

### **Introduction**

The new reforms in CAP of the European Union are directed to the adaptation period 2004-2013 of the newly audited members. The integration of the agricultural and ecological policy forms their basis, more particularly the development of systems of good plant protection practices in all fields of agricultural production including in the production of lucerne forage (Hajieva, 2004).

In connection with the control of weed infestation of lucerne, one of the main prerequisites for yield decrease and quality determination of its forage and in the context of the measures mentioned above, necessity rises for search and adaptation of different non-chemical methods that are friendly to the health of man means animals and environment (Dimitrova, 1998). The studies in a number of aspects of the problem are of interest in this respect, such as possibilities for use of allelopathy, introduction of cover crops, as well as the mixed stands of lucerne with perennial grass components pointed out by some authors as the most efficient against the resistant weed associations (Dimitrova and Tomov, 1993). The objective of this study was to make an economic analysis of the efficiency of some non-chemical methods of weed control used in lucerne forage production.

### **Material and methods**

The study was made with data of a field trial carried out under non irrigated conditions, on slightly leached moderately deep chernozem in the experimental field of the Institute of Forage Crops, Pleven, during the period of the 2001-2004. A part of the trial layout with selected variants according to the methodical scheme presented above was used for the analysis purposes (Table 1).

A system of natural and value characteristics used according to the methodical scheme of Stoykova and Dimitrova (1999) was applied to determine the economic efficiency of the tested non chemical methods of weed control in the lucerne forage production.

Table 1 Scheme of the trial.

Variants:*	Stand	Non chemical methods of weed control
V <sub>1</sub>	Lucerne	Pure stand - check
V <sub>2</sub>	Lucerne + wheat grass	Mixed stand with grasses
V <sub>3</sub>	Lucerne + smooth brome + oat	Mixed stand under oat cover
V <sub>4</sub>	Lucerne + oat	Pure stand under oat cover

\*V<sub>1</sub> – no weed control.

V<sub>2</sub> – mixed sowing of Lucerne with wheat grass as a grass component (local population, sowing rate 1,100 kg ha<sup>-1</sup>) at 1:1 component ratio was used as a non chemical method.

V<sub>3</sub> – mixed stand of Lucerne with smooth brome.

V<sub>4</sub> – pure Lucerne stand under oat cover (sowing rate 8 kg ha<sup>-1</sup>), in its capacity of a biological means exerting an inhibiting effect on the weeds.

## Results and discussion

The characteristics of the economic efficiency of the studied plant protection non chemical methods by variants are presented in Table 2.

Table 2. Economic efficiency of the application of some non chemical methods of weed control in lucerne forage production.

Economic characteristics	Variants			
	V <sub>1</sub> -check	V <sub>2</sub>	V <sub>3</sub>	V <sub>4</sub>
Average yield, kg ha <sup>-1</sup>	881	953	1127	1078
Gross output, levs* ha <sup>-1</sup>	88.1	95.30	112.7	107.8
Production costs, levs ha <sup>-1</sup>	47.8	51.10	55.20	49.8
Net income, levs ha <sup>-1</sup>	40.3	44.20	57.5	58
Profitability, %	84.31	86.50	104.17	116.47
Cost price, levs kg <sup>-1</sup>	0.05	0.05	0.05	0.05

\*1 euro=1.955 levs.

The observation on the results from the testing of V<sub>1</sub> representing a pure lucerne stand without weed control was indicative of the weed-depressive capacity of the Bulgarian lucerne variety Pleven 6. As it is well known, due to its slow rate of growth in the first year, lucerne has low competitive ability towards the weeds evenness and as a final result to low quality and productivity. This reflects on the economic results of lucerne forage production (Dimitrova and Stoykova, 1999). In this instance, the dry mass yield of 881 kg da<sup>-1</sup> obtained on average for the four year of growing was evidence of relatively good weed resistance of the variety. The net income of 40.30 levs and profitability of 84.31 % obtained per unit area at a cost price of 0.05 levs of 1 kg dry matter showed relatively good economic efficiency. The comparative economic analysis of V<sub>1</sub>-check and V<sub>2</sub> which allowed emphasize on the rate of the mixed lucerne stands with perennial grasses as a non chemical method of weed control is of interest. The application of variant V<sub>2</sub> where Lucerne was grown in mixture with hay type of wheat grass exceeded the net income obtained from the pure lucerne by 9.68 %, the profitability was 2.6 % higher and the forage mass, although with a low protein content, had balanced chemical composition (Chakarov, 1998). In other words, the conclusion is that the lucerne growing in mixed stands with perennial grasses with the purpose of suppressing the resistant weed populations without herbicide use and increasing the sward productivity is a good plant protection practice, particularly in mixtures with wheatgrass and smooth brome. In the search for new, non chemical alternatives of weed control the growing of lucerne under oat cover was outstanding. Good economic results were obtained from the use

of oat as a cover crop in the lucerne associations mixed with a grass component V<sub>3</sub>, as well as from the pure growing of lucerne under oat cover V<sub>4</sub>.

As compared to the pure lucerne without weed control (V<sub>1</sub>), lucerne grown in a mixed stand with smooth brome under oat cover as a biological weed depressive means (V<sub>3</sub>) brought additional net income of 42.68 percentage units and the profitability value reached to the impressive 17%. Among the tested non chemical methods of weed control in Lucerne forage production, the method of sowing pure lucerne under oat cover was outstanding as economically the most efficient one: the stand productivity increased by 36% resulted in additional net income of 17.70 levs ha<sup>-1</sup> and profitability of 47% - it was an increase of 16% compared to the check variant. These results indicated unambiguously the most efficient alternative to the chemical weed control from an economic and ecological point of view. Oat in its role as a biological means proves to be an allelopathic agent exerting an inhibiting effect on the weeds, excluding the herbicide use, which minimize risks for animal and human health, the environment and soil pollution.

On the basis of this study the following more important conclusions can be drawn up:

i) The results of lucerne growing in a pure stand for forage without chemical weed control (V<sub>1</sub>) depend to a great extent on the weed depressive capacity of the variety selected for this. The economic characteristics of the Bulgarian variety Pleven 6 indicate good efficiency in herbicide absence: profitability of 31% and cost price of 0.05 levs of kg forage dry matter. ii) The lucerne growing for forage without herbicides, but sown in a mixed stand with wheatgrass as a perennial grass component (V<sub>2</sub>) was distinguished for a good economic effect, the net income and profitability increase by 9.68 and 2.6 % respectively, compared to the check. iii) The lucerne growing in a mixture with perennial grasses (smooth brome) under oat cover (V<sub>3</sub>) is an economically efficient and environmentally friendly plant protection practice. The net income from such a stand compared to the pure lucerne increased by 48%, the profitability increases by 17% and the herbicide use are excluded from the technology for forage production. iii) The non chemical method of lucerne sowing under oat cover (V<sub>4</sub>) is economically and ecologically the most efficient. The profitability achieved in its testing is 41% higher than that of the pure lucerne without weed control

## Conclusion

It can be concluded from this study that in the production of lucerne forage there are some non chemical methods of weed control which, in addition to their ecological conformity, are also distinguished for a high economic effect meeting in this way the European standards of good plant protective practices.

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## Forage production and quality of some new legume cultivars in Serbia

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### Abstract

During 2003-2004 we evaluated new cultivars of forage legumes according to their standards. The evaluations were conducted at five locations: Kruševac, Zaječar, Sombor and Zemun which included three cultivars of alfalfa (*Medicago sativa* L.) and one cultivar of red clover (*Trifolium pratense* L.), birdsfoot trefoil (*Lotus corniculatus* L.) and sainfoin (*Onobrychis sativa* L.). Experiments were established in a randomized block design with five replications. The production of green, dry matter, and quality of forage was determined using laboratory analysis. Based on annual average and location dry matter yield of alfalfa ranged from 12.328 t ha<sup>-1</sup> to 13.776 t ha<sup>-1</sup> and concentration of crude protein from 18.9 to 20.6%. Red clover resulted in a dry matter yield of 10.08 t ha<sup>-1</sup> and crude protein concentration was 0.7 % higher than the standard cultivar. Birdsfoot trefoil resulted in an average yield of 10.51 t ha<sup>-1</sup> DM and sainfoin 11.734 t ha<sup>-1</sup>. Crude protein concentration of Birdsfoot trefoil and sainfoin was lower than in standard cultivars. Variances in cultivar dry matter production and concentration of crude protein were not statistically significant.

Keywords: yield, quality, new legume cultivars, Serbia.

### Introduction

In Serbia, breeding of perennial grasses and legumes has a long tradition. Today, there is an assortment of productive and adaptable cultivars of alfalfa, red clover and birdsfoot trefoil. Recently breeding work on sainfoin was started. Adaptable cultivars of forage species are of interest to livestock breeders, specifically, forage quality which are important in their production systems. Thus, cultivars are valued for their nutrition for domestic animals (Đukić *et al.*, 1996). Highly productive and quality cultivars of forage cultures used in livestock nutrition depend on type of livestock, breeding method and nutrition (White and Wight, 1984). Genetic potential for production of green mass or dry matter is becoming a limiting factor which can be improved to some extent in process of selection. Protein content is the most important quality parameter. Increasing only one percent will provide a significant contribution resulting in increases in value and quality of animal feed.

### Materials and methods

Testing of forage cultivars was carried out according to standard method in two year cultivar trials on four locations Kruševac, Zaječar, Sombor and Zemun during 2003-2004. Three alfalfa cultivars (*Medicago sativa* L.) and one new cultivar of red clover (*Trifolium pratense* L.), birdsfoot trefoil (*Lotus corniculatus* L.) and sainfoin (*Onobrychis sativa* L.) were tested. Samples taken in the second investigation year using standard laboratory methods determined: content of crude proteins, crude fibre, crude fats, crude ash and NFE. Obtained results were processed using variance analysis and statistical difference was tested using LSD test for level of significance 0.05 and 0.01.

### Results and discussion

In Table 1 production of dry matter of investigated cultivars of leguminous plants in relation to their standards is presented.

Table 1. Production of dry matter of tested cultivars. Alfalfa and standard.

Location /cultivar	Kruševac	Zaječar	Sombor	Zemun	Average year/loc.
Cultivar 1	13.304	17.768	13.146	10.886	13.776
Cultivar 2	11.818	16.770	11.042	9.682	12.328
Cultivar 3	13.434	14.958	10.966	11.324	12.671
NS Medijana	12.868	16.588	11.056	11.254	12.942
Red clover and standard			LSD 0.05 0.873; 0.01 1.079		
Cultivar 1	11.036	10.962	10.558	7.766	10.081
Kolubara	11.812	10.730	9.624	7.920	10.022
Sainfoin and standard			LSD 0.05 0.466; 0,01 0.589		
Cultivar 1	14.708	9.754	14.288	8.186	11.734
Standard	15.640	9.286	13.202	8.550	11.670
Bird's foot trefoil and standard			LSD 0.05 0.458; 0.01 0.608		
Cultivar 1	11.044	15.638	8.378	6.974	10.509
Bokor	11.116	15.280	6.872	7.044	10.078
			LSD 0.05 0.516; 0.010. 686		

Three new cultivars of alfalfa in relation to standard cultivar Medijana were not a statistically significantly in dry matter yield. The highest yield year-location was realized by cultivar 1 (Zaječar) 17.7 t ha<sup>-1</sup>. In his research De Falco *et al.*, (2003) investigated production and quality at different development stages and concluded that alfalfa at the bloom stage in first cut resulted in the highest dry matter of 5.3 t ha<sup>-1</sup>. The new cultivar of red clover didn't realized higher yield in Kruševac (11.04 t ha<sup>-1</sup>, by 0.06 t ha<sup>-1</sup> higher than average). Deprez *et al.*, (2004) tested 8 cultivars of red clover from Belgium which in the first year realized production of dry matter of 15.9-17.3 t ha<sup>-1</sup> and in the second 15.2-17.3 t ha<sup>-1</sup>, which is higher than a domestic cultivar. The new cultivar of sainfoin also achieved a higher yield (0.06 t ha<sup>-1</sup>) compared to the standard cultivar Soko-1, However the birdsfoot trefoil cultivar yield was not significantly different than the standard cultivar Bokor. Results obtained by Radović *et al.*, (2003), in a trial carried out in Kruševac with 8 domestic and foreign cultivars of birdsfoot trefoil, cultivar Bokor in 1997 produced dry matter of 11.28 t ha<sup>-1</sup>, and in the second year 10.50 t ha<sup>-1</sup>. In Table 2 forage quality of new cultivars and standards are presented.

Table 2. Parameters of quality of tested cultivars in the second year (%).

Cultivars	CP	CC	CF	Ash	NFE
1 alfalfa	19.44	30.03	2.60	9.67	38.26
2	20.56	28.03	2.54	9.28	39.67
3	18.88	30.25	2.23	8.75	39.97
Medijana	19.94	29.14	2.68	9.57	38.67
1 red clover	18.59	24.27	2.80	9.97	44.38
Kolubara	17.89	23.36	3.06	9.24	46.45
1sainfoin	17.28	27.05	3.30	7.32	44.52
Soko-1	17.36	28.67	3.13	7.47	43.88
1 birdsfoot.	19.13	25.35	3.38	8.87	43.27
Bokor	19.31	26.11	3.39	9.23	41.96

Content of crude protein determined for cultivar 2 was 20.56% which is 0.62% higher than standard cultivar. Cultivars 1 and 3 have somewhat lower percentage of protein than the standard cultivar. Zagni *et al.*, (2003) determined that cultivars Europe and Selena two-year average crude protein content ranged from 206.4 to 201.7 g kg<sup>-1</sup> and 191.4 to 193.3 g kg<sup>-1</sup> respectively. Crude fibre content in the two cultivars ranged from 310.9 to 264.7 g kg<sup>-1</sup> for both years. The crude protein content of the new cultivar of red clover was not statistically different from the control. Deprez *et al.*, (2004) evaluated dry matter production and forage quality of 8 cultivars of red clover which in the first year resulted in crude protein content of 2.9-3.0 t ha<sup>-1</sup> and in the second 2.8-3.1 t ha<sup>-1</sup>. New cultivars of sainfoin and birdsfoot trefoil realized somewhat lower yield of crude protein compared to standards. Crude fats and crude fibre were inversely proportional to content of crude protein. Radović *et al.*, (2003) investigated quality of 8 cultivars of birdsfoot trefoil in first cut in the second production year and determined concentration of crude protein from 205 to 234 g kg<sup>-1</sup>, and crude fibre ranging from 28 to 53 g kg<sup>-1</sup>. Kyuchukova and Naydenova (2003) determined several cultivars of sainfoin varied in crude protein content from 200 to 229 g kg<sup>-1</sup>, and content of crude fibre ranged from 200 to 234 g kg<sup>-1</sup>, and content of ash from 57 to 60 g kg<sup>-1</sup>.

### Conclusions

Evaluation of new domestic cultivars of perennial leguminous plants, alfalfa, red clover, sainfoin and birdsfoot trefoil, in two year investigation period on four locations in Serbia showed no significant difference in yield compared to standard cultivars. Forage quality of all cultivars was not statistically significant in crude protein. Domestic plant breeders need to continue in creating new cultivars improve dry matter yield and forage quality of standard cultivars.

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## ***Lotus corniculatus* under different defoliation frequencies and methods**

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### **Abstract**

The influence of harvest frequency and method (cutting 1 or 3 times, mulching 1 or 2 times per year) on the yield and sward composition of *Lotus corniculatus* L. in monoculture and in a mixture with *Festuca pratensis* L. was researched in field experiments in Prague during 7 vegetation years. The species *L. corniculatus* produced on average 7 t ha<sup>-1</sup> of dry mass under three cuts per year, 6.5 t ha<sup>-1</sup> under two harvests and 2.2 t ha<sup>-1</sup> under one harvest in the 2<sup>nd</sup> vegetation year. Yields decreased in later years. The proportion of *L. corniculatus* decreased from 99.6% (three cuts per year) or 96.9% (the twice mulched one) to 60% and 0 % respectively of the total yield of monoculture in the 7<sup>th</sup> year (a linear relation,  $r^2$  0.87-0.99). The *Lotus* share in the mixture increased till the 4<sup>th</sup> vegetation year but later it decreased similarly like in the monocultures (polynomial function). The number of *L. corniculatus* plants per m<sup>2</sup> was significantly lower under a lower harvest frequency. The reduced projective dominance of *L. corniculatus* decreased in a significant dependence on the time from 70 to 13% under 3 cuts. The research was supported by the grants QF4062 and MSM6046070901.

Keywords: *Lotus corniculatus*, cutting, mulching, yield, sward density, coverage.

### **Introduction**

*Lotus corniculatus* is one of the well adapted legumes under Czech conditions, but it is not used frequently for forage production on arable land, mainly due to its lower yield capacity. It is used only as a component in permanent grass swards. Novák (1998) researched overseeding of permanent grass stands by *L. corniculatus*. Under the current structural changes in agriculture *L. corniculatus* can find new use in extensively exploited swards e.g. for temporary setting arable land aside. Such swards are expected to maintain or improve soil fertility for future intensive and economically effective plant production - to protect it against soil erosion, weed infestation, improve its organic matter content, support nitrogen fixation etc. The winter coverage of legumes is generally lower than with grasses, but they fulfil other functions in grass swards, above all the nitrogen fixation can be required for the improvement of soil fertility. *L. corniculatus* has not been used for such purposes until now and there is no complete information available about its performance under extensive exploitation and mulching under such conditions. The main aim of the experiments was to evaluate *L. corniculatus* persistency, coverage in winter season and its development during 6 years of the swards vegetation under different managements.

### **Materials and methods**

Field plot experiments (3x10 m per plot) were established in Prague (chernozem, altitude 281 m a. s. l., average annual precipitation 472 mm, average annual air temperature 9.3°C). *Lotus corniculatus* L. cv. Lotar was sown as a monoculture (15 kg ha<sup>-1</sup>) in 1996 and in mixture (*Lotus* 6 kg ha<sup>-1</sup>) with *Festuca pratensis* L. cv. Otava (20 kg ha<sup>-1</sup>) in 1997. The swards were not fertilized, they were defoliated once or three times per year and the herbage removed, or, mulched once or twice per year by an AS 27/2 Enduro mulching machine. The total dry mass yield and the share of *L. corniculatus* in the harvested biomass, the number of plants and sprouts per m<sup>2</sup> and the plants coverage in winter (reduced projective dominance by a point method - 60 points, 5 replications) were measured during 7 vegetation years. The results were evaluated by the analysis of variance and the regression analysis by Statgraphics® Plus programme, version 4.0.

## Results and discussion

The species *L. corniculatus* produced on average  $7 \text{ t ha}^{-1}$  of dry mass in three cuts per year,  $6.5 \text{ t ha}^{-1}$  in two harvests per year and  $2.2 \text{ t ha}^{-1}$  in monoculture in the 2<sup>nd</sup> vegetation year. The results were similar to those of *Medicago media* (Svobodová *et al.*, 2001). Later the yields decreased. The influence of the harvest frequency on the legume competition capacity (Figure 1) was evident. The share of *L. corniculatus* in the dry mass yield was significantly lower under a lower harvest frequency. The results are in accordance with the results of McKenzie *et al.*, (2004). The regression lines were very similar for both of the once per year harvested variants (cut and mulched), both with the monoculture and with the legume grass mixture (Figure 1.).

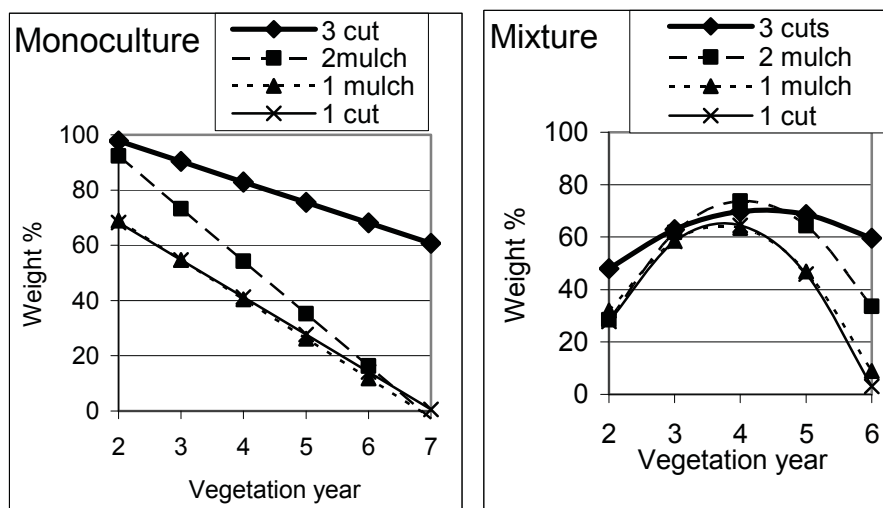


Figure 1. The weight share of *L. corniculatus* in the dry mass yield (%).

The share of the legume in the yield developed differently in monoculture and in the mixture with *F. pratensis*. In the monoculture it decreased linearly with the time ( $r^2 = 0.87-0.99$ ) and the steepest slope was found with the two times per year mulched variant. The share of the sown legume decreased from 99.6 % (three times per year cut variant) or 96.9 % (2 times per year mulched one) to 60%, and 0 % respectively in the 7<sup>th</sup> year of vegetation. In the mixture, the legume share was increasing till the 4<sup>th</sup> vegetation year but later it decreased similarly to the monoculture (polynomial of the second grade,  $r^2 = 0.70-0.87$ ). The differences in the curves behaviour under monoculture and mixed sowings can be explained by the competition conditions during the sward establishing (monoculture, or mixture with the grass) and the later development of its botanical composition (weed infestation, the grass development under different water conditions etc.). The winter coverage of *L. corniculatus* during all the vegetation years is presented in the Table 1. It decreased in dominance with time ( $r^2 = 0.6-0.75$  with the monoculture,  $r^2 = 0.78$  with the three times cut mixture). The correlation was very high with the monoculture ( $r = 0.77-0.88$ ) or 3 times cut mixture.

Table 1. *L. corniculatus* winter coverage (% of dominance) – logarithmical curves.

Sward type	Treatment	Vegetation year					r <sup>2</sup>
		2	3	4	5	6	
Monoculture	3 cuts	69.6	47.7	34.9	25.9	18.8	0.754
	2 mulch	30.7	18.0	10.6	5.3	1.2	0.591
	1 mulch	10.9	6.5	3.9	2.1	0.7	0.634
	1 cut	24.1	14.1	8.3	4.1	0.9	0.601
Mixture	3 cuts	57.3	36.8	24.9	16.4	9.8	0.785
	2 mulch	18.8	13.6	10.6	8.5	6.8	0.108
	1 mulch	4.3	6.0	6.9	7.6	8.2	0.012
	1 cut	6.2	6.5	6.6	6.7	6.8	0.001

The number of plants and sprouts was also significantly higher under 3 cuts per year. A lower harvest frequency influenced it negatively. These significant differences persisted until the 7<sup>th</sup> (6<sup>th</sup>) vegetation year (Table 2).

Table 2. Number of *L. corniculatus* plants and sprouts (pcs m<sup>-2</sup>) in monoculture and mixture.

Treatment	23.10.1998		15.11.99		8.7.2003	
	Plants per m <sup>2</sup> monoculture	Sprouts per m <sup>2</sup> monoculture	Plants per m <sup>2</sup> monoculture	Plants per m <sup>2</sup> mixture	Plants per m <sup>2</sup> monoculture	Plants per m <sup>2</sup> mixture
3 cuts	214.6	1404.2	154.2	83.3	41	35
2 mulch	91.7	845.8	63.3	91.7	39	36
1 mulch	68.8	543.8	45.8	79.2	21	23
1 cut	77.1	577.1	44.4	58.3	20	16
D <sub>min.</sub> (α=0.05)	59.5	323.9	54.0	-	20.9	11.8

## Conclusions

*L. corniculatus* development was significantly influenced by the harvest frequency without an evident effect of mulching compared with removal of cut herbage. Three or two harvests are desirable for long term maintenance of an acceptable share of the cultivated legume in the sward and its winter coverage.

## Acknowledgements

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# Influence of management on the representation of legumes in permanent grasslands

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## Abstract

This report presents an evaluation of the effect of the grassland management system on the proportion of legumes in permanent grassland communities. Evaluation of the overall representation of climbing and non-climbing legumes together in the two stands investigated (both of the association *Lolio-Cynosuretum* Tx.1937, Šumava Mts. foothills 650 m a.s.l. and 855 m a.s.l., Czech Republic) has shown that the best management method is a one-cut regime with fertilization cessation. The aim of this study is to find an optimum management that would support the representation of legumes in the stand.

Keywords: grasslands management, legumes, mowing, fertilization.

## Introduction

When presented in an optimal proportion legumes positively influence both the production and non-production functions of grassland communities (Laser *et al.*, 2004). The representation of legumes in permanent grasslands is markedly affected by a mutual interaction of environmental conditions and the management practices applied to the grassland (Klimesš, 1999; Hopkins and Hrabě, 2001; Pacurar and Rotar, 2004). The hypothesis to be tested is that a nature friendly management will be most suitable in this respect.

## Material and methods

Within the period of 2000-2004, possibilities of a suitable representation of legumes were studied in two mesophyte communities of permanent grassland (association *Lolio-Cynosuretum* Tx.1937), located in the Šumava Mts. (Bohemian Forest) foothills (650 m a.s.l. and 855 m a.s.l.). The soil type is sandy clay with a sufficient rich nutrient content and soil class is acid cambisol in both stands. The investigation was carried out using small-plot trials 70x3 m, organized in randomized blocks with four replicates of each treatment. The total cover of particular plant species and whole agro-botanical groups (grasses, legumes, other herbs) was estimated for all treatments at the time of first mowing. The plant cover was estimated in all treatments by the point-quadrat method (pin diameter 1 mm). Statistical analytical methods (ANOVA and dynamic models analysis) were used to evaluate the representation and cover dynamics of legumes, both in total and divided into two separate groups: non-climbing and climbing species. The non-climbing legumes included mainly *Trifolium repens* L. (White clover). The climbing legumes were represented mainly by *Lathyrus pratensis* L. (Meadow pea) and *Vicia cracca* L. (Bird vetch) in a fairly stable proportion which reached similar cover values. For more detailed description of the species composition and experimental design see Klimesš *et al.* (2003).

## Results and discussion

Evaluation of variances of legumes cover in the permanent grasslands studied indicates that the management of these coenoses significantly affects the cover dynamics of legume species. Both the

mowing frequency and fertilization affect differently climbing and non-climbing legumes (Figure 1). The overall evaluation of representation of legumes in the treatments studied indicates that the most suitable treatment consist of mowing without regular fertilization, which had the highest average cover ( $14.000 \pm 0.748$  %C) the lowest variation in cover between years and sites, as well as the best stabilization of cover with time (Figures 1 and 2). The climbing legumes are suitably represented in the total legumes cover on sites subjected to one mowing. Their cover, however, rapidly decreases after two mowings, with no substantial difference between fertilized and non-fertilized plots. In contrast, non-climbing legumes were conspicuously affected by fertilization after two mowings. The application of  $100 \text{ kg N ha}^{-1} + \text{PK}$  (see Fig 1) significantly reduced their cover from  $11.00 \pm 2.729$  to  $5.100 \pm 0.900$  %C. Similar outcomes, with respect to the management practices tested, were found in the cover dynamics of legume for both sites, which had very similar soil conditions and hosted the same plant association. This finding points to the possibility of extrapolation of the results concerning the representation of legumes. This conclusion is in agreement with numerous references for a somewhat wider spectrum of environmental conditions (Klimeš, 1999; Hopkins and Hrabě, 2001; Pacurar and Rotar, 2004). In addition to whole agro-botanical groups, investigation of their morphologically diverse sub-groups appears to be a useful methodological aspect of study of the given topic (Figures 1 and 2).

Legumes	nMo	Fertilization	% $\bar{C} \pm S_{\bar{C}}$													HG <sub>0.05</sub>	F	p	V <sub>c</sub> (%)
			2	4	6	8	10	12	14	16									
total	1	-	[Bar chart showing cover values]													*	3.007	0.045	16.431
	2	-	[Bar chart showing cover values]													*			67.676
	0	-	[Bar chart showing cover values]													* *			40.844
	2	NPK	[Bar chart showing cover values]													*			80.635
non-climbing	2	-	[Bar chart showing cover values]													*	5.196	0.005	78.436
	1	-	[Bar chart showing cover values]													* *			54.015
	2	NPK	[Bar chart showing cover values]													*			31.623
	0	-	[Bar chart showing cover values]													*			159.850
climbing	0	-	[Bar chart showing cover values]													*	3.920	0.017	55.000
	1	-	[Bar chart showing cover values]													*			49.397
	2	-	[Bar chart showing cover values]													*			201.992
	2	NPK	[Bar chart showing cover values]													*			227.261

nMo = number of mowing, C = cover (per cent),  $S_{\bar{C}}$  = standard error of mean, HG<sub>0.05</sub> = homogeneous groups at 0.05 level, V<sub>c</sub>(%) = coef. of variation,  $\Delta C'$  = medium theoretical value of coverage change year-to-year (in %C),  $k'_c$  = medium coef. of coverage change year-to-year, NPK =  $100 \text{ kg N ha}^{-1}$  in two doses,  $75 \text{ kg N ha}^{-1}$  in spring +  $25 \text{ kg N ha}^{-1}$  after 1st mowing

Figure 1. Results of the analysis of variation and dynamic characteristics of legumes coverage (%C).

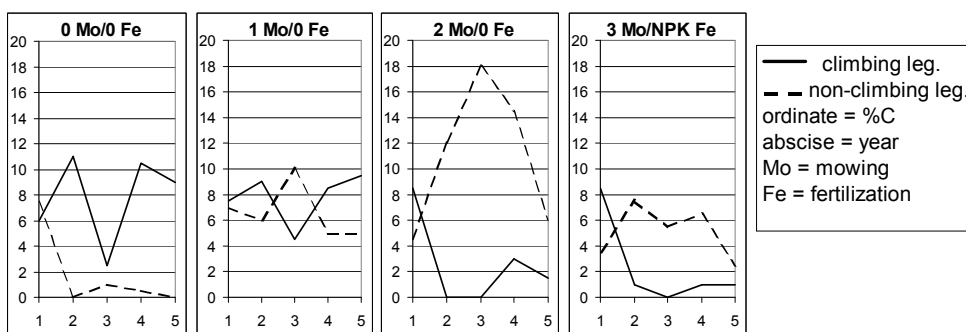


Figure 2. Development of the legumes cover (%C) in individual treatments.

## Conclusions

The most important conclusions include the necessity to differentiate between the effects of environmental conditions on the one hand and management practices on the other, on the cover dynamics of legumes in grassland communities. It is necessary to consider the different dynamics of climbing and non-climbing legumes, whose reactions differ. A new management approach, which takes into account the proportion of climbing legumes in the development of the overall agro-botanical group of legumes, is supported by the finding of a greater representation of climbing legumes under low-impact forms of management. These conclusions are relevant to grassland communities in which climbing legumes occur at fairly high frequencies (over 40 %), or even to those in which the cover of legumes is low. This study points out, besides a general approach to studies of legumes dynamics, the necessity to take into consideration the reaction diversity of separate legume groups or even species.

## Acknowledgements

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# The effect of bacterization and liming in the production of red clover

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## Abstract

The aim of this experiment was to examine the effect of bacterization and liming on the yield of red clover as well as on the microbiological activity in the soil rhizosphere. The lime (3 t ha<sup>-1</sup>) was applied before tillage. The seed of clover was inoculated with *Rhizobium leguminosarum* *bv. trifolii* (1ml 10<sup>8</sup> cells on 1 g seed) before seeding. The clover was grown on soil with pH values 5.1 and 6.2. The yield of raw forage, dry matter content, the total number of bacteria, number of *azotobacter* and the correlation between yield and microbiological activity were determined. The total yield of raw forage was higher in treatments with liming (1.5 t ha<sup>-1</sup>) and inoculation (3.5 t ha<sup>-1</sup>). The treatment where both bacterization and liming were applied gave produced the largest yield (4.5 t ha<sup>-1</sup>). The highest increase of the number of *azotobacter* and total number of bacteria occurred in the treatment where both liming and inoculation were applied. The correlation between yield of clover and number of *azotobacter* as well as total number of bacteria was positive.

Keywords: red clover, bacterization, liming, microbes.

## Introduction

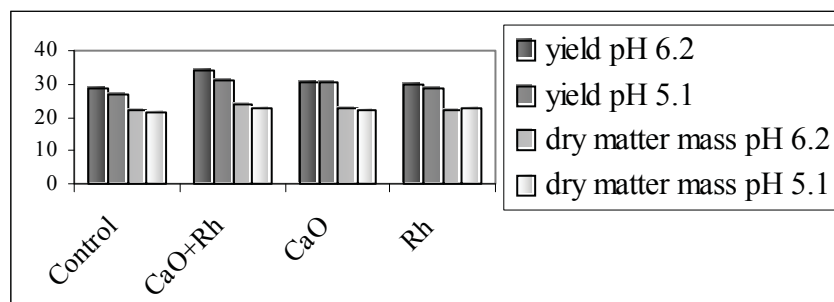
On the root of legumes, nitrogen fixing bacteria form nodules in which nitrogen fixation takes place (Giller and Cadisch, 1995). This nitrogen fixing bacteria are soil microorganisms; therefore, their number depends on various factors such as pH, moisture level, temperature, tillage, other microorganisms, etc. The effectiveness of a symbiosis is also determined by the reactions between the host plant and the strain of bacteria (Jarak, 2000). In agricultural soils, a certain amount of nitrogen fixing bacteria of clover, beans and peas can be found. However, the results are normally better when inoculation is applied in the production system (Hungria and Stacey, 1997). The aim of this investigation was to examine the effect of inoculation and liming on the yield of red clover and on the microbiological activity in the soil rhizosphere.

## Material and methods

The experiment with red clover (*Trifolium pratense* L.) was conducted in soils with two different pH values (pH 5.1 and pH 6.2). The size of the experimental plot was 6 m<sup>2</sup>. Before tillage, 5 t ha<sup>-1</sup> of CaCO<sub>3</sub> was applied. The red clover seed was inoculated with *Rhizobium leguminosarum* *bv. trifolii*. Experimental treatments were as follow: 1) control - no liming, no bacterization, 2) both liming and bacterization (CaO+Rh); 3) liming without bacterization (CaO); 4) bacterization without liming (Rh). The values determined were: the total yield of raw forage, dry matter mass of clover, the total number of microorganisms and the number of *azotobacter* in the rhizosphere of clover. The methods of Benson (2002) were applied.

## Results and discussion

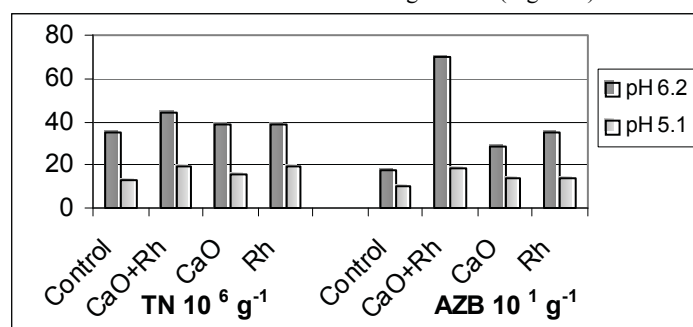
*Rhizobium leguminosarum* *bv. trifolii* is a bacterium which lives in symbiosis with clover and which fixes up to 450 kg N ha<sup>-1</sup> per year (Peoples *et al.*, 1995). This bacterium is tolerant of acidity, but its activity is higher in neutral soils ( Jarak *et al.*, 1994). The yield of raw forage of red clover was higher in the pH 6.2 soil and increased after liming and bacterization were applied (Figure 1). The percentage of the dry matter mass was approximately the same in all treatments and both types of soil.



	Yield		Dry matter mass	
LSD value	0,01	0,05	0,01	0,05
Treatments	1,60	1,15	1,22	0,88
pH	1,13	0,81	0,86	0,62

Figure 1. The total yield of raw forage (t ha<sup>-1</sup>) and of dry matter mass of red clover (%).

The total number of microorganisms and the number of azotobacter can be used as indicators of the fertility of soil (Alexander, 1977). Of all microorganisms in soil, bacteria are the most numerous. Their number is the largest in neutral soils; besides, their activity can increase at soils with lower acidity. In this investigation, the total number of microorganisms in the rhizosphere of red clover was higher in the pH 6.2 soil and in the variants with bacterization or liming or both (Figure 2).



	TN		AZB	
LSD value	0,01	0,05	0,01	0,05
Treatment	1,67	1,19	1,79	1,28
pH	1,18	0,84	1,27	0,91

Figure 2. The total number of microorganisms (TN) and number of *azotobacter* (AZB) in the soil rhizosphere of red clover.

As *Azotobacter* has the ability to fix nitrogen from the atmosphere thus with a higher number of *azotobacter*, the amount of nitrogen in soil will increase (Govedarica *et al.*, 2002). A high number of *azotobacter* indicates a fertile soil. In this investigation, the number of *azotobacter* was very low in the soil with higher acidity. In both types of soil, the number was the highest when both bacterization and liming were applied.

As microbial activity of soil is one of the most important factors which affects soil fertility, plant yield has normally a positive correlation with the number of microorganisms which release nutrients from the organic matter of soil (Lynch, 1983). In this investigation, the correlation between the total number of microorganisms, the number of *azotobacter* and the yield of raw forage of red clover was positive. The



yield of red clover is genetically determined, but it also depends on the fertility of soil, cultural practices, fertilization, etc. (Djukic, 2002). Both bacterization and liming has a positive effect on red clover yield, being the highest when the combination of both treatments was applied (Fig. 1 and 2). It was also confirmed that the yield depends on the microbiological activity of soil.

### Conclusions

The yield of raw forage of red clover was higher in the soil of lower acidity. Bacterization and liming had a positive effect on the yield of raw forage. The total number of bacteria and the number of Azotobacter in the soil rhizosphere under red clover increased in the treatments with bacterization and liming. There was a positive correlation between the yield of raw forage and the number of the groups of microorganisms studied.

### Acknowledgement

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# Role and potential of *Trifolium subterraneum* grown as a winter catch crop

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## Abstract

The objective of this study was to compare *Trifolium subterraneum* L. with four other winter catch crops (*Trifolium incarnatum* L., *Trifolium pratense* L., *Vicia villosa* Roth. and *Lolium multiflorum* Lam.) for growth potential and nitrogen accumulation during the autumn, winter and early spring. Results obtained from two field experiments in different locations show that *T. subterraneum* sown in August produced 2140 kg ha<sup>-1</sup> of herbage dry matter yield (HDMY) until the end of November. In comparison to other treatments this was the highest production. Early spring HDMY obtained from *T. subterraneum* was lower (1065 kg ha<sup>-1</sup>) than that obtained from *T. pratense* (2140 kg ha<sup>-1</sup>), *T. incarnatum* (2000 kg ha<sup>-1</sup>) and *V. villosa* (2405 kg ha<sup>-1</sup>). The amount of herbage nitrogen accumulated by *T. subterraneum* was high in the autumn (86.6 kg ha<sup>-1</sup>), due to very high HDMY. At the end of April the highest root and herbage nitrogen accumulation was obtained by *V. villosa* (120 kg ha<sup>-1</sup>), while *T. subterraneum* accumulated 71 kg N ha<sup>-1</sup>. The high autumn growth and nitrogen accumulation in *T. subterraneum* suggests it has potential as a winter forage and nitrogen catch crop in continental environmental conditions of Central Europe.

Keywords: catch crop, nitrogen accumulation, dry matter yield, *Trifolium subterraneum*.

## Introduction

The increasing concern about environmental protection and the challenge of environmentally 'safe' utilisation of natural resources force farmers to consider optimal utilisation of plant nutrients, of which nitrogen plays an important role. Crops harvested in summer are traditionally followed by catch crops. They can be used for forage, or can be grown only for nitrogen accumulation, with the aim of contributing nitrogen to the succeeding main crop. Some grasses and crucifers, which are traditionally used as forage catch crops, are very useful for this purpose. However, compared to grasses and crucifers the role and potential of legumes can be even more important. High quality forage legumes can take up residual soil nitrogen, and during the warmer autumn and spring periods can be effective in accumulating symbiotically fixed nitrogen. In continental part of Slovenia, *T. incarnatum*, *V. villosa* and sometimes *T. pratense* are the legumes traditionally used as catch crops sown in the summer and harvested at the beginning of May the following year. *T. subterraneum* is a relatively unknown legume in Slovenia. Experimental work with this species started in 2004 and will continue until 2006. The aim of the work was to investigate the effects of summer and autumn sowings of *T. subterraneum* on the growth potential and nitrogen accumulation during autumn, winter and early spring.

## Materials and methods

The first experiments were conducted from August 2004 to May 2005 at Hoče and Rogoza near Maribor (46° 34' N, 15° 38' E, 270-300 m a.s.l.), Slovenia. The soil of both experimental fields was brown. In August, before the start of the experiment, the soil pH (CaCl<sub>2</sub>) at Hoče was 5.4, P<sub>2</sub>O<sub>5</sub> content 28 mg and K<sub>2</sub>O content 70 mg per 100 g of dry soil (ammonium lactate extraction). At Rogoza the soil pH was 6.0, P<sub>2</sub>O<sub>5</sub> content was 22 mg and K<sub>2</sub>O content was 31mg per 100 g of dry soil. The yearly mean air temperature of the area is 10.7°C, the mean monthly minimum in January is (0.4 °C) and the average monthly maximum is in July (20.8 °C). The average rainfall in the area is ~ 1050 mm. January, February and March are the months with the lowest precipitation on average. During the other months

precipitation is relatively equally dispersed (Statistical Yearbook, 2004). The 10 day average air temperatures and precipitation during the experimental period are presented in Table 1.

Table 1. The average air temperature (°C at 2 m) and total precipitation (mm) for the first, second and third 10-day periods of each month during the experiments.

Month	The average air temperature			Precipitation		
	First	Second	Third	First	Second	Third
July 2004	20.4	19.8	21.1	45.7	13.1	39.0
August 2004	21.5	22.8	18.3	13.6	10.1	52.8
September 2004	16.7	15.9	13.8	12.0	23.2	63.8
October 2004	14.4	7.8	14.4	18.6	60.4	24.8
November 2004	8.5	5.1	2.7	15.9	44.2	0.2
December 2004	3.2	-1.8	1.5	4.6	0.2	38.5
January 2005	3.4	0.2	-2.0	2.0	3.6	8.7
February 2005	-4.6	1.4	-1.5	13.4	1.3	38.0
March 2005	-3.4	6.1	9.8	8.7	9.5	21.2
April 2005	9.6	10.8	12.6	40.5	46.5	13.2

The experimental design was a randomized block (four replicates) in a split-plot arrangement with different catch crops on main plots and different sowing dates on subplots (30 x 9 m). Catch crops were sown at sowing rates recommended for the Slovenian environment (Kramberger, 1999), i.e. *Trifolium subterraneum* – 25 kg ha<sup>-1</sup>, *Trifolium incarnatum* – 25 kg ha<sup>-1</sup>, *Trifolium pratense* – 20 kg ha<sup>-1</sup>, *Vicia villosa* – 95 kg ha<sup>-1</sup> and *Lolium multiflorum* 50 kg ha<sup>-1</sup>). The sowing was carried out on 16<sup>th</sup> August and 15<sup>th</sup> September in 2004. Catch crops were grown without applying any fertilizers or manures. The herbage yield was obtained from quadrates (50 x 50 cm) cut at 5 cm height and weighed on 18<sup>th</sup> November 2004. The remaining herbage was left over winter. In spring the yield was obtained by cutting and weighing herbage from new quadrates on 7<sup>th</sup> April 2005 and 26<sup>th</sup> April 2005. Roots were washed from soil on 7<sup>th</sup> April 2005 and 26<sup>th</sup> April 2005 and weighed. The herbage and root dry matter yields were obtained by drying samples at 70°C to constant mass. The herbage and roots were analyzed for N content by the Kjehldal method. The data were subjected to an analysis of variance. An F-ratio with P ≤ 0.05 was regarded as statistically significant. Comparison of treatments was made using Tukey HSD test.

## Results and discussion

As is shown in Table 2, mid August (sowing date 1) sown *T. subterraneum* grew vigorously in autumn, resulting in a very high herbage dry matter yield (HDMY) before winter (2140 kg ha<sup>-1</sup>). When sown in September (sowing date 2), *T. subterraneum*, like other clovers, did not produce a considerable quantity of HDMY (125 kg ha<sup>-1</sup>). A long winter with very low average temperatures (Table 1) had a greater negative effect on the yield of August sown *T. subterraneum* than on *T. pratense*, *T. incarnatum*, *V. villosa* and even the *L. multiflorum*. The amount of herbage produced by *T. subterraneum* on 7<sup>th</sup> April was only 545 kg dry matter ha<sup>-1</sup>, much less than that produced by the other catch crops. Its herbage production increased to only 1065 kg ha<sup>-1</sup> by the end of April. However, other catch crops, especially clovers, produced significantly higher yields. Like other catch crops sown in September, herbage production of *T. subterraneum* during the winter period also increased (Table 2). But the HDMY at the end of April of *T. subterraneum* sown in September (1025 kg ha<sup>-1</sup>) was comparable only to HDMYs obtained by *T. pratense* and *T. incarnatum* (390 kg ha<sup>-1</sup>, 895 kg ha<sup>-1</sup>). *V. villosa* and *L. multiflorum* produced significantly higher HDMY in April than *T. subterraneum*. *T. subterraneum* sown in August accumulated very high amount of herbage nitrogen (HN) in the autumn. The amount of 86.8 kg HN ha<sup>-1</sup> is not significantly different to HN accumulation by *V. villosa* of 95.4 kg ha<sup>-1</sup>. Other early sown catch

crops accumulated significantly less HN in autumn. Due to low HDMYs, the September sown catch crops accumulated little HN in autumn, with the exception of *L. multiflorum* which accumulated 24.9 kg ha<sup>-1</sup>. In April the HN accumulation and nitrogen accumulation by the roots and herbage varied significantly between catch crops and sowing dates. However, the amounts of nitrogen accumulated by *T. subterraneum* at the end of April (71 kg ha<sup>-1</sup>) were comparable to most treatments Only *V. villosa* (both sowing dates) and early sown *T. pratense* produced significantly more total HN.

Table 2. Late autumn and spring herbage dry matter production and nitrogen accumulation of different catch crops.

Catch crop	Sowing date	18 <sup>th</sup> Nov. 2004		7 <sup>th</sup> April 2005			26 <sup>th</sup> April 2005		
		HDMY <sup>1</sup>	HNA <sup>2</sup>	HDMY	HNA	NA <sup>3</sup>	HDMY	HNA	NA <sup>3</sup>
<i>Trifolium subterraneum</i>	16 Aug	2140 <sup>a*</sup>	86.8 <sup>a</sup>	545 <sup>cde</sup>	21.7 <sup>c</sup>	49.8 <sup>cd</sup>	1065 <sup>bc</sup>	40.2 <sup>c</sup>	71.7 <sup>bc</sup>
	15 Sept	125 <sup>e</sup>	4.6 <sup>d</sup>	335 <sup>de</sup>	11.9 <sup>c</sup>	32.8 <sup>cd</sup>	1025 <sup>bc</sup>	41.2 <sup>c</sup>	71.4 <sup>bc</sup>
<i>Trifolium pratense</i>	16 Aug	1070 <sup>bcd</sup>	40.9 <sup>bc</sup>	1090 <sup>bcd</sup>	44.3 <sup>bc</sup>	77.2 <sup>bc</sup>	2140 <sup>a</sup>	89.4 <sup>a</sup>	119.6 <sup>a</sup>
	15 Sept	50 <sup>e</sup>	1.6 <sup>d</sup>	100 <sup>e</sup>	3.6 <sup>e</sup>	8.9 <sup>d</sup>	390 <sup>c</sup>	15.0 <sup>c</sup>	28.3 <sup>d</sup>
<i>Trifolium incarnatum</i>	16 Aug	1540 <sup>abc</sup>	57.1 <sup>b</sup>	1190 <sup>bcd</sup>	42.6 <sup>bc</sup>	71.9 <sup>bc</sup>	2000 <sup>a</sup>	79.1 <sup>ab</sup>	98.4 <sup>ab</sup>
	15 Sept	70 <sup>e</sup>	2.2 <sup>d</sup>	465 <sup>cde</sup>	19.4 <sup>c</sup>	34.0 <sup>cd</sup>	895 <sup>bc</sup>	33.8 <sup>c</sup>	47.4 <sup>cd</sup>
<i>Vicia villosa</i>	16 Aug	2040 <sup>a</sup>	95.4 <sup>a</sup>	2260 <sup>a</sup>	120.6 <sup>a</sup>	144.7 <sup>a</sup>	2405 <sup>a</sup>	98.5 <sup>a</sup>	114.6 <sup>a</sup>
	15 Sept	207 <sup>de</sup>	6.6 <sup>d</sup>	1770 <sup>ab</sup>	93.9 <sup>ab</sup>	112.0 <sup>ab</sup>	2080 <sup>a</sup>	102.5 <sup>a</sup>	120.3 <sup>a</sup>
<i>Lolium multiflorum</i>	16 Aug	1920 <sup>ab</sup>	46.8 <sup>bc</sup>	1265 <sup>bc</sup>	31.3 <sup>c</sup>	55.6 <sup>cd</sup>	1475 <sup>ab</sup>	37.0 <sup>c</sup>	56.6 <sup>cd</sup>
	15 Sept	760 <sup>cde</sup>	24.9 <sup>cd</sup>	1130 <sup>bcd</sup>	30.1 <sup>c</sup>	53.0 <sup>cd</sup>	1525 <sup>ab</sup>	46.2 <sup>bc</sup>	65.4 <sup>bcd</sup>

<sup>1</sup>HDMY = herbage DM yield (kg ha<sup>-1</sup>).

<sup>2</sup>HNA = herbage nitrogen accumulation (kg ha<sup>-1</sup>).

<sup>3</sup>NA = nitrogen accumulation by plants (roots and herbage) (kg ha<sup>-1</sup>).

\*Treatment means within columns with different superscripts differ (P≤0.05).

According to the results of this first year in the project, *T. subterraneum* is a promising catch crop with high growth potential in autumn if it is sown in August. To the end of the growing season, the herbage yield could be sufficiently high that it can be used for forage. Early spring growth potential of *T. subterraneum* is lower than that of *T. incarnatum*, *V. villosa* and *T. pratense*. Consequently, it is less promising for winter forage catch crop. However, its autumn growth potential and potential for nitrogen accumulation during autumn, winter and early spring, with the possibility of transferring the accumulated nitrogen to succeeding main crops in field rotation, increases the role of *T. subterraneum*. Due to this fact, it deserves further investigation as a catch crop for forage and for nitrogen accumulation in continental environmental conditions of Central Europe.

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# Rational use of legume/grass swards for high-quality forage production

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## Abstract

Field trials (2001-2004) were established on stagnic luvisol and sod podzolic soils. Evolution of two alfalfa varieties, fodder galega, white clover and 7 grass species in pure and multi-species stands was carried out. Swards were cut two to four times during the growing season. The average dry matter (DM) yield of legume/grass stands was 5.85 to 11.04 t ha<sup>-1</sup>. By their quality parameters, the produced grasses may be helpful in making top-quality hay and silage.

Key words: grass, legumes, productivity, forage quality.

## Introduction

In Latvian agricultural practice, legumes are grown either in pure stands or in mixtures with species of grasses fertilised with nitrogen. Forage legumes are considered as high-quality forage because of their superior feeding value, symbiotic fixation of atmospheric nitrogen and positive contribution to soil structure (Frame, 1992; Adamovich and Adamovicha, 2003). Alfalfa and white clover is a traditional performing forage crop in Latvia. By the sown area it ranks second and is followed by red clover. The main aim of this study was to examine the optimum productivity of grass and legume/grass swards and determine the implications of the dynamics of cutting regime for the quality of forage.

## Materials and Methods

The field trials (2001 to 2004) were conducted on brown-lessive soil (pH<sub>KCl</sub> was 6.7, mobile P – 52, K – 128 mg kg<sup>-1</sup> of soil). Altogether 120 binary- and multi-species seed mixtures were composed of *Medicago varia* cv. “Skriveru”, cv. “Vernal”, *Galega orientalis* cv. ‘Gale’, *Trifolium repens* cv. ‘Priekuļu 61’ and 7 grass species: *Alopecurus pratensis*, *Dactylis glomerata*, *Festuca pratensis*, *Festuca rubra*, *Phleum pratense*, *Lolium perenne*, and *Poa pratensis*. The total seeding rate of each seed mixture was 1000 germinating seeds per m<sup>2</sup>. The ratio of legume/grass seeds in mixtures was as follows: 400:600 in mixtures, and in identical ratios of grass components in multi-species mixtures. The stands were cut 2 and 4 times during the growing season. The plots were fertilised with P 40 and K 150 kg ha<sup>-1</sup>, and two N-fertiliser treatments were applied – N 0 and N 90<sub>(45+45)</sub>. Chemical composition of plants was determined only for the first cut by the following methods: dry matter (DM) – drying; crude protein (CP) – modified Kjeldahl; crude fibre (CF), neutral detergent fibre (NDF) and acid detergent fibre (ADF) – by van Soest (1980). Silage quality was evaluated by determining the content of organic volatile acids (Lepper-Flig): lactic acid, acetic acid, and butyric acid. Fermentation coefficient was calculated for the fresh material (Weiß *et al.*, 1998).

## Results and Discussions

The productivity of grass swards without nitrogen fertilizer use was on average 1.06 t ha<sup>-1</sup> of DM yield. Nitrogen fertilizers in the amount of N 90<sub>45+45</sub> kg ha<sup>-1</sup> increased the level of productivity, and the average DM yield was 2.70 t ha<sup>-1</sup>. For four-cutting mode, the productivity of multi-species swards was higher – the average DM yield constituted 2.12 t ha<sup>-1</sup> (Table 1). The intensified use of swards resulted in a double reduction of productivity indicators.

Table 1. DM yield of legume/grass swards ( t ha<sup>-1</sup> )(average 2001-2004).

Regime of cutting (F <sub>A</sub> )	Nitrogen fertilizer, kg ha <sup>-1</sup> (F <sub>B</sub> )	-----Composition of swards (F <sub>C</sub> ) -----						Average	Average (F <sub>A</sub> )	Average (F <sub>B</sub> )
		in pure stand	---number of components in mixtures---							
			two	three	four	five	six			
Grass		-----Grasses mixtures-----								
2-fold	N-0	0.77	1.00	0.98	1.02	1.04	-	0.96	1.65	1.06
	N-90	2.21	2.49	1.90	2.44	2.60	-	2.33		2.70
4-fold	N-0	1.32	1.20	1.04	1.15	1.13	-	1.17	2.12	
	N-90	3.18	2.91	3.15	2.88	3.21	-	3.07		
Average F <sub>C</sub>		1.87	1.90	1.77	1.87	2.00	-		1.88	
White clover		-----White clover/grass mixtures-----								
2-fold	N-0	5.85	6.66	6.51	6.51	6.72	6.80	6.51	6.94	6.28
	N-90	6.63	7.36	7.62	7.43	7.61	7.60	7.38		6.97
4-fold	N-0	5.77	5.86	6.17	6.45	5.99	6.10	6.06	6.31	
	N-90	6.04	6.33	6.69	7.05	6.40	6.83	6.56		
Average F <sub>C</sub>		6.08	6.55	6.75	6.86	6.68	6.83		6.62	
Alfalfa		-----Alfalfa/grass mixtures-----								
2-fold	N-0	11.04	11.45	10.94	11.43	11.64	11.21	11.29	11.15	9.87
	N-90	10.83	11.20	10.89	11.47	10.56	11.13	11.01		9.70
4-fold	N-0	8.59	8.46	8.32	8.30	8.56	8.56	8.46	8.43	
	N-90	8.67	8.25	8.14	8.30	8.57	8.44	8.40		
Average F <sub>C</sub>		9.78	9.84	9.57	9.87	9.83	9.83		9.79	
Galega		-----Fodder galega/grass mixtures-----								
2-fold	N-0	9.13	9.95	10.30	10.29	10.35	9.97	10.00	9.26	8.24
	N-90	8.17	8.51	8.34	8.86	8.73	8.51	8.52		7.42
4-fold	N-0	7.14	6.62	6.22	6.66	6.15	6.13	6.49	6.40	
	N-90	7.07	6.11	5.97	6.80	5.71	6.25	6.32		
Average F <sub>C</sub>		7.88	7.80	7.71	8.15	7.73	7.72	7.83		

F<sub>A</sub>LSD<sub>0.05</sub>=0.20

F<sub>B</sub>LSD<sub>0.05</sub>=0.11

F<sub>C</sub> LSD<sub>0.05</sub>=0.13

Trial LSD<sub>0.05</sub>=0.43

Without nitrogen fertilizer use and two cuttings per annum, the average DM yield of pure alfalfa/grass swards was 11.04 t ha<sup>-1</sup>. The average DM yield of 9.87 t ha<sup>-1</sup> for mixed alfalfa/grass swards was observed when no nitrogen fertilizers were applied, and 9.70 t ha<sup>-1</sup> respectively for swards with nitrogen fertilizer application. The average productivity of white clover/grass swards at two-cutting mode without nitrogen fertilizers resulted in the average DM yield of 5.85 t ha<sup>-1</sup>. The average DM yield reached 6.63 t ha<sup>-1</sup> when mineral nitrogen fertilizers were used in the amount of N 90<sub>45+45</sub> kg ha<sup>-1</sup>. For mixed white clover/grass swards without nitrogen fertilizers, the average DM yield reached 6.28 t ha<sup>-1</sup> and 6.97 t ha<sup>-1</sup>, respectively. The productivity of white clover/grass swards in four-cutting regime reached the average of 6.31 t ha<sup>-1</sup>, which is 0.63 t ha<sup>-1</sup> less compared to the three-cutting mode. Fodder galega, due to the slow growth pattern, provided high DM yields only in the third to fourth production years. Inclusion of grass species in mixtures resulted in yield increases by 26 to 32 % already in the first production year. For pure galega, the following average yields of DM and CP were obtained in early flower: 8.17-9.13 t ha<sup>-1</sup> of DM and 1.74 t ha<sup>-1</sup> of CP. The productivity of binary fodder galega/grass swards was the following: the average yield of DM was 8.28 t ha<sup>-1</sup> in swards receiving no fertiliser N, and 7.31 t ha<sup>-1</sup> in swards where the fertiliser was split into two applications – at the beginning of the growing season and after cut 1. The yield quality for legume/grass swards was heavily dependent on the botanic composition and cutting intensity. In the mode of pasture, the average total content of protein

was 157-197 g kg<sup>-1</sup> of DM, NDF – 369-438, and ADF – 274-372 g kg<sup>-1</sup> of DM. For the swards in the cutting mode, the average quality indicators of first cutting were as follows: content of protein – 127-143 g kg<sup>-1</sup>, NDF – 469-552, and ADF – 329-387 g kg<sup>-1</sup> of DM. The DM content of obtained silage increased when DM of ensiling grasses was increasing during vegetation. A significant increase in silage of fodder galega and perennial ryegrass/white clover was found: by 32% and 34%, respectively (Table 2). Although NEL value in fodder galega was 6.22 MJ kg<sup>-1</sup>, its preserving was only at an 86.4% extent of the fresh material NEL value at the budding stage when obtained silage quality was unsatisfactory with plenitude of butyric acid.

Table 2. Fermentation quality of different herbage silages.

Botanical composition	DM, g kg <sup>-1</sup>	Total acids in DM, g kg <sup>-1</sup>	-----Proportion of acids, %---			Ammonia nitrogen, %
			Lactic	Acetic	Butyric	
Fodder galega	154,1	148,6	56	35	9	21,6
	294,9	123,8	65	30	5	10,9
Red clover/ timothy	171,9	122,0	67	29	4	13,2
	267,3	113,1	70	30	0	6,06
Perennial ryegrass/ white clover	179,4	138,6	62	33	5	13,3
	290,3	100,6	76	23	1	10,4

The fresh material wilting to 290±20 g kg<sup>-1</sup> positively affected fermentation processes providing stabilization of an adequate pH level and significantly decreased (p<0.05) the amount of total acids in silage prepared in different vegetation stages compared to silage without additives. The ammonia nitrogen content decreased in all investigated grasses silage due to the right fermentation processes, especially in red clover/timothy silage. Preserving of DM of investigated herbage using chemical conservation (the main component formic acid) and effective restricting of fermentation processes provided not only a corresponding pH value but significantly (p<0.05) decreased the content of total acids and ammonia nitrogen, too. The most important decrease of total acids was established in fodder galega silage prepared at the budding stage – at a 54.5% extent, and in grass/legume mixture silage – at a 53.1% extent, respectively.

## Conclusions

Alfalfa/grass and fodder galega/grass swards productivity are relatively low-affected by meteorological conditions. They have an extended long-term productivity, which results in a high DM yield (average yield of 5.85-11.04 t ha<sup>-1</sup>) with a high nutrition value. These varieties often suffer from dryness during the period of vegetation. Dry matter of legumes has a high content of crude protein with a high concentration of non-replaceable amino acids.

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## **Agronomic traits as forage crops of nineteen population of *Bituminaria bituminosa***

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### **Abstract**

Tedera (*Bituminaria bituminosa* L.) is one of the most interesting wild shrubby species for livestock from the Canary Islands. Although it is distributed all over the Mediterranean region is in these islands where the species show the highest variability. A group of 19 wild populations from the seven islands was selected. Data on biomass production, height and coumarin content during the first year of experimental crop are presented in this article. The results show significant differences between them. Also data on soil characteristics and tedera chemical composition from samples directly taken on natural population are given.

Keywords: biomass yield, nutritive value, coumarin, tedera.

### **Introduction**

*Bituminaria bituminosa* C.H.Stirton ( $\equiv$  *Psoralea bituminosa* L.) commonly known as tedera in the Canary Islands is one of the most important group of autochthonous plant, that are being studied as part of the work carried out on forage species from the archipelago. Tedera is spread all over the Mediterranean and Macaronesian region and is being also introduced in the Irano-Turanic, Siberian and Saharo-Arabian region (Coca, 2003). However, the higher variability of the species takes place at the Canary Islands, with a larger population showing important differences. Although tedera has been traditionally used at the archipelago as a dietary supplement for goat livestock (Méndez, 2000), in the present most of the exploitations are deficient in forage (large fibre) in favour of feedstuffs. In order to deal with this problem, the present study explores to possibilities to increase the local forage production, thus improving all aspects related to it. The results will be able to be applied in the future work on selection and breeding.

### **Materials and methods**

Soil, seeds and plants samples were collected from 19 populations from the seven islands. Seeds were used to establish the experimental plantation; it consisted in three random groups of five plants (15 plants) of the following population: Vilaflor (VIL), Teno (TEN), Hermigua (HER), Vallehermoso (VAL), Chamorga (CHA), Garabato (GAR), Ayagaures (AYA), Güimar (GÜI), Argaga (ARG), Valles Ortega (VOR), Masca (MAS), Tiñor (TIÑ), Dehesa (DEH), Tenteniguada (TENT), Pedro Álvarez (PAL), Barlovento (BAR), Fuencaliente (FUE), Vinámar (VIN), Andén Verde (AVE) and Famara (FAM); three Iberian Peninsula populations were added to compare: La Perdiz (PER), Llano Beal (BEA) and Mijas (MIJ). The experience was carried out during 2002 at the experimental field of I.C.I.A (Tejina, Tenerife) under infracanarian semiarid type climate. Biomass production (BP) and height (H) were measured, as well as coumarin content, psoralen (P) and angelicin (A), were measured according to Innocenti *et al.*, (1984) and adapted by Méndez *et al.* (2001). At the same time data on soil characteristics (pH, electrical conductivity (EC) and texture) as well as tedera chemical composition (crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL)) from natural population samples are given. The soils analysis were determined according to



Porta-Casanelles (1986) and chemical composition of the forage according to the A.O.A.C. (1990) and Goering and Van Soest method (1970).

## Results and discusión

There were significant differences in BP, H, P and A content (Table 1). The highest BP obtained was in the Iberian Peninsula population and in a group of canaries' tederas belonging to different varieties: var. *bituminosa* (TENT, BAR, TIÑ, DEH, and PAL) var *crassiuscula* (VIL) and var. *albomarginata* (FAM) (Méndez *et al.*, 1990-91). The populations VAL, CHA, VIN, AVE, HER TEN and FAM presented a low H and a low to intermediate BP. All of them had two common characteristic, a biotype without dominant stem and their natural distribution over the most ancient geological areas of the archipelago; however P and A contents were clearly different FAM from the other as previously reported (Méndez *et al.*, 2001). GÜI showing the highest H and one of the lowest B agrees with that suggested by Coca (2003), who considered it as different from the rest.

Table 1. Biomass production (BP), height (H) and coumarins content (angelicin (A) and psoralen (P)) from experimental tederas plant grown.

Population	BP (g pl <sup>-1</sup> )	H (cm)	P (mg kg <sup>-1</sup> )	A (mg kg <sup>-1</sup> )
BEA	2058 a	138 hijk	184 ab	344 ab
TENT	1898 ab	146 jk	71 ab	799 ab
BAR	1702 abc	92 defg	350 ab	540 ab
TIÑ	1398 abcd	99 fgh	216 ab	284 ab
MIJ	1326 abcd	139 hijk	-	-
VOR	1268 abcd	104 ghi	-	-
PER	1179 abcd	131 hijk	126 ab	233 ab
VIL	1164 abcd	140 hijk	528 b	387 ab
FUE	1103 abcd	93 efgh	-	-
DEH	1096 abcd	66 bcdef	285 ab	530 ab
FAM	1082 abcd	58 bcde	3 a	1 a
PAL	1040 abcd	90 defg	184 ab	327 ab
ARG	848 bcd	112 ghij	198 ab	515 ab
MAS	844 bcd	145 jk	118 ab	312 ab
GAR	794 bcd	106 ghi	226 ab	282 ab
AYA	760 cd	111 ghij	153 ab	409 ab
TEN	618 cd	36 ab	158 ab	370 b
GÜI	434 d	151 k	110 ab	573 ab
HER	410 d	76 cdefg	199 ab	283 ab
AVE	378 d	41 abc	130 ab	345 ab
VIN	360 d	55 abcd	316 ab	365 ab
CHA	354 d	17 a	525 b	550 ab
VAL	345 d	45 abc	252 ab	373 ab
Significance	***	***	*	*

\*\*\* Significant at 0.001 % level; \* Significant at 0.05 level.

Values followed with the same letter mean no significant differences between them.

As far as forage chemical composition is concerned (Table 2), there were no significant differences among populations. It has to be pointed out that the high CP values agree with the data obtained previously from experimental crop of the three main varieties (Ventura *et al.*, 2000).

Most of the soils (Table 2) had a tendency to neutral or light alkaline, except BAR (pH=4.7); two of them (VAL and HER) could be clearly considered as saline soil. Results on texture showed most of the soils with more than 50 % of sand, except TENT, PAL, FAM, and specially VIN with high clay content.

Table 2 Chemical composition of soil and teder forage samples from 19 populations.

Population	Soil			Forage			
	pH	EC dS m <sup>-1</sup>	USDA texture	CP (g kg <sup>-1</sup> )	NDF (g kg <sup>-1</sup> )	ADF (g kg <sup>-1</sup> )	LAD (g kg <sup>-1</sup> )
VAL	7.9	4.6	Loamy sand	-	-	-	-
CHA	7.7	0.8	Sandy loam	133	360	220	60
VIN	6.7	0.5	Clay loam	183	350	200	140
AVE	8.2	2.5	Sandy loam	122	390	260	110
HER	8.0	4.1	Sandy	105	380	250	70
GÜI	7.5	1.5	Loamy sand	164	410	270	80
TEN	7.7	1.8	Sandy loam	168	340	190	50
AYA	7.1	1.6	Sandy	114	390	250	170
MAS	6.7	0.2	Loamy sand	115	450	250	110
ARG	7.8	1.1	Sandy	-	-	-	-
PAL	6.2	0.5	Sandy clay loam	150	380	250	140
FAM	8.1	1.45	Sandy clay loam	115	420	270	90
DEH	7.7	0.29	Loamy sand	140	440	320	80
FUE	7.1	0.2	Sandy loam	131	440	290	90
VIL	7.7	0.1	Sandy	160	300	180	20
VOR	8.1	1.4	Sandy loam	150	410	270	100
TIÑ	6.4	0.2	Sandy loam	-	-	-	-
BAR	4.7	3.8	Sandy loam	150	450	280	60
TENT	6.7	1.3	Sandy clay loam	152	360	190	50

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# Effect of inoculation and addition of chestnut tannin on the protein quality of lucerne silages

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## Abstract

The main problem of lucerne (*Medicago sativa* L.) silage is the extensive proteolysis that occurs during conservation. This study has been conducted to evaluate the effectiveness of chestnut tannin and inoculation with lactic acid bacteria (LAB) on the fermentation and nitrogen fractions of lucerne silages harvested at early bloom stage, wilted to a DM content of 400 g kg<sup>-1</sup> and ensiled in round bales. The bales were sampled at regular intervals during the conservation period of 75 days. The LAB treatment improved the fermentation quality, while the tannin treatment did not produce any influence on the fermentation pattern. The addition of tannins and inoculation had only a slight effect on the nitrogen fractions, reducing NH<sub>3</sub>-N and NPN contents.

Keywords: lucerne, round bales, proteolysis, tannin, LAB.

## Introduction

The large amount of crude protein per hectare produced by lucerne can be exploited by early harvesting coupled with conservation by ensiling. However, it is known that extensive proteolysis occurs during the wilting and ensiling of the legumes, and that plant proteins are degraded to non-protein nitrogen (NPN) (Winters *et al.*, 2000). The main consequences of this process are the low utilization of silage nitrogen (N) by ruminants, the reduction of the nutritional value and the intake of the silage. The poor efficiency with which silage N is used in the rumen leads to the need of expensive supplemental protein in silage-based diets for dairy cattle (Broderick, 1995). Besides the forage specie, the most important factors affecting the level of proteolysis during ensiling are: the rate of silage pH decline, the wilting process and the use of silage additives. Condensed and hydrolyzable tannins are plant secondary metabolites that can protect proteins from protease attacks during conservation process. For this purpose, the use of different tannins as silage additives has recently been evaluated on chopped grass and legumes in laboratory silos (Salawu *et al.*, 1999; Cavallarin *et al.*, 2002). Little is known about the effectiveness of tannin treatments on bale silage. Since the herbage is not chopped and the release of nutrients from cells is restricted, the potential value for the protection of proteins from degradation could be reduced. The aim of this work was to study the effects of the addition of chestnut tannin (T) and the inoculation with lactic acid bacteria (LAB) on the ensiling of lucerne in small bale silage.

## Materials and methods

The trial was carried out in 2001, at the Experimental Farm of the University of Turin, northern Italy (44°50'N, 7°40'E) on a stand of lucerne (*cv.* Equipe) of third regrowth yielding 5.3 t DM ha<sup>-1</sup>. The herbage was cut at early bloom (3 July) with a roll-conditioner mower to reach a wilting level of 400 g kg<sup>-1</sup>. The wilted herbage was baled (60 cm diameter) from alternate windrows and individually wrapped with 6 layers of polyethylene film. The inoculant and tannin were sprayed onto the swath before baling with 10<sup>6</sup> cfu of *Lactobacillus plantarum* per gram of fresh matter (CSL, Italy, for legume crops) and 40 g kg<sup>-1</sup> DM of chestnut tannin (Silva Chimica, Italy) for the LAB and T treatments, respectively. An average of four round bales were harvested for each treatment. The silos were weighed immediately after being filled and were then stored for 75 days. Two bales were sampled for each treatment at 0, 1, 4, 10, 19, 30, and 75 days of conservation to evaluate the fermentation patterns. Sampling was performed

by coring the bale from its side to a depth of about 300 mm with a 50 mm diameter corer. The coreholes were plugged with a wooden stick and immediately sealed with silicone rubber, to avoid air damage and moulding. The herbage and silage samples were sub-sampled and immediately analysed for DM content by oven drying at 80°C for 24 h and for total nitrogen (TN) according to the Dumas method. The NPN was determined through the Kjeldahl method after precipitation with trichloroacetic acid, and ammonia nitrogen (NH<sub>3</sub>-N) using a specific electrode. The free amino acid nitrogen (Free AA-N) was determined according to Winters *et al.* (2002).

The chemical composition data were analysed for their statistical significance via ANOVA, with their significance being reported at a 0.05 probability level using the GLM of SPSS (v. 11.5, SPSS Inc.).

## Results and discussion

The main fermentation products and nitrogen fractions of the silages, at the end of the conservation period, are reported in Table 1. The wilted herbage reached a DM content of 425 g kg<sup>-1</sup> within 5 hours after mowing. The fermentation quality was good in all the silages, with no butyric acid and low ammonia nitrogen, due to the high DM contents reached during wilting. The LAB treatment greatly improved the amount of lactic acid, while the tannin treatment did not influence the acid concentrations or the pH. The TN content was not affected by any treatment with a mean value of 24.8 g kg<sup>-1</sup> DM. The NH<sub>3</sub>-N and NPN contents decreased slightly due to the tannin treatment. The free AA content was unaffected by either treatments. The DM losses were similar in all the treatments and always lower than 3% of the original DM. The evolution of the main fermentative and nitrogen fractions, during fermentation, are shown in Figure 1. The pH decreased faster in the LAB treatment than in the control, and the T treatment was in-between, while after 75 days the differences were smaller. The lactic acid production was inversely related to the pH, but the final values of the control and tannin treatment were lower than that of the LAB. A large production of acetic acid was observed in the LAB treatment. Among all the nitrogen fractions, only ammonia showed to be affected by the tannin, while the NPN and the free AA-N did not show any significant difference during fermentation.

Table 1. Chemical composition of the lucerne silages after 75 days of conservation.

	Treatment			significance
	Control	LAB	T	
DM (g kg <sup>-1</sup> )	415	428	432	NS
pH	5.14	4.82	5.21	NS
Lactic acid (g kg <sup>-1</sup> DM)	32.6 <sup>b</sup>	62.9 <sup>a</sup>	35.4 <sup>b</sup>	*
Acetic acid (g kg <sup>-1</sup> DM)	11.8 <sup>b</sup>	18.5 <sup>a</sup>	6.8 <sup>b</sup>	*
Butyric acid (g kg <sup>-1</sup> DM)	<0.01	<0.01	<0.01	NS
Ethanol (g kg <sup>-1</sup> DM)	4.7	4.9	6.4	NS
TN (g kg <sup>-1</sup> DM)	25.0	25.6	23.7	NS
NH <sub>3</sub> -N (g kg <sup>-1</sup> TN)	107 <sup>a</sup>	99.2 <sup>a</sup>	73.6 <sup>b</sup>	**
NPN (g kg <sup>-1</sup> TN)	612 <sup>a</sup>	590 <sup>a</sup>	537 <sup>b</sup>	*
Free AA-N (g kg <sup>-1</sup> TN)	322	383	333	NS
DM loss (%)	2.50	2.52	2.38	NS

Within rows, means not showing a common superscripts, differ significantly  
NS = not significant; \* = P<0.05; \*\* = P<0.01.

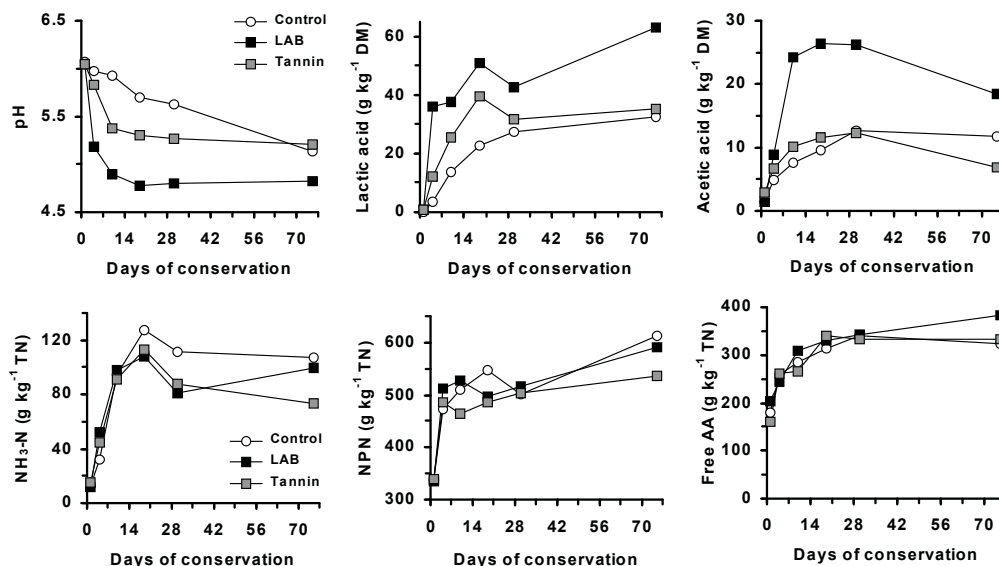


Figure 1. pH, fermentation products and N fractions during fermentation of lucerne silage.

### Conclusions

The DM content over 400 g kg<sup>-1</sup> reached with a fast wilting allowed silages with good fermentation quality to be obtained regardless of the treatments. The addition of tannins to the forage ensiled in wrapped bales did not improve the fermentation quality and it had only a slight effect on the nitrogen fractions, reducing NH<sub>3</sub>-N and NPN. The LAB inoculum sped up the pH decline, and increased the fermentation products.

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The work is attributable to the authors in equal part.

## Tropical legumes for forage

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### Abstract

A small-plot trial was conducted at the Rimski Šančevi Experiment Field in 2004 to compare yield and morphological characteristics of pigeon pea (*Cajanus cajan* (L.) Millsp.), hyacinth bean (*Lablab purpureus* (L.) Sweet), and cowpea (*Vigna unguiculata* (L.) Walp.) in a northern environment. These legumes, commonly grown in the tropics and sub-tropics, were sown in late April and harvested when first pods began to appear. Number of days from sowing to harvest ranged from 105 in ICPL 88020 pigeon pea to 148 in NI 147 cowpea, while two pigeon pea accessions failed to flower at this latitude of 45.2°N. The greatest stem length was in Xincharo cowpea (110 cm), while the greatest number of internodes was in Hunt pigeon pea (19.0). Associated with greatest number of lateral branches per plant (7.2) and individual plant mass (262.8 g), Quest pigeon pea had the highest yields of both fresh weight (52.82 t ha<sup>-1</sup>) and hay (14.79 t ha<sup>-1</sup>). Certain accessions of each of the three species produced enough biomass to make them competitive with traditional annual forage crops of the region. Observed variation in morphological characteristics suggests that there is potential to further develop these species as forage crops for the northern Balkans.

Keywords: pigeon pea, hyacinth bean, cowpea, yield, forage, northern Balkans.

### Introduction

As species typically grown in tropical and subtropical environments, pigeon pea, is completely unknown in Serbia and Montenegro, while hyacinth bean is grown rather sporadically as an ornamental plant. Cowpea (*Vigna unguiculata* (L.) Walp.) is not unfamiliar in this country (Erić *et al.*, 1996), but remained neglected and underutilized as forage and grain legume in favour of pea (*Pisum sativum* L.), common vetch (*Vicia sativa* L.), faba bean (*Vicia faba* L.) and French bean (*Phaseolus vulgaris* L.). The objective of our study was to determine the feasibility of growing pigeon pea, hyacinth bean and cowpea for forage in the northern Serbian Province of Vojvodina, as well as to compare their agronomic characteristics and to determine their breeding value for development of cultivars adopted to the northern Balkan Peninsula.

### Materials and methods

A small-plot trial was conducted at the Rimski Šančevi Experiment Field of the Institute of Field and Vegetable Crops in Novi Sad during 2004 at 45.2°N latitude and 19.6°E longitude. It included eleven accessions of three tropical legumes from the Annual Forage Legumes Genetic Collection (AFLGC) of the Forage Crops Department of the Institute of Field and Vegetable Crops (Table 1). The trial was established on 22 April 2004 with a crop density of 20 plants m<sup>-2</sup> for pigeon pea and hyacinth bean (Cameron, 1999; Rao *et al.*, 2003) and 30 plants m<sup>-2</sup> for cowpea (Skerman, 1977), with a plot size of 5 m<sup>2</sup> and three replicates. The growing season was characterised by an average temperature of 17.6 °C compared to the long-term average of 17.1 °C, and a precipitation sum of 432 mm compared to the long-term average of 374 mm. Nine legume accessions were cut when the first pods began to appear (Mišković, 1986), while Royes and NI 470 were rejected for remaining in the vegetative stage. Number of days from sowing to cutting, plant height (cm), number of lateral branches, number of internodes, individual plant mass on a wet weight basis (g) and yields of fresh weight and dry matter (t ha<sup>-1</sup>) were recorded. The results were processed by analysis of variance and the *LSD* test applied.

Table 1. Accessions of pigeonpea, hyacinth bean and cowpea grown for forage at Rimski Šančevi in 2004.

No. in AFLGC	Species	Accession	Country of origin
CAJ 001	<i>C. cajan</i>	ICPL 88020	Australia
CAJ 002	<i>C. cajan</i>	Hunt	Australia
CAJ 003	<i>C. cajan</i>	Quantum	Australia
CAJ 004	<i>C. cajan</i>	Quest	Australia
CAJ 005	<i>C. cajan</i>	Royes	Australia
CAJ 006	<i>C. cajan</i>	NI 470	Burundi
LAB 001	<i>L. purpureus</i>	NI 77R	Italy
VIG 003	<i>V. unguiculata</i>	Xincharo	Portugal
VIG 004	<i>V. unguiculata</i>	NI 147	France
VIG 005	<i>V. unguiculata</i>	NI 188	Suriname
VIG 006	<i>V. unguiculata</i>	NI 479	DR of the Congo

### Results and discussion

The number of days from sowing to pod development in pigeon pea (105 in ICPL 88020 to 122 days in Quantum and Quest) and in hyacinth bean (127 days), Table 1, are within the range previously reported for both species (Duke, 1997; ECHO, 1999). Cowpea required from 113 (NI 188) to 148 (NI 147) days for pod development. Stem length of pigeon pea ranged from 57 cm in ICPL 88020 to 94 cm in Hunt, substantially shorter than the same genotypes grown in more favourable conditions (Rao and Gill, 1995). The single accession of hyacinth bean reached a length of 105 cm, similar to that previously reported in the central USA (Anderson *et al.*, 1996), while cowpea length ranged from 74 (NI 479) to 110 cm (Xincharo). All accessions were prone to forming lateral branches. Branch number in pigeon pea ranged from 2.3 in ICPL 88020 to 7.2 in Quest, in hyacinth bean it was 4.2 and in cowpea branch number ranged from 3.8 in Xincharo to 5.0 in NI 188. With number of internodes ranging from 11.3 in ICPL 88020 to 19.0 in Hunt, pigeon pea accessions had greater variation for this characteristic than cowpea accessions, which ranged from 11.7 in NI 188 to 16.5 in NI 147. Hyacinth bean NI 77R had 12.7 internodes. Plant mass of pigeon pea ranged from 32.06 g in ICPL 88020 to 262.80 g in Quest, while mass of hyacinth bean was 169.62 g, and cowpea ranged from 35.16 g in NI 479 to 137.12 g in Xincharo. Associated with a long growing period, moderate plant height and great number of lateral branches, Quest had the highest yields of fresh weight and dry matter (52.8 t ha<sup>-1</sup> and 14.8 t ha<sup>-1</sup>) among pigeon pea accessions, demonstrating that this species has a great potential to provide forage production in the Balkans. Hyacinth bean NI 77R produced 34.1 t ha<sup>-1</sup> of fresh weight and 9.5 t ha<sup>-1</sup> of dry matter, similar to the yields over two years in its native environment (Shehu *et al.*, 2001). Xincharo was the most yielding of all cowpea accessions, producing 45.9 t ha<sup>-1</sup> of fresh weight and 12.9 t ha<sup>-1</sup> of dry matter, achieving similar results to those of mixtures of cowpea with maize, sorghum, sudangrass and sunflower in Croatia (Čížek, 1964).

Table 2. Agronomic characteristics of accessions of pigeonpea, hyacinth bean and cowpea grown for forage at Rimski Šančevi in 2004.

Accession name	Number of days from sowing to cutting	Stem length (cm)	Number of lateral branches	Number of internodes	Plant mass on a wet weight basis (g)	Yield of fresh weight (t ha <sup>-1</sup> )	Yield of dry matter (t ha <sup>-1</sup> )
ICPL 88020	105	57	2.3	11.3	32	6.4	1.8
Hunt	110	94	6.5	19.0	169	33.9	9.5
Quantum	122	74	3.7	14.7	108	21.7	6.1
Quest	122	84	7.2	15.3	263	52.8	14.8
NI 77R	127	105	4.2	12.7	170	34.1	9.5
Xincharo	139	110	3.8	14.7	137	45.9	12.9
NI 147	148	91	4.5	16.5	35	11.9	3.3
NI 188	113	80	5.0	11.7	117	39.2	11.0
NI 479	130	74	3.9	12.3	35	11.8	3.3
LSD	0.05	12	1.7	3.8	46	14.6	2.9
	0.01	17	3.6	5.2	62	19.2	4.0

## Conclusions

Pigeon pea, hyacinth bean and cowpea have potential as forage crops in the northern Balkan Peninsula, producing yields competitive with traditional annual forage crops. In order to confirm the preliminary results, the trial will be repeated and forage quality analyses performed. If proven reliable, breeding first Serbian cultivars of these three species could be aimed at high and quality yields of forage, short growing period and good yields of seed.

## Acknowledgements

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## Nine native leguminous shrub species: allometric regression equations and nutritive values

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### Abstract

Leguminous shrubs play an important role as feed for both wild and domestic herbivores, particularly in Mediterranean ecosystems. *Adenocarpus decorticans*, *Cytisus galianoi*, *Dorycnium pentaphyllum*, *Erinacea anthyllis*, *Genista cinerea*, *Genista scorpius*, *Genista umbellata*, *Genista versicolor* and *Ulex parviflorus* are all native legumes of the mountains of south-eastern Spain. Allometric regression equations (phytovolume vs total and edible browse phytomass) and the nutritive values (crude protein, dry and organic matter digestibility and metabolizable energy) are presented for these species. The correlation coefficient of the regressions equations between phytovolume and phytomass were high in all species ( $r^2 = 0.65$  to  $0.98$ ) except for *Genista versicolor*. Crude protein varied from 7.8 to 18.0%. The digestibility of dry and organic matter reached a maximum of 79.4 and 79.0% for *Adenocarpus decorticans* and a minimum of 27.7 and 24.1% for *Dorycnium pentaphyllum*. Metabolizable energy ranged from 3.6 to 11.7MJ kg<sup>-1</sup> DM. These results are useful for the management of silvopastoral ecosystems.

Keywords: phytovolume, phytomass, crude protein, digestibility, metabolizable energy.

### Introduction

The mountainous areas of south-eastern Spain are marginal lands characterized as ecologically fragile. Many of these areas have traditionally been used to breed sheep and goats. Leguminous shrubs are an abundant and important source of animal feed in these ecosystems. Nine widespread native legumes were selected to determine their pastoral value through their biomass and chemical composition. Information provided for these shrubs includes: i) biomass: the allometric regression equations (phytovolume vs total and edible browse phytomass) and ii) the chemical composition (crude protein, dry and organic matter digestibility and metabolizable energy). This data is useful for the management of silvopastoral ecosystems, including selection of fodder shrubs, shrubland improvement and accurate calculation of the carrying capacity.

### Materials and Methods

Study area.- The study was carried out in the Sierra Nevada National Park (south-eastern Spain, approximately 37° 03' N 2° 56' W; 1100 to 2200 m.a.s.l). The mean annual rainfall ranges from 527 to 700 mm; and the mean annual temperature from 10.5 to 14.7 °C. The vegetation series is a holm-oak forest (*Quercus rotundifolia* Lam.) with *Adenocarpus decorticans* L. (*Adenocarpus decorticans-Querceto rotundifoliae* sismetum).

Allometric regression equations.- Regression equations were established between apparent phytovolume (circular cylinder; Uso *et al.*, 1997) and aerial phytomass (both total and edible browse phytomass). For each species (*Adenocarpus decorticans* Boiss, *Cytisus galianoi* Talavera & Gibbs, *Dorycnium pentaphyllum* Scop., *Erinacea anthyllis* Link, *Genista cinerea* (Will) DC. in Lam & DC., *Genista scorpius* (L.) DC., *Genista umbellata* (L'Hér) Poirlet, *Genista versicolor* Boiss. and *Ulex parviflorus* Pourret) twenty to thirty plants of different sizes were harvested in spring. The height and mean diameter of each plant was measured to obtain the phytovolume. Edible browse phytomass was estimated by simulating the browsing of goats (Robles *et al.*, 2002). Samples of the total and edible

browse phytomass were dried until constant weight in a forced-air oven at 80 °C to transform fresh weight to dry weight.

Nutritive value.- Young stems, leaves and flowers were collected in spring. Two samples per species were oven dried until constant weight at 60 °C in a forced-air oven and analyzed for dry and organic matter (DM, OM) and crude protein (CP) following the recommendations of A.O.A.C. (1990). The digestibility of both dry (IVDMD) and organic matter (IVOMD) was determined *in vitro* using the rumen liquor-pepsin method (Tilley and Terry, 1963) as modified by Molina (1981), which uses inoculum from the rumen of autochthonous *segureña* sheep. Metabolizable energy (ME) was estimated from the digestibility of the organic matter using the following equation (see Robles *et al.*, 2002):  
 $ME (MJ kg^{-1} DM) = OM (g kg^{-1} DM) \times IVOMD (\%) \times 19 \times 0.82 \times 10^{-5}$

## Results and discussion

Allometric regression equations.- The relationship between total and edible browse phytomass of every legume was best expressed with an exponential equation (confidence level of 95 %). In a similar study, Uso *et al.* (1997) found that exponential models best explain the relationship between biomass and apparent volume.

Table 1. Parameter values of regression equations:  $y = a * x^b$ , y = total or edible browse phytomass (dry weight, kg DM per plant), x = volume (dm<sup>3</sup> per plant), a and b = estimated parameters (a intercept, b slope). r<sup>2</sup> = correlation coefficient; SEE = standard error of estimation, n = sample size.

Species	y	a	b	r <sup>2</sup>	SEE	n
<i>Adenocarpus decorticans</i>	Total	6.60588	0.942512	0,98	0.370	31
	Browse	2.61269	0.815397	0,98	0.311	
<i>Cytisus galianoi</i>	Total	6.62414	1.072210	0,87	0.359	20
	Browse	1.74470	0.843232	0,77	0.405	
<i>Dorycnium pentaphyllum</i>	Total	13.54246	0.849516	0,86	0.464	21
	Browse	2.28786	0.772380	0,84	0.454	
<i>Erinacea anthyllis</i>	Total	58.00098	0.806947	0,94	0.269	20
	Browse	8.433062	0.802182	0,88	0.384	
<i>Genista versicolor</i>	Total	52.04298	0.589333	0,43	0.860	20
	Browse	9.94003	0.507013	0,45	0.705	
<i>Genista cinerea</i>	Total	74.68879	0.515316	0,82	0.616	19
	Browse	12.10623	0.469741	0,82	0.553	
<i>Genista scorpius</i>	Total	22.99025	0.679505	0,92	0.237	21
	Browse	3.53315	0.639498	0,70	0.489	
<i>Genista umbellata</i>	Total	12.06912	0.760469	0,81	0.449	20
	Browse	2.85534	0.641064	0,65	0.581	
<i>Ulex parviflorus</i>	Total	13.21576	0.777866	0,86	0.451	22
	Browse	5.46700	0.587377	0,79	0.430	

p-level of regressions equations: p<0.00001.

The correlation coefficient of the regressions between phytovolume and phytomass were high in all species (Table 1), except for *G. versicolor*, which has no leaves, an irregular shape and a thorny stem. The highest statistical correlation coefficients found in this study were between phytovolume and total phytomass, consistent with previous work (Fernández, 1995).

Nutritive value- All chemical parameters varied widely among species (Table 2). The low CP content in *G. umbellata* and *E. anthyllis* was probably due to a higher proportion of mature stems and the lack of leaves. The low digestibility of *D. pentaphyllum* and *G. umbellata* might be attributed to the high

lignification of stem cell walls (Robles and Boza, 1993). *A. decorticans* showed the best analytical results confirming previous reports of its high palatability (Fernández, 1995).

Table 2. Nutritive value of leguminous shrubs. Chemical composition: dry matter (DM, g kg<sup>-1</sup>), organic matter (OM, g kg<sup>-1</sup> DM), crude protein (CP, g kg<sup>-1</sup> DM); *in vitro* dry matter digestibility (IVDMD, %), *in vitro* organic matter digestibility (IVOMD, %) and metabolizable energy (ME, MJ kg<sup>-1</sup> DM).

	DM	OM	CP	IVDMD	IVOMD	ME
<i>Adenocarpus decorticans</i>	378	953	180	79.4	79.0	11.7
<i>Cytisus galianoi</i>	328	953	159	67.1	66.2	9.8
<i>Dorycnium pentaphyllum</i>	469	950	113	27.7	24.1	3.6
<i>Erinacea anthyllis</i>	499	955	88	63.1	62.7	9.3
<i>Genista cinerea</i>	463	957	135	67.3	66.5	9.9
<i>Genista scorpius</i>	465	935	124	53.0	50.1	7.3
<i>Genista umbellata</i>	422	979	78	32.0	30.6	4.7
<i>Genista versicolor</i>	441	964	130	63.6	63.3	9.5
<i>Ulex parviflorus</i>	429	954	135	51.6	49.7	7.4

## Conclusions

The excellent  $r^2$  values obtained indicate the potential of these equations to predict total and edible browse phytomass from phytovolume values. *A. decorticans* is one of the best fodder resources in the mountainous south-eastern Spain due to its excellent nutritive value.

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# The effect of climatic factors on the visit of pollinators and the seed yield of alfalfa

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## Abstract

In the course of a two-year research the effect of meteorological factors on the visit of pollinators, pollination and seed yield of alfalfa (*Medicago sativa* L.) was studied. Pollinator activity and meteorological factors were monitored in the morning, at noon and in the afternoon during 10 days of flowering during summer regrowth. The temperature was recorded at three different heights above ground level (0.3 m, 0.6m and 1m). In the first year, characterized by abundant precipitations, the pollinators of alfalfa such as *Megachile rotundata*, *Nomia* *ssp.* and others were dominant (63.92%). In the second year, drier and warmer, honeybees prevailed (87.63%). The second year was much more favorable for seed production so the seed yield was considerably greater than in the first year. Seed yield and seed yield components (except for 1,000 seed weight) were significantly different between the studied years.

Keywords: climatic factors, pollinators, seed yield, seed yield components.

## Introduction

Production of alfalfa seed is highly dependant on meteorological conditions (Erić, 1995; Marković, 1997). Among meteorological factors, air and soil temperature and humidity during flowering have the greatest effect on seed yield, together with precipitations, light, velocity of the wind, etc. The optimal mean daily temperature for successful alfalfa pollination range from 25 to 30<sup>0</sup> C (Erić, 1995), and the optimal relative air humidity is from 30 to 50% (Žarinov and Ključ, 1995). Increased relative air humidity has a negative effect on the concentration of sugars in nectar which decreases the activity of honeybees (Ciurdaresku, 1974). The highest seed yields of alfalfa are achieved in dry and warm summers, while in damp and rainy years the yield is lower (Hoopingarner and Waler, 1992). The best regions for alfalfa seed production are those with average precipitations of 600-650 mm m<sup>-2</sup> (Bošnjak and Stjepanović, 1987). The activity of honeybees is affected primarily by wind. Wind speed of about 24 km h<sup>-1</sup> is limiting for honeybees activity (Martin, 1976). The objective of this paper was to determine how certain climatic factors affect the activity of pollinators and seed production of alfalfa.

## Materials and Methods

Our research was carried out in 1997 and 1998 at experimental field of Center for forage crops in Kruševac. The following meteorological factors were studied: air temperature, air humidity, precipitations and wind speed. All measurements were taken simultaneously with the counting of the pollinators in three terms (morning, noon and afternoon). The temperature was measured at three heights (30 cm, 60 cm and 1 m) above ground. Air humidity was measured by a mobile thermo-hygrograph placed in a meteorological shelter facility 250 m far from a trial field. Precipitation was determined by pluviometer while the wind speed was measured by anemometer. The number of all pollinators on 1 m<sup>2</sup> of flowering alfalfa seed crop in 30 min were counted every ten days. Seed yield was determined after harvest on three 2m<sup>2</sup> plots and seed yield components were determined on ten selected plants per plot. The number of seeds per pod and 1,000 seed weight were determined. The percentage of inflorescences forming pods and the percentage of florets forming pods was calculated. For all climatic factors mean, minimum and maximum values were determined, while seed yield and seed yield components were subjected to analysis of variance.

## Results and discussion

In the first experimental year the temperatures in the morning were lower while at the two other times of observation they were optimal for activity of pollinators (Table 1). In the second year, the temperatures recorded in the morning were suitable for pollinator activity while those at noon and in the afternoon were slightly higher than optimal. The difference between temperatures at different heights was, depending on the day, even greater than 1C<sup>0</sup>.

Table 1. Temperatures recorded during the trial in 1997 and 1998 (10 days analysis).

	Morning			Noon			Afternoon		
	0.3m	0.6m	1 m	0.3m	0.6m	1 m	0.3m	0.6m	1 m
1997									
Mean	22.9	23.57	23.4	27.4	27.7	27.3	26.9	27.0	26.8
Min	19.6	20.5	20.4	21.8	22.3	21.8	24.2	23.4	23.2
Max	25.8	27.8	26.8	31.5	32.8	32.7	30.8	31.7	31.4
1998									
Mean	24.8	24.8	24.6	31.9	31.9	31.7	30.9	30.9	30.8
Min	18.1	18.8	18.1	24.0	23.4	23.2	22.2	19.73	19.7
Max	29.0	28.6	28.2	37.8	37.3	36.9	35.7	35.5	35.8

Optimal humidity ranging from 50 to 70% was present only in the morning in both years of investigation. while humidity at noon and afternoon was lower than optimal. Air humidity at noon and afternoon was higher in the first year than in the second year. The first year was a rainy one. with rainfall occurring during the whole alfalfa flowering period (85 mm m<sup>-2</sup>). During measuring in 1998 the rain fell only once but abundantly (45 mm m<sup>-2</sup>). Velocity of wind was never limiting for pollinator activity.

Table 2. Meteorological factors in 1997 and 1998.

	Air humidity (%)			Precipitations (mm day <sup>-1</sup> )	Wind velocity (km h <sup>-1</sup> )		
	Morning	Noonday	Afternoon		Morning	Noonday	Afternoon
1997							
Mean	67.9	41.4	42.0	8.5	7.1	8.3	10.1
Min	56	29	29		3.0	3.6	4.2
Max	81	61	69		12.0	18.6	18.6
1998							
Mean	67.6	34.5	33.4	4.5	3.2	7.5	8.3
Min	60	23	21		1.8	4.2	1.2
Max	74	60	70		7.7	12.0	14.4

As regards pollinators. there was a marked difference between the first and the second year (Table 3). In the first year which was considerably more humid and cooler. there were 1,264 pollinators recorded daily on average. Other pollinators (63.92%) were much more active in comparison with honeybee (36.08%). In the second year. drier and warmer. we counted 159.2 pollinators daily with a strong prevalence of honeybees (7:1) with respect to other pollinators (*Megachile rotundata* Fab., *Nomia spp.* and others).

Table 3. Number of honeybees and other pollinators (10 days analysis).

Year	Pollinators								Total pollinators	Honeybees (%)	Others (%)
	Honeybees				Other pollinators						
	Times				Times						
I	II	III	Σ	I	II	III	Σ				
1997	17.4	16.8	11.4	456	26.6	32.8	21.4	808	1264	36.08	63.92
min	2	2	5		10	14	6				
max	37	33	34		45	49	49				
1998	43.5	52.3	43.7	$\frac{139}{5}$	9.3	5.1	5.3	197	1592	87.63	12.37
min	5	24	18		3	2	0				
max	72	69	68		18	11	13				

The second year proved to be more suitable for seed production than the first one. Seed yield and seed yield components in the second year had considerably greater values than in the first year, except for 1,000 seed weight (Table 4).

Table 4. Seed yield and seed yield components.

Trait-Year	Seed Yield (kg ha <sup>-1</sup> )	Inflorescences forming pods (%)	Florets forming pods (%)	Number of seeds per pod	1,000 seed weight (g)
1997	199.0	44.44	30.46	2.97	2.27
1998	409.2	83.71	44.02	3.49	2.28
Average	304.1	64.07	37.24	2.23	2.28
Lsd	0.05	71.7	8.96	0.17	0.24
	0.01	97.4	11.17	0.24	0.32

## Conclusions

Our two-year research on the effect of climatic factors on the activity of pollinators and seed production confirmed that the climatic factors studied, especially temperature and precipitations, had a great influence on the activity of pollinators and seed production of alfalfa. In the first year which was colder and more humid there were significantly more other pollinators in comparison with honeybee (2:1). Climatic conditions were rather unfavorable for seed production. In the second year which was dry and warm, honeybees were predominant. This year was also much better for seed production since seed yield was about double in comparison with the first year.

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# Effect of gut passage on viability and seed germination of legumes adapted to semiarid environments

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## Abstract

This study aims to analyze the effect of seed ingestion by sheep on seed viability and percentage of seed germination of legumes adapted to semiarid environments (one cultivated variety, *Medicago sativa* L. (MSCV); and two wild varieties: *M. sativa* (MSWV) and *Trigonella polyceratia* L. (TP)). Four treatments were applied to the seeds: Control, 24 h, 48 h and 72 h (untreated seeds and seeds recovered 24, 48 and 72 hours after ingestion, respectively). High recovery of seeds was found for MSWV and TP (around 34%), but MSCV only reached 2.1%. Percentage of germination decreased or did not increase for all treatments after ingestion in all species except for 48 h treatment in MSWV which was slightly enhanced. Viability only decreased for 24 h treatment for MSCV (60% lower than control treatment) and for 24 h and 72 h treatments for MSWV (around 30% lower than control in both cases). Our results suggest that ovine livestock can disperse a high quantity of viable seeds of TP and MSWV that may reach suitable places for germination and establishment. Thus, feeding the animals with these seeds and allowing them to graze in a certain area could help increase the population of these wild varieties.

Keywords: *Medicago sativa*, *Trigonella polyceratia*, wild variety, cultivated variety, seed dispersal.

## Introduction

Leguminosae is a family with great interest in Mediterranean semiarid environments, especially in degraded areas and abandoned lands. Legumes play an important role in the improvement of soil fertility and in the feeding of wild and domestic livestock. Animals are key elements for seed dispersal and recruitment of new populations for many of legume species (Malo and Suárez, 1995; Milton and Dean, 2001). The capability of livestock to disperse legumes by endozoochory might be useful as a 'seeding method' in degraded lands, fallows, etc. This capability is determined by the percentage of destroyed seeds due to digestive process and the ability of seeds to remain viable and germinate. This is a complementary study of that presented by Ramos *et al.* (2005), where we analyzed the percentage and pattern of seed recovery after ingestion by sheep of seeds of legumes adapted to arid and semiarid environments. In the current work we determine the percentage of germination and the viability of the seeds which were recovered in the previous 2005 assay.

## Materials and methods

Two varieties of *Medicago sativa* L. (one of them wild –MSWV- and the other cultivated -MSCV) and the species *Trigonella polyceratia* L. (TP) were used for the experiment. Seeds of each variety and species were offered to three individually penned sheep (56,000 seeds of MSWV, 42,200 of MSCV and 62,000 of TP). Faeces were collected 24, 48 and 72 hours after ingestion, dried at room temperature, weighed and crumbled. For each time interval and for each sheep, 30 subsamples of 2 g were taken and the number of intact seeds per subsample was determined by naked-eye. Percentage of seed recovery was determined by referring the mean number of seeds found in 2 g to the total mass of dung. Germination was assayed in a growth chamber with a photoperiod of 16 hours day/ 8 hours night, at 22/16° C. We tested four different treatments: 1) Control, 2) 24 h, 3) 48 h and 4) 72 h (i.e. seeds recovered 24, 48 and 72 hours after ingestion, respectively). Seeds from all treatments were washed and then disinfected by immersion in a 1% sodium hypochlorite solution for 10 min, and thoroughly rinsed with sterile distilled water. After that, they were placed in sterile plastic Petri dishes containing filter paper disks. All materials were previously sterilised. Dishes were initially moistened with 7 ml of a

suspension of Benomyl fungicide at 0.1%, being thereafter moistened as needed with sterile water. Each Petri dish contained 25 seeds, and there were 5 replicates per treatment. Due to the lack of seeds, 72 hours treatment could not be set for MSCV and only had three replicates for MSWV. Dishes were randomly repositioned within the chamber every 5 days. Germination, identified as visible radical protrusion, was recorded at two days interval during 30 days. Viability was tested with Tetrazolium test (2, 3, 5-triphenil tetrazolium; AOSA, 1970). Statistical analysis of percentage of germination and viability was performed for each species separately with a one-way ANOVA or Kruskal-Wallis when assumptions of normality and/or homocedasticity were not possible. Differences among treatments were detected by Tukey multiple comparison test, for ANOVA, or Nemenyi test, for Kruskal-Wallis.

## Results and discussion

High recovery of seeds was found for MSWV and TP (around 34%), but MSCV only reached 2.1%. Most seeds were released during the first 48 hours (see Ramos *et al.*, 2005, for further information). Germination percentage decreased for 24 h treatment in MSCV and for all treatments in TP, compared to Control treatment (Figure 1). Gut passage did not affect germination percentage for 72 h treatment in MSCV and for 24 and 72 h treatments in MSWV (Figure 1). A promotion of germination was only observed for 48 h treatment. Examples of enhancement of germination for legume seeds after ungulates gut passage are frequent in the literature (Russi *et al.*, 1992; Malo and Suárez, 1995) but no effect on germination after the digestive process is reported as well (Razanamandranto *et al.*, 2004).

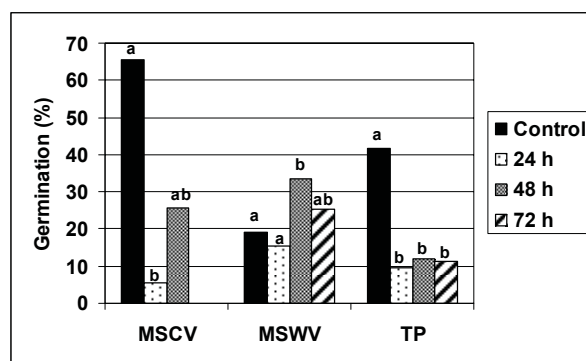


Figure 1. Percentage of germination of three legumes after gut passage. Different letters indicate significant differences detected by Nemenyi test ( $\alpha=0.05$ ) for MSWV and TP or by Tukey test ( $\alpha=0.05$ ) for MSCV.

TP had an equally high viability percentage for all treatments (around 95%; Figure 2). MSCV showed a sharp decrease in viability (around 60%) 24 h after ingestion compared to the control (Figure 2). Viability in MSWV was lower than in the control treatment (around 30%) for 24 h and 72 h treatments (Figure 2), but did not diminish for the 48 h treatment. The low viability of seeds after gut passage in MSCV and MSWV for 24 h and 72 h treatments might be explained by the difficulties to distinguish between intact seeds and seeds that had damaged embryos: despite they all looked healthy, some could have been affected by dung fermentation.



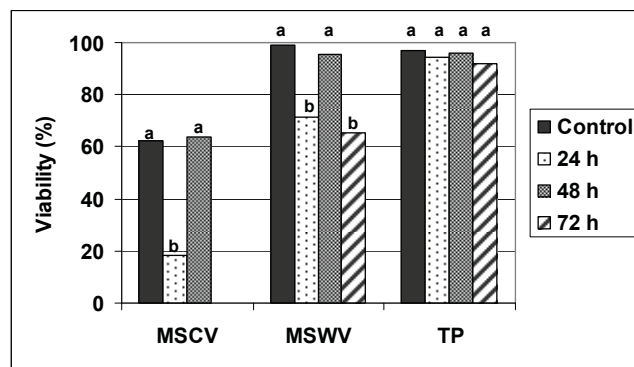


Figure 2. Percentage of viable seeds of three legumes after gut passage. Different letters indicate significant differences detected by Nemenyi test ( $\alpha= 0.05$ ) for MSCV or by Tukey test ( $\alpha= 0.05$ ) for MSWV and TP.

### Conclusions

We would like to highlight the following conclusions: a) Percentage of germination has a different behaviour for each species, which cannot be easily explained. b) A high amount of viable seeds of MSWV and TP are recovered from sheep dung. c) The dramatic decrease of germination in gut passage treatments for TP is not due to inviability of seeds, therefore they might germinate when appropriate conditions occur.

To conclude, this study shows that a great quantity of viable seeds of TP and MSWV can effectively be dispersed by livestock. Therefore, feeding the animals with these seeds and allowing them to graze for at least two days in a certain area can bring about an appropriate establishment of these wild varieties and species.

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# Alfalfa seed yield and its components as influenced by cutting schedule

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## Abstract

A two-year experiment has been conducted to determine the dependence of seed yield components and yield level on cutting schedule of alfalfa (*Medicago sativa* L.). Four cutting schedules have been examined: single cuts, early (5 May), medium (15 May), late (25 May) and two cuts (on 5 May and 5 June). The single, late cut achieved the best balance among the yield components and thus the highest seed yield (633 kg ha<sup>-1</sup>). The single early and medium cuts produced lower yields (368 kg ha<sup>-1</sup> and 378 kg ha<sup>-1</sup>, respectively). The seed production from the two-cut regime yielded 382 kg ha<sup>-1</sup>. Highest correlations with seed yield were recorded for the number of plants per unit area ( $r = -0.740$ ), number of productive stems per unit area ( $r = 0.747$ ), number of pods per raceme ( $r = 0.932$ ) and the number of seeds per pod ( $r = 0.660$ ).

Keywords: cutting schedule, *Medicago sativa*, pod, seed, stem.

## Introduction

The climatic conditions of Serbia favor the production of alfalfa seed from the second cut. The first cut is used for production of hay. With early first and second cuts, seed may be also produced from the third cut. Because alfalfa plants grow vigorously in the spring, they lodge easily after the stages of budding or flowering and in this case they produce a low seed yield. Spring cutting of the seed crops is therefore a common practice, with seed production on the first or second regrowth. Furthermore, cutting may be used to time the beginning and duration of the stage of flowering, i.e., to synchronize flowering with the maximum activity of pollinating insects, which is of key importance for seed yield performance in alfalfa.

## Materials and Methods

The experimental site was located in northern Serbia, 45°20' N, 19°51' E, at 80 m above sea level. This area has a continental semiarid to semihumid climate (Table 1).

Table 1. Basic climimatic parametars for the period 2001-2002.

Parametar	Year	Month							Sum/ Average
		March	April	May	June	July	August	September	
Precipitation (mm)	2001	73	127	75	233	56	30	162	756
	2002	11	26	87	27	33	55	46	285
Mean air temperature (°C)	2001	10,9	11,2	17,8	18,2	22,3	22,7	16,1	17,0
	2002	8,9	11,7	19,1	21,7	23,6	22,2	17,0	17,7

The trial was established in a randomized block design with four replications. Alfalfa was sown on 8 April 2000 with rows spaced at 25 cm and with a seeding rate of 15 kg ha<sup>-1</sup>. Each plot measured 2 x 5 m<sup>2</sup>, with eight rows per plot. In the second and third year of the crop (2001 and 2002), four harvest management systems with variable dates of initial harvest were applied. Single cuts for forage were performed on one of three dates: 5 May (budding, treatment c<sub>1</sub>), 15 May (start of flowering, treatment c<sub>2</sub>), or 25 May (full flowering, treatment c<sub>3</sub>); two cuts were performed on 5 May and 5 June (treatment c<sub>4</sub>). The regrowth from the single cuts or from the second of the two cuts was used for seed production.

Alfalfa seed was harvested with a single passage of a harvester, following desiccation with Diquat performed when about 70% of pods on normally developed plants were at the stage of physiological maturity. The yield of dried (at 10% moisture) and processed seed per plot was recorded. The results were subjected to analysis of variance. Significance of differences between mean values was tested by the LSD test. Pearson correlations were calculated between seed yield and yield components.

## Results and Discussion

The average seed yield was 440 kg ha<sup>-1</sup> (Table 2). Most variations in seed yield were caused by weather conditions between years. In 2002, which had favorable climatic conditions, seed yield was 4.3 times higher than in 2001, which had highly unfavorable conditions. In the region where this study was conducted variation in alfalfa seed yield is primarily affected by weather conditions (Erić, 1988). However, yield level may be stabilized to a certain extent by adjusting the cutting schedule.

Table 2. Seed yield depending on cutting schedule in 2001-2002 (kg ha<sup>-1</sup>).

Cutting schedule (C)	Year (Yr)		Average				
	2001	2002		LSD	C	Yr	C x Yr
c <sub>1</sub>	163	573	368				
c <sub>2</sub>	167	588	378				
c <sub>3</sub>	225	1041	633				
c <sub>4</sub>	109	656	382				
Average	166	715	440	0.05	43.51	30.77	61.53
				0.01	58.17	41.13	82.27

Table 3. Correlation coefficients between seed yield and yield components in alfalfa (n=32).

Character	Plants m <sup>-2</sup>	Shoots m <sup>-2</sup>	Fertile shoots m <sup>-2</sup>	Plant height	Stem DM	Racemes shoot <sup>-1</sup>	Florets raceme <sup>-1</sup>	Pods raceme <sup>-1</sup>	Seeds Pod <sup>-1</sup>
No. of shoots/m <sup>2</sup>	0.31	-							
No. of fertile shoots/m <sup>2</sup>	-0.43	-0.26	-						
Plant height	0.66	0.59	-0.39	-					
DM in the stem	-0.75	-0.60	0.68	-0.74	-				
No. of racemes/shoot	0.21	0.13	-0.11	0.18	-0.39	-			
No. of florets/raceme	-0.13	0.22	0.22	0.27	0.15	-0.06	-		
No. of pods/raceme	-0.83	-0.52	0.65	-0.74	0.89	-0.21	0.17	-	
No. of seeds/pod	-0.45	-0.48	0.58	-0.36	0.65	-0.27	0.17	0.60	-
Seed yield	-0.74	-0.44	0.75	-0.63	0.86	-0.27	0.19	0.93	0.66

The lowest seed yield, 368 kg ha<sup>-1</sup>, was obtained with the early cut treatment (c<sub>1</sub>), which was cut at the beginning of the bud stage. In this system, the regrowth of the seed crop coincided with a period of high soil moisture and low air temperatures, resulting in lush and dense growth which caused lodging. The highest seed yield, 633 kg ha<sup>-1</sup>, was achieved with the late cutting treatment (c<sub>3</sub>). This yield was significantly higher (by 66-72%) than those obtained with the other treatments. The late cut ensured the most favorable relationships among the yield components.

Regarding the correlation with alfalfa seed yield, the yield components, may be classified into three groups. Group 1 includes the yield components that showed a positive correlation coefficient with seed yield. These are the number of productive shoot per unit area ( $r = 0.747$ ), stem dry matter percentage ( $r = 0.862$ ), number of pods per raceme ( $r = 0.932$ ), number of seeds per pod ( $r = 0.660$ ). Group 2 includes the yield components that were negatively correlated with seed yield. This group

includes the number of plants per unit area ( $r = -0.740$ ), total number of shoots per unit area ( $r = -0.440$ ) and plant height at the stage of full flower ( $r = -0.628$ ). Group 3 includes the yield components that were not significantly correlated with seed yield. These are the number of racemes per shoot ( $r = -0.271$ ) and the number of florets per raceme ( $r = 0.193$ ) (Table 3).

The late cutting system ensured a low stand density, i.e., a reduced number of shoots per unit area. Simultaneously, this system produced the largest number and proportion of productive shoots. Regrowth rate was significantly faster with this treatment than in the others but the plants were shorter. Stem dry matter percentage was significantly higher in relation to the early and medium cut systems. Owing to these characteristics, plant sensitivity to lodging was reduced and conditions for alfalfa flowering and activity of pollinating insects improved, resulting in a high seed yield (Karagić, 2004).

The two-cut treatment ( $c_4$ ) tended to reduce growth vigor of the plants. As a result, this system achieved the lowest density and the shortest plant stature in the seed crop. Additionally, stem dry matter percentage was significantly higher than with the other systems, improving plant resistance to lodging. On the other hand, shortage of available soil water in the period of regrowth, intensive growth and budding of the second regrowth reduces the formation of alfalfa reproductive organs. Previous research has shown that, compared with optimum water supply, dry conditions halve the number of productive tillers, which significantly reduces seed yield (Goloborodko and Bodnarčuk, 1998).

### Conclusions

Climatic conditions have a large effect on alfalfa seed yield and yield components. Among these conditions, the total amount and distribution of rainfall play a decisive role. Variations in alfalfa seed yield level may be controlled to some extent by the cut system. Late cuts ensure a reduced stand density and maximum number of productive shoots. Also, plant height is reduced and dry matter content in the stem significantly increased in relation to the systems of early and medium cuts. Consequently, plant sensitivity to lodging is considerably reduced while conditions for alfalfa flowering and activity of pollinating insects are improved, which results in increased seed yield. The highest correlations with seed yield were recorded for the number of plants per unit area (negative correlation), the number of fertile stems per unit area, the number of pods per raceme and the number of seeds per pod (positive correlations).

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## Genus *Trifolium* in Greece: distributional aspects and life traits

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### Abstract

The importance of the genus *Trifolium* ssp. in contributing to the global economy has been well documented and recognised. It is estimated that there are over 250 taxa of *Trifolium* worldwide, many of them to be present in the Greek flora. Until now, there has been no systematic record of *Trifolium* species in Greece. Information is scattered in several Floras and phytosociological studies. The purpose of this paper was to evaluate published information about the *Trifolium* taxa found in Greece and to infer about their distributional patterns, phytogeographical connections, endemism, and life and growth traits. Major sources for this bibliographic research were the Floras and research studies previously conducted by the authors and other works. A total of 108 taxa (92 species, 16 subspecies, and 1 hybrid) were found in the records. Apparently, Greece is one of the most “*Trifolium*-rich” regions of the world.

Keywords: *Trifolium*, clovers, phytogeography, life-traits.

### Introduction

*Trifolium* ssp. (clovers) are important floristic elements of most of the natural ecosystems of the world. Their ecological significance, common to all legumes, is mostly found on the clover-*Rhizobium* symbiosis, enriching the soil with atmospheric nitrogen, and their beneficial role on soils (Taylor, 1985). In addition, they accelerate pollination processes, with their attractive-to-insects corollas, while they exhibit various blooming responses to competition (Vrahnakis, 2003). Clovers are well appreciated as forage material of high quality as they contain from 60-80% (w w<sup>-1</sup>) digestible dry matter (Knight and Watson 1977). Their ecological significance coupled with their high agronomic potential have stimulated the research in determining *Trifolium* species for breeding and selection purposes (Loi *et al.*, 2000; Lazaridou and Koutroubas, 2004). Consequently, the indigenous *Trifolium* spp. of a country is an important asset potentially used for many purposes (Papanastasis and Papachristou, 2000). The present paper researching the *Trifolium* ‘resources’ of Greece, summarizes the present taxonomic knowledge, and investigates their distributional patterns. We believe that the results will further support the aspect of Greece as a major centre for selection purposes.

### Materials and methods

Several bibliographic sources were used to accomplish the research. Sources included the typical floristic catalogues and keys (Flora Europaea vol. 2, Mountain Flora of Greece vol. 1, Med Checklist vol. 4, Flora of Turkey and East Aegean Islands vol. 3, and Zohary and Heller 1984). In addition, many floristic reports were used (among others Theodoropoulos, 1991, Eleftheriadou and Raus, 1996, Tsiripidis and Athanasiadis, 2003, Fotiadis, 2004). For the taxa located in these sources a data base was made that included synonyms, ecological and chorological information, life-forms, and habitats. *Trifolium* taxa were classified on the basis of their ecological, chorological and life-forms characteristics according to the widely accepted classification systems proposed by Oberdorfer (1990). This way, the chorological, ecological, and life-form spectra were produced. The results were evaluated on the basis of the importance of the species in terms of plant geography, endemism, adaptation to specific environmental conditions, growing patterns, and their potential to be proposed as understory species for Silvopastoral systems.

## Results and discussion

According to the processed information there are 108 taxa of *Trifolium* in Greece (92 species, and 1 hybrid) belonging to the 8 sections of the genus. There were many taxa synonyms used in the past. There are taxa with over 25 synonyms, while over eight genus were occasionally used to describe *Trifolium* species (e.g. *Amaresus*, *Amoria*, etc.). Eight species that were represented by subspecies (i.e. *T. hybridum* (3 subspecies), *T. incarnatum* (1), *T. medium* (1), *T. nigrescens* (2), *T. noricum* (1), *T. pannonicum* (1), *T. repens* (4), and *T. subterraneum* (4)) and the hybrid (*T. hybridum* ssp. *anatolicum* x ssp. *elegance*) were excluded from the analysis and only their subspecies were considered.

Ecologically, 75 *Trifolium* taxa out of 100 are annuals, 1 is biennial (*T. velenovskyi*), and 24 are perennials. Five perennials (i.e. *T. medium* ssp. *balcanicum*, *T. patulum*, *T. alpestre*, *T. ochroleucum*, and *T. physodes*) are phytosociologically typical forest taxa adaptive to oak and beech ecosystems. Consequently, these taxa should be potentially used as understory species in Silvopastoral systems. The rest grow in various ecosystems, while some of them are widely adapted to various habitats.

Concerning their life-forms, all annuals are therophytes, while the biennial and 22 perennials are hemicryptophytes and the rest 3 perennials are geophytes (*T. medium*, *T. pignanttii*, and *T. heldreichianum*). The reptant-type of growth of 12 taxa is of great agronomic importance as they are apparently well-resisted to grazing (i.e. *T. tomentosum*, *T. subterraneum* ssp. *oxaloides*, - ssp. *subterraneum*, - ssp. *yanniticum*, *T. patens*, *T. resupinatum*, *T. scabrum*, *T. repens* ssp. *orbelicum*, - ssp. *orphanideum*, - ssp. *prostratum*, - ssp. *repens*, and *T. fragiferum*).

Chorologically, 42 taxa are originated from the Mediterranean region, 26 are of sub-Mediterranean origin, 6 are Balcanic, 7 are sub-Balcanic, 9 are Eurasiatic, 3 are of Alpine origin, 1 is of Northern origin, and 6 are Greek endemics. The number of Balcanic and sub-Balcanic taxa increase from the south to the north of Greece. The majority of the 6 Greek endemics (*T. barbeyi*, *T. dolopium*, *T. parnassi*, *T. praetermissum*, *T. ottonis*, and *T. xanthinum* (?)) are grown on limestone in the peaks of high mountains or in islands. Surprisingly, only 9 taxa are of Eurasiatic origin, while the Northern taxon (*T. spadicum*), and the 3 Alpine taxa (*T. badium*, *T. noricum* ssp. *praetutianum*, and *T. pallescens*) are growing in the high mountains of northern Greece. *T. glomeratum*, which is present in the Greek flora, appears a remarkable distribution as it is also found in South Africa, while it remains unclear if it was introduced in Chile or it is indigenous.

Phytogeographically, the connections of *Trifolium* species to the east and the west Mediterranean region were investigated by elaborating 33 taxa spread to the east, and west Mediterranean region, the sub-Mediterranean and the Atlantic one. It was found that 64% of them are characterized as eastern Mediterranean (*osmed*) elements, and the 36% are Atlantic-west Mediterranean (*atl-wmed*) ones. This is in accordance with Zohary and Heller (1984) that asserted that the region of Anatolia (Turkey) is the Eurasiatic centre of diversity of the species.

## Conclusions

There are at least 108 *Trifolium* taxa in Greece; six of them are Greek endemics. The majority of them are annuals. Five of them are suggested to be used in Silvopastoral systems and 11 in intensive grazing systems. Forty-two of them are of Mediterranean origin. Greece and Turkey are apparently the *Trifolium*-richest countries of the world with over 100 taxa (species and subspecies) each.

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# Effect of presowing treatment of seeds with insecticides on productivity of alfalfa

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## Abstract

A pot experiment to determine the effect of presowing treatment of seeds with insecticides on the productivity of alfalfa grown for forage was carried out at the Institute of Forage Crops, Pleven, Bulgaria. Alfalfa (*Medicago sativa* L.) cultivar Obnova 10 was used. The insecticides Promet 400 SK (furathiocarb) at a rate of 3 L, and Carbodan 35 ST (benzofuran) at a rate of 1, 2 and 3 L were used per 100 kg seeds. It was found that the presowing treatment of seeds resulted in an increase of productivity of dry top mass from 9 to 14%. This measure exerted a positive effect as well as on the quantity of dry root mass, and the increase was from 12% to 23% as compared to the untreated control. The insecticide preparation Carbodan 35 ST applied for presowing treatment at a rate of 1 L 100 kg<sup>-1</sup> seeds showed the best positive effect in terms of the productivity of alfalfa grown for forage.

Keywords: alfalfa, insecticides, root mass, forage, seeds.

## Introduction

The presowing treatment of seeds with insecticides is considered an efficient and ecological means of controlling insect pests in some forage crops (Schiffers and Copin, 1993; Dochkova *et al.*, 2000). Alfalfa is a major forage crop in Bulgaria and an important source of proteins for ruminants. It is an economical source of biological nitrogen through its fixation by nodules (Peoples *et al.*, 1995). Dry matter yield of alfalfa could be increased from 25% to 35% under favourable conditions due to symbiotic nitrogen fixation (Starchenkov and Kot's, 1992; Kostov and Lynch, 1998). The aim of this study was to determine the effect of the presowing treatment of seeds with insecticides on the productivity of alfalfa grown for forage.

## Materials and methods

A pot experiment using leached chernozem soil from the region of Pleven, Bulgaria with 4 replicates per treatment was carried out during 2003 – 2004. Pots with a volume of 10 L were used. Four plants were grown in each pot. The alfalfa cultivar Obnova 10 was used. The insecticides Promet 400 SK (furathiocarb) at a rate of 3 L (standard) and Carbodan 35 ST (benzofuran) at a rate of 1, 2 and 3 L were applied per 100 kg seeds. The treatments were applied on the day of sowing. Background applications of phosphorus and potassium were added (P, 110 mg P kg<sup>-1</sup> soil; K, 110 mg K kg<sup>-1</sup> soil). Phosphorus was applied as triple super phosphate and potassium as potassium chloride. The quantity of top and root mass was recorded from one growth. Plant fractions were dried to a constant weight at 60°C. The experimental data were averaged and statistically analyzed using software SPSS for Windows 2000.

## Results and discussion

The presowing treatment of seeds resulted in an increase in the productivity of dry top mass of alfalfa (Table 1). In plants treated with Carbodan 35 ST applied at a rate of 1 L 100 kg<sup>-1</sup> seeds the increase was 14% compared to the untreated controls.



Table 1. Dry top mass and dry root mass of alfalfa after presowing treatment of seeds with insecticides.

Treatments	Dry top mass	+, Increase	Dry root mass	+, Increase
	(g plant <sup>-1</sup> )	(%)	(g plant <sup>-1</sup> )	(%)
Control	4.03	-	1.81	-
Carbodan 35 ST - 1 L	4.61	+ 14	2.23	+23
Carbodan 35 ST - 2 L	4.39	+ 9	2.04	+13
Carbodan 35 ST - 3 L	4.51	+ 12	2.02	+12
Promet 400 SK - 3 L	4.42	+ 9	2.08	+15
SE (P=0.05)	0.01		0.01	

The presowing treatment of seeds showed a positive effect on dry root mass as well. The increase varied from 12 to 23% and the highest increase was found with Carbodan 35 ST at a rate of 1 L 100 kg<sup>-1</sup> seeds. The root system of the plants was healthy, which could result in more favourable conditions for nodulation process. A significant positive correlation ( $r=0.93$ ) between number of nodules formed and quantity of dry top mass was found (data not shown). The correlation between quantity of dry root mass and dry top mass was also high ( $r=0.85$ ). Our data were in agreement with the results obtained by Ferreira and Castro (1995) for subterranean clover, and Kot's *et al.* (1995) for alfalfa. The total productivity of plants increased by 10% to 16% (Table 2). Root/top mass ratio was the highest (0.45) in the treatment with Carbodan 35 ST at a rate of 1 L 100 kg<sup>-1</sup> seeds.

Table 2. Total productivity of alfalfa after presowing treatment of seeds with insecticides.

Treatments	Total productivity	+, Increase	Root/ top mass
	(g plant <sup>-1</sup> )	(%)	
Control	6.13	-	0.42
Carbodan 35 ST - 1 L	7.14	+ 16	0.45
Carbodan 35 ST - 2 L	6.73	+ 10	0.43
Carbodan 35 ST - 3 L	6.83	+ 11	0.42
Promet 400 SK - 3 L	6.80	+ 11	0.44
SE (P=0.05)	0.16		0.01

## Conclusions

The presowing treatment of seeds with insecticides increased the productivity of dry top mass by 9 to 14% as compared to untreated control. This measure exerted a positive effect on the quantity of dry root mass, and the increase was from 12 to 23%. A significant positive correlation was found between number of nodules and dry top mass ( $r=0.93$ ). The insecticide preparation Carbodan 35 ST applied for presowing treatment at a rate of 1 L 100 kg<sup>-1</sup> seeds showed the highest positive effect as regard to productivity of alfalfa grown for forage.

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# The evaluation of genetic resources of forage legumes collected from natural grassland

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## Abstract

Activities in collecting and conservation of genetic resources in Latvia started only after gaining independence in 1993. The Latvian Gene Bank of Plants was created and inventory of the existing collections has been drawn up. Accessions of cultivated plant species and most significant hybrids developed in Latvia have been put in the Gene Bank. For last six years specialists from the Research Institute of Agriculture and the Latvian Gene Bank of Cultivated Plants have organised special expeditions. The aim of the expeditions was collecting and conservation accessions of local legume and grass species found in various districts of Latvia, as well as evaluation and description of these accessions and putting them for long term storage in the Latvian Gene Bank of Cultivated Plants. The summed up results give proof that there exist a great variety of forage legumes. By testing them in experimental plots a lot of valuable properties turn out. Because of that it is worth including them in the future plant breeding programmes.

Keywords: genetic resources, lucerne, red clover, alsike clover, expeditions.

## Introduction

Genetic resources of forage legumes in Latvia consist of registered varieties, valuable breeding material and wild ecotypes (Jansone *et al.*, 2005).

One of the main directions in the storage of genetic resources of forage plants in Latvia is the organization of scientific expeditions, the purpose of which is collecting wild plant kinds of cultivated plant species, describing their location and growing conditions (Rashal, 1998).

A special attention has been devoted to the identification, collection and storage of the species grown in natural surroundings. The next task is to evaluate the collected raw material, to describe it, as well as to use the plant seeds, together with their main properties, in the selection work immediately or to preserve them continuously at the Latvian Gene bank.

The following stage implies the sowing of the material in the cultivated fields and the evaluation of its properties under the specific conditions. It will help to use the selected genetic resources in the future scientific and plant breeding work.

## Materials and methods

In 2000, there was organized the first expedition for the collection of genetic resources of grasses. Up to now, 12 expeditions of this kind have taken place. Three of them were international, where the representatives from three Baltic states – Latvia, Lithuania, Estonia, have taken part. In total, 291 wild accessions have been collected, including 78 of lucerne (*Medicago sativa* L.), 136 of red clover (*Trifolium pratense* L.), 28 of alsike clover (*Trifolium hybridum* L.), and 49 of other legumes. The most important were the international expeditions, during the course of which the species which are not found in our country have been collected, like *Trifolium alpestris*, *Medicago falcata*, etc.

The collected accessions have been sown and evaluated in Skriveri experimental plots. From 2003 to 2005, 73 lucerne, 108 red clover, 21 alsike clover, and 22 various legumes samples (*Medicago ssp.*, *Trifolium ssp.*, *Vicia ssp.*, etc.) have been evaluated. This process is going on.

Some characteristics have been observed for alfalfa: winterhardiness, green colour of foliage, natural height at spring time and at bud stage for 1st and 2nd cut, time of beginning of flowering, colour of flowers, length of stems at full flowering, growth habit, lodging resistance, regrowth at spring and after

cutting, yield of DM and seeds, longevity etc. Equally were estimated accessions of red clover and alsike clover with some distinctives. For example, there were observed number of internodes, density of stem hairs, frequency of plants with white marks, resistance to *Sclerotinia trifoliorum* etc. for red clover. Each legume sample was sown in the area of: 1.5 m x 2.0 m. The distance between rows 0.6 m, between plots 0.9 m. The samples collected in the wild were compared with control species which were located repeatedly after every ten numbers. The experiments include two repetitions.

## Results and discussion

The winter of 2003/2004 was not favourable for legumes under the Latvian conditions. The most of the red clover samples, collected during the expeditions, wintered badly, got infected by *Sclerotinia trifoliorum* and perished. However, some samples were winterhardy and the number of ruined plants was less than that of control species. During the winter of 2005, in spite of changeable weather conditions, red clover comparatively did not suffer much, but because of the late and cool spring, it grew very slowly.

A number of the collected accessions turned out to be earlier in comparison with the early red clover species 'Skriveru agrais'. The sample which in comparison with the others stood out with very beautiful, brightly reddish rosy blossoms was very interesting and it could be also used for decorative purposes.

Like red clover, alsike clover, during the winter of 2004, was not winterhardy. In spring, it turned out that 35-90% plants had perished, despite the fact that while growing in the wild, these plants looked to be strong and healthy. We do not know if it was due the unfavourable winter or the unusual conditions, or the background of diseases in plant breeding nurseries. Several samples stood out in contrast to others with their intensive and steady blossoming. The hybrid clover No.03/2 was distinguished by its unusual colour of blossoms – bright redness.

As our Latvian winters are rather changeable, good winterhardiness is of great importance for lucerne. Some lucerne samples as No.64/43, No. 61/40, No.22/1, No.90 were distinguished by their winterhardiness and in this aspect it outdid the standard variety 'Skriveru'. The results of estimation for some accessions of lucerne are shown in Table 1.

The high growing intensity is one of the main advantages of growing lucerne. For this reason, there were some wild accessions, which stood out on the background of standard variety 'Skriveru'. According to the 10 grades scale their growing intensity exceeded 1-3 grades. More rapidly growing turned out to be the above mentioned samples – No.64/43 and No.22/1.

In comparison with other legumes, lucerne is growing and developing better during the next year if it has produced seeds or blossomed in the period of the 1st year. It was quite evident in this experiment, too. The plants, which produced seeds, since early spring grew more rapidly, and this tendency was preserved up to the blossoming stage.

Several samples were characteristic for thin stems, which are estimated positively. Though, quite often, it causes lodging after rain or wind.

Dry matter harvest of lucerne fluctuated in a wide amplitude. Nearly half of the estimated samples the harvest fell behind the harvest of the control variety. However, some samples outdid the variety 'Skriveru' up to 20%.

The growing of lucerne in Latvia is highly limited for its low harvest of seeds. The variety 'Skriveru' under favourable growing conditions, can give the harvest of seeds up to 400 kg ha<sup>-1</sup>. The year of 2004 was not favourable for growing lucerne because of cool and rainy weather. It was the cause for a low seed harvest. Under these conditions, the harvest of variety 'Skriveru' was 150 kg ha<sup>-1</sup>, which was estimated – 2 points according to the 3 grades scale. Several wild accessions had rather high harvest despite the unfavourable conditions, for example – No.49/28 which outdid the standard variety. In 2005, when the conditions for growing lucerne were more favourable, several samples outdid the standard variety too.

Table 1. Characterisation of selected accessions of alfalfa (2004- 2005).

Accession	Winter-hardiness (1-very low; 9-very high)	Regrowth intensity at spring (1-very low; 9-very high)	Longest stem at flowering, cm	DM (% to standard)	Yield of seeds (1-low; 3-high)	Lodging resistance (1-low; 3-high)
Skriveru (Standard)	8,5	5	102	100	2	3
Nº 66/45	7,0	5	112	95	1	1
Nº 64/43	9,5	8	117	115	2	1
Nº 62/41	9,0	5	98	101	2	1
Nº 61/40	9,5	6	103	115	2	1
Nº 57/36	9,0	6	107	103	2	1
Nº 55/34	7,0	6	97	101	2	2
Nº 49/28	9,0	5	108	99	3	3
Nº 34/13	8,0	5	95	113	1	2
Nº 22/1	9,5	7	90	119	1	1
Nº 24/3	9,0	5	109	114	1	1
Nº 90	9,5	6	102	115	2	2

### Conclusions

For successful usage of the collected accessions in the future breeding work, it is important to estimate and describe them because when changing the growing place, the plants may lose their advantages they have while growing in the wild.

Under the natural conditions, forage legumes are a very interesting raw material of selection, where useful features can be found for the formation of new cultivars.

Out of the collected lucerne samples, some of them were important for their fast growing and great productiveness of seeds even under unfavourable climatic conditions.

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# The influence of different legume crops management on the nitrogen circulation cycle in the agroecosystems

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## Abstract

A complex research was carried out at the Lithuanian Institute of Agriculture on an *Endocalcari-Endohypogleyic Cambisol* to identify the effects of legumes – red clover (*Trifolium pratense* L.), sown lucerne (*Medicago sativa* L.) grown as the main crops and undersown crop – red clover grown as catch crops on the intensity of the nitrogen circulation cycle in the agroecosystems. The underground biomass of red clover and sown lucerne accumulated 99.6 and 234.8 kg ha<sup>-1</sup> of nitrogen, respectively, or 66.2 and 156.5 kg ha<sup>-1</sup> of nitrogen fixed from the atmosphere. Sown lucerne was best at compensating by nitrogen (69.5 % fixed from the atmosphere) removed with cereal (w. wheat - w. wheat - s. barley) yield. Nitrogen content removed by spring barley after red clover, grown as a catch crop, was 90.7 % compensated by atmospherically fixed nitrogen.

Key words: sown lucerne, red clover, catch crops, nitrogen.

## Introduction

Biologization in agriculture is one of the chief factors maintaining soil productivity and assisting in finding solutions to environmental problems (Filip, 2002). A balance between organic matter synthesis and decomposition settles down in natural ecosystems, however due to anthropogenic action mineralization of organic matter intensifies (Crews and Peoples, 2004). Perennial legumes are one of the crops stabilizing decomposition processes. In many latest publications it is recommended that cereals should be grown after legumes, which would enable using the accumulated biological N for their nutrition in a more rational way (Schubert, 1995; Kumar *et al.*, 2001). Legumes can be used as catch crops to prevent or minimize post-harvest leaching of nutrients (Knappe *et al.*, 2002).

## Materials and methods

Two experiments were conducted to ascertain the effects of legumes grown as the main crops and catch crops on the nitrogen circulation cycle in the agroecosystems. The research “The effects of legumes grown as the main crop on the accumulation of nitrogen in cereals yield” was conducted following the experimental design: factor A. Preceding crops for cereal sequence (winter wheat- winter wheat-spring barley): red clover (*Trifolium pratense* L.), sown lucerne (*Medicago sativa* L.), vetch and oat mixture (*Vicia sativa* L., *Avena sativa* L.), factor B. Organic manure: 1. without manures, 2. 1st crop without manures, 3. 1st crop -green manure, 4. 1st crop- 40 t ha<sup>-1</sup> of farmyard manure. The research “The effects of catch crops differing in biological characteristics on the accumulation of nitrogen in cereals yield” following the experimental design: Catch crops: 1. without catch crops, 2. white mustard (*Sinapis alba* L.), 3. red clover (*Trifolium pratense* L.), 4. Italian ryegrass (*Lolium multiflorum* Lamk). The next year spring barley was grown after incorporation of catch crops biomass as green manure. Nitrogen content in the underground and overground biomass was determined by the Kjeldahl method, carbon – by analyser “Heraeus”. The share of nitrogen fixed by legume was calculated by multiplying nitrogen content by the coefficient (0.63) provided by Chopkins – PETERS.

## Results and discussion

The effects of legumes grown as the main crop on the accumulation of nitrogen in cereals yield. The largest amount of nutrients in the agrocenose was returned into the circulation cycle with the heaviest underground lucerne and clover biomass, while the lowest amount with the mixture of annual plants (Table 1). In the phytomass of clover and lucerne nitrogen fixed from the atmosphere accounted for the largest share. Seeking to balance nutrient transport in the ecosystem, the soil was additionally incorporated with overground biomass of legumes for green manure. The content of atmospherically fixed symbiotic nitrogen in plant overground mass incorporated as green manure was lower than that in underground mass.

Table 1. The role of legumes in introducing nitrogen in the biological circulation cycle.

Crop	Underground mass				Overground mass			
	Incorporation DM (t ha <sup>-1</sup> )	N (kg ha <sup>-1</sup> )		C:N	Incorporation DM (t ha <sup>-1</sup> )	N (kg ha <sup>-1</sup> )		C:N
		total	fixed			total	fixed	
Red clover	9.2±1.04	99.6±14.9	66.2±10.0	24	3.2±0.58	80.1±5.7	50.5±3.6	12
Sown lucerne	13.7±1.12	234.8±35.1	156.5±23.4	18	3.9±0.32	114.2±10.9	72.1±6.9	10
Vetch and oats mixture	5.3±0.51	38.8±7.5	25.9±5.0	35	3.8±0.21	36.0±3.4	22.7±2.1	32

± 16.09 standard error.

The highest content of symbiotic nitrogen was introduced with lucerne and clover overground biomass 3.2 and 2.2 respectively times more than with annual plants mixture. Carbon to nitrogen ratio for lucerne residues was the narrowest 18, and for vetch and oats mixture this ratio was the widest 35. Having incorporated in the soil nitrogen-rich plant overground mass, whose C:N ratio was narrower, organic matter mineralization sped up. Averaged data suggest that when cereals were grown after legumes for three years, large amounts of nitrogen (220.5 kg ha<sup>-1</sup>) were removed with cereal grain and straw yield from the agrocenose (Table 2).

Table 2. Compensating of nitrogen removed with cereals (w. wheat-w. wheat-s. barley) yield by legume crops biomass.

Treatment	Red clover		Sown lucerne		Vetch and oats mixture	
	Accumulated in cereals yield (kg ha <sup>-1</sup> )	Compensating with legume N (%)	Accumulated in cereals yield (kg ha <sup>-1</sup> )	Compensating with legume N (%)	Accumulated in cereals yield (kg ha <sup>-1</sup> )	Compensating with legume N (%)
1. Without manures	164.7	60.5	225.2	104.3	145.7	26.6
2. 1st crop without manures	201.7	49.4	271.5	86.5	192.2	20.2
3. 1st crop -green manure,	228.4	78.6	289.4	120.6	198.9	37.6
4. 1st crop- 40 t ha <sup>-1</sup> of FYM	236.9	107.3	277.5	140.3	213.4	90.6

LSD<sub>05</sub> (N accumulated in cereals) fact.A 7.74; fact.B 8.94; fact.AB 15.48.

Comparison of unfertilised treatments of individual preceding crops revealed that after lucerne the content of nitrogen removed with cereal yield was 36.7 and 54.6 % higher than after clover and vetch and oats mixture. Assessment of nitrogen circulation cycle of unfertilised cereal agrocenoses showed that after lucerne nitrogen was 69.5 % compensated by atmospherically fixed nitrogen, while after clover and annual plant mixture nitrogen compensation was lower (40.2 and 17.8 %, respectively). Having incorporated plant biomass as green manure, the difference of accumulated nitrogen in cereal yield, compared with fertilised with only mineral nitrogen fertiliser, made up: after clover 16.2 %, after lucerne 7.9 %, after mixture 4.6 %. Nitrogen accumulated in cereal yield by atmospherically fixed nitrogen was compensated by clover biomass 51.1 %, lucerne 79.0 %, mixture 24.4 %.

The effects of catch crops differing in biological characteristics on the accumulation of nitrogen in cereals yield. Of all catch crops grown, clover phytomass was significantly distinguished for nitrogen accumulation 114.6 kg ha<sup>-1</sup> or 76.4 kg ha<sup>-1</sup> atmospherically fixed nitrogen (Table 3).

Table 3. Effect of the catch crops with different biological properties on the amount of nitrogen accumulated in spring barley yield.

Catch crops	N incorporation with plant biomass (kg ha <sup>-1</sup> )		Accumulated in cereals yield		Compensating with plant biomass N (%)
	total	fixed	(kg ha <sup>-1</sup> )	Increase (%)	
Without catch crops	-	-	72.1	-	-
White mustard	48.3±16.09	-	89.8	+24.6	53.8
Red clover	114.6±21.25	76.4±14.17	84.2	+16.7	136.1
Italian ryegrass	57.0±18.78	-	78.9	+9.4	72.2
LSD <sub>05</sub> (N accumulated in cereals) 4.36.					

Such amount of biological nitrogen is valuable from the ecological viewpoint, since it can meet N needs of cereals grown after clover. However, white mustard and Italian ryegrass applied with low starter rates of nitrogen fertiliser (N<sub>30</sub>) accumulated twice as less nitrogen in their biomass (48.3 and 57.0 kg ha<sup>-1</sup>, respectively). The highest content of nitrogen was accumulated in barley yield when it was grown after clover and mustard 16.7 and 24.6 % more compared with the control treatment and 6.7 and 13.8 % more having incorporated biomass of Italian ryegrass. Nitrogen content removed by cereals after red clover was 90.7 % compensated by atmospherically fixed nitrogen, whereas white mustard and ryegrass biomass covered nitrogen expenditure by 53.8 and 72.2 %.

### Conclusions

Sown lucerne was best at compensating by bioogenic elements the nutrients removed with cereal (w. wheat - w. wheat - s. barley) yield: nitrogen – 69.5 % fixed from the atmosphere and 108.8 % biological nitrogen incorporated with underground plant mass. This coefficient for red clover and vetch and oats mixtures was significantly lower 40.2 and 17.8 %, respectively. Nitrogen content removed by cereals (s. barley) after red clover, grown as a catch crop, was 90.7 % compensated by atmospherically fixed nitrogen.

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## The yield and quality of mixed pastures established on hillslopes

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### Abstract

The experiment was carried out on the north-west-facing slope of west Lithuania. The pasture swards were composed of different clover (*Trifolium sp.*) and grass mixtures containing Lithuanian cultivars of white clover (*T. repens* L.), red clover (*T. pratense* L.), perennial ryegrass (*Lolium perenne* L.) and smooth-stalked meadow grass. Nitrogen fertilization, an annual rate of N<sub>240</sub>, was applied only to one of the treatments composed of grass mixture. The swards were grazed with young cattle 3-5 times per season. The weather conditions exerted an especially great effect on the sward yield. During the five years of swards use pure grass sward, fertilized with mineral nitrogen, produced the highest yield. White clover-grass sward was the least productive of all mixtures tested. Among the mixed swards the most productive were the sward mixtures including both white and red clover. The nutritive value of all swards was satisfactory and met the needs of productive cattle.

Keywords: grassland, dry matter yield, forage quality.

### Introduction

The recently increasing attention to environmental protection and ecologically clean foodstuffs in economically more advanced countries determined an interest in the swards of varied floral composition, especially in those with a higher content of white clover. Multi-component mixtures used in pastures have a host of advantages. They are characterised by a more stable yield and more flexible regime of management, the quality of herbage suits best nutritional needs of livestock, and the forage possesses dietary characteristics (Hopkins *et al.*, 1994). Legume/grass productive longevity, wide range of frequency of use, high forage quality, palatability and biological nitrogen fixation open wide prospects for the application of such swards in ecological farming (Frame, 1992). The objective of this work was to estimate and compare the DM, crude protein, digestible DM yields and quality of herbage of different mixed pasture swards arranged on hillslope of conditionally acid soil of West Lithuania.

### Materials and methods

The trial was carried out during the period 1999-2004 on the hillslope in West Lithuania's undulating region. Local soils are sandy clay loam *Dystric Albeluvisols* with pH<sub>KCl</sub> 5.5 at the beginning and pH<sub>KCl</sub> 5.2-4.1 at the end of experiment. Mean organic matter content in the 0-20 cm depth was 20.0 g kg<sup>-1</sup>, available P 136, K 115 mg kg<sup>-1</sup>. Clover/grass mixtures, consisting of 60 % of clovers (white and/or red) and 40 % of grasses, (*Lolium perenne* L. and *Poa pratensis* L. at initial ratio 1:1) were sown with barley as a cover crop. The treatments replicated four times were: 1. White clover (W. clover), grasses; 2. Red clover (R. clover), grasses; 3. W.clover 30 %, R. clover 30 %, grasses; 4. W.clover 30 %, R. clover 30 %, Ryegrass 40 %; 5. Grasses, N<sub>240</sub>; 6. Grasses (control). In the spring of each year the swards were fertilized by P<sub>60</sub>K<sub>60</sub>. The swards were grazed with cattle, when the sward height was about 10-15 cm. Prior to each grazing, one half of the plots was cut, about 1 kg of herbage was taken for DM yield, and its chemical composition analysis, the remaining part of herbage mass was spread and grazed. Cut and grazed parts were transposed every year. In the first year of use (2000) the plots were grazed five times, second and fifth years – four times, and for the 2002 and 2003 – only three times. The mean annual precipitation in 2000-2004 was 687.5, 825.9, 726.2, 614.0 and 761.9 mm, respectively. Distribution of precipitation during the sward growing season was different. September 2000, April, May, August 2002

and June-August 2003 were exceptionally dry. Analysis of variance (ANOVA), correlation and regression, were performed using the software Statistica, version 6.0 (StatSoft, Inc).

### Results and discussion

During the five years of swards use the highest yielding (6.20-12.68 t ha<sup>-1</sup>) was found to be pure grass sward fertilized with N<sub>240</sub> (Figure 1). The highest yielding of the mixed swards tested was a three-component mixture composed of white and red clover and perennial ryegrass (3.10-11.41 t ha<sup>-1</sup>). The yield of the pasture varied within the grazing season, the treatment and especially year of use. The dry matter yield in the first year of the swards use was the lowest in the control (2.56 t ha<sup>-1</sup>) and the highest in the grass sward fertilized by N<sub>240</sub> (6.60 t ha<sup>-1</sup>). From the second year of use, white clover/grass mixtures were inappreciably outyielded by the control treatment consisting of grasses.

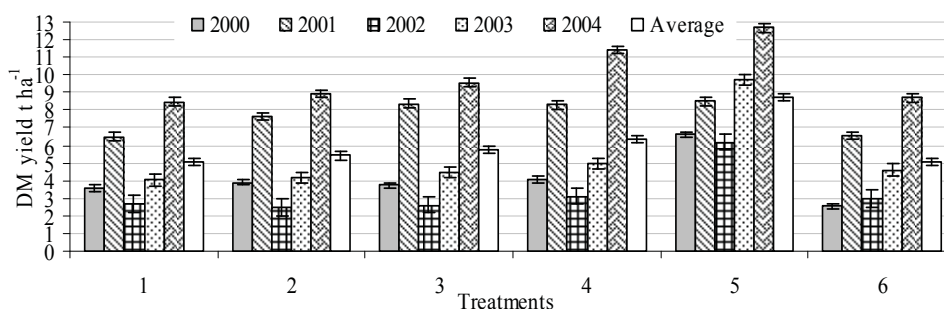


Figure 1. Annual dry matter yield of swards (t ha<sup>-1</sup>).

In 2002 herbage DM yield was the lowest of all the experimental period, while the highest yield was obtained in the fifth year of sward use (8.46 to 12.68 t ha<sup>-1</sup>). The fluctuation in DM yield most likely depends on the amount and distribution of rainfall during grazing season (Table 1). The productivity of fertilized grass sward (treatment 5) was the least related to the amount of rainfall: the correlation at 95 % probability level was statistically significant. The amount of rainfall significantly (at P<sub>01</sub>) influenced the productivity of mixed swards, red clover demonstrated a greater response to the amount of precipitation than white clover.

Table 1. The impact of rainfall rates on the total herbage yield and clover yield.

Treatment	Coefficient of correlation, equation of regression: x- rainfall rates mm, y- yield t ha <sup>-1</sup>		
	Total herbage yield	White clover yield	Red clover yield
1	0.975 <sup>**</sup> , y = -4.211 + 0.0274x	0.851, y = -3.861 + 0.0174x	-
2	0.990 <sup>**</sup> , y = -5.379 + 0.0319x	-	0.955 <sup>*</sup> , y = -4.748 + 0.0223x
3	0.993 <sup>**</sup> , y = -6.381 + 0.0359x	0.876, y = -3.248 + 0.0130x	0.913 <sup>*</sup> , y = -3.292 + 0.0162x
4	0.970 <sup>**</sup> , y = -6.845 + 0.0391x	0.708, y = -2.306 + 0.01x	0.980 <sup>**</sup> , y = -5.021 + 0.0223x
5	0.746, y = 0.9103 + 0.0231x	-	-
6	0.953 <sup>*</sup> , y = -4.619 + 0.0287x	-	-

Differences in DM yield in one year could be less than half in the other year due to amount and distribution of rainfall (Bryan *et al.*, 2000).

Annually and according to averaged data from five years of use, nitrogen-applied grass sward was distinguished by the highest crude protein concentration (Table 2).

Table 2. Averaged concentrations of crude protein and crude fibre in herbage DM g kg<sup>-1</sup>.

Quality component	Treatments						LSD <sub>05</sub>
	1	2	3	4	5	6	
Crude protein	169	175	174	175	234	173	12.189
Crude fibre	220	214	225	221	234	231	20.524

The average concentrations of crude protein of the other swards were very similar (169-175 g kg<sup>-1</sup>) and did not exceed the error limits. In all years of swards use there were found no significant differences in crude fibre concentrations between the treatments. Inappreciably higher crude fibre contents were found in grass swards. Variation of herbage mass quality depended on treatment, grazing and year. In the dry and hot third and fourth years of use the herbage was noted for the highest fibre and lowest protein content, which was especially obvious in the mixed swards (Zableckiene and Butkute, 2005). The regularities of crude protein and dry matter yield variation were mostly affected by herbage dry matter yield in treatments (Figure 2). The largest protein and digestible dry matter amounts were recorded in fertilized grass sward, and among mixed swards – in a three-component red clover, white clover and ryegrass sward (treatment 4). The effects of the year's conditions on these parameters were very important.

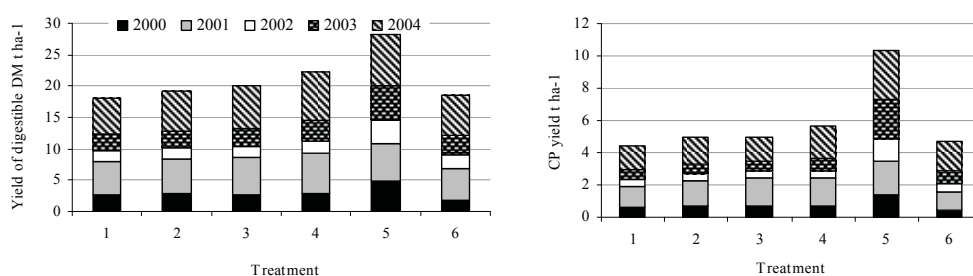


Figure 2. Annual dynamics of CP and digestible DM yield in the mixed pastures.

### Conclusions

The highest yields of herbage, protein and digestible dry matter were recorded in N<sub>240</sub>-fertilized grass sward, and among mixed and all N unfertilized swards – in a three-component red clover, white clover and ryegrass sward. The amount of rainfall significantly (at P<sub>01</sub>) influenced the productivity of mixed swards. Mixture composition of N unfertilized swards did not have any significant effect on crude fibre and crude protein concentrations. Nitrogen-applied grass sward was noted for the highest crude protein concentration.

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# White clover content in mixed swards installed in fields with different preceding crop

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## Abstract

This research focuses on the influence of ploughed down permanent (PG), temporary (TG) grassland and arable land (PA and TA) on the performance (DM-yield, digestibility) of a newly installed grass-clover sward and on the persistence of the white clover (*Trifolium repens* L.). The sward was installed during springtime and was managed under a simulated grazing cutting regime with a N dressing of 0, 100, 300 and 400 kg N ha<sup>-1</sup> yr<sup>-1</sup>. Results of the newly established swards were compared to results of 35-year old permanent grassland (PGG). PGG outyielded all other situations significantly in 2002, while in the first full harvest year PA was significantly higher than TG and PGG; in the second full harvest year PA was significantly higher than PG, TG and PGG. The initial clover content (July 2002) was significantly higher than the final value in October 2004, but clover content was always the highest in PA. We conclude that both the performance of a grass-white clover sward and the persistence of the clover in the sward are most successful if sown into arable land.

Keywords: white clover, soil N, grassland, arable land.

## Introduction

According to Loiseau *et al.* (2001) the risk of clover extinction could be avoided by sowing grass-clover swards on soils with a poor N-supplying capacity. The N-supplying capacity of the soil depends highly on the cropping system. Our experiment focuses on the effect of different preceding crops on the soil N-supplying capacity, on the yield performance, the clover content and clover evolution in a newly established grass-clover sward.

## Material and methods

The experiment was conducted on a sandy loam soil at the experimental farm of Ghent University in Melle (Belgium, 50°59' N, 03°49' E, 11 m asl). During spring 2002 a mixture of 40 kg ha<sup>-1</sup> perennial ryegrass (*Lolium perenne* L., cvs. 'Plenty' and 'Roy') and 4 kg ha<sup>-1</sup> white clover (*Trifolium repens* L., cv. 'Huia') was sown in both rotocultivated arable (A) land and grassland (G). Part of the arable land had been permanently arable land during 35 years (PA); part of it was temporary arable land in a 35 year lasting rotation of 3 year grassland followed by 3 year arable land (TA). Part of the grassland had been grassland for 35 years (PG), part of it was temporary grassland (TG) in a 35 year lasting rotation of 3 year arable land followed by 3 year grassland. The newly installed swards were compared to 35 year old swards (PGG). The swards were managed under a simulated grazing cutting regime with a N dressing of 0, 100, 300 and 400 kg N ha<sup>-1</sup> yr<sup>-1</sup>.

The experimental design was a split plot (4 replicates), the preceding crop and the nitrogen application rate being the main factor and the subfactor respectively. DM yield, N-content, digestibility (NIRS) of the forage and clover content (weight share on DM basis) were determined each cut. The residual soil nitrate-N (0-90cm) was measured with a nitrate specific electrode. According to the relative contribution of each cut to the total annual DM yield, one representative mixed sample of dry organic matter was constituted to determine digestibility.

## Results and discussion

The total annual DM yield of the grass-clover swards, averaged over the four N dressings during the period 2002-2004 is given in Table 1.

Table 1. DM yield (kg DM ha<sup>-1</sup>) of permanent grassland (PGG) compared to the yield of newly installed grass-clover swards on land with different preceding crops. Mean values over the four N-dressings. Period: 2002-2004. Duncan letters indicate significant differences within columns (LSD, p<0,05).

Preceding crop <sup>x</sup>	2002		2003		2004		Sum	
	kg ha <sup>-1</sup>	relative <sup>y</sup>	kg ha <sup>-1</sup>	relative	kg ha <sup>-1</sup>	relative	kg ha <sup>-1</sup>	relative
PA	10,807 <sup>b</sup>	86	14,469 <sup>a</sup>	142	14,349 <sup>c</sup>	113	39,626 <sup>c</sup>	111
TA	10,370 <sup>ab</sup>	83	12,780 <sup>b</sup>	126	13,806 <sup>bc</sup>	109	36,957 <sup>bc</sup>	104
PG	10,578 <sup>b</sup>	84	12,524 <sup>b</sup>	123	12,716 <sup>ab</sup>	100	35,818 <sup>ab</sup>	101
TG	9,467 <sup>a</sup>	76	10,495 <sup>c</sup>	103	13,231 <sup>ab</sup>	104	33,193 <sup>a</sup>	94
PGG	12,535 <sup>c</sup>	100	10,174 <sup>c</sup>	100	12,693 <sup>a</sup>	100	35,402 <sup>ab</sup>	100

<sup>x</sup>(PA: permanent arable land, TA: temporary arable land; PG: permanent grassland; TG: temporary grassland and PGG: undisturbed permanent grassland).

<sup>y</sup>Relative to PGG within columns.

The undisturbed permanent grassland (PGG) outyielded all other situations significantly during the sowing year (2002), owing to the extra spring cut. TG had the lowest performance: 24% less than PGG. In the first full harvest year (2003, with a very dry spring and summer), TG continued to be the significantly less productive within the resown swards while PA significantly outyielded TA, PG, TG and PGG. The poor results of PGG may be due to the superficial rooting system, typical for permanent grassland, which hampered water uptake during the very dry growing season. In the second full harvest year the yield on PA swards was significantly higher than the yield on TG, PG and PGG. PGG swards yielded 13% less than PA but did not significantly differ from PG or TG swards, which is remarkable: "Where is the plant breeding dividend?" Averaged over the three growing seasons, PA was the best option to install grassland and TG the less beneficial one.

During the year of establishment, PA and TA swards had the highest annual mean clover content for all N dressings. Averaged over all N dressings we obtained: PA 40%, TA 32%, PG 16%, TG 6% and PGG 8%. Averaged over the 0N and 100N dressings, clover content was: PA 69%, TA 59%, PG 28%, TG 11% and PGG 10%. The 300N and 400N treatments had a low clover content as expected (Figure 1). In the first full harvest year, 2003, annual mean clover content persisted in TA but increased in all other situations. Data averaged over the four N dressings: PA 49%, TA 30%, PG 23%, TG 22% and PGG 14%. In winter 2003-2004 clover content decreased significantly on the newly installed swards (0N and 100N) and on PA (300N and 400N), and did not recover during 2004. Clover content (four N dressings average) in July 2002 and October 2004 is given to illustrate clover evolution: PA 36% vs. 26%, TA 27% vs. 16%, PG 9% vs. 14%, TG 2% vs. 12% and PGG 3% vs. 17%. On PA and TA, clover content decreased; on PG, TG and PGG it increased.

In 2003 no significant differences were observed in digestibility. In 2004 PA had a significantly higher digestibility (averaged over four N dressings) compared to the other swards: 78.6% vs. 76.5%.

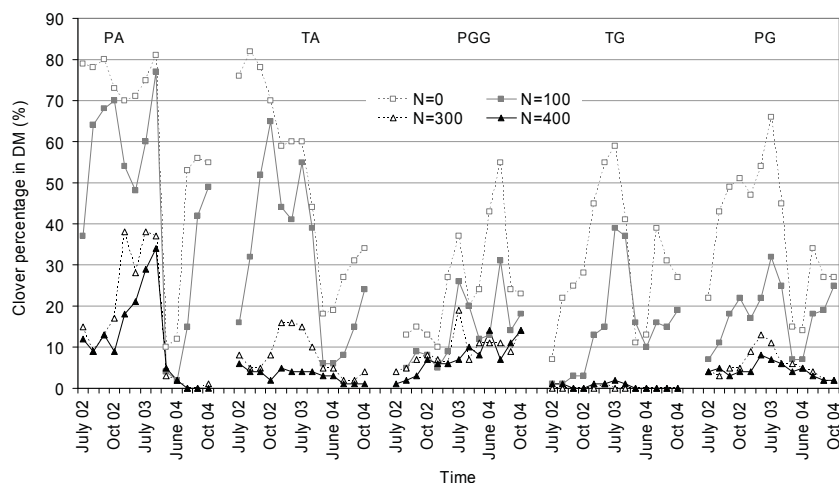


Figure 1. Evolution of white clover content in five different grass-clover swards fertilized with 0, 100, 300 and 400 kg N ha<sup>-1</sup>. Period: 2002-2004.

In 2002, herbage of the unfertilized PGG and TG swards had a significantly (42%) lower N content and (40%) lower N export than the other swards. At 100N, PG and PA exported significantly more than PGG but at higher N dressings PG and PA exported 20% and 15% less than PGG. In 2003 PA exported significantly more than the other swards at all N levels. In 2004 overall N export increased significantly with increasing fertilization, the unfertilized swards being not significantly different from the 100N swards.

The residual soil nitrate-N on all swards was below 90 kg N ha<sup>-1</sup> during the three years (except 400N PGG in 2004). In 2003 the residual soil nitrate-N showed a significant preceding crop effect. Averaged over four N dressings residual soil N was 10, 11, 12, 19 and 23 kg nitrate-N ha<sup>-1</sup> for TG, PA, TA, PG and PGG respectively. In 2004, PA, TA and TG showed similar residues for all N dressings (12 kg nitrate-N ha<sup>-1</sup>), a significantly lower value than for PG and PGG at 400N.

## Conclusions

Arable land offers the best opportunities to create a productive, clover rich, environmental clean grass-clover sward.

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## Timing of the last grazing of white clover/grass swards

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### Abstract

During the period 1998-2004 two field experiments were carried out on a light loamy gleyic cambisol. After harvesting of cover crop for forage, grasses were cut in the autumn of the sowing year, and in the years of use were last grazed at different times every two weeks, starting on September 1 and finishing on November 1. In the years of use, grasses were grazed 4-5 times per season. During the four years of use, a higher average dry matter yield  $6.54 \text{ t ha}^{-1}$  was produced by the sward sown with *Festulolium* and white clover (*Trifolium repens* L.). Perennial ryegrass (*Lolium perenne* L.) and white clover sward was less productive. The highest dry matter and digestible protein yield was obtained for both swards when the last grazing in the autumn had been performed in the middle of September or October. When the last grazing had been performed at the end of September or the first days of October, the herbage yield significantly declined. When grazing had been discontinued at this time, the herbage re-grew a little again and utilised nutrient reserves.

Keywords: *Trifolium repens*, *Lolium perenne*, *Festulolium*, grazing time.

### Introduction

While re-seeding swards it is very important to manage them in the way which would secure as long persistence of sown grasses as possible and stable, abundant and high quality herbage yield. Overwinter survival and longevity of grasses depend not only on the weather conditions but also on the state of grasses in the autumn, when their wintering shoots and roots are intensively accumulating reserves of nutrients (Stout, 1987; Sardana and Narwal, 2000). The long-term productivity of the sward depends on the species composition of grasses, frequency of use, nitrogen fertilisation, and timing of the last cut. The effects of these various factors on the sward can be very diverse in various geographic locations (Van Keuren, 1988; Taneja *et al.*, 1994).

When utilising pastures in the regions of more humid climate, it is very important to finish grazing at the right time so as to allow the sward to accumulate the necessary amount of nutrients and to prevent excessive trampling or poaching of the sward by livestock (mechanical damage). For pure swards dominated by alfalfa or red clover the last cut should be taken at the end of September or middle of October. For grassland swards dominated by awnless brome grass the best time of the last cut is end of September-beginning of October, for tall fescue the period of cutting may be longer – from middle of September to middle of October (Banikonienė, 1995). Consequently, the differences in the optimum time of the last cut for the swards of different type are relatively great and depend on many factors. The objective of the present study was to determine the time of the last cut in the sowing year and years of use of two pasture swards of *Festulolium* and ryegrass each sown with white clover.

### Materials and methods

During the period 1998-2004 field experiments were conducted on a gleyic light loamy cambisol. Soil pH varied between 6.4-6.8, humus content was 3.2-3.5%, available P 104-112  $\text{mg kg}^{-1}$  and K 118-130  $\text{mg kg}^{-1}$ . Two experiments were set up in 1998 and 2000. Vetch and oats mixture was undersown with two types of grass mixtures. Perennial ryegrass (cv. Zvilgė)  $15 \text{ kg ha}^{-1}$  was mixed with white clover (cv. Suduviai)  $2 \text{ kg ha}^{-1}$ . The other mixture was composed of *Festulolium* (*Lolium multiflorum* x *Festuca pratensis*) cv. Punia  $18 \text{ kg ha}^{-1}$  and white clover  $2 \text{ kg ha}^{-1}$ . After harvesting of vetch and oats mixture for forage, the swards in the autumn of the sowing year were cut and in the years of use were grazed at different time every two weeks on five dates starting on September 1 and finishing on November 1. The

swards were used for four years. In the spring of each year of use the swards were fertilised with P and K 60 kg ha<sup>-1</sup>. The annual rate of nitrogen fertiliser N 120 kg ha<sup>-1</sup> was applied at N 40 kg ha<sup>-1</sup> in spring and after the second and third grazing. Plot size was 2.5m × 10.0 m. The treatments were replicated 4 times and were grazed 4-5 times with a herd of dairy cows. Herbage yield was measured by cutting half of the plot. The botanical composition (grasses, clover, forbs) of the samples was measured after separation and is presented as dry matter weight. The data were statistically processed using analysis of variance.

## Results and discussion

In the autumn of the sowing year the swards were not grazed, they were cut at different time. Average dry matter yield of herbage when cut on September 1 totalled 0.84 t ha<sup>-1</sup>. When cut on the last date, the herbage yield increased to 1.53 t ha<sup>-1</sup>. The swards were dominated by sown grasses. *Festulolium* accounted for 56.1-74.1 %, perennial ryegrass for 52.9 – 69.3 %, white clover for 25.6 – 36.3 % of herbage dry matter yield. Herbage dry matter yields averaged over four years of the last grazing of the years of use are presented in Table 1. A delay in grazing until October 15 resulted in an increase in herbage dry matter yield for both swards. When grazing was performed on November 1, a significant reduction in the herbage yield occurred. Under Lithuania's conditions grass vegetation stops in the third ten-day period of October due to the weather becoming cold, often accompanied by the first snow.

Table 1. Herbage dry matter yield of the last grazing and annual dry matter yield t ha<sup>-1</sup>. Averaged data from two trials 1999 – 2004.

Last grazing date	Yield of the last grazing (t ha <sup>-1</sup> )	Annual yield (t ha <sup>-1</sup> )				Average
		Year of use sward				
		I	II	III	IV	
<i>Festulolium</i> + white clover						
1 September	0.80	7.21	5.74	5.02	6.16	6.03
15 September	1.49	8.92	5.78	5.62	5.85	6.54
1 October	1.32	7.82	5.46	5.32	5.65	6.06
15 October	1.53	8.28	5.93	6.00	5.85	6.50
1 November	0.93	7.31	5.58	5.61	5.91	6.10
Perennial ryegrass + white clover						
1 September	0.69	6.84	5.09	5.02	5.09	5.51
15 September	1.46	8.14	4.84	5.61	4.78	5.84
1 October	1.15	6.63	4.61	5.27	5.18	5.42
15 October	1.48	8.15	5.05	5.93	4.82	5.99
1 November	0.92	6.88	4.39	5.37	4.92	5.38
LSD <sub>0.05</sub>	0.35	0.73	0.33	0.38	0.48	0.27

The highest annual herbage dry matter yield was obtained in the first year of use. The sward composed of *Festulolium* and white clover tended to yield better. The highest herbage dry matter yield for both swards was obtained when grazing was finished on September 15 and October 15. In the swards of the second year of use the effect of the timing of the last grazing on the herbage yield in *Festulolium* sward was found to be insignificant, and in the sward of perennial ryegrass herbage yield significantly declined when grazing had been finished on October 1 and November 1. When the sward had been grazed on October 1, the herbage re-grew a little and was utilising nutrient reserves for re-growth. Grasses persist best in the sward and yield best when they are cut relatively early at the beginning of September or late, shortly before the end of the growing season. In the fourth year of use completion of grazing from September 15 to November 1 did not result in significant yield differences for both swards. Thus, younger swards respond more to the timing of the last grazing compared with older ones. Averaged data suggest that the highest yields of digestible protein 829 – 837 kg ha<sup>-1</sup> and metabolizable energy 58.6 – 62.3 GJ ha<sup>-1</sup> were obtained when grazing had been finished on September 15. Delay in the last grazing resulted in a significant deterioration of herbage nutritive value. When herbage had been cut on October 1, this gave already a significant reduction in crude protein content and an increase in crude fibre. When



grazing had been performed on November 1, crude protein content in herbage declined to 181- 185 g kg<sup>-1</sup> DM. Significant reductions occurred in herbage crude ash, P, K, Ca contents, while the content of crude fat remained similar. Completion of grazing at different time did not have any significant effect on sward botanical composition. In the fourth year of use festulolium accounted for 65.8 – 72.8 % of the dry matter yield, perennial ryegrass for 57.6 – 61.4 %. White clover accounted for 13.9 – 16.4 % in Festulolium sward, and in ryegrass sward for 9.8 – 14.1 %. A higher percentage of forbs was found in ryegrass sward (22.2-29.1%).

### Conclusions

In pastures dominated by perennial ryegrass and Festulolium with white clover it is not advisable to perform the last grazing at the end of September –beginning of October. For such swards it is better to finish grazing at the end of grass growing season in the middle of October. Younger swards (of the sowing year and first year of use) are more sensitive to the timing of the last grazing compared with older swards.

### Acknowledgments

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# Grazing influence on nutrient cycle and hay quality of coastal grassland plant associations

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## Abstract

The main goal of the work was to investigate pedo-ecological conditions of coastal soil development on coastal grassland and nutrient cycle in soil-plant systems. Emphasis was given to the influence of grazing on coastal grassland soil formation and plant associations. In Estonian coastal grasslands the most widespread plant associations are *Glauco-Juncetum gerardii*, *Festucetum rubrae* and *Phragmitetum australis*. Hay quality of different plant associations of coastal grasslands was investigated in the context of meet the requirements of grazing cattle. The content of mineral elements (Ca, Mg, and K) in soil and also in hay was satisfactory for beef cattle but the content of phosphorus was low.

Keywords: coastal grassland, soil, nutrient cycle, grazing.

## Introduction

Coastal grasslands are abundant on the islands and mainland of western Estonia where the landscape is flat. In the last 60–65 years, the total area of semi-natural grasslands and their importance as a forage resource for animal husbandry has radically changed in Estonia, depending on the management system practiced. The coastal grasslands have been traditionally grazed by cattle and horses for a long period of time. One reason for this was the relatively good quality of grass which met the requirements of livestock in extensive farming. Nowadays, the importance of grazing in coastal grasslands is emphasised mainly from a nature conservation point of view, as open coastal grasslands serve as a habitat for many endangered and rare species and as feeding sites for migratory birds.

Grazing influences nutrient cycling directly through removal of grass, urination and dung and indirectly through influences on the composition of vegetation and its effects on the soil (e.g. changes in soil aeration due to trampling). By uneven redistribution of mineral elements in time and space due to urination and dung (Peterson *et al.*, 1956) grazing alters the nutrient balance in the plant-soil system (Proulux and Mazumder, 1998) and acts as a creator of local level heterogeneity. The main goal of this work is to discuss the differences in nutrient cycle in soil-plant system of coastal grassland plant associations (both grazed and not grazed).

## Material and methods

The investigated coastal grasslands are situated on the Kassari Peninsula in South Eastern Hiiumaa. This area (approximately 400 ha) is grazed by horses and beef cattle. In the coastal grasslands investigated the most widespread plant associations are *Glauco-Juncetum gerardii*, *Festucetum rubrae* and *Phragmitetum australis*. The soils investigated on the island of Hiiumaa were categorized as *Hyposalic Fluvisols* and *Salic Fluvisols* on salt marches. Soil and plant samples were collected from investigated areas in the years 2001-2004. Soil samples were analyzed for P, K, Ca and Mg (Mehlich 3), pH (KCl), total C (Tjurin) and total N (Kjeldhal). Bulk density was determined in four repetitions (Methods of Soil, 1986). Plant material was analyzed for crude protein (CP), P, K, Na, Ca and Mg; metabolizable energy (ME) was calculated on the basis of ADF and NDF.

The analysis of variance was employed for data analysis and the Least Significant Difference ( $LSD_{0.05}$ ) and Standard Deviation (SD) are presented. One-way analysis of variance (ANOVA) procedures were

used to detect the differences between means of parameters examined in the grazed and non grazed areas.

## Results and discussion

The effect of grazing on plants and soil includes plant defoliation, nutrient removal and redistribution through excreta and mechanical impact on both soil and plants through trampling (Vallentine, 1990). The degree of soil compaction is influenced by stocking rate, duration and frequency of grazing, vegetation, soil type and moisture (Broersma *et al.*, 1998). Higher moisture contents in low-lying sites make these soils more vulnerable to trampling. In our investigated areas, the bulk density of humus horizon varied from 0.5–1.5 g cm<sup>-3</sup>. The greater value for bulk density was found in the area with heavy soil texture and greater grazing pressure where trampling was more intensive than in other areas. The content of plant available nutrients (Table 1) in the investigated grassland soils was relatively high in K, Na, Ca and Mg but phosphorus was low. The humus horizon of salt marshes (dominated by *Salicornia europaea*, *Honkenya peploides*, *Cakile maritima* and *Halimione pedunculata*) and habitat of *Phragmitetum australis* was significantly ( $p < 0.05$ ) richer in Na and Mg compared to the other soils covered by different plant associations.

Table 1. Chemical parameters of soil humus horizon by the different plant associations.

Indicators	Salt marshes	Grassland <i>Glauco–Juncetum gerardii</i>	Grassland <i>Festucetum rubrae</i>	Habitat of <i>Phragmitetum australis</i>	<i>LSD</i> <sub>0.05</sub>
pH (KCl)	7.4	5.8	5.2	5.3	
N, % (units)	0.03	0.50	1.09	1.53	0.13
C, %	0.8	5.4	14.2	25.3	0.46
P, mg kg <sup>-1</sup>	1.9	7.1	6.4	10.7	0.9
K, mg kg <sup>-1</sup>	496	366	428	1113	102
Na, mg kg <sup>-1</sup>	26058	7961	2982	44505	1458
Ca, mg kg <sup>-1</sup>	1637	2248	847	5318	119
Mg, mg kg <sup>-1</sup>	2154	943	313	3116	165

The hay quality was satisfactory for beef cattle but was inadequate for highly productive milking cows. Different plant associations contained 8.6-13.2%, crude protein while metabolizable energy varied 9.07-13.18 MJ kg<sup>-1</sup>. The content of mineral elements in forage was generally high, only with P content low. The best quality of hay was observed in plant association on salt marshes. Soil in grazed area contained less nutrients (K, Ca, Mg) than ungrazed area ( $p < 0.05$ ; Fig 1). Phosphorus in soil seemed not to be affected by grazing. Organic C accumulation in the humus horizon was lower under a grazing regime (25.7% and 27.1%) which is expected due to decreased input of leaf litter in grazed areas. The decrease in soil organic matter, which is a major source of nutrient mineralisation, may gradually lead to a lower soil nutrient store and availability (Kooijman and Smit, 2001).

Plant N content in grazed area (24 g kg<sup>-1</sup>) was higher than in ungrazed areas (14 g kg<sup>-1</sup>). There were significant differences ( $p < 0.05$ ) in mineral elements in plants as well as nutrient content in soil humus horizon between grazed and ungrazed areas (Figure 1).

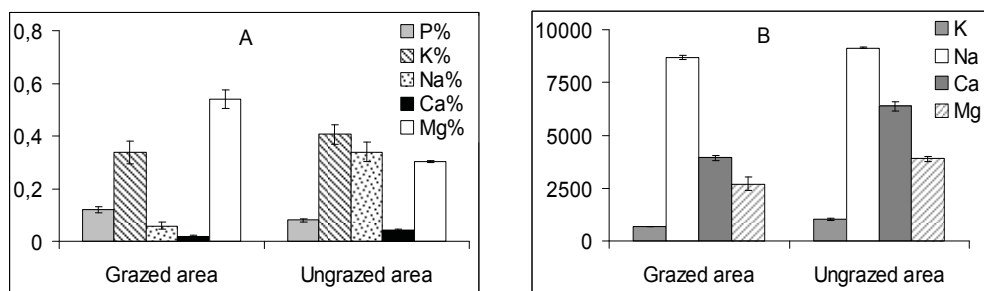


Figure 1. Plant chemical parameters (A) and content of nutrients ( $\text{mg kg}^{-1}$ ) of soil humus horizon (B) of grazed and ungrazed areas ( $M \pm SD$ ).

## Conclusions

Grazing is the key factor to maintain biodiversity in coastal areas and suppress the spread of reed. Hay quality of studied coastal grasslands was satisfactory to meet the requirements of beef cattle and horses thus permitting continued grazing in coastal areas. The content of plant available nutrients in the soils and forage studied was relatively high in K, Na, Ca and Mg but the content of phosphorus was very low. Grazing disturbance includes also soil compaction, decreasing of C accumulation in soil, and acceleration of the nutrient cycling.

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## Role of clovers in the productivity of *Festuca arundinacea* and *Phalaris arundinacea* swards

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### Abstract

The productivity of different management systems in temporary swards of red and white clovers with *Festuca arundinacea* Schreb., and *Phalaris arundinacea* L. was compared on a sod podzolic Orthieutric Albeluvisol (Jib2) light loam on medium loam. In red clover/grass swards the content of clover was on average 34.7-43.5%, the highest in the cutting system. In white clover/grass swards the content of clover was lower (23.8-31.0%), the highest in the cut + grazed system. Cut and cut + grazed swards were most productive (6.00-7.03 and 5.84-6.13 t ha<sup>-1</sup> of dry matter (DM), respectively). The yield of grazed sward (4.28-5.07 t ha<sup>-1</sup> DM) was significantly lower. Grazed herbage had the highest crude protein content (169-186 g kg<sup>-1</sup>) and the lowest content of crude fibre 212-247 g kg<sup>-1</sup>. Only cut pure *Festuca arundinacea* and *Phalaris arundinacea* did not meet the requirements of high productivity cattle for crude protein (106 g kg<sup>-1</sup>). When grazing cattle at pasture maturity stage, the intake of grass in mixed-used swards was 80-86 % and that in grazing swards 63-74%.

Keywords: clovers, *Festuca arundinacea*, *Phalaris arundinacea*, management systems, productivity, intake.

### Introduction

Tall fescue (*Festuca arundinacea* Schreb.) has a bunch-type growth habit and a strong root system. Reed canary grass (*Phalaris arundinacea* L.) is rhizomatous and can withstand long flooding periods and severe colds. These grasses perform well both on wet and dry soils. However, contradictory opinions about the above-mentioned grasses can be found in literature. Moreover, they consider them suitable not only for cutting but also for grazing (Koc and Gokkus, 2003, Nagy, 2003, Negovanović *et al.*, 2003). Clovers can be used to improve the feeding value of grasses. Our experiments were designed to investigate the effect of clovers on differently managed tall fescue and reed canary grass swards.

### Materials and methods

The field experiments were set up in a bi-factorial block design with four replicates. The soil of the experimental site was Orthieutric Albeluvisol (Jib2), pH 5.4-6.2, with available P<sub>2</sub>O<sub>5</sub> content ranging between 179-266 and K<sub>2</sub>O 190-360 mg kg<sup>-1</sup>.

Factor A represented management regimes: cutting (3 cuts), grazing (4 grazings), cut + grazed (1 cut + 2 grazings). Factor B was the mixture sown: *Festuca arundinacea* cv. Baltika and *Phalaris arundinacea* cv. Pervenec (50+50 %) and mixtures with *Trifolium pratense* cv. Vyliai or *Trifolium repens* cv. Atoliai (60+20+20 %). The swards in the first, second and third years of use were fertilised with 60 kg ha<sup>-1</sup> of P<sub>2</sub>O<sub>5</sub> and 90 kg ha<sup>-1</sup> of K<sub>2</sub>O. Nitrogen fertiliser was applied in the first and second year 120 kg N ha<sup>-1</sup>, and 60 kg ha<sup>-1</sup> in the third year.

Soil agrochemical characteristics in the 0-20 cm soil layer were determined using the following methods: pH<sub>KCl</sub> - by electrometric method, hydrolytic acidity - by Kappen method, available phosphorus and potassium - by A-L method. Plant chemical composition was estimated by the following methods: nitrogen – after Kjeldhal, crude protein - according to the amount of nitrogen, multiplying it by 6.25, phosphorus - by colorimetric method, potassium - by flame photometry. Yield data were processed by mathematical statistical methods.

## Results and Discussion

Botanical composition of the herbage yield depended on the biological characteristics of grasses, management regime and the weather conditions. Sown grasses prevailed in the swards. Forbs accounted for 10 - 25 %. *Festuca arundinacea* + *Phalaris arundinacea* pure and sown in mixtures with clover spread very differently. In all sward management regimes *Festuca arundinacea* suppressed *Phalaris arundinacea* from the first year of use. In red clover/grass swards the content of clover was on average 34.7-43.5%, the highest in the cutting system. In white clover/grass swards the content of clover was lower (23.8-31.0%), the highest in the cut + grazed system (Table 1).

Table 1. Quality of the swards under different management regimes.

Swards (factor B)	Management regimes (factor A)			Mean of factor B
	cutting	grazing	cut + grazed	
	DM yield t ha <sup>-1</sup>			(LSD <sub>0.05</sub> =0.36)
Trifolium pratense +	7.03	4.28	5.84	5.72
Trifolium repens +	6.23	5.07	6.13	5.81
<i>Festuca arundinacea</i> + <i>Phalaris arundinacea</i>	6.00	4.39	6.04	5.47
Mean of factor A (LSD <sub>0.05</sub> =0.36)	6.42	4.58	6.00	
	Clover % DM			(LSD <sub>0.05</sub> =2.85)
Trifolium pratense +	43.5	34.7	38.5	38.9
Trifolium repens +	23.8	29.1	31.0	28.0
Mean of factor A (LSD <sub>0.05</sub> =4.03)	33.6	31.9	34.8	
	Crude protein g kg <sup>-1</sup> DM			(LSD <sub>0.05</sub> =5.872)
Trifolium pratense +	126	183	131	147
Trifolium repens +	133	186	144	154
<i>Festuca arundinacea</i> + <i>Phalaris arundinacea</i>	106	169	131	135
Mean of factor A (LSD <sub>0.05</sub> =5.872)	122	179	135	
	Crude fibre g kg <sup>-1</sup> DM			(LSD <sub>0.05</sub> =7.91)
Trifolium pratense +	259	212	270	247
Trifolium repens +	265	224	258	249
<i>Festuca arundinacea</i> + <i>Phalaris arundinacea</i>	288	247	278	271
Mean of factor A (LSD <sub>0.05</sub> =7.91)	270	228	269	
	Phosphorus g kg <sup>-1</sup> DM			(LSD <sub>0.05</sub> =0.11)
Trifolium pratense +	2.87	4.07	3.20	3.37
Trifolium repens +	3.20	4.23	3.33	3.59
<i>Festuca arundinacea</i> + <i>Phalaris arundinacea</i>	2.83	4.03	2.27	3.38
Mean of factor A (LSD <sub>0.05</sub> =0.11)	2.97	4.11	3.27	
	Potassium g kg <sup>-1</sup> DM			(LSD <sub>0.05</sub> =1.63)
Trifolium pratense +	31.1	37.3	28.5	32.3
Trifolium repens +	30.6	35.1	30.6	32.1
<i>Festuca arundinacea</i> + <i>Phalaris arundinacea</i>	29.6	37.9	33.0	33.5
Mean of factor A (LSD <sub>0.05</sub> =1.63)	30.4	36.8	30.7	
	Intake %			(LSD <sub>0.05</sub> =5.62)
Trifolium pratense +	-	72	86	79
Trifolium repens +	-	74	81	78
<i>Festuca arundinacea</i> + <i>Phalaris arundinacea</i>	-	63	80	72
Mean of factor A (LSD <sub>0.05</sub> =3.97)		70	82	

LSD<sub>0.05</sub> - least significant difference at P ≤ 0.05.

The swards in the cutting and cut + grazed regimes gave the highest DM yield and that corresponding to grazed swards were significantly lower. Under cutting regime the highest yield was found to be the mixture of *Trifolium pratense* + grasses. The yields of pure grasses and *Trifolium repens* + grass mixture were significantly lower, 1.03-0.8 t ha<sup>-1</sup> DM, respectively. Under grazing management *Trifolium repens* + grass mixture produced 5.07 t ha<sup>-1</sup> dry matter, 0.68-0.89 t ha<sup>-1</sup> higher-yielding than the other swards studied. Under combined management regimes the production of the swards were not statistically different.

Chemical composition of perennial grasses depends on botanical composition and growth stage (Brencienė, 1995). The cut grass mixture did not match the needs of highly productive cattle for crude protein. Clover tended to improve chemical composition of grass mixture. *Trifolium repens* + grass mixtures managed in different ways accumulated higher contents of crude protein compared to *Trifolium pratense* + grass mixtures. The herbage yield of grazed swards contained much less crude fibre compared to cut and combined-used swards. The lowest and almost identical crude fibre yield was identified for cut *Trifolium pratense* + grass mixture and for combined-used *Trifolium repens* + grass mixture.

Differently managed *Trifolium repens* + grass mixtures accumulated significantly more phosphorus than the swards of other botanical composition. Young herbage of pure grass mixture showed higher contents of potassium compared to grass/clover mixtures. Grazed swards showed the highest contents of phosphorus and potassium.

The highest intake was determined for grass mixtures with clover. The intake of combined-managed grass mixture was significantly better than that of grazed one.

### Conclusions

Clover + grass mixtures were higher yielding than pure grass mixtures by 0.25 – 0.34 t ha<sup>-1</sup> DM. The herbage yield of mixtures with clover had 9-14 % higher protein content and 9-10 % lower crude fibre content. The intake of mixtures with clover was better than that of pure grasses.

Due to the high competitive power of *Festuca arundinacea* and *Phalaris arundinacea*, their proportions in mixtures, especially that of tall fescue, should be smaller, because luxuriantly growing grasses suppress legumes, which results in poor supply of grasses with biological nitrogen and in the deterioration of the feeding value of mixtures.

### Acknowledgements

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## Fatty acid content of three grass/clover mixtures

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### Abstract

Linoleic acid (C18:2) and linolenic acid (C18:3) in feeds are the precursors of conjugated linoleic acid (CLA) in milk and meat. Moreover, the linolenic acid content in forage also increases the content of omega-3 fatty acids in these products.

In 2003 and 2004 we investigated the fatty acids of three grass/clover mixtures of the first to the third cut. Mixture A contained only grasses, mainly ryegrass and cocksfoot. In mixture B there were grasses and red clover and mixture C contained grasses, clover and alfalfa. In the different mixtures the botanical composition changed from cut to cut and from the first to the second year. The results showed that the average amount of linolenic acid (C18:3) was 64.1, 64.3 and 60.7% in the three mixtures A, B and C. The average content of linoleic acid (C18:2) was 15.4, 16.0 and 17.2% and of that palmitic acid (C16:0) 14.0, 13.8 and 14.8%, respectively. The amount of stearic (C18:0) and oleic acid (C18:1) was lower than 3%.

Keywords: grasses, clover, alfalfa, mixtures, fatty acids.

### Introduction

Forages are important sources of precursors of fatty acids, such as linolenic acid and conjugated linoleic acid (CLA), in milk and meat from ruminants.

According to Bauchart *et al.* (1984) the five major fatty acids in grass are linolenic acid (C18:3), linoleic acid (C18:2), oleic acid (C18:1), stearic acid (C18:0) and palmitic acid (C16:0). Their concentration varies depending on the plant species, growth stage, temperature and light intensity (Hawke, 1973).

Four trials were conducted at our research station in order to assess the effect of botanical composition of grass and conserved forage (hay and silage) on the chemical composition of milk, especially on the fatty acid profile of milk fat. The results with grass and hay of the second cut were published by Morel *et al.* (2005). In this paper the fatty acid concentrations within the three mixtures and different cuts are shown.

### Material and methods

Three different mixtures were sown in spring 2002. Mixture A contained only grasses: ryegrass, cocksfoot, meadow fescue and timothy. Mixture B contained the same grasses as mixture A, plus clover, especially red clover. Mixture C contained alfalfa, red clover, cocksfoot, ryegrass and timothy. In 2003 and 2004 samples were taken from the first three cuts in May, June and July. The forage of the second and third cut was about four to five weeks old. Dry matter (DM), ash, crude protein, cell wall constituents as well as fatty acids were analysed. Because only one sample per mixture of the first and third cut was analysed, no statistical analysis was carried out and only the values were compared.

### Results and discussion

In the different mixtures the botanical composition changed from cut to cut and from the first to the second year (Figure 1). In the second year, all mixtures contained also some herbs. In mixture A and B the proportion of ryegrass decreased and the proportion of cocksfoot increased in 2004 in comparison to 2003. In mixture B the proportion of red clover increased from cut to cut, mainly in the second year. Due to the drought in the first year, alfalfa developed very well in mixture C and also in the second year its proportion varied between 55 and 63%.



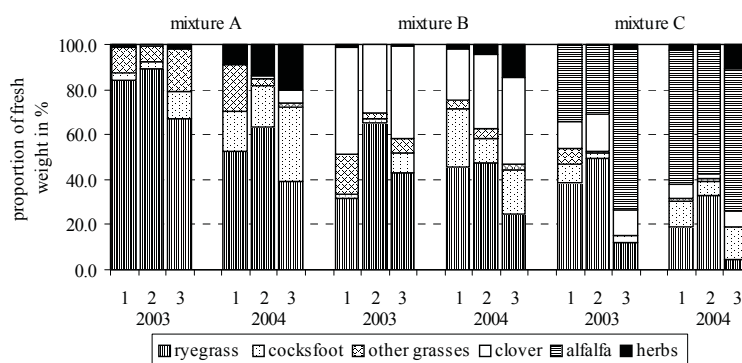


Figure 1. Changes in the botanical composition of the three mixtures within the cut and years.

Ash, crude protein and cell wall constituents of the three mixtures are shown in Table 1. The crude protein content increased in all mixtures from cut to cut in both years. But in 2003, the differences between the first and third cut were bigger than in 2004. Especially the forage of the second cut from 2003 had high crude fibre contents. The crude fibre, respectively ADF contents were higher in mixture C with alfalfa.

Table 1. Ash, crude protein, crude fibre, ADF and NDF contents ( $\text{g kg}^{-1}$  DM) of the three mixtures.

Mixture	Year	Cut	Ash	Crude Protein	Crude Fibre	ADF	NDF
A	2003	1	70	93	250	268	470
		2	95	113	275	304	513
		3	99	230	234	270	496
	2004	1	74	100	260	292	499
		2	117	123	243	272	471
		3	106	141	240	282	477
B	2003	1	89	139	252	283	463
		2	112	149	268	313	470
		3	99	220	222	266	462
	2004	1	86	131	254	294	484
		2	116	170	236	282	453
		3	113	174	245	282	467
C	2003	1	103	147	265	305	453
		2	108	143	309	359	499
		3	98	209	256	300	382
	2004	1	96	164	263	316	436
		2	125	210	241	287	419
		3	114	226	259	307	409

In all three mixtures the linolenic acid content (C18:3) was the dominant fatty acid (Table 2). The values varied between 8.6 and 12.8 g per kg DM. In 2004 in all mixtures the highest values were found in the second cut and the lowest values in the third cut. In 2003 no systematic trend was carried out. In mixture A the C18:3 concentrations were higher in 2003 in comparison to 2004. This is partly due to the higher proportion of cocksfoot, and in line with Dewhurst et al. (2001), who found higher

concentrations of C18:3 in ryegrass in comparison to cocksfoot. The average amount of linolenic acid in total fatty acids was 64.1, 64.3 and 60.7% for the three mixtures. The mixture C with the highest proportion of legumes (alfalfa) had the lowest amount of C18:3. Morand-Fehr and Tran (2001) showed that the stage of maturity influences the concentration of the fatty acids; however, it is difficult to see any differences between grasses and legumes.

The values of linoleic acid (C18:2) varied between 2.1 and 3.1 g per kg DM and the average amount was 15.4, 16.0 and 17.2% for the three mixtures. Similar values were also found for palmitic acid (C16:0). The average amount was 14.0, 13.8 and 14.8%. The three mixtures had low concentrations of stearic (C18:0) and oleic acid (C18:1) and their amount was lower than 3%.

Table 2. Fatty acid concentrations (g kg<sup>-1</sup> DM) of the three mixtures.

Mixture	Year	Cut	C16:0	C18:0	C18:1	C18:2	C18:3	Total FA
A	2003	1	2.1	0.2	0.5	2.4	9.3	15.7
		2	2.3	0.2	0.4	2.3	10.0	15.5
		3	2.4	0.3	0.5	2.5	12.2	18.9
	2004	1	1.9	0.2	0.4	2.3	8.9	13.7
		2	2.1	0.2	0.3	2.1	9.5	14.5
		3	1.9	0.2	0.3	2.2	8.8	13.4
B	2003	1	2.0	0.2	0.5	2.4	9.0	14.2
		2	2.4	0.3	0.4	2.6	10.3	16.2
		3	2.4	0.3	0.5	2.8	10.1	17.4
	2004	1	2.0	0.2	0.4	2.5	10.2	15.3
		2	2.2	0.2	0.3	2.5	12.8	18.3
		3	2.0	0.2	0.3	2.4	8.7	13.6
C	2003	1	2.3	0.2	0.4	2.8	10.3	16.3
		2	2.3	0.3	0.3	2.4	8.6	14.1
		3	2.5	0.4	0.4	2.6	9.2	17.2
	2004	1	2.2	0.2	0.4	2.8	9.2	15.2
		2	2.4	0.2	0.4	2.6	12.2	18.1
		3	2.3	0.3	0.4	3.1	8.8	14.8

### Conclusions

In all three mixtures, linolenic acid (C18:3) was the dominant fatty acid and the average amount was over 60%, followed by linoleic (C18:2) and palmitic acid (C16:0). Generally speaking the fatty acid composition of the three mixtures was similar.

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# Influence of clover species in mixtures with grasses on fatty acid composition of mixtures

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## Abstract

White clover (WC, *Trifolium repens* L.) or red clover (RC, *Trifolium pratense* L.) was grown in mixture with grasses. Samples of the mixtures and pure clovers were taken during three cuts, and the fatty acids (FA) compositions were determined. The clover species had no significant effect on the individual FA contents of the leys (g kg<sup>-1</sup> dry matter (DM)), nor on the total FA content. Pure clovers had lower content of all individual FA (8.2 vs. 12.4 g kg<sup>-1</sup> DM) than mixtures; the contents of all FA decreased with increasing percentage of clover in the mixture, but the decrease was weaker for C18:3n-3 than for the other FA. Although pure WC and RC had similar FA contents, the relative proportions (percentage of total FA content) of C16:0 and C18:3n-3 differed; RC had a higher proportion of C18:3n-3 and a lower proportion of C16:0. These results are partly in contradiction with previously reported.

Keywords: fatty acids, temperate clovers, grass-legume mixtures.

## Introduction

Recent studies indicate that milk fat from cows fed clover-based silages has higher levels of beneficial n-3 FA than milk from cows fed pure grass silages (Dewhurst *et al.*, 2003). This could partly be ascribed to a higher content of C18:3n-3 in clover than in grass (Dewhurst *et al.*, 2001). As the C18:3 concentration of milk fat is influenced by the C18:3n-3 concentration of forages (Hebeisen *et al.*, 1993), it is interesting to examine agronomic practices that can affect the FA composition of forages. Few studies have compared clover species with respect to FA content. Boufaïed *et al.* (2003) found higher content of total FA (TFA) and C18:3n-3 in white clover (*Trifolium repens* L.) than in red clover (*Trifolium pratense* L.), and higher content of TFA but lower content of C18:3n-3 in legumes than in grasses. However, the study of Boufaïed *et al.* (2003) was carried out in pure stands of legumes and grasses. The objective of the present study was to investigate how the clover species affect the FA content of the herbage when grown in a mixture with grasses.

## Materials and methods

Red clover (RC) or white clover (WC) was grown in mixture with timothy (*Phleum pratense* L.), meadow fescue (*Festuca pratensis* Huds.) and perennial ryegrass (*Lolium perenne* L.) in three replicates (field size ± 2.3 ha) at the University of Life Sciences, Norway (59°N, 10°E) and cut for silage three times in 2004. The leys were established in 2003. For each of three plots, distributed evenly on a line diagonally through each field (replica), a sample was taken by harvesting 0.5 m<sup>2</sup> at a stubble height of 6 cm. The harvested material was split into two sub samples, one for the botanical analysis and one for the FA analysis (Mix). In addition, pure clover samples (Pure) were also taken by harvesting the mixture adjacent to each harvested plot at a stubble height of 6 cm and immediately hand separating the clovers from the other species in field. The samples for the FA analysis were kept in airtight plastic bags, frozen within an hour after harvest, stored at -20°C, and subsequently freeze-dried and prepared for gas chromatography of FA methyl esters according to Sukhija and Palmquist (1988), with slight modifications. The results were analysed by the analysis of variance between treatment differences using the mixed procedure of SAS (SAS, 1998).

## Results and discussion

In this paper, results are presented as averages over three harvest dates (cuts). There was a significant effect of the harvest date on FA contents and composition. However, the effect of clover species was consistent across harvest dates.

Table 1. Fatty acid (FA) content ( $\text{g kg}^{-1}$  DM) and relative proportion (% of total FA) in grassland herbage containing either white clover (WC) or red clover (RC) in mixture with grasses (Mix) and in pure clover (Pure) hand-separated from the same stand; averaged over three cuts and replicates ( $n=9$ ).

Fatty acid	WC		RC		s.e.d.	Significance		
	Mix	Pure	Mix	Pure		Species	Mix vs. Pure	Interaction
$\text{g kg}^{-1}$ DM								
Total	11.7	8.03	13.2	8.34	0.92	NS	***	NS
C16:0	2.28	1.80	2.29	1.57	0.17	NS	***	NS
C18:1	0.21	0.14	0.20	0.10	0.03	NS	**	NS
C18:2n-6	1.77	1.46	1.96	1.69	0.16	NS	*	NS
C18:3n-3	5.62	4.05	6.40	4.55	0.51	NS	**	NS
% of total FA								
C16:0	20.6	24.1	18.2	18.9	0.54	***	**	**
C18:1	1.9	1.5	1.5	0.9	0.22	*	*	NS
C18:2n-6	16.0	20.2	16.0	20.8	0.80	NS	***	NS
C18:3n-3	49.0	48.9	51.2	55.7	1.37	*	*	*

s.e.d., standard error of the difference for interaction effect.

Significant differences between means at \*  $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$ ; NS, not significant.

There were no significant differences in the dry matter (DM) yield between the two leys. The percentage of clover in the mixture was significantly ( $P < 0.001$ ) higher in the RC ley (53%) than in the WC ley (35%). There were no significant differences between the clover species in the content of total and individual FA (Table 1). These results are in contrast to Boufaïed *et al.* (2003), who found higher content of TFA in WC than in RC. However, Boufaïed *et al.* (2003) samples were taken from the spring growth of pure stands harvested at blooming that were 3 weeks later for RC than for WC, while our samples were taken from mixed stands harvested at the same date. Pure clovers had lower content of total ( $8.2 \text{ g kg}^{-1}$  DM) and individual FAs ( $P < 0.001$ ) than the mixtures ( $12.4 \text{ g kg}^{-1}$  DM) (Table 1). This indicates that the FA concentrations were higher in the grasses than in the legumes. A linear regression between percentage of clover in the mixture and the content of FA in the mixture supported this result, as the content of total and individual FAs decreased with increasing clover content in the mixture (TFA =  $23.8 - 0.20 * C$ ,  $R^2 = 0.56$ ,  $P < 0.001$  for RC and TFA =  $17.3 - 0.16 * C$ ,  $R^2 = 0.18$ ,  $P < 0.05$  for WC, where C is the clover percentage of the DM yield). This is also in contrast with the results of Boufaïed *et al.* (2003), who found a higher content of most FA, except C18:3n-3, in legumes than in grasses. C18:3n-3, C18:2n-6 and C16:0 were the most abundant FA, averaging 51, 18 and 20% of TFA, respectively. These results are consistent with that reported previously for forage plants (e.g. Boufaïed *et al.*, 2003). Although clover species did not affect the herbage FA content, the FA proportion expressed as percentage of TFA was affected by clover species. The proportion of C16:0 was higher ( $P < 0.001$ ) in WC than in RC (24.1 and 18.9 %, respectively), while that of C18:3n-3 was higher ( $P < 0.05$ ) in RC (55.7 and 48.9 %, respectively) (Table 1).

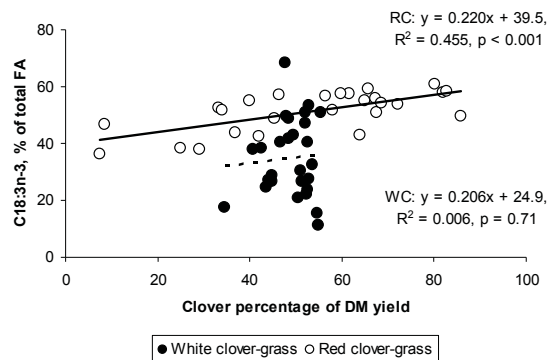


Figure 1. The relationship between clover percentage of DM yield and the proportion of C18:3n-3 (% of total FA) in white clover-grass herbage (WC) and red clover-grass (RC) herbage; samples from 3 cuts, 3 replicates and 3 plots within replicate.

The proportion of C16:0 was higher in pure clovers than in mixtures, particularly for WC (Table 1). Pure RC had a higher proportion of C18:3n-3 than the mixture, what was not found in WC (Table 1; indicated also by the significant interaction between species and mixtures vs. pure). The latter result was also reflected in a positive relationship between the clover percentage in the mixture and the proportion of C18:3n-3 found in RC mixture but not in WC mixture (Figure 1). Therefore, even though the content of all FA decreased with increasing clover percentage in the mixture, the reduction was less pronounced for C18:3n-3 in RC than for the other FA.

### Conclusions

Clover species did not affect herbage FA content (g FA kg<sup>-1</sup> DM). However, the proportion of C18:3n-3 was higher and that of C16:0 lower in RC than in WC leys. The content of FA decreased generally with increasing clover content in the mixture, but the proportion of C18:3n-3 increased with increasing clover proportion in the RC mixture. More research is needed to clarify the cause of discrepant results between studies.

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# The role of *Trifolium repens* in maintaining pasture ground cover on a peat-muck soil

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## Abstract

Grazing studies were conducted in two experiments established in 1996. One of the aims of these studies was to determine the role of *Trifolium repens* L. in the pasture surface cover on a peat-muck soil. The mixtures sown in two experiments contained *Phleum pratense* L., *Dactylis glomerata* L., *Trifolium repens* L. and *Poa pratensis* L. (experiment A) or *Lolium perenne* L. (experiment B). The protective function of *Trifolium repens* on the peat-muck soil manifested itself in better ground cover. This role of white clover was dependent on the proportion of grass components in the sward, their rate of development and competitive abilities. Existence of this species in the sward with *Poa pratensis* was very significant ( $r = 0.90$ ) for ground cover at the beginning of studies. In the sward with *L. perenne*, *T. repens* contributed to organic soil protection, particularly after detrimental influence of harsh winter conditions.

Keywords: *Trifolium repens*, surface cover, pasture, peat-muck soil.

## Introduction

Grasslands located on organic soils make up about one third of the total grassland area in Poland. From an ecological point of view maintaining permanent peat grasslands as carbon sinks is very important (Freibauer *et al.*, 2004). After harsh winters we observe gaps in the surface cover. Together with the age of sod, increasing content of *Poa pratensis* and also management of the sward do not always improve ground cover. Thus, to overcome this limitation and to protect the organic soil surface against degradation, introduction of fast growing species to postboggy habitats (peatland after dewatering) seems to be needed to limit invasion of indigenous grasses (e.g. *Poa trivialis*) and weeds. White clover is not a typical grassland species on peat-muck soils. Its presence in the pasture can be beneficial if it persists in the sward for more than six or seven years. The aim of this paper was to compare the importance of maintaining *Trifolium repens* in the surface cover at the beginning of study (Krzywiec, 2000) and in the seventh and eighth year of pasture utilization on a peat-muck soil.

## Methods

The grazing studies were conducted in two experiments, established on a peat-muck soil (Mt II – middle decayed peat) in 1996 (Krzywiec, 2000). A randomized block design with four replications was used. The mixtures sown in two experiments contained *Phleum pratense* (20%), *Dactylis glomerata* (10%), *Trifolium repens* (35%) and *Poa pratensis* (35% - experiment A) or *Lolium perenne* (35% - experiment B). Swards composed only of the above grasses without white clover were used as a control. The swards were fertilized with 40 kg N, 35 kg P and 100 kg K ha<sup>-1</sup> year<sup>-1</sup> and grazed rotationally with Limousin cattle four times during the grazing season. Ground cover of the pasture and the proportion of individual species in the surface cover were determined in autumn (at the end of growing period) and in spring with Levy's point method.

## Results and discussion

Ground cover of the pasture with grass-clover sward ranged between 91-100% in experiment A and 98-100% in experiment B at the end of the sowing year and was better than with a grass only (respectively: 90 and 85-97%) sward (Warda and Krzywiec, 1998). The protective function of *Trifolium repens* on a

peat-muck soil manifested itself in better pasture cover. However, the role of white clover was dependent on the proportion of the main grass components in the sward, their rate of development and competitive abilities. Presence of fast growing *Lolium perenne* at the beginning of the study was conducive to better ground cover (B) but it limited the quantity of *T. repens* in the sward ( $r = -0.75$ ). Slow development of *Poa pratensis* was most favourable for encouraging the white clover content. The relationship between the content of grass dominant species and *T. repens* was explained by correlation coefficients (Table 1). Existence of *T. repens* in the sward with *Poa pratensis* (autumn 1996) was very significant ( $r = 0.90$ ) for pasture cover.

Table 1. Correlation coefficients between the content of grass dominant species and *Trifolium repens* in a sod.

Relationship	Autumn			Spring		
	1996	2002	2003	1997	2003	2004
<i>Poa pratensis</i> x <i>Trifolium repens</i>	0.90	-0.63	-0.36	-0.38	-0.21	-0.57
<i>Lolium perenne</i> x <i>Trifolium repens</i>	-0.75	0.37	-0.59	0.52	0.84	-0.16

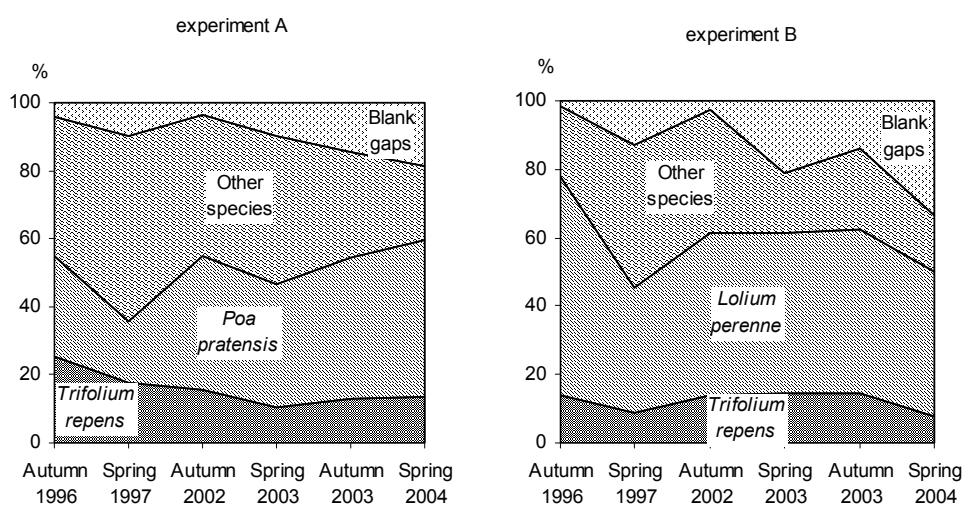


Figure 1. Pasture ground cover and the proportion of *Trifolium repens* in the sward.

Ground cover of the pasture decreased in the successive years, particularly in experiment B (Figure 1). It was often observed in spring probably as a result of the detrimental effect of harsh winter conditions on the maintenance of *L. perenne*. In such cases, white clover spreading through the sward by branching stolons filled gaps and protected the organic soil surface against degradation (spring 2003 –  $r = 0.84$ ). After eight years of pasture management, *T. repens* continued to make an important contribution to the sward. According to the results of earlier studies (Warda, 2004), the presence of white clover, filling gaps in the sward can be one of the factors limiting the rate of soil organic matter wastage. Fluctuations in the content of white clover in the pasture surface cover between autumn and spring also depended on white clover cultivar (Table 2).

Table 2. Fluctuations of *Trifolium repens* content (%) in the pasture surface cover between autumn and spring, depending on the sward type and white clover cultivar.

Treatment	Sward with						Mean for the treatment
	<i>Poa pratensis</i>			<i>Lolium perenne</i>			
	1996/1997	2002/2003	2003/2004	1996/1997	2002/2003	2003/2004	
Grasses(G) + <i>T. repens</i> cv. Anda	11.0	0.0	7.3	3.0	-4.6	-6.3	1.7
G + <i>T. repens</i> cv. Armenia	-1.0	-10.0	-14.5	-7.0	0.8	-7.5	-6.5
G + <i>T. repens</i> cv. Astra	-5.0	-15.0	3.3	1.0	-9.6	-10.0	-5.9
G + <i>T. repens</i> cv. Rema	-11.0	-7.0	-3.3	1.0	-0.8	-13.2	-5.7
G + <i>T. repens</i> cv. Romena	-23.5	8.7	6.8	-18.0	7.0	-9.6	-4.8
G + <i>T. repens</i> cv. Alice	-22.0	-11.2	0.0	-8.0	2.5	0.7	-6.3
G + <i>T. repens</i> cv. Santa	-2.0	-7.1	2.1	-5.0	0.8	-5.5	-2.8
G + <i>T. repens</i> Polish cvs mixture	-10.0	0.8	0.1	-10.0	8.3	-2.9	-2.3
Mean	-7.9	-5.1	0.2	5.4	0.5	-6.8	

Among the studied cultivars, the large-leaved *T. repens* cv. Romena was the most persistent in the pasture under organic soil conditions during the whole study period. However, independently on the sward type, the largest variation in proportion within the sward were observed after the first winter (1996/1997), presumably as a result of the loss of less resistant young plants.

### Conclusions

Under grazing management, the protective function of *Trifolium repens* on a peat-muck soil manifested itself in better ground cover of the pasture and depended on the proportion of main grass components in the sward, their rate of development and competitive abilities. Existence of white clover in the sod with *Poa pratensis* was very significant for the surface cover at the beginning of the study. In the sward with *Lolium perenne*, *Trifolium repens* contributed to organic soil protection, particularly after the detrimental effect of harsh winters conditions on young sward components.

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# Silage quality of faba-bean alone or with triticale growing organically.

## I: Effect of wilting

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### Abstract

The aim of this work has been to evaluate the effect of wilting on ensilability parameters, chemical composition and effluent production in winter intercrops and their silages. An intercrop of triticale (*x Triticosecale* Wittm.) and fava bean (*Vicia faba* L.) grown organically was carried out in experimental assays during winter 2004. The total yield was divided in half, with one half prepared for ensiling immediately and the other half wilted for 24 hours before ensiling. Both parts were subdivided in half again to provide laboratory silages of an intercrop of fava bean and triticale and fava-bean alone. The results showed that 24 hours of wilting were enough to improve the fermentability coefficient without significant changes on forages chemical composition and to avoid effluent production and the lost of nutrients during the fermentation process. Wilting forages induced an important fall in the proportion of ammonia nitrogen ( $p < 0.001$ ), with the ammonia synthesis lower in the intercrop than in silage made from only fava bean ( $p < 0.05$ ). The pattern of fermentation was not affected by wilting.

Keywords: intercrop, wilting, ensilability, chemical composition, effluents, organic culture.

### Introduction

Fava bean (*Vicia faba* L.) has an important role in organic as well as conventional farming due their lower dependence on N fertilization. Moreover, it has a good ensilability pattern due to its low buffering capacity (BC) and adequate content of water soluble carbohydrates (WSC) for the fermentation process (Argamentería *et al.*, 2004). However, when it is harvested at the immature legume-seed stage, the dry matter (DM) content is low and as a consequence the effluent production during the silage process is high. In recent years, cereal-legumes winter intercrops have been used in wet areas of North West Spain (Martínez *et al.*, 2002) to increase the DM of the crops, with the best results obtained from triticale (*x Triticosecale* Wittm.) and fava bean intercrops, despite these bi-crops reducing digestibility and crude protein (CP) concentrations (Argamentería *et al.*, 2004). The use of triticale, harvested at the phenological stage of initial grain development, with high energy and protein concentrations, does not increase the DM content sufficiently in the intercrop. For this reason, it is necessary to wilt the intercrop before silage making. The aim of the current work has been to evaluate the effect of wilting on ensilability, nutritive and fermentative value and also effluent production in intercrop silages of triticale and fava bean grown under organic management.

### Materials and methods

A winter intercrop of triticale and fava bean grown organically was conducted in the North West of Spain, during the winter of 2004 on an experimental plot area of 360 m<sup>2</sup>. This intercrop was established on an old low-input mixed sward (*Lolium perenne* and *Trifolium repens*) used for grazing and without NPK fertilization. The seeding rate was 159 grains m<sup>-2</sup> for the triticale and 26 seeds m<sup>-2</sup> for the fava bean.

After 14 weeks of growth samples were collected in a single cut system in spring 2005, with a ratio of 6.3:1 in the number of triticale to fava bean plants. Plants from the existing sward were discarded. Samples of the intercrop were analyzed for ensilability characteristics and chemical composition. The total yield was divided in half, with one half prepared for ensiling immediately and the other half wilted for 24 hours. Before ensiling, both samples were subdivided in half again to provide laboratory silages

of both intercrop triticale and fava bean (TFB) and fava-bean alone (FB) following the hand separation of triticale. Four types of experimental silages (fava-bean and intercrop, wilted (W) and non wilted (D)) were made according to Martínez and de la Roza (1997) with three replicates of each silage. The silages were opened after 80 days and effluent production was recorded during this period. At the end of the fermentative process the silages were opened and, immediately a sub-sample was measured to determine the pH, ammonia-N by UVvis and lactic acid and volatile fatty acids by HPLC in the extract. The rest of the silage was freeze-drier and analyzed for DM, ash and CP (AOAC, 1984), neutral detergent fiber (NDF; Van Soest *et al.*, 1991) and cellulase digestibility to estimate metabolizable energy (ME) by ARC (1980).

## Results and discussion

The results in Table 1 show an increase in the DM content of the wilted forages, with few changes occurring in their chemical composition. The increase in DM was higher in the intercrop than fava bean. With 24 hour of wilting, the fermentability coefficient (FC; Schmidt *et al.*, 1971 cited by Weissbach, 1999) increased, due to the WSC proportion increasing and the BC falling markedly.

Table 1. Forages characteristics of fava bean and intercrop with triticale, freshly and wilted.

	Fava bean		Triticale + Fava bean	
	Fresh	Wilted	Fresh	Wilted
Ensilability characteristics				
DM (g kg <sup>-1</sup> )	183	280	206	361
WSC (g kg <sup>-1</sup> DM)	94	142	201	251
BC (meq NaOH kg <sup>-1</sup> DM)	319	221	218	127
FC	21	33	28	52
Chemical composition and estimated energy				
CP (g kg <sup>-1</sup> DM)	173	166	117	111
NDF (g kg <sup>-1</sup> DM)	519	491	550	534
ME (MJ kg <sup>-1</sup> DM)	9.3	8.9	9.5	9.5

DM. dry matter; WSC: water soluble carbohydrates; BC: Buffer capacity; FC: Fermentability coefficient; CP: Crude protein; NDF: Neutral detergent fiber; ME: Metabolizable energy.

Twenty-four hours of wilting was enough to avoid effluent production during the fermentation period. The effects of forage type and wilting on the chemical composition are shown in Table 2. Wilting did not affect the silage pH, though TFB pH was lower than FB pH (4.12 vs 4.36 respectively,  $p < 0.05$ ). The wilting period did not affect the chemical composition of the silages, except the DM content (283 vs 200 g kg<sup>-1</sup> respectively,  $p < 0.001$ ). The differences due to the type of forage show higher values of ash (60 g kg<sup>-1</sup>DM) and CP (203 g kg<sup>-1</sup>DM) and a lower proportion of DM (208 g kg<sup>-1</sup>DM) and NDF (490 g kg<sup>-1</sup>DM) in FB than TFB silages (51, 129, 275 and 575 respectively,  $p < 0.001$ ). The interaction in DM content could be explained by the proportional increase in the DM of FB which was higher than in the TFB. Wilting increased the ash content in FB due to loss of the leaf fraction, with the ME of the TFB unaffected by wilting.

Wilting led to an important decrease in the proportion of ammonia-N (15 vs 33 g NH<sub>3</sub> kg<sup>-1</sup>N,  $p < 0.001$ ) without any effect on CP content, with ammonia synthesis lower in TFB than in the FB silages ( $p < 0.05$ ). The fermentation characteristics were not affected by wilting. The TFB had a lower proportion of both lactic and propionic acids and higher proportion of acetic acid than FB, with the lactate to acetate ratio lower in the TFB despite their better FC.

Table 2. Effect of wilted on fava bean and intercrop triticale-fava bean silages.

	FB		TFB		s.e.	Significance		
	D	W	D	W		Forage	Wilted	Forage* Wilted
pH	4.36	4.36	4.11	4.14	0.048	*	NS	NS
DM (g kg <sup>-1</sup> DM)	178	238	222	328	2.2	***	***	***
Ash (g kg <sup>-1</sup> DM)	59	61	53	49	0.7	***	NS	*
CP (g kg <sup>-1</sup> DM)	199	206	131	127	2.9	***	NS	NS
NDF (g kg <sup>-1</sup> DM)	492	488	574	577	6.9	***	NS	NS
ME (MJ kg <sup>-1</sup> DM)	9.3	8.8	8.9	8.8	0.10	NS	NS	NS
Ammonia (g NH <sub>3</sub> kg <sup>-1</sup> N)	30	18	37	13	1.1	NS	***	*
Lactic acid (g kg <sup>-1</sup> DM)	60.2	53.8	45.6	34.2	6.11	NS	NS	NS
Acetic acid (g kg <sup>-1</sup> DM)	48.9	45.2	68.7	53.1	4.00	NS	NS	NS
Propionic acid (g kg <sup>-1</sup> DM)	11.6	9.7	2.8	3.6	1.08	**	NS	NS
Butyric acid (g kg <sup>-1</sup> DM)	7.2	3.1	5.1	4.7	0.65	NS	NS	NS

\*, \*\* and \*\*\* significant at 0.05, 0.01 and 0.001% levels respectively. NS p>0.05.

## Conclusions

The results of the present study indicate that to wilt the forage 24 hours before making silage improves the fermentability coefficient without significantly changing the chemical composition of the forage. In addition, this period of wilting is sufficient to avoid effluent losses during fermentation process and also reduce ammonia nitrogen synthesis without changes in CP content.

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# Nutritional interest of growing mixtures of grasses and legumes with and without tannins

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## Abstract

This study aimed at assessing the effect of growing grass and legume species in association rather than separately, on yield, chemical composition and estimated organic matter digestibility (OMD). Five mixtures each composed of two grasses and two legumes with and without tannins were simultaneously grown at the same place without N fertilisation. Grasses and legume species differed among mixtures. Four mixtures consisted mainly of legumes (60 to 80 %) dominated by sainfoin or red clover, and the fifth of grasses (73%). Total nitrogen (N), soluble N, cell-wall contents and pepsin-cellulase digestibility were determined on the mixtures, the grass and legume parts of them and on the pure species grown alone. Dry matter and N yield of the mixtures were higher than that of pure grass crops and close to that of pure legume crops. Mixtures had a higher and less soluble N content than that calculated from N content of the pure species grown alone. Tannins in sainfoin and *Lotus* reduced soluble N only in mixtures. Cell-wall content (NDF) in mixtures was 2 points lower than that calculated from pure crops and estimated OMD was higher only in the grass rich mixture.

Keywords : grasses, legumes, mixture, pure crop, forage quality.

## Introduction

The new economical constraints towards more sustainable farming systems enhanced mixed cropping of grasses and legumes to combine the agronomic benefits of both forage families. These mixtures allow a high and regular production of a good quality feed, with low N fertilisation and consequently a better N use at both the land and animal level.

The aim of this work was to assess the effects of growing grasses and legumes as mixtures rather than separately on biomass yield, chemical composition and nutritive value for the ruminants. The effect of tannins contained in some legumes on protein value was of a particular interest.

## Materials and methods

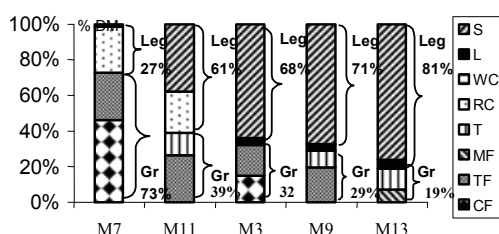


Figure 1. The botanical composition (%DM) of the 5 grass-legumes mixtures.

The cropping was done in Provins (Brie champenoise, France) on a silt calcareous soil (pH 7.9) with a mean annual rainfall of 650mm. Five mixtures (figure 1) chosen for their persistency, each composed of

four species - 2 grasses (Gr) and 2 legumes (Leg) with or without tannins- and the same species grown alone were harvested, at the first growth cycle, (may 2004), on the third year of cropping. The components were sainfoin (S), *Lotus corniculatus* (L), white clover (WC), red clover (RC), timothy (T), meadow fescue (MF), tall fescue (TF), cocksfoot (CF). The M7 mixture had a high proportion of grasses (73%), the others had an increasing proportion of legumes (61 to 81%).

The mixtures were hand - separated into species, dried (60°C), ground (1mm screen) and analysed as were the same species grown as pure crop. Total N (Kjeldahl method), soluble N (Ns) in artificial saliva, fibre contents (NDF) were determined in all the samples. PEG 4000 was used to assess the tannin protective effect on protein. The *in vivo* organic matter digestibility was estimated by a pepsin-cellulase method. Data were analysed by ANOVA using the GLM procedure of SAS, with mixture (M3 to M13), nature of forage (grass, legume, mixture), measured or calculated (from pure crops) data, as factors. When the treatment effect was significant ( $p < 0.05$ ), the means were compared by the Duncan multiple-range test.

## Results and Discussion

Dry matter (DM) yield (figure 2) of grasses grown as pure crops was about half that of legumes (2.7 vs 6.6 t ha<sup>-1</sup> respectively). The DM yield of mixtures (mean 5.8 t ha<sup>-1</sup>) was close to that of legume pure crops. Then DM yields of legume rich (>60%) mixtures were close together (6.0 t ha<sup>-1</sup>) and higher than M7 mixture (4.8 t ha<sup>-1</sup>,  $p < 0.05$ ). The 'mixture effect' is difficult to assess because the yield of species in the mixtures is unknown. For the sainfoin rich mixtures (M3, M9, M13), the theoretical DM yields calculated from the yield of pure crops using the observed proportion in the mixtures were higher than those measured. This agrees with the results of Springer *et al.* (2001).

No difference or the opposite was observed for grass rich mixtures (M7, M11). So mixing grasses and legumes might result in opposite effect depending on the proportion of the two plant families and the nature of species.

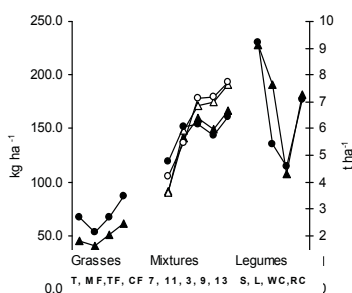


Figure 2. Effect of mixed cropping for grasses and for legumes in 5 mixtures on measured dry matter (●) and N (▲) yields of pure crops of these forages; comparison of dry matter and N yields of the mixtures to the calculated ones (○, △). See Figure 1 for the botanical nature of the forages

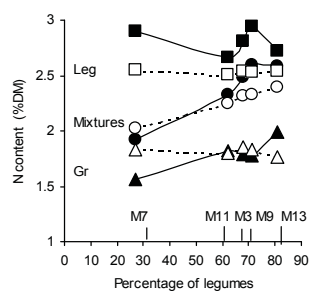


Figure 3. Effect of grass-legume mixed cropping in the 5 mixtures, on N content of grasses (△, ▲) and legumes (□, ■) when grown alone (△, □) or in mixtures (▲, ■); consequence on N content of the mixtures comparing the observed values (●) to the calculated ones from pure crops (○). See Figure 1 for the botanical composition of the mixture.

Calculated N contents (from pure crop composition, DM basis) among the grass (mean 1.8%) and the legume parts (mean 2.5%) of the mixtures (Figure 3) showed small variations. In all the mixtures the measured N content of the legume part was higher than the calculated one ( $p < 0.05$ ); for the grass part, the difference between the calculated and measured N content varied according to the mixture. Measured N content of mixtures increased with the proportion of legumes, more quickly than the calculated one, the difference being positive except for M7 ( $p < 0.05$ , fig 3). This positive effect of mixing might be the result of the decrease in DM yield and probably of biological interactions between grasses and legumes. Variations in N yield among mixtures paralleled that of DM yield (Figure 2).

Variations in measured Ns content (%N) among the grass parts of mixtures (20% to 29%, mean 23.4%) resulted from differences both in the species and their proportions in the mixtures. Soluble N in legume parts was slightly higher (mean 25.1%,  $p > 0.05$ ) and less variable. Calculated Ns contents were higher than measured values ( $p < 0.05$ ) and similar between grasses and legumes (27.9% and 29.0% respectively). Soluble N contents among the mixtures were similar (mean 25%) and 3 points lower ( $p < 0.05$ ) than values calculated from pure crops. This low but systematic decrease in Ns is a positive factor for the N value as was the increase in N content. For legumes, this 'mixture effect' might be explained by the protection of proteins by the tannins in sainfoin and *Lotus* highlighted by PEG addition.

The NDF contents (% DM, results not shown) of the grass parts of the mixtures were similar both among mixtures and between measured (mean 58.3%) and calculated values from pure crops (mean 59.0%). Conversely, NDF contents of the legume parts exhibited small variations among mixtures when calculated (mean 40.3%); observed values were lower (mean 37.6%). Measured NDF contents of the mixtures (mean 46.0%) were systematically lower than calculated values (48.6%,  $p < 0.05$ ). Globally the chemical composition of legumes was more altered by mixed cropping than that of grasses. Legumes appear younger in mixtures than in pure crops. It might suggest that their association with grasses slow down their growth.

The mean OMD of the grass parts of the mixtures calculated from pure crops (69.5%) were similar to that measured (70.7%) except for M7 (4 points increase). For the legume parts the calculated values were similar among mixtures (mean 70.3%) and lower than the measured values (mean 72.9%,  $p < 0.05$ ), this difference being mainly due to the 8 points decrease in M7. Consequently mean measured OMD of the mixtures (71.7%) tended to be higher than the calculated value (69.6%,  $p > 0.05$ ), this 'mixture effect' originating mainly from the grass rich M7 mixture. Conversely, Emile and Traineau (1991) observed a slight decrease in OMD when associating bromegrass with lucerne or red clover.

## Conclusion

As frequently shown, the largest effect of mixing grasses and legumes was observed on DM and N yields that increased compared to grass pure crops. However, the 'mixture effect' calculated from pure crop yield was negative, except for the grass rich mixture. Mixing grasses and legumes improved the nutritive value of the mixtures through its effect on total and soluble N content, on NDF content and on OMD. Additional research is needed to have a better knowledge of the factors involved in the mixing effect.

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# Assessment of palatability of different grass and legume species, and of their combinations

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## Abstract

Forage palatability is a relative characteristic accumulating all forage attributes related to intake and digestibility. In this context, the palatability of cocksfoot (*Dactylis glomerata* L.), crested wheatgrass (*Agropyron cristatum* (L.) Gaertn.), sainfoin (*Onobrychis viciifolia* Scop.), white clover (*Trifolium repens* L.) and bird's foot trefoil (*Lotus corniculatus* L.) was compared through the preference of cows for them during grazing. The above mentioned grass and legume species were grown alone and in all 10 possible two-component mixtures between them each of plots of 12 m<sup>2</sup> in four replications. The preferences of twelve cows for the different swards were assessed by visual observation, every 5 min. of each animal during the first hour after the animals were taken to the grazing paddock along four consecutive days, six hours per day. During the observed period the cows spent 48.7% of the time grazing legumes grown alone and their mixtures, 10.7% of the time grazing pure and mixtures grasses, and 40.6% of the time grazing grass:legume combinations. The ratio between time spent for grazing legumes, grass:legume mixtures and grasses was 5: 4: 1 which is similar to the grazed forage quantity of the respective legumes, grass: legume mixtures and grasses.

Keywords: palatability, grazing, grasses, legumes.

## Introduction

Forage intake is a value related to palatability, morphological, chemical and physical properties of the forage and depending on the animal properties and its responses to diets. In the presence of more than one forage the animals have the possibility of choosing and giving preference to the forages that have better acceptability, olfactory, or visual senses (Minson and Bray, 1986; Black *et al.*, 1989). The forage palatability is of importance when establishing new varieties of different grass species for grazing by ruminants. Gillet *et al.* (1983) assessed forage palatability by a test of consumption, or the so called crib cafeteria, where the animals had access to several cut green forages put in the crib. The obtained results in the crib cafeteria for the intake of the different forages were considered as relative values allowing comparisons between them. The preference of animals during grazing of different species give information of palatability but depends on many factors (Parsons *et al.*, 1994; Penning *et al.*, 1994).

The objective of this study was to compare the palatability of cocksfoot (*Dactylis glomerata* L.) and crested wheatgrass (*Agropyron cristatum* (L.) Gaertn.), and legumes sainfoin (*Onobrychis viciifolia* L.), white clover (*Trifolium repens* L.) and bird's foot trefoil (*Lotus corniculatus* L.), grown alone or in mixture between them by the preference of cows assessed by minutes eating each forage during the first hour of grazing. For the sake of brevity we called this method grazing cafeteria.

## Materials and methods

The trial was carried out at the Institute of Forage Crops in Pleven. Pure swards of cocksfoot, crested wheatgrass, sainfoin, white clover, bird's foot trefoil and their two-component mixtures between them each in four replications (plots of 12 m<sup>2</sup>), i.e. a total of 60 plots were used. Twelve cows were observed in paddock while grazing the third growth at the age of 42 days from the last cutting of swards. The swards in this study were grouped as follows: pure legumes as legume, legume: legume mixtures as legumes, pure grasses and grass: grass mixtures as grass, and grass:legume mixtures. The observations were made during grazing of third growth at the age of 42 days from the last cutting. During 4 consecutive days 12 cows, which grazed on the natural pasture before and after the trial were allowed to

graze for 6 h on the paddock with plots and their feeding behavior during the first hour was assessed by visual observation. Every 5 min the time spent by each animal grazing a determined plot was recorded. The total grazing time was considered 100% and the relative proportion of grazing time for each legume and grass in pure or mixed stands was determined. At the beginning and at the end of the trial dry mass yield of each sward were determined. The amount of grazed forage was determined by the difference between dry mass yield at the beginning and at the end of the four days trial. The amount of grazed forage from each sward was presented in relative value as a proportion of the total grazed forage.

## Results and discussion

The data on relative distribution of grazing time across the different sward types are represented in Fig. 1. The grazing time on grass:legume mixtures was the longest and representing 40.6% of the total grazing time, and that on pure grasses and their mixtures between them the shortest, accounting for only 10.7%. The cows spent over twice more time grazing pure legumes (23.9%) than grasses, which indicates the higher palatability of the legumes. The grazing time on pure legumes (23.9%) was almost equal to that on legume: legume mixtures (24.8%), which indicates that the different combinations had no effect on legume palatability. The total time spent grazing pure and mixtures legumes (48.7%) was longer than that grazing grass: legume mixtures (40.6%).

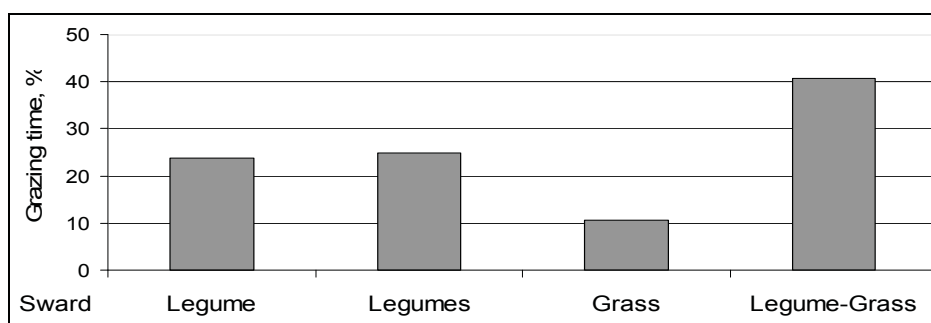


Figure 1. Time spent by cows in grazing: legume - pure legumes, legumes – legume mixtures, grass – pure grasses and grass mixtures, legume-grass – legume/grass mixtures.

The ratio of grazing time of pure and mixture legumes, grass: legume mixtures and grasses was 5:4:1 respectively. The results of grazed forage during 4-day and 6 hours per day (24 hours in total) grazing are represented on Fig. 2. Grazed quantity of legumes, either pure (33.3%) or in mixture (32.4%) was the maximal, and that of grasses the minimal (10.0%). Intake of pure legumes including sainfoin, white clover and bird's foot trefoil accounted for 33.3% of the total grazed forage, whereas intake of wheatgrass, cocksfoot and their mixture was 10% only. Grass: legume mixtures took an intermediate position with 24.3% of the total grazed forage. The ratio of the grazed forage during grazing period was 6.5:2.5:1.0 for pure and mixtures legumes, grass: legume mixtures, and pure and mixtures grasses respectively, and it is similar to the ratio of respective grazing time obtained in the first hour of the grazing period.



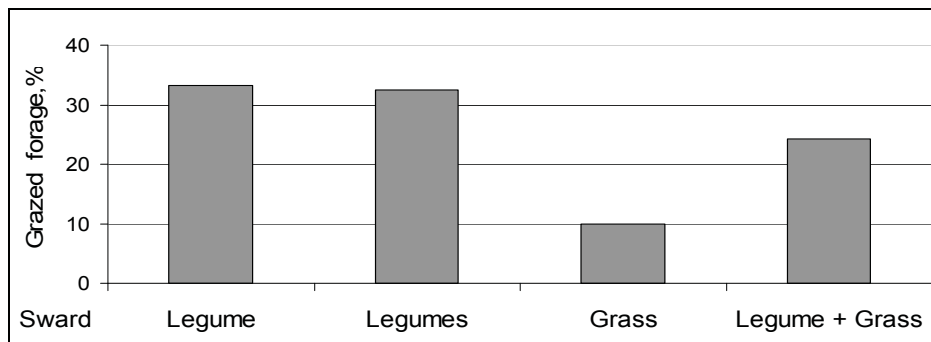


Figure 2. Grazed forage by cows, % of the total.

### Conclusion

The results obtained in the first hour of the grazing period suggest that animals prefer legumes, either pure or in mixture, to grasses. The cows spent over twice more time grazing pure legumes than pure grasses, which indicates the higher palatability of the legumes. The different two-component combinations between sainfoin, white clover and bird's foot trefoil had no effect on their palatability. The ratio of grazing time of pure and mixture legumes, grass:legume mixtures and grasses was respectively 5:4:1 which is approximately similar to the ratio of the grazed forage quantity from the same swards which was 6.5:2.5:1.0.

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## Silage quality of faba-bean alone or with triticale growing organically. II: Effect of *Lactobacillus buchneri*

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### Abstract

The purpose of this study was to evaluate the effect of *Lactobacillus buchneri* as an additive on the chemical composition and fermentative characteristics of winter intercrop cereal-legume silages. For this purpose, the field experiment was developed during winter 2004 in the wet area of North West of Spain. An intercrop of triticale (x *Triticosecale* Wittm.) and fava bean (*Vicia faba* L.) grown organically was carried out in an experimental plot. After harvest, four types of laboratory silages were made: intercrop triticale + fava bean or fava-bean alone with or without additive. The results showed that the use of *L. buchneri* as an additive increased the protein value of silage ( $p < 0.01$ ) without affecting  $\text{NH}_3\text{-N}$  synthesis. Nevertheless, this additive increased acetic and propionic acids ( $p < 0.001$ ) and, at the same time lactic acid content decreased ( $p < 0.001$ ).

Keywords: ensilability, intercrop, additive, chemical composition, effluent production, organic culture.

### Introduction

Nowadays, there is great interest in the use of biological additives for silages that can be used in organic farming, according to European regulation n°1804/1999. In this sense, there are several commercial products made with heterofermentative *Lactobacillus* cultures, for example *Lactobacillus buchneri*, which have been demonstrated to inhibit fungal growth (Nishino and Hattori, 2005). Moreover, these additives have been used to improve the aerobic stability of silages after long-term storage (Kung and Ranjit, 2001), to decrease losses during the fermentation process and to improve animal production (Kung *et al.*, 2003). The current study was designed to evaluate the effectiveness of *L. buchneri* as a biological inoculant on the losses during the fermentation process, chemical composition and fermentative characteristics of fava bean alone or in triticale + fava bean bi-crop silages. The crops were grown organically and harvested at the phenological stage of initial grain development for triticale and immature pod-stage for fava bean.

### Materials and methods

A field assay was developed in an inner valley of North West of Spain, during the winter 2004. A winter bi-crop of triticale (x *Triticosecale* Wittm.) and fava bean (*Vicia faba* L.) grown organically was carried out in a experimental plot of 360 m<sup>2</sup>. This intercrop was established at random on an old low-input mixed sward (*Lolium perenne* and *Trifolium repens*) previously used for grazing and without NPK fertilization. The seeding rates were 159 grains m<sup>-2</sup> for triticale and 26 seeds m<sup>-2</sup> for fava bean. Samples were chopped by a conventional forage harvester after 14 weeks of growth, with a ratio of 6.3:1 in the number of triticale to fava bean plants. After discarding the plants from the existing sward, the fresh samples of triticale and fava beans were analyzed for ensilability characteristics and chemical composition. Four types of laboratory silages were made: bi-crop triticale + fava bean (TFB) or fava-bean alone (FB), with (A) or without (D) additive, according to Martínez-Fernández and de la Roza (1997) and with three replicates per treatment. The additive used was *Lactobacillus buchneri* NCIMB strain 40788 (Lallemand Animal Nutrition, BP 59, Cedex, France) at a rate of  $1 \times 10^5$  cfu g<sup>-1</sup> of fresh forage. The laboratory silages were opened after 80 days, immediately a subsample was analysed to determine the pH, ammonia-N by UVvis, and lactic acid and volatile fatty acids by HPLC in the extract. The rest of silage was freeze-drier and analyzed for dry matter (DM), ash and crude protein (CP)

(AOAC, 1984), neutral detergent fiber (NDF; Van Soest *et al.*, 1991) and cellulase digestibility to estimate metabolizable energy (ME) by ARC (1980).

## Results and discussion

The ensilability characteristics, chemical composition and estimated ME of the forages before ensiling are shown in Table 1. As expected, the bi-crop had higher DM and lower CP proportions than fava bean alone. The fermentability coefficient (FC; Schmidt *et al.*, 1971 cited by Weissbach, 1999) was better in bi-crop as consequence of their higher proportion of water soluble carbohydrates (WSC) and lower buffer capacity (BC). The proportion of total DM losses was higher in FB than TFB (211 vs 171 g kg<sup>-1</sup> respectively, p<0.01), these losses are directly related to effluent production (97 vs 67 L t<sup>-1</sup> for FB and TFB respectively). The losses were higher in silages with the additive compared to silages without additive (228 vs 154 g kg<sup>-1</sup> respectively, p<0.001).

Table 1. Forage characteristics of fava bean and intercrop with triticale.

	Fava bean	Intercrop
Ensilability characteristics		
DM (g kg <sup>-1</sup> )	232	284
WSC (g kg <sup>-1</sup> DM)	118	226
BC (meq NaOH kg <sup>-1</sup> DM)	270	173
FC	27	40
Chemical composition and estimated energy		
CP (g kg <sup>-1</sup> DM)	169	114
NDF (g kg <sup>-1</sup> DM)	505	542
ME (MJ kg <sup>-1</sup> DM)	9.1	9.5

DM: Dry matter; WSC: Water soluble carbohydrates; BC: Buffer capacity; FC: Fermentability coefficient; CP: Crude protein; NDF: Neutral detergent fiber; ME: Metabolizable energy.

The results of chemical composition, estimated ME and fermentative characteristics according to additive incorporation and type of forage are showed in Table 2.

Table 2. Effect of *Lactobacillus buchneri* as additive on fava bean and intercrop silages.

	FB		TFB		s.e.	Significance		
	D	A	D	A		Forage	Additive	Forage * Additive
pH	4.12	4.61	4.01	4.23	0.018	***	***	**
DM (g kg <sup>-1</sup> DM)	196	186	262	235	10.2	**	NS	NS
Ash (g kg <sup>-1</sup> DM)	58	62	48	54	0.53	***	***	NS
CP (g kg <sup>-1</sup> DM)	193	213	122	136	2.2	***	**	NS
NDF (g kg <sup>-1</sup> DM)	479	501	553	597	5.5	***	**	NS
ME (MJ kg <sup>-1</sup> DM)	9.4	8.8	9.0	8.6	0.09	NS	*	NS
Ammonia (g NH <sub>3</sub> kg <sup>-1</sup> N)	19.5	28.0	25.6	24.0	2.41	NS	NS	NS
Lactic acid (g kg <sup>-1</sup> DM)	89.6	24.3	52.8	27.0	2.07	***	***	***
Acetic acid (g kg <sup>-1</sup> DM)	31.6	62.5	44.0	77.8	1.80	**	***	NS
Propionic acid (g kg <sup>-1</sup> DM)	5.7	15.6	2.0	4.4	0.70	***	***	*
Butiric acid (g kg <sup>-1</sup> DM)	6.7	6.6	5.8	3.9	0.67	NS	NS	NS

\*, \*\* and \*\*\* Significant at 0.05, 0.01 and 0.001 % levels respectively. NS p>0.05.

The silage pH was affected by the additive (4.07 vs 4.42 for D and A respectively, p<0.001), and TFB silage pH was lower than FB (4.12 vs 4.36 respectively, p<0.001). The differences due to the addition of *L. buchneri* showed higher values of ash (58 vs 53 g kg<sup>-1</sup>DM; p<0.001), CP (174 vs 157 g kg<sup>-1</sup>DM; p<0.01) and NDF (549 vs 516 g kg<sup>-1</sup>DM; p<0.01), but lower energetic value (8.7 vs. 9.2 MJ ME kg<sup>-1</sup>DM; p<0.05) than silages without additive. Nevertheless, the *L. buchneri* did not affect NH<sub>3</sub>-N synthesis despite the bigger proportion of CP. The fermentation characteristics were affected by the

additive, as expected with the heterofermentative *Lactobacillus* culture metabolism the propionic and acetic acids synthesis, whose combined action is antifungal, increased ( $p < 0.001$ ) during the fermentation process, with a decrease in lactic acid content ( $p < 0.001$ ). Similar results have been reported with both cereal (Kung and Ranjit, 2001) and legume (Kung *et al.*, 2003) silages.

### Conclusions

The results showed that the use of *Lactobacillus buchneri* as an additive increased the protein value of silage without affecting  $\text{NH}_3\text{-N}$  synthesis. In addition, this additive caused a change in the amount of final fermentation products, with changes from lactic acid to acetate and propionate according to metabolism of heterofermentative biological cultures.

### Acknowledgements

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## Field surveys of *Fusarium* root rot in organic red clover leys

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### Abstract

The prevalence of the root rot complex of red clover in forage and green- manure leys was determined on organic farms in seven provinces in south and central Sweden during 2003 and 2004. The results show that the injuries caused by root rot are widely distributed already in the seedling year. Average disease severity index (DSI) for internal root symptoms was 12 the seedling year, 28 in first year leys and 61 in second year leys.

Keywords: *Trifolium pratense*, organic leys, *Fusarium* root rot, persistence.

### Introduction

Red clover (*Trifolium pratense* L.) is the most important legume crop in Swedish forage production, and the major component in green- manure crops on many organic farms. However, red clover is infected by several fungal pathogens affecting growth, persistence and overwintering capacity. Clover rot (*Sclerotinia trifoliorum* Erikss) and stem nematode (*Ditylenchus dipsaci*) have been well known for a long time, and are handled with by resistant cultivars, whereas the importance of *Fusarium* root rot was practically unknown in Sweden in the early 1970s (Rufelt, 1986). Root rot is caused by several soilborne pathogens where *Fusarium spp* are most important. A nationwide survey undertaken in the mid 1970s showed that the occurrence of root rot was very frequent (Rufelt, 1979). During the past few years organic farmers and advisors have reported about poor establishment and sustainability in red clover leys. Recently premature ripeness of red clover seed crops are reported as well as poor growth the second ley year caused by severely infected roots (Wallenhammar, unpublished). In a co-operation project 2003 undertaken in three provinces in Sweden the prevalence of root rot of red clover in forage leys and green- manure leys was determined. This project was extended in 2004 to seven provinces in south and central Sweden, in order to facilitate a sustainable forage production.

### Material and methods

In 2003 sampling was mainly undertaken in second year leys, whereas sampling in 2004 was evenly distributed between 15 first and second year leys in the beginning of July. At the end of October five fields of the seedling year were sampled. Sampling was carried out on ten positions, where four plants were dug out, along the diagonal of the field. In all 5,567 plants were dug out and examined in 2004. The red clover plants were transported to a laboratory where they were rinsed in running water. The external root symptoms were read as lesions. The plants were splitted with a scalpel. The internal root symptoms were read as the degree of discolouration in the vascular tissues following Rufelt (1979). A disease severity index (DSI) was calculated. Information on crop rotation, fertilizing, soil type, management system etc was collected by a questionnaire.

## Results and discussion

The results from 2003 show a general distribution of root rot since 93 and 97 % of the plants respectively were infected in the provinces of Örebro and Östergötland, while 78 % of the plants investigated in Skåne were infected. The corresponding DSI was 51, 59 and 18 respectively. The results from the survey 2004 show that the injuries from root rot are yet widely distributed on organic farms throughout the provinces investigated. 96 % of the plants in second year leys on average showed internal root symptoms (Table 1). Average DSI was determined to 61. First year leys also showed high levels of infection since 71 % of the plants showed internal root symptoms. DSI ranged from 19 to 33. External root symptoms are presented in Table 2.

Table 1. Internal root symptoms seedling year, first year ley and second year ley 2004.

Province	<i>Seedling year</i>		<i>First year ley</i>		<i>Second year ley</i>	
	Infected plants %	Disease severity-index (DSI)	Infected plants %	Disease severity-index (DSI)	Infected plants %	Disease severity-index (DSI)
Örebro län	76	24	94	34	100	77
Östergötland	28	7	73	21	99	64
Skåne	1,5	5	80	31	90	56
Värmland	21	9	62	19	91	53
Halland	49	12	96	29	100	62
Uppsala	44	18	69	26	93	46
Södermanland	29	9	80	33	96	71
Average	35	12	79	28	96	61

The readings of the plants in their seedling year showed a greater variation in infection level ranging from 1.2 to 76 % infected plants. Average DSI was determined to 12.

Table 2. External root symptoms seedling year, first year ley and second year ley 2004.

Province	<i>Seedling year</i>		<i>First year ley</i>		<i>Second year ley</i>	
	Infected plants %	Disease severity-index (DSI)	Infected plants %	Disease severity-index (DSI)	Infected plants %	Disease severity-index (DSI)
Örebro län	97	32	91	35	100	77
Östergötland	31	9	69	25	99	64
Skåne	2	8	80	31	91	55
Värmland	19	8	82	40	100	76
Halland	93	25	99	36	100	63
Uppsala	19	7	63	24	100	74
Södermanland	5	1,25	89	44	99	76
Average	38	13	82	34	98	69

Information on field data (results not shown here) showed that many farmers grow ley on large acreages, the share in crop rotation ranged from 10 to 100 %. Many growers are pleased with the clover stand, despite large infections occurred, while several farmers have observed a degeneration of their clover leys.

Many of the soil-borne fungi infecting red clover are considered as weak pathogens (Rufelt 1986). However, when disease-enhancing abiotic factors are prevailing, or when frequent cutting and

harvesting occur, disease can be severe. This survey reveals that the root rot complex is a serious problem in Swedish organic clover leys. The results are well in line with the findings reported by Rufelt (1979) when average DSI of internal root symptoms was 6.1 the seedling year, 38 the first year ley and 61.6 the second year ley. However, these results did not prove that the root rot complex is a fungal disease. It was clear that the fungi isolated were pathogenic, but the results did not exclude that environmental stress was the primary cause of root rot.

Pathogen isolations from red clover and white clover collected on organic farms in central Sweden showed presence of a number of pathogenic fungi dominated *F. avenaceum* and *F. culmorum* (Lager and Gerhardson, 2002). Isolates of *F. culmorum* and *F. avenaceum* from white- and red clover roots reduced shoot dry weight of timothy and meadow fescue, (Lager and Wallenhammar, 2002). Furthermore Lager (2002) showed that the growth of particularly wheat, peas but also barley and oats were significantly decreased in red-clover rotations.

Several soil-borne pathogens that normally induce sublethal root rots, have wide host ranges. Hence, the build-up of soil-borne inoculum is inevitable in organic crop rotations increasing the pressure on red clover.

### Conclusions

The survey shows that injuries caused by the root rot complex are widely distributed on organic farms in south and central Sweden thus affecting the persistence of red clover. Recent greenhouse studies indicate that the pathogens affecting red clover have wide host ranges increasing the pressure on red clover in prevailing crop rotations. Access to red clover varieties with some degree of resistance to the root rot complex is necessary in order to improve the persistence of red clover stands.

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# The impact of perennial forage legumes in simple mixtures with cocksfoot

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## Abstract

Cocksfoot-legume mixtures are suitable for a wide range of conditions, including soils of low general fertility. The impact of two legumes species: *Trifolium repens* L. (white clover) and *Lotus corniculatus* L. (birdsfoot trefoil) in simple mixtures with *Dactylis glomerata* L. (cocksfoot), was studied at 3 levels of fertilization: N<sub>0</sub>, N<sub>50</sub>, N<sub>100</sub>. The forage yields registered by the white clover-cocksfoot mixtures exceeded the yields of pure cocksfoot culture with: 1.7 t DM ha<sup>-1</sup> at N<sub>0</sub>, with 0.9 t DM ha<sup>-1</sup> at N<sub>50</sub> and with 0.1 t DM ha<sup>-1</sup> at N<sub>100</sub>. The forage yield gains, in the case of birdsfoot trefoil – cocksfoot mixtures, were: 3.0 t DM ha<sup>-1</sup> at N<sub>0</sub>, 1.1 t DM ha<sup>-1</sup> at N<sub>50</sub> and 0.3 t DM ha<sup>-1</sup> at N<sub>100</sub>. Besides the forage yield gains, the presence of legumes assured a higher forage quality and a related higher animals performances.

Keywords: *Dactylis glomerata*, *Trifolium repens*, *Lotus corniculatus*, simple mixtures, N fertilization

## Introduction

The enrichment of soil with nitrogen, fixed biologically by bacterial symbiotic activity of legumes, reduces the amount of N fertilizers and the risks of pollution in the conditions of an ecological agricultural practice. At the same time, the presence of legumes in mixtures with grass increases the forage quantity and quality (Breazu *et al.*, 2002, Razec *et al.*, 2001).

This study aimed to compare the influence of two of the most important legumes, *Trifolium repens* – white clover and *Lotus corniculatus* – birdsfoot trefoil in simple mixtures with *Dactylis glomerata* – cocksfoot, an important grass species for the temperate grasslands.

## Materials and methods

The study was performed during 2001-2004, at the Grassland Research and Development Institute, Brasov, at an altitude of 560 meters about sea level, on a sandy loam chernozem soil, with a pH of 5.8, and with a content of 41 ppm P and 164 ppm K. Two varieties of cocksfoot were used: Poiana and Regent, each in pure stands and in mixture with birdsfoot trefoil – cv. Doru and white clover – cv. Carpatin. The seed rates was: 25 kg ha<sup>-1</sup> for cocksfoot, 5 kg ha<sup>-1</sup> for birdsfoot trefoil and 3 kg ha<sup>-1</sup> for white clover. The nitrogen levels were: N<sub>0</sub>, N<sub>50</sub> (applied in spring, at the beginning of growing season) and N<sub>100</sub> (50 kg ha<sup>-1</sup> applied in spring and 50 kg ha<sup>-1</sup> after the first cut). The studies were conducted on 12 m<sup>2</sup> plots, with four replications, distributed at random, for each grass cultivar (Poiana, Regent), on each type of culture (simple culture, mixture with white clover and mixture with birdsfoot trefoil), on each level of fertilization (N<sub>0</sub>, N<sub>50</sub>, N<sub>100</sub>).

The experimental period was very dry for this geographical area, the rainfall in the second and in the third year of experiments being about 300 mm lower than the average over 59 years. Also higher temperatures and sunburn had negative effects on plant growth and regeneration. Consequently, 2 cuts in the year of establishment, 3 cuts in the second year and only 2 cuts in the third and fourth experimental years were obtained. The yields were 40-50% lower than in normal climatic years. Botanical analyses, for the determination of grass / legumes ratio, were made at every cut. For statistical interpretation the analysis of variance was used.



## Results and discussion

Table 1 gives the average DM yields, during the 4 years of experimentation, for three levels of fertilization and shows that the yields of the mixtures were always higher than the pure stands in all experimental variants.

Table 1. Dry matter yields (t DM ha<sup>-1</sup>) of cocksfoot, in pure culture and in a mixture with legumes, at different levels of fertilization (N<sub>0</sub>, N<sub>50</sub>, N<sub>100</sub>) and years of exploitation (I-IV).

Culture type	Variety	N <sub>0</sub>					N <sub>50</sub>					N <sub>100</sub>					M
		I	II	III	IV	M	I	II	III	IV	M	I	II	III	IV	M	
Pure culture	Poiana	4.1	3.0	1.5	0.9	2.4	4.0	6.2	3.5	1.0	3.7	5.0	7.9	3.4	1.5	4.5	3.5
	Regent	3.2	2.4	1.5	0.7	2.0	4.6	6.1	2.2	1.1	3.5	6.0	8.2	3.1	1.4	4.7	3.4
	Mean	3.7	2.7	1.5	0.8	2.2	4.3	6.2	2.9	1.0	3.6	6.0	8.0	3.3	1.4	4.6	3.5
Cocksfoot + white clover	Poiana	4.5	7.3	2.6	1.2	3.9	4.2	8.0	3.7	1.6	4.4	5.2	8.0	3.4	1.5	4.5	4.3
	Regent	3.7	7.8	3.0	1.2	3.9	4.1	8.0	4.0	1.8	4.5	5.0	8.1	3.4	1.7	4.8	4.4
	Mean	4.1	7.6	2.8	1.2	3.9	4.2	8.0	3.9	1.7	4.5	5.1	8.6	3.4	1.6	4.7	4.4
Cocksfoot + birdsfoot trefoil	Poiana	3.6	9.0	5.5	2.8	5.2	3.4	7.6	4.2	2.3	4.4	4.8	6.0	4.8	1.9	4.9	4.8
	Regent	3.5	7.7	6.0	2.9	5.0	4.1	7.6	5.7	2.4	5.0	5.2	7.8	4.5	2.2	4.9	5.0
	Mean	3.6	8.4	5.8	2.8	5.2	3.8	7.6	5.0	2.3	4.7	5.0	7.9	4.7	2.0	4.9	4.9

DL 5% = 0.260 for the type of culture (A).

DL 5% = 0.184 for the level of fertilization (B).

DL 5% = 0.451 for A x B.

The yields obtained by the cocksfoot – white clover mixtures exceeded the yields of cocksfoot pure culture with 1.7 t ha<sup>-1</sup> DM at N<sub>0</sub>, with 0.9 t ha<sup>-1</sup> DM at N<sub>50</sub> and with 0.1 t ha<sup>-1</sup> DM at N<sub>100</sub>. In the case of mixtures with birdsfoot trefoil the yields were higher with: 3.0 t ha<sup>-1</sup> DM at N<sub>0</sub>, with 1.1 t ha<sup>-1</sup> DM at N<sub>50</sub> and with 0.3 t ha<sup>-1</sup> DM at N<sub>100</sub>, the highest differences being at the unfertilized variants. It must be pointed out that, on average, the cocksfoot – birdsfoot mixture at N<sub>0</sub> exceeded the yields registered in all the other variants, even at N<sub>50</sub> and N<sub>100</sub>. On average, the yields of mixtures with birdsfoot trefoil were higher than with white clover, in the drought conditions of experimental years, birdsfoot trefoil being more resistant.

The highest yields and also the highest differences between the pure culture and grass – legume mixtures were registered in the second year. The gains achieved by the mixture with white clover were: 4.9 t ha<sup>-1</sup> DM, at N<sub>0</sub> 1.8 t ha<sup>-1</sup> DM at N<sub>50</sub> and 0.6 t ha<sup>-1</sup> DM at N<sub>100</sub> while in the case of mixtures with birdsfoot trefoil the gains were: 5.7 t ha<sup>-1</sup> DM at N<sub>0</sub>, 1.4 t ha<sup>-1</sup> DM at N<sub>50</sub> and at N<sub>100</sub> the pure culture yield was with 0.1 t ha<sup>-1</sup> DM lower, cocksfoot using efficiently the N fertilizers.

The differences, from a competition point of view, between the two middle late cocksfoot varieties used in experiments were very low. The productivity was strongly affected by the drought conditions of the experimental years. The analysis of variance showed that the differences between the type of culture, between the levels of fertilization and the interaction of these factors are statistically assured. The percentage of legume in mixtures, in connection with the level of fertilization is presented in Table 2.

Table 2. The percent of legumes in mixtures with cocksfoot, on different levels of N fertilization (N<sub>0</sub>, N<sub>50</sub>, N<sub>100</sub>) and years of exploitation (I-IV).

Culture type	Variety	N <sub>0</sub>					N <sub>50</sub>					N <sub>100</sub>				
		I	II	III	IV	M	I	II	III	IV	M	I	II	III	IV	M
Cocksfoot + white clover	Poiana	1.8	34	33	36	30	11	17	5	13	12	22	6	3	18	12
	Regent	29	30	15	20	24	11	19	6	17	13	14	3	2	11	8
	Mean	24	32	24	28	27	11	18	6	15	13	18	5	3	15	10
Cocksfoot + birdsfoot trefoil	Poiana	23	48	61	77	52	9	31	31	66	34	6	8	7	39	15
	Regent	23	59	58	80	55	6	31	20	65	31	6	5	12	32	14
	Mean	23	54	60	79	54	8	31	26	66	33	6	7	10	36	15

On average, the percentage of legume in mixtures with cocksfoot were: 27% white clover and 54% birdsfoot trefoil at N<sub>0</sub>, 13% white clover and 33% birdsfoot trefoil at N<sub>50</sub> and 10% white clover and 15% birdsfoot trefoil at N<sub>100</sub>. Therefore, the percentage of legumes was strongly influenced by the level of fertilization and the species. In the unfertilized variants, white clover was maintained at about the same level, except in the second year, while the birdsfoot proportion increased from 23% in the first year, to 79% in the fourth year, the species having a higher resistance to drought. The level of 50 kg ha<sup>-1</sup> had a high negative influence over the both legumes species. In the first year, the percentage of birdsfoot trefoil was very reduced, but in the following years, the percentage increased. For white clover, there was better establishment in the first year (11%), but the percentage was very reduced in the third year (6%). This was the most dry experimental year (the rainfall was 310 mm lower than the long-term average). At N<sub>100</sub> the negative effect of drought was further emphasized for both species. In the fourth experimental year the rainfall was nearer to the average and as result, an increase of legumes was observed.

### Conclusions

The presence of legumes in mixtures with cocksfoot provides higher yields compared with pure cultures and at the same time a better forage quality. Legumes, maintained at an optimal level, can replace N fertilizers with positive effects on a decrease in environment pollution.

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# New white clover cultivars in Norway – effects on yield, feed quality and persistence

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## Abstract

In this study four Norwegian white clover cultivars were compared with the Danish variety Milkanova under farm site conditions in the different climatic conditions of Norway. A series of field experiments were established with five different white clover cultivars sown in grass mixtures. The swards were defoliated at a sward height of 20 cm to simulate grazing.

There were no clear differences in yield production or clover content between the cultivars in the southerly/lowland regions when averaged over three years. Milkanova is a relatively high yielding variety, but has a poor overwintering capacity. The grass/clover mixtures containing Milkanova had the highest yield in the first years of the experiment, but the yields declined and tended to be lower than other cultivars in the third year, with significantly lower clover content. In the alpine/northerly regions, grass/clover mixtures containing Milkanova had lower yield, and lower clover content from the start of the experiment. Both protein, ash and NDF contents were closely linked with white clover content in the swards; with more protein and ash less NDF in plots containing more clover. Feed quality was lower in 3<sup>rd</sup> cut than in 1<sup>st</sup> cut.

Keywords: white clover, grass mixture, ash, NDF, crude protein.

## Introduction

Increased demand for grassland species suitable for grazing purposes and more focus on feed quality as well as organic agriculture has led to more interest in white clover (*Trifolium repens* L.) in Norway. Previously, the Danish cultivar, Milkanova, was the only commercially available white clover cultivar on the market, and its lack of winter hardiness restricted the use of this species especially in the alpine or northern regions of Norway. With the release of new winter hardy Norwegian cultivars (Rapp, 1996), farmers see the potential of using more white clover, both for improved feed quality and for N-fixing purposes. However, although these new cultivars have been evaluated in testing programmes, yield production, feed quality and persistence have still not been evaluated under farm conditions in the different regions of Norway.

## Materials and methods

Five cultivars of white clover were sown at a seeding rate of 5 kg ha<sup>-1</sup> in a mixture with grasses, 20 kg ha<sup>-1</sup>, in plots of 1.5 x 7m<sup>2</sup> with three replicates. The grass mixture consisted of smooth meadowgrass (*Poa pratensis* L.), meadow fescue (*Festuca pratensis* Huds.) and timothy (*Phleum pratense* L.) and in the Region South, perennial ryegrass (*Lolium perenne* L.) was included. A treatment with pure grass was also included. The field experiments were established in 1997 and 1998 on 9 farms spread throughout Norway. The farms were grouped into two regions according to climatic conditions: southern and western lowland (Region South) and alpine/northern (Region North). The trial was fertilized with 62 kg N ha<sup>-1</sup>, 26 kg P ha<sup>-1</sup> and 96 kg K ha<sup>-1</sup> divided into two dressings in Region North, or 93 kg N ha<sup>-1</sup>, 39 kg P ha<sup>-1</sup> and 144 kg K ha<sup>-1</sup> in three dressings in Region South. The swards were defoliated at a 5cm stubble height when the sward height was approximately 20 cm to simulate grazing. This resulted in three to six cuts per year depending on the length of the growing season. Dry matter (DM) yields were measured after drying yield samples (60°C, 48h). N-contents and aspects of feed quality were analysed in samples from first and third cut by the use of near infrared reflectance

spectroscopy (NIRR). Clover content of the swards was estimated visually before each harvest of the plots.

## Results and discussion

There were no differences between cultivars in DM yields in the first year after establishing the fields (Table 1). In the third year after establishment, grass mixtures containing the Danish variety Milkanova tended to have lower yields and significantly lower clover content in both regions. Milkanova has a low ability to withstand winter stresses, and may therefore have low persistence in the swards under Norwegian conditions (Rapp and Junttila, 2000). In Region 1 the cultivar 'Snowy', which is bred from populations from lowland/western part of Norway, had the highest yields and highest clover content over time. It also performed well in Region 2, but there were no differences in clover persistence between the Norwegian cultivars in this region.

Table 1. Total dry matter (DM) yields and clover content in grass/clover mixtures of 1<sup>st</sup> and 3<sup>rd</sup> years leys in Region 1 (5 farms, south/lowland) and Region 2 (4 farms, alpine/north).

Clover Cultivars	Region 1				Region 2			
	grass/clover yields (t ha <sup>-1</sup> )		clover content (%)		grass/clover yields (t ha <sup>-1</sup> )		clover content (%)	
	1 <sup>st</sup> year	3 <sup>rd</sup> year	1 <sup>st</sup> year	3 <sup>rd</sup> year	1 <sup>st</sup> year	3 <sup>rd</sup> year	1 <sup>st</sup> year	3 <sup>rd</sup> year
Ho9240	6.97 <sup>a</sup>	7.13 <sup>a</sup>	15 <sup>a</sup>	20 <sup>ab</sup>	6.21 <sup>a</sup>	5.88 <sup>a</sup>	33 <sup>a</sup>	26 <sup>a</sup>
Norstar	7.12 <sup>a</sup>	6.98 <sup>a</sup>	19 <sup>a</sup>	14 <sup>bc</sup>	6.21 <sup>a</sup>	5.47 <sup>a</sup>	35 <sup>a</sup>	25 <sup>a</sup>
Snowy	7.43 <sup>a</sup>	7.04 <sup>a</sup>	18 <sup>a</sup>	24 <sup>a</sup>	6.35 <sup>a</sup>	5.64 <sup>a</sup>	30 <sup>a</sup>	28 <sup>a</sup>
Kv9001	7.22 <sup>a</sup>	7.05 <sup>a</sup>	17 <sup>a</sup>	19 <sup>ab</sup>	6.30 <sup>a</sup>	5.87 <sup>a</sup>	31 <sup>a</sup>	27 <sup>a</sup>
Milkanova	7.35 <sup>a</sup>	6.81 <sup>a</sup>	24 <sup>a</sup>	12 <sup>c</sup>	5.77 <sup>a</sup>	5.11 <sup>a</sup>	19 <sup>b</sup>	20 <sup>b</sup>
Grass without clover	5.93 <sup>b</sup>	6.20 <sup>b</sup>	1 <sup>b</sup>	5 <sup>d</sup>	5.54 <sup>a</sup>	4.36 <sup>b</sup>	16 <sup>b</sup>	4 <sup>c</sup>

Means (averages of 9 farms, 3 replicates) followed by differing letters are significantly different ( $P < 0.05$ , Student Newman Keul-test).

There were no effects of variety or region on feed quality, but feed quality varied significantly between cut 1 and cut 3 (Table 2). Cut 3 had lower yields, lower digestibility, but more clover and higher content of ash than cut 1. Huhtanen (2003) also found a reduced digestibility of the regrowth in comparison with the first cut even though the NDF content was lower or similar in the regrowth. The slightly higher content of clover in cut 3 probably did not compensate for this.

Table 2. Difference between 1<sup>st</sup> and 3<sup>rd</sup> cut in dry matter yields (DM), clover %, crude protein (CP), digestibility, neutral detergent fibre (NDF) and ash.

	DM yields (t ha <sup>-1</sup> )	Clover (%)	CP (g kg <sup>-1</sup> )	Digestibility (g kg <sup>-1</sup> )	NDF (g kg <sup>-1</sup> )	Ash (g kg <sup>-1</sup> )
Cut 1	2.38 <sup>a</sup>	14.5 <sup>b</sup>	152 <sup>a</sup>	803 <sup>a</sup>	475 <sup>a</sup>	67 <sup>b</sup>
Cut 3	1.86 <sup>b</sup>	22.1 <sup>a</sup>	168 <sup>a</sup>	779 <sup>b</sup>	457 <sup>a</sup>	82 <sup>a</sup>

Means (averages of 9 farms, 2 or 3 years) followed by differing letters are significantly different ( $P < 0.05$ , Student Newman Keul-test).

Regression analyses revealed significant relationships between white clover content and NDF, protein, ash in the forage (Table 3), with less NDF and more protein and ash as the clover content increased.

Table 3. The effects of increasing white clover content on crude protein (CP), NDF and ash content in 1<sup>st</sup> and 3<sup>rd</sup> cut, evaluated by regression analysis.

Regression	Intercept	Regression coefficient	P-value regression	R <sup>2</sup>
Crude protein g kg <sup>-1</sup> = 135 +1.1 clover	135***	1.1***	0.001	0.22
NDF g kg <sup>-1</sup> =501-1.7 clover	501***	-1.7***	0.001	0.20
Ash g kg <sup>-1</sup> =67+0.3 clover	67***	0.3***	0.001	0.18

Values marked with \*\*\* are significantly different from 0 with P<0.001.

### Conclusion

The most commonly used variety, Milkanova, is less winter hardy, and therefore not recommendable for use in the northerly/alpine regions. It also exhibits low persistence in southerly/lowland regions of Norway, and should be replaced by the Norwegian variety, Snowy. The Norwegian varieties were similar in yields and persistence, but Norstar, which is bred for alpine/northern regions, did not perform better than Snowy, which is bred for lowland conditions. Increased white clover content gives improved feed quality with positive effects both on protein and ash content in the forage. Increased clover content also lead to decreased NDF content in the forage.

### Acknowledgements

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# Changes of perennial grasses agrocenosis depending on the soil reaction and nutrients

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## Abstract

Experiments were carried out in the Vežaičiai Branch of the Lithuanian Institute of Agriculture. The aim of this research was to estimate the structure of agrocenosis and state of perennial grasses in soils with different agrochemical characteristics. The soil acidity varied from 3.9 till 6.7, available P<sub>2</sub>O<sub>5</sub> from 47 till 317 mg kg<sup>-1</sup>, available K<sub>2</sub>O from 100 till 360 mg kg<sup>-1</sup>, and the amount of humus from 17.1 till 23.6 mg kg<sup>-1</sup>. Such range of soil ecological conditions was created through the application of different amounts of lime, mineral and organic fertilizers. Dry matter (DM) yield of perennial grasses and their density significantly increased as soil nutrient content increased. Damage by diseases to red clover was 1.4 times larger in more productive, neutral soils than in poor soils, but in these soils red clover was 1.7 times less damaged by pests.

Keywords: perennial grasses, soil nutrients, soil reaction.

## Introduction

It is estimated that plant density and competitive capacity are affected by nitrogen fertilization in crop rotations. Sowing red clover along with barley fertilized with nitrogen resulted in poor growth of the legume due to the high density and luxuriant barley growth which shades the cover clover. Besides, under these conditions plants are constantly exposed to attack by pathogenic organisms, especially pathogenic fungi (Salonen *et al.*, 2001; Sigvald, 2000). Some pests, as *Phytonomus meles* F. and *Ph. nigrirostris* F. spread more rapidly in high density perennial pastures, because they like places which are warm and without direct sunshine. On the contrary, *Apion seniculus* Kby. causes less damages in red clover in neutral soils fertilized with high rates of potassium and phosphorus. Red clover is slightly tolerant to acid soil conditions, but optimal pH<sub>KCl</sub> is between 6.0 and 6.5. There is estimated strong dependence between red clover productivity elements (Muntean, 2003, Salonen *et al.*, 2001).

The objective of this research was to investigate the changes in the botanical composition of swards, yield of perennial grasses as well as their pathological state in soils with different pH<sub>KCl</sub> and amount of nutrients.

## Materials and methods

Experiments were carried out in the Vežaičiai Branch of the Lithuanian Institute of Agriculture in a Dystric Albeluvisol (texture-morain loam) soil. The following crop rotation was assayed: 1) fodder beet (*Beta vulgaris* L.), 2) spring barley (*Hordeum vulgare* L.) and perennial grasses: red clover (*Trifolium pretense* L.) 'Liepsna' + timothy (*Phleum pretense* L.) 'Gintaras II', 3) first year perennial grasses, 4) winter wheat (*Triticum aestivum*), 5) oat (*Avena sativa* L.). There were two experiments. In the first experiment, in a completely randomized plot design, there were 48 treatments and in the second one 24. Seeding rate of perennial grasses was 160 kg ha<sup>-1</sup>. The seed proportion of red clover and timothy was 80:20. Soil was limed with different amounts of CaCO<sub>3</sub> (92.6%): 1.9, 3.3, 5.8, 14.7 and 49.6 t ha<sup>-1</sup>. After liming soil reaction was measured: pH<sub>KCl</sub> increased from 3.9 to a maximum of 6.7. In both field experiments every plot, with different soil reactions, was fertilized with increasing rates of mineral fertilizer according to the following scheme: unfertilized, P<sub>30</sub>K<sub>30</sub>, P<sub>60</sub>K<sub>60</sub> and P<sub>90</sub>K<sub>90</sub>. Furthermore, along the crop rotation and for two times in the intercropping periods different amounts of manure, ranging

form 20 to 120 t ha<sup>-1</sup>, were applied. In this way it was created a full range of nutrients in the soil: available P<sub>2</sub>O<sub>5</sub> 47-317 mg kg<sup>-1</sup>, available K<sub>2</sub>O 100-360 mg kg<sup>-1</sup> and organic matter (OM) content 17.1-23.6 mg kg<sup>-1</sup>.

Fungi and pests of above-ground parts of red clover were investigated: in a time of stem splitting and blooming and root diseases early in spring. Incidents of foliar diseases and pests were accounted using the leaf number damaged by diseases and pests expressed by percentage.

The data were analyzed statistically according to a normal distribution (Student's distribution). Correlation analysis (the Spearman's coefficient r) was calculated between diseases and pests incidence, swards density, yield and amount of soil nutrients.

## Results and discussion

The results show that in soils with pH<sub>KCl</sub> 3.9-4.9 forbs are the largest proportion of botanical composition (Table 1). Forbs proportion in perennial pasture agrocenosis is significantly reduced by increasing the amount of nutrients in soil (r=0.642). The biggest density of red clover occurred in soils with the highest amount of macroelements and OM (r=0.545), however, soil fertility had no any significant influence on timothy density (r = 0.312). Perennial grass dry matter yield was 1.7-2.4 times higher in the soils with these characteristics: P<sub>2</sub>O<sub>5</sub> 157-317 mg kg<sup>-1</sup>, K<sub>2</sub>O 206-360 mg kg<sup>-1</sup> and OM 1.98 – 2.36%, in comparison with perennial grass yield in the soil where nutrients were in the range: P<sub>2</sub>O<sub>5</sub> 47-156 mg kg<sup>-1</sup>, K<sub>2</sub>O 100-205 mg kg<sup>-1</sup> and OM 17.1-19.7 mg kg<sup>-1</sup>.

Table 1. The influence of soil nutrient levels into swards botanical composition and state in soils with pH<sub>KCl</sub> 3.9-4.9. Average data of 1998 – 1999 and 2005.

No	Soil nutrients, (mg kg <sup>-1</sup> )		Organic Matter (mg kg <sup>-1</sup> )	Botanical composition of swards (% DM)			Yield of perennial grasses (t DM ha <sup>-1</sup> )	Damaged above- ground parts of red clover (%)	
	P <sub>2</sub> O <sub>5</sub>	K <sub>2</sub> O		Clover	Timothy	Forbs		By diseases	By pests
1	47-101	100-152	17.1-18.4	28.0	15.3	56.8	1.95	42.0	59.4
2	102-156	153-205	18.5-19.7	30.0	19.9	50.2	2.60	41.8	48.9
3	157-211	206-258	19.8-21.0	30.0	18.0	52.1	4.39	55.4	60.0
4	212-266	259-311	21.1-22.3	37.3	14.5	48.3	4.59	55.5	56.9
5	267-317	312-360	22.4-23.6	36.6	16.8	46.7	4.70	50.5	55.6
	LSD <sub>0.05</sub>			8.22	4.74	9.58	0.61	12.50	10.45

Note: LSD<sub>0.05</sub> - least significant difference at P ≤ 0.05.

During the study period the red clover was harmed by eight kinds of pathogenic microorganisms. According to the data, red clover was 1.2-1.3 times more damaged by fungal diseases in soils rich in nutrients (Table 1). It was found a mild positive correlation between foliar diseases in red clover and the amounts of nutrients in which plants grew (r = 0.598). During investigation period red clover were damaged by 5 species of pests. The level of nutrients in soils with pH<sub>KCl</sub> 3.9 - 4.9 had not significant influence into pest attacks (r=0.255).

In comparison to more acid soils (pH<sub>KCl</sub> 3.9 – 4.9), in soils with pH<sub>KCl</sub> 5.7-6.7 the largest proportion of botanical composition was constituted by red clovers (Table 2). In fact, the contribution of red clover to botanical composition in swards did not increase significantly by increasing the amount of nutrients in soil (r=0.465). On the contrary, timothy and forbs contribution to botanical composition was not reduced significantly by the increasing levels of P<sub>2</sub>O<sub>5</sub> from 47 till 317 mg kg<sup>-1</sup>, K<sub>2</sub>O from 100 till 360 mg kg<sup>-1</sup> and OM from 17.1 till 23.6 mg kg<sup>-1</sup> (r = -0.355 and -0.322, respectively). Perennial grass dry matter yield was 1.4 times higher in soil richer in nutrients (r=0.545), although red clover in such soils undergone more damages caused by foliar fungal attacks.

Table 2. The influence of soil nutrient levels into swards botanical composition and state in soils with pH<sub>KCl</sub> 5.7-6.7. Average data of 1998 – 1999 and 2005.

No	Soil nutrients, ( mg kg <sup>-1</sup> )		Organic Matter ( mg kg <sup>-1</sup> )	Botanical composition of swards ( % DM )			Yield of perennial grasses ( t DM ha <sup>-1</sup> )	Damaged above- ground parts of red clover ( % )	
	P <sub>2</sub> O <sub>5</sub>	K <sub>2</sub> O		Clover	Timothy	Forbs		By diseases	By pests
1	47-101	100-152	17.1-18.4	52.5	13.7	33.8	3.46	38.0	66.4
2	102-156	153-205	18.5-19.7	53.5	20.1	27.4	4.46	45.8	55.8
3	157-211	206-258	19.8-21.0	53.8	15.7	30.5	4.55	56.0	60.5
4	212-266	259-311	21.1-22.3	54.8	11.3	33.9	4.58	54.3	47.4
5	267-317	312-360	22.4-23.6	59.9	13.4	26.7	4.98	54.5	39.1
	LSD <sub>0.05</sub>			5.95	6.86	5.24	0.48	14.55	13.85

Note: LSD<sub>0.05</sub> - least significant difference at P ≤ 0.05.

It was obtained a relatively strong positive correlation between red clover diseases incidence and amount of soil nutrients (r=0.644). On the contrary, pests damages were 1.7 times larger in soils with the lowest amount of nutrients and OM in comparison with the plants which grew in the soils with the highest amount of nutrients (r = 0.752).

### Conclusions

Different soil reaction significantly influenced the botanical composition of swards: in the soils with pH<sub>KCl</sub> 3.9-4.9 prevailed forbs and in the soils with pH<sub>KCl</sub> 5.7-6.7 red clover.

Perennial grass dry matter yield was significantly influenced by increasing amount of soil nutrients. Red clover growing in soils with the highest amount of P<sub>2</sub>O<sub>5</sub>, K<sub>2</sub>O and OM (pH<sub>KCl</sub> 5.7 – 6.7) was 1.4 times more damaged by fungal diseases compared to that growing in the soils with the lowest amount of nutrients, but those plants were 1.7 times less damaged by pests.

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## Performance of several forage legumes submitted to different management systems

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### Abstract

Forage legumes are an alternative to fertilized swards for the production of high quality forage. Although white clover is the most important forage legume in grassland, the performance is limited in grazing systems by low summer drought resistance as well as due to reduced dry matter yield in comparison to other forage legumes like red clover and lucerne in cutting systems. The objective of this study was to compare the performance of different forage legume species submitted to different management systems, i.e. grazing, 4-cut and 5-cut system. The experiment was established in 2003 as binary swards with perennial ryegrass as the companion grass in a split-plot design with three replicates. Legume species were arranged within each management system as completely randomised blocks. Results of agronomic performance in 2004 (dry matter yield, botanical composition, phenological development) are presented for white clover (*Trifolium repens* L.), red clover (*Trifolium pratense* L.), lucerne (grazing type, *Medicago sativa* L.), birdsfoot trefoil (*Lotus corniculatus* L.) for the grazing system; and in addition to the former ones lucerne (hay type), kura clover (*Trifolium ambiguum* M. Bieb.) and pure stands of perennial ryegrass (*Lolium perenne* L.) (with and without cattle slurry) for the cutting systems.

Keywords: dry matter yield, forage legumes, grazing, cutting frequency.

### Introduction

White clover is the most important forage legume for grassland systems because of its high persistence under grazing and its feeding value in legume based swards. However, its low resistance towards longer summer droughts and its lower dry matter yield compared to other forage legumes like red clover or lucerne may limit its utilization. Although several studies investigated possible alternative forage legumes to white clover, there is a lack of studies which examined simultaneously different forage legumes submitted to different management systems. Therefore, the objective of the present study is to investigate several forage legumes as potential alternatives to white clover, which were submitted to grazing-, 4-cut- and 5-cut- system. Depending of legume species and management system differences in yield and botanical composition are expected.

### Materials and methods

The experiment was conducted at the experimental farm 'Lindhof' of the University of Kiel. The average annual precipitation is 774.4 mm and the average annual temperature 8.7 °C. The experiment was established 2003 as a split-plot design with three replicates and carried out in 2004. White clover, red clover, birdsfoot trefoil, lucerne (hay type and grazing type) and kura clover were established as binary swards with perennial ryegrass. Perennial ryegrass was established also as pure sown swards (with cattle slurry: 200 kg N ha<sup>-1</sup> yr<sup>-1</sup> and without slurry). The swards in the cutting systems were harvested either 4 times (silage cut) or 5 times (simulated grazing). For the grazing system white clover, red clover, birdsfoot trefoil and lucerne (grazing type) were used and grazed by Limousin heifers, with a total of 5 grazing cycles in 2004. White clover was the control group in all management systems. At each sampling time, swards were cut to 5 cm height and DM yield established by drying samples at 60°C. The botanical composition of the harvested herbage was obtained after separation in grass, clover

and herbs. The phenological development was determined by counting the amount of reproductive tillers in proportion to the total of tillers sampled.

## Results and discussion

During the growing period there was for nearly all legume species an increase in the proportion of the legumes in the sward. At the beginning of the growing period the proportion of red clover (48.8%) and lucerne (53.7%, grazing type) was significantly higher compared to the white clover swards (25.5%) over all management systems. Birdsfoot trefoil (25%) showed similar legume proportion to white clover swards. Comparing legumes within the management systems, there was a greater proportion of legumes in the grazing and simulated grazing system compared to the silage cut system at the beginning of the growing period, except for birdsfoot trefoil. In the end of the growing period a higher proportion of lucerne (62.3%, grazing type) and birdsfoot trefoil (60.5%) was observed in the silage cut system in comparison to white clover (29.6%). These differences may be explained by a lower cut frequency in the silage cut system (4 silage cuts vs. 5 simulated grazing cuts). In the grazing system the botanical composition of white clover was 30 to 40% during the whole growing period, while the proportions of red clover and lucerne (grazing type) tended to be lower. Such results for white clover were also observed by Schils *et al.* (1999). Significant differences in the grazing system could only be observed for birdsfoot trefoil, which was reduced to 10% of the botanical composition at the end of the growing season.

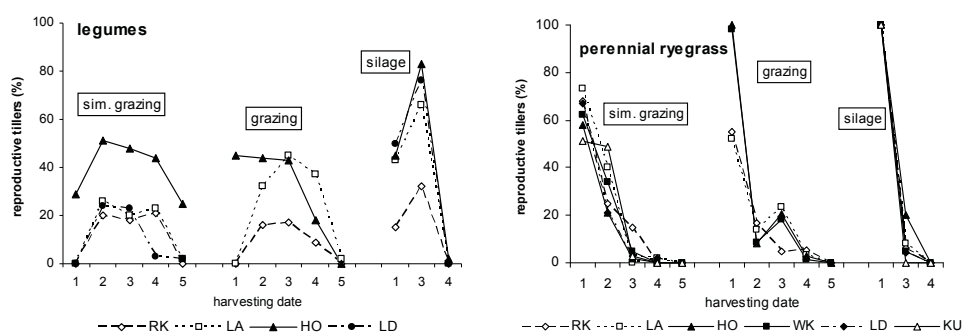


Figure 1. Proportion of reproductive tillers (%) of the different forage legumes and perennial ryegrass under different management systems and harvesting dates (RK: red clover; LA: lucerne, grazing type; HO: birdsfoot trefoil, LD: lucerne, hay type, KU: kura clover, WK: white clover).

At the beginning of growing period there was an increase in reproductive tillers for all legume species followed by a decrease until the end of the growing season. Because there were only 2 animal groups available for the grazing system, it was not possible to start with all 4 legume species at the same time. Therefore birdsfoot trefoil and white clover based pastures were older at the first grazing cycle as shown in Figure 1.

The perennial ryegrass showed similar proportions of reproductive tillers within the different management systems. As shown in Figure 1 there were a high proportion of reproductive tillers at the first harvests of all systems followed by a decrease afterwards.

Table 1a. Total dry matter yield (g DM m<sup>-2</sup>) of the different legumes/grass swards and the pure swards of perennial ryegrass submitted to different cutting systems (RK: red clover; LA: lucerne, grazing type, HO: birdsfoot trefoil, LD: lucerne, hay type, KU: kura clover, WK: white clover, DW+: perennial ryegrass with slurry, DW-: perennial ryegrass without slurry).

Species/System	WK	RK	LA	HO	LD	KU	DW+	DW-
Silage	969.5 <sup>a</sup>	1,072.8 <sup>a</sup>	1,199.8 <sup>a*</sup>	1,084.0 <sup>a</sup>	1,115.4 <sup>a</sup>	892.9 <sup>a</sup>	478.4 <sup>a*</sup>	390.2 <sup>a*</sup>
Simulated grazing	897.9 <sup>a</sup>	728.8 <sup>b</sup>	844.8 <sup>b</sup>	727.6 <sup>b</sup>	801.2 <sup>b</sup>	756.1 <sup>a</sup>	450.4 <sup>a*</sup>	347.0 <sup>a*</sup>

<sup>a, b</sup> means within the same column with different superscripts differ significantly ( $P < 0.05$ )

\* means within the row differ significantly ( $P < 0.05$ ) to white clover SE=55.6

Table 1b. Total dry matter (g DM m<sup>-2</sup>) of the different legumes/grass swards submitted to different cutting systems and rotational grazing.

Species/System	WK	RK	LA	HO
Silage	969.5 <sup>ab</sup>	1,072.8 <sup>a</sup>	1,199.8 <sup>a*</sup>	1,084.0 <sup>a</sup>
Simulated grazing	897.9 <sup>b</sup>	728.8 <sup>b</sup>	844.8 <sup>b</sup>	727.6 <sup>b</sup>
Grazing	1,114.8 <sup>a</sup>	954.9 <sup>a</sup>	758.6 <sup>b*</sup>	1,040.9 <sup>a</sup>

<sup>a, b</sup> significant differences ( $P < 0.05$ ) between the systems

\* significant different ( $P < 0.05$ ) to white clover within legume species SE=65.4

LA was the only sward, which produced a significant higher yield under silage cut-system compared to white clover (Table 1a). As shown in table 1b the lucerne (LA, grazing type) has in the grazing system a significant lower yield than white clover (758.6 vs. 1,114.8 g DM m<sup>-2</sup>). There were no significant differences between white clover and the other legume species in the simulated grazing system. The pure sown swards of perennial ryegrass had significant lower yields under all cutting systems in comparison to the white clover swards. Comparing the different cutting systems red clover, lucerne (both hay and grazing type) and birdsfoot trefoil showed for the silage cut system significant higher yields than the simulated grazing system. The results for both lucerne types within cutting systems are in agreement with the results of Kallenbach *et al.* (2002). No significant differences between cutting systems were observed for white clover and kura clover.

## Conclusions

Red clover could be an alternative to white clover swards in the year after establishment also for grazing systems. For the different cutting systems all legume/grass stands are comparable to white clover. Only lucerne showed higher dry matter yields in the silage cut-system. The pure ryegrass swards with application of cattle slurry were not an alternative for white clover swards. The results suggest that different legume/grass mixtures can provide high yields in comparison to white clover swards, which may be useful in ley farming systems.

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# Effect of *Trifolium repens* cultivars on yield and chemical composition of pasture sward

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## Abstract

In 2003-2004 the effects of 8 cultivars of *Trifolium repens* L. used in a grass-legume mixture were investigated for the effects on dry matter yield and chemical composition of the sward. The seed mixtures consisted of *Trifolium repens* – 35%, *Festuca pratensis* Huds.– 40% and *Lolium perenne* L.– 25%. The following parameters were determined for 5 periods of regrowth: dry matter yield, the proportion of white clover in the yield and the sward chemical composition: content of nitrogen, carbon and sulphur with the assistance of the elementary analysis using the Vario max apparatus of the Elementar Company. It was found that Romena cultivar exhibit the highest competitiveness in relation to grasses used in the applied mixtures. The highest annual dry matter yields were found in the treatments with new breeding strains AND 1602 (10,114 kg ha<sup>-1</sup> DM). White clover cultivars used in experimental mixture with meadow fescue and perennial ryegrass were also found to influence the sward chemical composition.

Keywords: grass-clover mixtures, herbage quality, *Trifolium repens*.

## Introduction

The appropriate proportion of white clover (*Trifolium repens*) in the pasture sward guarantees high nutritive value of forage for ruminants as the result of the increased content of protein, minerals and the improvement of sward digestibility and palatability. In addition, since it substitutes nitrogen fertilisation of meadows and pastures, it plays an important role both in sustainable and low-input systems of feed production (Nilsdotter-Linde *et al.*, 2004). One of the major research problems requiring investigation at the moment is the suitable selection of cultivars of white clover for grass-clover mixtures which will guarantee its appropriate proportion in the sward throughout the vegetative season, especially in dairy farms because only the stability of pasture sward will allow the achievement of high milk production (Woodfield *et al.*, 2004).

The aim of the performed investigations was to assess the sward yields and quality of grass-clover mixtures depending on the sown cultivar of white clover.

## Materials and methods

During 2003-2004 at the Experimental Station of Cultivar Testing in Nowa Wieś Ujska near Poznań (mean annual rainfall of approximately 500 mm) studies were conducted to evaluate the effects of white clover cultivar on dry matter yields and chemical composition of grass-clover swards under simulated pasture utilisation. The experiment was established on Histosol soil, in a random block design, on 10 m<sup>2</sup> plots in four replications. The seed mixtures consisted of *Trifolium repens* – 35%, *Festuca pratensis* cv. Aureus – 40% and *Lolium perenne* cv. Solen – 25% were sown in May 2003 at the density of 1200 seeds m<sup>-2</sup> using the Øyjord drill. The following five Polish cultivars of white clover were analysed: Astra, Aura, Dara, Rawo, Romena as well as three breeding strains: ABM 9 BD (NL), AND 1602 (PL), WOM 302 (PL). Effectively, the following quantities of seeds were sown: *Trifolium repens* – 2.9-3.9 kg ha<sup>-1</sup> (depending on the cultivar), *Festuca pratensis* – 10.5 kg ha<sup>-1</sup> and *Lolium*

*perenne* – 11.4 kg ha<sup>-1</sup>. Nitragine was applied directly into the soil in the year of sowing. During the period of vegetation, five cuts were harvested using the plot harvester Hege 212. The first cut was performed in spring, when the perennial ryegrass was 15 cm high. The second and third cuts were harvested at 21 days intervals, the fourth and fifth – after 28-35 days. The following parameters were determined in individual combinations: dry matter yield, the proportion of white clover in the yield and the sward chemical composition. For chemical analyses, mean samples of the plant material were collected from each plot, dried in a forced-draught oven at 50 °C and ground. The sward quality was assessed with the assistance of the elementary analysis using the Vario max apparatus of the Elementar Company.

Tests of the main effects were performed by F-tests. Means were separated by the LSD and were declared different at the  $p < 0.05$  level.

## Results and discussion

Cultivars of white clover were characterized by different growth and development dynamics in the sward during the vegetation season. Their mean proportion in the sward yield of the first regrowth was 10.3%, 2<sup>nd</sup> – 19.1%, 3<sup>rd</sup> – 14.3%, 4<sup>th</sup> – 8.7%, 5<sup>th</sup> – 4.2% (Table 1).

Table 1. Effect of *Trifolium repens* cultivars on dry matter (DM) yield and proportion of white clover in pasture sward.

Cultivar	DM yield (kg ha <sup>-1</sup> )					Total	Proportion of white clover in sward (%)					M
	Regrowth						Regrowth					
	14. May	7. June	30. June	27. July	6. Sept.		14. May	7. June	30. June	27. July	6. Sept.	
Astra	2,167	1,513	1,888	2,599	1,674	9,841	13.8	20.0	17.4	8.5	5.2	13.0
Aura	2,224	1,463	1,949	2,625	1,775	10,035	11.0	19.3	14.9	8.2	3.9	11.5
Dara	2,422	1,433	1,902	2,532	1,594	9,883	7.4	16.6	16.2	7.7	2.2	10.0
Rawo	2,341	1,408	1,967	2,571	1,653	9,940	9.4	16.3	9.1	7.8	3.7	9.3
Romena	2,226	1,390	1,884	2,818	1,701	10,019	12.8	22.3	17.8	12.7	5.5	14.2
ABM 9 BD	2,135	1,395	2,101	2,543	1,772	9,944	8.7	19.8	9.0	8.5	4.2	10.1
AND 1602	2,205	1,613	1,888	2,745	1,663	10,114	10.3	19.4	16.3	7.5	5.1	11.7
WOM 302	2,504	1,451	2,030	2,493	1,590	10,068	8.8	19.2	14.0	8.3	3.6	10.8
LSD <sub>0.05</sub>	197.8	39.7	111.5	ns	ns	50.6	-	-	-	-	-	2.13

The obtained results of experiments confirm the phenomenon of a greater competitiveness of white clover in the examined mixtures in relation to grasses during the period of late spring and summer and smaller competitiveness at the beginning of vegetation and in the autumn. (Warda and Krzywiec, 1998). Out of the examined cultivars of white clover in pasture mixtures with perennial ryegrass and meadow fescue, cv. Romena (large-leaved form) whose mean share in the sward during the vegetative season amounted to 14.2% deserves to be singled out. Its higher proportion in sward was connected not only with leaf size, but also with other specific biological properties e.g. stolon density. Cultivar Rawo (medium-leaved form) showed a distinctly lower competitiveness (mean share 9.3%) in relation to grasses, although it was characterised by the best stability of its proportion in the sward during vegetative period (lowest range between its highest and lowest share in five consecutive regrowths). The results in Table 1 shown, that the highest annual dry matter yields were found in the treatments with new breeding strains AND 1602 (10,114 kg ha<sup>-1</sup> DM). The lowest annual yield level of 9,841 kg ha<sup>-1</sup> DM was determined in the mixture containing the Astra cultivar. This means that white clover cultivar has very little impact on total yield. The higher yield of AND 1602 seems to be driven by the greater yield at regrowth 2, this means that AND 1602 indicated seasonal variation in yielding. This effect needs more investigations in the future.

White clover cultivars used in experimental mixture with perennial ryegrass and meadow fescue were also found to influence the sward chemical composition (Table 2).

Table 2. Effect of *Trifolium repens* cultivars on chemical composition of pasture sward – means in growing season.

Cultivar	N (g kg <sup>-1</sup> DM)	C (g kg <sup>-1</sup> DM)	C/N	S (g kg <sup>-1</sup> DM)
Astra	34.8	429.6	12.4	2.3
Aura	35.5	427.8	12.1	2.3
Dara	34.6	428.7	12.4	2.5
Rawo	34.0	429.0	12.7	2.4
Romena	35.2	428.3	12.2	2.4
ABM 9 BD	35.0	424.8	12.2	2.4
AND 1602	35.9	431.5	12.1	2.1
WOM 302	35.0	428.1	12.3	2.1
LSD <sub>0.05</sub>	ns	2.24	ns	0.24

The highest nitrogen content in the sward of the examined mixtures was recorded when AND 1602 constituted its leguminous component, while the lowest – when Rawo cultivar was employed as the mixture component. Although not statistically significant, the low clover % in Rawo swards corresponded with the lowest N content of herbage, while the high clover % of Romena swards corresponded with some of the highest herbage N contents. Also the carbon content was found the highest when AND 1602 was used, while its content was the lowest in the case when ABM 9 BD was the legume constituent. C content was significant between cultivars but the range was only 2% (highest divided by lowest), so biological impact is likely to be negligible. Variation in the content of nitrogen and carbon affected the C/N ratio. Its lowest value was recorded in mixtures containing the Aura and AND 1602 cultivars. The sulphur concentration in the sward ranged from 2.1 to 2.5 g kg<sup>-1</sup> DM.

White clover cultivars used in experimental mixture with meadow fescue and perennial ryegrass were found to influence the sward yield and its quality. However, their impacts on total yield, mean proportion in sward and chemical composition of herbage were little.

### Conclusions

Because of their unique morphological and biological properties, cultivars of white clover determine the yield and quality of fodder obtained from grass-clover mixtures. Out of the examined cultivars of white clover, the following deserve positive assessment: Romena – for its highest proportion in the sward and AND 1602 – for the increased dry matter yield and improvement of the sward chemical composition in mixtures with perennial ryegrass and meadow fescue.

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# The effect of legumes on the accumulation of nitrogen in herbage yield on succeeding spring wheat

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## Abstract

Introducing of suitable legumes in cropping systems is important due to their ability to enrich soil with inorganic nitrogen. The experiment was aimed to assess the possibilities of nitrogen accumulation in swards yield and the impact of herbage swards on the yield of succeeding crop. Perennial legumes and grasses were sown with and without a cover-crop of barley (*Hordeum vulgare* L.) or peas (*Pisum sativum* L.) for whole crop and barley for grain. Red clover (*Trifolium pratense* L.) and lucerne (*Medicago sativa* L.) were sown in mixtures with perennial ryegrass (*Lolium perenne* L.) and ryegrass as monoculture and either fertilised with nitrogen or not. After second year of swards use spring wheat (*Triticum aestivum* L.) was conventionally drilled after the afore-mentioned pre-crops. Lucerne/grass sward sown with peas for whole crop accumulated the highest nitrogen amount in the two harvest years. The yield of clover/grass sward was inappreciably dependent on cover-crop. In most cases legume/grass swards produced earlier and higher yield, than pure grass sward even fertilised with 240 kg nitrogen ha<sup>-1</sup> in two years of use. The wheat grain yield was higher after legume/grass sward than after ryegrass without or even with nitrogen fertiliser.

**Keywords:** clover, lucerne, protein, yield, cover-crop, spring wheat.

## Introduction

Development of a more sustainable agricultural production system is an important aim nowadays. There is a pressing need to develop cereal growing methods that require much lower inputs of N fertilizer and other agrochemicals than conventional farming, but are economically viable and environmentally acceptable. Introduction of ley/arable rotations could be an effective tool for a significant further reduction of the use of external mineral N-input and an increase of the N use efficiency (Nevens *et al.*, 2004). Therefore legumes are of great importance in the rotations as soil improvers and as valuable preceding crops. The complex of factors and their interactions influence the potential of legumes, which is greatly dependent on legume species and successful management (Kadziulis, 2001; Maikstienė and Arlauskienė, 2004).

The aims of the studies were to assess the possibilities of N accumulation in swards yield and to explore organic N of legumes as a potential N source for subsequently grown spring wheat.

## Materials and methods

Field studies were conducted on a loamy *Endocalcari-Epihypogleyic Cambisol* in Dotnuva (55° 24' N). Soil pH varied between 6.5 - 7.0, humus content was 2.5-4.0 per cent, available P 50-80 mg kg<sup>-1</sup> and K 100-150 mg kg<sup>-1</sup>. Legume/grass mixtures were sown with and without a cover crop of barley (*Hordeum vulgare* L.) or peas (*Pisum sativum* L.) for whole crop or of barley for grain. Red clover (*Trifolium pratense* L.) and lucerne (*Medicago sativa* L.) were sown in mixtures with perennial ryegrass (*Lolium perenne* L.), and ryegrass was also sown as a monoculture and either fertilised with N or not. The layout was a randomised block design with four replicates, and plot size of 2.5x13 m, P and K were applied according to the need based on soil analysis. The yields of swards were taken-off at flowering stage of the legumes. The swards were cut twice a year in the first year. Barley and peas as whole crops were harvested at the wax and grain 'greasy' stage, respectively, and for one treatment - barley for grain - at complete ripeness stage. Succeeding crop spring wheat (*Triticum aestivum* L.) was harvested at

complete ripeness stage. Nitrogen in the dry matter (DM) was determined by the Kjeldahl method. The yield data were statistically processed using analysis of variance.

## Results and discussion

Legume species and cover crop in the sowing year affected the total forage yield. The highest forage yield was obtained from lucerne/grass and red clover/grass swards sown with barley for whole crop (Table 1). The lucerne-based swards were higher-yielding in the second year compared with red clover/grass. DM yield was positively related to the content of legumes.

Table 1. Impact of sward composition and sowing method on the DM yield over two years.

Treatments: sward + cover crop	2003	2004	Legumes in DM (%)
	DM yield (t ha <sup>-1</sup> )	DM yield (t ha <sup>-1</sup> )	
Red clover, perennial ryegrass	2.87	6.05	59
Red clover, perennial ryegrass + Bgr	5.47	5.60	71
Red clover, perennial ryegrass + Bwc	6.00	5.87	59
Red clover, perennial ryegrass + Pwc	4.79	5.71	49
Lucerne, perennial ryegrass	4.04	9.19	86
Lucerne, perennial ryegrass + Bwc	6.72	7.46	61
Lucerne, perennial ryegrass + Pwc	5.33	9.08	81
Perennial ryegrass + N <sub>60+180</sub>	2.18	8.43	0
Perennial ryegrass + N <sub>0</sub>	2.02	2.85	0
LSD <sub>0.05</sub>	0.858	0.919	

Cover crop: Bgr-barley for grain, Bwc-barley for whole crop, Pwc-peas for whole crop.

Table 2. Wheat grain yield responses to legumes in pre-crops.

Treatments: sward + cover crop	2003	2004	2005	
	N in yield	N in yield	N in roots of legumes	Wheat grain yield
	(kg ha <sup>-1</sup> )	(kg ha <sup>-1</sup> )	(g kg <sup>-1</sup> )	(t ha <sup>-1</sup> )
Red clover, perennial ryegrass	82	184	23	2.92
Red clover, perennial ryegrass + Bgr	107	182	21	3.09
Red clover, perennial ryegrass + Bwc	79	183	21	3.20
Red clover, perennial ryegrass + Pwc	126	151	22	3.27
Lucerne, perennial ryegrass	121	372	20	3.85
Lucerne, perennial ryegrass + Bwc	86	321	20	3.78
Lucerne, perennial ryegrass + Pwc	148	338	21	3.81
Perennial ryegrass + N <sub>60+180</sub>	53	188	0	2.46
Perennial ryegrass + N <sub>0</sub>	50	76	0	2.09
LSD <sub>0.05</sub>	11.3	19.2	1.29	0.212

Cover crop: Bgr-barley for grain, Bwc-barley for whole crop, Pwc-peas for whole crop.

The N accumulation in the harvested forage is shown in Table 2. It was the highest in lucerne/grass mixture sown with peas cover crop in the sowing year and lucerne/grass in the second year leys. The amount of nitrogen in red clover-based swards was lower than in lucerne, and similar to that in N fertilised perennial ryegrass. The differences of N yield were dependent upon proportion of legumes in



swards, which was also reported by other authors (Kristensen *et al.*, 1995). It is well known that grass/legume mixtures create very good conditions for subsequent crops. The main reason is a large amount of organic matter ploughed-in. On average, about 110 -200 kg N ha<sup>-1</sup> after legume/grass swards were introduced into the soil together with considerable quantities of potassium, phosphorus and other nutrient elements (Kadziulienė, 2004; Kryszak, 2004). Wheat performance did respond to previous swards and benefited from the lucerne/grass swards. Generally, wheat following legume/grass swards produced a higher grain yield than pure grass sward even fertilised with 240 kg nitrogen ha<sup>-1</sup> in two years of use.

### Conclusions

The lucerne-based swards sown with peas or barley for whole crop produced the highest DM yield over two years of use. The amount of nitrogen accumulated in the yield of lucerne -based swards was 60-70 per cent higher than that in red clover swards. Spring wheat following legume/grass swards produced higher grain yield than sown after grasses fertilised with 240 kg N ha<sup>-1</sup> yr<sup>-1</sup>.

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# The impact of incorporating white clover into crop rotations with cereal grains

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## Abstract

The experiment was designed to assess alternative means of incorporating of white clover into crop rotation and its impact on cereal grain yield. In the first year white clover (*Trifolium repens* L.) was sown alone, with a cover-crop of barley (*Hordeum vulgare* L.) for forage and compared with barley for grain without clover. In the second year winter wheat (*Triticum aestivum* L.) was no-till drilled into growing clover or conventionally drilled after the afore-mentioned pre-crops. In the third year triticale (*X Triticosecale* Wittn.) was conventionally drilled. In terms of grain yield, winter wheat sown into growing white clover significantly lagged behind the conventionally sown wheat. In autumn before sowing wheat, inorganic N concentration in the soil was generally greater after ploughed-down white clover. In no-till drilled wheat, soil inorganic N concentration was found to be the lowest, and this trend persisted until wheat ripening. Winter wheat sown after ploughed-down white clover yielded 300-480 kg grain ha<sup>-1</sup> more than the wheat grown conventionally after barley (without clover). The yield of triticale was higher after wheat grown after pre-crops with white clover and slightly lower after wheat grown together with white clover.

Keywords: white clover, cover-crop, winter wheat, triticale.

## Introduction

Unlike conventional farming, organic farming has to rely on efficient nutrient circulation within the farm to maintain soil fertility and high production. Unfortunately, in Lithuania many organic farms are involved solely in crop production, farming without livestock. Hence, there is a need for alternative strategies for nutrient management. A feasible way for reducing the need for N fertilisation in cereals is the use of legumes as preceding crops, or other alternative cropping systems. The potential for N incorporation into the soil by the legume-*rhizobium* association is very high, but it can be very variable depending on the conditions. Type of legume is very important. Forage legumes tend to fix more N and leave a larger N residue in the soil compared with grain legumes (Kadziuliene, 2004; Lapinskas, 1998; Ledgard, 2001). Furthermore, the benefit for the following crop depends on the recovery efficiency of this N. This efficiency is affected by the rate and extent of N mineralized and accumulated in the soil prior to and during the leaching period, and uptake by the crop. Management of the preceding sward thus greatly influences the yield of the succeeding crop (Høgh-Jensen and Schjoerring, 1996). Environmental problems and excess cereal production caused researchers to devise a method of growing cereals where much of the N requirement is supplied by a permanent, perennial white clover understorey. If winter wheat is no-till drilled into defoliated clover it is possible to achieve a whole crop silage yield approximately equal to that in conventionally grown crops (Bergkvist and Clements, 2001). To test the potential of white clover as nitrogen supplier to subsequent wheat and triticale crop, within context of an organic farming system, a three-year (crop rotation) trial was initiated.

## Materials and methods

A field experiment was conducted on a loamy *Endocalcari-Epihypogleyic Cambisol* in Dotnuva (55° 24' N). Soil pH varied between 6.5 - 7.0, humus content was 25-40 g kg<sup>-1</sup>, available P 50-80 mg kg<sup>-1</sup> and K 100-150 mg kg<sup>-1</sup>. The layout was a randomised block design with four replicates and plot size of 2.5x13 m, P and K were applied according to the need based on soil analysis. The pre-crops for winter wheat were barley for grain, white clover with barley cover crop, white clover for forage, and white clover

whose first cut was used for forage and second (last) cut in autumn was used for green manure. In the autumn wheat was no-till or conventionally drilled into pre-crop treatments as outlined in Table 1. In the following year wheat and clover developed together and wheat was harvested for grain. In autumn all crops were ploughed and triticale was conventionally drilled and harvested at the ripe grain stage the following summer. Soil samples were collected at 0-30 and 30-60 cm depths for inorganic N analysis in the autumn before sowing winter wheat, the following spring, and through grain ripening. Grain yield and soil N data were statistically processed using analysis of variance.

## Results and discussion

Both pre-crop and sowing method affected grain yield of winter wheat. A significantly ( $p < 0.05$ ) higher wheat grain yield was obtained having used conventional sowing compared with no-till sowing (Table 1). Clements *et al.* (1997) suggest that grain yield in winter wheat bi-cropped with clover was quite variable and only about 50 per cent of conventional yields. In our experiment wheat after white clover ploughed-down and with conventional sowing was higher yielding ( $0.48$  and  $0.30 \text{ t ha}^{-1}$ ) than after conventionally sown barley. Grain yields were also variable after no-till drilling. Wheat after the white clover yielded  $0.56 \text{ t ha}^{-1}$  more than after white clover with a barley. The effect of white clover incorporated before sowing on grain yield of winter wheat can be quite negligible, which is probably due to the large losses of N through leaching following the incorporation of legumes early in autumn (Bergkvist and Clements, 2001; Bergkvist, 2003).

Table 1. The impact of pre-crop and sowing method on wheat (2003) and triticale (2004) grain yield .

Wheat pre-crops + sowing method	W. clover (DM) or barley grain <sup>1)</sup> ( $\text{t ha}^{-1}$ )	Wheat ( $\text{t ha}^{-1}$ )	<i>Triticale</i> ( $\text{t ha}^{-1}$ )
Barley, CD	3.21 <sup>1)</sup>	4.59	2.99
White clover, NT	1.41	3.01	3.79
W. clover, barley cover crop, NT	2.86 <sup>1)</sup>	2.45	3.46
White clover, green manure, CD	1.23	4.89	3.78
White clover, CD	1.27	5.07	3.18
LSD <sub>05</sub>		0.641	0.493

CD- conventional drilling; NT- no-till drilling.

In the first autumn, inorganic N concentration in the soil was similar in treatments with and without white clover (Table 2), but generally greater after ploughed-down white clover. In the following spring, soil inorganic N concentration was similar or lower than in the autumn. However, in the soil under no-till drilled wheat, soil inorganic N concentration was found to be the lowest, and this trend persisted until wheat ripening. In all cases the amount of  $\text{NO}_3^-$  was 2 to 3 times higher than that of  $\text{NH}_4^+$ . Low concentrations of inorganic N during wheat booting and heading suggests an intensive uptake of N by plants, and low N concentration in the subsoil indicates minimal N leaching. Inclusion of white clover in the cropping system had a measurable effect on the grain yield of triticale following wheat that had been grown in ploughed-down white clover. The effect of a white clover pre-crop over two years of cereal (wheat and triticale) production resulted in a  $0.67$  to  $1.09 \text{ t ha}^{-1}$  increase in grain yield.

Table 2. The impact of pre-crops and sowing methods on the content of inorganic N in the soil.

Wheat pre- crops + sowing method	Inorganic N in the soil (mg kg <sup>-1</sup> )									
	Autumn		Spring		Booting		Heading		Ripeness	
	NO <sub>3</sub> <sup>-</sup>	NH <sub>4</sub> <sup>+</sup>	NO <sub>3</sub> <sup>-</sup>	NH <sub>4</sub> <sup>+</sup>	NO <sub>3</sub> <sup>-</sup>	NH <sub>4</sub> <sup>+</sup>	NO <sub>3</sub> <sup>-</sup>	NH <sub>4</sub> <sup>+</sup>	NO <sub>3</sub> <sup>-</sup>	NH <sub>4</sub> <sup>+</sup>
0-30 cm										
Barley, CD	2.96	1.00	4.95	0.44	1.35	0.65	1.06	0.72	4.11	0.10
White clover, NT	2.51	1.20	3.49	0.52	1.18	0.92	1.09	1.23	4.22	0.11
W.clover, barley cover crop, NT	2.43	1.32	3.02	0.63	1.28	0.68	1.54	1.01	3.87	0.30
W. clover, green manure, CD	1.07	1.59	4.51	0.32	1.29	0.73	1.31	1.04	5.38	0.12
White clover, CD	3.99	1.47	4.26	0.22	1.05	0.66	1.63	0.88	4.17	0.11
LSD <sub>05</sub>	1.04	0.34	0.99	0.31	0.42	0.26	0.40	0.17	1.12	0.13
30-60 cm										
Barley, CD	5.42	0.74	4.80	0.11	2.93	0.66	1.60	0.44	3.57	0.00
White clover, NT	6.88	0.97	3.75	0.14	3.07	0.58	1.77	0.8	4.00	0.00
W.clover, barley cover crop, NT	4.40	0.79	2.22	0.14	1.67	0.31	1.54	0.72	4.05	0.00
W. clover, green manure, CD	7.13	0.93	4.50	0.05	4.27	0.40	1.89	0.57	3.85	0.03
White clover, CD	9.87	0.96	4.00	0.36	2.69	0.43	2.15	0.67	4.38	0.00
LSD <sub>05</sub>	2.05	0.22	1.27	0.36	1.70	0.22	0.41	0.19	0.73	0.06

\* CD- conventional drilling; NT- no-till drilling.

### Conclusions

Grain yield of winter wheat sown into growing white clover significantly lagged behind that of conventionally sown wheat. In autumn before sowing wheat, inorganic N concentration in the soil was generally greater after ploughed-down white clover. In spring in no-till drilled wheat, soil inorganic N concentration was found to be the lowest, and this trend persisted until wheat ripening. Winter wheat sown after ploughed-down white clover yielded 300-480 kg grain ha<sup>-1</sup> more than wheat grown conventionally after barley (without clover). The yield of triticale was also higher following wheat grown after pre-crops that included white clover.

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## Production and persistency of red clover (*Trifolium pratense*) varieties when grown in mixtures

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### Abstract

In the Netherlands organic and conventional dairy farmers are taking an increasing interest in grass and red clover mixtures for ley pastures (cutting only). A constraint to the adoption of such mixtures is the persistency of the red clover (*Trifolium pratense* L.) varieties presently used in the Netherlands; Rotra, Barfiola, Violetta and Merviot. The latter being the most persistent under practical circumstances. Testing of red clover varieties in Switzerland showed a high degree of persistency of the so-called 'mattenklee' varieties such as Astur and Pica (Suter *et al.*, 2004). We compared eight red clover varieties including 2 'mattenklee' varieties in mixtures with perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.), and a mixture containing white clover and perennial ryegrass only. Red clover mixtures out yielded the white clover mixtures by 5.4 t dry matter (DM) ha<sup>-1</sup>. ULC 1715186 and Astur were the most productive red clover varieties. The development of the clover content of Astur showed that this variety scored highest on persistency.

Keywords: red clover, varieties, yield, persistency.

### Introduction

Red clover, in combination with perennial ryegrass and white clover, can achieve high yields with a low degree of fertilisation. A mixture of perennial ryegrass with red and white clover out yields a mixture of perennial ryegrass with only white clover by 1 to 1.5 t DM ha<sup>-1</sup> (Wit *et al.*, 2004). This makes such mixtures interesting crops for organic dairy farmers as well as intensive conventional dairy farmers in the Netherlands. In such systems the mixture fits perfectly in a crop rotation with maize. Red clover usually yields well for two to four years, after which the majority of nitrogen fixation is taken over by white clover. The production and persistency of these systems depend on the red clover variety and frequency of harvesting. A constraint to the adoption of grass/red clover mixtures is the persistency of the red clover varieties presently used in the Netherlands; Rotra, Barfiola, Violetta and Merviot. This paper presents the production and persistency results of a comparison of eight red clover varieties in a mixture of perennial ryegrass and white clover in comparison with a mixture of perennial ryegrass with only white clover over two years.

### Material and Methods

The experiment was established on a commercial dairy farm in the north of the Netherlands, on a clay soil. The following red clover varieties were compared; Barfiola, Rotra, Tedi, Lemmon, Astur, Merviot, ULC 1715/86 and Pica. Red clover varieties were sown in a mixture with 30 kg ha<sup>-1</sup> perennial ryegrass, 3 kg ha<sup>-1</sup> white clover (Alice). The amount of red clover seed was based on the number of seeds in 5 kg ha<sup>-1</sup> Barfiola. The mixtures were sown on plots of 3 m x 8 m each replicated three times. The trial was sown in August 2002 after a crop of barley and peas. In 2003 and 2004 the plots were fertilised with 25 m<sup>3</sup> ha<sup>-1</sup> of slurry. Dry matter percentage and clover content in the dry matter were determined by taking 7 m x 1.5 m strips just before each cut. In 2003 as well as in 2004 the plots were cut four times.

### Results and discussion

In the first year, red clover developed slowly. This may be explained by the high soil nitrogen level left by the previous crop of barley and peas. Another explanation could be that the experimental plots were

invaded with chickweed (*Stellaria media* L.). Despite the slow establishment, high total yields were achieved in both years (on average 15.5 t DM ha<sup>-1</sup> in 2003 and 17.3 t DM ha<sup>-1</sup> in 2004; Table 1). However DM production of red clover in the second year almost tripled compared to the first year. When looking at DM production of red clover in 2003, it can be seen that the varieties Pica and Lemmon were less productive than ULC 1715/86 and Rotra (which formed the middle group) whilst Barfiola, Astur, Tedi and Merviot produced the highest clover yields.

Table 1. Production and persistency of red clover varieties.

Group	Variety	White clover	Yield dry matter (t ha <sup>-1</sup> )				% red* clover 4 <sup>th</sup> cut
			1 <sup>st</sup> year		2 <sup>nd</sup> year		
			Total	Clover	Total	Clover	
Diploid 'Mattenklee'	Pica	Alice	15.5 <sup>b</sup>	3.4 <sup>b</sup>	17.6 <sup>bc</sup>	10.5 <sup>b</sup>	41 <sup>a</sup>
Tetraploid 'Mattenklee'	Astur	Alice	16.3 <sup>b</sup>	5.7 <sup>c</sup>	18.8 <sup>d</sup>	15.2 <sup>cd</sup>	74 <sup>b</sup>
Diploid 'Akkerklee'	Merviot	Alice	15.6 <sup>b</sup>	5.5 <sup>c</sup>	18.0 <sup>cd</sup>	13.2 <sup>c</sup>	42 <sup>a</sup>
Diploid	Lemmon	Alice	15.9 <sup>b</sup>	3.3 <sup>b</sup>	18.3 <sup>cd</sup>	11.0 <sup>bc</sup>	41 <sup>a</sup>
Tetraploid	Barfiola	Alice	16.5 <sup>b</sup>	6.3 <sup>cd</sup>	17.0 <sup>b</sup>	11.7 <sup>bc</sup>	52 <sup>a</sup>
Tetraploid 'Akkerklee'	Rotra	Alice	15.5 <sup>b</sup>	4.6 <sup>bc</sup>	17.4 <sup>bc</sup>	12.7 <sup>bc</sup>	44 <sup>a</sup>
Tetraploid	Tedi	Alice	16.4 <sup>b</sup>	5.6 <sup>c</sup>	17.8 <sup>c</sup>	11.8 <sup>bc</sup>	51 <sup>a</sup>
Tetraploid	ULC 1715/86	Alice	16.5 <sup>b</sup>	4.5 <sup>bc</sup>	18.8 <sup>d</sup>	14.3 <sup>cd</sup>	55 <sup>a</sup>
White clover		Alice	11.7 <sup>a</sup>	0.1 <sup>a</sup>	11.9 <sup>a</sup>	4.4 <sup>a</sup>	55 <sup>a*</sup>
<i>Average</i>			<i>15.5</i>	<i>4.33</i>	<i>17.3</i>	<i>11.6</i>	<i>50</i>

\* In the variant 'white clover' percentage white clover was measured instead of percentage red clover. Within a column, values that are followed by the same letter differ not significantly ( $P < 0.001$ ).

In 2004, the production groups contained almost the same cultivars, with the difference that ULC 1715/86 and Astur yielded the highest production and Barfiola decreased in production in comparison with the other varieties (differences between groups were found to be significant ( $P < 0.001$ )). In the plots with only grass/white clover, hardly any clover was found in 2003. This was probably due to the heavy infestation of chickweed (*Stellaria media*), which affected the establishment of white clover much more than the establishment of red clover. In both years, DM production of the plots with only grass/white clover was significantly lower than that of plots containing red clover, even when the white clover percentage recovered in the second year. In the last cut of the second year, percentages of red clover decreased, whereas white clover percentage increased.

The 'Mattenklee' variant Astur showed the highest persistency, with 74% red clover in the last cut (Table 1). This was significantly higher than any of the other varieties ( $P < 0.001$ ). The frequency of harvesting was equal for all variants in this experiment, four cuttings annually. Therefore, differences in persistency of the red clover varieties cannot be declared by harvesting frequency. The average harvesting frequency of four cuttings is, under Dutch circumstances, especially in case of high yields per cut, positive for the development of red clover (optimal three cuttings annually) in comparison with white clover (optimal five cuttings annually).

## Conclusions

In general, it can be concluded that grass mixtures containing both red and white clover gave higher yields when compared with grass mixtures with only white clover. Within the red clover varieties, Astur and ULC 1715/86 gave the highest DM production over the two years of the experiment. Astur was the most persistent red clover cultivar in the study.

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## Controlling broad-leaved dock (*Rumex obtusifolius*) in grass clover mixtures

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### Abstract

This article describes three experiments on the control of broad-leaved dock. Experiment 1: Dock seeds were ensiled in grass silages of different dry matter percentages; 23, 34 and 60% respectively. All silages showed a decline of seed vitality in time. Grass clover with dock seeds should be ensiled at a low dry matter percentage or remain in the silage bin for a longer period than 8 weeks. Experiment 2: In a potassium fertilisation trial on grass clover the development of dock was followed. After two years of potassium fertilisation, the number of dock and the root mass was not significant different between the fertilised and the unfertilised plots. It is concluded that potassium fertilisation at a low potassium status does not positively influence the dock development. Experiment 3: In a resown grass clover, dock seedlings were cut at three frequencies; 2, 4 and 6 weeks. After 3 months the number of seedlings had decreased the same in all treatments. However the root biomass of the seedlings was significantly affected. It is concluded that frequent cutting has a negative effect on root biomass but should be practised for a longer period than 12 weeks to have an effect on seedling numbers.

Keywords: broad-leaved dock, grass clover, silage, potassium fertilisation, cutting frequency.

### Introduction

For organic dairy farmers in the Netherlands the control of broad-leaved dock in grass clover is a major problem. Research on broad-leaved dock control focuses on three major topics; 1) controlling of the seed dispersion on the farm, 2) controlling docks in a permanent pasture and the control of docks at the time of reseeding a pasture (Van Eekeren and Jansonius, 2005). This article describes three experiments, covering facets of these major topics:

Experiment 1: Importation of seed on a farm via silage from natural grasslands looks one of the sources through which infestation of a farm with docks takes place. The objective of this research is to test the vitality of seeds in grass silage with different dry matter percentages.

Experiment 2: Soil potassium concentrations has a positive effect on number of docks (Humphreys *et al.*, 1999). With a low potassium status, potassium fertilisation has a positive effect on grass clover development. The objective of this research is to see the effect of potassium fertilisation on dock development.

Experiment 3: Once a resown grass clover ley is established young dock seedlings are difficult to cope with. Cutting frequency has been shown to have effect on first year dock plants in a grass clover mixture in pots (Humphreys *et al.*, 1999). The objective of this trial was to study the effect of cutting frequency on dock seedlings under field conditions.

### Material and methods

Experiment 1: Dock seeds were ensiled in pots with wilted grass of three different dry matter percentages. The vitality of ensiled seeds was measured each 2 weeks for 8 weeks, on basis of a tetrazolium test (Hampton and TeKrony, 1995).

Experiment 2: In an ongoing potassium fertilisation trial on grass clover, number of adult broad leaved dock plants were counted in June 2005. The fertilisation trial which started in 2003 is situated on a sandy soil, in a block design with 4 repetitions. Phosphate status of soil is high and potassium status of soil is low. The treatments in which dock plants were counted received either 0 kg K<sub>2</sub>O per ha<sup>-1</sup> or 480



kg K<sub>2</sub>O per ha<sup>-1</sup> on a yearly base. No other fertilizer was applied. Plots are cut 4 to 5 times per year. Besides number of plants the root biomass from 10 adult plants per plot was determined.

Experiment 3: In April 2004 a cutting trial was established on a resown grass clover, heavily infested with broad-leaved dock seedlings. Three cutting frequencies were practised; 2, 4 and 6 weeks interval. Plots size was 1.5 m x 5 m, layed-out in a randomised block design in 5 repetitions. The treatments lasted for 12 weeks. Before each harvest and after 24 weeks the number of seedlings were counted. After 12 weeks the root biomass of 10 plants per plot was determined.

## Results and discussion

Experiment 1: The vitality of seeds decreased significantly over time ( $p < 0,001$ ) (see Figure 1). The dry matter percentage of the silage had a significant effect on the vitality of the seeds ( $p = 0,004$ ). Even the silage with the highest dry matter (60% dry matter) showed a decline of seed vitality within 8 weeks after ensiling (Figure 1). Research carried out in Austria showed also a decrease of the germination percentage in silages with a dry matter percentages of 18 and 35% dry matter. However the germination percentages in a dryer silage (47% dry matter) remained stable (Pötsch, 2000).

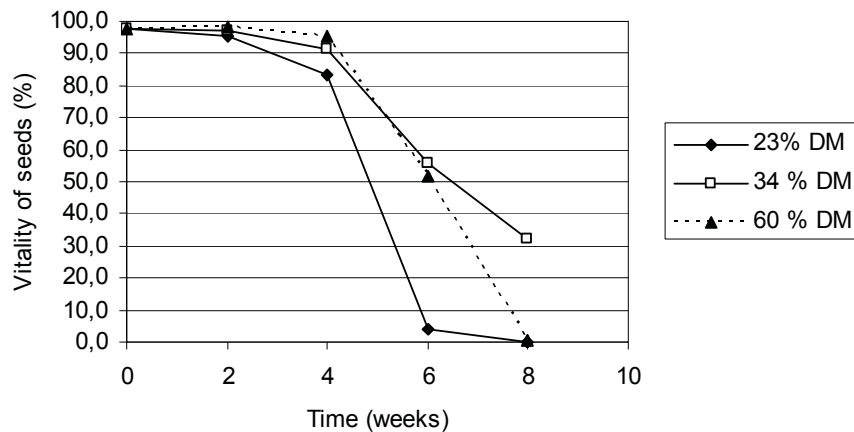


Figure 1. Development of dock seeds vitality in silage with different dry matter percentages.

Experiment 2: Fertilisation on grass clover gave a higher clover percentage and total dry matter production. The number and the root mass of adult broad leaved dock plants on the plots with potassium fertilisation is lower but differences are not significant due to variation within the plots (Table 1). Humpreys *et al.* (1999) found a positive effect of soil potassium concentration on the number of broad leaved dock plants. In the described experiment with a low soil potassium status, potassium fertilisation did not have an effect on the number of docks or root biomass.

Table 1. Number of dock plants and root mass under two potassium fertiliser rates.

Fertilisation	No. of adult plants m <sup>-2</sup>	Root mass G DM per plant
0 kg K <sub>2</sub> O per ha <sup>-1</sup>	0.81 (ns)	87.1 (ns)
480 kg K <sub>2</sub> O per ha <sup>-1</sup>	0.46 (ns)	66.2 (ns)

Experiment 3: The number of seedlings decreased steadily during the trial period but no difference between the treatments was observed (Figure 2). However, an increased cutting frequency had a significant ( $p=0,004$ ) diminishing effect on the root mass after 12 weeks of treatments; 0.40 g DM per plant at 2 weeks cutting interval, 0.62 g DM per plant at 4 weeks interval and 0.97 g DM per plant at 6 weeks interval. Again this did not affect the number of plants after 25 weeks. Apparently frequent cutting to control broad leaved dock seedlings has to be continued for a longer period. To make this practical for farmers this would probably mean continues grazing with young stock.

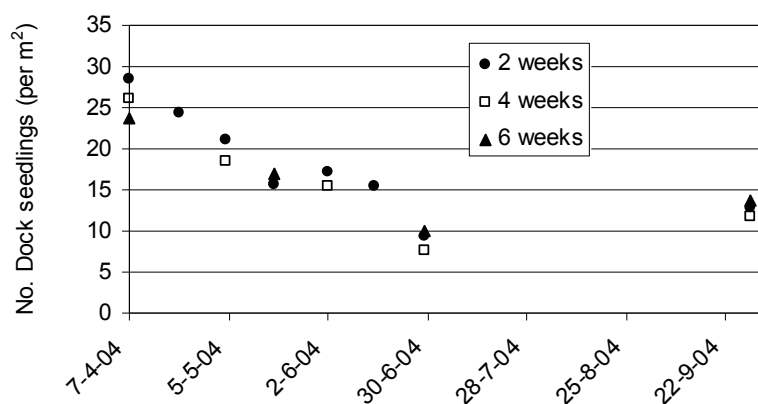


Figure 2. Development of number of dock seedlings under 3 cutting frequencies.

### Conclusions

Grass clover with dock seeds should be ensiled at a low dry matter percentage or remain in the silage bin for a longer timer than 8 weeks. Potassium fertilisation at a low potassium status does not positively influence the dock development. Frequent cutting has a negative effect on root biomass but should be practised for a longer period than 12 weeks to influence the number of seedlings.

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## ***Trifolium repens* and *Trifolium pratense* under P and K fertilization**

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### **Abstract**

The influence of PK fertilization on the production of white and red clover (*Trifolium repens* L. and *Trifolium pratense* L., respectively) was studied. The herbage samples were obtained along eleven years in a mountain meadow (type *Arrhenatheretalia* Las Salas, North West Spain) harvested three times each year.

As far as the effect of P fertilizer is concerned, it could be observed that the white clover production was increased in the three harvests performed each year, whereas that corresponding to the red clover was significantly decreased.

Regarding K fertilisation, white clover production was increased in all the harvests, whereas red clover production was not significantly modified by this element.

Keywords: white clover, red clover, management.

### **Introduction**

The meadows located in valleys, particularly the uplands of Spain, produce the principal source of forage for feeding ruminant animals in mountain areas. Fertilisation and irrigation can intensify management of these mountain meadows (Rodríguez *et al.*, 1996), thus being possible to obtain herbage productions higher than 10 000 kg DM ha<sup>-1</sup> yr<sup>-1</sup>. However, the application of mineral fertilizers changes the botanical composition, and hence the nutritive value of the herbage. For example, nitrate fertilization affects negatively two of the most important legumes present in these meadows, the red and white clover (García *et al.*, 2005). On the contrary, it was generally assumed that P and K fertilizers favoured legumes development. However, it has been demonstrated that the effects of both fertilizers are not the same for all the legumes (García *et al.*, 2005). Therefore, the aim of the present study is to analyse the effect of different levels of P and K fertilizers on the production (kg DM ha<sup>-1</sup>) of both, red and white clover, obtained in mountain meadows harvested three times each year.

### **Material and methods**

The study was conducted in an irrigated meadow of 3600 m<sup>2</sup> located in north-west of Spain (Las Salas, León; 42° 55' 60N, 5° 5' 60W) at an altitude of 1010 m. According to the Köppen Climate Classification System the area of study is characterised by a Temperate-Mediterranean climate (FAO-SDRN, 1997) with a dry summer season (types Csb-Cfb of the classification). Under these agro-climatic conditions, the growing season of the herbaceous vegetation starts in late March, once the average temperature is above 5°C. The precipitations are scarce (some years less than 30 mm) during the driest months (July and August), so the meadow has to be irrigated every 10-15 days at this time. Regarding the soil characteristics the texture is clay-mud, pH is slightly acid (6.2), with moderate levels of organic matter (122.7 g kg<sup>-1</sup>), nitrogen (6.24 g kg<sup>-1</sup>) and calcium (4,700 mg L<sup>-1</sup> CaO), but it is poor in phosphorus (87.5 mg L<sup>-1</sup> P<sub>2</sub>O<sub>5</sub>) and potassium (170 mg L<sup>-1</sup> K<sub>2</sub>O). According to FAO classification (FAO-ISRIC-ISSS, 1998) the soil is a Fluvisol Gleyic.

From a botanical point of view, this meadow is a plant community classified within the vegetation type *Arrhenatheretalia* (Rivas-Martínez *et al.*, 2002).

This experiment was carried out along eleven years. It consisted in 16 plots of the meadow (7 × 3.5 m each one) fertilized according to all the possible combinations of four doses of each fertilizer (P<sub>2</sub>O<sub>5</sub>: 0, 80, 160 and 240 kg ha<sup>-1</sup> yr<sup>-1</sup>; K<sub>2</sub>O: 0, 60, 120 and 180 kg ha<sup>-1</sup> yr<sup>-1</sup>). All these doses were applied in a

non-fractionated way during the last days of the winter season. The meadow was harvested three times each year, the first cut taking place during the early June and the two subsequent regrowths during July and September, respectively.

The herbage obtained in each plot was harvested and weighted separately during each harvest season. Then, a representative sample was taken from each plot to hand-separate white and red clover. The total production (kg DM ha<sup>-1</sup>) of both legumes obtained during each harvest season was calculated. Finally, the means were compared by using GLM and Duncan procedures contained in SAS software (SAS, 1989).

## Results and discussion

Compared to the control plot (P<sub>2</sub>O<sub>5</sub>: 0 kg ha<sup>-1</sup> yr<sup>-1</sup>; K<sub>2</sub>O: 0 kg ha<sup>-1</sup> yr<sup>-1</sup>) white clover production was increased with phosphoric fertilization up to 256, 169, and 207% during the first, second and third cut, respectively (P<0.01, Table 1). It has to be mentioned that during the first and third cuts the production was increased according to the fertilizer dose, but there were no significant differences of the dose as far as the second harvest was concerned.

Also, K fertilization had a positive effect on the white clover production, which was increased 83, 123 and 114% during the first, second and third harvest, respectively. Regarding the first cut no significant differences were observed among doses; on the contrary, significant differences in white clover production were demonstrated between extreme doses in both regrowths.

Table 1. Effect of the different doses (kg ha<sup>-1</sup> yr<sup>-1</sup>) of phosphorus and potassium on white and red clover production (kg DM ha<sup>-1</sup> yr<sup>-1</sup>) (n=44).

	Levels of P <sub>2</sub> O <sub>5</sub>				Levels of K <sub>2</sub> O				s.e.d.
	0	80	160	240	0	60	120	180	
White clover									
Harvest 1	206c	563b	686ab	781a	343b	577a	625a	678a	68
Harvest 2	257b	652a	667a	753a	329c	525b	671ab	798a	80
Harvest 3	185c	436b	429b	568a	241c	344bc	454ab	575a	66
Red clover									
Harvest 1	102a	95a	51ab	24b	96	82	43	51	25
Harvest 2	85a	59ab	68ab	30b	72	57	56	58	19
Harvest 3	68a	41ab	32b	16b	57	36	26	39	15

Different letters in the same line and harvest season indicate significant differences between means (P<0.01).

These results are similar to those described in the bibliography (Pérez, 1989; Rodríguez *et al.*, 1980), where a positive effect of both, P and K fertilization, on the white clover production has been observed. This is due to the fact that these fertilizers promote the extension of the roots, increase the number and area of leaves, and the length of the petioles. The effect of K is particularly important during both regrowths (Bailey and Laidlaw, 1998, Mosquera *et al.*, 2004).

On the other hand, red clover production was negatively affected by P fertilization, thus decreasing around 76, 65 and 65% during the first, second and third harvests, respectively. The main decrease in red clover production during the first and second harvests (P<0.01, Table 1) compared to the control plot (P<sub>2</sub>O<sub>5</sub>: 0 kg ha<sup>-1</sup> yr<sup>-1</sup>; K<sub>2</sub>O: 0 kg ha<sup>-1</sup> yr<sup>-1</sup>) was observed when the highest doses were applied. With regard to the third cut, the medium and highest doses of P produced a significant decrease in the red clover production. On the contrary, K fertilization had no significant effects on red clover production.

Red clover behaviour in a natural environment where different species grow up would be related not only to its morphological, physiological and agronomic characteristics, but also to its competition skills with other species. When there is enough N available for the plant, the ratio shoot/root is increased by the P fertilization. This fact could be very dangerous for the root development, and hence for the whole plant, as the red clover depends on the roots as a source of nutrients much more than white clover (Hellsten and Huss-Danell, 2001).

Moreover, it has to be stressed that, compared to the white clover, red clover has smaller capacity of regrowth (García and Calleja, 2003); in addition it has difficulties to form seeds before the second harvest, which is critical for its own persistence (Sakanoue, 2004).

Regarding the red clover, the lack of response observed with potassium was also described in different meadows of this mountain area (Rodríguez *et al.*, 1980). Moreover, unlike different meadows located in the North of Europe with more intensive management practices (Klitsch, 1965), our soils would be able to supply the doses of potassium required by our plants (Arnold and Close, 1961).

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## Assessment of white clover genetic diversity

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### Abstract

Research into morphological traits and biological characteristics of white clover (*Trifolium repens* L.) *hollandicum* and *giganteum* forms suggests a great diversity not only between the forms but also within the forms. The cultivars Sūduviai and Nemuniai belong to the *hollandicum* x *giganteum* form and according to earliness and abundance of inflorescences occupy an intermediate position, and according to herbage yield are equal to the clover of the *giganteum* forms. Having conducted DNA tests of different clover forms with 30 tetra-, tri- and dinucleotide motif primers, no DNA fragments specifically marking these forms were obtained. However, a slight variation of DNA profiles between varieties was identified. Using different primers, 122 DNA fragments of which only 29 were found to be polymorphic, were obtained in the DNA profiles of the varieties. Higher polymorphism of DNA fragments was identified while testing profiles of separate individuals. DNA profiles of separate individuals of the varieties Bitūnai, Sūduviai and Nemuniai, amplified by 9 primers were found to possess 55 fragments, of which 42 were polymorphic.

Keywords: white clover, morphological traits, DNA analysis.

### Introduction

White clover is the chief legume used in pasture mixtures. It is characterised by a high nutritive value, rapid re-growth, wear tolerance and longer persistence in swards compared with red or alsike clover. These indicators depend not only on agroclimatic conditions but also on genetic origin. Not only morphological-physiological tests but also genetic tests are necessary to get a versatile assessment of the vast diversity of clovers. Recently attention has been drawn to the phenomenon of polymorphism within species and variety. Plant diversity is especially widely revealed by using molecular markers in tests. Unlike morphological markers, molecular markers are more varied, less dependent on the environment, informative at any plant development stage. The objective of our study is to determine the suitability of DNA fingerprints (inter-SSR) method for the assessment of clover forms, varieties and separate individuals.

### Materials and methods

For morphological analyses clover was grown in an experimental nursery with a 50x50cm nutritional area. The clover was tested for 14-16 morphological or biological traits. Assessment in points was done using the standards developed by the International Plant Genetic Resources Institute (IBPGR): 1 – 9 (IBPGR, 1992) or 3 – 7 (UPOV, 1985) point system, where 1-3 – very low or low, 5 –medium, 7-9 – very high value of the trait.

DNA was extracted from young leaves of 30 bulked plants per variety and from 20 individual plants from the varieties Bitūnai, Sūduviai, Nemuniai using a protocol of Doyle and Doyle (Doyle and Doyle, 1990). Polymerase chain reactions (PCR) were carried out in 25 µl volume in an Eppendorf Master Cycler Gradient thermocycler. Amplification products were analysed in 1.5% agarose gel and electrophoresis was carried out in 1xTAE buffer. GeneRuler™ DNA Ladder Mix (Fermentas) was used as the DNA fragment size marker. The gels were analysed in UV light by staining with ethidium bromide.

## Results and discussion

Clover belonging to *giganteum* form (Atoliai, Regal) surpasses *hollandicum* plants (except for Huia) according to the indicators determining herbage yield (plant height, leaf size, stolone thickness). The clover of *Hollandicum* form (Bitūnai, Huia, Nora) is earlier maturing, forms more inflorescences and re-grows more rapidly. The average height of plants of *giganteum* form varieties (Atoliai, Regal) was 13.7 cm, and within varieties between individual plants it ranged from 7.6 to 25.4 cm, whereas for *hollandicum* form varieties - it was 8.1 cm and ranged from 2.5 to 15.2 cm. To combine positive traits of both clover forms, mixed form *hollandicum* x *giganteum* varieties Sūduviai and Nemuniaiai have been developed in Lithuania. These cultivars take an intermediate position in terms of earliness and abundance of flowers, are equivalent to the cultivars of *giganteum* form in terms of herbage yield, and produce the same seed yield as the clover of the *hollandicum* form (Bitūnai, Nora). Sūduviai and Nemuniaiai demonstrate better re-growth than the clover of the *giganteum* forms; and Nemuniaiai is noted for tolerance of clover rot (Table 1).

Table 1. Description of morphological traits and biological characteristics of white clover varieties.

Variety	Systematic form	Leaf length	Petiole length	Petiole thickness	Stolone thickness	Date of flowering	Seed yield	Height	Profuseness of flowering	Growth rate	Resistance clover rot
Sūduviai.	1 x 2	7	5	7	5	5	5	7	5	5	3
Atoliai	2	7	6	7	7	7	3	7	3	3	3
Bitūnai	1	3	4	3	3	3	5	5	7	5	3
Nemuniaiai	1 x 2	7	5	7	5	5	5	7	7	5	1
Huia	1	5	5	5	5	3	3	5	5	5	7
Nora	1	3	4	3	3	3	5	5	5	5	3
Regal	2	7	6	7	7	7	3	7	3	3	3

\*1 –form *hollandicum*, 2 –form *giganteum*, 1 x 2 –form *hollandicum* x *giganteum*.

These cultivars take an intermediate position in terms of earliness and abundance of flowers, are equivalent to the cultivars of *giganteum* form in terms of herbage yield, and produce the same seed yield as the clover of the *hollandicum* form (Bitūnai, Nora). Varieties Sūduviai and Nemuniaiai demonstrate better re-growth than the clover of the *giganteum* forms; and Nemuniaiai is noted for tolerance of clover rot.

Having conducted DNA tests with 30 tetra-, tri- and dinucleotide motif primers of the varieties belonging to different morphological forms of DNA profiles between varieties was identified. Using different primers, 122 DNA fragments were obtained, of which only 29 were found to be polymorphic. Similar results were obtained while investing DNA profiles of white clover varieties using other methods (Kolliker *et al.*, 2001). A much higher diversity of DNA profiles was identified for separate individuals of the varieties Bitūnai, Sūduviai and Nemuniaiai (Table 2).

Table 2. ISSR products generated by 9 primers in Lithuanian white clover varieties.

Primer	Total number of reproducible bands	Total number of polymorphic bands	Number of polymorphic bands		
			Bitūnai	Sūduviai	Nemuniai
(AGAC) <sub>4</sub> GC	5	5	5	4	5
AC(GACA) <sub>4</sub>	5	4	3	3	3
(ACTG) <sub>4</sub> GA	4	3	2	3	3
(GACA) <sub>4</sub> TC	2	2	1	1	2
(CTC) <sub>5</sub>	9	4	3	3	4
(AC) <sub>8</sub> CG	5	3	2	1	2
(AC) <sub>8</sub> T	8	6	4	4	5
(CA) <sub>7</sub> GA	7	7	6	6	5
(AC) <sub>8</sub> G	10	8	8	6	7
Total	55	42	34	31	36

The fewest fragments were obtained using (GACA)<sub>4</sub>TC primer. Other tetranucleotide motif primers used generated formation of 4-5 fragments, of which all or larger part were polymorphic. Among separate individuals there were identified from 7 to 11 types of DNA profiles. Nine fragments were obtained by trinucleotide CTC motif primer, of which 44.4% were polymorphic. Dinucleotide motif primers also revealed a high DNA polymorphism between separate individuals of the varieties tested. Fifty five fragments were amplified by the primers used, of which 42 were polymorphic. Experimental evidence of Czechish researchers suggests that DNA analysis by RAPD method has also shown a higher DNR polymorphism between separate individuals within one variety rather than between different varieties (Dolanska and Čurn, 2004).

### Conclusions

The cultivars Sūduviai and Nemuniai belong to the *hollandicum x giganteum* form and according to earliness and abundance of inflorescences occupy an intermediate position, and according to herbage yield are equal to the clover of the *giganteum* forms. The variation of DNA profiles (fingerprints) between cultivars and between individuals within one cultivar was identified, however, no fragments specific to *hollandicum* and *giganteum* forms were obtained.

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# The impact of white clover and birdsfoot trefoil in simple mixtures with tall fescue

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## Abstract

The forage legumes *Trifolium repens* L. (white clover) and *Lotus corniculatus* L. (birdsfoot trefoil) are the most important components of mixtures with grasses, for sustainable agriculture practices. The impact of legumes on forage yield was studied in simple mixtures with *Festuca arundinacea* Schreb., a suitable species for dry conditions, at different levels of nitrogen fertilization.

The positive effect of legumes was more striking at  $N_0$ , the mixture yields exceeding the pure grass culture with 1.3 t dry matter (DM)  $ha^{-1}$  in the case of white clover mixtures and with 2.7 t DM  $ha^{-1}$  in the case of birdsfoot trefoil mixtures. At  $N_{50}$ , the yield gains were 0.6 t DM  $ha^{-1}$  in the mixtures with white clover and 1.9 t DM  $ha^{-1}$  in the mixtures with birdsfoot trefoil. At  $N_{100}$  the yields achieved in the mixtures with white clover and in the tall fescue pure culture were equal, while in the mixtures with birdsfoot trefoil the yield gain was 1.2 t DM  $ha^{-1}$ . The yields achieved by the grass/legume mixtures exceeded the yields of pure tall fescue culture fertilized with  $N_{50}$  and in the case of birdsfoot mixtures, even the pure culture fertilized with  $N_{100}$ .

Keywords: *Festuca arundinacea*, *Trifolium repens*, *Lotus corniculatus*, simple mixtures, nitrogen fertilization levels.

## Introduction

The presence of herbage legumes species in mixtures with grass and the maintenance of an adequate proportion, assures a high forage quality and improved the feeding value, decreasing in the same time the soil pollution, by the reduction of N-fertilizer inputs. (Breazu *et al.*, 1987; 2002).

The paper presents a study of the impact of two of the most important herbage legumes species: white clover (*Trifolium repens*) and birdsfoot trefoil (*Lotus corniculatus*) on the production of tall fescue (*Festuca arundinacea*), a suitable species for dry conditions, in relation with different N-fertilization levels.

## Materials and methods

The experiments were carried out during 2001 – 2004, at the Grassland Research and Development Institute, Brasov, on a levigated chernozem of sandy loam texture soil, with a pH of 5.8, with a 41 ppm P and 164 ppm K content.

The productivity of two tall fescue varieties: Brio (late) and Adela (middle early) was determined in pure culture and in simple mixtures with two legumes species: white clover (cv. Carpatin) and birdsfoot trefoil (cv. Doru). The seed rate was 25 kg  $ha^{-1}$  for tall fescue, 3 kg  $ha^{-1}$  for white clover and 5 kg  $ha^{-1}$  for birdsfoot trefoil.

Three nitrogen fertilizer levels were used:  $N_0$ ,  $N_{50}$ ,  $N_{100}$ . For  $N_{50}$  variants, the fertilizer was applied in spring, at the beginning of growing season and for  $N_{100}$  variants 50 kg  $ha^{-1}$  were applied in spring and 50 kg  $ha^{-1}$  after the first cut. The plots were subdivided in 4 replications on each level of fertilization, for each culture type and for each variety, the harvestable area of each plot being 12  $m^2$ . At every cut, botanical determinations were made. Analysis of variance was used for statistical interpretation.

## Results and discussion

The tall fescue dry matter yields in pure culture and in mixture with white clover and birdsfoot trefoil, on four years of experiments at three levels of fertilization are presented in Table 1. It must be mentioned that the period of experiments was very dry for this geographical zone, the rainfall in the third year of experiments was 310 mm lower than the yearly average, with higher temperatures and sunburn. Consequently, 2 cuts in the first year of experiments, 3 cuts in the second year and only 2 cuts in the third and fourth year were obtained. The production was much lower than the biological potential.

Table 1. Tall fescue dry matter (DM) yield ( $\text{t ha}^{-1}$ ), in pure culture and in mixture with legumes, at different level of fertilization ( $N_0$ ,  $N_{50}$ ,  $N_{100}$ ) and years of exploitation (I-IV).

Culture type	Variety	$N_0$					$N_{50}$					$N_{100}$					M
		I	II	III	IV	M	I	II	III	IV	M	I	II	III	IV	M	
Tall fescue pure culture	Brio	3.8	3.4	2.8	1.4	2.9	4.2	6.2	4.1	1.5	4.0	5.5	8.1	3.9	1.8	4.8	4.0
	Adela	3.7	3.2	3.1	1.8	3.0	4.4	5.9	4.5	1.6	4.1	5.6	7.7	3.6	1.6	4.6	4.0
	Mean	3.8	3.3	3.0	1.6	3.0	4.3	6.0	4.3	1.5	4.1	5.6	7.9	3.8	1.7	4.7	4.0
Tall fescue + white clover	Brio	4.1	8.0	3.8	1.7	4.4	4.2	8.6	3.6	2.0	4.6	4.6	8.7	3.9	2.3	4.9	4.6
	Adela	4.0	7.6	3.2	1.7	4.1	4.2	8.6	4.1	2.0	4.7	3.7	7.9	3.7	2.2	4.4	4.4
	Mean	4.1	7.8	3.5	1.7	4.3	4.2	8.6	3.9	2.0	4.7	4.2	8.3	3.8	2.3	4.7	4.5
Tall fescue + birdsfoot trefoil	Brio	4.2	9.5	6.8	2.8	5.8	4.3	10.1	6.7	2.9	6.0	4.7	9.8	5.9	3.2	5.9	5.9
	Adela	4.0	8.3	6.5	3.1	5.5	3.9	9.7	7.1	3.0	5.9	4.0	9.8	6.5	3.1	5.9	5.8
	Mean	4.1	8.9	6.7	2.9	5.7	4.1	9.9	6.9	2.9	6.0	4.4	9.8	6.2	3.1	5.9	5.9

DL 5% = 0.264 for type of culture (A).

DL 5% = 0.186 for the level of fertilization (B).

DL 5% = 0.457 for A x B.

The results showed that at the same level of N fertilization, the yields achieved in the mixtures are higher than in grass pure culture. In the climatically conditions of the experimental years, birdsfoot trefoil gave increased yields in comparison with white clover.

The positive effect of legumes is more significant in the unfertilized variants, the yield gain being in average  $1.3 \text{ t ha}^{-1}$  DM in the mixtures with white clover and  $2.7 \text{ t ha}^{-1}$  DM in the mixtures with birdsfoot trefoil. The gain was higher in the case of cv. Brio ( $1.57 \text{ t ha}^{-1}$  DM in the mixture with white clover and  $2.9 \text{ t ha}^{-1}$  DM in the mixture with birdsfoot trefoil), compared with cv. Adela ( $1.1 \text{ t ha}^{-1}$  DM in the mixture with white clover and  $2.5 \text{ t ha}^{-1}$  DM in the mixture with birdsfoot trefoil). At  $N_{50}$  level, the yield gains were, in average,  $0.6 \text{ t ha}^{-1}$  DM for white clover mixtures and  $1.9 \text{ t ha}^{-1}$  DM for birdsfoot mixtures. At  $N_{100}$  level the yields of pure culture and white clover mixtures were equal but in the variants with birdsfoot trefoil the gain was of  $1.2 \text{ t ha}^{-1}$  DM.

From the analysis of variance it results that the differences between the type of culture (pure stand, mixture with white clover and mixture with birdsfoot trefoil), the differences between the levels of fertilization ( $N_0$ ,  $N_{50}$  and  $N_{100}$ ) and the interaction of these factors are statistically assured. Regarding the percent of legumes in the mixtures, there were differences between the species.

Table 2. The percent of legumes in mixtures with tall fescue, on different level of N fertilization ( $N_0$ ,  $N_{50}$ ,  $N_{100}$ ) and years of exploitation (I-IV).

Culture type	Variety	$N_0$					$N_{50}$					$N_{100}$				
		I	II	III	IV	M	I	II	III	IV	M	I	II	III	IV	M
Tall fescue + white clover	Brio	62	48	26	15	38	63	45	15	16	35	55	36	6	6	26
	Adela	78	54	25	25	46	66	41	11	15	33	27	26	4	3	15
	Mean	70	51	26	20	42	65	44	13	16	35	41	31	5	5	21
Tall fescue + birdsfoot trefoil	Brio	50	61	75	66	63	29	53	51	59	48	22	31	3	42	32
	Adela	51	62	69	66	62	45	49	54	54	52	39	30	41	41	38
	Mean	51	62	72	66	63	37	51	53	53	50	31	31	37	42	35

In the first year of exploitation, at all the fertilization levels, the white clover percent exceeded those of birdsfoot trefoil. In the second year, as a result of especially dry climatic conditions, the percent of birdsfoot increased, this species being more resistant to drought. At  $N_0$  the white clover percent decreased from 70% in the first year to 20% in the fourth year, while the birdsfoot trefoil percent increased from 51% to 66%. At  $N_{50}$  the white clover percent decreased from 65% in the first year to 16% in the fourth year, while the birdsfoot trefoil percent increased from 37% to 53%. At  $N_{100}$  the white clover percent decreased from 41% in the first year to 5% in the fourth year, while the birdsfoot trefoil percent increased from 31% to 42%.

### Conclusions

It must be noticed that the yields achieved by tall fescue in mixtures with legumes at  $N_0$  exceeded the yields of pure culture fertilized with  $N_{50}$  and in the case of birdsfoot trefoil even those obtained at  $N_{100}$ . The addition of legumes to the grass improves forage quality and replaces the amount of N fertilization that is needed.

Tall fescue / birdsfoot trefoil mixture is very suitable for drought conditions.

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# Screening of symbiotic bacteria associated to the leguminous plant *Biserrula pelecinus*

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## Abstract

*Biserrula pelecinus* L. is an annual legume native to the Mediterranean basin, commonly used in pastureland. It is ideal in agricultural systems on acidic and sandy soils, providing high quality feed for livestock. In addition, its ability to establish symbiosis with nitrogen-fixing bacteria makes this species a good candidate to improve pasture quality. However, the nature of its symbiotic interaction with bacteria from the *Mesorhizobium* and *Sinorhizobium* species is sparsely known. This study evaluated the symbiotic interaction of 22 strains of *Biserrula* root-nodule bacteria collected from two different locations in the Iberian Peninsula with seedlings of *B. pelecinus*. The 22 strains were characterized using BOX-PCR. A total of 17 different strains were identified (7 from Spain and 10 from Portugal). We proved that different strains confer different characteristics of performance in terms of higher biomass production, as well as, better development on the host plants. Thus, the appropriate selection of inoculants might help enhance pasture production in the field.

Keywords: *Biserrula pelecinus*, rhizobial diversity, biologic nitrogen fixation.

## Introduction

*Biserrula pelecinus* L., unique in its genera, is an annual legume adapted to acidic and sandy soils (Howieson *et al.*, 1995). The plants develop a deeply rooted system that enhance their survival during dry autumns and springs when droughts are frequent (Carr *et al.*, 1999). Consequently, biserrula does not tolerate soils with bad drainage (Howieson *et al.*, 1998). To date, there are two cultivars of this species, 'Casbah' and 'Mauro', developed by CLIMA (*Centre for Legumes in Mediterranean Agriculture*). The study of this species in Portugal is being conducted by ENMP (Estação Nacional de Melhoramento de Plantas, Elvas), including the analysis of biodiversity of grassland legumes and a selection programme to find ecotypes adapted to poor and bad-structured soils within the Portuguese region of the Alentejo. Preliminary studies indicate that *B. pelecinus* presents a high specificity to its associated rhizobia. Howieson *et al.* (1995) observed that all strains isolated from *B. pelecinus* do not nodulate species of the common agricultural legumes and that species *R. leguminosarum* bv. *trifolii*, *R. leguminosarum* bv. *viciae*, *S. meliloti* and strains of *Bradyrhizobium* spp. do not nodulate biserrula. Through taxonomic and molecular analysis it has been possible to determine that bacteria nodulating *B. pelecinus* belongs to the genera *Mesorhizobium* (Nandasena *et al.*, 2001).

## Materials and Methods

Nodules of *Biserrula pelecinus* were collected in two locations: Avila, Spain (latitude 40° 27' 27"; longitude 5° 19' 33"; altitude 1062 m); and Elvas, Portugal (latitude 39°32'45" N; longitude 7°35'43"W; altitude 268 m). Isolation and morphological characterization of the bacterial strains was carried out as Vincent (1970). Identification of different rhizobial strains was performed through molecular techniques, namely BOX-PCR using primer BOX-A1R (0.4 µM) and PCR constituents: 1U of Taq polymerase (Fermentas); 0.24 mM of each dNTP; and 20 ng of DNA. The amplification programme consisted of an initial cycle at 95°C (2 min.); 30 cycles at 94°C (40s), then 40s at 58°C, 1min. at 72°C, and a final cycle of 3 min. at 72°C. The PCR products were separated by electrophoresis in 2% agarose and visualized under UV light. After identification, the inoculant strains were authenticated and further

submitted to evaluation of their infectiveness and effectiveness in their specific host, *B. pelecinus*. *B. pelecinus* seeds were surface sterilized and germinated in 7% agar. Seedlings were transplanted to trays containing a sterilized mixture of vegetal soil and sand. The trial was conducted in a glasshouse under controlled conditions of temperature (22°C day night<sup>-1</sup>), humidity (80mm day night<sup>-1</sup>) and photoperiod (10h day and 14h night). Two different treatments were applied (i) inoculated plants and (ii) non-inoculated plants (negative control). For each inoculant as well as for the control, eight replicates were produced. In parallel, the inoculant strains were grown in liquid medium YMB during 4 days in order to reach the logarithmic phase of bacterial growth. Plant development was documented during 60 days. Table 1 present the average results of the variables recorded per isolate (values calculated to a number of plants between three and eight since some seedlings did not resisted till the end of the experiment). The statistical *T-student* test was performed, at a significance level of 5%, comparing results of negative control (treatment ii) with results from each isolate used (treatment i).

## Results and Discussion

From the Spanish and Portuguese collections, respectively, 9 and 13 different bacterial strains were isolated. The Spanish strains were identified as 11 to 29; the Portuguese strains were identified as 31 to 313.

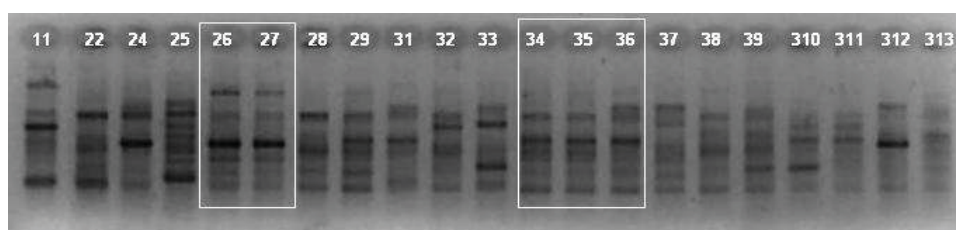


Figure 1. Amplification patterns using primer BOX-A1R.

Figure 1 presents the genetic patterns of all bacterial isolates obtained by PCR amplification. Patterns of inoculums 26-27 and 34-35-36 (framed in white) are similar, indicating that distant geographical locations hold identical rhizobial strains, which might explain the wide distribution of *B. pelecinus* as well as its potential to colonize new areas. It has important implications from both the economic and ecological point of view. On one side, *B. pelecinus* should not present marked difficulties if used to improve pasturelands due to the apparent broad presence of its associated symbiont. On the other hand, *B. pelecinus* might act as a strong invasive where not desired, so its use in agriculture has to be under strict controls. Table 1 presents the average results of the variables measured after the inoculation experiment on biserrula seedlings for Spanish and Portuguese isolates. For Spanish isolates, even though 25 produced fewer nodules than isolates 24 and 29, it resulted in higher biomass, suggesting that it may be more efficient in nitrogen fixation as observed in other leguminous species (Rodríguez-Echeverría *et al.*, 2003; Pérez-Fernández and Lamont, 2004). Similarly, plants inoculated with this isolate showed a significant (Table 1) higher shoot and root lengths and higher number of branches, indicating its greater contribution to plant performance. For the Portuguese isolates, 35 and 310 resulted in the best biomass production. Regarding effective nodules, isolates 35, 39 and 312 were the more infective and effective in comparison with the remaining ones. In conclusion, our results indicate that the right selection of *B. pelecinus* and its rhizobial symbionts would help in programmes of pastureland improvement, always using native strains.

Table 1. Average results of variables measured on seedlings of *Biserrula pelecinus* on the inoculation experiment. Asterisks indicate significant differences obtained on the *T-student* test.

Inoculum	Number of effective nodules		Shoot length (cm)		Root length (cm)		Number of branches		Dry biomass (grams)	
Control (-)	0,00		3,63		4,67		5,00		0,0070	
11	6,00	ns	5,00	ns	3,00	ns	6,00	ns	0,0158	Ns
22	4,00	ns	4,33	ns	5,17	ns	5,67	ns	0,0229	Ns
24	2,40	***	6,30	**	6,00	ns	9,00	ns	0,0346	**
25	2,25	ns	7,00	**	9,25	**	11,00	ns	0,0438	**
27	4,67	ns	4,17	ns	5,67	ns	6,00	ns	0,0188	Ns
28	2,00	ns	4,00	ns	2,75	ns	5,00	ns	0,0170	Ns
29	6,20	ns	5,90	**	5,90	ns	8,40	**	0,0372	**
31	2,75	ns	5,75	**	5,00	ns	6,75	ns	0,0292	**
32	2,80	**	6,30	**	5,90	ns	9,00	ns	0,0333	**
33	3,00	ns	4,50	ns	6,00	ns	6,00	ns	0,0146	Ns
35	4,38	ns	6,75	**	7,00	ns	9,50	ns	0,0450	***
37	1,60	ns	6,40	**	5,20	ns	9,40	ns	0,0413	Ns
38	1,67	ns	4,83	ns	6,00	ns	7,00	ns	0,0273	**
39	4,75	ns	5,38	**	5,75	ns	8,75	ns	0,0290	Ns
310	3,75	ns	7,13	***	6,13	ns	11,25	ns	0,0444	***
311	3,50	ns	5,50	ns	6,50	ns	7,00	ns	0,0225	**
312	5,00	ns	5,83	**	6,83	ns	7,33	ns	0,0355	Ns
313	2,50	ns	6,50	**	6,00	ns	8,00	ns	0,0368	**

Ns, not significant; \*\*, significant at 95%; \*\*\*, significant at 99%.

### Acknowledgements

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# The yield of grass-legume mixtures under different conditions of growth

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## Abstract

In order to reduce animal production cost, there is increasing interest in pasture system with lower inputs. The effect of different fertiliser systems on grass and grass/legumes mixture was studied during three years under different climatic conditions. The first and second year of the experiment were affected by strong drought. Three fertiliser systems were compared: 40 t ha<sup>-1</sup> manure in Year 1; 20 t ha<sup>-1</sup> manure in Year 1 + mineral fertiliser (50 kg ha<sup>-1</sup> N annually and 50 kg ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub> and 50 kg ha<sup>-1</sup> K<sub>2</sub>O in years 1 and 3); mineral fertiliser (100 kg ha<sup>-1</sup> N yr<sup>-1</sup> and 50 kg ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub> and 50 kg ha<sup>-1</sup> K<sub>2</sub>O in years 1 and 3). In drought conditions there was a close relationship between potential of growth and the quantity of nutrients available in the soil. The yield was low, with a maximum of 5.81 t ha<sup>-1</sup> DM. This increased up to 12.12 t ha<sup>-1</sup> DM in conditions where water was non-limiting. The total annual yield was higher under the mixed manure-fertiliser system. The drought affected white clover (*Trifolium repens* L.) more than birdsfoot trefoil (*Lotus corniculatus* L.), which accounted for over 25 % of the yield in every year.

Keywords: fertiliser system, grass, legumes, mixtures, manure, nitrogen nutrition index.

## Introduction

For increasing ruminant productivity, it is necessary to produce sufficient forage of optimal quality, which can be achieved by good management of sown grassland. Many data are available about the effect of fertilisers on the growth processes of grass/legumes mixtures and on forage quality (Frame 1992, Baars 2001, Razec *et al.*, 2002) but it is still necessary to study these effects under novel conditions. The objective of this study was to analyse the effect of fertiliser systems (organic, organic + mineral and mineral) on the yield and mineral N nutrition of grass and grass/legumes mixtures. The hypothesis was that the fertiliser system and grass/legume balance are additional factors influencing the level of yield and maintenance of an optimum N nutritional level.

## Materials and methods

The experiment was carried out, during 2002-2004 on a sandy loam chernozem soil with a good supply of mineral nutrients and a pH<sub>(H2O)</sub> of 6.5. Three mixtures were sown in spring 2002; grass species (A<sub>1</sub>), grass species/white clover (A<sub>2</sub>) and grass species/white clover + birdsfoot trefoil (A<sub>3</sub>), replicated three times. The trials were cut two or four times per year, depending on climatic conditions. Three fertiliser system were compared (Table 1): 40 t.ha<sup>-1</sup> manure once for four years (B<sub>1</sub>); 20 t.ha<sup>-1</sup> manure and NPK (B<sub>2</sub>); mineral fertilisation NPK (B<sub>3</sub>). Solid cattle manure was used. The manure, P and K were applied in autumn. Total dry matter (DM) yield, proportion of legumes and total N content were determined. For the first cut, the level of nutrition was determined by the nitrogen nutrition index (NNI) to examine differences between factors. The NNI was calculated by the method of Lemaire *et al.* (1989). Differences between years were correlated with rainfall during the growing season.

Table1. Total annual inputs of nutrients from manure and mineral fertilisers.

Year	Manure 40 t ha <sup>-1</sup> (B <sub>1</sub> )			Manure 20 t ha <sup>-1</sup> + NPK (B <sub>2</sub> )			Mineral NPK (B <sub>3</sub> )		
	Equivalent N	P	K	N	P	K	N	P	K
2002	260	79	120	180	57	101	100	22	41
2003	-	-	-	50	-	-	100	-	-
2004	-	-	-	50	22	41	100	22	41
Sum	260	79	120	280	79	142	300	44	82

## Results and discussion

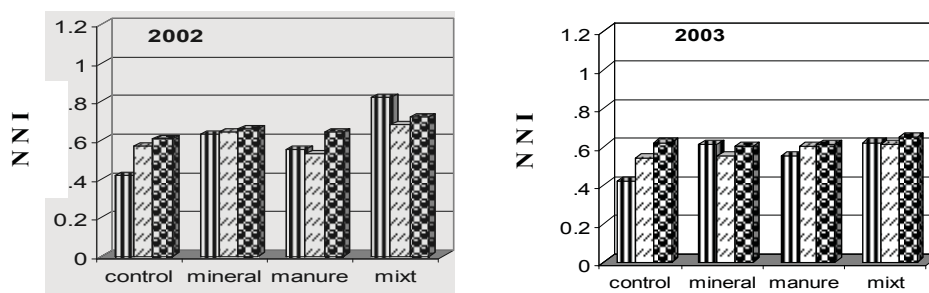
Between years of study there was a big difference of yield, due to climatically conditions. The total DM yield was lower in 2002 and 2003 than in 2004. The quantity of rainfall had a big influence on DM accumulation during vegetative growth in each year (Table 2). Drought conditions reduced the effect of manure and inorganic fertiliser and it was possible to obtain only two cuts in the first and second years of the study. White clover growth was slow under drought conditions in summer, which affected DM yield and forage quality. In contrast, birdsfoot trefoil showed good resistance to drought conditions, which accounted for over 25 % of the yield in every year. Better climatic conditions resulted in a higher proportion of legumes in the total DM yield in 2004. The annual total DM yield was higher under A<sub>3</sub>B<sub>2</sub> and it reflected the nutrients available during the growth season. P and K were the most important nutrients for growth and survival legumes. The highest N concentration was from the A<sub>3</sub>B<sub>2</sub> treatment, with a maximum value of 27.4 mg kg<sup>-1</sup> (mean 21.4 mg kg<sup>-1</sup>). The A<sub>1</sub>B<sub>1</sub> plot produced the lowest concentration of N at 18.6 mg kg<sup>-1</sup> (mean 19.1 mg kg<sup>-1</sup>). The different of N content in plants can be explain by level of fertilizer (Smith *et al.*, 2005) and content of legumes in DM.

Table 2. Annual DM yield and legume content of mixtures with different fertilising systems.

Fertilising system	Grasses			Grasses + <i>T. repens</i>			Grasses + <i>T. repens</i> + <i>L. corniculatus</i>		
	2002	2003	2004	2002	2003	2004	2002	2003	2004
Annual yield DM t ha <sup>-1</sup>									
Solid cattle manure	3.25	4.58	8.53	3.69	2.80	8.17	3.59	4.33	10.46
Manure + NPK	3.67	5.81	10.56	3.64	4.75	11.30	4.21	5.73	12.12
NPK	4.42	5.35	10.17	4.75	4.45	12.03	4.23	4.88	11.97
Difference between rainfall in year and long-term mean mm)	-----			-140	-232	6	-----		
Legume yield DM t ha <sup>-1</sup>									
Solid cattle manure	-	-	-	0.39	0.31	1.32	0.36	0.54	2.41
Manure + NPK	-	-	-	0.42	0.58	2.21	0.51	0.77	3.78
NPK	-	-	-	0.27	0.28	1.20	0.17	0.35	2.04

Differences: LSD 5% = 0.1264 t ha<sup>-1</sup> (between two mixtures means), LSD<sub>B</sub> = 0.3872 t ha<sup>-1</sup> (between two fertilising means), LSD = 0.118 t ha<sup>-1</sup> (between two years means), LSD<sub>AB</sub> = 0.2619 t ha<sup>-1</sup> (between two means in interactions).

The NNI was affected of climatically conditions too. For all mixtures the NNI was optimum (0.8-1) only in the third year of the study (Figure 1).





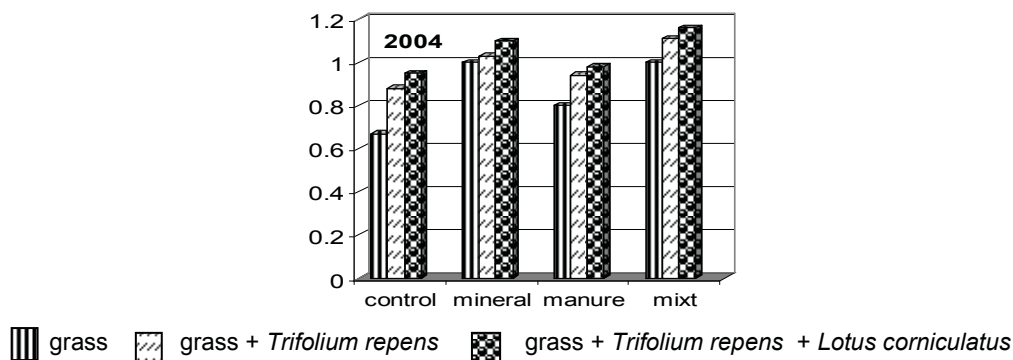


Figure 1. Nitrogen nutrition Index according to fertilizing system and year of growth for first cycle.

### Conclusions

The comparison of relationships between mixtures and fertiliser systems provided information about nutrient requirements for growth process. Growth was related to the quantity of nutrients taken up during the spring growth period. Also, climatic conditions during vegetative growth had a big influence on DM accumulation. For reducing the influence of climatic changes on annual DM yield, it is recommended to include in the mixture one or two species with good drought resistance (e.g. birdsfoot trefoil).

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## Mineral nitrogen nutrition of *Festuca arundinacea* in mixtures with legume species

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### Abstract

The influence of legumes species, *Trifolium repens* L., *Trifolium pratense* L. and *Lotus corniculatus* L., and fertiliser N level (0, 50, 100, 150 kg N ha<sup>-1</sup>) on the efficiency of N use were studied during a 3-year experiment. The first and second year were affected by drought. *T. repens* was most affected in the second year of the study, when the legume accounted for 12-18% of dry matter (DM) yield. *T. pratense* was most dominant in the first year, accounting for 80% of the yield, and then decreased to 8-12%. *L. corniculatus* performed well in drought conditions. It contributed 28-49% of the yield in the first year and increased to 81% in the third year. Yield was affected more by the proportion of legume than level of fertiliser. For the mixtures, the N yield was 517, 403 and 391 kg N ha<sup>-1</sup> for *L. corniculatus*, *T. pratense* and *T. repens*, respectively. The N yield for *Festuca arundinacea* Schreb. was 369 kg ha<sup>-1</sup>. There were large differences between years. DM yields were highest in the third year. For mixtures, an optimum NNI (0.8-1) could be achieved with 50 kg fertiliser N ha<sup>-1</sup> but for the pure *F. arundinacea* sward, this required 100-150 kg N ha<sup>-1</sup>.

Keywords: N fertiliser, grass, perennial legumes, N harvest, nitrogen nutrition index (NNI).

### Introduction

European Union rules referring to animal products include standards that farmers must adhere to for their products to be accepted onto the European market. In this context, it is necessary to reduce N inputs to minimise sources of environmental pollution. Many papers have shown positive effect of N fertilisers on grass growth, by linear increases in biomass yield up to fertiliser rates of 300-350 kg N ha<sup>-1</sup> (Frame 2000). But at the same time, the results showed the negative effect of high N levels on the proportion of legumes in mixed swards (Sousana and Arrequi, 1995). In Romania there is increasing interest in promoting perennial legumes to reduce N inputs and to provide solutions for organic agriculture, which is being more widely adopted in the country.

### Materials and methods

The study was carried out between 2002 and 2004 on a chernozem cambic soil of medium N content (2.0 g kg<sup>-1</sup>), good P and K status and a pH<sub>(H2O)</sub> of 6.7. Swards were *F. arundinacea* as a pure crop and in mixtures with *T. repens*, *T. pratense* and *L. corniculatus*, replicated in three times. All received a range of fertiliser N levels (0, 50, 100 and 150 kg N ha<sup>-1</sup>). Dry matter (DM) yield, harvested N yield and nitrogen nutrition index (NNI) (Lemaire *et al.*, 1997) were determined for each cut. There was a big difference between annual climatic conditions. Thus, 2002 and 2003 (first and second years of the study) were strongly affected by drought, with rainfall 140 and 232 mm, respectively below the annual long-term mean of 568 mm. Rainfall in 2004 was close to normal.

### Results and discussion

In general, *F. arundinacea* performed well in mixture with all species of legumes but the proportion of legumes was dependent on species and climatic conditions. In the first year, proportion of legumes was range between 38-58 % for *T. repens*, 87-92 % for *T. pratense* and 27-53 % for *L. corniculatus*. In the second year *T. repens* and *T. pratense* species were strong affected of drought. Thus, their proportion in DM yield was reduced to 8-12 % for *T. repens* and 12-19 % for *T. pratense*, instead *L. corniculatus* was ranged between 34-40 % and in third year this species increase up to 81 %, due to its good drought resistance (figure 1).

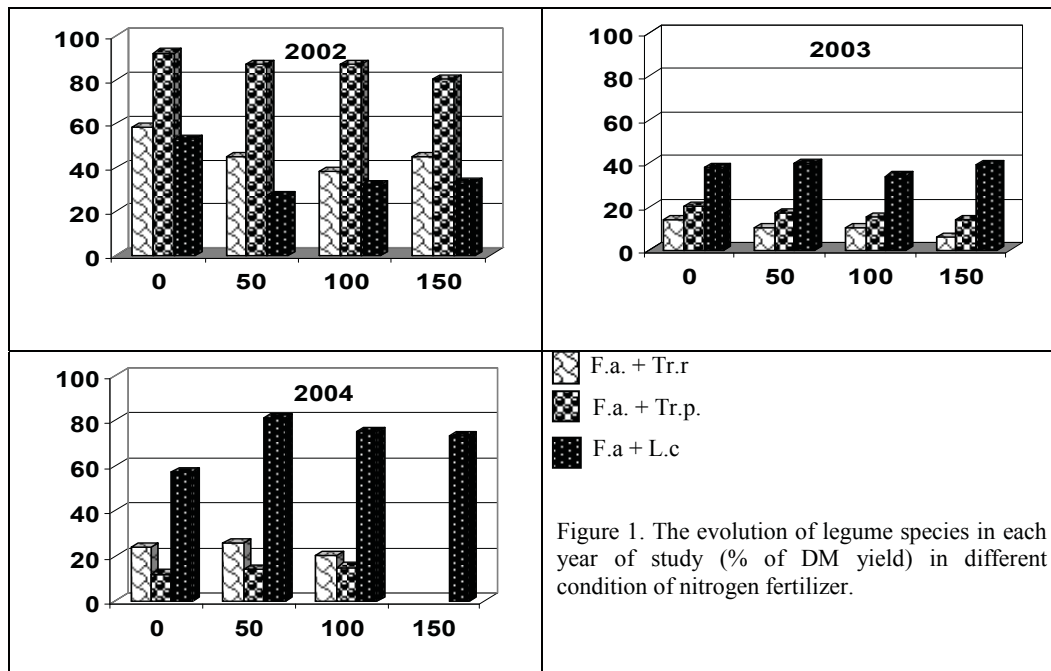


Figure 1. The evolution of legume species in each year of study (% of DM yield) in different condition of nitrogen fertilizer.

DM yield was a result of interactions between study factors, climatic conditions and year of study. The results provide evidence of the positive influence of legumes (Razec *et al.*, 2002) but are different for each species. Thus, *T. repens* contributed 2.17 t ha<sup>-1</sup> DM above the yield of the pure crop, *T. pratense* 3.82 t ha<sup>-1</sup> and *L. corniculatus* made the biggest contribution with 5.92 t ha<sup>-1</sup>. Nitrogen fertiliser level had a different effect on DM accumulation. For the pure *F. arundinacea* sward and mixtures with *T. pratense* and *L. corniculatus*, DM accumulation increased up to the 150 kg ha<sup>-1</sup> fertiliser N level, whereas *T. repens* produced a low yield at this level. Strong drought conditions in 2003 affected DM accumulation. *F. arundinacea* and the mixtures with *T. repens*. and *T. pratense* achieved only 38-55% of the DM yield obtained under normal rainfall conditions in 2004. The yield of *L. corniculatus* decreased by only 11-19%. There was a positive relationship between N yield and the mixture structure, DM yield, N fertiliser level and proportion of legume in the mixture. In the first year *T. pratense* achieved a higher harvested N yield (316 kg N ha<sup>-1</sup>) than for *T. repens* or *L. corniculatus* mixtures. The pure *F. arundinacea* sward registered a maximum of 198 kg N ha<sup>-1</sup> from the treatment receiving 150 kg fertiliser N ha<sup>-1</sup>. In the second and third year of the study, *L. corniculatus* registered the maximum N yield, at 269 and 517 kg ha<sup>-1</sup>, respectively. The results (Table1) indicate that there was a bigger influence of the proportion of legumes on N yield in the first year of study. In the following years, N yield was influenced more by N fertiliser level. In mixtures, NNI was optimum (0.8-1) when legumes contributed more than 25% of DM yield. For the pure *F. arundinacea* sward it was necessary to fertilise with 100-150 kg N ha<sup>-1</sup>.

Table 1. Dry mater (DM) yield and N harvest in relation to legume species with different rates of N.

Mixture / Year of study	N fertiliser ( kg ha <sup>-1</sup> )	DM ( t ha <sup>-1</sup> )			N harvest ( kg ha <sup>-1</sup> )		
		2002	2003	2004	2002	2003	2004
	0	5.23	3.79	6.71	148	119	264
<i>Festuca arundinacea</i> pure crop	50	6.03	4.01	8.14	163	123	261
	100	7.48	5.45	9.23	189	152	298
	150	8.09	5.62	12.2	199	155	369
	0	9.04	4.73	7.85	215	138	306
<i>Festuca arundinacea</i> + <i>Trifolium repens</i>	50	10.89	5.08	12.40	244	145	276
	100	10.26	5.17	13.50	234	147	383
	150	9.83	6.37	12.90	227	169	391
	0	14.54	5.37	7.96	296	150	330
<i>Festuca arundinacea</i> + <i>Trifolium pratense</i>	50	15.04	5.71	10.70	303	157	367
	100	15.04	5.86	12.20	303	160	403
	150	15.99	7.40	12.00	316	187	403
	0	8.57	13.28	14.90	207	278	479
<i>Festuca arundinacea</i> + <i>Lotus corniculatus</i>	50	8.41	12.62	16.32	204	269	486
	100	8.98	12.28	17.50	214	264	497
	150	7.79	12.67	19.50	235	270	517
LSD 0.05		1.14	0.48	2.68	8	6	14

### Conclusions

Each species of legume made a different contribution to botanical composition, DM yield, N harvest and NNI by biological N, legume species characteristic and in particular by its resistance to drought. *L. corniculatus* was particularly drought resistant. In the pure grass sward, 100-150 kg fertiliser N ha<sup>-1</sup> assured an optimum NNI. For mixtures, the 50 kg ha<sup>-1</sup> N rate was enough to achieve an optimum NNI for all legume species, provided the mixture contained more than 25% legumes in the DM yield. Satisfactory N nutrition increased DM yield and determined an optimal morphological structure in plants.

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## **Session 3**

### **Production and quality aspects of different animal feeds**



## Grazing versus indoor feeding: effects on milk quality

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### Abstract

Changing societal drivers and consumer demands request systems that provide desired products through improved, sustainable production processes.

In this paper dairy product supply chains were analysed, with emphasis on milk quality in relation to feed. Milk fatty acids were analysed in milk produced in different regions, various seasons, and with different feeding systems and also in forages and concentrates. A rapid screening method for conjugated linoleic acid (CLA) in milk fat was developed. Milk from cows on fresh green forage, especially grazed grass, had a much higher unsaturated:saturated fat proportion with more poly-unsaturated FA (beneficial for heart diseases) and more conjugated linoleic acid (CLA isomer rumenic acid, C18:2 c9,t11, possible anti-cancer effects) than milk from silage-fed cows. The FA composition of milk has recently become less favorable than before, e.g., in the 1960s, due to different feeding practices and nobody is aware because it was never monitored, but essential FA and CLA levels have dropped substantially. With low-fat dairy products, human intake of these is declining even further, as ruminant products are the main source of CLA intake.

Farmers from some dairy cooperatives in The Netherlands that produce milk from grazed grass now receive a premium on top of their milk price, so compared with farmers that keep their cows indoors year-round, these primary producers benefit from the higher market value at the end of the production chain.

Keywords: forage, silage, feeding system, seasonal change, milk fatty acids

### Introduction

Changing societal drivers (landscape values, animal welfare) and consumer demands (tasty healthy products) request systems that provide desired products through improved, sustainable production processes.

High-fat diets, especially those rich in saturated fats, can elicit detrimental effects on cardiovascular disease risk factors such as blood low density lipoprotein (LDL) cholesterol (Williams, 2000). Dairy products contribute 15-20% of the intake of total fat, 25-33% of saturated fat, and about 15% of dietary cholesterol in the US population (Havel, 1997). Milk fat contains approximately 70% saturated, 25% monounsaturated, and 5% polyunsaturated fatty acids (Grummer, 1991), but this can be modified by changing the animal diet. It is thought that lauric acid (C12:0), and especially myristic (C14:0) and palmitic acid (C16:0) raise total and LDL cholesterol, whereas stearic acid (C18:0) is neutral relative to the monounsaturated fatty acid (MUFA) oleic acid (C18:1) (Grundy and Vega, 1998). Cardiovascular risk might be reduced by lowering the intake of undesirable saturated FA or by making alterations in the quality of the fat consumed. Manipulation of the fatty acid composition of milk fat is receiving attention because dietary saturated fatty acids, especially myristic and palmitic acid, can induce hypercholesterolemia in humans (Denke and Grundy, 1992). Ashes *et al.* (1992) reported that feeding encapsulated canola seeds (60% oleic acid) significantly reduced the proportions of lauric, myristic, and palmitic acid in milk fat while increasing oleic acid. The resulting fat-modified milk contained 51% saturated, 10% polyunsaturated, and 39% monounsaturated fatty acids. When men and women consumed fat-modified dairy products, their plasma total cholesterol was reduced 4.5% compared with intake of conventional dairy products (Noakes *et al.*, 1996).

Ruminant products contain some polyunsaturated fatty acids (PUFA), including omega-3 FA (Dewhurst *et al.*, 2003), with associated health effects for the consumer. The main omega-3 FA in milk is  $\alpha$ -linolenic acid (C18:3 n-3). Another category of

PUFA are conjugated linoleic acids (CLA); the main isomer in milk being rumenic acid (C18:2 *cis*-9, *trans*-11). MUFA in milk consist mainly of C18:1 *cis*-9 (oleic acid) and also C18:1 *trans*-11 (trans vaccenic acid, TVA). Rumenic and vaccenic acid are both *trans*-11 FA produced by rumen micro-organisms and are unique for ruminant fat. They could also be termed omega-7 *trans* fatty acids (Ellen and Elgersma, 2004), to distinguish them from *trans* FA in general, which have a negative health effect. Rumenic acid has been associated with anticarcinogenic properties in rats (Corl *et al.*, 2003; Ip *et al.*, 1999) and possibly in humans (Aro *et al.*, 2000; Belury, 2002). Also other potential beneficial effects of CLA for human health were mentioned, but more work is needed to elucidate the safety and efficacy of isomers and required doses (Belury, 2002). Ingested TVA can be converted into rumenic acid in monogastrics, including humans (Salminen *et al.*, 1998; Santora *et al.*, 2000). Turpeinen *et al.* (2002) estimated that on average in humans 19% of dietary vaccenic acid is endogenously converted into rumenic acid. Banni *et al.* (2001) demonstrated that feeding vaccenic acid to rats (2% in the diet) not only increased tissue levels of rumenic acid, but also had a protective effect against the development of mammary tumours. This implies that, when calculating the dietary intake of rumenic acid, it is reasonable to add 20% of the vaccenic acid present in the diet. CLA contents in products, calculated on the fat fraction, are comparable with those of the milk(fat) of the raw milk from which these products are obtained (Lavillonière *et al.*, 1998; Dhiman *et al.*, 1999b).

Milk produced by farmers therefore plays a key role. As milk FA composition is mainly related to the FA composition of the feed of the animals (Khanal and Olson, 2004), effects of forage, feed and feeding systems will be discussed here.

### Lipids in animals

In contrast to short- and medium-chain FA, ruminants cannot endogenously synthesise these long-chain C18 FA that are desired in meat and milk. Therefore, long-chain FA have to be ingested by the ruminant with the feed.

Linoleic acid (C18:2 *cis*-9, *cis*-12) and  $\alpha$ -linolenic acid (C18:3 *cis*-9, *cis*-12, *cis*-15) are C18 substrates for rumen biohydrogenation (Fig. 1). In herbage, C18:3 predominates, whereas maize contains mainly C18:2. However, the FA content of maize is much lower than of grass. The FA profile of lipids in oils seeds used in concentrates is highly variable. In groundnuts, rapeseed and sesame seed C18:1 is the major FA, cottonseed, soybeans and sunflower seeds are high in C18:2 and linseed is high in C18:3. Currently used cheap concentrate feed ingredients such as coconut meal and palm kernels, however, contain mainly saturated fats (C14:0 and C12:0, respectively). Animal diets could also be supplemented with fish oil, containing predominately FA of 20 or 22 carbons as the major FA, but this might negatively affect milk taste and harm the image of dairy farming and dairy products while plant sources, especially forage, would represent the most natural and environmentally sustainable source.

There are several possible ways to reach the objective of increasing the concentration of desired FA in ruminant products, either to increase the amount (intake) or the concentration of substrate in the feed, to reduce the extent of biohydrogenation in the rumen, or to enhance the activity or amount of the  $\Delta^9$  - desaturase enzyme that converts TVA into rumenic acid in the udder (Figure 1).



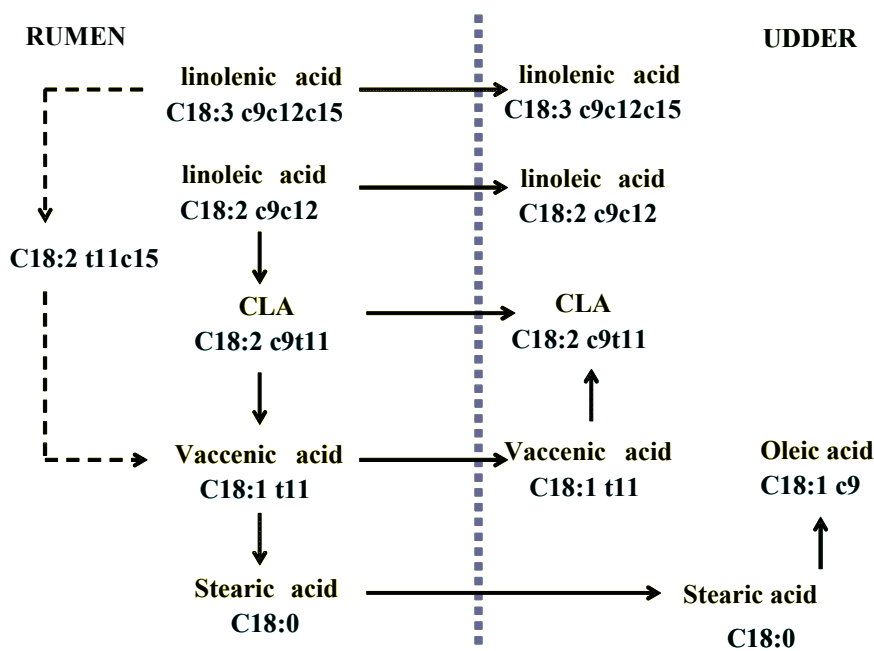


Figure 1. Simplified scheme of biohydrogenation and desaturation pathways of C18 fatty acids in rumen and udder of dairy cow.

Most linoleic and linolenic acid are biohydrogenated in the rumen. A varying proportion of PUFA, ranging from less than 2 to over 20% is recovered as TVA, an important product of the biohydrogenation of both linoleic and linolenic acid.

Lipolysis and biohydrogenation have been extensively studied *in vitro*, notably with soybean oil and to a lesser extent with linseed oil. The FA composition of linseed oil more or less resembles that of fresh grass, in that lipids in fresh grass also contain a high proportion of linolenic acid.

In the experiments with linseed oil of Chow *et al.* (2004), an accumulation of the intermediates C18:2 *trans*-11, *cis*-15 and TVA were observed. Because of its similarity with linseed oil, the C18:2 *trans*-11, *cis*-15 is most likely also an important intermediate in the rumen of grass-fed cows. It is likely that this is reflected in the FA profile of milk produced by grass-fed dairy cows. Both C18:2 (from maize and other grains) and C18:3 can be converted to TVA in the rumen and might lead to CLA in the udder by action of the  $\Delta^9$ -desaturase enzyme, but only C18:3 gives rise to the intermediate C18:2 *trans*-11, *cis*-15 in the rumen (Fig. 1). Comparing winter diets with diets on fresh grass revealed that in grass-fed cows the C18:2 *trans*-11, *cis*-15, when expressed as proportion of the sum of C18:2 + C18:3, ranged between 0.30 and 0.46, whereas on winter diets this was only between 0.029 and 0.042 (B. Vlaeminck, pers. communication). Changing from a grass and maize silage based winter diet to fresh grass elevated the level of this intermediate.

### Lipids in plants

Dairy cows' diets are usually composed of a mix of fresh forages (mainly leaf blades), conserved forages (herbage leaf blades and also stems in spring; seeds and stems in case of forage maize) and concentrates (seeds), all of which contain lipids of vegetable origin. Diet composition differs greatly among seasons and regions.

Some information is available in the literature about the lipid concentration and composition of forages and factors influencing them. The lipid fraction in leaves of herbs and grasses ranges from 30 – 100 g kg<sup>-1</sup> DM, much of which is contributed by lipids in the chloroplasts (Bauchart *et al.*, 1984). Sources of variation in lipid concentration are plant species, growth stage, temperature and light intensity (Hawke, 1973). There are five major fatty acids in grasses and approximately 95% consists of C18:3, C18:2 and C16:0 (Hawke, 1973). Fresh grass contains a high proportion (0.50-0.75) of its total fatty acid content in the form of  $\alpha$ -linolenic acid, C18:3. Levels of  $\alpha$ -linolenic acid vary with plant and environmental factors such as stage of maturity, genetic differences (Elgersma *et al.*, 2003a, b), season and light intensity (Dewhurst and King, 1998).

Quantifying the concentrations and composition of fatty acids in grasses in response to environmental factors could help to design management strategies to increase precursors for beneficial FA in products from ruminants. However, there is a lack of standardized procedures for sampling, storing, extracting and analysing lipids in leafy material. Drying, freeze-drying or thawing prior to extraction may affect the results and hampers comparison of data among the various laboratories. A ring test was set up in the autumn of 2005 for analyzing fresh and ensiled grass in different laboratories, and results are awaited.

Lipids in plants are not static entities, but are continuously subject to turnover, meaning that lipid degradation is a normal process in the living plant and that lipases are normally present. At the short term this will not have big influences on the FA composition of the lipid fraction in plants. There are at least three occasions when the lipid fraction in plants or plant parts may significantly be modified, during senescence, after detachment (grazing or cutting) and during storage.

In detached plants, immediately after cutting and perhaps during the early stages of ingestion and ensiling, the metabolism of plant cells can continue. Also, there is activity of the enzymes of dead tissue. The processes of respiration and proteolysis are best known, however also lipolysis occurs.

In ruminants grazing fresh pastures, the first stages of lipolysis could be mediated by plant lipases (Lee *et al.*, 2003). These enzymes are widely present in plants and their regulation might be altered due to the double stress of elevated temperature and anoxia imposed on the plant metabolism of intact plant cells after ensiling or ingestion by ruminants.

Dewhurst and King (1998) studied the effect of ensiling on the fatty acids in the material. Wilting prior to ensiling reduced the content of total fatty acids by almost 30%, with for linolenic acid even 40% loss. These authors suggest that the ensiling process itself has little influence, provided compaction and sealing of the silos is good. This may not always be the case in big bale silages. Adding silage additives (formic acid, formalin) resulted in much smaller losses, which was also found for formic acid by Doreau and Poncet (2000). Hay making reduced total FA by over 50%, with loss of linolenic acid to an even higher percentage (Doreau and Poncet, 2000). Similar observations were seen in haylage (70% DM) by Elgersma *et al.* (2003c).

Some work has been carried out to investigate the effect of nitrogen fertilization on FA concentration in the grass. Boufaïed *et al.* (2003) applied 120 vs 0 kg N ha<sup>-1</sup> on *Phleum pratense*, resulting in significant increases of C16:0 (18%), C18:2 (12%) and C18:3 (40%) in the herbage and an overall increase of 26% in the concentration of FA.

Elgersma *et al.* (2005) hypothesized that the protein concentration in the herbage, the leaf blade proportion of the canopy and regrowth period of the sward might affect the concentration of fat and the proportions of FA in the herbage. Regrowth period affected the total FA concentration, and significantly lower concentrations of C18:3 and C16:1 were found after a longer period of regrowth. N application resulted in higher concentrations of all FA. The FA composition was not affected by N application, but a longer regrowth period significantly decreased the proportion of C18:3 and increased those of C18:2 and C16:0. A strong positive overall linear relation was found between the concentrations of total FA and C18:3 with the N concentration in the herbage. These results were confirmed by further experiments (Witkowska *et al.*, 2006).

#### **Effect of feeding system on milk FA composition**

Cows on pasture (Dhiman *et al.*, 1999a; Elgersma *et al.*, 2004a) grazing lush green grass (Dewhurst *et al.*, 2003; Elgersma *et al.*, 2003d; Khanal and Olsen, 2004) at a high herbage allowance (Elgersma *et al.*, 2004b) produce milk with the highest concentrations of PUFA.

Experiments showed a quick response of the CLA content in milk to changing cows from indoor feeding to pasturing (Kelly *et al.*, 1998) and *vice versa* (Elgersma *et al.*, 2004a). The maximum effect is reached within about 5 days (Chilliard *et al.*, 2001).

Grazing cows at a high herbage allowance can select and ingest upper layers of the canopy, where young leaf blades predominate. It was therefore hypothesized that the protein concentration in the herbage, the leaf blade proportion of the canopy and regrowth period of the sward might affect the concentration of fat and the proportions of FA in the herbage. A high herbage allowance results in milk with high concentrations of CLA, probably because it enables selection of leafy plant parts with higher lipid concentrations.

As fertilization and herbage allowance can be managed easily by farmers, it might represent an interesting and feasible way to obtain higher concentrations of fatty acids in herbage. Also decisions on the timing of cutting or grazing, i.e., the regrowth stage at which herbage is harvested, can be a practical means of influencing herbage quality. Scollan *et al.* (2005) stated grassland offers considerable scope to help creating product differentiation in increasingly competitive markets.

Alternatively, also the concentrate composition could be modified (e.g., Ashes *et al.*, 1992). Last but not least, apart from milk FA composition, also the milk production level and the milk fat concentration have to be taken into account.

### Regional and seasonal variation in milk FA composition

Milk fat consists of a range of FA, but for this overview we will focus on proportions of unsaturated fatty acids (UFA), omega-3 FA, and the concentration of CLA, as these are the desired categories of FA.

Seasonal variation in milk FA composition has long been recognized and is caused by difference in feed between summer and winter. Nałęcz-Tarwacka (unpublished results) studied 429 cows of 23 farms in central Poland during two years. The proportion of UFA was 344 g kg<sup>-1</sup> in summer when all cows had green grass, and 308 g kg<sup>-1</sup> in winter when they were fed maize silage, grass silage and brewers' grain. The omega-3 FA declined from 9 to 8 g kg<sup>-1</sup>, and the CLA concentration from 7.2 to 5.4 g kg<sup>-1</sup>.

In another study, Reklewska *et al.* (2003) found CLA concentrations in milk of Black-and-White cows in Poland to be higher in summer (8.4 g kg<sup>-1</sup>) and autumn (8.9 g kg<sup>-1</sup>) than in winter (6.3 g kg<sup>-1</sup>). They also compared CLA in milk from cows on a total mixed ration (TMR) and on pasture, respective values were 6.1 and 11.7 g kg<sup>-1</sup>. A further study compared seasonal changes in cows indoors, on three different feeding systems (Table 1).

Table 1. CLA concentrations (g kg<sup>-1</sup> of milk fat) of cows on different feeding systems in Poland, depending on the season (Reklewska *et al.*, 2003).

Feeding system	Summer	Winter
Indoor-pasture ecological	10.0	7.1
Indoor-pasture conventional	5.0	6.9
Indoor TMR	6.7	0.4

Remarkably, contrary to the general trend of lower CLA concentrations in winter, cows on the conventional indoor-pasture system had a higher value in winter; the pasture in summer was ad libitum and in winter less pasture was offered.

Also cattle breed could play a role: Żegarska *et al.* (2001) reported milk compositions of summer milk from cows on pasture and found a higher CLA concentration for Polish red (11.9 g kg<sup>-1</sup>) than for Black-and-White (9.4 g kg<sup>-1</sup>).

Individual animal variation should be included as well. Animals on the same diet showed a three-fold difference in milk conjugated linoleic acid (CLA) content (Kelsey *et al.*, 2003).

Elgersma *et al.* (2004a) found a range in CLA contents in milk of six individual cows of 14-36 g kg<sup>-1</sup> of milk fat on grazed grass, and 4.0 – 5.8 g kg<sup>-1</sup> at the end of a 2-week transition period to a 1:1 grass/maize silage diet. In a second experiment, the CLA contents in milk fat of 12 cows ranged from

12.4 to 27.8 g kg<sup>-1</sup> on grazed grass, and from 4.0 to 8.6 g kg<sup>-1</sup> four days after transition to maize silage. In general, CLA levels differed among cows, but patterns in response to diet changes were similar. The ranking of cows for CLA concentration in their milk at the various sampling days was rather similar Elgersma *et al.* (2004a). Also Lock and Garnsworthy (2002) found consistency in the ranking of individual cows for *cis*-9, *trans*-11 C18:2 concentration over time on the same diet or when cows were switched between diets. This would offer scope for selecting high-CLA animals for breeding purposes, provided the trait is at least moderately heritable.

### **FA analysis - methodology**

The concentration of CLA in fat is usually determined by gas chromatography (GC), which is a costly and time-consuming analysis. Scientific progress is partly hampered by the labour intensive, slow and costly standard analysis of FA using gas chromatography.

A screening method (details are confidential and cannot be provided here) was developed to predict the CLA concentrations in milk fat by using an alternative analysis. The correlation between both methods was very high ( $R^2 = 0.998$ ) across a range of CLA concentrations (2 – 32 g kg<sup>-1</sup> fat) (Elgersma and Wever, 2005). The method is more rapid and could be used for screening purposes.

For lipid analyses in herbage, various methods are used. As mentioned previously, a ring test has been initiated to investigate effects on FA data.

### **From farm to factory to consumer – marketing and future perspectives**

It was discovered by chance that during the last decades, the FA composition of milk has become less favorable in The Netherlands and nobody is aware because it was never monitored, but essential FA and CLA concentrations have dropped substantially. The total FA production from cows could be higher as a result of higher milk fat concentrations, which would have to be taken into account for a total picture, next to the FA concentrations. However, with low-fat dairy products being marketed, human intake of these FA is declining even further, as ruminant products are the main source of CLA intake. Health effects of CLA need further study in humans, but the beneficial effect of unsaturated fats in general seems commonly accepted. CLA concentrations are correlated with those of unsaturated fatty acids and could perhaps be used as indicators for the FA composition of milk.

In the Netherlands, the findings mentioned in this review could perhaps undermine the currently increasing trend for cows to be kept indoors year round.

Farmers from a small Dutch dairy cooperative that produce milk from grazed grass now receive a premium on top of their milk price. So in this case primary producers benefit from the higher market value at the end of the production chain.

### **Conclusions**

This review showed that milk quality can be changed by farmers by feeding strategy and there are longer-term options for animal breeding. There are possibilities for a chain-approach from farmer to industry to consumer and small-scale examples are presented, but there are still questions regarding health claims in humans. Also, monitoring milk quality is no routine procedure yet. Research and standard protocols for sampling, storage and lipid analysis in forages are needed. An integrated chain approach is essential for putting science into practice.

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# Potential of fodder trees and shrubs as animal feeds in the Mediterranean areas of Europe

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## Abstract

Fodder trees and shrubs are indispensable sources of animal feed in southern Europe, particularly in areas with dry to semi-dry Mediterranean climate. This is because they can alleviate the feed shortages or even fill up the feed gaps in the winter and especially in the summer period when grassland growth is limited or dormant, due to unfavourable weather conditions. They are spontaneous species, essential components of natural communities. They cover large areas and constitute grazing lands for all domestic animals, mainly goats. Productivity and nutritive value vary widely among species and provenances. In general, they have low protein content, high fiber and ash and low to moderate digestibility. Their feeding value however does not always relate to their chemical composition due to the presence in most species of anti-nutritional factors such as tannins, alkaloids, saponins, etc., which limit nutrient utilisation and reduce animal performance. A number of native and exotic species have been selected for artificial plantations. The most important of them include *Medicago arborea* L., *Atriplex halimus* L., *A. nummularia* Lindl., *Chamaecytisus proliferus* Link subsp. *palmensis* (Christ.) G. Kunkel, *Amorpha fruticosa* L., *Colutea arborescens* L., *Morus alba* L., *Robinia pseudoacacia* L. and *Gleditsia triacanthos* L. All these species are nutritionally superior to most spontaneous species and are recommended as strategic resources to complement natural grasslands.

Keywords: natural shrublands, artificial plantations, feed gaps, goats, digestibility, strategic resources

## Introduction

A significant part of southern Europe amounting to about 693,000 km<sup>2</sup> is characterised as Mediterranean (Le Houerou, 1993). Animal production in this part of Europe is constrained by several factors, such as the rugged and highly dissected landscape, the rich but very variable vegetation and the complex land use systems (Talamucci and Chaulet, 1989). The most important factor though that affects herbage production and consequently animal performance is the Mediterranean climate, characterised by the alternation of cool and rainy winters with hot and dry summers.

Compared to temperate parts of Europe, where temporary or permanent grasslands are the main, if not the only, forage resources, Mediterranean areas have a variety of such resources with grasslands playing a less important role due to the limited area they occupy (Le Houerou, 1993; Papanastasis and Mansat, 1996). In addition, grasslands have a highly seasonal growth, due to the Mediterranean climate, involving two minima, a big one in the summer and a small one in the winter period. These minima are virtually feed gaps that create serious problems to animal production and survival, especially in the arid and semi-arid zones (Papanastasis and Mansat, 1996). On the contrary, woody plant communities such as shrublands and woodlands occupy much larger areas and play a significant role in animal production (Talamucci and Chaulet, 1989; Le Houerou, 1993).

Fodder trees and shrubs are common components of Mediterranean vegetation and are well adapted to the Mediterranean climate, particularly to summer drought. For this reason, they can fill up the feed gap of the summer period as well as the winter one if they are evergreen or seasonally dimorphic species (Kebaili and Papanastasis, 2005).

In the past, fodder trees and shrubs were considered poor feeds for the animals and effort was made to control or eradicate them from grasslands. Over the last 25-30 years, however, this point of view has



changed and substantial research has been done which shows that fodder trees and shrubs are important forage resources for the Mediterranean areas. On the other hand, a number of nutritional problems have been identified in several species. In this paper, the potential of fodder trees and shrubs as animal feeds is analysed and discussed and their integration into the Mediterranean production systems is explored.

### Biogeography of fodder trees and shrubs

#### Spontaneous species

Spontaneous species of fodder trees and shrubs are found all over southern Europe. They are parts of natural plant communities distributed along the coastal zone as well as in areas influenced by the Mediterranean climate. These communities may be shrublands or woodlands and cover an estimated area of 396,000 km<sup>2</sup> (Le Houerou, 1993). In addition, grasslands contain a small proportion of shrubs.

Shrublands are composed of a great variety of shrubs, which result in various vegetation types. They include maquis or matorral, i.e. dense shrub communities with evergreen sclerophyllous shrubs; garrigues, i.e. open shrub communities with evergreen or deciduous shrubs; and phrygana, i.e. dwarf shrub communities with seasonally dimorphic species (Papanastasis, 2000).

Woodlands are characterised by the tree overstorey composed of several species. Their understorey may be composed of only herbaceous or shrubby species but quite often a midstorey exists with shrubs and the understorey is composed of only herbaceous species. Based on the overstorey species, these woodlands could be dense (>40% tree cover) and dominated by coniferous or broadleaved species, or open (<40% tree cover) and dominated by broadleaved species, evergreen or deciduous (e.g. dehesas of Spain)

#### Cultivated species

Due to the relatively slow growth and poor feeding value of most spontaneous species, several studies have been made by Mediterranean scientists to select the most promising of them on the basis of desirable ecological, agronomic and nutritional characteristics, so that artificial plantations are established to meet specific needs of Mediterranean production systems (Olea *et al.*, 1992; Dupraz, 1999; Papanastasis *et al.*, 1999). Some of these studies also included introduced highly promising multipurpose species. The most important species that come out of these studies are the following:

1. *Medicago arborea* L. (tree medic) is a leguminous shrub, very palatable, winter growing shrub but sensitive to winter frosts and dormant in the summer (Papanastasis *et al.*, 1999). Much variability in production traits was found in natural populations, indicating the possibility of increasing the forage yield through early selection and breeding (Amato *et al.*, 2004).
2. *Atriplex halimus* L. (Mediterranean saltbush) is a perennial C<sub>4</sub> shrub, native in Mediterranean Europe, with an excellent tolerance to drought and salinity (Le Houerou, 1992). The same author also reported that there are two quite different groups extremely heterogeneous in terms of morphology, ecology, productivity and palatability to herbivores; one grown in northern (subsp. *halimus*) and the other in the southern shores of the Mediterranean basin (subsp. *schweinfurthii*).
3. *Atriplex nummularia* Lindl. (oldman saltbush) is an Australian species, with three subspecies, but virtually all the introductions in the Mediterranean Europe belong to the subsp. *nummularia*. Its major problem is its high sensitivity to overgrazing (Le Houerou, 1992).
4. *Chamaecytisus proliferus* Link subsp. *palmensis* (Christ.) G. Kunkel (tagasaste) is a native to Canary Islands, fast growing perennial leguminous shrub, well suited to sandy soils with a pH 5-7 (Olea *et al.*, 1992).
5. *Amorpha fruticosa* L. (false indigo) is a leguminous shrub originating in Central and Eastern North America. It produces increased amounts of grazable material of high quality during summer (Papachristou *et al.*, 1999).
6. *Colutea arborescens* L. (bladder senna) is a leguminous, deciduous shrub, native to the Mediterranean basin. It is a productive species prolifically sprouting after pruning (Papanastasis *et al.*, 1997). Its fodder quality and palatability are high.
7. *Morus alba* L. (white mulberry) is a deciduous, multipurpose tree with leaves valuable to animals for direct browsing (Talamucci and Pardini, 1993) or as fodder (Papanastasis *et al.*, 1999). Considerable

breeding work has been done in Japan and several varieties have been released including 'Kokuso 21' (Machii *et al.*, 2000).

8. *Robinia pseudoacacia* L. (black locust) is a leguminous, deciduous, fast growing and multipurpose tree species, also used as feed for livestock with a value equivalent to that of *Medicago sativa* L. In Mediterranean Europe, its foliage was found to be a very important source of feed to livestock (Papachristou *et al.*, 1999; Papanastasi *et al.*, 1999). It has several botanical varieties but the most important is *monophylla*. In a breeding effort to select clones of this variety, it was found feasible to combine high biomass production, high regeneration ability after cutting, reduced thorn length and high crude protein (CP) content of leaves (Dini-Papanastasi, 2004a).

9. *Gleditsia triacanthos* L. (honey locust) is a fast growing leguminous tree species, native of the eastern USA. It is a multipurpose tree, but its main use is for its pods, which contain high sugar content. In France, several clones have been selected, with pods of high quality for sheep feeding (Dupraz, 1999; Papanastasi *et al.*, 1999). Some of these grafted clones were tested with success for several years in northern Greece (Dini-Papanastasi, 2004b).

## Production characteristics

### Natural communities

Forage production in shrublands is affected by several factors. One such factor is their structure, namely shrub density and height. According to Talamucci (1987), a Tuscan high maquis produces 400-1,000 kg/ha of available fodder, while a dense *Cistus* maquis produces no more than 200kg/ha of fodder. Open shrublands can accommodate herbaceous growth as well resulting in higher available forage for grazing animals than dense shrublands (Platis and Papanastasi, 2003).

Another factor that affects forage production in shrublands is the age of shrubs since the last disturbance, especially burning. Several studies have shown a faster annual growth of *Q. coccifera* shoots in the early years after fire than later (Long *et al.*, 1978; Cañellas and San Miguel, 1991). This reduction in annual growth with age results in reduced forage production in aged shrublands compared to the younger ones.

Forage production however is also affected by environmental factors. According to Le Houerou (1993), maquis and garrigue production is inversely related to aridity; it ranges from 600 to 1,800kg/ha dry matter in the semi-arid zones and from 900 to 3,000 kg/ha dry matter in the sub-humid and humid zones. Also, phrygic rangelands dominated by *Cistus creticus* L., *Cistus salvifolius* L. and *Ononis spinosa* L. in western Crete, Kyriakakis and Papanastasi (1993) have found that forage production was highest (1,100 g/m<sup>2</sup>) in the middle elevation zone (600 m a.s.l.) followed by the high zone (1,200 m a.s.l.) with 925 g/m<sup>2</sup> and the low zone (25 m a.s.l.) with 597 g/m<sup>2</sup>. Shrublands are more productive than grasslands when grown in comparable environments (Papanastasi, 2000).

In forests, forage production is related to crown density and to the age of tree stand. Open and young stands have higher forage production than dense and old ones (Armand and Etienne, 1995; Braziotis and Papanastasi, 1995; Papanastasi *et al.*, 1995). In addition to understory production, fruit yield may also be significant in several silvopastoral systems, such as the *Q. coccifera* open forest in Crete (Papanastasi and Misbah, 1998).

### Artificial plantations

Artificial plantations have in general higher forage production than natural communities, because the species are more productive and are normally established in better soils. This production however is also affected by the particular species or accession involved, shrub or tree density, plant age and by environmental factors.

When the appropriate accessions were used in Sicily, *M. arborea* produced about 0.5 kg/plant/year of edible biomass (leaves, twigs and fruits) (Stringi *et al.*, 1987). *Atriplex* species can produce 1 to 3 kg of edible DM per plant and year in the semi-arid zone; under a mean annual rainfall of 200-400mm and appropriate management, *A. halimus* and *A. nummularia* can produce 2,000 to 4,000 kg/ha/year (Le Houerou, 1992).

Some deciduous species growing in more favourable environments can attain much higher production. For example, the Japanese variety of *M. alba* 'Kokuso 21' produced up to 2 kg DM/plant of leaves on

good sites in southern France, but in poorer sites the production was much lower (0.4 kg DM/plant) (Armand and Meuret, 1993). On the other hand, forage production of fodder trees and shrubs changes widely from one year to the next due to environmental factors such as air temperature and precipitation (Papanastasis *et al.*, 1997). Compared to cultivated herbaceous species, however, whose yield is available in the winter period, fodder trees and shrubs in general produce less annual yield but are available during the summer period (Table 1).

Table 1. Annual DM yield (t /ha) and availability periods of some forage species in central Italy: minimum and maximum of a three years period (modified from Talamucci and Pardini, 1999).

Species	Annual DM yield (t ha <sup>-1</sup> ) (min – max)	Availability period
Herbaceous species		
<i>Lolium rigidum</i>	5.2 – 6.3	Nov – Apr
<i>Phalaris aquatica</i>	5.9 – 7.2	Nov – Apr
<i>Festuca arundinacea</i>	6.4 – 8.5	Oct - May
<i>Bromus willdenowii</i>	6.0 – 7.8	Oct - May
<i>Lotus corniculatus</i>	4.2 – 5.8	Mar – Jun
<i>Trifolium subterraneum</i>	6.0 – 7.1	Nov – Apr
<i>Medicago polymorpha</i>	5.1 – 5.9	Nov – Apr
Woody species		
<i>Morus alba</i>	4.2 – 5.3	Jul – Oct
<i>Amorpha fruticosa</i>	4.0 – 4.8	Jul – Oct
<i>Robinia pseudoacacia</i>	5.5 – 6.1	Jul - Sep
<i>Colutea arborescens</i>	3.7 – 4.2	Jul – Oct
<i>Medicago arborea</i>	3.9 – 5.0	Aug – Sep / Jan -Feb

Finally, forage production of fodder trees and shrubs is affected by plant density. It has been found that dense plantations produce more forage per unit area although with lower production per plant (Ainalis and Tsiouvaras, 1998). With a spacing of 1.0 x 1.5 m, a plantation of *M. arborea* produced 3t/ha of edible biomass in Sicily (Amato *et al.*, 2004).

## Feeding value and animal performance

### Feeding value

The feeding value of spontaneous and cultivated fodder trees and shrubs in southern Europe is widely variable, depending on species or cultivars, plant parts, phenological stage, environmental conditions and management (Yiakoulaki and Nastis, 1987; Cabiddu *et al.*, 2000; Ventura *et al.*, 2002). The feeding value of some spontaneous and cultivated species is presented in Table 2. It is obvious that cultivated species are generally superior to most spontaneous species grown in natural shrublands. They contain sufficient crude protein levels to meet the demands of small ruminants for maintenance and lactation (NRC, 1981). The low to moderate values of organic matter digestibility of some species are probably a result of the high presence of secondary compounds such as lignin, tannins, etc. Woody species, used as sole feed for goats, supplied the energy but not the protein requirements for maintenance, because of the low protein digestibility caused by the high level of tannins (Perevolotsky *et al.*, 1993; Decandia *et al.*, 2004a).

Table 2. Concentration (% DM) of crude protein, NDF, ADL and *in vitro* digestibility of some spontaneous and cultivated fodder trees and shrubs in Mediterranean areas.

Species	CP	NDF	ADL	IVOMD
Spontaneous species				
<i>Pistacia lentiscus</i> <sup>1</sup>	6.0	43.3	24.0	52.3
<i>Quercus suber</i> <sup>1</sup>	7.0	55.1	36.0	54.3
<i>Carpinus orientalis</i> <sup>2</sup>	13.8	37.2	nd	55.7
<i>Fraxinus ornus</i> <sup>2</sup>	10.9	35.3	nd	52.4
Cultivated species				
<i>Medicago arborea</i> <sup>3</sup>	15.5	43.3	9.0	64.9
<i>Chamaecytisus proliferus</i> var. <i>palmensis</i> <sup>3</sup>	17.4	43.8	7.7	63.8
<i>Robinia pseudoacacia</i> <sup>4</sup>	20.6	43.2	6.6	57.8
<i>Atriplex halimus</i> <sup>5</sup>	14.6	31.2	13.1	74.5
<i>Atriplex nummularia</i> <sup>5</sup>	22.1	36.9	13.7	74.7

<sup>1</sup>Ammar *et al.*, 2005; <sup>2</sup>Yiakoulaki and Nastis, 1987; <sup>3</sup>Ventura *et al.*, 2002; <sup>4</sup>Papachristou and Papanastasis, 1994; <sup>5</sup>van Niekerk *et al.*, 2004

Species such as *M. arborea*, *M. alba*, *R. pseudoacacia* and *C. proliferus* subsp. *palmensis* are some of the most nutritious and palatable species (Stringi *et al.*, 1987; Ríos *et al.*, 1989). Moreover, the deciduous species maintain their nutrients during summer and can be used as supplements for animals (Papanastasis, 2000). An important role in animal nutrition is also played by fruits. The pods of *G. triacanthos*, for instance, can be used as supplements to sheep (Bruno-Soares and Abreu, 2003). The inclusion of 300 g DM/head/day of *Q. suber* acorns during the suckling period and the first three weeks of lactation in a complete diet of Sarda sheep neither reduced animal performance nor caused poisoning (Sitzia *et al.*, 2005).

#### Anti-nutritional factors

Feeding value of fodder trees and shrubs is restricted by the presence of secondary compounds or metabolites, which are generally termed as anti-nutritional factors. These compounds are not involved in primary metabolism but may have different roles, such as protecting plants from disease and herbivore intake, toxicity and mimicking hormone actions. Among them, tannins are the most widely occurring components. They are complex phenolic compounds contained in approximately 80% of the woody dicotyledonous plants (Rhoades, 1979). They are often classified into hydrolysable and condensed tannins and are considered to have either adverse or beneficial effects in animal nutrition depending on their concentration, animal species, physiological state of the animal and composition of the diet (Makkar, 2003).

Condensed tannins are usually associated with anti-nutritional effects in ruminants while hydrolysable tannins are more prone to cause toxicity (Kumar and Vaithianathan, 1990). High levels of tannins in the diet reduce digestibility and intake (Decandia *et al.*, 1999) as well as palatability, probably due to astringency feeling, which is caused by the interaction of tannins with salivary proteins and oral mucosa. However, tannins in low concentrations (2-4%) induce beneficial effects, which are associated with suppression of bloat in ruminants (Jones *et al.*, 1973) and protection of dietary proteins in the rumen.

Other compounds such as alkaloids, saponins, etc., present in fodder trees and shrubs, can also limit nutrient utilisation and reduce animal performance or even cause toxic symptoms. Several experiments have shown that the adverse effects of condensed tannins can be overcome by complexing them with the polymer polyethylene-glycol (PEG). For example, the daily administration of PEG to goats grazing on woodlands resulted in increased digestibility and intake (Silanikove *et al.*, 1996). Also, PEG-

supplemented Sarda goats spent more time foraging on tanniferous than herbaceous plants, ingested more dry matter and digested more proteins than unsupplemented animals (Decandia *et al.*, 2000a; b).

### **Animal performance**

The effect of browse to ruminant performance was mentioned by Aristotle and Theophrastus in the classical antiquity (Thanos, 1994). According to them, sheep are fattened on young *Olea europea* L. shoots or pods of *Cytisus aeolicus* Guss., while *M. arborea* can increase milk production.

During the last decades, several studies have shown the importance of using fodder trees and shrubs in increasing milk production and animal body weight gains (e.g. Borens and Poppi, 1990; Becholie *et al.*, 2005). However, although some species produce high quality fodder, not all ruminants can utilize it or its utilization is associated with high consumption of water. For example, *C. proliferus* subsp. *palmensis* could not be used as a supplementary feed for crossbreed dairy cows without decreasing feed intake and milk yield (Varvikko and Khalili, 1993). On the contrary, sheep supplemented with the same species at the rate of 30% of the total diet increased dry matter intake and body weight gain (Becholie *et al.*, 2005).

It seems that when browse leaves are given as a sole feed, small ruminants consume them in amounts that are inversely related with their content in secondary compounds and especially tannins. The administration of agents that deactivate tannins, such as PEG, has significantly improved intake and animal performance. It has been found that Sarda goats fed with fresh branches of *Pistacia lentiscus* L. and supplemented with PEG showed an increase of *in vivo* CP digestibility (Decandia *et al.*, 1999). Additionally, liveweight gains of Mamber goats during pregnancy, higher birth weight of kids and a considerable increase of milk yield were recorded in Anglo-Nubian and Sarda goats (Decandia *et al.*, 2000a, b) receiving PEG as a supplement.

### **Modes of utilisation**

#### **Natural communities**

Natural shrublands and woodlands can be directly grazed by livestock. However, their contribution to animals' diet widely varies among animal species and breeds, phenological stages of vegetation, regions of the Mediterranean zone and environments. Although goats are considered as opportunistic feeders with a very flexible foraging behaviour, they are the most appropriate animals to utilize the high-fibre, low-nitrogen forage produced on shrublands and woodlands. Also, since goats are also grazers, they can utilise the herbaceous component present in most situations. The high level of degradable protein in the herbage can counteract the negative effects of tannins on the environment in the rumen. For example, local goats in Greece grazing three shrublands with varying shrubby and herbaceous vegetation cover selected a diet with higher CP content and had higher intake levels in the pasture with the greater proportion of herbaceous species (Yiakoulaki and Nastis, 1995). Similar results were found in Sarda goats grazing shrublands with different herbage cover (Decandia *et al.*, 2004b). Cattle and especially sheep are less efficient in utilizing natural woody resources. Hardy local breeds of beef cattle however have been found to make good use of maquis and woodlands in Israel (Henkin *et al.*, 2005).

#### **Artificial plantations**

Cultivated fodder trees and shrubs can be used by all kinds of animals if included in artificial plantations. These plantations though have a high cost of establishment and present several difficulties in their management (Papanastasis *et al.*, 1999; Le Houerou, 2000), particularly in communally used grasslands. For this reason, such plantations should be used as strategic resources to complement natural grasslands during the critical periods of the year. There are four possible ways to integrate fodder trees and shrubs in grassland systems:

1. Browsing fodder reserves. Shrubs are established in a specific area of a private or a communal grassland farm for direct browsing by livestock. Animals should graze these stands during the critical periods and only for part of the day. In other words, they should be fenced and used as "reserves" (Le Houerou, 2000). The critical problem in these reserves is their grazing management. Various species

- have different acceptance by animals, which will result in selective grazing (Papachristou and Papanastasis, 1994).
2. **Feeding fodder reserves.** In this case, shrubs or trees are also planted in specific areas for indirect rather than direct use by animals. This means that branches of the fodder trees and shrubs are cut-and-carried to the animals in the barn at the end of the day for supplemental feeding (Papachristou *et al.*, 1999). In southern France, several farmers use ancient trees or newly established shrubs of *M. alba* to feed their animals with green leaves at the end of the day as a 'dessert' (Armand and Meuret, 1993).
  3. **Introduction into cereal fields.** Grazing of cereal stubble after crop harvesting in summer is a common practice in several parts of southern Europe. In these stubble fields, the contribution of fodder shrubs to goats' diet is important (Yiakoulaki and Papanastasis, 2003). Fodder shrubs have been established in southeast Spain in cereal fields in widely spaced (10-20m) rows, in order to improve their grazing capacity (Le Houerou *et al.*, 1991). Experiments in this region have shown that the stocking rate was increased to 3.3 ewes/ha/year, which is 3 times higher than without shrubs (Correal *et al.*, 1990; Otal *et al.*, 1991).
  4. **Silvopastoral systems.** Fodder trees can be planted far apart (8-10m) in rows or grids in grasslands so that an overstorey is created for production of foliage or, mainly, fruits to be used by the animals at the end of the summer or during the autumn and winter periods. A 7 years-old orchard of selected varieties of *G. triacanthos* produced more than 200kg/ha of high quality pods, providing a very valuable feed to animals during the critical winter months on top of the understorey grassland (Papanastasis *et al.*, 1999).

## Conclusions

The following conclusions may be drawn from this review:

1. Fodder trees and shrubs are both spontaneous and cultivated in the Mediterranean areas of Europe and constitute indispensable feed resources for livestock during the whole year but especially during the long and dry summer period.
2. Woody plant communities such as shrublands and woodlands are much more widespread in the Mediterranean areas of Europe than grasslands. Their forage production is variable but very often higher than that of grasslands. Artificial plantations with cultivated species are less common but more productive than natural stands.
3. Feeding value of fodder trees and shrubs is variable but cultivated species are superior to the spontaneous ones. In general, both groups contain sufficient protein levels to meet small ruminant demands for maintenance, but the organic matter digestibility is low to moderate due to the presence of secondary compounds, especially tannins.
4. Performance of animals fed with fodder trees and shrubs depends on the particular animal and plant species. Maximum performance can be achieved if the adverse effects of tannins are overcome by using additives or if shrubs are combined with herbaceous species.
5. Natural woody communities can be grazed directly by livestock, with shrublands being most appropriate feed resources for goats. Cultivated species, on the contrary, can be used as fodder reserves for grazing or feeding during the critical summer period in mixed production systems based on temporary or permanent grasslands.

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## Sheep on lowland pastures. A predator safe grazing alternative in Norway?

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### Abstract

In Norway, about two million head of sheep annually graze on unimproved range, mainly in the mountains. The past ten years, total losses of animals during grazing have increased from 3.7% to 6% simultaneously with increasing numbers of predators. On the other hand, large and safer grazing areas along the coast have been abandoned due to changes in the agricultural structure. A study was initiated to evaluate the value of different lowland pastures as an alternative to the traditional highland pasture, by comparing lambs performance. Ewes of Norwegian Land Race nursing twin lambs grazed on three different study areas: highland, cultivated coastal lowland and island pastures. Lambs average daily weight gain (ADG) from birth to slaughter (carcass weight) of 126, 132 and 126 g d<sup>-1</sup> for the three study areas, respectively, were not significantly different. It is concluded that starting spring grazing early on lowland pastures maintain a high growth rate in lambs during the summer. Therefore, using safer grazing areas in the lowland might be a possible management strategy to avoid heavy losses of sheep due to predators during the grazing period.

**Keywords:** weight gain, preventive measures, abandoned pastures;

### Introduction

Traditionally, sheep in Norway are kept in mountainous areas for summer grazing. In 2003, a total of two million heads of sheep were released to outlying pastures, mainly in the mountains. Lamb meat produced on mountain pasture is considered cheap to produce due to low labour costs. But, the number of large predators (lynx (*Lynx lynx*), Scandinavian brown bear (*Ursus arctos*), wolves (*Lupus lupus*) and wolverines (*Gulo gulo*)) has increased over the past 10 years. In the same period, number of sheep lost during grazing has increased from 3.7% to 6%. Therefore, more shepherding of the sheep is required. Several preventive measures have been tried to combine large predators with sheep farming (Hansen *et al.*, 2002). It seems that the best way to reduce losses of sheep to predators are to separate domestic sheep and predators in time and space. Moving sheep to predator-free grazing areas in the lowland could be an alternative. Animals' impact on abandoned meadows is well documented (Mitchell *et al.*, 1997) but few scientific works describe the animal performance when grazing these areas (Eilertsen and Lind, 2005). The aim of this study was to evaluate coastal lowland pastures vs. a highland pasture by comparing lambs performance.

### Materials and methods

In 2001 a three-year grazing experiment with sheep was initiated at Tjøtta Rural Development Centre (65°50'N, 12°28'E) in Northern Norway. Three study areas were used, a highland pasture, a grass dominated cultivated lowland pasture and an island pasture characterized by an old-field succession. The highland study area of approximately 15 km<sup>2</sup> ranged from 250 to 700 m above sea level. The area was hardly exposed to large predators. The quality of the pasture was characterized as less good to good (Rekdal, 2001). The lowland study area (Nordoya) of approximately 2.5 km<sup>2</sup> was located along the coastline and had been grazed by sheep and cattle for decades. This pasture was characterized as a very good grazing area (Rekdal, 2001). The third study area consisted of four small abandoned islands rising up to 20 meter above sea level. The total area of the islands was 0.23 km<sup>2</sup> and the quality was characterized as good (Rekdal, 2001).

The sheep (Norwegian Land Race) were all selected from the same herd at Tjøtta Farm. Only ewes from 2 to 6 years of age nursing twin lambs were included in the experiment. Lambs were born indoors during April and May. Each year, approximately 30 randomly selected ewes with lambs were sent to the islands (total animal density was 390 sheep km<sup>-2</sup>) for spring and summer grazing. A total of 40, 28 and 22 lambs were included in the experiment in the three study years, respectively. All other ewes and lambs were spring grazing at Nordoya. This herd was separated randomly in two equivalent groups each year in mid June. Approximately 300 ewes and lambs were transported by boat and released to the highland pasture (20 sheep km<sup>-2</sup>) for summer grazing including 46, 35 and 43 lambs, respectively, in the experiment. The remaining ewes and lambs (approximately 250) stayed at Nordoya for summer grazing with 32, 44 and 112 lambs, respectively included in the experiment. The total animal density at Nordoya was estimated to 500 sheep km<sup>-2</sup>, including 70 breeding cows and 35 heifers. In late August and early September all sheep were collected from the three grazing areas. Lambs were slaughtered when reaching a live weight above 42 kg. All lambs were weighted with a mobile spring weight at birth (birth weight), in mid June (spring weight), in late July (summer weight) and in late August/early September (autumn weight). Lambs ADG were calculated for spring grazing (birth to spring), early summer grazing (spring to summer) and late summer grazing (summer to autumn) using live weight and from birth to slaughter using birth weight and carcass weight.

## Results and discussion

Neither ADG from birth to slaughter nor ADG on spring grazing was significantly different between grazing areas (Table 1).

Table 1. Average daily gain (ADG) (g d<sup>-1</sup>) on spring grazing, early summer grazing, late summer grazing and from birth to slaughter (carcass weight) average of 2001, 2002 and 2003.

	Highland	Nordoya	Island	Significance
Spring	<sup>1</sup>	329 <sup>1</sup>	329	NS
Early summer	226 b	318 a	345 a	*
Late summer	284 a	283 a	185 b	*
Birth to slaughter	126	132	126	NS

<sup>1</sup> All lambs were grazing on Nordoya for spring grazing.

\* Significant at  $p < 0.05$ .

During the three years, the ADG on early summer grazing was significantly lower on highland pasture than on both Nordoya and the islands ( $P < 0.05$ ). The ADG on late summer grazing did not differ between lambs from the highland and Nordoya whereas lambs from the island pasture had a significantly lower ADG (Table 1). A high growth rate in lambs during spring grazing is important to maintain a high growth rate during the summer (Bekken, 1995). In the lowland, all the plants initiates and develops more or less at the same time and are available to the animals early in the summer. Nutritive values in forage plants changes during the summer being highest in the spring and lowest in the autumn. It is apparent that the nutritive value often becomes the limiting factor in the growth of grazing sheep late in the grazing season. The lambs on Nordoya maintained a high ADG throughout the summer, which indicates that the qualities of the plants are kept at a high level. It is likely to believe that the advantage of mixed grazing with sheep and cattle reported by Gudmundsson (2001) was an important reason for the high ADG throughout the summer.

The mountain grazing area has a large variety of different plants available to sheep. Plants develop slowly in the mountains due to late snow melting and low temperatures. Sheep take advantage of this and gradually graze new plant shoots from the lower mountain parts tracking phenological gradients to the higher areas. We found that the ADG was higher on late summer grazing than on early summer grazing. We assumed that the quantity of available pasture was limited for sheep on early summer

grazing, indicating that the spring begins later in the highland area than expected. Gudmundsson (2001) finds that ADG drops quickly from the middle of August.

To utilize the grazing potential on islands, it is important to have sheep onto them as soon as possible in the spring. The island pasture in this experiment had been invaded by plant species characteristic for old-field succession. Several studies (Mitchell *et al.*, 1997) report that abandoned areas become more open, the number of plant species increases and the composition of species changes when grazed by herbivores. The effect on lamb's growth weight when sheep are grazing in pastures exposed to old-field succession is only reported in few scientific works (Eilertsen and Lind, 2005). They show that lambs ADG is high. On small and limited areas the influence of the weather seems to be more sensitive to the total amount of available plants than on larger pastures with varying exposition and elevation. Sheep management systems involving small islands must be followed carefully to prevent a drop in the ADG late in the summer. In our experiment, the island pasture got short of food available to the animals during August 2003 and the sheep were not moved in time. We believed that this was the explanation of the significant lower ADG on late summer grazing. By using the ADG from birth to slaughter as a measure for the quality the results show that pasture quality and animal density on the study areas were satisfying.

### **Conclusion**

It is concluded that early spring grazing on lowland pastures maintain a high growth rate in lambs during the summer with the right management. Coastal lowland pastures are alternatives to traditional mountain pastures.

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## Utilization of autumn grass/clover: pellets or silage?

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### Abstract

In The Netherlands, most silage is produced in spring. Protein supplementation is often needed when this silage is fed in winter. In grass/clover swards, the clover content is usually at its highest in late summer/autumn, resulting in relatively high protein content. However, due to weather conditions the probability of loss of quality during silage production increases during this season. An alternative is artificial drying of the cut grass/clover and using the resulting pellets as a concentrate. In a feeding trial at organic research farm Aver Heino, three forms of protein supplementation were compared: autumn grass/clover silage (SIL), grass/clover pellets (PEL) and protein rich concentrates (PRC). PEL was a good substitute for PRC. Total dm intake was 19.9 and 19.5 kg cow<sup>-1</sup> d<sup>-1</sup>, respectively. Milk yield was 23.7 and 23.3 kg cow<sup>-1</sup> d<sup>-1</sup>, respectively. For SIL, total dm intake was 17.7 kg cow<sup>-1</sup> d<sup>-1</sup>; milk yield was 21.6 kg cow<sup>-1</sup> d<sup>-1</sup>. The costs for production of SIL, PEL and PRC were 0.17, 0.21 and 0.25 € kg<sup>-1</sup> dm, respectively. Milk yield minus feeding costs was lowest for SIL, 4.68 € cow<sup>-1</sup> d<sup>-1</sup>, compared to 4.89 and 5.05 € cow<sup>-1</sup> d<sup>-1</sup> for PEL and PRC, respectively.

Keywords: utilisation grass clover, nutrition dairy cows, economy.

### Introduction

In organic dairy farming in The Netherlands, grass/clover swards are the main source of nitrogen (N) and the main source of roughage in summer (grazing) and winter (silage and hay). A limitation is the growth cycle of clover, being slow to start growth in spring but growing profusely in late summer and autumn (Schils *et al.*, 1999). This results in a relatively low protein content in the first cut, which is often used for silage production for the winter period. In late summer and autumn the protein content increases, and in many cases high enough to result in inefficient N-use. To improve N-efficiency, many farmers limit the grazing time in this period, supplementing the ration with roughage with lower protein content, like maize silage or spring grass/clover silage. In this case, part of the grass/clover yield can be ensiled for the winter season. However, during late summer and autumn probability increases of sub-optimal weather conditions for ensiling. Another option is to artificially dry herbage from these cuts, and use the resulting grass/clover pellets instead of autumn silage or (expensive) organic high-protein concentrates.

### Material and methods

The Animal Sciences Group of Wageningen UR conducted a comprehensive study to explore the possibilities of artificial drying of autumn grass/clover in an organic dairy system. On its research farm for organic dairy farming, Aver Heino, half of an autumn grass/clover cut was wilted and ensiled (SIL), while the other half was artificially dried and pelleted (PEL). The resulting products were compared for forage quality. In a feeding trial with three groups of 15 lactating dairy cows these products were compared with a third treatment of protein rich concentrates (PRC), with similar protein supplement levels (total of 250 g digestible protein available in the intestine, DPI). The three groups were fed a basal diet of spring grass/clover silage (first cut) and maize silage. The total feed intake and resulting milk yield, protein and fat contents were measured.

The energy demand of ensiling or pelleting autumn grass/clover was compared to that of commercially available concentrates. Furthermore, an economic comparison was made, based on the results of the feeding trial, with feed costs and milk profits. A full Dutch report on the results is published by Klop *et al.* (2005).

## Results

In a pre-trial period of four weeks all cows got the same diet, resulting in an intake of 7.4 kg dm spring grass/clover silage, 4.5 kg maize silage, 4.5 kg concentrates, 1.1 kg SIL, 0.90 kg PEL and 0.67 kg PRC (kg DM cow<sup>-1</sup> d<sup>-1</sup>). The cows were milked by a robot, on average 2.26 times d<sup>-1</sup>. This frequency did not differ between treatments, because of actively herding cows to the robot when they had a long milk interval.

The quality of the grass/clover autumn silage and pellets is given in Table 1. Table 1 also gives the results of feed intake, milk yield and milk composition during the main trial period of ten weeks. The cows clearly showed a lower intake of SIL, compared to PEL and PRC, resulting in a lower energy balance and milk production. Also, N efficiency was slightly lower for SIL, as seen by a higher urea content of the milk and a lower N balance.

Table 1. Results of treatments with ensiled autumn grass/clover (SIL), artificially dried and pelleted autumn grass/clover (PEL) and protein rich concentrates (PRC) in a feeding trial with dairy cows.

	SIL	PEL	PRC	SED	Significance
Product quality					
dm content (% of product)	42.3	94.8	90.3		
crude protein content (% of dm)	19.1	21.6	19.9		
NE <sub>L</sub> (kJ kg <sup>-1</sup> dm)	5280	6000	7180		
DPI (g kg <sup>-1</sup> dm)	61	94	122		
Intake (kg dm cow <sup>-1</sup> d <sup>-1</sup> )					
spring grass/clover silage	6.4 b	9.0 a	9.0 a	0.2	P<0.001
maize silage	3.3 b	4.3 a	4.5 a	0.1	P<0.001
autumn grass/clover silage	4.0				
autumn grass/clover pellets		2.6			
protein rich concentrates			2.0		
regular concentrates	4.1	4.0	4.0		
Total intake NE <sub>L</sub> (MJ cow <sup>-1</sup> d <sup>-1</sup> )	108 b	124 a	125 a	2.3	P<0.001
Total intake DPI (g cow <sup>-1</sup> d <sup>-1</sup> )	1224 b	1417 a	1422 a	25	P<0.001
Milk yield (kg cow <sup>-1</sup> d <sup>-1</sup> )					
Fat (%)	4.65	4.43	4.65	0.10	NS
Protein (%)	3.31 b	3.35 ab	3.44 a	0.05	P=0.039
Lactose (%)	4.62	4.61	4.67	0.02	NS
Urea (mg 100 g <sup>-1</sup> milk)	24.2 a	20.8 b	20.4 b	0.5	P<0.001
Energy balance (NE <sub>L</sub> , % of requirement)					
Energy balance (NE <sub>L</sub> , % of requirement)	97 b	106 a	106 a	2	P=0.001
Protein balance (DPI, % of requirement)					
Protein balance (DPI, % of requirement)	99	99	100	2	NS
N efficiency (protein production as % of protein intake)					
N efficiency (protein production as % of protein intake)	26.7	27.4	28.7	0.9	P=0.098

Values with differing letters are significantly different; SED = standard error of differences of means.

For making silage or pellets energy is required. Energy demand was estimated at 0.9 MJ kg<sup>-1</sup> dm for silage, 11.9 MJ kg<sup>-1</sup> dm for pellets and 7.8 MJ kg<sup>-1</sup> dm for concentrates. The difference in energy demand between these products was mainly attributed to the drying process. Where no energy for

drying was used for silage, 9.5 MJ kg<sup>-1</sup> dm was used for pellets and 3.0 MJ kg<sup>-1</sup> dm for concentrates. For field work, harvesting, transport, grinding and pelleting the energy costs were lower for grass/clover pellets compared to concentrates.

The total financial costs for production of SIL, PEL and PRC were estimated at 0.17, 0.21 and 0.25 € kg<sup>-1</sup> dm (or 0.032, 0.035 and 0.039 € MJ<sup>-1</sup> NE<sub>L</sub>), respectively. For SIL, the costs per MJ are calculated for a well conserved silage. The total costs include costs for the land and cultivation for SIL and PEL, assuming a yield of 10,000 kg dm ha<sup>-1</sup>. For PEL, an EU subsidy of approximately € 0.068 kg dm<sup>-1</sup> (De Bont *et al.*, 2002) reduces the artificial drying costs to 0.11 € kg<sup>-1</sup> dm.

A farm economics model study for two typical farms showed milk yield minus feeding costs was lowest for SIL, 4.68 € cow<sup>-1</sup> d<sup>-1</sup>, compared to 4.89 and 5.05 € cow<sup>-1</sup> d<sup>-1</sup> for PEL and PRC, respectively.

### Discussion and conclusion

Artificial drying and pelleting of own surplus autumn grass/clover results in better feed quality, animal performance and economic return compared to ensiling autumn grass/clover, even when the ensiled product is of good quality. Pellets also appear to be a good alternative to protein rich concentrates.

However, the economic return is strongly related to factors the farmer can not fully influence: the market price for surplus grass/clover, the quality of the ensiled product, costs of drying, pelleting and transport, subsidies on drying, costs of concentrates, costs of milk quota, etc.

Artificial drying has a high energy demand. For this reason some farmers do not consider this procedure to fit within an organic system. In fact, the difference between the energy costs of drying own grass/clover and commercial concentrates also depends on the source of the products used in the concentrates. There is a large difference between using lupine from Southern Europe, or soy from South America. Also, the use of regional products for animal feed is considered increasingly important in organic farming. In this respect, artificial drying of own grass/clover is preferred.

### Acknowledgements

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# The role of natural grasslands and croplands in the diet of wild geese

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## Abstract

This paper presents the total number and annual distribution of wild geese population on Puszta Hortobágy, Hungary. Two migrating goose species, White-fronted Goose (*Anser albifrons*) and Bean Goose (*Anser fabalis*) contribute most to the total number of geese population. Feeding place selection of geese depend on the season. In autumn and winter, croplands largely provided food for geese flocks. Due to selections among fields and parts of the field, sometimes relatively high grazing pressure for the whole area may be severely multiplied in some cases. Grazing pressure on a field may be controlled by disturbing the geese flocks on the grazing field.

Keywords: wild geese, grazing, grassland, crop land, grazing pressure.

## Introduction

Hortobágy is the largest grassland in Central Europe and (Latitude: 47°50' – 48° N, Longitude: 21° E) serves as an important stop for migrating flocks of wild geese. The habitats on the Puszta may serve as feeding, resting and flocking together places for the migrating wild goose species. In recent years, attention has been paid to the effects of wild geese stocks on agricultural lands. These effects may cause crop, sward and soil damages. The degree of the effect on agricultural lands may depend on the total number of wild geese in a given area, the stock density on a given plot of land, the time spent on a given area and different weather conditions. Since 1989, we have made regular observations of the total number, species composition, daily routine feeding, resting and flocking together place selection of migrating wild geese flocks.

## Material and methods

The size of our observation area is about 20,000 ha, located in the northern and middle part of the Hortobágy; consisting of fishponds, natural grasslands and croplands. Observations were made 2-3 times a week in the migrating seasons. Geese were observed on resting and feeding sites during the day, and on sites where they spent the night. Goose monitoring was made according to the standard international method (Gilbert, 1998). In autumn of 2004 and spring of 2005, small (1 m<sup>2</sup>) sample plots were selected on a winter wheat field after the first visit of large wild geese flocks. On the plots, we recorded: number of plant shoots and leaves removed by geese; number of excreta droppings on the plots.

## Results

In November and March, we recorded a peak in goose number (Figure 1), when migrating geese moved from North to South in autumn and in spring from South to their Northern nesting places. There is a high variation among years and between seasons, which is influenced by weather conditions, feeding and resting place availability and the differences in the migrating routes of the goose flocks. The wild geese population consisted of White-fronted Goose (*Anser albifrons*) mostly. Of the migrating flocks, Bean Goose (*Anser fabalis*) is the second most common species. The area also has a nesting species, the Graylag Goose (*Anser anser*). The geese flocks selected different places for resting, feeding and spending the night. As resting places, fishponds or large waterlogged fields were preferred by geese.

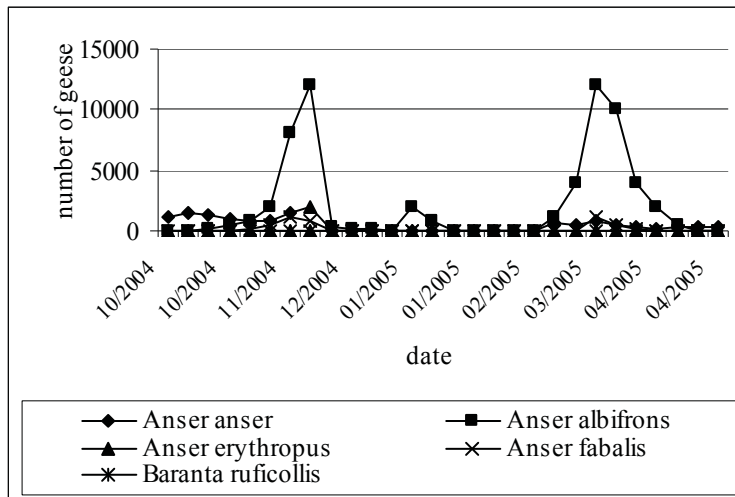


Figure 1. The goose number and its distribution among species on Hortobágy in the time of migration 2004/05.

Feeding places differed according to the season of the year. In spring, geese preferred fresh grass, so they mostly grazed on grasslands. In autumn and winter, goose flocks looked for arable lands (wheat, barley) where they could graze young shoots of emerging plants. If goose flocks were not disturbed on feeding sites, they continued to visit the selected places until they found available food. If a goose flock were to be disturbed by farmers on the fields during grazing time, they changed feeding sites. The distribution of the goose flocks was uneven on the grazed fields. On some sample plots of wheat fields, the goose population density was extremely high (rate of defoliation up to 100%), while on the others, e.g. on edges of the field, geese did not graze at all. The number of excreta droppings also changeable, but sometimes extremely high population density on the grazing fields (0-35 pieces m<sup>-2</sup>) (Figure 2.).

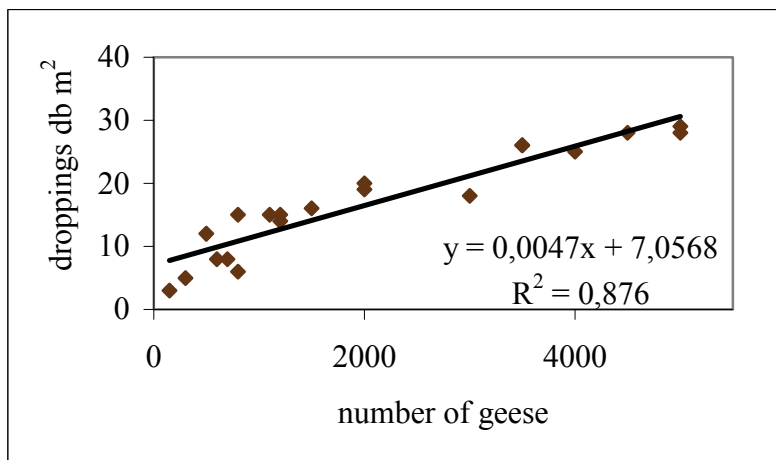


Figure 2. The correlation between goose number and droppings.

## Discussion

According to investigations of daily food consumption, daily food intake of wild goose species is about 0.15-0.30 kg DM depending on the food and the live weight of the goose (Sterbetz, 1979; Walterné, 1998). As a result, large populations of wild geese flocks may cause grazing pressure on agricultural lands, if weather conditions are not favorable. Their feeding place selection depends on available food supply. In spring they preferably graze on natural and cultivated grasslands in Hortobágy. In autumn and winter they have to move to croplands to take food. Our observations showed, however, that geese flocks select feeding areas and visit them day by day, so grazing pressure was high on the selected fields (150-200 geese-days ha<sup>-1</sup>) than the whole area (Kuyken, 1969). The winter crops can recover from total defoliation in spring after the geese move to north.

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# Sulla and chicory production and quality under sheep grazing management

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## Abstract

The role of sulla (*Hedysarum coronarium* L) and chicory (*Cichorium intybus* L) when included, as forage crops, in a dairy sheep system was evaluated in a trial carried out in Sardinia during 2000-2003. Herbage mass and quality of these perennial species in the year of sowing (SU1, CH1) and in the first year of re-growth (SU2, CH2) and milk production under grazing management were assessed. SU1 and CH1 were grazed after 112 and 109 days from sowing, respectively, when herbage mass was 1.55 and 1.24 t DM ha<sup>-1</sup>. On average, SU2 and CH2 were first utilised on 17<sup>th</sup> December and 4<sup>th</sup> November and yielded 1.43 and 1.47 t DM ha<sup>-1</sup>, respectively. The forages maintained high quality during the growing season. No differences were found in milk production (sulla 1.78 and chicory 1.67 kg ewe d<sup>-1</sup>).

Keywords: herbage mass; sheep milk.

## Introduction

Sulla (*Hedysarum coronarium*), a short perennial legume, is widely used as green forage under grazing management in animal production systems. It grows in Mediterranean environment during autumn - spring period and its inclusion in sheep diet improve milk production (Molle *et al.*, 2003). Thanks to a moderate level of condensed tannins sulla can improve the efficiency of protein use in ruminants (Barry and Mc Nabb, 1999) and can also reduce the gastrointestinal nematode burden in sheep (Niezen *et al.*, 1995). Chicory (*Cichorium intybus*) is a perennial daisy species well adapted to a temperate and Mediterranean climate. In New Zealand, chicory enhances pasture quality by improving the seasonal distribution of high quality herbage (Foster *et al.*, 2002). Like sulla, chicory has the potential to reduce the gastrointestinal nematodes (Molan *et al.*, 2003). The aim of this trial was to evaluate forage production and quality of sulla and chicory under a sheep grazing management in rain-fed Mediterranean conditions. In addition their complementary use as grazed forage when crops of different age are introduced, was also assessed.

## Materials and methods

The trial was carried out during 2000-2003 at the Bonassai Experimental Farm (40° 39' 46''N, 8° 21' 46''E) in the Northern west of Sardinia on a flat clay-loam calcareous soil, (pH 7.5). The climate is Mediterranean with a long-term average rainfall of 590 mm. Four swards, 1 ha each, 2 of sulla (1 year old, SU1, and two year old SU2) and 2 of chicory (1 year old, CH1, and two year old CH2), belonging to a 6 ha sheep system trial (Sitzia and Fois, 2005), were monitored. Sulla (cv Grimaldi) and chicory (cv Spadona) were sown, at the end of October with a seeding rate of 35 and 40 kg ha<sup>-1</sup>, respectively. At sowing all plots were fertilised with 100 kg ha<sup>-1</sup> of P<sub>2</sub>O<sub>5</sub>, in addition chicory received 70 kg ha<sup>-1</sup> of N. Sulla seed was inoculated with a specific rhizobia (*Rhizobium hedisari*) before seeding. The plots were rotationally grazed (grazing period of 7-14 days) during autumn-spring season, with an average stocking rate of 9.5 Sarda dairy ewes per ha. The ewes were machine milked twice a day. Daily milk yield, hay and concentrate consumption were measured. Before grazing herbage mass on offer (HM, 12 quadrats per ha of 0.5 m<sup>2</sup> each), its crude protein (CP) and NDF content (AOAC, 1984) and in vitro DM digestibility (IVDMD) (Tilley and Terry, 1963) were assessed. Herbage mass and quality were statistically analysed by GLM to test the effect of sward type on forage production and utilization.

## Results and discussion

The average three years rainfall was 575 mm and was representative of the climatic long-term average rainfall. The 2 forage species showed different performance in the year of sowing or in the regeneration year. In fact the grazing season started in autumn with the utilization of the second year swards as follows: 4<sup>th</sup> November for CH2 and 17<sup>th</sup> December for SU2, whereas the first grazing utilization in CH1 and SU1 was in winter (17<sup>th</sup> February). No differences were found in HM at the first grazing utilisation for all swards: 1.47, 1.43, 1.24, 1.55 t DM ha<sup>-1</sup> in CH2, SU2, CH1 and SU1, respectively. Seasonal distribution of HM was significantly different between swards in spring when standing biomass of sulla and in particular of SU2 was higher than the others (Table 1).

Table 1. Seasonal herbage mass on offer (HM).

		CH1	SU1	CH2	SU2
HM autumn	t DM ha <sup>-1</sup>	-	-	1.32±0.27a	1.39±0.34a
HM winter	t DM ha <sup>-1</sup>	1.10±0.44 a	1.57±0.44 a	0.92±0.38 a	1.56± 0.25a
HM spring	t DM ha <sup>-1</sup>	1.94±0.29 bc	2.63±0.25 b	1.86±0.25 c	3.81±0.21 a

Different letters within rows are significantly different (P<0.05).

In our experiments chicory production was lower than that found by Landau *et al.* (2005), who report 4 t DM ha<sup>-1</sup> in spring, whereas sulla HM was in good agreement with that found by Molle *et al.* (2003). Both of these studies were carried out in conditions that were similar to our experiments. The forages maintained a high quality during the growing season and CH1 and SU1 had the same nutritive value when grazed by ewes. Within the second year swards, SU2 offered a forage with a higher CP content than CH2. IVDMD was the same in SU1 and CH1 and was higher than in CH2 and SU2 (Table 2).

Table 2. Average chemical composition and IVDMD of the studied forages.

		CH1	SU1	CH2	SU2
CP	g kg DM	220.9± 12.9 ab	215.8± 12.1b	208.6± 8.5b	243.3± 7.6 a
NDF	g kg DM	314.9± 16.6 b	343.2± 15.4 b	335.7± 10.9 b	392.8± 9.8 a
IVDMD	% DM	78.86± 1.75a	78.91± 1.63a	74.44± 1.15b	74.97± 1.03b

Different letters within rows are significantly different (P<0.05).

Grazing periods in CH2 and SU2 were greater than in SU1 and CH1 (8.0 vs 3.5 times, P<0.05) whereas no differences were found in the number of grazing days per each one. During the trial no differences were found in the average hay and concentrate consumption, and milk yield (Table 3). During the grazing season the forages covered 73% of the total energetic requirements of ewes with the remaining 27% provided by supplements.

Table 3. Grazing management, supplements consumption and milk yield of sheep.

		CH1	SU1	CH2	SU2
Grazing period	days	7.4±1.1 a	8.0±1.0 a	8.6±0.7 a	8.4±0.6 a
Average stocking density	n° head ha <sup>-1</sup>	66±3.7 a	56±3.5 b	66±3.7 a	53±2.2 b
Hay consumption	g head day <sup>-1</sup>	381±120 a	376±112 a	465±79 a	449±71 a
Concentrate consumption	g head day <sup>-1</sup>	260±39 a	240±37 a	256±26 a	257±23 a
Milk yield	g head day <sup>-1</sup>	1693±101 a	1804±95 a	1637±79 a	1758±64 a

Different letters within rows are significantly different (P<0.05).

## Conclusions

Sulla and chicory, cultivated under Mediterranean conditions, can serve as high-quality pasture for sheep. The good seasonal distribution, in particular, of chicory, and the high quality of herbage mass

during the growing season, in particular, of sulla swards, can support the milk yield of dairy ewes during the first period of lactation and in late lactation. The second year swards afforded grazing utilization of more than 2 months allowing the system to cover more than 70% of the total energetic requirements of ewes from pasture.

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# Recovery of n-alkanes in manure of dairy cows fed fresh grass supplemented with maize silage

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## Abstract

Grass intake of grazing dairy cows can be estimated using the n-alkane technique. For a reliable application of this technique, the assumptions concerning the faecal recovery of the individual alkanes are crucial. However, no information is available on the recovery of diets of grass supplemented with maize silage. Also, it is not clear how the pattern of faecal excretion of alkanes is changed when diets are composed of more ingredients. This paper describes the results of an experiment in which rations with and without maize silage were offered to dairy cows indoors. Individual alkane recovery was estimated by total faecal collection and faecal grab samples were taken three times a day. Faecal recoveries of some alkanes (C31, C32 and C33) were higher than those mentioned in the literature while other alkanes (C25 and C27) had lower recovery values when grass was supplemented with maize silage. Moreover, the recovery of the synthetic alkane C32 was considerably higher than those of C31 and C33. Alkane excretion was not constant over the day and the excretion pattern of synthetic alkanes deviated considerably from that of the natural alkanes.

Keywords: n-alkane technique, faecal recoveries, grass intake, maize silage, alkanes.

## Introduction

Grass intake of grazing dairy cows can be estimated using the n-alkane technique (Mayes *et al.*, 1986). For a reliable application of this technique, a similar recovery of the individual alkanes used to estimate feed intake is crucial. The method has been shown to deliver reliable estimations of dry matter intake during grazing (Malossini *et al.*, 1994; Reeves *et al.*, 1996). However, no information is available on the alkane faecal recovery on animals fed on grass supplemented with maize silage. Also, it is unclear how the pattern of faecal excretion of alkanes is changed when diets are composed of more ingredients. It might be that more daily faecal samples are needed to cover the excretion pattern. This paper describes the results of an experiment in which (1) grass and (2) grass/maize silage was offered to dairy cows indoors. The alkane faecal recoveries of dairy cows fed on diets composed of grass and maize silage were evaluated and compared with those obtained in similar studies. Furthermore, the effect of faecal sampling time was explored.

## Materials and methods

The experiment lasted for three weeks and was performed with four productive dairy cows in a facility with quantitative manure collection. A two-week adjustment period was applied prior to the 4-day measuring period. A ration of fresh grass and concentrates (treatment G) was compared to a ration of fresh grass, maize silage and concentrates (treatment M) (Table 1). Feeds and manure were analysed as to the relevant alkanes, including the synthetic dosed alkanes C32 and C36. The determination of n-alkanes was based on saponification of the material followed by liquid-liquid extraction of n-alkanes and separation and quantification by GC-FID, as described by Vulich *et al.* (1995). Individual alkane recovery was estimated by total faecal collection. On the last day of the experiment also grab samples were taken three times a day to measure the daily alkane excretion pattern.

Table 1. Dry matter intake (kg d<sup>-1</sup>) for two treatments.

	Grass	Maize silage	Concentrates	Alkane concentrates
Treatment G	15.2	0	4.5	0.9
Treatment M	7.6	6.0	4.5	0.9

## Results and discussion

The n-alkane content of maize silage was much lower than that obtained for grass, especially in the case of C31, the most abundant alkane in the offered grass (Table 2).

Table 2. N-alkanes in components of the ration (mg kg<sup>-1</sup>).

	C25	C26	C27	C28	C29	C30	C31	C32	C33	C35	C36
Grass	32.4	0	50.9	0	141.8	12.6	246.5	7.7	76.9	10	0
Maize silage	2.2	0	6.9	0	13.2	1.5	16.5	2.2	9.6	2.3	0
Concentrates	4.1	0	11.9	1.4	56.5	2.1	26.9	2.3	2.8	0	0
Alkane concentrates	2.6	0	1.9	0	2.3	0	2.5	892.7	0	0	866.7

The faecal recoveries of C31, C32 and C33 for both treatments were higher than the often-used values of Dillon (1993) (Table 3). Moreover, the faecal recovery of the synthetic alkane C32 was considerably higher than those of C31 and C33. The high faecal recoveries on the G treatment might be caused by the specific grass composition, since the offered fresh grass was relatively dry (24% DM) and had high sugar and low protein contents. The high faecal recoveries on the M treatment might be caused by the inclusion of maize silage in the diet. The faecal recoveries of Dillon were found on rations of fresh grass only. The faecal recoveries of shorter alkanes (C25 and C27) decreased significantly by supplementation with maize silage.

Table 3. Faecal recoveries of alkanes for treatment G (ration of fresh grass and concentrates), treatment M (ration of fresh grass, maize silage and concentrates) and according to Dillon (1993).

	C25	C27	C29	C31	C32	C33	C35	C36
Treatment G	60.4	74.9	86.2	93.5	96.6	91.5	87.9	82.0
Treatment M	49.3	63.7	77.3	89.7	97.6	92.2	na	83.4
Dillon (1993)	na*	67.8	77.2	81.4	85.7	85.3	89.5	87.3

\* na = not available.

Alkane excretion was not evenly distributed over the day (Table 4). In general, the content of natural n-alkanes in individual faecal samples was lower at 06.00 and 20.00 hrs than at 12.00 hrs. Furthermore, the excretion pattern of the synthetic alkanes C32 and C36 deviated considerably from that of the natural alkanes. Where the natural alkanes showed highest values at noon, the synthetic alkanes showed highest values in the early morning. The excretion pattern might be related to the periods of intake and passage rate of the different ration components and digestion fractions. It implicates that manure sampling is extremely important when using the n-alkane technique for measuring intake, especially in rations containing more roughage components.



Table 4. Effect of sampling time on n-alkanes in manure (mg kg<sup>-1</sup>). Total = the average content of n-alkanes in all manure gathered during the four days of the experiment.

	Total	20.00 hrs, day 3	06.00 hrs, day 4	12.00 hrs, day 4	LSD*
C25	36.6a	32.8b	31.4b	36.7a	3.0
C27	76.0a	68.3bc	66.3c	74.7ab	6.4
C28	8.7	7.9	16.4	9.1	ns*
C29	259a	231b	230b	247ab	19
C30	21.0a	18.8b	18.8b	20.6a	1.4
C31	450a	407b	402b	442a	31
C32	144b	138bc	152a	132c	7.3
C33	138a	127b	125b	137a	8.8
C35	23.0	16.9	16.2	18.1	ns
C36	106b	105b	116a	101b	4.9
C31/C32	3.11ab	2.91b	2.64c	3.30a	0.24
C32/C33	1.13b	1.16b	1.33a	1.05b	0.12

\*LSD = least significant difference, ns = not significant (P>0.05).

### Conclusions

Diet composition (rations and grass quality) may affect the faecal recoveries of n-alkanes. More insight is needed to use the n-alkane technique reliably for measuring grass intake of dairy cows supplemented with silage maize or concentrates.

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## Factors affecting the fatty acid patterns of *Lolium perenne*

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### Abstract

Fresh forages provide the most natural and environmentally sustainable source of fatty acids (FA) for ruminants. Several factors influence the concentration and proportion of FA in herbage. This study evaluated the influence of N application levels (0, 45 and 100 kg N ha<sup>-1</sup>) and regrowth interval on the FA composition in *Lolium perenne* L. during three periods in 2004. In each experiment, the  $\alpha$ -linolenic acid (C18:3) concentration decreased with an extended regrowth interval, whereas levels of C16:0 and C18:2 increased. Higher N application increased total FA and C18:3 concentrations. A positive correlation between herbage N and C18:3 concentrations was established throughout the entire period. Moreover, total FA, and C18:3 concentration and proportion gradually increased with N fertilization level, from May to October. Grassland management can affect levels of FA in herbage, which is important for the quality of ruminant products.

Keywords: *Lolium perenne*, herbage, nitrogen, fertilization, regrowth interval, fatty acids.

### Introduction

The lipid fraction in leaves of forage species ranges from 30 – 100 g kg<sup>-1</sup> dry matter (DM). High fatty acid (FA) concentrations occur during primary growth, leafy regrowth, and late in the season (Bauchart *et al.*, 1984). Besides, Hawke (1973) reported a positive correlation between the amounts of FA and the chlorophyll concentration in a leaf tissue. The chlorophyll concentration is affected by the solar radiation and by the nutrition of the plant. The primary nutrient, nitrogen, is a necessary part of all proteins, enzymes, and metabolic processes involved in the synthesis and transfer of energy. Consequently, with a sufficient supply of N, levels of all plant proteins as well as the density or mass of chloroplast and perhaps associated lipids raise, giving a plant the “lush” appearance. Still, the nature and interactions between factors influencing the FA levels in herbage remain unclear. As forage is a cheap and environmentally sustainable source of FA, these relationships are important to enhance levels of beneficial FA in ruminant products. Thereby, this study evaluated the influence of N application levels and regrowth interval on FA composition of *Lolium perenne* during three periods in 2004.

### Material and methods

The experiment was conducted at an experimental field of Wageningen University, the Netherlands. The experimental set-up was a randomized block design with three replicates (Elgersma *et al.*, 2005). Three N treatments (0, 45 and 100 kg N ha<sup>-1</sup>) were applied before three subsequent periods (*May – June*, *Aug. – Sept.*, and *Oct.*) in 2004. During each period, three harvest dates were chosen to simulate various cutting regimes (early, normal, and late). Fresh herbage was sampled immediately with dry ice and stored in a freezer (-18°C), before analysis of FA. Other random samples of the herbage were taken to assess the DM content and morphological characteristics. Samples used for DM determination were analyzed for total nitrogen and water-soluble carbohydrates. Multiple regression analyses were carried out, according to a model taking account of the effects of period, N fertilization and regrowth interval, as well as period x N fertilization, period x regrowth interval, N fertilization x regrowth interval and period x N fertilization x regrowth interval. Moreover, regression analyses were conducted between chemical variables and FA concentrations to establish overall relationships based on measured plant characteristics.

## Results and discussion

The most distinct differences in all analyzed herbage chemical characteristics were reported between material collected in *May – June* (reproductive development stage), *Aug. – Sept.*, and *Oct.* (vegetative development stage). The highest WSC and lowest N and FA concentrations occurred in the first period, while during the following months the WSC concentrations decreased, and N and FA concentrations increased ( $P < 0.05$ ; Figure 1 and 2) (Hawke, 1973).

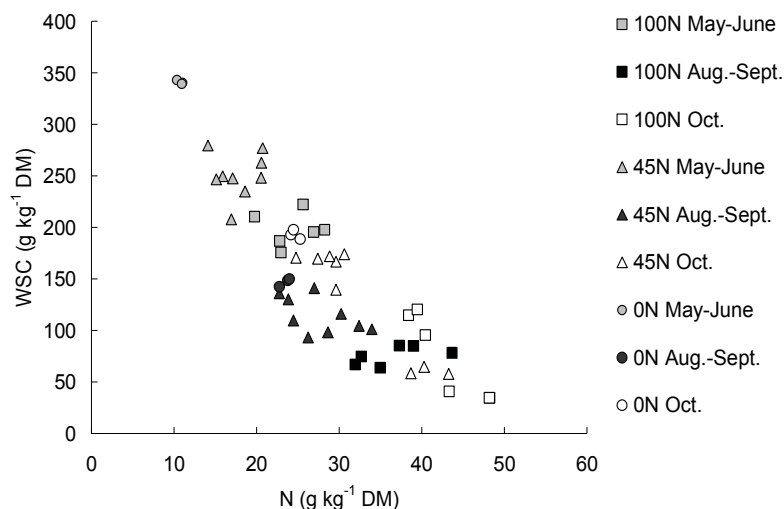


Figure 1. The relation between WSC and N ( $\text{g kg}^{-1}$  DM) of *Lolium perenne* during three periods in 2004;  $N = 367.44 - 7.49 * \text{WSC}$ ;  $R^2 = 0.79$ .

The differences in the herbage nutritive value observed during the entire experiment might be explained in three ways. Firstly, the nutritive value of morphological forage fractions and their proportions derives the nutritive value of the forage. Since, in *May – June* the leaf blade proportion was smallest, the concentrations of N and FA were lower, and WSC higher than in the following months. Secondly, an accumulation of DM with the regrowth interval negatively influences the nutritive value of the herbage. High herbage yields coupled with a considerable increase in the yield with an extended regrowth interval (45N-20d:  $1.9 \pm 0.2 \text{ t ha}^{-1}$  vs. 45N-32d:  $4.2 \pm 0.8 \text{ t ha}^{-1}$ ) were only reported in *May – June*, when weather conditions were favourable for herbage growth. In subsequent periods, the yields were approximately 50% lower, as the growth was limited by either high temperature (*Aug. – Sept.*) or low solar radiation (*Oct.*). Thirdly, the photosynthetic rate decreases with the herbage age, leading to reduced synthesis of metabolic components, such as proteins, chlorophyll, and FA (Hawke, 1973). Therefore, with the extended regrowth interval, the concentrations of N, total major FA and C18:3 decreased ( $P < 0.01$ ), while the concentrations of WSC, C16:0 and C18:2 increased ( $P < 0.001$ ). Besides, higher N fertilization levels increased concentrations of FA ( $P < 0.01$ ). The N fertilization promotes photosynthesis, by increasing the leaf area (light harvesting part of the plant) and the synthesis of metabolic components, such as chloroplast and proteins. Pigment-protein complexes, composed of proteins, chlorophylls, and FA are constituents of chloroplast thylakoid membranes (Evans, 1996), and are directly responsible for the harvesting of the solar radiation. We can speculate, based on the previously reported positive relationship between chlorophyll and FA (Hawke, 1973), that this relationship should be valid for all main constituents of these complexes. This might be an explanation for the positive correlation between N and C18:3 concentrations established throughout the entire experiment (Figure 2).

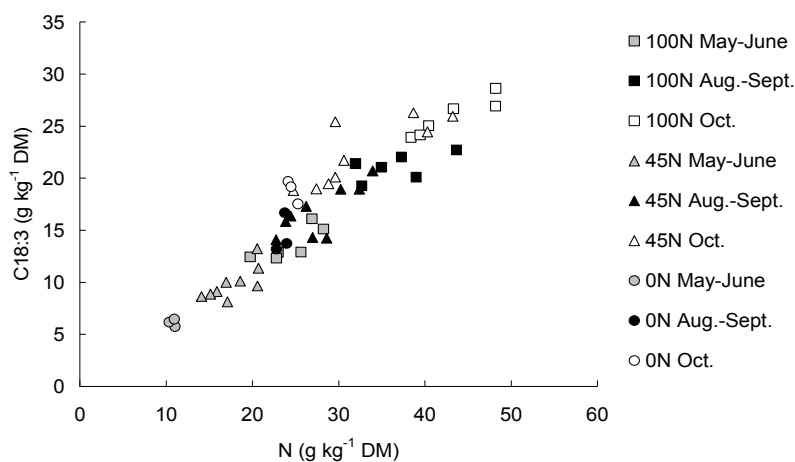


Figure 2. The relation between N and C18:3 concentration ( $\text{g kg}^{-1}$  DM) of *Lolium perenne* during three periods in 2004;  $\text{C18:3 concentration} = 0.49 + 0.59 * \text{N}$ ;  $R^2 = 0.87$ .

### Conclusions

Grassland management can enhance levels of important FA in herbage. Frequent cutting of the sward results not only in a higher nutritive value of forage, but also in higher levels of FA. It seems to be especially important in spring, when the rate of herbage growth is highest. N fertilization also seems to be an attractive way for enhancing levels of FA in herbage. Nonetheless, large seasonal variations in the concentrations of WSC, N, and FA in the herbage exist. Further research is required to explore these relationships on more fundamental plant level.

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# Characterization of acorn yield (*Quercus rotundifolia*) using multiphase regression models

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## Abstract

To optimize acorn-feeding season, the knowledge of several aspects related to production and quality of acorns is needed, taking into account that acorns are the key during fattening period of Iberian pigs in Spanish "dehesas". Information about available resources and their spatial and temporal distribution is of great interest to organize acorn feeding seasons. The aim of this study is to characterize the acorn yield in a dehesa located in NW Toledo province using multiphase regression models (piecewise linear regression or breakpoint of regression). Analysis of cumulative falling through time with this kind of models was satisfactory, resulting in a single breakpoint in 2001/02 and two breakpoints for the rest of the seasons, defining various phases of differing acorn falling rate throughout the feeding season.

Keywords: acorn production, Iberian pig, holm oak, Castilla-La Mancha.

## Introduction

Acorns are a key resource for the fattening of the Iberian pig in the Spanish "dehesa" system. Information on the annual production available and its distribution throughout the feeding season is of crucial importance in order to develop predictive models, determine the carriage capacity and to optimize the use of this limited resource. However the study of this type of variable is frequently problematic as they do not easily adjust to the functions of distributions generally employed for this type of analysis. Multiphase regression models have proven useful for the analysis of complex variables in different scientific fields (Barret *et al.*, 2003; Tilman *et al.*, 1994) and may thus be a useful instrument in this case. The aim of this study is to characterize the acorn yield in a dehesa located in NW Toledo province using multiphase regression models (piecewise linear regression or breakpoint of regression).

## Materials and methods

The study area is situated in the "Centro de Investigaciones Agropecuarias "Dehesón del Encinar", Oropesa, Toledo, in an area that forms part of the surface destined for the feeding of Iberian pigs. The area comprises 130ha of dehesa with smooth hills and low density of trees. The vegetation is dominated by holm oaks, with cork oaks in the more humid areas.

Throughout four consecutive years (seasons) (2001-02, 2002-03, 2003-04 and 2004-05) we collected the production of 30 holm oaks from two different patches of five and eight hectares in size respectively. The oaks had been selected according to the results of a previous characterization of the oak woodlands (López-Carrasco *et al.*, 2004), and were excluded from the feeding of Iberian pigs from September to March. All acorns from each tree were collected using nets extended below the tree and weighing the total of the fallen acorns in three week intervals during the seasons 2001-02, 2002-03 and in two week intervals during the seasons 2003-04 and 2004-05, throughout the period from October to March. The development of the accumulated fresh (wet) weight of the acorns for each year was analysed using a multiphase regression model according to Kim *et al.*, (2004).

## Results and discussion

The results related to the breakpoints between the different phases of the feeding season are presented in Table 1. For each season the day in which the slope of the regression line changes and the percentage of acorn yield accumulated in this time period are indicated. Significant differences between the

fragmented lines obtained with the t-student test are given. Table 2 contains the formulas that describe the periods that the model defines for each of the study years.

Table 1. Breakpoints (days) and accumulated acorn yield available (%).

	2001/02		2002/03		2003/04		2004/05	
	days	% accum.	days	% accum.	days	% accum.	days	% accum.
Breakpoint 1	61	92	37	39	34	14	47	19
Breakpoint 2			64	95	77	96	89	94
Slope1/slope2	Z= -6.594 P>  t = 0.00017		Z= 72.188 P>  t = 0.00000		Z =249.625 P>  t = 0.00000		Z =15.273 P>  t = 0.0001	
Slope2/slope3			Z = -323.759 P>  t = 0.00000		Z = -356.474 P>  t = 0.00000		Z = -21.042 P>  t = 0.00003	

Table 2. Descriptive formulas for the periods defined by the breakpoints.

	2001/02	2002/03	2003/04	2004/05
BP 1	Y = -0.132 + 0.017* D (0.086) (0.002) if D < 61	Y = -0.0329 + 0.0125* D (0.001) (0.0001) if D < 37	Y = -0.0130 + 0.005* D (0.0003) (0.00003) if D < 34	Y = -0.027 + 0.005* D (0.008) (0.0004) if D < 47
BP 2	Y = 0.895 + 0.001* D (0.114) (0.001) if D > 61	Y = -0.332 + 0.020* D (0.0032) (0.0001) if 37 < D < 64	Y = -0.534 + 0.019* D (0.0034) (0.0001) if 34 < D < 77	Y = -0.695 + 0.019* D (0.0587) (0.0008) if 47 < D < 89
BP 3		Y = 0.969 + 0.0003* D (0.0011) (0.00001) if D > 64	Y = 0.998 + 0.00001* D (0.0006) (0.000005) if D > 77	Y = 0.940 + 0.0004* D (0.0256) (0.0002) if D > 89

BP: breakpoint; D: days

The permutation test (Kim *et al.*, 2000) verified the existence of a single breakpoint in the season of 2001/2002 (first test:  $p < 0.0002$  and second test:  $p < 0.865$ ), while two breakpoints were identified for the seasons of 2002/2003, 2003/2004 and 2004/2005 (first test:  $p < 0.0002$  and second test:  $p < 0.05$ ) (Figure 1). The total number of permutations was 4500. The model gave statistically significant results for all seasons analysed. The feeding season varied in length, the shortest being the season 2002/03 during which 95% of the yield was obtained during only 2 months in contrast to the period of 2.5 months in 2003/04 and three months in 2004/05. Also, the distribution of the quantity of acorns available varied, being more homogenous in the season 2002/03 during which 40% of the yield was available in the first period (37 days) while, during a similar period in 2003/04 (until day 34) only 14 % and in 2004/05 (until day 47) only 19% of the total yield was available. The regression coefficient of each fragmented line represents the daily acorn harvest. In order to facilitate comparison between seasons the value has been multiplied by 100. In the seasons with two breakpoints the yield was lower in the first period (the daily acorn harvest rate was 1.2 in 2002/03, 0.5 in 2003/04 and 2004/05) in comparison to the second period during which the yield was similar for the three seasons (the daily acorn harvest rate was 2 in 2002/03 and 1.9 in 2003/04 and 2004/05) (Table 2). The described differences between feeding seasons evidence the complex nature of the use of this resource. The results indicate that especially the production of the first month of the season is of importance and conditions the carriage capacity for livestock. Thus stocking rate will have to be varied according to the available production, which underlines the necessity for a good characterisation of the production although a larger study period than the one presented here should be employed as a basis.

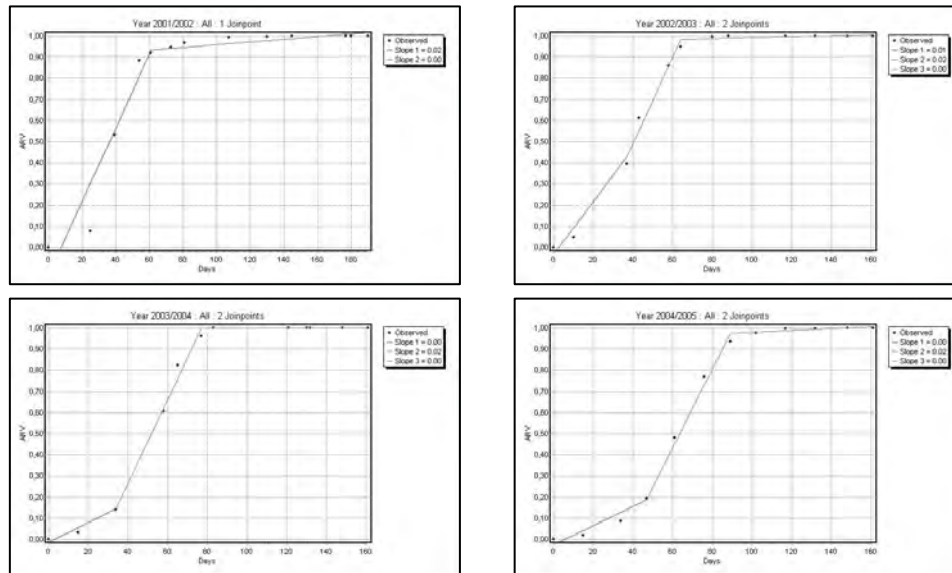


Figure 1. Multiphase model for acorn yield.

## Conclusions

The multiphase regression model allowed us to characterise and simplify a complex polygon as the distribution of the acorn yield over the season. Except for the first seasons studied with a single breakpoint, all other seasons showed two breakpoints, defining thus three periods of differing length and acorn yield. The sum of the first and second period explains more than 90% of the total production.

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# Effects of shading on species richness, above-ground biomass production and litter in an agroforestry system

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## Abstract

Shading is the main factor that affects species richness and herbage production in agroforestry systems. The effect of shading in four spacing treatments (10m x 10m, 7m x 7m, 4m x 4m, 2m x 2.5m), on species richness, on the above-ground biomass production of herbaceous vegetation and on litter was studied in a wild cherry (*Prunus avium* L.) agroforestry system. The research was conducted in the area of Laggadia, Pella prefecture, Northern Greece during 2005. The wild cherry trees were 13 years old. The experimental area had been protected from grazing since 1998. Moderate shading in the 2m x 2.5m spacing treatment resulted in a higher number of species and in lower litter accumulation compared to the other spacing treatments. Light shading slightly increased the above-ground biomass production in the 7m x 7m spacing treatment.

Keywords: agroforestry systems, herbage production, litter, spacing, wild cherry.

## Introduction

It is well documented that the stability of the ecosystems is closely related to species diversity. Biodiversity is enhanced in agroforestry systems by the gradients of environments that are created within (vegetation structure, shading, moisture) according to Mosquera-Losada *et al.* (2005). Shading effect on floristic diversity is related to the individual species. Cool season grasses are benefited by light to moderate shading, while warm season species and almost all legumes are suppressed (Pieper, 1990). Trees might critically modify the microclimate. Trees primarily reduce light, which is considered the most critical factor for understory growth in agroforestry (Sibbald *et al.*, 1991). Shading affects directly the photosynthesis and indirectly the dynamics of water and nutrient use efficiency and their partitioning between trees and understory vegetation (Bergez *et al.*, 1997). The presence of trees in Mediterranean grassland ecosystems ensures greater herbage production value when moderate, rather than low or heavy shading exists (Koukoura and Nastis, 1989, Kyriazopoulos *et al.*, 1999). Little experimentation has been undertaken to study the effect of various spacing treatments in different establishment phases of agroforestry systems on herbaceous vegetation. The purpose of this study was to investigate the effects of shading from various tree densities on species richness, herbage production and litter in a well established agroforestry system.

## Materials and methods

The study was conducted in the area of Laggadia, Pella prefecture, northern Greece close to the borders of FYROM (40°45'N, 22°58'E at 600 m altitude). The climate of the area is classified as subhumid Mediterranean, with a mean air temperature of 14.3°C and an annual rainfall of 535 mm. The soil of the study area is classified as *chromic luvisol* according to the FAO soil system classification (Kyriazopoulos, 2001). The area is situated at the lower limits where *Fagus sylvatica* L. occupies in the alliance of *Fagion moesiaca*, association *Fagetum submontanum* (Athanasiadis, 1986). Wild cherry trees (*Prunus avium* L.) were planted in a 3 ha natural grassland with 15% slope, in 1993. Four tree densities were tested: 100, 200, 625 and 1600 trees per ha, corresponding to four spacing treatments: 10m x 10m, 7m x 7m, 4m x 4m and 2m x 2.5m. The experimental area has been protected from grazing since 1998.



Species number was measured from ten randomly selected 0.5m x 0.5m sampling quadrats in each spacing treatment during 2005. In addition herbage production from the same sampling quadrats was determined by clipping all vegetation at 1 cm above ground at the end of the growing season (late June) and oven-drying the above ground biomass at 65°C for 48h. Litter was also selected from the same sampling quadrats and oven-dried at 65°C for 48h.

The experimental design was simple factorial (Snedecor and Cochran, 1989) with spacing treatment as the factor. The data were subjected to analysis of variance using the MSTAT program (Freed, 1991).

## Results and discussion

Species richness, as described by the number of species, increased as shading increased and it was significantly higher in the 2m x 2.5m spacing treatment compared to the other spacing treatments (Table 1). The dominance of the cool season perennial grasses in the 10m x 10m spacing treatment resulted in a very low number of species. Vrachnakis *et al.* (2005) have found that species richness decreased under heavy shade in forests. This suggests that there might be a shading limit which affects species richness. The result of this experiment indicates that dense spacing treatments in agroforestry systems create a more stable environment.

Table 1. Mean of species richness, herbage production and litter of four spacing treatments in an agroforestry system.

Spacing Treatment	Species richness (Species quadrat <sup>-1</sup> )	Herbage (g m <sup>-2</sup> )	Litter (g m <sup>-2</sup> )
10m x 10m	6.9	219.8	356.8
7m x 7m	9.7	275.5	434.8
4m x 4m	9.8	199.2	362.7
2m x 2.5m	14.9	190.4	228.8

\*Means in the same column with the same letter are not statistically significant at the 0.05 level.

Above-ground biomass production (Table 1) was significantly higher in the 7m x 7m spacing treatment (light shading) compared to the 10m x 10m spacing treatment (very light shading) and to the 4m x 4m and the 2m x 2.5m spacing treatments (moderate shading). In the Mediterranean zone, soil water use efficiency, which is the main limiting growth factor, might be improved under light shading. Lower herbage production under moderate shading in the 2m x 2.5m spacing treatment is primarily due to the reduced photosynthetic rate under low light (Blenkinsop and Dale, 1974). Kyriazopoulos (2001) also found higher herbage production under light shading compared to very light shading and heavy shading. Litter accumulation (Table 1) was significantly lower in the 2m x 2.5m spacing treatment compared to the rest of the spacing treatments. Lower herbage production, soil temperature and the higher soil water content in this treatment probably favoured better decomposition conditions causing limited litter accumulation. Obviously further research is needed.

## Conclusions

This study shows that moderate shading in the 2 m x 2.5 m spacing treatment increased species richness and decreased litter accumulation. Herbage production was higher under lighter shading in the 7mx7m spacing treatment.

## Acknowledgements

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# The role of high quality forages in Alpine dairy production over winter

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## Abstract

Forages with high nutritional and hygienic qualities are needed for the in-door feeding period to achieve high dry matter (DM) intake and milk yield in Alpine farms. Local forage resources, harvested as haylage in wrapped round bales, can also offer the opportunity of producing typical cheeses during winter. The DM yield and nutritional quality evolution of some permanent meadows (from 1500 to 1800 m a.s.l.) have been evaluated, and the forages have been harvested as haylage and hay and fed to forty “Valdostana” dairy cows at the Alpine Research Centre in Sauze d’Oulx (Italy). Round bale silages and hays were harvested in two different moments of the first growth cycle. The higher quality of forages obtained at the earlier harvest coupled with the haylage technique permitted a reduction in the use of purchased lowland concentrates and an improvement in the milk yield making the milk quality nor clostridial spore content worse.

Keywords: mountain meadows, round bale silage, hay, forage quality.

## Introduction

In the Alpine environment, where the winter feeding period can last 7 months or longer, successful forage conservation is crucial to milk production. Alpine dairy farms, generally characterised by small dimensions and low technology levels, are involved in the production of typical or Protected Denomination of Origin (PDO) cheeses (Buchin *et al.*, 1999). Summer production, which is considered to be of higher quality, represents the Alpine environment and the utilisation of forages from native pastures, but in fact represents less than 20% of the annual production. Most cheeses are therefore produced in winter and in the early spring period, when animals are in the peak of lactation. The winter forage system is based on local hay traditionally harvested late in the season or hay purchased from the plain. A recent Italian survey on 7000 hay samples showed that the quality of the mountain hay produced in the first growth cycle is particularly low with values of 633 g kg<sup>-1</sup> DM for NDF and 89 g kg<sup>-1</sup> DM for CP (Borreani *et al.*, 2005). As a result winter milk production has to be supported with “anonymous” concentrates or maize silage purchased from the plain, thus breaking the basic link between the products and the original land. Forages with high nutritional and hygienic qualities are needed over the rest of the year to achieve high DM intake and milk yield. Wrapped silage offers many advantages over hay making: large quantities of forage can be conserved in a short time, forage conservation is less weather dependent and silage is well suited to mechanisation (Charmley, 2001).

The main objective of this study was to compare the effects of two conservation methods (hay vs. haylage) of natural permanent meadows on forage quality, on milk production in dairy cows, and on the chemical characteristics of milk.

## Materials and methods

A four year study (2001-2004) was conducted at the “V. Vezzani” Experimental Research Centre in Sauze d’Oulx (1850 m a.s.l.) in the Susa Valley (Turin, Italy). During the 2001-2003 period, herbage samples were collected from 4 native meadows (from 1500 to 1800 m a.s.l.) 4 to 5 times at progressive morphological stages from early May to the middle of July to evaluate the evolution of pre-harvest forage quality. An earlier harvesting time was chosen for the haylage in comparison to the traditional haymaking in 2003, in order to improve the quality of the conserved forage. The hay and the haylage were produced on 5.5 ha of native grassland located at 1500-1600 m a.s.l.. The haylage was harvested

from 10 to 19 June (Trials S1 and S2), and the hay from 30 June to 16 July (Trials H3 and H4). The forage was baled with a 120 cm diameter baler and the silage bales were individually wrapped (6 layers) with a conventional polyethylene film. Hay and haylage bales were stored safely for 7 months. A herd of forty dairy cow of the “Valdostana” breed, producing on average 4000 kg milk  $y^{-1}$  cow $^{-1}$ , was used in the feeding experiment. The cows were divided into two groups and were balanced for parity, stage of lactation, and individual daily milk yield. The crossover design was used, with 10 days of adaptation followed by 2 experimental weeks. The silage diet (S) included haylage fed ad libitum and 3.5 kg of concentrate (21% crude protein), while the hay diet (H) included hay fed ad libitum and 4.4 kg of concentrate. Forage samples were analysed for crude protein (CP = total N x 6.25), NDF, ADF, and organic matter digestibility (OMD). The lactic and monocarboxylic acids (acetic and butyric acids), pH and ammonia N were determined on the silage samples.

## Results and discussion

The DM yield of the four native meadows increased on average from 1.5 to 6.0 t  $ha^{-1}$  from May to the middle of July. In the middle of June, the DM yield reached 4.0 t  $ha^{-1}$ , which makes harvesting for bale haylage profitable. The pre-harvesting quality of the herbage decreased with advancing maturity. The NDF increased from 378 to more than 600 g  $kg^{-1}$  DM, while the CP content decreased from 168 to less than 90 g  $kg^{-1}$  DM from May to July. In the middle of June, the NDF was slightly higher than 500 g  $kg^{-1}$  DM and the CP content was around 120 g  $kg^{-1}$  DM. The meadows were cut in four moments, two in June (early cut, S1 and S2) to produce haylage and two in July (traditional cut, H3 and H4) to produce hay. The variations in the NDF and CP of the herbage from cutting to baling, due to wilting and harvesting operations are reported in Table 1. During wilting, the CP of the haylage decreased by 12 and 4 g  $kg^{-1}$  DM and NDF increased by 61 and 79 g  $kg^{-1}$  DM, for S1 and S2, respectively. For the hay harvesting, 15 days later, the decreases in CP were of 3 and 4 g  $kg^{-1}$  DM and the increases in NDF were 76 and 50 g  $kg^{-1}$  DM, for H3 and H4, respectively. The low pre-harvesting quality of the herbage and the losses during harvesting resulted in a lower hay quality than that observed in hays in the Italian Alps (Borreani *et al.*, 2005). Despite higher harvesting losses, the CP and NDF of the haylage remained around 110 and less than 600 g  $kg^{-1}$  DM, respectively. The fermentation quality of the bales was good with no butyric acid and low ammonia N content, due to the high DM content reached during wilting (Table 2). A total of 47 and 49 bales of hay and haylage, respectively, were obtained for the feeding trial. The dry matter intake of the forages of the two diets were 12.5 and 11.0 kg  $d^{-1}$ , for the S and H treatments, respectively. Therefore the S diet was composed of 78% native grassland haylage and 22% concentrate, while the forage contribution to the H diet was reduced to 72%. The S treatment received 23% less concentrate than the H treatment, due to the better quality of the haylage in terms of CP and NDF (11.3 vs. 7.7% of DM and 58.8 vs. 67.0% of DM for the S and H treatments, respectively). The milk yield and other performance traits are reported in Table 3. When the cows were fed the S diet, they yielded 1.3 kg  $d^{-1}$  more milk than when fed the H diet, while the milk components and clostridial spores did not differ in the two diets.

Table 1. Dry matter content (DM, g  $kg^{-1}$ ), crude protein and NDF (g  $kg^{-1}$  DM) at cutting and after baling of haylage (S) and hay (H) produced from native meadows (1500-1700 m a.s.l.).

Trial	Harvesting date	DM yield (t $ha^{-1}$ )	At cutting			After baling		
			DM	CP	NDF	DM	CP	NDF
S1	10-11 June	3.93	220	120	541	564	108	602
S2	17-19 June	4.44	179	122	499	579	118	578
H3	30 June –3 July	5.57	264	96	589	884	93	675
H4	13-16 July	5.38	331	81	634	882	77	684

Table 2. Fermentation quality of round bale haylage of native meadows (1500-1700 m a.s.l.).

Trial	DM at ensiling (g kg <sup>-1</sup> )	pH	Lactic acid (g kg <sup>-1</sup> DM)	Acetic acid (g kg <sup>-1</sup> DM)	Butyric acid (g kg <sup>-1</sup> DM)	NH <sub>3</sub> -N (g kg <sup>-1</sup> TN)
S1	564	5.20	10.5	1.5	0.17	47
S2	579	5.06	13.0	2.7	<0.01	54

Table 3. Milk yields, milk composition and spore content of the milk.

	Diets	
	S	H
Milk yield (kg d <sup>-1</sup> )	14.9	13.6
4% FCM	13.1	11.8
Milk fat (%)	3.19	3.10
Milk protein (%)	2.92	2.87
Milk lactose (%)	4.85	4.80
SBC (cfu x 1000 mL <sup>-1</sup> )	141	131
Clostridial spores (MPN l <sup>-1</sup> )	924	1036

H, hay; S, haylage; FCM, fat corrected milk; MPN, most probable number; cfu, colony forming units.

### Conclusions

Improving the quality of local forages in an Alpine environment, thanks to the correct management of round bale haylage (> 50% DM content) led to a reduction in the purchased concentrate of the diet, and an increasing milk yield which allowed a closer link to the land of dairy productions. Furthermore, neither the milk quality nor the clostridial spore content showed any differences between the treatments. Future works will deal with the microbiological and sensory quality of the cheese production.

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# Grazing behaviour of four types of suckler cows and their calves on mountain pastures

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## Abstract

In response to the decreasing stocking rates of dairy cattle on mountain pastures, extensive beef production is used as a solution to keep the landscape open. In order to adequately manage these areas, it is crucial to understand the feeding behaviour of the animals because the opportunities for feed selection and, as a consequence, the heterogeneity of the vegetation increase. The objective of this study was to analyse the feeding behaviour of different types of suckler cattle on extensive mountain pastures. Daylight observations of the cows and their calves were conducted on an unfertilised pasture at low stocking rate in Swiss Jura in June and July 2005. Four types of suckler cattle were compared : Limousine, Herens, Angus and crossbred Red Holstein x Limousin. Differences were found between types especially in grazing time. Calves had a different feeding behaviour compared to the cows.

Keywords: feeding behaviour, suckler cows, calf, mountain pasture.

## Introduction

Understanding the feeding behaviour of domestic herbivores in natural conditions is difficult because a lot of factors may interact, such as the vegetation, the weather, the duration of daylight or the type of animals. In this study, we focused on the animal factor by comparing the feeding behaviour of different types of suckler cattle (cows and calves).

## Materials and methods

The study was conducted in natural conditions on an unfertilised mountain pasture in Swiss Jura (1120 m a.s.l., dominant vegetation : *Festuca rubra* L. / *Agrostis tenuis* L). Four cattle types were compared : Angus (An), Limousin (Li), F1 (crossbred Limousin x Red Holstein) and Herens (Hr). A herd of 12 suckler cows (3 per type) with their calves was used. It was managed as a single herd in a rotational grazing system including 3 paddocks of 7.4 ha each, giving thus a stocking rate of 0.6 -600 kg-animal unit per ha.

The activities (grazing, ruminating, no-mastication activities) of each of the 24 animals as well as their position (standing, lying) were recorded visually at 10-min frequencies from sunrise to sunset. Drinking and suckling activities were also recorded. The observations were repeated in June (rotation 2) and July (rotation 3) on the first paddock two days after the beginning of the rotation. Also, four cows (one per type) were located individually at 10-min frequencies on a map by using a grid of 25x25m.

The two days of observations took place under sunny conditions succeeding to a rainy day. The air temperature varied from 9.3°C to 17.3°C on the first day (15th of June) and from 13.0°C to 22.7°C on the second (25th of July). Observations were conducted from 5:00 to 22:30 in June and from 05:30 to 22:10 in July corresponding to 106 and 101 observations per animal and day respectively. The characteristics of the animals are given in Table 1.

Table 1 . Animal characteristics<sup>a</sup>.

	Cows								Calves							
	June				July				June				July			
	An	F1	Li	Hr	An	F1	Li	Hr	An	F1	Li	Hr	An	F1	Li	Hr
Age (months)	31.4	33.2	35.3	30.9	32.7	34.5	36.6	32.3	5.9	6.5	6.6	6.8	7.2	7.9	7.8	8.1
Live weight (kg)	632	588	612	447	646	598	608	459	218	265	196	203	264	319	245	246
ADG <sup>b</sup> (g/day)	429	857	417	607	402	159	-98	293	1143	1339	1131	1214	1130	1317	1179	1057

<sup>a</sup> Data are mean of 3 animals. An: Angus, F1: crossbred Limousin x Red Holstein, Li: Limousin, Hr: Herens.

<sup>b</sup> Average daily gain calculated from 17 May to 14 June and from 14 June to 25 July for June and July, respectively.

### Results and discussion

The duration of each activity of the cows at pasture during daylight time is given in figure 1. The cows spent 49.4% and 50.4% of the time for grazing in June and July, respectively, corresponding to 8.6 h in average. The Hr cows, which are smaller, grazed significantly less than the three other types. Rumination was probably concentrated during the night as only 2.7 to 3.1 h was recorded. Compared to June, the cows ruminated more in the standing position in July, which might be due to the higher temperature.

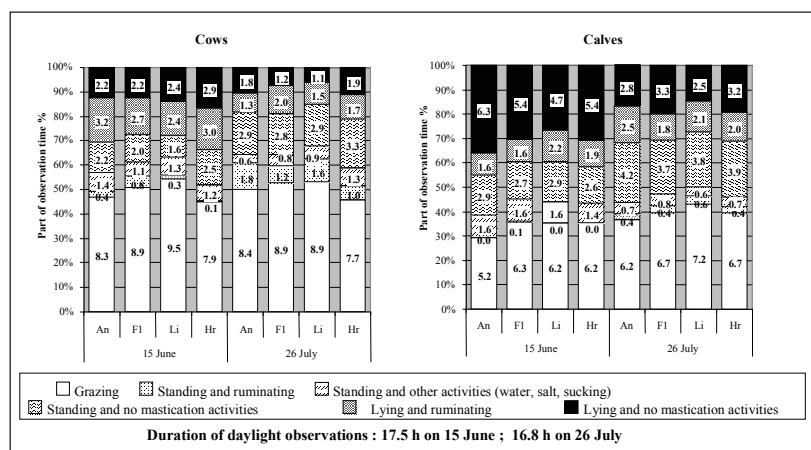


Figure 1: Activities of suckler cattle of four types during daylight time at pasture

The calves spent less time grazing than the cows: in average 6.0 h in June and 6.7 h in July. The An calves had the shortest grazing time. In July, the grazing and ruminating times of the calves increased both by 0.5 to 1.3 h. The suckling activities are given in table 2. All calves received milk at least once per day. The An tend to suckle the most frequently. However, they had the shortest suckling periods at the opposite of the Li, which had the longest. The duration of the meal is not correlated with milk intake but influenced by milk availability and how hungry the calf is, as reported by De Passillé (2001). No significant correlation was found between the number of suckling, the grazing time and the ADG of the calves.

Table 2 . Suckling activities of the calves of four cattle types at pasture<sup>a</sup>.

	June				July			
	An	F1	Li	Hr	An	F1	Li	Hr
Number of suckling periods per day	3.7	2.3	3.0	2.3	3.3	2.3	3.3	1.7
Average duration of suckling (min)	8.5	10.1	13.8	9.8	10.4	12.1	13.1	9.8

<sup>a</sup> Data are mean of 3 calves. An: Angus, F1: crossbred Limousin x Red Holstein, Li: Limousin, Hr: Herens.

The 24 animals had synchronised activities as shown on figure 2. In June and July, the herd had two major meals: after sunrise and before sunset. In June, the cows realised 3 intermediary meals during the day whereas most of calves had only two. In July, cows and calves had two intermediary meals but the time between the two major meals was shorter due to shorter daylight. Synchronisation between calf and dam activities was studied by counting for each couple, how much time the calf was simultaneously classified in the same activity as its dam: it occurred in 52.8% and 53.7% of the observations in June and July, respectively. There were nearly no differences between the cattle types. The same was done for the position: calves and their dam were simultaneously lying or standing in 84.4% and 82.4% of the observations in June in July. The spatial use of the paddock was different between the two observation periods (figure 3). In June the herd stayed at the bottom of the paddock. It went to the top for the night while in July, it explored nearly the whole paddock during the day. No differences could be seen in the exploration of the paddock between types since the herd always remained grouped. The distance covered by the cows was estimated between 2.9 km and 3.5 km in June and between 3.2 km and 3.7 km in July.

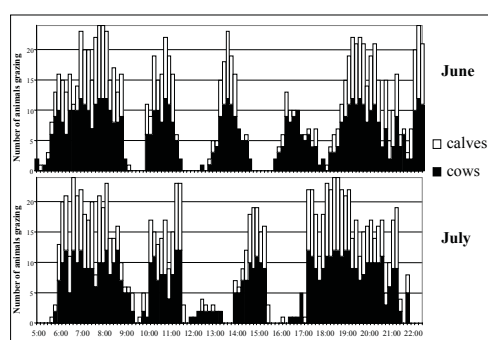


Figure 2: Daily rhythm of the cows and the calves in June and July

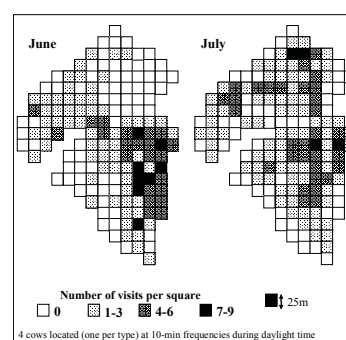


Figure 3: Spatial exploration of the paddock in June and July

## Conclusions

Some differences in feeding behaviour could be found between the four cattle types studied. However, they were probably reduced by social factors inside the herd. The differences in feeding behaviour between cattle types and between the two observation periods were more marked for the calves. The proportion of milk in the ration of the calves was probably the main cause of these differences. In order to better understand the interactions between animal and vegetation more detailed observations are required.

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# Meadow fescue infestation with *Neotyphodium uncinatum* and influence of endophyte on growth of microorganisms in vitro

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## Abstract

The objective of this research were to investigate the occurrence of *Neotyphodium uncinatum* in meadow fescue (*Festuca pratensis* Huds.) cultivars cultivated in Poland and to determine an endophyte inhibition effect on mycelium growth of chosen microorganisms *in vitro*. The endophyte mycelium was identified in three cases (45%, 52% and 65%). All the infected seed samples contained a living mycelium of *N. uncinatum*. At 10 °C the development of mycelium of *G. graminis*, *F. equiseti*, *F. culmorum* and *F. graminearum* was inhibited by the endophyte. The average growth inhibition zone [mm] was, respectively: 2.4%, 1.0%, 1.0% and 1.0%. At 20 and 30 °C the mycelium growth was inhibited only in *G. graminis* (3.2%, 3.7%, respectively) and *F. culmorum* (2.0%, 2.9%, respectively). As for other microorganisms no interaction was noted between the mycelia.

Keywords: *Neotyphodium*, endophyte, *Festuca pratensis*, microorganisms, pathogens.

## Introduction

Grasses, just like many other plants, are frequently infested with symbiotic microorganisms – endophytes, which usually develop in the plant without showing any symptoms. Endophytic fungi of *Neotyphodium* and *Epichloë* genera are the most-thoroughly investigated and most beneficial for the host plant. Offering ‘shelter’ to such organism, the plant receives e.g., a greater competitiveness, a better growth, lower susceptibility to stress caused by drought, a greater resistance to pests feeding and to infection with pathogens (Malinowski *et al.*, 2005).

The associations of greatest importance include *Neotyphodium lolii* in perennial ryegrass (*Lolium perenne* L.), *Neotyphodium uncinatum* in meadow fescue (*Festuca pratensis* Huds.) and *Neotyphodium coenophialum* in tall fescue (*Festuca arundinaceae* Schreb.). They are of interest not only due to the benefits for the plant but, to the presence of substances toxic to the farm animals when feed infested plants with the endophyte. A favorable effect of the endophyte on the plant is, in turn, due to other chemicals it produces; the most thoroughly-investigated are peramine and lolines, being the main factors which condition an increased resistance to various biotic and abiotic stress factors. Occurrence of *N. uncinatum* in meadow fescue cultivars cultivated in Poland and determining an endophyte inhibition effect on mycelium growth of chosen microorganisms *in vitro* were investigated.

## Materials and methods

The research used meadow fescue seeds provided by breeders and seed companies. The endophyte mycelium was dyed with bengal rose (Saha *et al.*, 1988) and microscopically examined to detect *N. uncinatum*. The number of seeds with the endophyte mycelium was expressed as a percentage of seed sample infection. To investigate the mycelium viability and to isolate the endophyte, from each infected lot, 150 seeds were sown into pots. After 6 weeks of growth, the lowest-located leaf sheaths were examined; the occurrence of the endophyte mycelium with the microscope (Saha *et al.*, 1988). From infected plants, the endophyte was isolated from leaf sheaths onto the PDA medium (Eggestein *et al.*, 1996). After about 3 weeks the endophyte colonies grown were split off and secured for further research.

The effect of *N. uncinatum* on the growth of selected microorganisms was researched following the method by White and Cole (1985). The fungi tested were obtained from *Festuca* genus rhizosphere,

roots and grass haulms. Five mm-in-diameter PDA medium plugs with the endophyte mycelium were placed in the middle of the plate and incubated at 25 °C, in the dark until the growing colony reached the diameter of 10-15 mm. After 14 days of incubation the growth inhibition zone was measured. The experiment was set up at 10, 20 and 30 °C, 5 replications in each (1 plate = 1 replication).

## Results and discussion

A total of 26 seed samples of 7 meadow fescue cultivars were accumulated. The endophyte mycelium was identified only in three cases. The percentage of infection was, however, relatively high and ranged from 45 to 65%. All the infected seed samples contained a living mycelium of *N. uncinatum* which was isolated. The infection of plants with the live mycelium did not exceed 32%, which could have been due to inadequate seed storage over a long period (Rolston *et al.*, 1986). The literature reports show that the infection of meadow fescue with *N. uncinatum* varies a lot. A low percentage of infection is especially characteristic of certified seeds of registered cultivars of different grass species and on plantations used for a short time (Cappelli and Buonauro, 2001). Pfannmöller *et al.* (1994) report on a very low infection of fescues as out of 153 samples tested, only 17 contained the endophyte. Meadow fescues seed samples showed the greatest number of infection (9) and its greatest extent (70%). The results of earlier research performed by the author (Paňka and Sadowski, 2002) into the infection of perennial ryegrass also coincide with the present results of a low infection of certified seeds.

A considerably higher percentage of infection is observed in natural grass communities and on plantations used for many years (Faeth *et al.*, 2001).

In total there was tested the effect of *N. uncinatum* towards 15 fungi: *Bipolaris sorokiniana*, *Drechslera* spp., *Fusarium avenaceum*, *F. culmorum*, *F. equiseti*, *F. graminearum*, *F. oxysporum*, *F. poae*, *Gaeumannomyces graminis*, *Gliocladium catenulatum*, *Gliocladium roseum*, *Pythium* spp., *Rhizoctonia solani*, *Trichoderma viride*, most of which represent dangerous grass pathogens. At 10 °C the development of mycelium of *G. graminis*, *F. equiseti*, *F. culmorum* and *F. graminearum* was inhibited by the endophyte. The average growth inhibition zone [mm] was, respectively: 2.4%, 1.0%, 1.0%, and 1.0%. At 20 and 30 °C the mycelium growth was inhibited only in *G. graminis* (3.2%, 3.7%, respectively) and *F. culmorum* (2.0%, 2.9%, respectively). *Fusarium poae*, *G. catenulatum*, *G. roseum*, *R. solani* and *T. viride* were not inhibited by the endophyte - on the contrary, they made its further growth impossible to a different extent, and their mycelium grew over *N. uncinatum* mycelium freely, especially at 10 and 20 °C. As for *Pythium* sp. no interaction was noted between the mycelia. They developed without obstacles growing over one another freely. At 20 and 30 °C endophyte and *F. oxysporum* and *Drechslera* sp. interacted with one another, the border of mycelia coming together was clear.

The occurrence of the endophyte can lower the susceptibility of the host-plant to infection with pathogens and pest feeding (Malinowski *et al.*, 2005). Schmidt (1994) observed a greater resistance of meadow fescue with the endophyte to the infection with some pathogens developing on plants after emergence and noted that plants with the endophyte were more susceptible to infection with pathogens causing the pre-emergence root-rot of seedling. In the present research was noted that an inhibiting effect of *N. uncinatum* on the growth of mycelium of some pathogens in the laboratory experiment, which suggests that under field conditions such effect can be also visible. All that is confirmed by earlier reports of the present author, involving a plot experiment with meadow fescue infected with *Neotyphodium uncinatum* (Paňka *et al.*, 2004). Riccioni *et al.* (1994), investigating the effect of *Acremonium coenophialum* and other *Acremonium* sp. isolated from tall fescue on the growth of *Drechslera erythrospila* and *D. graminea* on the PDA medium, demonstrated both an inhibiting effect and no effect at all. The present research revealed no effect of the endophyte on the development of *Drechslera* sp., and *T. viride*, *G. catenulatum* and *G. roseum* – fungi showing an antagonistic activity towards many pathogens, while White and Cole (1985) noted a poor, inhibiting effect of *N. coenophialum* isolate researched on the growth of *T. harzianum*.

## Conclusions

The infection of the meadow fescue cultivars researched with *Neotyphodium uncinatum* was low. It showed the greatest inhibiting effect on the growth of *Fusarium equiseti* mycelium and *Gaeumannomyces graminis*, especially at 30 °C. The results obtained, suggests that under field conditions a similar interaction will be visible. The effect of the endophyte on the development of other microorganisms depends on its genotype and the species as well as many other factors. For that reason the final effect of the occurrence of the endophyte is a product of the interaction of the observed factors and it is characteristic of a given association of the endophyte/host plant.

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# Persistence of nine fodder grasses and red clover in phenologically differentiated meadow mixtures

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## Abstract

During the years 1990-1998, research plots on a mineral soil at Falenty investigated the persistence of nine fodder grasses and their cultivars and two red clover cultivars in conditions of intensive utilisation. They were planted in four phenologically differentiated grass-clover mixtures. Persistence was assessed by the proportion of the each of the sown species in the sward at the first cut of each year. Among the grass species examined, the most persistent were *Poa pratensis* L., *Bromus inermis* Leyss. and *Festuca arundinacea* Scheb. cv. Rahela. *Arrhenatherum elatius* L. showed moderate persistence in the long term while *Dactylis glomerata* L. was persistent and dominant in the medium term. Of the short-lived species, *Trifolium pratense* L. and *Lolium perenne* L. were characterised by a relatively high contribution to total herbage over a short period. *Festuca pratensis* Huds., *Festuca rubra* L., and to a lesser extent *Phleum pratense* L., were also short-lived. Choice of cultivar within a species had little influence on persistence, except for *Festuca arundinacea*. Persistence also appeared to be related to weather conditions particularly low rainfall.

Keywords: persistence, climatic conditions, grass mixtures.

## Introduction

In average habitat conditions the persistence of grass species is from 5 to 10 years depending on soil type. The aim of plant breeding is to obtain grass species and cultivars with increased persistence and vitality even in hard soil conditions. This will increase the persistence of plant communities and reduce the need for grasslands renovation (Falkowski *et al.*, 1997). In Poland the testing of grass species and their cultivars usually covers too short a period to evaluate persistence of perennial grasses. The persistence of grass and legumes in mixed sowings is very important (Rutkowska *et al.*, 1995). The aim of this study was the evaluation of the persistence of nine grass species and red clover cultivated in four grass-clover mixtures under long term intensive cutting management and mineral NPK fertilisation.

## Material and methods

The research was conducted at Falenty during the years 1990-1998 in plots on a mineral soil. The experiment was established in spring 1989 in a split-plot design with two factors: grass-clover mixture (4 phenological types—see Table 1) and fertilisation (3 levels of N fertilisation: 120, 180 and 240 kg ha<sup>-1</sup> with stable P and K fertilisation) with 4 replicates. Each plot had a size of 20 m<sup>2</sup> (10 m x 2 m). The seeds mixtures were sown into soil prepared with full cultivation method. The mixtures components were: *Dactylis glomerata*, *Festuca arundinacea*, *Festuca pratensis*, *Arrhenatherum elatius*, *Phleum pratense*, *Bromus inermis*, *Lolium perenne*, *Poa pratensis*, *Festuca rubra* and *Trifolium pratense*. Utilisation was by 3 cuts per annum with evaluation of botanical composition of the swards starting in 1990. The persistence of each grass species was determined by its share in the total sward at the first cut of each year.

Table 1. The proportion (%) of each specie/cultivar in the sown seed mixture and then in the herbage at the first cut of each year. Mean of 3 N-fertilisation levels.

Species, variety	(%)	Year									
		1989	1990	1991	1992	1993	1994	1995	1996	1997	1998
Early mixture											
<i>Dactylis glomera</i> , Brudzyńska	10	15.1	19.9	21.9	67.2	46.2	34.0	22.4	11.0	14.8	8.5
<i>Festuca arundinacea</i> , Brudzyńska	15	5.4	10.5	3.8	3.9	2.5	10.0	18.4	7.2	2.4	1.4
<i>Festuca pratensis</i> , Skra	25	19.3	8.9	9.9	1.4	1.1	1.0	0.3	0.0	3.0	0.8
<i>Arrhenatherum elatius</i> , Skrzyszowicka	10	0.0	9.3	23.0	10.1	22.3	19.0	17.3	5.3	8.2	14.2
<i>Lolium perenne</i> , Argona	10	22.4	15.2	6.1	0.5	0.4	0.8	1.2	0.6	0.9	0.1
<i>Poa pratensis</i> , Skrzyszowicka	10	3.9	3.0	8.3	9.0	24.2	25.0	27.4	54.3	40.4	44.5
<i>Festuca rubra</i> , Nakielska	10	7.6	1.3	14.8	3.7	2.7	2.7	2.7	1.6	1.8	0.4
<i>Trifolium pratense</i> , Nakielska	10	15.1	31.0	9.9	1.3	0.5	0.0	0.0	0.0	0.0	0.0
Other species	-	11.2	0.9	2.3	2.9	0.1	7.5	10.3	20.0	28.5	30.1
Moderately early mixture											
<i>Dactylis glomerata</i> , Nakielska	10	17.7	13.4	28.8	39.4	34.0	25.0	16.8	11.6	9.6	1.6
<i>Festuca arundinacea</i> , Rahela	15	4.5	9.9	17.5	19.8	30.3	32.0	35.4	30.7	24.7	41.7
<i>Festuca pratensis</i> , Motycka	25	14.1	8.3	8.1	5.6	3.8	4.9	6.0	1.0	4.6	3.7
<i>Arrhenatherum elatius</i> , Wiwena	10	0.0	14.2	17.6	24.3	14.3	11.0	8.1	12.3	22.7	0.2
<i>Lolium perenne</i> , Argona	10	21.1	9.9	9.0	0.7	1.3	1.3	1.3	1.3	0.3	0.3
<i>Poa pratensis</i> , Skrzyszowicka	10	3.4	1.3	6.7	6.1	11.5	13.5	15.4	24.3	17.2	17.6
<i>Festuca rubra</i> , Nakielska	10	6.0	9.1	3.5	2.1	1.4	2.3	3.7	1.8	2.8	0.3
<i>Trifolium pratense</i> , Nike	10	17.2	33.2	7.8	0.4	0.7	0.0	0.0	0.1	0.0	0.0
Other species	-	16.0	0.7	1.0	1.6	2.7	10.0	13.3	16.9	18.1	34.6
Moderately late mixture											
<i>Dactylis glomerata</i> , Nera	10	10.5	23.8	42.3	61.9	56.9	41.0	22.7	13.2	13.5	12.4
<i>Festuca pratensis</i> , Skrzyszowicka	20	17.5	14.6	4.5	1.8	0.3	0.7	3.4	6.0	0.9	0.2
<i>Phleum pratense</i> , Skrzyszowicka	15	5.8	4.3	5.7	8.0	6.8	7.0	7.1	10.5	3.3	7.8
<i>Bromus inermis</i> , Brudzyńska	15	3.8	10.9	18.2	14.8	16.5	16.5	16.6	21.6	43.0	30.1
<i>Lolium perenne</i> , Maja	10	23.3	16.8	15.4	1.2	0.8	1.4	2.1	0.0	0.3	1.3
<i>Poa pratensis</i> , Beata	10	3.8	7.1	5.6	5.8	12.0	16.6	21.2	30.0	15.5	20.9
<i>Festuca rubra</i> , Brudzyńska	10	6.7	6.0	1.9	1.4	2.0	2.5	3.0	0.5	0.8	1.0
<i>Trifolium pratense</i> , Nike	10	18.4	15.4	5.1	2.2	0.5	0.5	0.5	0.0	0.0	0.0
Other species	-	10.2	1.1	1.3	2.9	4.2	13.8	23.4	18.2	22.7	26.3
Late mixture											
<i>Dactylis glomerata</i> , Baza	10	15.9	27.8	28.9	40.7	46.0	33.0	20.4	6.6	3.0	0.8
<i>Phleum pratense</i> , Bartowia	25	11.3	4.1	12.6	8.4	10.4	8.5	6.5	3.9	10.6	0.0
<i>Bromus inermis</i> , Brudzyńska	25	3.7	12.2	27.5	34.8	27.7	21.0	15.3	39.8	38.0	44.6
<i>Lolium perenne</i> , Arka	10	25.2	29.0	9.1	2.2	2.6	2.1	1.5	0.8	2.1	1.9
<i>Poa pratensis</i> , Beata	10	4.0	8.8	11.8	8.8	7.3	14.0	20.3	25.8	13.4	10.4
<i>Festuca rubra</i> , Brudzyńska	10	6.4	3.2	2.6	1.0	3.6	3.6	3.6	0.7	3.1	0.0
<i>Trifolium pratense</i> , Nike	10	19.3	14.2	6.5	0.5	0.0	0.0	0.2	0.0	0.2	0.0
Other species	-	14.2	0.7	1.0	3.6	2.4	17.8	32.2	22.4	29.6	42.3

## Results and discussion

The share of a particular grass species or red clover in the sown seeds mixture was different to that of the herbage in the first year (Table 1). Changes became more intensive in following years with one or two species dominating and covering most of the plot surface within each grass mixture. However this dominance changed with time, for example, in the early mixture *Dactylis glomerata* was dominant (67%) by Year 4 but *Poa pratensis* was dominant (45%) by Year 10. The content of other species was relatively high in the first year (13%), very low over Years 2-5 (2%), and then steadily increased to be 33% by Year 10.

The most persistent grasses in the long term (Year 10) were *Poa pratensis* (e.g. 40-45% in early mixture) and *Bromus inermis* (45% in late mixture and 30% in moderately late mixture), and the cluster grass *Festuca arundinacea* cv. Rahela (42% in moderately early mixture). *Dactylis glomerata* was also a persistent grass up to 4-5 years (39-67%), but then declined to low levels (1-12%) by Year 10. *Arrhenatherum elatius* was moderately persistent. *Trifolium pratense* and *Lolium perenne* were short-lived (2-3 years) but contributed significantly more to herbage production in the first 2 years than the proportion they were sown at *Festuca pratensis*, *Festuca rubra*, and to a lesser extent *Phleum pratense*. Generally there was little difference between the persistence of particular cultivars within a species, except *Festuca arundinacea* cv. Rahela that had considerably better persistence than cv. Brudzyńska. This is in agreement with Kamiński (2000), that persistence is a species character but may be considerably modified by plant breeding. The persistence of grass species and their botanical composition in grass-clover mixtures depends on weather conditions, especially on moisture conditions as determined by precipitation. Generally, the weather conditions were not favourable for grasses with all the trial years being dry, except for 1997. The mean climatic rainfall coefficient (Vinczeffý, 1984) for the entire growing season in the years 1990-1998 was 0.100. The driest years were 1992 and 1993 (climatic coefficients were 0.082 and 0.089, respectively). Under the conditions of soil water deficiency, mostly in the first years of the trial, grass species with smaller water requirements showed dominance, i.e. *Dactylis glomerata* (40-60% in 1992 and 1993), *Festuca arundinacea* cv. Rahela (heavy development after 1993) and *Bromus inermis*. *Arrhenatherum elatius* also appeared to be a useful species to cultivated in dry conditions (over 20% in swards in 1992 and 1993).

## Conclusions

The results of this study show that persistence of grasses and clover within complex mixtures will change over time and may be influenced by climatic conditions, particularly rainfall, and in some cases choice of cultivar. These results did not confirm earlier findings that different grass mixtures under similar utilisation, in this case cutting utilisation, causes the equalization of their composition and hence development of quite similar plant communities.

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## Ergovaline in populations of endophyte infected *Festuca rubra* subsp. *pruinosa*

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### Abstract

*Festuca rubra* subsp. *pruinosa* is a grass whose preferred habitat are coastal cliffs, a harsh environment where plants grow in cavities and are exposed to salt water spray. This grass species is systemically infected by the fungal endophyte *Epichloë festucae* and the interaction is asymptomatic. Endophyte infected grasses produce several alkaloids toxic to herbivores. In a previous work, we showed that an average of 69% of *Festuca rubra* subsp. *pruinosa* plants from cliff ecosystems (in the North Atlantic coast of Galicia region –Spain-) were infected by the endophyte *Epichloë festucae*. The main objective in this study was to determine whether endophyte infected plants of *F. rubra pruinosa* produce the ergovaline alkaloid, which is toxic to large herbivores. For this purpose infected plants of *F. rubra pruinosa*, from four populations and at two different harvests were analysed for the presence of ergovaline. An average of 80% of the analysed plants, which were endophyte infected, contained ergovaline. The alkaloid was detected in the four populations. The concentration ranged from 0.05 – 0.58  $\mu\text{g g}^{-1}$  in the first harvest and between 0.06 and 1.90  $\mu\text{g g}^{-1}$  in the second. The mean ergovaline content increased from the first (0.14  $\mu\text{g g}^{-1}$ ) to the second harvest (0.35  $\mu\text{g g}^{-1}$ ).

Key words: fungal endophytes, ergovaline, alkaloids, grasses.

### Introduction

*Festuca rubra* subsp. *pruinosa* is a grass whose preferred habitat are coastal cliffs. The environment of these areas is very inhospitable for plant growth mainly due to two characteristics. First, the soil is scarce, and plants grow in cracks and cavities in the rock. Second, the water stress derived from environmental salinity and often from salt-water spray.

Fungal endophytes of the *Epichloë* and *Neotyphodium* genera systemically infect aerial plant tissue of several grass species. Most of these plant-fungus interaction are asymptomatic and the fungus is seed-transmitted. The fungal endophyte *Epichloë festucae* infects several grass species such as *Festuca rubra*, *F. gigantea*, *F. glauca*. Endophytic fungi synthesize several biologically active metabolites, notably alkaloids. These alkaloids help in defending the host grass against a range of grass herbivores. The type and amount of alkaloids produced in infected grasses varies with the grass and fungus species involved in the association. For example, *F. rubra* subsp. *pruinosa* infected by *Epichloë* produces ergovaline, toxic to grazing vertebrates, and peramine, a feeding deterrent to insects (Leuchtman *et al.*, 2000).

Four populations of *F. rubra pruinosa* from the northern coast of Galicia, in northern Spain, were analysed for the presence of fungal endophytes, and we found that an average of 69% of plants were infected asymptotically by the endophytic fungi *Epichloë festucae* (Zabalgoeazcoa *et al.*, in press). So far, there are no references about the alkaloids produced by this grass-fungus association. The objective of this study was to determine whether the alkaloid ergovaline is produced by infected plants of *F. rubra pruinosa*. For this purpose several plants from different populations and at two different harvests were analysed.

### Materials and methods

*Festuca rubra* subsp. *pruinosa* plants were collected in sea cliff populations in the North Atlantic coast of Galicia (Spain). Four different locations were selected: Cedeira (CED), Torre de Hércules (TDH), Pantín (PAN), and Estaca de Bares (EDV). At each location 15-20 plants were collected leaving a space

of at least five meters between plants. Plants were transported to the laboratory (in Salamanca, western Spain) and transplanted into individual pots (in a mixture with peat, sand and perlite). The detection of fungal endophyte infection by *Epichloë festucae* was carried out by microscopic analysis of stem pith scrapings, as well as by isolation of the fungus from plant stems and leaf sheaths (Bacon and White, 1994).

In autumn season, infected and non-infected plants were transplanted to an experimental farm, in the province of Salamanca. Plants were irrigated during their establishment but not thereafter, and they were never fertilized. The experimental farm is located in an area characterized by a semiarid continental climate, with low precipitation and high temperatures in spring and summer. Plants were grown several months to obtain enough biomass for chemical analysis of ergovaline. The first sampling was done at the end of May, at heading stage. At this time dry leaves were not present. The second harvest was done one month later, at anthesis stage. At this time, the few dry leaves present were removed. In most plants, the inflorescence was present in a 50% of tillers, but there were plants in an earlier maturity stage where tillers elongated showing the inflorescence were not present. Plant samples were freeze-dried and ground. Ergovaline concentration was determined following the chromatographic method of Hill *et al.*, (1993) with the modifications suggested by Yue *et al.* (2000). Ergotamine tartrate (Sigma) was used as internal standard; ergovaline standard was provided by Dr Forrest Smith (School of Pharmacy, Auburn University).

A two-way analysis of variance (ANOVA) was used to determine the effects of plant population and harvest on the ergovaline content of plants.

### Results and discussion

The percentage of plants producing ergovaline ranged from 60% in CED population to 100% in EDV population (Table 1). The alkaloid was detected, at both sampling dates, and we did not find plants producing ergovaline only in one harvest. Ergovaline was not detected in non-infected plants. There was a significant effect ( $P < 0.05$ ) of harvest on the ergovaline content. The mean concentration of populations increased twice from the first to the second harvest. In CED and PAN populations, the mean in the second harvest was three times the content in the first harvest (Table 1). At both sampling dates, EDV was the population with the lowest ergovaline mean content, and PAN the population with the highest one (Table 1). However, differences between plant populations were not statistically significant ( $P > 0.05$ ). The greatest ergovaline concentration ( $1.9 \mu\text{g g}^{-1}$ ) was detected in PAN population, at the second harvest.

Table 1. Percentage of plants with ergovaline and concentration range ( $\mu\text{g g}^{-1}$  dry matter) in *Festuca rubra* subsp. *pruinosa* populations infected by *Epichloë festucae*, at two harvests.

Population	Plants with ergovaline (%)	Harvest 1			Harvest 2		
		Mean	Range	SE	Mean	Range	SE
CED	60	0.11	0.06-0.14	0.025	0.32	0.12-0.56	0.130
EDV	100	0.08	0.05-0.15	0.024	0.19	0.09-0.38	0.067
PAN	80	0.21	0.06-0.58	0.124	0.65	0.13-1.9	0.418
TDH	80	0.16	0.07-0.28	0.048	0.24	0.06-0.46	0.093
mean	80	0.14	0.05-0.58	0.055	0.35	0.06-1.9	0.177

The range of concentrations in *F. rubra* subsp. *pruinosa* was higher than that found in *F. rubra* subsp. *rubra* populations from semiarid grasslands (Vázquez de Aldana *et al.*, 2004). The trend of increasing ergovaline content with date (maturity stage) is similar to that reported in other infected grass species (Cagas *et al.*, 1999). At each harvest, we found that plants without inflorescences (e.g. CED19 =  $0.14 \mu\text{g g}^{-1}$ ) can have greater ergovaline content than more mature plants with inflorescences (e.g. TDH4 =  $0.07 \mu\text{g g}^{-1}$ ). This suggests that differences in the alkaloid concentration



due to plant and fungal genotype could be stronger (more important) than differences due to the maturity stage of the plant.

The ergovaline alkaloid is responsible for fescue toxicosis in livestock, a syndrome mainly characterized by a decrease in weight gain. Most research workers regard  $0.40 \mu\text{g g}^{-1}$  ergovaline in diet as the critical toxic level for cattle (Bony and Delatour, 2001). We found that PAN population in the second harvest had a mean ergovaline content above that level. In the area where plants were collected there are no large grazing herbivores. Therefore, a question arises: which is the function of the ergovaline alkaloid in *F. rubra pruinosa*? In the harsh environmental conditions where plants grow, the cost of harboring the endophyte and the cost of producing this secondary metabolite should bring benefits for these plants, otherwise the percentage of infected plants (an average of 69 %) would be much lower. The alkaloid contents in infected plants can increase under abiotic stress conditions. Thus, it has been reported an increase in ergovaline under water deficit in endophyte infected *Festuca arundinacea* (Belesky *et al.*, 1989). This suggests that ergovaline alkaloid may provide an advantage to water-stress and therefore could be related to salinity tolerance.

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# Effect of intensity of grassland management on biodiversity and botanical composition

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## Abstract

The aim of this work was to evaluate the effect of different levels of utilization (cutting) and fertilization on the botanical composition of small experimental plots over three years (2003-2005). The trial examined four methods of utilization: intensive (4 cuts per year – first on 15<sup>th</sup> May, followed by cuts at 45 d intervals), middle intensive (3 cuts per year – first on 30<sup>th</sup> May, followed by cuts at 60d intervals), low intensive (2 cuts per year – first on 15<sup>th</sup> June, next cut after 90 days), extensive (2 cuts per year– first on 30<sup>th</sup> June, next cut after 90 days). Each type of utilization was furthermore divided in four levels of fertilization (without fertiliser, P<sub>30</sub>K<sub>60</sub>, N<sub>90</sub>P<sub>30</sub>K<sub>60</sub>, N<sub>180</sub>P<sub>30</sub>K<sub>60</sub>). The dominant species in the permanent sward were *Poa pratensis* L., *Dactylis glomerata* L., *Lolium perenne* L., *Trifolium repens* L. and *Taraxacum sect. Ruderalia* Schrk., In the first year of monitoring there were considerable changes in the botanical composition due to the fertilization level. During the second year differences in botanical composition were detected between the types of the grassland utilization

Keywords: species, botanical composition, fertilization, utilization.

## Introduction

The study of the botanical composition of stands with various types of utilization is an important factor in the maintenance and development of permanent grasslands in the Czech Republic. The dominant type of permanent grassland in many European countries is the intensively exploited stand and these stands are ecologically less valuable. Some factors (fertilization, cutting frequency) can effect the botanical composition of the permanent grassland. In the paper there is assessed influence of the fertilization and the intensity of utilization on botanical composition of the permanent grasslands.

## Materials and methods

A small plot trial, with a range of cutting intensities and various levels of mineral fertilization, was established at the Research Institute for Cattle Breeding in Rapotín (Czech Republic) in 2003. The experiment was located on an east facing slope with a declination of 5.1 – 6.2 ° at a height of 390 – 402 m above sea level. The soil type was considered to be a cambisol (horizons Ao-Bv-B/C-C).with the characteristics of a sandy-loam soil.

The average daily during the experimental period was 6.0 °C and the total annual rainfall was 544 mm. Before the establishment of the trial the sward was utilized as an animal pasture.

The experiment examined four methods of utilization: intensive (4 cuts per year–first cut on 15 May followed by cuts at 45d intervals); middle intensive (3 cuts per year–first cut on 30 May followed by cuts at 60d intervals); low intensive (2 cuts per year–first cut on 15 June a further cut after 90d); extensive (2 cuts per year – first cut on 30 June with a further cut after 90 days).

Each method of utilization was sub-divided in four levels of fertilizer; without fertilization; P<sub>30</sub>K<sub>60</sub>; N<sub>90</sub>P<sub>30</sub>K<sub>60</sub> and N<sub>180</sub>P<sub>30</sub>K<sub>60</sub>.

There were four replicates of each treatment. The botanical composition of each sub-plot was analysed before the first sampling by means of the projective dominance method. The evaluation of the influence of the fertilization and of the intensity of utilization was carried out by means of the z-test (Hendl, 2004).

## Results and discussion

Considerable changes in botanical composition took place during the first year of the experiment. The influence of the intensity of utilization on the number of the grass, legume and native species is shown in Table 1. The level of intensity of utilization had a significant effect on the number of the grass species in the stand. The higher intensity of utilization decreased the number of grass species in the stand. Similar, significant effects were also noted for legume and native species. Table 2 shows the fertilization effect on the number of grass, legume and native species. Reductions in fertilization decreased the number of grass species and increased of the number of legume species present in the plots. The increase in the number of the native species in the unfertilised treatments was statistically insignificant. Bakker (1989) and Bassignana *et al.* (2002) reported a positive effect of increasing management frequency on biodiversity of unfertilised grasslands. The plots that received PK fertilization without any nitrogen fertilization were characterised by a decrease in the number of the grass species and an increase in the number of the legume species present. The increase of the number of the native species was statistically insignificant. Jančovič *et al.* (2004) reported a positive effect of PK fertilization on the herbal character of the stand and the spread of the dicotyledonous plants. Hein *et al.* (1999) also found that higher nitrogen fertilizer doses decreased the level of diversity and that PK fertilization stimulated the expansion of legumes. Although the number of herb species increased in the unfertilized and PK fertilized treatments there was an increase in the number of the valued dicotyledonous species such as *Plantago lanceolata* L.

Table 1. Influence of the intensity of utilization on the number of the grass, legumes and native species occurred in the permanent grasslands in locality Rapotín.

Var.	Grasses				Legume				Herbs			
	z				z				z			
	2003	2004	2005	(2005-2003)	2003	2004	2005	(2005-2003)	2003	2004	2005	(2005-2003)
1	7.0	5.3	5.3	3.47 <sup>+++</sup>	1.1	1.4	1.7	2.84 <sup>++</sup>	8.9	10.5	11.9	3.75 <sup>+++</sup>
2	8.3	5.8	7.1	2.59 <sup>++</sup>	1.1	1.4	1.6	2.47 <sup>+</sup>	8.6	9.0	10.4	1.75
3	8.4	7.6	7.3	2.41 <sup>+</sup>	1.1	1.3	1.2	0.35	8.9	8.9	8.8	1.03
4	8.6	7.8	7.5	2.405 <sup>+</sup>	1.4	1.6	1.3	0.00	10.8	8.4	8.8	2.02 <sup>+</sup>

Legend: 1 - 4 cuts per year; 3 - 2 cuts per year; 2 - 3 cuts per year; 4 - 2 cuts per year  
Significant at + ( $\alpha=0.05$ ); ++ ( $\alpha=0.01$ ); +++ ( $\alpha=0.001$ ).

Table 2. Influence of the fertilization on the number of the grass, legumes and native species occurred in the permanent grasslands in locality Rapotín.

Var.	Grasses				Legume				Herbs			
	z				z				z			
	2003	2004	2005	(2005-2003)	2003	2004	2005	(2005-2003)	2003	2004	2005	(2005-2003)
Without fertilis.	8.1	7.1	6.8	2.93 <sup>++</sup>	1.3	1.4	1.8	2.667 <sup>++</sup>	9.8	9.3	10.1	1.75
P <sub>30</sub> K <sub>60</sub>	8.4	6.8	6.9	2.94 <sup>++</sup>	1.2	1.6	2	3.32 <sup>+++</sup>	9.0	10.1	11.1	1.87
N <sub>90</sub> P <sub>30</sub> K <sub>60</sub>	8.1	6.5	6.9	2.405 <sup>+</sup>	1.1	1.5	1.1	0.00	9.1	9.4	9.6	1.11
N <sub>180</sub> P <sub>30</sub> K <sub>60</sub>	7.6	6.3	6.4	2.59 <sup>++</sup>	1.1	1.1	0.9	0.35	9.3	8.1	9.0	-0.25

Legend: A – zero fertilisation; B – PK fertilisation; C – N<sub>90</sub>PK fertilisation; D – N<sub>180</sub>PK fertilisation  
Significant at + ( $\alpha=0.05$ ); ++ ( $\alpha=0.01$ ); +++ ( $\alpha=0.001$ ).

## Conclusions

In the period under consideration it was noticed that increasing the intensity of utilization and fertilization decreased the number of grass species. The results show the increase of the number of legumes with the higher intensity of utilization. The number of legume species increased in the lower fertilization regimes (unfertilized and PK treatments). We can also conclude that the higher intensity of utilization induced an increase in the number of native species in the stand.

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# Utilization of excessively wet meadows and protection of their diversity

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## Abstract

Investigations carried out on meadows of wet sites in river valleys of western Poland allowed assessing the impact of utilisation on the floristic diversity of meadow communities and indicate possibilities of their protection. For this purpose, species wealth, botanical structure, phytosociological variability and floristic diversity were determined in the identified communities. In the examined communities, the authors found from 18 (*Phalaridetum arundinaceae*) to 232 (*Alopecuretum pratensis*) plant species. The highest Shannon-Wiener index floristic diversity was calculated for the *Molinietum coeruleae* association (4.6) while the lowest were for the *Phalaridetum arundinaceae* and *Ranunculo-Alopecuretum geniculati* associations (1.20). The obtained results show that the high floristic diversity of plant communities as well as the natural value of meadows in periodically excessively wet sites was achieved thanks to their extensive and sustainable utilization.

Keywords: excessively wet sites, utilization, floristic diversity.

## Introduction

River valleys, due to their diversified geomorphological structure, exhibit considerable variability of site conditions, especially connected with moisture content, on a relatively small area (Świć, 2004; Kryszak and Grynia, 2006). This leads to the development of many sites of different floristic composition favourable for the establishment of grass communities of varying structure and floristic wealth, frequently of unique natural value. Thanks to them, the role of river valleys increased in importance as ecological corridors of great value, important to sustain biodiversity (Kryszak *et al.*, 2005).

The aim of this study was to assess the floristic diversity of meadow communities occurring in Western Poland, and which are periodically excessively wet, in relation to the intensity of their utilisation.

## Materials and methods

Geobotanical investigations were carried out in years 1997 to 2003 in western Poland in the valleys of the Warta, Barycz and Obra rivers. The evaluation was performed on the basis of 1500 floristic surveys taken by the Braun-Blanquet method. The evaluation of the impact of the wetness of sites on the identified communities was carried out on the basis of:

The intensity of their utilization – method of utilization, number of cuts or grazings as well as the level of the applied NPK fertilization ( $\text{kg ha}^{-1}$ ),

The floristic composition - the following parameters were determined: species wealth, botanical structure, phytosociological variability and floristic diversity (Magurran, 1996), and also the yield of dry matter ( $\text{t ha}^{-1}$ ).

## Results and discussion

Moisture is one of the major factors affecting the way of utilization of meadows and pastures. Irregular regimes of waters in rivers and the high level of ground waters restrict the intensive of agricultural utilization of meadows along rivers and, consequently, results in long-term maintenance of natural vegetation in river valleys. Therefore, traditional extensive utilization of these areas continued for centuries in fossilized biocenotic systems characterized by considerable floristic wealth (Table 1).

Table 1. Duration of flooding (Df) and the level of ground waters (Gwl) vs. utilization intensity of meadow communities.

Water regime		Utilization	Fertilization (kg NPK ha <sup>-1</sup> )	DM yield (t ha <sup>-1</sup> )	Plant community
Df (days)	Gwl (m)				
90	0.1-0.3	1 cut	brak	10.8	<i>Phalaridetum arundinaceae</i>
60	0.7-1.1	2 cuts	90	8.4	<i>Phalaridetum arundinaceae alopecuretosum pratensis</i>
40	0.9-1.3	3 cuts	120	6.5	<i>Alopecuretum pratensis</i>
30	1.0-1.5	2 cuts + grazing	60	5.4	<i>Alopecuretum pratensis</i> var. with <i>Poa pratensis</i>
non	0.25-1.3	1 cut	non	2.0	<i>Molinietum coeruleae</i>
non	0.6-1.6	1 cut	non	2.5	<i>Deschampsietum caespitosae</i>
non	0.9-1.3	free grazing, 1 LU ha <sup>-1</sup>	non	3.1	<i>Lolio-Cynosuretum</i> var. with <i>Deschampsia caespitosa</i>
non	1.0-1.7	1 grazing	non		<i>Holcetum lanati</i>
non	0.3-0.7	free grazing 0.5 LU ha <sup>-1</sup>	non	1.2	<i>Ranunculo-Alopecuretum geniculati</i>

The increasing fodder requirements frequently resulted in some attempts to regulate water regimes of rivers, among others, by the construction of water reservoirs or – in the case of marshy sites – by carrying out drainage. This kind of changes of site conditions allowed more intensive utilization of meadows and pastures, leading to changes in vegetation and development of high-yielding grass communities, albeit of lower natural value. In the result of such actions, numerous sub-associations or variants of associations developed, frequently of simplified floristic composition and reduced value of the Shannon-Wiener index (Table 2).

Table 2. Floristic diversity (H') of the examined meadow-pasture communities.

Plant community	Number of plant species:		H'
	total	in phytosociological surveys	
<i>Phalaridetum arundinaceae</i>	18	8 (4-12)	1.19
<i>Phalaridetum arundinaceae alopecuretosum pratensis</i>	22	12 (9-15)	1.45
<i>Alopecuretum pratensis</i>	232	45 (37-53)	2.73
<i>Alopecuretum pratensis</i> var. with <i>Poa pratensis</i>	78	29 (22-36)	1.03
<i>Molinietum coeruleae</i>	96	32 (25-39)	4.6
<i>Deschampsietum caespitosae</i>	88	17 (7-28)	1.74
<i>Lolio-Cynosuretum</i> var. with <i>Deschampsia caespitosa</i>	51	26 (11-21)	1.35
<i>Holcetum lanati</i>	43	18 (12-29)	1.56
<i>Ranunculo-Alopecuretum geniculati</i>	21	11 (9-15)	1.20

Extensive utilization, both by means of cutting or grazing, supports floristic diversity in grass communities and, consequently, protects threatened plant communities, plant species as well as breeding grounds and feeding areas of birds which prefer wet meadows. One-cut utilization of meadows, grazing with the stocking rate not exceeding 2 large animals per hectare or utilized alternatively (cutting/grazing) allow for the appearance in the sward of late-developing plant species, among others from the *Gentiana* genus as well as *Dianthus superbus*, *Molinia coerulea*, *Lathyrus palustris*. In addition, delayed cutting of meadows contributes to the protection of bird species threatened by extinction, even on the international scale, e.g. *Crex crex*.

Excessively wet sites, because of the occurring floristic and faunal peculiarities, are frequently part of the NATURA 2000 program, as exemplified by sections of the Warta and Barycz river valleys. On these areas, the main objective is the protection of breeding sites of water-mud fowl and fodder production is

of secondary importance. Therefore, extensive utilization of these areas helps maintain natural values of meadows but the worse fodder value should be financially compensated (Hodge and Ortiz-Miranda, 2004; Hopkins and Holz, 2005; Nösberger and Kessler, 1997; Pervanchon *et al.*, 2004).

The obtained results show that the high floristic diversity of plant communities as well as the natural value of meadows in periodically excessively wet sites was achieved thanks to their extensive and sustainable utilization.

### Conclusions

In order to maintain the floristic but also faunal wealth and diversity of meadows and pastures situated on excessively wet sites it is necessary to maintain their extensive utilization and to compensate the farmers for losses associated with the reduced fodder value.

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# Effect of different management systems on quality parameters of forage from mountainous grassland

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## Abstract

Data from comprehensive field studies in eight different Austrian grassland regions and data from exact field trials were analysed concerning aspects of floristic diversity and forage quality. Special attention was paid on the impact of different management measures, which are offered within the Austrian agri-environmental program. The mentioned measures, mainly focusing on the reduction of farm external resources, only showed a marginal influence on forage quality. This clearly indicates that farmers efficiently use their natural nutrient resources, which are an essential part of a low input strategy. Nutrient balances on a farm gate scale show in general a well balanced result and confirm a sustainable, environmentally friendly management. But on some farms a N-surplus is existing and has to be considered critically.

Keywords: forage quality, nitrogen balance, floristic diversity, agri-environmental programs.

## Introduction

In contradiction to some intensive European grassland regions, homegrown forage from pastures and meadows is the main feed basis for grassland and dairy farms in Austria.

This paper is dealing with the effects of different management systems on parameters of yield, quality and botanical composition of forage from mountainous grassland. Measures like organic farming, reduction and renunciation of yield increasing substances are censoriously considered and discussed, regarding the above mentioned parameters.

## Materials and methods

The presented results originate from comprehensive field studies, carried out in eight representative grassland regions of Austria in the period from 1997 to 2002. At all, more than 2200 forage samples were taken and analysed for their content of crude nutrients, minerals, trace elements, heavy metals, digestibility of organic matter and energy content (Poetsch and Resch, 2005). Beside detailed recordings of farm structure data and aspects of management, investigations on soil properties, floristic diversity, forage quality and feed intake have been carried out. Nutrient balances have been calculated both on a farm gate and on a field scale, mainly focusing on nitrogen.

## Results and discussion

Forage from pastures and meadows amount up to 95 % of the feed ration for dairy cows and therefore its quality is of great importance (Gruber and Poetsch, 2005). About half of the total permanent grassland is used in a very extensive way with low stocking rates and is cut or grazed once or twice a year. Permanent grassland in more favourable regions of the Austrian mountains can be at least used three times per year (silage cut, hay cut, second cut hay or alternatively grazing in the autumn). Only in some small, very productive lowland areas even up to five cuts per year can be harvested.

It is well known, that beside other aspects forage quality strongly depends on the composition of plant communities and on the stage of vegetation. Concerning the most relevant grassland types for production there is a negative correlation between the floristic diversity and the energy concentration of forage. Forage from species rich grassland types is therefore of lower quality than that from grassland with a smaller number of plant species (Table 1). Up to a medium production level of about 5,000 to



6,000 kg milk per cow and year (the official average Austrian milk production level is at 5,432 kg) forage from species rich grassland can be used in a very efficient way. But with an increasing production level, the requirements for the quality of the feed ration significantly rise up and displace forage from more extensive grassland types.

Table 1. Relationship between floristic diversity (species number) and energy value of forage from selected grassland types (Poetsch and Blaschka, 2003).

Grassland type	n	Ø	min.	max.	Ø MJ NEL (kg DM <sup>-1</sup> )
Extensive pastures	120	54	6	115	5,13
Cultural pastures	73	46	24	86	5,50
Mowing pastures	105	38	18	64	5,64
One cut meadows	235	46	8	91	4,55
Two cut meadows	693	38	14	88	5,39
Three cut meadows	328	32	13	58	5,69
Four cut meadows	28	29	7	52	5,60
Ley farming areas	15	32	23	48	5,89

For Austria and some other countries with mountainous production conditions, special programs and support are necessary to balance the natural disadvantages. Low input strategies are efficiently supported by the Austrian environmental program for agriculture “OEPUL”, which covers the whole country and is with 82% well accepted by the farmers. Some special measures with different premiums are offered within this program, mainly focusing on the reduction of yield increasing substances like mineral nitrogen or pesticides both on grassland and arable land.

Table 2. Forage quality parameters of selected grassland types with different management measures (Poetsch and Resch, 2005).

Grassland type	Parameter	Without OEPUL		Reduction of yield increasing products		Abdication of yield increasing products		Organic farming	
		Ø	s	Ø	s	Ø	s	Ø	s
Two cut meadows n=592	XP g kg DM <sup>-1</sup>	147,0 <sup>a</sup>	40,9	150,9 <sup>a</sup>	32,8	145,9 <sup>a</sup>	35,4	143,7 <sup>a</sup>	31,6
	XF g kg DM <sup>-1</sup>	252,9 <sup>a</sup>	37,4	244,7 <sup>a</sup>	36,2	250,4 <sup>a</sup>	37,4	248,2 <sup>a</sup>	37,5
	MJ NEL kg DM <sup>-1</sup>	5,38 <sup>ab</sup>	0,71	5,43 <sup>ab</sup>	0,49	5,29 <sup>a</sup>	0,65	5,50 <sup>b</sup>	0,58
Three cut meadows n=322	XP g kg DM <sup>-1</sup>	143,7 <sup>a</sup>	27,5	143,5 <sup>a</sup>	22,4	160,5 <sup>b</sup>	34,5	149,4 <sup>a</sup>	30,3
	XF g kg DM <sup>-1</sup>	256,2 <sup>a</sup>	30,2	264,9 <sup>a</sup>	32,4	243,8 <sup>b</sup>	31,4	245,5 <sup>b</sup>	34,5
	MJ NEL kg DM <sup>-1</sup>	5,73 <sup>a</sup>	0,48	5,53 <sup>a</sup>	0,41	5,69 <sup>a</sup>	0,66	5,74 <sup>a</sup>	0,61
Four cut meadows n=129	XP g kg DM <sup>-1</sup>	173,7 <sup>a</sup>	27,0	167,1 <sup>a</sup>	34,5	169,9 <sup>a</sup>	29,4	173,2 <sup>a</sup>	28,5
	XF g kg DM <sup>-1</sup>	246,4 <sup>a</sup>	31,7	236,1 <sup>a</sup>	34,9	234,0 <sup>a</sup>	31,6	226,8 <sup>a</sup>	29,7
	MJ NEL kg DM <sup>-1</sup>	5,62 <sup>a</sup>	0,52	5,47 <sup>a</sup>	0,43	5,49 <sup>a</sup>	0,57	5,61 <sup>a</sup>	0,53

XP: crude protein; XF: crude fiber.

The results presented in Table 2 deal with the impact of selected OEPUL-measures on aspects of forage quality concerning two, three and four cut meadows, which are the main types for forage production on dairy farms in the mountains (Poetsch, 2000). With the exception of the two cut meadows, which are of great importance for organic grassland farms in mountainous regions, no significant differences in the energy concentration were found concerning the discussed management measures. These results indicate, that farmers efficiently use the natural nutrient resource on farms namely manure and care for sufficient phosphorus supply to improve the growth of legumes.

Table 3. Farm gate balances for nitrogen in different grassland regions of Austria.

Test region	n farms	kg N ha reduced agricultural used area <sup>-1</sup>			
		Ø	median	min.	max.
Ennstal	78	+ 7,2	+ 3,8	- 47,6	+ 84,3
Pongau	25	+ 6,9	+ 6,6	- 23,7	+ 43,7
Kitzbüchel	29	+ 6,0	+ 5,4	- 29,1	+ 37,8
Oberkärnten	19	- 7,4	- 6,6	- 51,4	+ 41,7
Hallein	16	+ 9,6	+ 2,4	- 21,0	+ 80,5

Balances for nitrogen were set up on a farm gate scale, including external N-input (concentrates, fertilizer, livestock and fodder purchase, bedding material, N-deposition and biological N-fixation) and N-output (livestock sales, milk, plant products and unavoidable, gaseous N-losses). The calculated N-saldo was then referred to the fertilized grassland area and showed a well equalized result on an average of all farms in the test regions (Table 3). But looking at the range of the results, a strong variation can be noticed between a deficit of 51 kg N ha<sup>-1</sup> and a surplus amounting to 84 kg N ha<sup>-1</sup>.

### Conclusions

Forage quality is of great importance for Austrian grassland and dairy farms, which are mainly located in disadvantaged, mountainous regions. In contradiction to intensive grassland regions in Europe a high floristic diversity can be observed on the different grassland types. Species rich, extensive grassland tends to result in lower forage quality, which with an increasing milk production level and rising requirements leads to a displacement of these ecologically valuable areas.

Milk and grassland farming in mountainous regions of Austria strongly meets ecological and environmental demands and fulfils multifunctional tasks for the public. The continuance of the milk quota system is a fundamental assumption for a productive land management system in alpine and mountainous grassland. Alternative forms of grassland utilization like energy production via biogas or heating plants are additional options but will not be able to ensure an area-wide land management.

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## Vegetation dynamics of three perennial swards in NW Greece

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### Abstract

The impact of three treatments on agronomic characteristics of three perennial swards was investigated under rainfed conditions. The experimental fields were established at the Ioannina Research Station in NW Greece, where treatments studied were: a) sward type (*Trifolium repens* L. + *Lolium perenne* L., *T. pratense* L. + *L. perenne*, *L. perenne* only), b) mode of usage (cutting or sheep grazing) and c) mineral fertilization (0, 40 and 80 kg N ha<sup>-1</sup> and additional for *L. perenne*/cutting treatment 160 and 360 kg N ha<sup>-1</sup>) in three replicates each. Samples were analyzed for total dry matter (DM) and DM fractionation according to botanical categories. Dry matter production at the establishment year (two harvests) ranged from 482-641 g DM m<sup>-2</sup> yr<sup>-1</sup> whereas at the first productive year total dry matter production ranged from 2907-3265 and 1520-1613 g m<sup>-2</sup> yr<sup>-1</sup> for grazing (7 harvests) and cutting (4 harvests) treatments respectively. It is concluded that sown swards can effectively support sheep farming in the area.

Keywords: perennial swards, grass, legume, grazing, cutting.

### Introduction

Grazing on sown swards can effectively support major part of a productive ruminant's nutritional requirements. This is more important in Mediterranean areas where natural grazing lands are productive for short periods in the year (spring and autumn) (Hadjigeorgiou *et al.*, 2003). Moreover, market demand for organic products is guiding animal farmers to increase the use of grazed material. Knowledge of the vegetation dynamics in sown swards is crucial for their effective management, but this type of information is limited in Mediterranean environments (Koutsotolis, 1991; Mosimann and Lehmann, 2002; Hadjigeorgiou and Thanopoulos, 2004).

### Materials and Methods

The experimental fields were established at the Ioannina Research Station in NW Greece (annual rainfall: 1100 mm, soil type: clay, organic matter: 3% and pH (1:1): 6.0), where three sward types were developed: A) *Trifolium repens* + *Lolium perenne*, B) *T. pratense* + *L. perenne* and C) *L. perenne* only. These swards were further varied through mineral fertilization (0, 40 and 80 kg N ha<sup>-1</sup> and additional for *L. perenne*/cutting treatment 160 and 360 kg N ha<sup>-1</sup>) in three replicates each, and utilized either by grazing or cutting regimes. No irrigation or herbicide treatments were applied. *Rhizobium* inoculation was not used because efficient strains have been found in the area (Thanopoulos and Kefalogiannis, 1993). Therefore, treatments studied were: a) sward type (A, B or C), b) mode of usage (cutting or sheep grazing) and c) mineral fertilization (0, 40 and 80 kg N ha<sup>-1</sup> and additional for *L. perenne*/cutting treatment 160 and 360 kg N ha<sup>-1</sup>). Swards A and C were harvested at 4 cm, while sward B at 6 cm. Four metal exclusion cages per replication were used to collect samples in grazing treatments. Harvesting vegetation above and below the operational cutting plane provided the samples. Samples were split into sub samples, one was dried (80° C for 48 h) for total DM determination and the second was botanically separated into standing dead matter, *L. perenne*, *T. repens*, *T. pratense* and indigenous vegetation material before fractional DM determination. Data were analysed through a GLM model, where the factors Sward type, N-Fertilization, Harvest and the interaction Sward type × Harvest were fitted.

## Results and Discussion

The first (establishment) year (Table 1) dry matter productivity ranged from 241.4 to 320.5 g m<sup>-2</sup> per harvest. The main component of the produced herbage was the indigenous vegetation that was significantly higher in sward A. However, indigenous vegetation was reduced drastically at the second harvest. Red clover in the grass-legume sward (sward B) gave significantly higher DM production than white clover (sward A).

Table 1. Average dry matter productivity, per harvest, of the experimental swards during the first year (two cutting harvests) and the respective components, followed by the significance of the investigated factors.

	Total DM (g m <sup>-2</sup> )	Standing dead matter (g m <sup>-2</sup> )	Clover (g m <sup>-2</sup> )	Perennial ryegrass (g m <sup>-2</sup> )	Indigenous vegetation (g m <sup>-2</sup> )
Sward A	320.5	33.2	10.8	42.5	232.4
Sward B	241.4	33.4	45.3	40.7	119.4
Sward C	254.6	28.7	-	51.8	175.2
Sward	*	ns	***	ns	***
N-fert.	ns	ns	ns	ns	ns
Harvest	***	***	ns	*	***
Sward × Harvest	**	ns	ns	ns	**

The second year the sown species improved their contribution to sward productivity (see Tables 2 and 3). In both utilization regimes red clover in the mixture performed significantly better than white clover. Moreover, perennial ryegrass was more productive as monoculture and less suppressed in mixture with white clover (Table 2).

Table 2. Average dry matter productivity, per harvest, of the experimental swards during the second year (four cutting harvests) and the respective components, followed by the significance of the investigated factors.

	Total DM (g m <sup>-2</sup> )	Standing dead matter (g m <sup>-2</sup> )	Clover (g m <sup>-2</sup> )	Perennial ryegrass (g m <sup>-2</sup> )	Indigenous vegetation (g m <sup>-2</sup> )
Sward A	396.9	100.5	57.1	91.6	147.7
Sward B	380.1	84.8	89.3	108.0	97.9
Sward C	403.3	95.1	-	152.7	155.5
Sward	ns	ns	**	***	**
N-fert.	ns	ns	ns	ns	ns
Harvest	***	***	***	***	***
Sward × Harvest	ns	ns	ns	*	ns

Total DM production was satisfactory during the first year, but the second year was particularly high due to favourable climatic conditions (above average, evenly distributed rainfall and mild temperatures). In both the study years and for both utilization treatments indigenous vegetation was reduced more efficiently by red clover (sward B), probably due to its erect development habit. Nearly in all treatments and for both years there were significant differences in total DM production between harvests. The general pattern was the higher production occurring in spring-early summer period, while the lower in winter. Nevertheless, for an effective nutritional management of sheep, it is important that these swards were still producing during summer. Generally nitrogen fertilization had no significant impact on any of the established swards, probably due to the high soil fertility, which was further increased through the mechanical incorporation of the natural vegetation during field preparation.

Table 3. Average dry matter productivity, per harvest, of the experimental swards during the second year (seven grazing harvests) and the respective components, followed by the significance of the investigated factors.

	Total DM (g m <sup>-2</sup> )	Standing dead matter (g m <sup>-2</sup> )	Clover (g m <sup>-2</sup> )	Perennial ryegrass (g m <sup>-2</sup> )	Indigenous Vegetation (g m <sup>-2</sup> )
Sward A	466.4	157.4	66.2	159.6	83.1
Sward B	450.3	138.8	93.7	155.7	62.0
Sward C	415.3	154.5	-	194.4	66.3
Sward	*	ns	***	ns	ns
N-fert.	ns	ns	*	ns	ns
Harvest	***	***	***	***	***
Sward × Harvest	***	ns	***	ns	ns

### Conclusions

Rain fed perennial swards in Mediterranean areas, where adequate rainfall is observed, could play a strategic role in ruminant animal nutrition by filling most of the production gaps found in natural grasslands and support the organic systems of production.

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# Effectiveness of the use of indicator species in predicting the floristic richness in two Dolomitic pastures

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## Abstract

The possibility of using indicator species that are characteristic of specific environmental conditions represents an important aspect in the ecological planning of landscape. We considered 50 botanical surveys that were carried out on a sub-alpine pasture. We identified some potential indicator species of floristic richness, and then the optimal number  $n$  of these indicators that could be used for a reliable prediction. In the present work we tried to evaluate, for different  $n$  values, the effectiveness of the indicators in the individuation of the surveys with high floristic richness. We considered different values  $s$  of floristic richness as thresholds for the definition of the precise surveys that had to be individuated. Subsequently, the possibility of using the indicator species for the prediction of floristic richness in a higher spatial scale has been tested considering 40 botanical surveys that were performed in a neighbouring pasture. The results underlined that, for a selected  $n$ , the higher the threshold value  $s$  that was considered, the better was the general accuracy of the estimate, while a decrease of the precision level was encountered. With increasing  $n$  values, a general decrease of the accuracy was observed, followed by an increase of the precision.

Keywords: biodiversity, indicator species, floristic richness, biodiversity monitoring.

## Introduction

The quantification of biodiversity is fundamental for monitoring and conservation, and it requires tools that are both practical and economically sustainable. The knowledge of total species richness in a region, for groups such as plants, birds or insects, is a useful starting point for further research on ecosystem function and species extinctions under habitat loss, as well as being of inherent interest (Krishnamani *et al.*, 2004). Species richness also is an intuitive measure of obvious significance for managers and the public (Mac Nally and Fleishman, 2002). The use of indicator species that can give information about floristic richness is one of the most appropriate methods in those situations where planners want to individuate some precise areas (Fleishman *et al.*, 2000). This tool can limit the sampling work necessary for the identification of coenosis that are in need of protection or some other specific land use. The main aim of this work was to contribute to the practical possibilities of using indicator species in finding areas with specific environmental characteristics.

## Materials and methods

The study area is located in the municipality of Livinallongo del Col di Lana (Belluno, NE of Italy). The pastures of Malga Chertz and Malga Castello were considered. Malga Chertz has a surface of 184 ha, at 1677 to 2060 m a.s.l. and Malga Castell has a surface of 217 ha, at 1,750 to 2,200 m a.s.l. (Susan, 2003). The two pastures are neighbouring, although this area is morphologically and lithologically very complex with acid substrata often adjacent to dolomitic and calcareous soils. In these pastures, 90 botanical surveys (50 at Malga Chertz and 40 at Malga Castello, respectively) were carried out during 2002. The Braun-Blanquet approach was used, though with some modifications with the replacement of abundance-dominance class with the per cent cover value for each species. The plot size was 100 m<sup>2</sup>. Each survey was repeated three times during the vegetative period because of the different phenology of the species present. Applying binary logistic regression to all the species recovered in the botanical surveys carried out at Malga Chertz, 31 species were found that could be considered as potential

indicator species, being statistically more frequent in the species-rich surveys than in the species-poor ones (Susan and Ziliotto, 2006). Subsequently, some subsets of  $n = 3, 5, 8, 10, 15, 18, 20$  indicator species have been randomly drawn from these 31 species. For each  $n$ , 10 replicates have been considered, with  $k = 1, 2, 3, \dots, 10$  indicating the single replicate for the same  $n$ . For all the 70 subsets (identified by  $n$  and  $k$ ), the 50 botanical surveys have been further divided into  $i = 1+n$  classes, including from 0 to  $n$  indicator species. Then, three different values  $s$  of floristic richness were considered and were used as threshold values for the characterization of the precise number of surveys that had to be individuated. The capability of the subsets, for a certain  $n$  value to find the surveys composed by at least  $s$  species has been analysed. Finally, in order to assess the capacity of a certain  $n$  of predicting specific richness on a higher spatial scale, the same approach has been applied to the surveys carried out at Malga Castello.

## Results

The surveys of Malga Chertz were characterized by the number of species included, between 26 and 55. With reference to the three threshold values that have been considered ( $s >45, >50, >52$ , respectively) it was highlighted that for a specific  $n$ , the percentage of surveys having all the  $n$  species (with  $i = n$ ) and characterized by a floristic richness of at least  $s$  species that were individuated, increased with increasing  $s$  (Table 1). In fact, when  $n = 3$ , it was underlined that passing from  $s = 45, 50$  and  $52$ , the percentage of surveys including all the indicators and at least  $s$  species (on a total of 21, 8 and 3 surveys, respectively) increased from 50% to 65% and 93%. When  $s$  increased, the precision of the estimate, expressed as the percentage of surveys including all the  $n$  species and with floristic richness greater than  $s$  among all the surveys with  $i = n$ , decreased, passing from 79.37% to 42.24% and 25.27%. The use of subsets with higher  $n$  resulted in a decrease of accuracy, even though an increasing tendency for higher  $s$  values was still observed.

Table 1. Malga Chertz: accuracy level (percentage of surveys with  $i = n$  and with floristic richness higher than  $s$ , calculated in the totality of surveys with at least  $s$  species) and precision (percentage of surveys with  $i = n$  and with floristic richness higher than  $s$ , calculated in the totality of surveys with  $i = n$ ) of the estimate for different values of  $n$  and  $s$ . Near all the  $s$  values, in brackets, the number of species having at least  $s$  species is reported. All values are calculated as mean of the 10 replicates.

MALGA CHERZ							
Threshold value (s)		>45 (21)		>50 (8)		>52 (3)	
Subset size (n)	Aver. no. of surveys	Accuracy	Precision	Accuracy	Precision	Accuracy	Precision
3	14.1 (6-22)	50.00	79.37	65.00	42.24	93.33	25.27
5	8.1 (2-17)	31.43	86.65	48.75	50.80	83.33	35.66
8	4.5 (2-7)	19.52	94.05	35.00	69.00	63.33	49.71
10	3.5 (1-5)	16.67	100.00	30.00	70.67	56.67	52.50
14 out of 15	4.2 (2-7)	18.10	92.07	30.00	59.38	50.00	34.79

This decrease was contrasted by a progressive increase in precision. Therefore, for  $n = 10$ , all surveys (100%) including the  $n$  indicator species were characterized by at least  $s$  species, but these surveys represented only 16.67% of all the surveys with at least  $s$  species. The use of the indicator species on the pastures of Malga Castello highlighted similar results (Table 2). The surveys performed in this pasture were characterized by a number of species included between 14 and 60. Despite the close proximity of the two pastures, two of the indicators of Malga Chertz, *Onobrychis viciifolia* Scop. and *Dactylis glomerata* L., were not present in the surveys of Malga Castello. As a consequence of this, the number of surveys having all the  $n$  species was clearly lower, because of the inability to find some species. In fact, while in the case of Malga Chertz, for a certain  $n$ , the higher fraction of surveys was included, on average, in the class  $i = n$ , in the case of Malga Castello it occurred for lower values of  $i$  ( $n-1, n-2, n-6$

for  $n=3$ ,  $n=5$ ,  $8$ ,  $10$  and  $n=15$ , respectively). Moreover, the accuracy and precision levels were also lower.

Table 2. Malga Castello: accuracy level (percentage of surveys with  $i=n$  and with floristic richness higher than  $s$ , calculated in the totality of surveys with at least  $s$  species) and precision (percentage of surveys with  $i=n$  and with floristic richness higher than  $s$ , calculated in the totality of surveys with  $i=n$ ) of the estimate for different values of  $n$  and  $s$ . Near all the  $s$  values, in brackets, the number of species having at least  $s$  species is reported. All values are calculated as mean of the 10 replicates.

MALGA CASTELLO							
Threshold value ( $s$ )		>45 (15)		$\geq 50$ (6)		>50 (3)	
Subset size ( $n$ )	Aver. no. of surveys	Accuracy	Precision	Accuracy	Precision	Accuracy	Precision
2 out of 3	14.1 (10-20)	48.00	51.73	56.67	25.16	60.00	13.64
3 out of 5	10.4 (6-15)	38.00	55.18	30.00	27.92	50.00	14.54
6 out of 8	4 (0-8)	18.66	79.30	26.67	44.76	26.67	24.01
8 out of 10	3.8 (1-6)	20.00	83.00	30.00	44.33	40.00	26.83
9 out of 15	3.9 (1-7)	18.00	83.02	30.00	55.17	43.33	38.36

## Conclusion

The use of indicator species in the individuation of coenosis with high floristic richness gave some similar results in the case of Malga Chertz and Malga Castello. Low  $n$  value brought to higher accuracy levels, while precision increased with increasing subset size. These tendencies are preserved also if the  $s$  threshold is higher, but the estimates are less precise and more accurate. In the case of Malga Chertz, if the individuation of only a part of all those areas with high floristic richness is requested, the use of  $n=10$ ,  $15$  is recommendable, because it gives more precise results. With the use of smaller subsets ( $n=3$ ,  $5$ ,  $8$ ) it is possible to find a higher number of coenosis, with a higher range in floristic richness. Even in this case it is possible to focus on a limited number of coenosis, and this aspect makes the field work easy, reducing the potential number of areas that need further detailed studies.

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# The effect of feeding two grassland herbs on the growth performance of weaned piglets

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## Abstract

Twenty-five individually-penned weaned piglets (35 days old) were used in a 35-day growth trial. They were offered a nutritionally balanced cereal-based diet (wheat, barley and oats) *ad libitum* with different amounts (0, 40, 80 or 160 g/kg air-dry feed) of either chicory (*Cichorium intybus* L.) or ribwort (*Plantago lanceolata* L.). The herbs, harvested at the vegetative stage, were dried and milled before use. Feed consumption was recorded on a weekly basis and pigs were weighed at the start and thereafter weekly until the end of the experiment. With the exception of animals fed the highest inclusion of ribwort, there were no significant differences in the daily growth rates (650 vs. 521 g day<sup>-1</sup>) over the entire experimental period. This was associated with a lower feed intake (1,138 vs. 967 g day<sup>-1</sup>), mainly in the beginning of the experiment, of the piglets fed the diet with highest ribwort inclusion. There were no significant differences in the feed to weight gain ratio between diets. It is concluded that some herbs, chicory in particular, can be included in moderate amounts in pig diets without any negative effects on performance.

Keywords: chicory, ribwort, piglets, intake, growth rate, feed conversion.

## Introduction

Pigs are omnivores and have a need for fibre in their diet in order to stay healthy (Low, 1985). As diets for organically raised pigs should be based on home-grown crops and domestically produced feedstuffs, forages/pastures can play a vital role in organic production systems, both as feed components and as a means to promote normal behaviour of the animals. Chicory (*Cichorium intybus* L.) and ribwort (*Plantago lanceolata* L.) are herbs that are deep-rooted, drought resistant and rich in minerals (Foster, 1988); they may complement traditional forage crops both as sward components and feed sources. The growth rates of calves and lambs grazing mixed swards containing chicory and/or ribwort have been good or enhanced (Fraser *et al.*, 1988; Rumball *et al.*, 1997), but herbs have not been evaluated as forage crops for growing piglets.

## Materials and methods

The herbs (Puna chicory and Lancelot ribwort) were grown in a University's field at Uppsala (59°49'N, 17°39'E) and were harvested at the vegetative stage (September) with a stubble height of *c.* 5 cm. They were dried at 30° C for a week and milled through a 5 mm screen before mixing with the other feed ingredients. The control diet was composed of ground cereals (wheat, barley and oats) supplemented with protein, amino acids, mineral and vitamins to meet the nutritional requirements of piglets. The treatments studied were different amounts (g/kg air-dry feed) of either chicory or ribwort; 0 (Control), 40 (C40/RW40), 80 (C80/RW80) or 160 (C160/RW160).

Twenty-five 35-day old weaned castrated male piglets (Landrace x Yorkshire) from five different litters were used in a 35-day growth trial. They were purchased from a herd free from diseases according to the A-list of the International Office of Epizootics ([www.oie.int](http://www.oie.int); 30-Jan-2004), that is Aujeszky's disease, atrophic rhinitis, transmissible gastro-enteritis, porcine epidemic diarrhoea, porcine reproductive and respiratory syndrome and salmonellosis. The piglets were weighed at the beginning of

the experiment ( $11.7 \pm 0.8$  kg) and thereafter weekly until the end of the experiment. They were individually penned and their feed intake was registered on a weekly basis. Feeding was restricted during the first days of the experiment, but thereafter was *ad libitum*. With the exception of the highest inclusion rates (C160 and RW160), which were replicated four times, all treatments had three replicates, while the control had five. The GLM procedure of SAS (2001) was used to test the effect of experimental diets on body weight, daily gain and feed consumption, using diet and litter as factors in the model.

## Results and discussion

All piglets were healthy throughout the experiment and showed no signs of discomfort. With the exception of the animals fed the highest inclusion of ribwort (RW160), there were no significant differences between treatments in daily gain over the entire experimental period ( $P < 0.01$ , Table 1). The litter had a significant effect on the initial BW, the daily gain during weeks 2 and 4 and on the feed consumption during weeks 1, 2 and 3, as well as over the entire experimental period.

Table 1. Body weight (BW, kg) of piglets at the start and at the end of the trial, and the weekly evolution of daily gain ( $\text{g day}^{-1}$ ) and feed consumption ( $\text{g day}^{-1}$ ).

	Control	C40	C80	C160	RW40	RW80	RW160	SD	Treatment	Litter
n	5	3	3	4	3	3	4			
Initial BW	11.0	12.0	11.9	12.4	12.4	11.4	11.4	0.8	NS	**
Final BW	34.8	35.4	34.9	34.7	34.2	33.7	29.6	2.0	*	NS
Daily gain										
Week 1	270	231	203	140	186	263	76	67	*	NS
Week 2	567	523	565	593	558	584	388	104	NS	*
Week 3	774	787	816	801	783	719	606	74	*	NS
Week 4	757	808	710	671	699	684	635	64	*	*
Week 5	1,031	986	994	977	885	928	902	92	NS	NS
Week 1-5	680	667	658	636	622	636	521	43	**	NS
Feed consumption										
Week 1	401	397	376	314	331	412	270	72	NS	***
Week 2	836	831	863	871	838	864	660	109	NS	***
Week 3	1,200	1,273	1,287	1,281	1,220	1,211	992	107	*	*
Week 4	1,428	1,490	1,480	1,431	1,415	1,353	1,276	80	*	NS
Week 5	1,809	1,804	1,879	1,806	1,738	1,692	1,637	101	NS	NS
Week 1-5	1,135	1,159	1,177	1,140	1,109	1,106	967	71	*	**

\*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ , ns = non significant.

The lower daily gain on RW160 was the result of a lower feed consumption ( $P < 0.05$ ) on this diet compared to the other diets. This effect was seen from the beginning of the trial and resulted in a lower body weight (BW) of approximately 4 kg ( $P < 0.05$ ) of the piglets fed this diet compared to the other treatments at the end of the trial. There were no significant differences in feed conversion ratio ( $\text{kg feed kg gain}^{-1}$ ) between treatments and the ratio over the entire experimental period was  $1.76 (\pm 0.08)$ . This variable was higher than that measured in piglets of the same age and breed ( $1.38 \pm 0.08$ ) fed *semi-ad libitum* on a cereal-based diet (Pedersen and Lindberg, 2004) with similar nutritional properties. It is possible that some feed had been wasted due to the *ad libitum* feeding strategy in the current study and that the actual feed intake was lower than what was recorded. In addition, the calculated content of neutral detergent fibre (NDF) in the control diet in the current study was approximately  $125 \text{ g kg}^{-1}$  dry matter (DM) compared to approximately  $90 \text{ g NDF kg}^{-1}$  DM in the study by Pedersen and Lindberg (2004). Thus, the poorer feed conversion ratio in the current study could partly be due to higher NDF content in the diets.

Interestingly, the herbs fed in the amounts used in this study, chicory in particular, did not appear to have any detrimental impact on piglet performance. This suggests that herbs can be included to a greater extent in piglets' diets than grasses and/or legume forages (Lindberg and Andersson, 1998). A low feed consumption during the first week after weaning is a well-known phenomenon in pig production (Fowler and Gill, 1989). This was also observed in the present experiment, but feed consumption soon recovered in all treatments except RW160. However, in general, the feed consumption and daily gain were high in the present study compared with other reports (Fowler and Gill, 1989; Pedersen and Lindberg, 2004). This may be explained by the age at weaning and a good health status, in combination with a palatable feed given *ad libitum*.

### Conclusion

It is concluded that herbs like ribwort and chicory in particular can be included in moderate amounts in piglets' diets without apparent negative effects on performance.

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## Diet quality and intake during the grazing season in beef cows on permanent pastures

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### Abstract

Feed intake, diet quality and animal performance (milk production, weight of both cows and calves) were studied in beef cows at grazing from May to November. Two groups of 8 Charolais cows with calves (calving dating from January to February) continuously grazed a permanent pasture either at high stocking rate (HSR: between 1.6 and 0.9 cows ha<sup>-1</sup>) or at low stocking rate (LSR: 0.7 cows ha<sup>-1</sup>). Feed intake, diet digestibility and sward characteristics were measured on five occasions every six weeks. Sward height varied from 3.3 to 7.7 cm and from 5.9 to 13.1 cm on HSR and LSR treatments, respectively, while biomass ranged from 1.76 to 3.52 t ha<sup>-1</sup> and from 2.61 to 4.15 t ha<sup>-1</sup>, respectively. Diet digestibility was constantly lower on LSR treatment (0.70 vs. 0.73, p<0.001). However, neither organic matter intake (13.5 vs. 13.0 kg) nor digestible organic matter intake (9.5 kg) were significantly different between the two treatments, except in September where intake was higher on LSR treatment (14.0 vs. 11.2 kg OM, and 9.5 vs. 8.1 kg DOM, p<0.05). Both treatments allowed similar milk production and calf growth (1,080 g d<sup>-1</sup>), but cows on HSR treatment lost weight in autumn.

Keywords: beef cows, intake, diet digestibility, grazing, permanent pasture.

### Introduction

Grazing management aims to provide herbage in quantity and of sufficient quality to satisfy animal requirements while sustaining the grassland. Extensive management for environmental purposes, such as enhancing biodiversity, can be achieved by lowering grazing pressure or stocking rate. This results in the development of pasture heterogeneity, with an increased herbage biomass but of lower nutritive value. Thus, it is essential to understand the interplay between animal intake and vegetation dynamics in order to evaluate the resource value for the animals, and to manage that resource (Baumont *et al.*, 2005). When compared at high and low stocking rates during the grazing season, sheep adapted their foraging behaviour to maximise diet digestibility, and were able to maintain a comparable intake of digestible matter (Garcia *et al.*, 2003). The aim of this trial was to investigate the ability of beef cows to maintain diet quality and intake, as well as their production performance, on permanent pastures managed at high or low stocking rates during the grazing season.

### Materials and methods

The experiment was conducted in 2004 in the experimental farm of Monts-Dore (France) on an upland (1,200 m) permanent pasture dominated by *Festuca rubra* and *Agrostis tenuis*. Sixteen Charolais cows (4 to 7 years old) with their calves were split into two groups of eight with similar initial weights (682 ± 70 and 119 ± 15 kg for cows and calves, respectively). One group continuously grazed a plot of 11.4 ha. That corresponds to a low stocking rate (LSR: 0.70 cow ha<sup>-1</sup>), leading to some under-exploitation of the vegetation in spring. The other group grazed at a higher stocking rate (HSR: mean grazing pressure of 1.23 cow ha<sup>-1</sup>) a plot that varied from 5 to 9 ha, the surface being adjusted to maintain the sward vegetative and not higher than 8 cm.

Both groups grazed continuously from May to November with five experimental periods: P1, 17-28 May; P2, 28 June-9 July; P3, 16-27 August; P4, 20 September-1 October; P5, 25 October-5 November. During each period, sward height was characterised by stick measurements and sward biomass was

measured from 10 x 0.1m rectangles cut at ground level with a hand mower (8 and 4 rectangles on LSR and HSR, respectively). Before cutting, 2 quadrats (0.14 x 0.14 m) were sampled on each rectangle to assess morphological sward structure by hand-sorting and to assess sward organic matter (OM) digestibility by pepsin-cellulase method (Aufrère and Demarquilly, 1989).

For each experimental period the daily organic matter intake of the cows was measured using ytterbium oxide (Yb<sub>2</sub>O<sub>3</sub>) as an indigestible marker to estimate faecal output, and faecal nitrogen content to estimate OM diet digestibility (Garcia *et al.*, 2003). The grazing time was recorded using 'Ethosys' collars (IMF Technology GmbH, Frankfurt, Germany). Before and after each experimental period, the cows and calves were weighed, and milk production was estimated by weighing the calves before and after suckling (Le Neindre, 1973).

The data were analysed by analysis of variance with treatment (HSR vs. LSR) and period (P1 to P5) effects and their interactions. As the different experimental periods were not independent, the period was considered as a repeated measure using the mixed procedure in SAS 8.1 software (SAS, Cary, NC).

## Results and discussion

Under both treatments, the highest sward height and biomass were obtained at the beginning of July during P2 at the end of the reproductive growth (Table 1). LSR treatment induced a significantly higher sward height and biomass. Digestibility of sward samples cut at the ground level decreased significantly during the grazing season for both treatments, and was closely related to the digestibility of the green leaves and to the proportion of dead material in the sward ( $R^2 = 0.85$  for multiple regression).

Table 1. Treatment (T) and period (P) effects on sward height, biomass and OM digestibility.

	Treatment	Period					s.e.d.	Effect		
		P1	P2	P3	P4	P5		T	P	TxP
Height (cm)	HSR	5.9	7.7	5.3	4.5	3.3	0.93	***	**	**
	LSR	7.0	13.1	10.1	7.5	6.3				
Biomass (T DM/ha)	HSR	2.69	3.52	2.59	1.76	2.16	0.36	*	***	NS
	LSR	2.61	4.15	3.20	2.91	2.78				
OM Digestibility (g/g)	HSR	0.68	0.60	0.59	0.60	0.54	0.013	NS	***	**
	LSR	0.66	0.61	0.58	0.57	0.56				

s.e.d.: standard error of difference; NS = non-significant; \* =  $P < 0.05$ ; \*\* =  $P < 0.01$ ; \*\*\* =  $P < 0.001$ .

Diet digestibility estimated by faecal nitrogen content decreased during the grazing season (Table 2) and was closely related to sward digestibility ( $R^2 = 0.83$ ). From P2 to P5, the digestibility of the diet selected by the animals was significantly higher on the HSR treatment. Faecal output was significantly increased after P1 for both treatments (Table 2). Faecal output was significantly higher under LSR treatment, and reached a plateau between P2 and P5. Voluntary intake varied significantly with period, and was closely related to sward biomass ( $R^2 = 0.76$ ). Neither organic matter intake nor digestible organic matter intake were significantly different between the two treatments, except in September (P4) where intake was higher on the LSR treatment. Grazing time was always higher on HSR treatment, reaching significance for P2, P3 and P4.

Table 2. Treatment (T) and period (P) effects on OM diet digestibility, intake parameters and grazing time.

	Treatment	Period					s.e.d.	Effect		
		P1	P2	P3	P4	P5		T	P	TxP
OM Digestibility of the diet (g/g)	HSR	0.79	0.73	0.72	0.72	0.69	0.006	***	***	***
	LSR	0.78	0.71	0.68	0.68	0.66				
Faecal output (kg OM/day)	HSR	2.8	4.1	4.0	3.1	3.6	0.3	**	***	***
	LSR	2.7	4.5	4.4	4.6	4.2				
Intake (kg OM/day)	HSR	13.0	15.1	14.0	11.2	11.7	0.85	NS	***	**
	LSR	11.9	15.7	13.8	14.0	12.3				
Digestible OM intake (g/day)	HSR	10.2	11.1	10.0	8.1	8.1	0.62	NS	***	**
	LSR	9.2	11.2	9.4	9.5	8.1				
Grazing time (min/day)	HSR	551	571	596	588	577	22	*	*	NS
	LSR	518	517	549	544	558				

s.e.d.: standard error of difference; NS = non-significant; \* = P<0.05; \*\* = P<0.01; \*\*\* = P<0.001.

Milk production of the cows reached 8 kg after P1 and decreased thereafter in accordance with data by Petit and Agabriel (1989). No significant treatment effect was detected on milk production nor on the average daily weight gain of the calves (1,090 and 1,070 g day<sup>-1</sup> for HSR and LSR, respectively). However, the weight of the cows was significantly lower under HSR treatment after P3 and until the end of the trial (745 vs. 788 kg, P<0.001).

Under HSR, cows maintained a higher diet quality but intake was limited by low herbage availability, in particular in the autumn not fully compensated by the increase in grazing time. Under LSR, the quality of the ingested diet was lower, but the cows maintained a sufficiently high level of organic matter intake to allow a similar intake of digestible organic matter. Intake was probably limited by the amount of matter in the digestive tract, as indicated by the plateau in faecal output. As a consequence, animal production was little affected by stocking rate: both grazing management strategies allowed similar calf growth, but the cows lost weight in autumn under the HSR treatment.

## Conclusion

At high or low stocking rates, beef cows are able to maintain to a certain extent their intake of digestible matter, either with increased grazing time when grass availability is low, or with increased flow of non digested material when sward quality is low. It gives a certain amount of flexibility in managing beef cows at grazing.

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# Changes in species composition of grasslands induced by some agronomic practices

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## Abstract

Grassland management plays a key role in the performance of species in swards. This has been little documented in intensive farming with complex mixtures including numerous grass and legume species. An agronomic field trial was established in spring 2003 at INRA Lusignan, France. Twenty mixtures with 2 to 8 species were grown under two defoliation regimes and two levels of nitrogen fertilisation. Botanical composition was assessed twice a year. After two years, changes in species frequency appeared to be related to several factors. In mixtures of grass species, defoliation rhythm and nitrogen fertilisation are key factors for the proportion of *Festuca arundinacea* L. and *F. rubra* L. Nitrogen had little effect on the proportion of *Dactylis glomerata* L. and *Lolium perenne* L.. In grass and legume mixtures, an infrequent defoliation regime leads to a higher proportion of *Trifolium pratense* L. and *Medicago sativa* L.. Contrarily, proportion of *Trifolium repens* L. and *Lotus corniculatus* L. increased under frequent cuttings. This study shows that proportion of species in mixtures changed rapidly in the first growing cycles.

Keywords: botanical composition, defoliation frequency, grassland, nitrogen fertilisation.

## Introduction

Forage production and quality of temporary grasslands depend on the initial species diversity, fertilisation level and defoliation frequency. These last two factors will play a key role in the changes of species abundance in swards along seasons. Recent studies (Mariott *et al.*, 2004; Jeangros and Bertola, 2002) have focussed on the impact of transition from intensive to extensive farming practices (lower cutting frequency, reduction or suppression of N, P, K fertilisation). The originality of the present study was to investigate changes in multi-species grassland swards under two defoliation frequencies (simulating grazing and hay-making) and two fertilisation regimes (simulating intensive and extensive conditions). After three years of study, changes in contribution of species to sward aerial biomass were investigated as a function of the agronomic practices.

## Materials and Methods

Six grass mixtures and 14 grass-legume mixtures, including from two to eight species (Table 1), were sown in spring 2003 at INRA, Lusignan, France, in two separate experimental designs in a split-plot design with three replicates with N regime as sub-block and defoliation frequency as a sub-sub-block. Individual plot size was 9.4 m<sup>2</sup>. On long-term average, Lusignan is characterised by an annual rainfall of 830 mm with a strong summer drought. No irrigation was provided to the trial. Two defoliation regimes were applied with either a frequent cutting regime every 25 days or an infrequent regime every 45 days. Two nitrogen fertilisation levels were provided in both mixture designs. Under high (N+) and low (N-) fertilisation treatments, mixtures of grass species received 250 and 60 kg ha<sup>-1</sup>yr<sup>-1</sup> respectively, distributed over the whole growing season, while mixtures of grasses and legumes received 50 kg N ha<sup>-1</sup> yr<sup>-1</sup> in early spring and no nitrogen respectively. Every year, P and K were applied at a rate of 110 kg ha<sup>-1</sup>. All plots were mechanically harvested at a 5 cm height. Botanical composition was assessed twice a year, in spring (late April or early May) and in autumn (late October). Samples were collected with hand clippers on sub-plots of 0.18 m<sup>2</sup>. Species were manually separated and dried in an oven at 60°C during 72 hours to determine contribution of each species to total sward biomass. When present, weeds were bulked and species were not identified.

Table 1. List of mixtures sown in spring 2003.

Species \ Mixtures	Mixture					Mixture															
	2.1	2.2	2.3	3.1	4.1	5.1	2.4	3.2	4.2	4.3	5.2	5.3	5.4	5.5	5.6	6.1	6.2	7.1	7.2	8.1	
Number of species	2	2	2	3	4	5	2	3	4	4	5	5	5	5	5	6	6	7	7	8	
Number of grasses	2	2	2	3	4	5	1	2	3	3	4	3	3	3	3	5	4	3	5	6	
Number of legumes							1	1	1	1	1	2	2	2	2	1	2	4	2	2	
<i>Lolium perenne</i> <sup>(1)</sup>	■		■	■	■	■	■	■	■		■	■	■	■	■	■	■	■	■	■	
<i>Lolium perenne</i> <sup>(2)</sup>															■		■		■		
<i>Lolium x boucheanum</i>															■						
<i>Dactylis glomerata</i>	■	■		■	■	■		■		■	■	■	■	■		■		■	■	■	
<i>Festuca arundinacea</i>		■	■	■	■	■		■		■	■	■	■	■		■		■			
<i>Festuca pratensis</i>					■	■			■	■						■				■	
<i>Phleum pratense</i>																	■		■	■	
<i>Poa pratensis</i>									■						■		■		■	■	
<i>Festuca rubra</i>						■			■							■	■		■	■	
<i>Medicago sativa</i>												■									
<i>Trifolium repens</i>							■	■	■		■	■			■	■	■	■	■	■	
<i>Trifolium pratense</i>												■	■	■		■	■	■	■	■	
<i>Medicago lupulina</i>											■						■				
<i>Lotus corniculatus</i>									■				■				■				

<sup>(1)</sup> Late-heading variety; <sup>(2)</sup> Early-heading variety.

For calculating changes in proportion of species, values observed in autumn 2003 was taken as a control ( $F_0$ ) and proportions observed in each plot in the subsequent sampling dates  $d$  ( $F_d$ ) were corrected as  $P_d = F_d / F_0$

$P_{ijkl}$  is the corrected proportion of a species in  $i^{\text{th}}$  fertilisation level ( $i=1$  for N+ and  $i=2$  for N-),  $j^{\text{th}}$  defoliation regime ( $j=1$  for frequent defoliation),  $k^{\text{th}}$  replicate,  $l^{\text{th}}$  species mixture where it is included and  $d^{\text{th}}$  sampling date. Response of species to fertilisation and defoliation in sampling date was calculated for each replicate as follows.

Response to fertilisation:  $\ln(P_{1.k.d} / P_{2.k.d})$ ; Response to defoliation:  $\ln(P_{.1k.d} / P_{.2k.d})$

Criteria developed to measure responses to fertilisation and defoliation do not meet assumptions of classical analysis of variance. Thus, adequate statistical tests are under development.

## Results and discussion

Years 2003 to 2005 were characterised by an early summer drought that prolonged till autumn in 2004. Along the first year, from April 2003 to April 2004, total rainfall reached 746 mm with high summer temperatures in 2003. High temperatures did not damage sward structure. The second growing season (April 2004 to April 2005), rainfall only reached 508 mm. In all plots, proportion of weeds remained very low with average values of 0.1% in spring and autumn 2004 and 0.4% in spring 2005. This increase may suggest a significant increase of weeds in seed bank in the following years. In grass mixtures, proportions of *Lolium perenne* and *Dactylis glomerata* were little affected by the N fertilisation in the first two years while infrequent cutting negatively affected proportion of *Lolium* (Figure 1). Proportion of *Dactylis glomerata* doubled between autumn 2003 and spring 2005 under infrequent cutting. *Festuca arundinacea* and *F. rubra* showed contrasting responses. Proportion of *F. arundinacea* increased under high N fertilisation and infrequent cutting. On the opposite, frequent cuts and low N supply facilitated increased proportion of *F. rubra*. Straightforward changes in proportions occurred progressively over the three sampling dates performed up to now.



1)

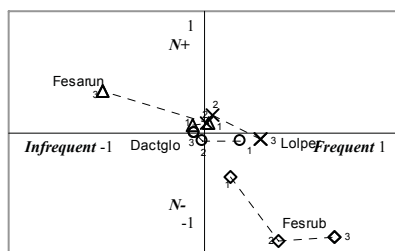


Figure 1. Influence of defoliation regime (x axis) and N fertilisation (y axis) on response of species in grass mixtures in spring 2004 (1), autumn 2004 (2) and spring 2005 (3).

2)

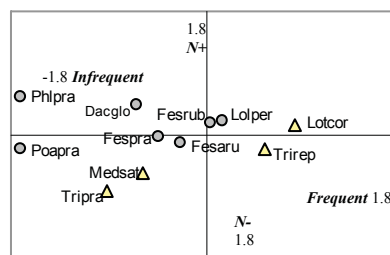


Figure 2. Influence of defoliation regime and fertilisation on species response in grass-legume mixtures in spring 2005.

Abbreviations: Dacglo, *Dactylis glomerata*; Fesaru, *Festuca arundinacea*; Fespra, *Festuca pratensis*; Fesrub, *Festuca rubra*; Lolper, *Lolium perenne*; Lotcor, *Lotus corniculatus*; Medsat, *Medicago sativa*; Phlpra, *Phleum pratense*; Poapra, *Poa pratensis*; Tripra, *Trifolium pratense*; Trirep, *Trifolium repens*.

Presence of legume in mixtures, especially *Trifolium repens* modified behaviour of grass species, mainly *F. arundinacea* and *F. rubra* (Figure 2). *Phleum pratense* and *Poa pratensis* were better adapted under infrequent defoliation. When present, *Trifolium repens* contributed very high proportion of aerial biomass, reaching up to 80% in some mixtures in spring 2005. This may be due to the aggressiveness of the variety Aran that was chosen for this trial. Response of grasses to N supply in this design was small as it may be expected from the low N supply in N+ treatment, but, on the N axis, grasses and legumes ranked as expected. Among legumes, infrequent cutting combined with lack of nitrogen fertilisation favoured development of *Trifolium pratense* and *Medicago sativa*, forage legumes with erected stems. *Trifolium repens* proportion increased under frequent cutting as it may be expected for this species well adapted to intensive grazed grasslands. Frequent defoliation also contributed to an increased proportion of *Lotus corniculatus*. After three years of trials, two species disappeared from the mixtures, namely *Medicago lupulina* and *Lolium x boucheanum*.

## Conclusion

In temporary grasslands, marked changes in species proportion occurred in the first seasons and partly depended on exploitation regimes. Changes will be further analysed over the next three years. This study is part of a large project that aims at relating changes in species proportion and changes in dry matter production and quality.

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# The effect of grazing intensity across spatial scales of plant species richness

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## Abstract

The method of additive partitioning was used to study species richness across three spatial scales (quadrat, paddock and field) in two grazing intensities. The total species richness ( $\gamma$ ) found in a collection of samples at any spatial scale can be partitioned into the average number of species that occur within a sample ( $\alpha$ ) and the average number of species absent from a sample ( $\beta$ ). Thus,  $\beta$  species richness is a measure of the extent to which the richness of two or more spatial units differs. The results of a three-year experiment showed a higher total species richness in the lower stocking intensity compared to the higher stocking intensity. These treatment specific differences were mainly due to the increased spatial complexity and thereby  $\beta$  diversity on paddock scale (patchiness) and on field scale (habitat heterogeneity) developing in the extensively stocked pasture. Therefore, restoring spatial components of diversity should be recognized as a goal in management aiming at maintenance or even enhancement of plant species richness.

Keywords: spatial diversity, additive partitioning, grazing, habitat heterogeneity, beta-diversity, species richness.

## Introduction

Spatial patterns of species diversity change over multiple scales. The pattern observed within a sample quadrat might be very different from those found over broader areas such as paddock, field, landscape or region. The additive partitioning of species diversity is a promising approach for analyzing patterns of diversity sampled from hierarchically scaled studies (Godfray and Lawton, 2001). As was shown by Lande (1996), Whittaker's (1960) term's of  $\alpha$ ,  $\beta$  and  $\gamma$  diversity can be applied to additive partitions of total species richness or diversity into components. For this, the total species diversity ( $\gamma$ ) found in a collection of samples at any spatial scale can be partitioned into the average number of species that occur within a sample ( $\alpha$ ) and the average number of species absent from a sample ( $\beta$ ; Veech *et al.* 2002). Thus,  $\beta$  diversity is a measure of the extent to which the diversity of two or more spatial units differs. Additive partitioning can be applied not only across different scales, but the same approach can also be used to examine changes in diversity over time (Gering *et al.* 2003, Magurran 2004). By this, diversity partitioning can improve surveys by identifying the primary sources of the total species diversity of a field and target conservation efforts accordingly (Veech *et al.* 2002). Only little information is available on which components of botanical diversity in temperate grasslands are changeable by management measures. Yet, this information is crucial for performing successful biodiversity targeted management.

This paper presents the results of an experiment analyzing the effects of different grazing intensities on plant species diversity on different spatial and temporal scales. Specifically, the following questions were asked. (1) How important is the effect of grazing intensity on total species richness? (2) Is the effect of grazing intensity on species richness at the patch (quadrat) scale different to the effect of grazing intensity at the paddock scale?

## Material and methods

The experiment was carried out from 2002 – 2004 at Relliehausen (51° N, 9° E), 200 m a.s.l. in Solling Uplands on moderately species rich mesotrophic hill grassland, vegetation type *Lolio-Cynosuretum*. The

following two continuous stocking treatments of growing steers were imposed: moderate stocking (MC) with a target compressed sward height of 6 cm and lenient stocking (LC) with a target sward height of 12 cm.

Vegetation data were sampled in ten random permanent quadrats of 1 m<sup>2</sup> in each paddock of 1 ha. Each treatment was replicated three times in a block design resulting in a total of 30 quadrats of 1 m<sup>2</sup> per treatment. Presence of plant species were recorded in the quadrats three times in the years 2002 to 2004. In this paper, only the recordings of the first observation (May/June) in the years 2002 and 2004 are considered. In some cases, it was difficult to identify plants down to species level. Those were treated as species aggregate. This applies for *Agrostis*, *Cerastium*, *Crepis*, *Geranium*, *Poa*, *Sonchus*, *Veronica* and *Vicia* species. Therefore, the true species number is underestimated.

A hierarchically nested design to sample data on plant species richness was used. The following hierarchical levels (corresponding to spatial scales) were represented in this design: experimental field, treatment, paddock and quadrat. The highest level was represented by the field site. The total observed species richness at the field site was partitioned into scale-specific diversity components using the additive partitioning approach (Lande, 1996). Within the context of this study,  $\gamma$  is the total plant species richness. The total richness is partitioned into the average diversity within sampling unit ( $\alpha$ ) and between sampling unit ( $\beta$ ) so that  $\gamma = \alpha + \beta$ , and  $\beta$  diversity can be estimated by  $\beta = \gamma - \alpha$  (Wagner *et al.*, 2000). In this study,  $\alpha_1$  represents species richness within quadrats,  $\beta_1$  between quadrats,  $\beta_2$  between paddocks (replications),  $\beta_3$  between treatments. The total species richness at the field site found in the pooled sampling unit of 60 quadrats is  $\gamma_3$ . Thus, the total plant species richness in this study can be described by the following formula:  $\gamma_3 = \alpha_1 + \beta_1 + \beta_2 + \beta_3$ . Additive partitioning was conducted on species richness, which is defined as the number of species in a sampling unit. Statistical analyses were performed by repeated-measures ANOVA separately for  $\alpha_1$ ,  $\beta_1$  and  $\beta_2$  components of species richness, testing the effects of treatment and year.

## Results and discussion

In total, 55 plant species were recorded on the experimental site. The  $\alpha_1$  richness comprised about 11 species at the 1 m<sup>2</sup>-quadrat scale (Fig. 1), and was not affected by treatment or year. At the start of the experiment in 2002, the richness components  $\beta_1$  and  $\beta_2$  had about the same proportion as  $\alpha_1$  in both treatments, but the  $\beta$  components developed differently during the course of the experiment. While  $\beta_1$  richness increased slightly in both treatments,  $\beta_2$  richness significantly decreased in the MC treatment and increased in the LC treatment. This temporal divergent development for  $\beta_2$  richness resulted in a significant treatment effect ( $P=0.046$ ). The decrease in  $\beta_2$  richness in the MC treatment was due to the loss of some plant species which had been present only in one of the three paddocks per treatment. Correspondingly, the gain in  $\beta_2$  richness in LC was caused by new emerging species which occurred only in one paddock. Those were mainly annual ruderals and two perennial grassland forbs. These treatment-specific reactions might be caused by different species pools (Tews *et al.*, 2004) adjacent to the three blocks in combination with different sward structure in the two treatments. While the MC treatment was characterized by a dense sward, the tiller density in the LC treatment was much lower. During the course of the experiment, the dominance of *Lolium perenne* L. increased favoured by frequent defoliation and the nitrogen cycling by excrement deposit in the MC treatment, and this resulted in a high tiller density. It can be assumed that this dense sward in MC has restricted the colonization of invading species via diaspores while a more open and in some areas less competitive sward in LC has favoured the establishment of new species.

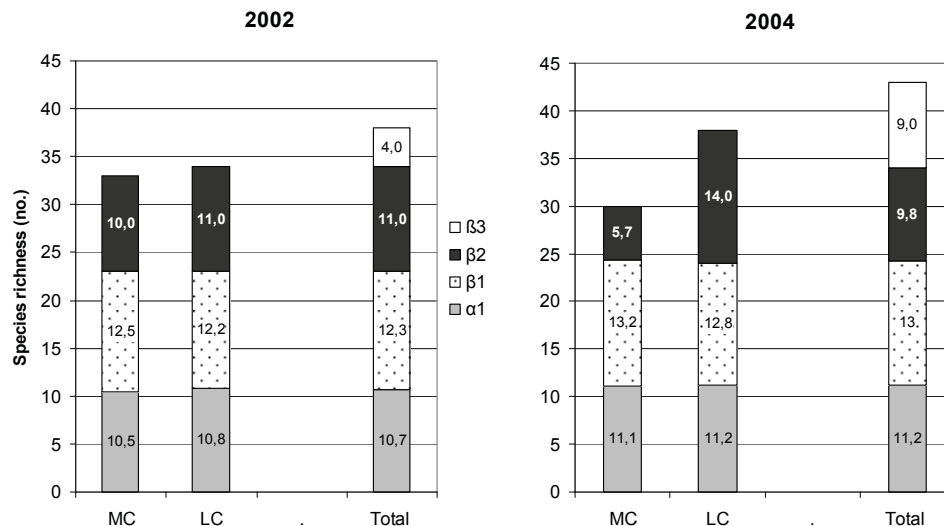


Figure 1. Components of spatial plant species richness in moderate (MC) and lenient (LC) continuous stocking in 2002 and 2004 calculated by the additive partitioning method. Values of total species richness ( $\gamma$ ) at the field site in each year are given by the top line of the bar 'Total',  $\alpha_1$  represents richness within quadrats,  $\beta_1$  between quadrats,  $\beta_2$  between paddocks,  $\beta_3$  between treatments.

Due to a high surplus of forage on offer on more extensively grazed paddocks, the grazing animals can impose higher selectivity resulting in lightly and strongly grazed subareas which differ substantially in their structure (Tews *et al.*, 2004). Long-term monitoring is needed to test whether this structural diversity will further increase on the extensively grazed swards and by this favour higher species richness.

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# Effects of goat breed and stocking rate on biodiversity of Atlantic heathlands

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## Abstract

The effects of goat grazing management on plant and animal biodiversity of heather-gorse shrublands were studied. Three treatments with three replicates were established: Cashmere breed at low stocking rate (CL), Cashmere breed at high stocking rate (CH) and local breed at high stocking rate (LH). After three grazing seasons, there was a higher reduction of shrub cover and higher increase of herbaceous in LH than in CH, as well as in CH than in CL. The highest plant diversity was found in CH and the lowest in CL. In relation to fauna data there were only significant effects of the stocking rate on grasshopper abundance and of sampling period on all groups. Only few significant correlations were found between vegetation data and the abundance and diversity of different animal groups, mainly related to gorse, heather and herbaceous cover during certain seasons.

Keywords: biodiversity, birds, goat grazing, heathland, invertebrates.

## Introduction

Grazing herbivores greatly influence on the composition and structure of the vegetation and consequently may affect the wild fauna living in such communities. This paper is part of an EU project (QLK5-2001-00130 FORBIOBEN) to examine the effects of stocking rate and breed of grazing livestock on the biodiversity (floral and faunal) of natural or semi-natural grassland systems (Rook *et al.*, 2004). In this case we compare two goat breeds, one commercial (Cashmere) and one local (Celtiberic) at high stocking rate, and two stocking rates (high or low) in the case of Cashmere breed, grazing on Atlantic heathlands.

## Materials and methods

The experimental farm is located in the north-west of Asturias (Spain) at 950 m a.s.l. The vegetation is an acidophilous heather-gorse community (*Erica* spp, *Ulex gallii*). Three grazing treatments with three replicates were established in 2002 on nine 0.6 ha plots: Cashmere breed at low stocking rate (CL), Cashmere breed at high stocking rate (CH) and Local breed at high stocking rate (LH).

Vegetation cover was assessed by recording 1000 contacts with a sward-stick in a 50 m long transect per plot. Controls were achieved at the beginning (May-June), at the middle (August) and at the end (October) of the grazing season (2002-2005). Butterflies and grasshoppers samplings consisted of two 5 m wide transects of 50 m length per plot, sampled every two weeks in 2003 and 2004 (when weather permitted their capture). Bird data were obtained through direct observations at dawn, every two weeks from 2003 to the spring of 2005. Ground-dwelling arthropods were captured by means of pit-fall traps (12 per plot) for a period of 4 days, three times per year. They were identified into family level and grouped into trophic groups (carnivorous, herbivorous, detritivorous and omnivorous). Only data from year 2003 are considered since the identification of taxa is still progressing.

A General Linear Model was applied to study the treatment and season effects and the relationships between vegetation and animal data were analysed by bivariate Spearman correlations.

## Results and discussion

Vegetation dynamics were significantly affected by goat breed and stocking rate (Celaya *et al.*, 2005). The cover of shrubs decreased more and herbaceous percentage increased more in LH compared to CH, as well as in CH compared to CL. Consequently dead matter percentage increased more under Local goat grazing than under Cashmere goat grazing. The highest plant diversity indexes were found in CH and the lowest in CL where the dominance of woody plants continued unaltered after three grazing years, being intermediate in LH.

No significant treatment effects were observed on abundance, diversity and species richness of birds and butterflies, whereas in the case of grasshopper abundance it was higher in CH compared to CL ( $p < 0.001$ ). The sampling season had a significant effect over all the groups mentioned above (Jáuregui *et al.*, 2005). In the other countries of the same project, on herbaceous grasslands, higher abundance and species richness of butterflies was found at the lower stocking rate (WallisDeVries *et al.*, 2005).

In relation to ground-dwelling arthropods, data from year 2003 are summarized in Table 1. Neither the stocking rate nor the goat breed had significant effects on the abundance of arthropods considered as a whole as well as when the different trophic groups are analysed separately. The global abundance of arthropods and the abundance of carnivorous, detritivorous and omnivorous varied significantly during the different sampling periods.

Table 1. Abundance, family richness and diversity index ( $H'$  Shannon) of ground-dwelling invertebrates (arthropods) and abundance of the different trophic groups in each treatment (means of three plots) and sampling season of 2003 (a: spring; b: summer; c: autumn).

	Season	CL	CH	LH	Treatment effect	Season effect
Arthropod abundance	a	173.67	196.67	297.00	ns	***
	b	100.67	94.33	89.00		
	c	51.33	57.67	88.33		
Family richness	a	18.33	17.00	17.00	ns	*
	b	12.67	14.00	14.00		
	c	9.67	9.00	14.33		
Diversity ( $H'$ )	a	2.46	2.55	1.98	ns	ns
	b	2.07	2.44	2.76		
	c	2.08	1.96	3.15		
Herbivorous abundance	a	8.00	8.00	9.00	ns	ns
	b	4.67	7.00	7.67		
	c	3.67	3.33	5.00		
Carnivorous abundance	a	111.33	139.00	252.33	ns	**
	b	84.33	81.00	75.00		
	c	33.00	40.00	66.33		
Detritivorous abundance	a	51.33	46.33	33.67	ns	***
	b	10.67	5.67	6.33		
	c	13.67	13.33	15.67		
Omnivorous abundance	a	3.00	3.33	1.00	ns	**
	b	1.00	0.67	1.00		
	c	1.00	1.00	1.33		

Significance level: \*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ ; ns not significant ( $p > 0.1$ ).

Although significant correlations were found between plant cover and abundance and species richness of the different animal groups, these relationships were confounded with the effects of sampling period, as vegetation differed not only between the grazing treatments but also between seasons. Restricting the analysis to each season (Table 2), gorse cover percentage was negatively correlated to bird abundance and species richness during spring and to grasshopper abundance during autumn. Heather percentage was positively correlated to diversity of grasshoppers along summer and negatively to carnivorous abundance. On the contrary, the abundance of this trophic group was positively correlated to herbaceous cover percentage during spring and autumn. Percentage of dead litter was positively correlated to bird

abundance and species richness during spring. No significant relationships were found between plant cover and the abundance of herbivorous, detritivorous and omnivorous arthropods.

Table 2. Correlations between abundance and species richness of birds and invertebrates (grasshoppers and carnivorous ground-dwelling arthropods) and the main components of the plant cover in each sampling season of 2003 (a: spring; b: summer; c: autumn).

	Season	Parameter	n	Gorse (r sig)	Heather (r sig)	Herbaceous (r sig)	Litter (r sig)
Birds	a	Abundance	27	-0.485 **	ns	ns	0.403 *
		Richness	27	-0.456 *	ns	ns	0.484 *
	b	Abundance	18	ns	ns	ns	ns
		Richness	18	ns	ns	ns	ns
	c	Abundance	18	ns	ns	ns	ns
		Richness	18	ns	ns	ns	ns
Grasshoppers	a	Abundance	18	ns	ns	ns	ns
		Richness	18	ns	ns	ns	ns
	b	Abundance	18	ns	ns	ns	ns
		Richness	18	ns	ns	ns	ns
	c	Abundance	18	-0.719 ***	ns	ns	ns
		Richness	18	ns	ns	ns	ns
Carnivorous	a	Abundance	9	ns	-0.937 ***	0.728 *	ns
	b	Abundance	9	ns	-0.611 (*)	ns	ns
	c	Abundance	9	ns	-0.731 *	0.756 *	ns

Significance level: (\*)  $p < 0.1$ ; \*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ ; ns not significant ( $p > 0.1$ ).

## Conclusion

At the moment, with the available preliminary information (one complete year), no clear effects of the different grazing managements with goats have been observed on the fauna, despite significant differences were found in vegetation canopy structure and composition.

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# Effects of goats grazing on vegetation cover in natural protected areas

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## Abstract

Between years 2002 and 2004 monitoring of the state of ecosystems under grazing conditions was carried out, in a natural protected space located in a mountainous area in the northwest part of the island. Survey consisted in fifty one transects. Monitoring of transects was done every year and vegetation cover was calculated from the results obtained. Annual data were analysed with ANOVA statistical test to show whether the vegetation was affected or not by grazing in the area. Differences in vegetation cover between areas with and without grazing were significant only in years with lower cover. Grazing is affecting then vegetation cover in some areas of the Park, although this effect depends on the year, decrease of vegetation in those areas could contribute to erosion and loss of soil.

Keywords: methodology, vegetation cover, grazing, Canary Islands.

## Introduction

The effects of grazing in ecosystems has been in the past a very controversial query. Many authors have noticed the complexity of the existing relationship between plants and animals. This makes grazing in natural ecosystems a problem not easy to solve. Considering that the main goal of the livestock management of land is the sustainability of the ecosystem (assembly of productivity, stability, sustainability and equity), the variables of stocking rate and carrying capacity are of light utility in situations with a high variety in space and climate, as it is in the case of natural ecosystems in Mediterranean environments. In this paper we consider these thoughts by introducing concepts related to the stability of an ecosystem used for grazing. Our aim is to create patterns of the relationship between plants and animals that permits to create a base for range management. In this case we choose the vegetation cover as a variable, due to its relationship with erosion processes. The main objective is to determine how the vegetation cover of the grazing units responds in an environment with year-to-year variations.

## Materials and methods

This research was carried out on a natural protected space located on the northwest part of Tenerife (Canary Islands). The vegetation cover was determined by fifty one permanent transects (Daget and Poissonet, 1971) within a working area of 12,566 ha twenty five transects were settled in grazing areas and twenty six in areas not used for grazing. The study was carried out between 2002 and 2004. The grazing areas were established by doing interviews to nine goats farmers for milk production, with a stocking rate of approximately 0.4 animal units per ha. The information obtained was compared to the files of subsidies for grazing for the year 2003 (Real Decreto 708/2002).

The area that was monitored was divided in management units according to the macro-orientation (north and south), because of the high effects of the trade winds in the Canary Islands, the slope, the different types of vegetation and the altitude zone. All together four management units, two grazing areas within abandoned cultivation space (*Hypparrhenia hirta*, *Phalaris caerulea*, *Medicago spp.* and *Trifolium spp.*) and two areas of coastal scrub (*Euphorbia spp.*) in two different altitude zones (zone one: 350-600 meters and zone two: over 600 meters) were obtained.



The statistical analysis consisted in the variability analysis with measures repeated in 2002, 2003 and 2004. As reference level the Wilks' Lambda was taken. The dependant variable was the vegetation cover defined as the percentage of surface not covered by any vegetal. We defended the hypothesis that there are no significant differences within the depending variable due to grazing.

### Results and discussion

In the results obtained by the ANOVA of repeated measures a significant effect on the vegetation cover during the year was observed (Table 1). This shows a relevant general instability due to different combinations of the environmental conditions every year. The most interesting effect is the interaction between year and grazing use (p-level: 0.027\*). This means that most of the differences between grazing areas and non grazing areas are generated according to the year.

Table 1. Result of ANOVA analysis (Wilks' Lambda).

Effect	F	P – level
Year	8.008	0.001***
Year*grazed use	9.979	0.027*
Year*management unit	1.586	0.145
Year*management unit*grazed use	2.238	0.049*

\*significant at 0.05% level, \*\*\* significant at 0.001% level.

The significant interaction year-grazing use, are produced because in years with minimum average values of cover the differences between the marginal average of the grazing areas and the non grazing areas have increased (Figure 1).

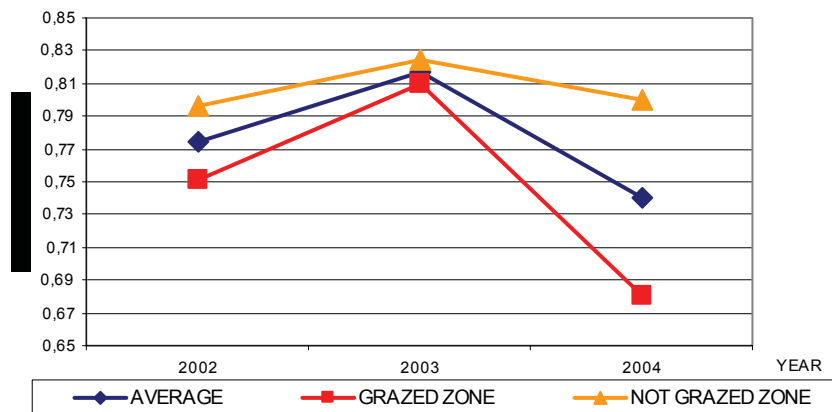


Figure 1. Total average, grazed and no grazed zone within years 2002 and 2004.

In periods of maximum cover the differences are very little. So we can say that it is not only the farmer who adjusts the productivity circle to the annual evolution of productivity (Bermejo, 2003) but in a long term approach, the stocking rate adjusts to the minimum values of productivity of each of the units. There is an adaptation of the grazing use to the variation year-to-year of the productivity that generates two extreme situations: i) remaining the year of high productivity and ii) significant differences in the vegetation cover in dry years.

Although the management can be considered as correct due to the adaptation to the natural dynamic of the ecosystems, it is necessary to determine the magnitude of the differences of vegetation cover that each unit can afford, so that the level of usage of the forage biomass during these years can be sustainable and it does not generate long lasting differences in productivity between the areas used for grazing and the areas not used for this purpose. This idea is decisive because once a limit is oversized erosive processes may begin due to the loss of vegetation cover. (Abel, 1993; Aitken *et al.*, 2002). The most important effect at the interaction year\*management unit\* grazing use (p-level: 0.049\*) shows a different behaviour of the management units within the area that leads to a replica of the temporal effect (effects within a year).

So the location and temporary bases of the grazing management within the area are based on the principle of adjustment to the minimum productivity periods. This has two immediate consequences: on one hand the loss of efficiency of both, the relationship of stocking rate and carrying capacity and also of the methodology of the use factor for the rational planning of the grazing usage of the area (Blench, 2001; Agreil *et al.*, 2002; Nösberger and Staszewski, 2002) in a land with a high variability of climate and lands. On the other hand is necessary to concentrate the research on the long term consequences of this type of results concerning the productivity potential and the erosion processes in grazing areas.

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# Relationship between *in vivo* digestibility and *in situ* degradability of grass silage

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## Abstract

In 1997 and 1998 silage from a pasture composed by *Lolium perenne* L. and *Trifolium pratense* L. was harvested at different maturity stages [jointing (26th March), start of spiking (10 April and spiking 2 April)] preserved with 3.5 L of formic acid  $t^{-1}$  of fresh pasture and ensiled in clamp silos. Silages were offered to three non lactating cows placed in a metabolic chamber for *in vivo* apparent digestibility of dry matter (DMD), organic matter (OMD), neutral detergent fiber (DNDF), nitrogen (DN) and effective *in situ* degradability (DMd, OMd, NDFd y Nd) following a latin square design. *In situ* degradability was a good predictor of *in vivo* digestibility.

Keywords: silage, maturity, cow dairy, prediction.

## Introduction

Biological methods are used as a tool to simulate animal digestibility and digestion processes. The most widely used biological methods are: (i) *in vivo*; (ii) *in vitro* (Tilley and Terry, 1963); (iii) *enzymatics* (Riveros and Argamentería, 1987); (iv) *in situ* (Meherz and Orskov, 1977); and (v) *in vitro gas production* (Menke and Steingass, 1988). *In vivo* methodologies are expensive as they require large amounts of feed and specialised. On the other hand, *in situ* methodologies require surgically manipulated animals, but a lower amount of feed.

The aim of this work was to estimate digestibility of silages harvested at different stages of maturity and measured using *in vitro*, *in vivo* and *in situ* techniques.

## Materials and methods

Silage from *Lolium perenne* and *Trifolium pratense* was prepared as clamp silage at different maturity stages. Following mowing, the herbage was pre-dried for 24 h, chopped to a length of 10 to 12 cm and preserved with 3.5 L of formic acid  $tonne^{-1}$  of fresh herbage. Days of harvest for each year and maturity stage were: 26 March (jointing); 10 April (start of spiking) and 25 April (spiking). Three multiparous non-lactating Friesian cows (average body weight  $608 \pm 23$  kg, two of them ruminally fistulated) were used in each nutritional balance. Cows were individually fed with the different silages together with 150 g sodium bicarbonate, without concentrate and allowing for water and mineral salts during the whole period.

Animals were placed in a metabolic chamber for individual ingestion control and collection of faeces and urine during three periods of 25 days (10 days adaptation and 15 days sampling) for each silage and year, following a 3x3 latin square design: 3 cows x 3 silages. Degradability was estimated using the *in situ* technique (Ørskov and McDonald, 1979), with two replicates per silage, year and cow, using an outflow rate of  $k = 0.06 h^{-1}$ .

## Results and discussion

Chemical composition of different silages is shown in Table 1. The highest dry matter content (DM) in 1998 is due to optimal conditions for the initial drying. Crude protein (CP) concentration decreased by  $1.35 g kg^{-1}$  and *in vitro* digestibility ( $De_v$ ) by  $4.97 g kg^{-1}$  DM, while there was an increase of  $2.38 g kg^{-1}$  DM for neutral detergent fibre (NDF) and  $1.35 g kg^{-1}$  (DM) a day for each 1 day delay in harvest date. CP and  $De_v$  values are similar to those obtained by Rinne *et al.* (2002)

Maturity decreased crude protein and increased neutral detergent fiber (NDF) ( $P < 0.001$ ) by  $-0.129$  and  $0.228$  percentage units from 28 March (first day of consumption). Fermentation characteristics (pH and  $N-NH_3$ ) are considered adequate for these silages.

Table 1. Chemical composition of silages.

	Year 1997			Year 1998			Significance		
	Jointing	Star of spiking	Spiking	Jointing	Star of spiking	Spiking	Std	Silage	Year
DM*	196.0	218.0	248.0	201.0	234.0	276.0	1.18	***	***
Ash*	95.7	94.3	91.9	100.5	96.5	94.0	0.17	***	***
CP*	170.4	129.4	123.1	182.4	147.4	135.8	1.22	***	***
NDF*	540.3	578.6	638.0	527.0	575.0	632.0	1.67	***	***
pH	4.32	4.09	3.96	4.1	3.98	3.9	0.05	***	***
N-NH <sub>3</sub> /N	128.4	106.3	81.9	146.7	125.1	98.1	0.73	***	***
DMd	707	647	521	701	662	549	2.06	***	***
OMd	695	597	480	692	674	487	18.62	NS	***
NDFd	465	385	305	478	395	323	2.21	***	***
Nd	830	759	538	809	733	532	2.64	***	***

DM: Dry matter; CP: crude protein; NDF: neutral detergent fibre; \* Values expressed as  $g\ kg^{-1}$  DM; DMd: Effective degradability of dry matter; OMd: Effective degradability of organic matter; NDFd: Effective degradability of neutral detergent fibre; Nd: Effective degradability of N; \*\*\* ( $P < 0.001$ ); \*\* ( $P < 0.01$ ); NS: non significant.

*In vivo* digestibility of dry matter (DMD), organic matter (OMD), neutral detergent fibre (NDFD) and N (ND) decreased as maturity increased ( $P < 0.001$ ) (Table 2). Thus, DMD, OMD, NDFD and ND decreased  $0.54$ ;  $0.73$ ;  $0.47$  and  $0.54$  percentage units with respect to ensiling at jointing. A similar decrease was observed on *in situ* degradability with average values of  $-0.56$ ;  $-0.70$ ;  $-0.52$  and  $0.94$  for DMD, OMD, NDFD and ND respectively. The fact that the slope of ND was different to Nd, may be explained by the overestimation of soluble N coming from non-protein nitrogen by the effective degradability assay. The previously commented slopes are within the range observed using the *in vitro* digestibility of the organic matter ( $-0.50$ ) from jointing to spiking.

Table 2. *In vivo* digestibility and *in situ* degradability of the different silages.

	Jointing	Star of spiking	Spiking	Std	Silage	Year
DMD*	787	670	625	7.4	***	NS
OMD*	807	705	588	9.6	***	***
ND*	718	623	552	7.8	***	***
NDFD*	786	689	639	7.4	***	***
DMd*	704	654	535	14.9	***	***
NDFd*	472	390	314	13.6	***	***
OMd*	614	635	483	22.3	***	NS
Nd*	820	746	535	25.2	***	***

\* Values expressed as  $g\ kg^{-1}$  DM; \*\*\* ( $P < 0.001$ ); \*\* ( $P < 0.01$ ); NS: non significant.

Table 3 indicates the relationships between *in vivo* digestibility of DM, OM, NDF and N with respect to effective degradability (DMd, OMd, NDFd and Nd), soluble fractions of the degradation kinetics (aDM, aOM, aCP) and slowly degradable fractions (bNDF). More than 65% of the variation in *in vivo*

digestibility of the parameters considered are explained by the *in situ* technique. Although the soluble fraction of dry matter (aDM), organic matter (aOM) and crude protein (aCP) explain only 45%, 67% and 78% of the variation; no relationship was observed with the soluble fraction of neutral detergent fibre.

Table 3. *In vivo* digestibility estimation from *in situ* technique degradability.

Independent variable	Y = a + bx	R <sup>2</sup>	se	n
DMD	Y = 48.8 + 0.669 aDM	0.45	5.32	24
	Y = 22.94 + 460.3 cDM	0.67	4.10	24
	Y = 16.34 + 0.84 DMd	0.76	3.50	24
NDFD	Y = 19.9 + 0.66 bNDF	0.78	3.44	24
	Y = 21.79 + 914.7 cNDF	0.48	5.29	24
	Y = 36.42 + 0.87 NDFd	0.65	4.38	24
OMD	Y = 55.36 + 0.68 aOM	0.67	5.39	24
	Y = 140.28 - 1.14 bOM	0.70	5.13	24
	Y = 31.57 + 352.1 cOM	0.91	2.82	24
	Y = 28.85 + 0.68 OMd	0.65	5.65	24
ND	Y = 39.92 + 0.50 aCP	0.78	3.54	24
	Y = 46.68 + 106.5 cCP	0.75	3.76	24
	Y = 26.79 + 0.519 Nd	0.75	3.78	24

aDM; aNDF; aOM; aCP: soluble fraction; bDM; bNDF; bMO; bCP: slowly degradable fractions; cDM; cNDF; cOM; cPB: degradation rate (h<sup>-1</sup>).

### Conclusions

The *in situ* digestibility can be used as a tool to estimate *in vivo* digestibility of herbage silages produced under Atlantic climatic conditions.

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## Grass silage intake estimation *in vivo* and *in situ* techniques

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### Abstract

In 1997 and 1998 silage from a pasture composed by *Lolium perenne* L. and *Trifolium pratense* L. was harvested at different maturity stages [jointing 26th March, star spiking 10 April and spiking 2 April], preserved with 3.5 l of formic acid tonne<sup>-1</sup> of fresh pasture and ensiled in clamp silos. Silages were offered to three non lactating cows placed in a metabolic chamber for estimation of *in vivo* apparent digestibility of dry matter (DMD), organic matter (OMD), neutral detergent fibre (NDFD), nitrogen (ND) and effective *in situ* degradability (DMd, Omd, NDFd and Nd) following a latin square design. As a consequence of a delay in harvest, dry matter intake decreased by 0.9 g DM kg<sup>-1</sup>W<sup>0.75</sup> and N intake by 5.63 g d<sup>-1</sup>. *In situ* degradability resulted as a more consistent method than *in vivo* technique to estimate N losses in feces and urine and opposite to ingestion of dry matter.

Keywords: grass silage, dry cow, intake, N excretion, prediction.

### Introduction

Grass silages are important constituents of dairy cattle rations. The nutritional value of forages is a function of nutrient content and voluntary consumption (Meissner *et al.*, 1989). Digestibility and intake are major indices of the nutritive value.

The most important benefit of *in situ* evaluation of digestibility is that it evaluates the digestibility of feeds taking into account diurnal variation in rumen pH and other potential effects related to energetic and protein supplementation. *In situ* digestibility uses data in a kinetic model, resulting in a good proximity to *in vivo* digestion processes. Nevertheless, results obtained by the *in situ* technique show a greater variability than those determined by the *in vitro* technique (Siciliano-Jones, 2002).

The aim of the present work is to evaluate if *in vivo* digestibility and *in situ* degradability values can be used to estimate dry matter consumption and N excretion in the faeces and urine of dairy cattle fed grass silages of different digestibility.

### Materials and methods

*Lolium perenne* and *Trifolium pratense* was preserved as clamp silage at 15 day intervals. Following harvesting, forage that had been wilted for 24 hours, was chopped to an approximate length of 10-12 cm and preserved with 3.5 L formic acid per 1000 kg of fresh forage. Harvest days for each year were 26 March (at jointing stage), 10 April (start of spiking) and 25 April (spiking). Three multiparous Friesian non lactating cows (2 of them with fistulated rumen) were used in each nutritional balance. Average weight was 608±23 kg, with silages corresponding to the previously mentioned harvest days and offered with 150 g of sodium bicarbonate, with ad libitum supply of water and mineral salts.

Animals were placed in metabolic chambers to permit the individual measurement of intake and the separate collection of faeces and urine during three 25-day periods per silage and year, following a latin square 3x3 design: 3 cows x 3 silages. Each period consists of 10 days adaptation and 15 days experimental.

### Results and discussion

Ingestion and consumption prediction: Dry matter ingestion and nitrogen balance are shown in Table 1. The delay in the harvest day decreased silage consumption (P<0.001), with a 0.90 g DM d<sup>-1</sup> decrease from 26 March (jointing) to 25 April (spiking); which means -12.2 g DM per 10 g kg<sup>-1</sup> decrease in *in*

*in vivo* organic matter digestibility, which is similar to values observed by Rinne *et al.* (2002), who reported a decrease of 14.5 g kg<sup>-1</sup>.

Table 1. Dry matter intake and nitrogen balance.

	Joining	Start Spiking	Spiking	Std	Silage	Year	Interaction
g DM kg <sup>-1</sup> W <sup>0.75</sup>	101.6	91.76	74.37	0.74	***	***	***
Ingested N (g d <sup>-1</sup> )	358.4	246.4	189.02	7.49	***	***	NS
Faecal N (g d <sup>-1</sup> )	100.8	92.7	83.7	0.92	***	***	NS
Urine N (g d <sup>-1</sup> )	107.8	92.7	87.9	0.89	***	***	NS
Excreted N (g d <sup>-1</sup> )	179.6	155.1	143.2	1.56	***	***	NS
Fixed N (g d <sup>-1</sup> )	148.4	58.8	19.7	3.24	***	***	***

\*\*\* (P<0.001); NS: p<0.05.

The relationship between dry matter consumption (expressed as g kg<sup>-1</sup> W<sup>0.75</sup>) and *in situ* degradability is shown in Figure 1. The prediction of dry matter ingestion by different variables is found in Table 2, showing that *in situ* degradability, DMd, NDFd and Nd can be used for estimation. In contrast, *in vivo* digestibility, DMD and DNDF showed a lower correlation coefficient.

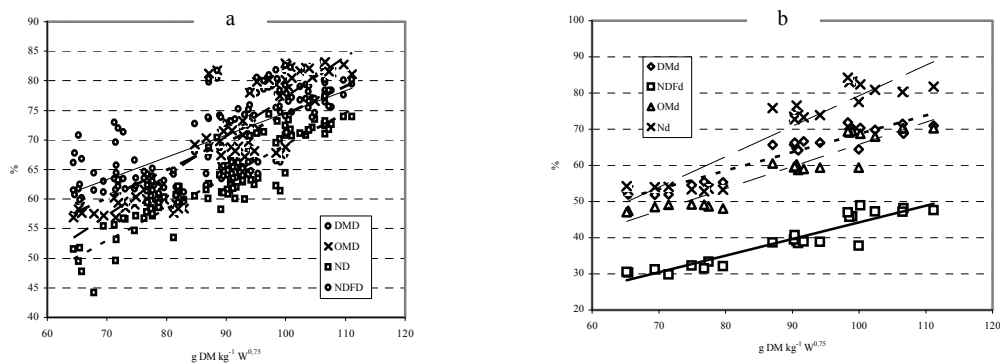


Figure 1. Relationship between dry matter consumption (g DM kg<sup>-1</sup> W<sup>0.75</sup>) and *in vivo* digestibility (a) and *in situ* degradability (b).

Table 2. Prediction of dry matter ingestion by *in situ* degradability and *in vivo* digestibility.

Independent variable	Y = a + bx	R <sup>2</sup>	Se	N
<i>In situ</i> Degradability				
g DM kg <sup>-1</sup> W <sup>0.75</sup>	Y = -21.74 + 1.75 DMd	0.89	4.45	24
g DM kg <sup>-1</sup> W <sup>0.75</sup>	Y = 30.32 + 0.97 OMd	0.61	8.63	24
g DM kg <sup>-1</sup> W <sup>0.75</sup>	Y = 14.53 + 1.90 NDFd	0.87	4.95	24
g DM kg <sup>-1</sup> W <sup>0.75</sup>	Y = 17.99 + 1.01 CPd	0.85	5.26	24
<i>In vivo</i> Digestibility				
g DM kg <sup>-1</sup> W <sup>0.75</sup>	Y = -11.41 + 1.45 DMD	0.68	6.97	24
g DM kg <sup>-1</sup> W <sup>0.75</sup>	Y = 3.54 + 1.22 OMD	0.82	5.28	24
g DM kg <sup>-1</sup> W <sup>0.75</sup>	Y = 8.94 + 1.14 NDFD	0.42	9.41	24
g DM kg <sup>-1</sup> W <sup>0.75</sup>	Y = -4.95 + 1.48 ND	0.80	5.47	24

N excretion and its estimation: Overall, delaying harvesting reduced N ingestion by about 5.63 g d<sup>-1</sup>; the decline was larger between the start of spiking and jointing (7.46 g d<sup>-1</sup>), due to the reduced crude protein content of silage (r<sup>2</sup>=0.75 P<0.001). With regard to total N excretion (faeces + urine), crude protein content in silage was the parameter that was best related to excreted total N (g d<sup>-1</sup>) = 108.09 + 0.197 ingested N (g d<sup>-1</sup>); r<sup>2</sup>=0.88 ±5.12. Nevertheless, the *in vivo* methodology explains more of the variation in excreted N than did the *in situ* methodology (Table 3).

Tabla 3. Prediction of excretion of the N (faeces + urine) to the degradability *in situ* and digestibility *in vivo*.

Independent variable	Y = a + bx	R <sup>2</sup>	Se	N
<i>In situ</i> Degradability				
g N d <sup>-1</sup>	Y = 35.81 + 1.95 DMd	0.76	8.14	24
g N d <sup>-1</sup>	Y = 92.14 + 1.11 OMd	0.55	11.25	24
g N d <sup>-1</sup>	Y = 67.72 + 2.35 NDFd	0.90	5.21	24
g N d <sup>-1</sup>	Y = 83.37 + 1.08 CPd	0.67	9.63	24
<i>In vivo</i> Digestibility				
g N d <sup>-1</sup>	Y = 23.66 + 1.97 DMD	0.87	5.30	24
g N d <sup>-1</sup>	Y = 53.67 + 1.52 OMD	0.87	5.23	24
g N d <sup>-1</sup>	Y = 48.31 + 1.58 NDFD	0.57	9.8	24
g N d <sup>-1</sup>	Y = 44 + 1.83 ND	0.85	5.77	24

### Conclusions

*In situ* degradability can be used as a consistent method to estimate dry matter ingestion in grass silages. In contrast, N excretion (faeces + urine) is more consistent than the *in situ* methodology.

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## Size – forage biomass relationships of four shrub species from the Anaga Rural Park (Tenerife, Canary Islands)

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### Abstract

Twenty two log-log regression equations between forage biomass and size were determined in four species from semiarid shrublands of the Anaga Rural Park in Tenerife (Canary Islands). All the models show good fit, homoscedasticity and predictability. The shrub species were: *Erica arborea* L., *Globularia salicina* Lam., *Rumex lunaria* L. and *Teline microphyla* (DC.) PE.Gibbs & Dingwall. The equations obtained would permit a determination of the appropriate goat carrying capacity for sustainable management of subtropical and semiarid rangelands in the Anaga Rural Park.

Keywords: Canary Islands, forage biomass, goats.

### Introduction

Anaga Rural Park is a semiarid area in north-western Tenerife (Canary Islands) which has one of the best preserved cardonal-tabaibal sub-tropical shrub formations. Nevertheless, the conservation of this natural resource is at risk, especially due to the rapid increase in local population and tourism in the last thirty years. Livestock activities, including goat farming in particular, have not been a problem since the Anaga Rural Park was regulated by administrative laws to preserve rural development and local culture. Due to this conservation effort over the past years, the intensity of livestock farming has been constant and in equilibrium with the environment (Mata *et al.*, 2002). Different studies have shown the advantages and the problems of goat farming in sub-tropical areas of the Canary Islands, especially the importance of carrying capacity (Bermudo, 2002). For appropriate management, the first step is the determination of forage biomass production of the different species used by range animals (Holecheck *et al.*, 1995). In this paper we show different predictive equations based on log-log regressions for the determination of productivity in forage of four species of shrubs: *Erica arborea* L., *Globularia salicina* Lam., *Rumex lunaria* L. and *Teline microphyla* (DC.) PE.Gibbs & Dingwall at the bioclimatic level of fayal-brezal inside Anaga Rural Park (Tenerife, Canary Islands) (Mata *et al.*, 2002).

### Materials and Methods

Anaga Rural Park is located in the north of Tenerife Island (Figure 1) and covers endangered and protected forests of laurisilva, fayal-brezal and cardonal-tabaibal formations. The rough topography inside the Park, expressed by variations in altitude of more than 700 m, causes considerable microclimate differences in rainfall that produce a very complex mosaic of vegetation. In fact, previous research had shown nine agroecological units in Anaga Rural Park (Mata *et al.*, 2002). The four shrub species analysed in this paper (*Erica arborea* L., *Globularia salicina* Lam., *Rumex lunaria* L. and *Teline microphyla* (DC.) PE.Gibbs & Dingwall) are distributed in the Thermo (cardonal-tabaibal) and Meso (fayal-brezal and laurisilva) Mediterranean bioclimatic levels (Rivas *et al.*, 1993). Average temperatures oscillate between 15-17° C and rainfall between 800-1000 mm. Three different areas protected from goats were selected for forage biomass determination. Inside each area, randomly stratified (Cochran, 1993) sampling was carried out in order to cover the wide range in plant size. Measured variables were: maximum height (H), maximum diameter (D<sub>1</sub>), minimum diameter (D<sub>2</sub>), canopy area (A), canopy volume (V) and dry matter (DM) of forage biomass. A and V were carried out according to the following equations:

$$A = \pi 0,785 * (D_1 * D_2) \quad (\text{Eqn. 1})$$

$$V = A * H \quad (\text{Eqn. 2})$$

The forage biomass of each plant was stored in paper bags and desiccated in an air-forced electric oven to 65°C for 72 h. The bags were weighed on electronic scales with a level of accuracy of ±0.001g. Log-log regression models were applied to each species (Patón *et al.*, 2002).



Figure 1. Location of Anaga Rural Park (in green) in Tenerife island.

## Results and Discussion

The broad range of measurements within each of the variables for all four species (Table 1) enables an effective test of log-log regressions (Table 2). All the models have good predictive values, homoscedasticity and normality of residuals and therefore are perfectly applicable to the estimation of biomass production. This can aid in sustainable goat range management in Anaga Rural Park (Tenerife, Canary Islands). Using the proposed models together with nutritional information of the shrub species, we can calculate the Metabolizable Energy (ME) per hectare in these ecosystems. This information is the key for carrying capacity determination of shrublands in Anaga Rural Park

Table 1. Range of variables for the four shrub species.

Species	DM (g)	H (cm)	D <sub>1</sub> (cm)	D <sub>2</sub> (cm)	A (dm <sup>2</sup> )	V (m <sup>3</sup> )
<i>Erica arborea</i>	18.7 - 640.1	72 - 270	46 - 250	29 - 237	12 - 464	0.08 - 10.6
<i>Globularia salicina</i>	6.3 - 1433.8	25 - 256	24 - 270	22 - 228	4 - 484	0.01 - 12.09
<i>Rumex lunaria</i>	1.7 - 940.6	15 - 215	12 - 270	12 - 190	1 - 397	0.002 - 8.33
<i>Teline microphylla</i>	2.3 - 813.2	35 - 188	35 - 170	30 - 150	9 - 194	0.03 - 3.64

Table 2. Log-log regression models and major statistical tests:  $R^2$ , ANOVA F-test, Shapiro-Wilk normality test of residuals and Breusch-Pagan test for homoscedasticity. \*\*\*: p-value < 0.001, ns: non-significant differences.

Species	n	Equations	$R^2$	F-test	Shapiro-Wilk	BP
<i>Erica arborea</i>	34	$\log(\text{DM}) = 1.024 \cdot \log(D_1)$	0.983	1306.4 ***	0.969 ns	0.0015 ns
<i>Globularia salicina</i>	39	$\log(\text{DM}) = 1.279 \cdot \log(A)$	0.993	3710.0 ***	0.968 ns	0.0072 ns
<i>Rumex lunaria</i>	29	$\log(\text{DM}) = 0.719 \cdot \log(V)$	0.978	825.3 ***	0.966 ns	0.0155 ns
<i>Teline microphylla</i>	37	$\log(\text{DM}) = 0.757 \cdot \log(V)$	0.978	1184.0 ***	0.925 ns	0.2614 ns

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# A parallel genetic algorithm for the prediction of metabolizable energy of grasslands

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## Abstract

Metabolizable energy (ME) is a basic parameter in the analysis of the carrying capacity of *dehesas*; however, determination of ME involves considerable time and expense. We developed a model to estimate ME based on parallel genetic algorithm (PGA) using data from the Spanish scientific literature. The resulting PGA model to determine ME was based on two parameters: neutral detergent fiber (NDF) and acid detergent fiber (ADF). Observed and predicted ME showed a good correlation ( $r=0.928$ ,  $p < 0.001$ ). This mathematical model can be used to estimate ME of grasslands more rapidly and inexpensively compared with laboratory estimates.

Keywords: metabolizable energy, grasslands, genetic algorithms, *dehesa*.

## Introduction

Metabolizable energy (ME) is a basic parameter in the analysis of the carrying capacity of grassland ecosystems. The determination of ME, however, is costly in time and money in studies that generate a large number of samples. Recent research demonstrates that it is possible to find good predictive models for wide geographical areas with a high level of accuracy based on non-linear regressions (Paton, 2003). In this paper we developed a parallel genetic algorithm (PGA) model for the determination of ME in Mediterranean and Atlantic grasslands of the Iberian Peninsula. The model is based on data from the Spanish scientific literature published since 1976. The dataset included 220 samples of green forage and wild grasses collected in 15 localities (Figure 1), in different seasons, under very different environmental conditions (Atlantic, Mediterranean, coastal, mountainous, semiarid, arid and continental climate).



Figure 1. Geographic areas of the dataset used in the genetic algorithm model.

## Material and methods

An extensive search of scientific literature (e.g., Internet websites, congresses, proceedings and peer-reviewed journals) was carried out to obtain data on the nutritional value of grasslands. Sixty-four documents with relevant information on neutral detergent fiber (NDF), acid detergent fiber (ADF), crude protein (CP) and ME based on *in vitro* organic matter digestibility (IVOMD) analysis were selected. Data include nutritional information from different geographic areas, climates, altitudes, types of soils, management conditions, botanical composition and seasons. A summary of parameters used in the model is shown in Table 1.

Table 1. Summary of parameters used in the model. CP: crude protein, NDF: neutral detergent fiber, ADF: acid detergent fiber, ME: metabolizable energy.

Parameter	Range	Average $\pm$ standard deviation
CP	2.80 – 28.90	9.60 $\pm$ 5.00
NDF	30.10 – 82.60	63.13 $\pm$ 11.37
ADF	15.10 – 56.13	37.25 $\pm$ 6.60
ME	5.13 – 13.99	8.18 $\pm$ 1.90

The best two correlated (based on Spearman's non-parametric test) chemical parameters (transformation or combination) were selected as independent terms in the PGA model. After the elimination of less than 10% of observations as outliers, the PGA was recalculated. The variability of estimates (ANOVA test), normality of residues (Shapiro-Wilk's test), heteroskedasticity (Breusch-Pagan's test) (Jobson, 1991), residuals skewness and kurtosis were determined. Residuals differences from zero were analyzed by means of Student's t-test. The PGA model was validated by a Chow test using two random 50% data samples tested against the whole dataset (Jobson, 1991). Estimated and observed ME was analyzed by Spearman's correlation index. All the statistical analyses were calculated with an R (R Development Core Team, 2003) and XLDLAS (Sigvaldason, 1997) program in a UNIX (FreeBSD 5.4) workstation.

## Results and Discussion

The variables ADF ( $r=-0.926$ ,  $p < 0.001$ ) and ADF\*NDF ( $r=-0.882$ ,  $p < 0.001$ ) were most highly correlated with ME. The equation obtained, after the elimination of 8.89% of the observations as outliers, was:

$$ME = 97.680 - 51.087*ADF^{0.0635} - 11.917*(NDF*ADF)^{0.100}$$

This model shows a good correlation between observed and predicted ME ( $r=0.928$ ;  $p < 0.001$ ). The residuals were not different from zero ( $t=-1.142$ ;  $p=0.254$ ) and were normally distributed ( $W=0.972$ ;  $p > 0.05$ ). Residual skewness ( $s=0.275$ ) and kurtosis ( $k=1.017$ ) were in the interval  $[-1.96, +1.96]$  (Jobson, 1991). The Chow-test showed non-significant differences of each 50% of the data as regards the global model ( $cw=1.05$ ,  $p > 0.05$ ). The results indicate that PGA increases the fit of traditional predictive models based on linear regression, for most grasslands in the Iberian Peninsula.

## Acknowledgements

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## A biomass prediction model for grasslands of Canary Islands

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### Abstract

Between 2001 and 2004, 220 modified (30 m) point quadrats were carried out in five protected areas in the Canary Islands. Contacts (phytovolume) of each plant species and three biomass (DM) samples were recorded in each transect. In each transect, average biomass productivity was related to total contacts in order to decrease the sampling time. This economy in sampling would permit us more complex studies at the regional scale. DM-contacts relationship was established using a Parallel Genetic Algorithms (PGA) procedure. PGA is a part of Artificial Intelligence that uses an iterative procedure of analyses, usually with better results than non linear regressions. The PGA model shows a reasonably good fit ( $r=0.71$ ,  $p<0.001$ ), but results suggest that an increase in the number of transects and a reduction in transect length (1m) would provide a better fit for biomass analysis.

Keywords: parallel genetic algorithms, grasslands, productivity.

### Introduction

Since 1997 many different studies on the environmental impact of extensive goat farming in Canary Islands have been reported (Mata *et al.*, 2000 a,b; Mata and Bermejo, 2000). Actually, nine evaluations in different protected areas of 4 islands (La Gomera, Tenerife, La Palma and El Hierro) are in development. The evaluations cover 50,000 ha with distinct orographic, climatic and botanic diversity. One of the main aspects in these studies is the determination of biomass (DM) of natural grasslands. Therefore, it is necessary to optimise sampling methods that are related to biomass analysis. Previous studies develop by Buyolo *et al.* (2004) in the Mediterranean grasslands of the Extremadura region (Southwestern Spain) showed a high correlation between observed and predicted biomass ( $r=0.998$ ) using Parallel Genetic Algorithms (PGA) (a part of Artificial Intelligence) when a large number of quadrats were taken ( $n=396$ ). In this paper we discuss the possibility of this method being applied to the natural grasslands of Canary Islands for range management purposes.

### Material and Methods

220 Point-Quadrats of 20 m (transects based in contacts) were realized in five protected areas of Tenerife (Anaga and Teno Rural Parks) and La Gomera (Valle Gran Rey Rural Park, Lomo del Carretón Natural Monument and Majona Natural Park) between 2000 and 2004. In each Point-Quadrat (Daget and Poissonet, 1971) three random biomass samples were recorded constituting a sampling area of 1m<sup>2</sup>. A PGA model was realized using the average biomass (of three random samples) as the dependent variable and the total contacts of each Point-Quadrat as the independent term. After 156 initial tests with different combinations of parameters following the methodology of Spears (1998), PGA was fitted with 3,000 iterations, a mutation and perturbation rate of 10<sup>-3</sup> and a crossover rate of 0.5. After a first analysis the 10% of outliers were suppressed and a second and definitive analysis was realized. Spearman's correlation between predicted and observed biomass and its statistical differences (Student's t test) were analysed. The validation of the model was determined by a Chow test (Jobson, 1991) comparing two 50% random subsamples taken from the whole data. In all the analyses the programmes R (R Development Core Team, 2003) and XLDLAS (Sigvaldason, 1997) under a UNIX FreeBSD 5.4 workstation were used.

## Results and Discussion

Function finally obtained was:

$$DM = -3.712 + 0.550 * CONTACTS^{0.935}$$

Spearman's correlation was  $r=0.706$  ( $p < 0.001$ ) and differences between observed and predicted DM were not significant ( $t=-0.324$ ;  $p = 0.746$ ). Standardized residuals were normally distributed with skewness (0.373) and kurtosis (-0.231) between  $[-1.96+1.96]$  as a  $N(0,1)$ . The Chow test shows that there were no differences between subsamples ( $cw=5.48 \times 10^{-4}$ ,  $p=1$ ). The results obtained show that this model can not be definitive due to the low correlation index and data. Nevertheless, the tendencies in the results show that it may be possible to obtain a better fit with correlations close to 0.8-0.9 if we doubled the amount of data collected and this would provide a useful model for predictive purposes (Figure 1). Our experience with similar models in other environmental conditions suggests the most probable cause of error was the length of transect and variability between samples in the analysis of biomass. We suggest that transects of 1 m with a single estimation of biomass could provide a solution for a more accurate predictive model of biomass. This is the methodology followed by Buyolo *et al.* (2004) with excellent results for Mediterranean grasslands from Monfragüe Biosphere Reserve. Nevertheless, for botanical composition analysis in Mediterranean and arid rangelands a majority of range managers use larger transects that obviously require two or three biomass estimations. Certain studies demonstrate the relevance of the methodological studies to adapt sampling techniques to local characteristics of species composition and distribution of grasslands (Papanastasis, 1977). Future research combining different types of transects and genetic algorithms for data analysis of biomass-contacts relationship, would provide a best useful methods for range management purposes in Mediterranean and subtropical grasslands.

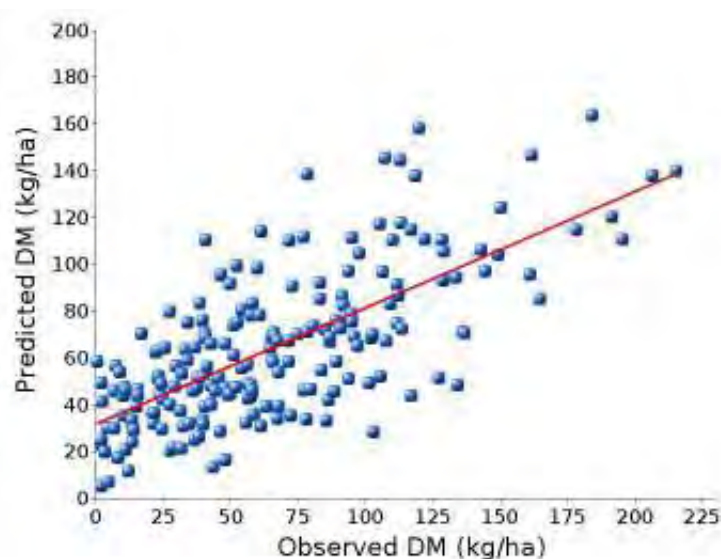


Figure 1. Relationships between predicted and observed dry-matter (DM) using a Parallel Genetic Algorithm (PGA) model for Canary Islands grasslands.

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# Effect of sward height and ploidy of perennial ryegrass on N-surplus at grazing with dairy cows

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## Abstract

With the aim to reduce N-surplus on pasture a grazing trial with 64 dairy cows was carried out in two 30 days periods in 2004. In the rest of the season the sward was cut for silage. In a 2x2 factorial design, the cows grazed paddocks with two different compressed sward heights, 6 and 10 cm, and two different grass types, diploid and tetraploid perennial ryegrass (*Lolium perenne* L.), both mixed with white clover (*Trifolium repens* L.). White clover constituted 23-50 % of herbage dry matter with the highest proportion at low sward height and diploid grass. The cows grazed day-only (7.5 h) and were restrictively fed in the barn. The ploidy level of grass had limited effect on herbage quality, milk production and N-flow. At low sward height the digestibility of organic matter and content of crude protein was higher than at high sward height. This led to a higher amount of N in urine, as the milk yield per cow was unaffected. N-balance (input-output) on field level was slightly lower at low sward height due to a relatively higher increase in N-intake than in N-excretion on pasture. The total annual yield (herbage for silage and milk yield) on field level was highest at low sward height.

Keywords: grazing, dairy cows, sward height, ploidy of ryegrass, N-surplus.

## Introduction

N-surplus is often high on field level under grazing with dairy cows especially due to a high concentration of protein in the herbage in relation to the concentration of degradable carbohydrates. We expected that N-surplus under day-only grazing could be decreased by increasing sward height, using tetraploid perennial ryegrass instead of diploid, decreasing the N content in supplements, and synchronizing rumen carbohydrate and protein degradation. This hypothesis was tested in a trial, and results concerning sward height and ploidy level of grass are presented in this paper.

## Materials and methods

A grazing trial with 64 Danish Holstein Friesian dairy cows was carried out in two 30 d periods during the season 2004, I) May 19-June 17 and II) August 4-September 2, on sandy clay. The experimental design was a 2x2x2x2 factorial design with two treatments on pasture (grass ploidy and sward height (SH)) and two treatments in the barn (urea level and supplementation strategy). On pasture the cows were split in four groups (2 sward heights x 2 ploidy) of 16 cows each. Each group grazed three plot-replicates, meaning that each plot was grazed every third day. The sward was composed of a medium and a late variety of perennial ryegrass within ploidy level and white clover. The ryegrass was either diploid (v. Canasta and Cancan) or tetraploid (v. Calibra and Maurice). The sets of varieties were selected to have the same growth profile. The compressed sward heights were planned to 6 and 10 cm, respectively. The sward height was measured weekly by 50 measurements per plot by a plate raising meter, and available area was adjusted accordingly. The different sward heights at the beginning of the grazing periods were obtained by cuts at different dates before the periods. The season was thus composed of cut 1, grazing period I, cut 2, grazing period II and cut 3. The pasture was irrigated at drought stress. Herbage quality was determined in hand collected samples twice per period. Proportion of clover was determined in samples cut at 2 cm height at the beginning of each period.

Cows were on pasture for 7.5 h pr day, and to minimise variation in intake on barn, all cows were afterwards restrictively fed a mixed ration in the barn, consisting of maize silage (3.2 kg dry matter (DM)), mineral mix (250 g), vitamins (30 g), fodder salt (50 g) and sodium sulphate (50 g). Depending

on urea level, either 0 or 145 g urea was supplied with the mixed ration, and depending on supplementation strategy in the barn, cows were fed either barley (3.6 kg) prior to the grazing and soy hulls (3.6 kg) immediately after grazing or vice versa. All 64 cows were therefore offered the same amount of energy in the barn. Daily energy and N intake on pasture was calculated based on energy demand for maintenance, growth and milk yield (Strudsholm *et al.*, 1999), expected feed utilization (0.87), energy intake in the barn, and N and energy content in herbage. N-excretion in faeces was estimated based on total DM and N intake (Kristensen *et al.*, 1997), and N-excretion in urine was calculated as  $N_{\text{intake}} - (N_{\text{faeces}} + N_{\text{milk}})$ . Deposition on pasture of N from urine and faeces was calculated based on correcting N-excretion in faeces and urine for part of the day spent on pasture (7.5h/24h).

## Results and discussions

Clover content in herbage was relatively high and was in period I highly affected by the treatments with the highest content in diploid-low SH (41% of DM) and lowest in tetraploid-high SH (23% of DM) (Table 1). In period II clover content was higher than in period I and not significantly affected by ploidy, but as in period I, affected by SH. In general the herbage quality was highly affected by the sward height and not by the ploidy of grass. In period I the average sward height in high SH was higher than planned due to a high growth rate and increasing amount of rejected grass during the period. The proportion of grass stem was therefore high, which gave a relatively high content of water soluble carbohydrates (WSC). Surprisingly, content of WSC was similar for diploid and tetraploid swards in both periods. At low SH the concentration of neutral detergent fibre (NDF) was lower, and in vitro digestibility of organic matter (IVOMD) and the concentration of crude protein (CP) was higher than at high SH (Table 1). Grazing intensity (cows per ha) was highest at high SH in period I and at low SH in period II, respectively (Table 2). The initial sward heights were obtained by different harvest times and the grazing area was regulated in accordance with the sward height. Due to very different growing conditions in the two periods caused by the actual climate this affected the grazing intensity differently. It was expected that the intake under grazing would be higher at high SH because of the restricted barn feeding. However, the milk yield per cow was nearly unaffected by the treatments, and on average the cows during grazing ate 104 and 78 MJ NE d<sup>-1</sup> in period I and II, respectively. The cows were on pasture 7.5 h and cows on low SH spent 73% and 66% of the time grazing in period I and II, and cows on high SH spent 68% and 69% of the time grazing in period I and II (data not shown). Thus herbage allowance at low SH was not a limiting factor for the intake. The higher content of crude protein in both grass and clover at low SH (Table 1) led to a higher N-intake under grazing and by that a higher N-excretion in urine, as the milk protein yield was not affected. On average N-excretion in urine was 0.13 kg N ha<sup>-1</sup> d<sup>-1</sup> higher at low SH than at high SH (data not shown).

In the grazing periods N-output from pasture was N-intake under grazing, and N-input was N-excretion. N-input from N-fixation was not estimated, but it has been substantial because of the high clover content in herbage. In general N-balance was negative, which means that the amount of N removed by grazing was higher than N deposited on the pasture. In period I the N-intake was nearly the same for the two sward heights. The higher herbage intake at high SH was counterbalanced by the higher content of N in the herbage at low SH. This resulted in nearly the same N-balance. In period II N-intake was considerable higher at low SH because the growing conditions favoured the growth in that treatment, and that resulted in a better N-balance. Thus the N-balance was affected both by the sward height and the growing conditions.

For the area, which was grazed all the time in the two periods, the total annual yield was 5.3 and 2.6 t DM ha<sup>-1</sup> at cutting (cut 1, 2 and 3) and 11.0 and 11.6 t energy corrected milk (ECM) ha<sup>-1</sup> (grazing period I and II) at low and high SH, respectively. The total yield was thus considerably higher at low SH.

Table 1. Sward height, clover content and herbage quality in the two periods at low and high sward height (SH) and diploid and tetraploid perennial ryegrass.

Period I (May 19-June 17)		Sward height cm	Clover content % of DM	Herbage quality			Grass CP % of DM	Clover CP % of DM
				IVOMD % of OM	WSC % of DM	NDF % of DM		
Low SH	Diploid	5.7	41.1	79.5	8.3	25.4	22.6	30.8
	Tetrapl.	5.1	29.0	80.2	10.1	24.2	23.4	30.8
High SH	Diploid	13.0	35.0	77.9	15.2	33.8	13.3	27.1
	Tetrapl.	13.8	23.1	77.4	15.6	32.5	13.2	24.6
Ploidy level		NS	**				NS	NS
Sward height		**	*				***	***
Period II (Aug.4-Sep.2)								
Low SH	Diploid	6.6	49.9	73.9	8.5	29.8	22.1	29.9
	Tetrapl.	6.6	46.8	74.4	8.4	29.0	22.1	29.7
High SH	Diploid	10.0	44.8	71.2	9.1	34.6	18.1	26.5
	Tetrapl.	9.3	39.7	72.9	8.6	33.0	18.3	26.9
Ploidy level		NS	NS				NS	NS
Sward height		***	*				***	***

Table 2. Grazing intensity, milk yield, N-intake, N-excretion and N-balance at field level calculated on daily basis.

Period		Cows ha <sup>-1</sup>		Milk yield		N-intake		N-excretion		N-balance	
		I	II	kg ECM ha <sup>-1</sup> d <sup>-1</sup>		kg N ha <sup>-1</sup> d <sup>-1</sup>		kg N ha <sup>-1</sup>		kg N ha <sup>-1</sup>	
				I	II	I	II	I	II	I	II
Low SH	Diploid	6.8	7.8	183	187	2.60	2.68	0.86	0.95	-1.73	-1.73
	Tetrapl.	6.4	7.9	176	186	2.69	2.57	0.87	0.95	-1.83	-1.63
High SH	Diploid	7.7	7.2	213	166	2.74	2.13	0.86	0.80	-1.88	-1.32
	Tetrapl.	8.1	6.8	224	167	2.79	2.27	0.86	0.82	-1.93	-1.45

N-balance: N-excretion (urine + faeces) on pasture - N-intake under grazing

### Conclusion

Low compared with high sward height led to a higher total annual yield (cutting and grazing), a better herbage quality and a slightly better N-balance on field level. Ploidy of perennial ryegrass had limited effect.

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## **Ruminant livestock characterization on the basis of grazing and forage resources**

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### **Abstract**

A ruminant livestock characterization in Aragon (Spain) is presented, based on grazing resources (cultivated and uncultivated land), animal species (sheep, goat, cattle), animal production types (beef, milk) and stocking rates. This characterization is municipal district-scaled. A Hierarchical Cluster Analysis was carried out on 59 variables (44 of uncultivated pasture resources, 9 of agricultural grassland resources and 6 referred to livestock) and 729 cases (all the municipal districts). With the similarity level used, the Cluster Analysis generated 12 groups (conglomerates). A high diversity of livestock production models based on the grazing and forage resources is found in Aragon.

Keywords: agricultural grasslands, grazed forests, shrub pastures, natural grasslands, Cluster analysis.

### **Introduction**

This work aims to make an information layer over the pasture resources cartography that could describe how livestock is linked to these resources and how exploits them. The authors have recently published the cartography of the agricultural grassland (Maestro *et al.*, 2004) and uncultivated pasture resources (Barrantes *et al.*, 2005).

### **Materials and methods**

A matrix with 59 variables (44 of them are of uncultivated pasture resources, 9 are variables of agricultural grassland resources, and 6 are referred to livestock) and 729 cases (all the municipal districts of Aragon) has been built (Table 1a). The resources variables were calculated from the Maestro *et al.* (2004) and Barrantes *et al.* (2005) works. The livestock data were obtained from the 1T (DGA, 1999). A hierarchical cluster, where the groups were formed according to the method of Ward (1963), was carried out by means of the SPSS 12.0 software.

### **Results and discussion**

With the similarity level used, the cluster generated 12 groups (conglomerates). Due to spatial limitation, results are summarized in Table 1b (where the main vegetation types and the highest and lowest values of the variables are shown in bold type) and Figure 1.

Table 1. (a) Variables used: areas of vegetation types/ Uncultivated area of the municipal district (UA) (in %); Cultivated area (CA)/ Total area of the district (TA) (in %); Agricultural type of the municipal district (qualitative); Stocking rate (heads km<sup>-2</sup> TA). (b) Main characteristics (variables) that explain the differences among groups. The main vegetation types and the highest and lowest values of the variables are shown in bold type.

(a)		Vegetation types of Uncultivated lands										Stocking rate (heads km <sup>-2</sup> TA)					
Grazed forests (dense -d- and open -op-)		Grazed shrublands					Grasslands										
<i>Abies alba</i>		<i>Buxus sempervirens</i>					<i>Lygeum spartum</i> (Ls)										
<i>Betula pendula</i>		<i>Cistus ladanifer</i>					Prepyrenaean xerotrophic grasslands (Xg)										
<i>Fagus sylvatica</i>		<i>Echinospartum horridum</i> (Eh)					Iberian xerotrophic grasslands (Xg)										
<i>Juniperus phoenicia</i> (d, op)		<i>Erinacea anthyllis</i> (Ea)					Pyrenaean alpine grasslands (Ag)										
<i>Juniperus thurifera</i> (Jt) (d, op)		<i>Genista scorpius</i> (Gs)					Iberian alpine grasslands (Ag)										
<i>Pinus halepensis</i> (Ph) (d, op)		Gs+ <i>Thymus sp</i> (GT)															
<i>Pinus nigra</i> (Pn) (d, op)		Gypsophilous shrub (G)															
<i>Pinus pinaster</i> (Pp) (d, op)		Halophilous shrub (H)															
<i>Pinus sylvestris</i> (Ps) (d, op)		<i>Juniperus communis</i> subsp. <i>nana</i>															
<i>Pinus uncinata</i> (Pu) (d, op)		<i>Quercus coccifera</i> (Qc)															
<i>Quercus faginea</i> (Qf) (d, op)		Spiny shrub (Ss)															
<i>Quercus ilex</i> (Qi) (d, op)		<i>Rhododendron ferrugineum</i>															
<i>Quercus pyrenaica</i> (Qp) (d, op)		<i>Rosmarinus officinalis</i> (Ro)															
Riversides (R) (d, op)		Ro + Gs															

(b)		Agricultural type of the district and grassland resources										Stocking rate (heads km <sup>-2</sup> TA)					
		Meadows - Forage crops (f)										Sheep (S)					
		Winter Cereal (c): stubble, fallow										Goats (Gt)					
		Irrigated lands (i): alfalfa, forage maize, sown meadows, etc.										Beef cattle (BC)					
		Olive-Almond (o): herbaceous stratum under canopy										Dairy cattle (DC)					
		oc: mixture of Olive-Almond (o) and Winter Cereal (c)										Total cattle (TC)					
		ic: mixture of Irrigated lands (i) and Winter Cereal (c)										Total Livestock Units (LU)					
		cf: mixture of Winter Cereal (c) and Meadows - Forage crops (f)															
		Vineyards - Sweet fruits (v): very restricted grazing resources															
		vc: mixture of Vineyards - Sweet fruits (v) and Winter Cereal (c)															

Group	Agricultural type				Vegetation types of Uncultivated lands										Stocking rate (heads km <sup>-2</sup> TA)						
	CA/TA (%)		Other		Forest (d)		Forest (op)		Shrublands		Grasslands										
	Main	Other	Main	Other	Main	Other	Main	Other	Main	Other	Main	Other	S	Gt	BC	DC	TC	LU			
1	76.1	ic	c	v	<b>Ph</b>	R	Qi	<b>Qi</b>	Ph	R	<b>GT</b>	Qc	G	<b>Ls</b>	-	<b>90.9</b>	1.2	0.3	0.9	1.2	15.0
2	83.3	i	vc	-	<b>R</b>	Ph	Qi	<b>R</b>	Qi	Ph	<b>GT</b>	H	G	<b>Ls</b>	-	<b>99.2</b>	1.0	<b>2.6</b>	<b>3.8</b>	<b>6.4</b>	<b>21.4</b>
3	65.8	i	ic	vc	<b>Ph</b>	R	Qi	<b>Ph</b>	R	Qi	<b>G</b>	GT	Qc	<b>Ls</b>	-	<b>83.3</b>	0.9	0.7	0.5	1.2	13.8
4	69.4	c	-	-	<b>Ph</b>	Qi	R	<b>Ph</b>	Qi	Jt	<b>GT</b>	G	Qc	<b>Ls</b>	-	62.0	1.0	0.3	0.3	0.6	10.0
5	47.9	c	-	-	<b>Qf</b>	Pn	Ps	<b>Qi</b>	Qf	Ps	<b>Ea</b>	Gs	-	<b>Xg</b>	-	76.1	<b>0.4</b>	0.2	0.2	0.4	11.9
6	64.8	c	-	-	<b>Qi</b>	Qf	Pn	<b>Qi</b>	Qf	Ps	<b>Gs</b>	GT	Ea	<b>Xg</b>	Ls	63.5	<b>0.6</b>	0.2	0.1	0.3	9.8
7	24.1	c	oc	cf	<b>Pn</b>	Qi	Ps	<b>Qi</b>	Qf	Jt	<b>Gs</b>	Qc	Ro	<b>Xg</b>	Ag	45.5	1.5	0.5	0.1	0.6	7.7
8	12.0	f	c	cf	<b>Ps</b>	Pn	Qf	<b>Ps</b>	Qf	Qi	<b>Eh</b>	Gs	Ea	<b>Ag</b>	Xg	45.1	1.6	<b>4.8</b>	0.3	<b>5.1</b>	12.1
9	46.2	o	c	vc	<b>Ph</b>	Pn	-	<b>Ph</b>	-	-	<b>Qc</b>	GT	Ss	<b>Xg</b>	-	<b>30.6</b>	<b>2.6</b>	0.2	<b>0.0</b>	0.2	<b>5.1</b>
10	62.5	v	c	o	<b>Qi</b>	Ph	Pp	<b>Qi</b>	R	Ph	<b>Gs</b>	GT	Ro	<b>Xg</b>	-	48.3	0.9	<b>0.0</b>	0.4	0.4	8.1
11	37.6	c	v	vc	<b>Ph</b>	Qi	Pp	<b>Qi</b>	Ph	-	<b>Ro</b>	Gs	-	-	-	56.2	<b>1.8</b>	<b>0.0</b>	<b>0.1</b>	<b>0.1</b>	8.8
12	53.1	oc	vc	o	<b>Ph</b>	Qi	Ps	<b>Qi</b>	Ph	-	<b>GT</b>	Gs	Ro	-	-	47.8	1.1	0.1	0.2	0.3	7.6

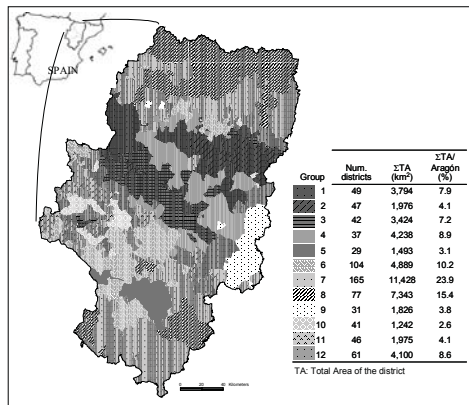


Figure 1. Cartography of the obtained groups. Denser points or diagonal lines and darker colours of the legend are related to higher stocking rates of goat, beef cattle and sheep, respectively. The boundaries among municipal districts are not shown.

### Conclusions

A high diversity of livestock production models based on the grazing and forage resources is found in Aragon (Spain), correlated with its high heterogeneity of geology, topography, climate, soil and landscape. In some cases movements of livestock (transhumance) or forages (i.e. hay or dehydrated alfalfa) occur. On the other hand, it seems clear that the Cluster method employed has proved to be suitable for the aim of this work.

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# Effect of partial neutralization of grass silage on feed intake by lambs

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## Abstract

Effect of partial neutralization of direct cut silage on dry matter (DM) intake was studied with forty-two lambs. The silage offered *ad libitum* was either untreated or partially neutralized with sodium bicarbonate (NaHCO<sub>3</sub>). A low (8 g kg<sup>-1</sup> fresh weight) and high (16 g kg<sup>-1</sup>) level of NaHCO<sub>3</sub> was used. To avoid silage fermentation NaHCO<sub>3</sub> was added to the silage just before feeding. The silages were supplemented either with barley grain or with a mixture of barley grain and commercial protein concentrates. There were distinct differences in the voluntary silage DM intake. The consumption of untreated silage averaged 0.51 kg DM day<sup>-1</sup>. Adding high level NaHCO<sub>3</sub> increased the DM intake more than the addition of low level (108 vs. 40 g, linear effect of NaHCO<sub>3</sub> increment; P=0.02). A protein concentrate had no effect on silage intake (P=0.24). The neutralization with 8 and 16 g NaHCO<sub>3</sub> raised the pH of silage from 3.95 to 4.41 and 5.45, respectively. The amount of sodium gained from NaHCO<sub>3</sub> surpassed more than fivefold the sodium requirement of sheep. Still, no health problems were encountered. Silage neutralized with NaHCO<sub>3</sub> proved to be acceptable to the lambs.

Keywords: direct cut silage, feed intake, forage, lamb, sodium bicarbonate.

## Introduction

In Finland, dry hay is the most common herbage for sheep during winter. Due to the long indoor feeding period (on average 240 days) cost of winter roughage plays a major role. Weather in the early growing season is often changeable, while during late season conditions are already too moist to dry hay outdoors. Drying hay indoors is more expensive than silage production. Since the feed unit price of silage is lower than that of hay, the utilization of silage should be increased in order to reduce feeding costs in meat production. Besides high contents of moisture, short chain fatty acids and lactic acid, low acidity is considered to reduce the intake of ensiled forages (Shaver *et al.*, 1985). Sodium bicarbonate (NaHCO<sub>3</sub>) has been used as an additive in silage-based diets to offset the deleterious effects of low rumen pH. NaHCO<sub>3</sub> may also be beneficial in increasing DM intake when silage is a major component in the diet. However, NaHCO<sub>3</sub> has not improved feed intake in all conditions. Findings have differed depending on animal species and on the amounts added (Farhan and Thomas, 1978). The main purpose of this study was to determine the effect of NaHCO<sub>3</sub> on silage intake in lambs receiving formic-acid silage.

## Materials and methods

The study was carried out at MTT (Agrifood Research Finland) in Jokioinen (60°54'N, 23°30'E, 107 m above sea level). A grass mixture of timothy (*Phleum pratense* L.) and meadow fescue (*Festuca pratensis* Huds.) was harvested on 22 June. Forage was ensiled using an acid based additive (80% formic acid, 2% phosphoric acid) at a rate of 4.0 l t<sup>-1</sup> into a bunker silo. Forty-two Finnsheep lambs, aged 97 days (SD 5.0) with an average initial weight of 28.9 kg (SD 3.1) were allocated according to weight and age into four feeding groups. Individually penned lambs were fed *ad libitum* their respective experimental rations for 84 days. Rations consisted of four treatments as follows: 1. Untreated silage as a control, 2. Silage treated with the low level of NaHCO<sub>3</sub> (8 g kg<sup>-1</sup> fresh weight), 3. Silage treated with the high level of NaHCO<sub>3</sub> (16 g kg<sup>-1</sup> fresh weight) and 4. Silage treated with the high level of NaHCO<sub>3</sub>



(16 g kg<sup>-1</sup> fresh weight) and commercial protein concentrates substituted for 50 % barley grain. NaHCO<sub>3</sub> was mixed with the silage manually immediately prior to feeding. In addition, the lambs were daily fed with 0.5 kg of barley grain (CP 120 g kg<sup>-1</sup> DM), except the lambs in treatment 4 which received 0.25 kg of barley and 0.25 kg of commercial protein concentrates (CP 232 g kg<sup>-1</sup> DM). The lambs had free access to water, salt and minerals.

Conventional feed analysis was performed with the standard method used at MTT's Animal Production Research. Silage DM content was corrected with equations given by Huida *et al.* (1986). *In vivo* digestibility of untreated and treated silages was determined by the total collection method with the lambs. Feed intake was recorded daily and live weight measured at the beginning, at 2-week intervals and at the end of the study. Correlation between observations from the same animal was taken into account by repeated measurement ANOVA model. The rest of variables was measured once and analysed using one-way ANOVA. All analyses were performed using SAS MIXED-procedure. The effect of NaHCO<sub>3</sub> was tested using orthogonal polynomials. The effect of protein supplement was tested by comparing treatments 3 and 4.

## Results and discussion

Untreated silage contained 167, 304, 688, 84 g kg<sup>-1</sup> DM crude protein, crude fibre, digestible organic matter (D-value) and amino acids absorbed in the small intestine (AAT), respectively. The metabolizable energy content was 11.1 MJ kg<sup>-1</sup> DM. As expected, forage organic matter content was at its lowest in the silage neutralized with the high level of NaHCO<sub>3</sub> (891 g kg<sup>-1</sup> DM). Neutralization tended to decrease the digestibility of organic matter. That was 75.7, 74.8 and 73.7 % in the untreated, low and high level NaHCO<sub>3</sub> silage, respectively.

Fermentation quality of all silages was good. The extent of fermentation was indicated by the low pH of 3.95 in the untreated silage. The neutralization of silage with the low level of NaHCO<sub>3</sub> raised the pH to 4.41 and high level to 5.45. Because the NaHCO<sub>3</sub> was added to the silages just before feeding, the silage fermentation was not altered. Ammonium nitrogen averaged 4.4 and 5.2 % of the total nitrogen in the untreated and high level NaHCO<sub>3</sub> silage, respectively. Butyric acid (<0.2 g kg<sup>-1</sup>) was encountered only at the opening of the silo.

In agreement with Phillip (1983), partial neutralization with NaHCO<sub>3</sub> increased the DM intake by lambs (Table 1). The incremental increase was linear (P=0.03) i.e. every gram of NaHCO<sub>3</sub> increased the DM intake by 6.4 g, with the highest increase from feeding silage with the higher level of neutralization. Silage DM intake averaged 36.2, 38.9, 43.1 and 46.1 g kg<sup>-1</sup> W<sup>0.75</sup> in the untreated, low level NaHCO<sub>3</sub>, high level NaHCO<sub>3</sub> and high level NaHCO<sub>3</sub> silage with protein supplement, respectively. The higher addition of NaHCO<sub>3</sub> increased the DM intake by 21 % and the lower by 8 %. These increases are of a similar magnitude to those reported earlier in adult sheep (Sormunen-Cristian, 1992). The effect of NaHCO<sub>3</sub> infused straight into the rumen maybe greater than when fed with the silage. Live weight gains of lambs fed silage treated with a high level of NaHCO<sub>3</sub> tended to be greater than those in other treatments. However, the differences in live weights during the entire experiment were not statistically significant (Table 1).

The lambs received 5, 11 and 12 g of sodium from NaHCO<sub>3</sub> additive in low level NaHCO<sub>3</sub>, high level NaHCO<sub>3</sub> and high level NaHCO<sub>3</sub> silage with protein supplement, respectively. In addition the average sodium intake from salt was 5 g day<sup>-1</sup>. Sodium requirement for sheep is 0.09 – 0.18 % in the sheep's total diet. In our study the amount of sodium surpassed more than fivefold the sodium requirement, but no health problems were observed.

Table 1. Effect of partial neutralization of grass silage and protein supplement on the daily intake and live weight gain of lambs.

	Grass silage				S.E.	Significance		
	Untreated	8 g NaHCO <sub>3</sub>	16 g NaHCO <sub>3</sub>	16 g NaHCO <sub>3</sub> + Protein Suppl				
Silage intake (g DM)	513	553	621	674	31.7	0.02	0.72	0.24
Concentrates (g DM)	435	430	429	440	3.5	0.22	0.68	0.04
Total DM intake (g)	987	1022	1090	1154	33.2	0.03	0.70	0.18
Total ME energy (MJ)	11.7	12.0	12.6	12.9	0.36	0.09	0.69	0.54
Total AAT (g)	91	93	98	103	2.8	0.10	0.68	0.23
Live weight gain (kg)	10.5	11.9	12.4	14.0	0.81	0.12	0.67	0.17

DM = Dry matter, ME = Metabolizable energy, AAT = Amino acids absorbed in the small intestine, S.E. = Standard Error, P1 = Linear trend of NaHCO<sub>3</sub>, P2 = Quadratic trend of NaHCO<sub>3</sub> and P3 = Effect of protein supplement.

### Conclusions

The results suggested that NaHCO<sub>3</sub> additive had beneficial effect on intake when grass silage made up about 60 % of the total diet.

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# The effect of grassland quality on live-weight gains of heifers

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## Abstract

Utilisation of grassland by replacement heifers was studied. Effects of herbage quality on live-weight gains were assessed. A diet containing grassland herbage *ad libitum* and a concentrate supplement was offered. Improved quality of herbage and a higher rate of concentrate increased the live-weight gains.

Keywords: heifers, grassland, herbage quality, live-weight gains.

## Introduction

A decline of socio-economic conditions in agriculture is related to problems in animal husbandry at farms. In Slovakia, heifer rearing is one of the weakest aspects of cattle husbandry. The success in heifer's rearing is conditioned by availability of good quality feed throughout the season allowing an adequate body development and good health. In upland and mountain regions, there is a high proportion of grassland in relation to total agricultural land, being the basis of forage for ruminants. The productivity and quality of grassland depends not only on the site conditions, sward management techniques or grazing management, but also on the botanical composition (proportions of grasses, legumes and other herbs and the relationships between them). The botanical composition and structure of sward have an impact on the quality of herbage, and consequently, on animal performance (Šúr, 1994). Therefore, great attention should be paid to the botanical composition of grazed areas and good quality species for grazing swards have to be provided. A restoration of pastures by oversowing with quality grass and legume species is a prerequisite of their feeding value improvement (Novák and Kubinec, 2002). Hrazdira (1998) reported that the oversown cultivars had been markedly more productive than the original sward ecotypes.

## Materials and methods

Research Trials (A and B) were carried out with 16 Slovakian Spotted heifers aged from 3 months onwards and with 155 kg mean live weight. Throughout the season, rotational paddock grazing was applied with free access to drinking water and without housing. Trial A was performed on permanent grassland (PG) and oversown grass/clover mixture sward (OG) was grazed at Trial B. The heifers in the pasture were divided into 2 groups (Group 1 and 2) of 8 animals for the Trial A and Trial B. Both Groups in each Trial have access to mineral links and received 1000 g head<sup>-1</sup> day<sup>-1</sup> and 500 g head<sup>-1</sup> day<sup>-1</sup>, respectively, of a commercially produced concentrate (*DKZ HD 03B*). Live-weight gains were measured and recorded once a month during the research trials. Herbage was sampled for laboratory analyses to determine dry matter (DM) content, organic nutrients – nitrogen (crude protein, CP = 6.25 x N), fat, ash, fibre and minerals (P, K, Na, Ca, Mg). Consequently, the nutritive value was determined as PDIE (protein digested in the small intestine when energy is limiting), PDIN (protein digested in the small intestine when nitrogen is limiting), NEL (net energy for lactation), NEV (net energy for fattening) and ME (metabolisable energy) using the equations published by the Ministry of Agriculture of the Slovak Republic (MPSR) in the Annex 8 of MPSR Decree No. 39/1/2002-100.

## Results and discussion

The quality is an important factor in the determination of sward potentiality, related to animal units supported per unit of area. Hence, the quality indicates the range of sward - animal relationships and the

adjustment of grazing animals, according to their needs, along the grazing season. The content of nutrients and the nutritive value of herbage at swards A (PG) and B (OG) are given in Table 1. The quality of sward B (OG) was significantly better than that of sward A (PG).

Table 1. Nutrient content and nutritive value of sward A (PG) and B (OG) along the grazing cycles.

Grazing cycles	Cycle duration (from-to)	Dry mater	Crude protein	Fibre (g kg <sup>-1</sup> DM)	PDIN	PDIE	NEL			ME (MJ kg <sup>-1</sup> DM)
							NEV	NEV	ME	
Sward A										
I	7 May-12 June	328.86	115.74	230.76	72.89	72.85	4.61	4.19	8.12	
II	13 June-28 July	380.87	116.31	239.38	73.98	72.57	4.58	4.16	8.07	
III	29 July-27 Aug	337.14	138.69	214.02	88.22	73.33	5.15	4.89	8.87	
IV	28 Aug-29 Sept	341.57	146.36	186.43	93.90	73.75	4.87	4.58	8.42	
Sward B										
I	5 May-6 June	218.22	134.26	198.04	80.96	72.97	5.87	5.79	9.88	
II	7 June-21 July	230.37	143.73	205.57	86.71	73.64	5.85	5.75	9.86	
III	22 July-29 Aug	258.03	146.60	214.25	88.84	73.81	5.79	5.68	9.76	
IV	30 Aug-1 Oct	244.41	154.83	176.08	94.99	74.10	5.70	5.59	9.61	
Tukey										
(P<0,05) +	Cycle	-	-	+	++	-	-	-	-	-
(P<0,01) ++	Sward	++	+	++	++	-	++	++	++	++

Mean daily live-weight gains over the grazing season are given in Table 2. At the Trial A (PG), mean daily live-weight gains were 0.895 kg head<sup>-1</sup> in Group 1 and 0.830 kg head<sup>-1</sup> in Group 2. At the Trial B (OG), mean daily live-weight gains were 0.997 kg head<sup>-1</sup> in Group 1 and 0.930 kg head<sup>-1</sup> in Group 2. At both the sward types, heifers of Groups 1 showed higher live-weight gains than those in Groups 2, although the differences were not statistically significant. The recorded live-weight gains were in agreement with those reported in the literature, recommending 600 – 1000 g for the heifers to the age of 12 months. A comparison between the sward types showed statically significant higher gains for Trial B than for trial A.

Table 2. Daily live-weight gains at heifers (kg head<sup>-1</sup> d<sup>-1</sup>).

Trials (sward types)	A (PG)					B (OG)					
	Cycles	I	II	III	IV	Ø	I	II	III	IV	Ø
Group 1		0.795	0.871	0.865	0.955	0.895	0.853	0.970	1.039	1.035	0.997
Group 2		0.718	0.806	0.841	0.910	0.830	0.775	0.892	0.983	0.987	0.930
Tukey (P<0.05) group				-					-		
Tukey (P<0.05) sward						+					

## Conclusions

The heifers with higher rates of concentrate supplementation showed higher live-weight gains (although not significant) in both sward types, A (PG) or B (OG). It was concluded that optimum live-weight gains were achieved at grazing good quality sward (with sufficient CP at 12-14 %) supplemented by *DKZ HD 03 (B)* concentrate rate of 500 g head<sup>-1</sup> day<sup>-1</sup>. This conclusion was in agreement with the data reported in literature specifying up to 1 kg of concentrate supplement per head per day as a sufficient rate for optimum live-weight gains. The better quality of sward B herbage (higher values of CP, PDI and energy parameters) resulted in increased live-weight gains. Consequently, it is recommended to improve pastures for grazing ruminants by the introduction of productive, ecologically appropriate good quality grasses and legumes to the original sward. This could allow to obtain good quality replacement heifers, covering their specific requirements, but also to contribute to maintain the traditional heifer production in the upland and mountain regions.

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# Prediction of forage rumen protein degradability from an *in situ* degradability database

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## Abstract

In most feed evaluation systems, forage protein value evaluation relies on the estimation of forage protein degradability in the rumen (deg). To improve this estimation, a database of 375 deg measurements for fresh forages using the *in situ* technique (rumen degradability in nylon bags) was built. Deg decreases as forage maturity stage increases, and deg is higher for the 1<sup>st</sup> growth than for the following growth cycles. Analysis of the database using a mixed model to take into account laboratory effects revealed that deg can be predicted from the crude protein content of the forage, with a quadratic effect, and from botanical family and vegetation cycle effects (mean deg = 75.3; R<sup>2</sup> = 0.87; RSD = 3.5). Furthermore, deg values of conserved herbage can be predicted from the corresponding values for fresh herbage for hays (n = 29; R<sup>2</sup> = 0.71; RSD = 3.6), and for fermented herbage (direct cut, wilted or big bale silage) in combination with dry matter content (n = 32; R<sup>2</sup> = 0.53; RSD = 4.2).

Keywords: forage, protein degradability, rumen, prediction.

## Introduction

The degradability of crude protein (deg) obtained from a standardized *in situ* method (nylon bag technique, Michalet-Doreau *et al.* 1987) is one of the key parameters for calculating the protein value of feeds expressed in Protein truly Digestible in the small Intestine, (PDI; Vérité and Peyraud, 1989). Due to the lack of experimental data, the deg values for forages have been set to fixed values, for example 73% of crude protein for all fresh forages. However, it was shown that deg is affected by botanical family, plant species, growth cycle and level of nitrogen fertilization, and decreases with plant maturity stage (Le Goffe, 1991; Antoniewicz *et al.*, 1995; Peyraud and Astigarraga, 1998; Aufrère *et al.*, 2000). The aim of this study was to develop equations for predicting forage deg values in order to improve the estimation of PDI values. For this purpose, we built a database of deg measurements for fresh forages and for conserved forages studied in correspondence with fresh ones.

## Materials and methods

The database for fresh forages was built around the following criteria: (i) Deg had to be measured in cows with the nylon bag technique (Michalet-Doreau *et al.*, 1987 or Dulphy *et al.*, 1999 for data obtained at the INRA); (ii) Botanical family, species, growth cycle number and crude protein content (CP in g/kg of dry matter (DM)) of the forage had to be known. Data came from the INRA (11 trials, 214 samples) and from European literature (8 publications, corresponding to 10 trials and 138 samples). Data from the Swiss Research Federal Station of Posieux (16 samples, R. Daccord, personal communication) and from the Irish Teagasc-Moorepark research station (7 samples, J. Murphy, personal communication) were incorporated into the database. The database contains 375 deg values for fresh forages. Data obtained outside the temperate part of Europe were excluded.

For a part of the fresh forages in the database deg were also measured on the corresponding hays or silages. Twenty-nine comparisons between fresh forages and hays and 32 comparisons between fresh forages and fermented forages without additive (8 direct cut, 16 wilted and 8 big bale silages) were used to investigate whether the deg of conserved forages can be predicted from the deg of the fresh forage. Eight direct cut and 8 wilted silages were also prepared with additive (8 with acid formic, 4 with acid

salt and 4 with bacteria) allowing testing the effect of the presence of the additive on deg. Statistical analysis of the data for fresh forages was performed with a 'trial' effect to take into account differences in methodologies (bags types, fresh, freeze dried or desiccated samples, etc.) and in scientific goals (effects of plant maturity stage, level of nitrogen fertilization, etc.) between trials and authors. The trial effect was considered as a random effect (St. Pierre, 2001). We tested the botanical family, the plant species and the growth cycle as fixed effects and the CP content as a co-variable. The relationship between deg of fresh forages and deg of conserved forages was analysed by multiple regression.

## Results and discussion

The main grass species in the database is Perennial rye grass (*Lolium perenne* L.) (37% of samples) and the main legume is Alfalfa (*Medicago sativa* L.) (13%). Cocksfoot (*Dactylis glomerata* L.), Italian rye grass (*Lolium italicum*), and Bromegrass (*Bromus* sp.) are the other significant grasses, while white and red clover (*Trifolium repens* L. and *Trifolium pratense* L.) are the other significant legumes species.

Table 1. Database of protein degradability values for fresh forages (n = 375).

		n	deg (% of CP)	CP (g kg <sup>-1</sup> DM)
Grasses	1 <sup>st</sup> growth	60	80.9 ± 6.5	150 ± 52
	Other growths	141	73.9 ± 8.5	197 ± 61
Legumes	1 <sup>st</sup> growth	43	81.4 ± 4.9	185 ± 46
	Other growths	33	81.4 ± 4.8	205 ± 49
Natural grasslands	1 <sup>st</sup> growth	31	73.6 ± 9.0	158 ± 54
	Other growths	67	67.2 ± 8.3	194 ± 42

The main significant effects were CP content, botanical family and growth cycle (first growth vs. others). Deg was strongly related to forage CP content, with a quadratic effect. Grass and legume proteins were significantly more degradable than proteins of natural grasslands (p<0.0001; Table 1). Grasses and natural grasslands proteins were significantly more degradable during the first growth cycle than following cycles (p<0.0001). However, the cycle effect was not significant for legumes. Thus, to predict the deg of fresh forage, we propose the following equation:

$$\text{deg} = 51.2 + 0.14 \text{ CP} - 0.00017 \text{ CP}^2 + 8.8 (\text{grasses} - 1^{\text{st}} \text{ growth}) + 4.6 (\text{grasses} - \text{other growths}) + 6.8 (\text{legume forages} - \text{all growths}) + 4.4 (\text{natural grasslands} - 1^{\text{st}} \text{ growth}) + 0 (\text{natural grasslands} - \text{other growths})$$

with deg (%) and CP (g.kg<sup>-1</sup> DM); (n = 375; R<sup>2</sup> = 0.87; RSD = 3.5)

The plant species effect was significant, but did not explained variability of deg more than the botanical family effect. Testing another chemical characteristic of forage such as NDF content did not improve the model. The equation considers that whatever the source of CP content variation (level of nitrogen fertilisation, plant maturity stage, etc.), the variation in deg is similar, which is consistent with the results of Van Vuuren *et al.* (1991). Significant relationships were found between deg of conserved forages and deg of the corresponding fresh ones. The protein degradability of hay is lower than that of the corresponding fresh forage (71.5 vs. 79.4). From these comparisons, the following equation was calculated:

$$\text{deg}_{\text{hay}} = 17.1 + 0.68 \text{ deg}_{\text{fresh forage}} \quad (\text{n} = 29; \text{r}^2 = 0.71; \text{RSD} = 3.6)$$

The protein degradability of fermented forages varies with the intensity of the wilting process. Deg was much higher for direct-cut silages prepared without additive at a DM content of 20%, than for the corresponding fresh forage (88.7 vs. 80.5). Deg for wilted silages at a DM content of 28% was also higher than for the corresponding fresh forage (79.1 vs. 71.3). However, the deg of big bale silage at a DM content of 54.5% was close to the deg of the corresponding fresh forage (79.5 vs. 82.2). The following equation, including a DM content effect, was calculated:

$$\text{deg}_{\text{fermented forage}} = 46.5 + 0.56 \text{ deg}_{\text{fresh forage}} - 0.25 \text{ DM}_{\text{content}} \quad (n=32; r^2=0.53; \text{RSD}=4.2)$$

When the silage was prepared with additive, soluble nitrogen content was reduced, leading to a reduced deg:

$$\text{deg}_{\text{with additive}} = 0.96 \text{ deg}_{\text{without additive}} \quad (n = 16; r^2 = 0.83; \text{RSD} = 1.8)$$

## Conclusions

Using this database, it was possible to develop equations to predict the protein degradability of fresh herbage and conserved forages. These equations have satisfactory accuracy and can be used to improve PDI estimation for herbage from European temperate climate areas.

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# Changes in forage nutritive value and forage nutrient production caused by various intensity of grassland management

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## Abstract

The aim of this study was to assess the influence of different grassland management on the dry matter production and the nutritive value of the forage. The small plot trial was managed during 2003 and 2004 in four levels of the cutting intensity and in four levels of the fertilization. The model cattle load was 0.1 and 2 LU ha<sup>-1</sup>. The lowest value of the dry matter production (6.55 t ha<sup>-1</sup>) was ascertained in the intensive utilization. The fertilizer application significantly increased ( $P < 0.01$ ) dry matter production compared to nil fertilization (from 5.83 to 8.27 t ha<sup>-1</sup>). The results show decrease of concentration of energy (NEL, 5.83 to 5.07 MJ kg<sup>-1</sup> DM) and PDIN (111.0 to 77.1 g kg<sup>-1</sup> DM), PDIE in connection with decrease of the utilization intensity. With the application of the different utilization and fertilization it is possible to influence significantly the amount and the quality of the fodder from grasslands.

Key words: grasslands, cutting frequency, forage quality, fertilizer level.

## Introduction

Grasslands in the Czech Republic (CR) comprise 22.2 % of the agrarian land (4,280,000 ha). In relation to the stagnation of the agricultural products consumption it could be expected other expansion of the permanent grassland areas. In this situation it is necessary to find out objectively the most suitable methods of the grassland management for the next time. The permanent grassland management by means of the cattle breeding is the most rational alternative. For its practical realization in the necessary range it is important to use also the foreign knowledge about the feed intake.

For example Gruber *et al.* (2000) ascertained in forage from grassland with three different types of utilization these values of the voluntary intake: 10.4, 13.0, 15.2 kg DM on head. For the animals' requirements' filling it is important to provide the necessary quality of the feedstuffs. Pozdíšek *et al.* (2002) point out that these parameters are depended on the species and varieties and also on the type of the management. With the suitable frequency of the utilization it is possible to achieve the energy concentration (NEL) on the value of 6.1 MJ kg<sup>-1</sup> DM. On the contrary the lower frequency of the utilization (older stand) causes the decrease of this value up to 5.1 MJ kg<sup>-1</sup> DM.

## Material and Methods

In 2003 it was founded the long-term small plot trial on the permanent grassland sites in the locality Rapotín. It consists of 16 treatments, in 4 replications, with a 10 m<sup>2</sup> harvest plot size. The grassland vegetation on the experimental stands was classified as *Arrhenatherion*.

It was managed with four levels of the intensity of utilization:

I<sub>1</sub> = intensive (1<sup>st</sup> cut until May 15<sup>th</sup>, 4 cuts per year – cuts at 45 day interval),

I<sub>2</sub> = medium intensive (1<sup>st</sup> cut between 16<sup>th</sup> and 31<sup>st</sup> May, 3 cuts per year at 60 day interval),

I<sub>3</sub> = low intensive (1<sup>st</sup> cut between 1<sup>st</sup> and 15<sup>th</sup> June, 2 cuts per year at 90 day interval) and

I<sub>4</sub> = extensive (1<sup>st</sup> cut between 16<sup>th</sup> and 30<sup>th</sup> June, 1 or 2 cuts per year, second cut after 90 days).

Each type of the utilization was furthermore divided in four levels of fertilization:

F<sub>0</sub> = no fertilization, F<sub>PK</sub>=P<sub>30</sub>K<sub>60</sub>N<sub>0</sub>; F<sub>PKN90</sub>=P<sub>30</sub>K<sub>60</sub>+N<sub>90</sub>, F<sub>PKN180</sub>=P<sub>30</sub>K<sub>60</sub>+N<sub>180</sub>.

It was measured the annual dry matter production for all plots. The samples from these plots (all variants and revisions, 352 in total) collected during 2003 and 2004 were analyzed in laboratories of the Research institute for cattle breeding, Ltd., Rapotín. By means of the Weenden analysis there were estimated the values of nitrogen compounds, fat, crude fibre and ash. Furthermore, it was counted the quantity of the nitrogen free extract in dry matter of each sample. Forage quality in terms of crude protein (CP), fibre, NEL (net energy of lactation), NEV (net energy of fattening), PDIE (ingested digestive protein allowed by energy), PDIN (ingested digestive protein allowed by nitrogen) was predicted by means of the regression equations for the organic matter digestibility (Pozdíšek *et al.*, 2001) and by means of the equations that mentions Petrikovič *et al.*, (2000). The results were statistically evaluated with two-factor analysis of variance with one observation in the subclass; the differences between the averages were tested by the Tuckey test ( $DT_{0.05}$ ,  $DT_{0.01}$ ).

## Results and Discussion

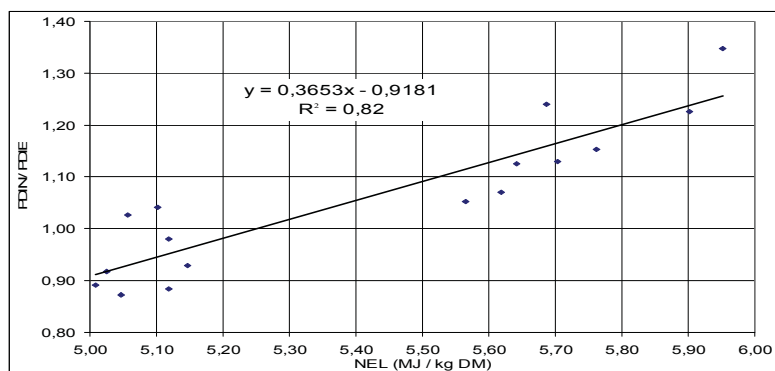
Table 1 show that the overall average dry matter production during two years (2003 and 2004) was for sixteen treatment combinations  $7.05 \text{ t ha}^{-1}$ . The dry matter production was the lowest in the intensive utilization ( $6.55 \text{ t ha}^{-1}$ ) (see Table 1A). It conforms to results of Gruber *et al.* (2000). They state, on the basis of the evaluation of the long-term trials at BAL Gumpenstein, that the graded cutting frequency causes the decrease of the yield from grasslands, especially four-cut utilization ( $8.65$ ,  $8.05$ ,  $6.51 \text{ t ha}^{-1}$  DM, with 2, 3 and 4 cut-utilization).

Table 1. Overall average dry matter production and forage quality at different levels of intensity of utilization and fertilization during 2003 – 2004.

Treatments intensity of utilization and fertilization	DM ( $\text{t ha}^{-1}$ )	NEL ( $\text{MJ kg}^{-1}$ DM)	NEV ( $\text{MJ kg}^{-1}$ DM)	CF ( $\text{g kg}^{-1}$ DM)	CP ( $\text{g kg}^{-1}$ DM)	PDIN ( $\text{g kg}^{-1}$ DM)	PDIE ( $\text{g kg}^{-1}$ DM)	PDIN/ PDIE
<b>A</b>								
I <sub>1</sub>	6.55	5.83	5.72	239	173	111.0	91.3	1.214
I <sub>2</sub>	7.54	5.63	5.47	254	156	100.3	88.7	1.122
I <sub>3</sub>	7.46	5.09	4.80	297	120	78.1	82.0	0.949
I <sub>4</sub>	6.66	5.07	4.77	298	118	77.1	81.1	0.936
AVG	7.05	5.40	5.19	272	142	91.6	85.8	1.055
$DT_{0.05}$	0.59	0.11	0.13	10	4	2.6	1.1	0.024
$DT_{0.01}$	0.71	0.13	0.16	12	5	3.2	1.3	0.030
<b>B</b>								
F <sub>0</sub>	5.83	5.37	5.16	273	131	84.8	84.5	0.991
F <sub>PK</sub>	6.35	5.37	5.16	274	133	86.2	85.0	1.005
F <sub>PKN90</sub>	7.76	5.42	5.21	271	144	92.7	86.2	1.062
F <sub>PKN180</sub>	8.27	5.45	5.23	272	161	102.8	87.3	1.164
AVG	7.05	5.40	5.19	272	142	91.6	85.8	1.055
$DT_{0.05}$	0.59	0.11	0.13	10	4	2.6	1.1	0.024
$DT_{0.01}$	0.71	0.13	0.16	12	5	3.2	1.3	0.033

The fertilizer application over two years significantly increased ( $P < 0.01$ ) dry matter production compared to nil fertilization, i.e. from  $5.83$  to  $6.35 \text{ t ha}^{-1}$  in F<sub>PK</sub> fertilised treatments, or to  $7.76 \text{ t ha}^{-1}$  in N- fertilization of  $90 \text{ kg ha}^{-1}$ , or to  $8.27 \text{ t ha}^{-1}$  in N-fertilization of  $180 \text{ kg ha}^{-1}$ , respectively. The energy (NEL) and PDIN, PDIE concentration decreases in connection with decrease of the utilization intensity. The highest concentration of energy was acquired in 4-cut utilization. Figure 1 shows relation between energy concentration (NEL) and PDIN/PDIE ratio. PDIN/PDIE ratio with the sufficient concentration of

NEL was ascertained in the medium intensity of utilization and in the  $F_{PK}$  fertilization or in the low nitrogen dose, respectively.



PDIN -Protein truly Digestible in the small Intestine dependent on N-substances  
 PDIE –Protein truly Digestible in the small Intestine dependent on food energy

Figure 1. Relation between concentration of nettoenergy and PDIN:PDIE ratio.

### Conclusion

By means of the various types of the grassland utilization and fertilization it is possible to influence significantly the amount and the quality of the fodder. These findings are important for the cattle nutrition security and for the sufficient grassland management. It is necessary the other knowledge enlargement.

### Acknowledgements

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# The interspecific plant competition affects the production and the nutritive value of grassland species

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## Abstract

Five grassland species were cultivated in monoculture and in mixture with a highly competitive cultivar of *Dactylis glomerata* L. This setup aimed to evaluate the impact of species interactions on the productivity and on the nutritive value of these grassland species differing in their growth strategies. All 5 species exhibited larger above ground organs when submitted to competition with the cultivar and this was associated to an increase in the proportion of structural tissue (NDF) and to a decline of their nutritive value. Above ground production in competition increased for tall species but decreased for the small ones. For small species, frequent defoliation regime balanced the negative effect of competition.

Keywords: plant traits, enzymatic digestibility, yield, cutting frequency, nitrogen, grasses.

## Introduction

In grasslands, interspecific plant relationships structures plant communities (Herben and Huber-Sannwald, 2002), modifies species relative abundance and affects their agronomic characteristics (Marriott and Carrère, 1998). To assess the impact of the interspecific competition on the productivity and on the nutritive value of grassland species, a field trial was setup in order to compare the functioning of five grass species, morphologically and functionally distinct, sown in monoculture or in mixture with a *Dactylis glomerata* cv.

## Materials and methods

The seeds of *Dactylis glomerata* (Dg), *Festuca arundinacea* Schreb.(Fa), *Festuca rubra* Gaud. (Fr), *Poa pratensis* L. (Pp) and *Trisetum flavescens* Beauv. (Tf), collected in semi-natural grasslands were sown, in 2002 at Theix (870 m a.s.l., Massif-Central, France), in monocultures and in mixtures with a highly competitive cultivar of *Dactylis glomerata* cv *Lupré* (Cv). The plots were managed with two cutting frequencies (3 and 6 cuts per year, C- and C+) and at two levels of inorganic N supply (120 and 360 kg N ha<sup>-1</sup>yr<sup>-1</sup>, N- and N+) in a complete block design with 3 replicates. Measurements were done during two consecutive years in 2003 and 2004. At each cut, the vegetation was mown at 6 cm, collected, dried and weighted. The annual dry matter yield (DMY) was the sum of the 3 (C-) or 6 (C+) cuts performed each year. The aggressivity index (Ag) allowed evaluating the relative competitiveness of the species and was calculated by equation 1:

$$Ag = 0.5 * [Mij/Mii)-(Mji/Mjj)] \quad (Eq 1)$$

with Mij the DM of the native species in mixture, Mii the DM of native species in pure sward, Mji the DM of the competitor in mixture and Mjj the DM of the competitor in pure sward.

Plant traits were measured in spring and summer, after 3 weeks of regrowth, on 10 tillers collected at random: elongated plant height (PHe), sheath length (SL). After rehydration, the length (LL), area (LA), fresh mass (LFM) of the youngest fully expanded leaf were measured. Leaf dry matter content (LDM) and specific leaf area in DM (SLA) were then calculated.

Forage quality was assessed via near infrared reflectance spectroscopy by the content of neutral detergent fibre (NDF) (Van Soest and Wine, 1967) using the Ankom apparatus (Ankom® Tech. Co., Fairport, NY, USA) and the enzymatic dry matter pepsin cellulase digestibility (EDMD) (Aufrère and Demarquilly, 1989). The cell wall content and the nutritive value between pure and mixed swards were

compared on the purely vegetative regrowth on samples taken at the autumn cut, to avoid artefact linked to differences in the phenological development of species during spring.

## Results and discussion

On average over the two years, the annual DMY of the mixtures was greater than the mean of the DMY of the cultivar and of the ecotypes grown in monoculture (Figure 1). The mixture Cv-Dg and Cv-Fa, exhibited a higher DMY than that of the species Cv, Dg or Fa grown in monoculture. For the three other species, the DMY of the mixture was greater than the DMY of the ecotype in monoculture but smaller than that of the competitor.

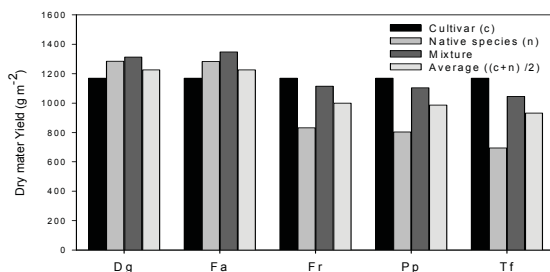


Figure 1. Average dry matter yield ( $\text{g m}^{-2} \text{y}^{-1}$ ) of the cultivar and of the native species in pure and mixed sward.

The aggressivity index of the five ecotypes considered (Figure 2) was shown to be significantly different ( $p < 0.001$ ). The aggressivity of Dg was positive, nearly null for Fa and negative for the three other species. In this third case, this implicates that the species were largely dominated by the competitor. The cutting frequency influenced significantly ( $p < 0.001$ ) the Ag index for all the ecotypes but Dg. Ag was greater under frequent cutting regime.

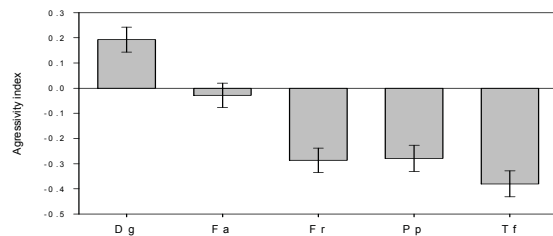


Figure 2. Aggressivity index of the five species. Positive values mean that the ecotype was dominant toward the cultivar associated. The vertical bars shown the standard error,  $n = 24$ .

In mixture, all species but Dg responded to competition by modifications in plant morphology, mainly by an increase in size of their above ground organs (tiller, sheath or lamina) (Table 1), or by alterations of lamina thickness (increase of the SLA).

These modifications in plant morphology were associated to an increase in NDF contents (Table 2), which means an increase in the proportion of structural tissue in the total biomass. As a consequence, the digestibility (DMD) of ecotypes was significantly smaller in mixture than in monoculture (Table 2). This appeared mainly under frequent cutting regime (C+), while this effect was significant only for Pp under infrequent cutting regime (C-). Under high level of nitrogen supply (N+), we observed difference in the DMD of ecotypes between pure and mixed sward for all the species but Dg; this differences remain under N- only for Fa (data not shown).

Table 1. Average value of five plant traits : elongated plant height (PHe, mm), sheath length (SL, mm), lamina length (LL, mm), lamina dry matter content (LDM, %) and specific leaf area (SLA, cm<sup>2</sup>g<sup>-1</sup>) measured on the five ecotypes in pure and mixed swards (n=24).

	Dg			Fa			Fr			Pp			Tf		
	pure	mix	p	pure	mix	p	pure	mix	p	pure	mix	p	pure	mix	p
PHe	396	413	NS	360	332	*	202	201	NS	223	266	***	207	228	*
SL	100	109	NS	65	56	*	40	38	NS	51	58	*	69	65	NS
LL	272	279	NS	291	265	NS	143	148	NS	160	182	**	131	148	*
LDM	19.5	19.6	NS	22.2	21.0	**	27.0	23.8	***	24.9	23.2	**	21.7	19.1	***
SLA	280	282	NS	185	209	**	103	132	***	197	217	*	291	369	***

Significance level \* p<0.05, \*\* p<0.01, \*\*\* p<0.001.

Table 2. Neutral detergent fibre content (NDF, g kg<sup>-1</sup>, mean ± s.e., n=24) and enzymatic dry matter digestibility (EDMD, g kg<sup>-1</sup>, mean ± s.e., n=24) for five grass species cultivated monoculture or in mixture with a competitor (*Dactylis glomerata* cv *Lupre*).

	NDF			EDMD		
	Pure sward	Mixture	p	Pure sward	Mixture	p
<i>Dactylis glomerata</i>	555 ± 6.3	593 ± 6.2	***	702 ± 12.2	667 ± 10.2	*
<i>Festuca arundinacea</i>	540 ± 7.4	590 ± 4.6	***	675 ± 11.5	625 ± 6.0	***
<i>Festuca rubra</i>	549 ± 8.4	604 ± 4.9	***	641 ± 14.6	577 ± 11.4	**
<i>Poa pratensis</i>	535 ± 9.2	591 ± 7.4	***	678 ± 18.3	612 ± 16.3	**
<i>Trisetum flavescens</i>	508 ± 9.4	574 ± 5.5	***	750 ± 16.5	680 ± 13.3	**

Significance level \* p<0.05, \*\* p<0.01, \*\*\* p<0.001.

The comparison of the morphology and performances of each species in pure and mixed sward has shown the major role of interspecific competition for light. Competition induces an increase in size of above ground organs (sheath, lamina) in order to facilitate the capture of incident radiation for small species, or in order to dominate the neighbours for tall species. In mixtures, Aerts (1999) considers that functional traits for successful development are those that allow species to dominate their neighbours. As a consequence, under lenient perturbation regime (infrequent cuts), swards will be dominated by the tallest species, whereas under frequent cutting, regular defoliation will limit the competition for light which allows the coexistence of a larger number of species. Our results suggest that the negative effect of competition for small species (Fr, Pp, Tf) is counterbalanced by frequent defoliation regimes. Nevertheless greater plant size caused by interspecific competition induces a greater content in structural tissue (NDF), which implicates a decline in the nutritive value of the herbage.

### Acknowledgements

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# Biomass production and protein content of semiarid grasslands in western Spain over a 20-years period

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## Abstract

The ‘dehesas’ are savanna-like semiarid grasslands typical of western Spain. Over a 20-years period, from 1986 to 2005, biomass production and protein content of ‘dehesa’ pastures in the province of Salamanca (western Spain) was analysed. Herbage samples were collected at the end of spring, in several locations (between 5 and 30, depending on years), at two different topographic positions (upper and lower slope zones).

Biomass production ranged between 50 – 214 g DM m<sup>-2</sup> in the upper slope zone, and between 148 – 419 g DM m<sup>-2</sup> in the lower slope zone. The mean over the 20-years period was 125 g DM m<sup>-2</sup> in the upper zone and 287 g DM m<sup>-2</sup> in the lower zone. The protein content ranged between 79.8 – 111.3 g kg<sup>-1</sup> in the upper zone, and between 85.7 – 144.2 g kg<sup>-1</sup> in the lower zone. The mean across years was 96.5 g kg<sup>-1</sup> in the upper and 116.4 g kg<sup>-1</sup> in the lower zone.

Interannual variations in biomass production and protein content of upper and lower zones were correlated. A significant correlation ( $P < 0.05$ ) was found between biomass production and annual precipitation.

Keywords: dehesas, protein, herbage production, interannual variations.

## Introduction

Grasslands of the western of the Iberian Peninsula form part of the ‘dehesas’, a semiarid ecosystem which occupy more than 6,000,000 ha. The ‘dehesa’ system occurs on gently undulating hills and features low-density *Quercus ilex* L. subsp. *rotundifolia* with a botanically complex herbaceous component. The main land use is extensive grazing by beef and bullfighting livestock.

Mediterranean ecosystems are characterized by strong seasonal and inter-annual variations in the biomass production. At a regional scale (spatial scale) mean aboveground net primary production of grasslands of widely different systems is strongly correlated to annual precipitation (Sala *et al.*, 1988). Much less is known about the control of temporal, inter-annual variations of productivity at a given site, and relationships are weak.

The objective of this study was to analyse the interannual variations of biomass production and protein content of semiarid grasslands in western Spain, over a 20-years period and to evaluate relationships between production, protein and climatic variables.

## Materials and methods

Thirty gentle slopes were selected within the ‘dehesa’ grasslands of Salamanca province (western Spain). From 1986 to 1993 years, herbage samples were collected in those 30 locations. From 1994 to 2005 five slopes, of the 30 previously selected, were chosen and herbage samples were collected at two different dates 15 days separating. In all cases, two slope positions were differentiated on each location: upper and lower zones. Sampling was done at the end of the growing season, when plants were at the flowering-fruiting stage; those obtained from the upper zones were at a later stage of ripening than those from the lower zone. Sampling was done by cutting the herbaceous vegetation in four randomly selected quadrants (0.25 m<sup>2</sup>). Dry matter production was determined by drying at 60 °C for 48 h in a forced-air oven. Ground samples were analysed for crude protein by the Kjeldahl method.

Analysis of variance with sampling year and slope zone as factors was applied to data. A correlation analysis was used to assess relationships between biomass production, protein content and the following

climatic variables: annual precipitation, seasonal and monthly precipitation, mean annual daily and mean seasonal temperature. Total annual precipitation and mean annual daily temperature were calculated from 1 October of the year previous to sampling to 30 June of the sampling year, which is the month when samples were collected.

## Results and discussion

The yearly mean values of biomass production at two slope positions, over the 20-years period, are shown in Figure 1. There was a significant effect of sampling year ( $P < 0.001$ ), slope zone ( $P < 0.001$ ) and their interaction ( $P < 0.01$ ). In the upper zone, the biomass production ranged from 50.3 g m<sup>-2</sup> (in 1997 year) to 214.4 g m<sup>-2</sup> (in 1998). In the lower slope zone, ranged from 147.8 g m<sup>-2</sup> (in 2002) to 416-419 g m<sup>-2</sup> (in 1990 and 1996 respectively). The mean over years was significantly ( $P < 0.001$ ) greater in the lower zone (287.9 g m<sup>-2</sup>) than in the upper (125.5 g m<sup>-2</sup>).

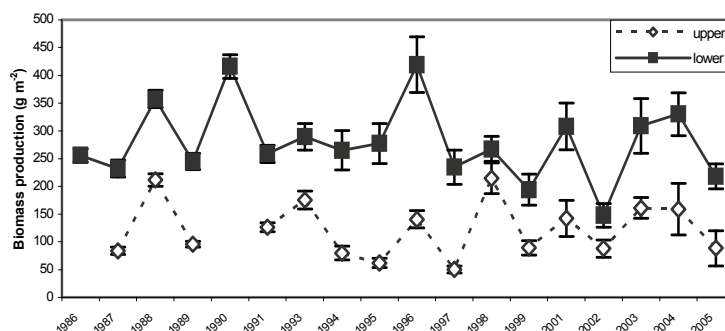


Figure 1 - Biomass production (g m<sup>-2</sup>) of 'dehesa' pastures in the upper and lower slope zones over a 20-years period (1986-2005). Values are means  $\pm$  SE.

There was a significant relationship of the biomass production between the upper and the lower zones across sampling years ( $r^2 = 0.788$ ,  $P < 0.001$ ,  $n = 16$ ). This suggests that interannual variations in the biomass production follow similar patterns in both slope zones.

Of all climatic variables considered, the biomass production across sampling years was only significantly related to annual precipitation ( $r^2 = 0.54$ ,  $P < 0.05$ ,  $n = 16$ ). Interannual variations of a single shortgrass steppe site across 52-years series have been related to precipitation of the same year (Lauenroth and Sala, 1992) and to previous year production and precipitation (Martin *et al.*, 2001). In both studies, the percentage of the variation in production among years explained was greater than in ours, but this could be due to differences in the time period.

The protein content of herbage in the upper and lower zones is shown in Figure 2. Analysis of variance showed a significant effect of sampling year ( $P < 0.001$ ), slope zone ( $P < 0.001$ ) and their interaction ( $P < 0.001$ ). In the upper zone, the protein ranged between 79.8 g kg<sup>-1</sup> (in 1996 year) and 111.3 g kg<sup>-1</sup> (in 1993). In the lower zone the minimum was 85.7 g kg<sup>-1</sup> (in 1988) and the maximum 144.0 g kg<sup>-1</sup> (in 1991 and 1993 years). The mean protein content, over the whole sampling period, was significantly ( $P < 0.001$ ) greater in the lower zone (116.4 g kg<sup>-1</sup>) than in the upper (96.5 g kg<sup>-1</sup>).



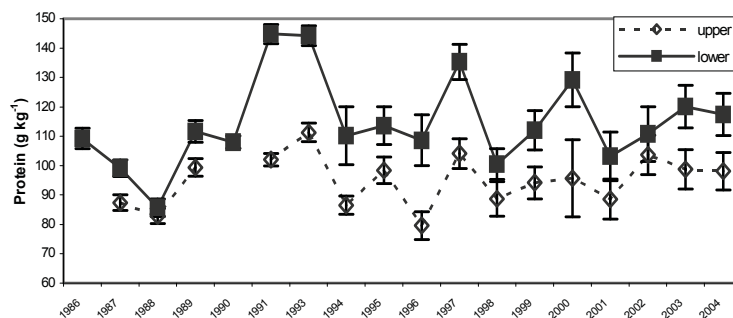


Figure 2 – Protein content (g kg<sup>-1</sup> DM) of 'dehesa' pastures in the upper and lower slope zones over a 20-years period (1986-2005). Values are means ± SE.

A significant correlation was found in the protein content between upper and lower zones ( $r^2= 0.79$ ;  $P<0.001$ ;  $n= 16$ ), which indicates similar patterns of variation in both slope zones.

The protein content was negatively related to annual precipitation ( $r^2= -0.56$ ;  $P<0.05$ ;  $n=16$ ). This could be explained because the proportion of legumes in the herbage is negatively related to the proportion of grasses ( $r^2= -0.70$ ;  $P<0.01$ ;  $n=16$ ). As the protein content of grasses is much lower than that of legumes (Vázquez de Aldana *et al.*, 2000), the increase in precipitation which leads to an increase in production, means also a decrease in the proportion of legumes, and therefore lower protein content in the herbage.

### Conclusions

Interannual variations in biomass production and protein content of upper and lower zones were correlated. Significant positive correlation was found between biomass production and annual precipitation and negative between protein content and annual precipitation.

### Acknowledgements

We are grateful to the owners of the 'dehesas' where sampling was done during all these years, specially to owners of 'Berrocal de la Espinera', 'Los Valles', 'Llen', 'Membribe' and 'Valeros'. Thanks to L. García Criado and J.C. Estévez for technical assistance.

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# Long-term effects of fertilisation on herbage composition, yield and quality of an *Arrhenatherion*-type meadow

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## Abstract

Species-rich meadows of the *Arrhenatherion* alliance, traditional lightly fertilised hay-meadows of the Swiss Plateau, are ecologically valuable. On such a meadow, we studied the long-term effects of 17 fertilisation treatments with varying amounts of mineral nitrogen (0 – 60 kg N ha<sup>-1</sup> yr<sup>-1</sup>), potassium (0 – 110.7 kg K ha<sup>-1</sup> yr<sup>-1</sup>) and phosphorus (0 – 23.2 kg P ha<sup>-1</sup> yr<sup>-1</sup>) on the yield, the nutrient content in the herbage and the botanical composition. After 10 years, herbage yield was reduced in similar proportion by the omission of N, P or K in the fertilisation. The P and K content in the herbage were strongly reduced by the input reduction of the corresponding nutrient, whereas the crude protein content was rather higher without N fertilisation. The proportion of legumes in the sward was high without N fertilisation and lowest without P or K fertilisation. Some forbs, especially *Galium album* (Miller), seemed to be favoured by low K availability combined with N and P fertilisation, but the number of plant species per plot was similar in all the treatments. Balanced low inputs of N, P and K were therefore appropriate to maintain an ecologically valuable sward.

Keywords: nitrogen, potassium, phosphorus, botanical composition, yield, nutrient content.

## Introduction

Until the middle of the last century, the hay meadows of the Swiss Plateau were traditionally cut two to three times per year and fertilised with small quantities of manure. Under such management, species-rich plant communities of the *Arrhenatherion* alliance developed. Once considered as productive fertile grassland, these ecologically and aesthetically valuable plant communities nowadays remain only in small areas due to intensification. The conservation strategy includes their recognition as ecological compensation areas and the integration of the harvested structure-rich hay into animal production. We aim at optimizing the fertilisation of this type of meadows in order to promote an ecologically interesting assemblage of plant species while maintaining a certain level of herbage yield and quality.

## Materials and methods

Five levels of mineral nitrogen (N), phosphorus (P) and potassium (K) were applied in different combinations (Table 1) during 10 years on a permanent meadow of the *Arrhenatherion* alliance. The plots were arranged in a randomized complete block design with 4 replicates. The meadow was mowed three times per year, with a first cut on the 15<sup>th</sup> of June. P and K availability in the Cambisol of the experimental site (altitude 500 m, average rainfall 1030 mm yr<sup>-1</sup>) was sufficient at the beginning of the experiment, as shown by extraction with CO<sub>2</sub>-saturated water. The forage was ground-dried on the plots to allow natural renewal of the soil seed bank. The crude protein (CP), energy (NEL), P and K contents of the harvested parts of the plants were analysed after aliquot samples from the three harvests had been mixed together. The presence of each species of vascular plant was listed and the botanical composition in terms of yield proportions was determined according to the method of Daget and Poissonet (1969).

## Results and discussion

The amount of applied N, P and K in the  $N_3P_3K_3$  treatment (Table 1) was calculated for a target dry matter (DM) yield of  $6.5 \text{ t ha}^{-1} \text{ yr}^{-1}$ , according to the Swiss standard recommendations for fertilisation (Walther *et al.*, 2001). On that site, this fertilisation rate, however, allowed to sustain a higher production level of around  $8 \text{ t ha}^{-1} \text{ yr}^{-1}$  (Figure 1A). With a fertilisation rate of 33 % ( $N_1P_1K_1$ ) and 67 % ( $N_2P_2K_2$ ) of the recommendations, the yield reached 70 % and 90 %, respectively, of the yield in  $N_3P_3K_3$ . After 10 years without applications of one of the nutrients ( $N_0P_3K_3$ ,  $N_3P_0K_3$ ,  $N_3P_3K_0$ ), the yield did not differ significantly between these three treatments and was considerably lower than in  $N_3P_3K_3$ .

Table 1. Quantities of N, P and K applied per ha and year in the fertilisation treatments.

	Fertilisation treatments														
	$N_0P_0K_0$	$N_1P_1K_1$	$N_2P_2K_2$	$N_3P_3K_3$	$N_4P_4K_4$	$N_0P_3K_3$	$N_1P_3K_3$	$N_2P_3K_3$	$N_3P_0K_3$	$N_3P_1K_3$	$N_3P_2K_3$	$N_3P_3K_0$	$N_3P_3K_1$	$N_3P_3K_2$	$N_3P_3K_4$
kg N ha <sup>-1</sup>	0	15	30	45	60	0	15	30	60	—	45	—	—	45	—
kg P ha <sup>-1</sup>	0	5.8	11.6	17.4	23.2	—	17.4	—	0	5.8	11.6	23.2	—	17.4	—
kg K ha <sup>-1</sup>	0	27.7	55.3	83.0	110.7	—	83.0	—	—	83.0	—	0	27.7	55.3	110.7

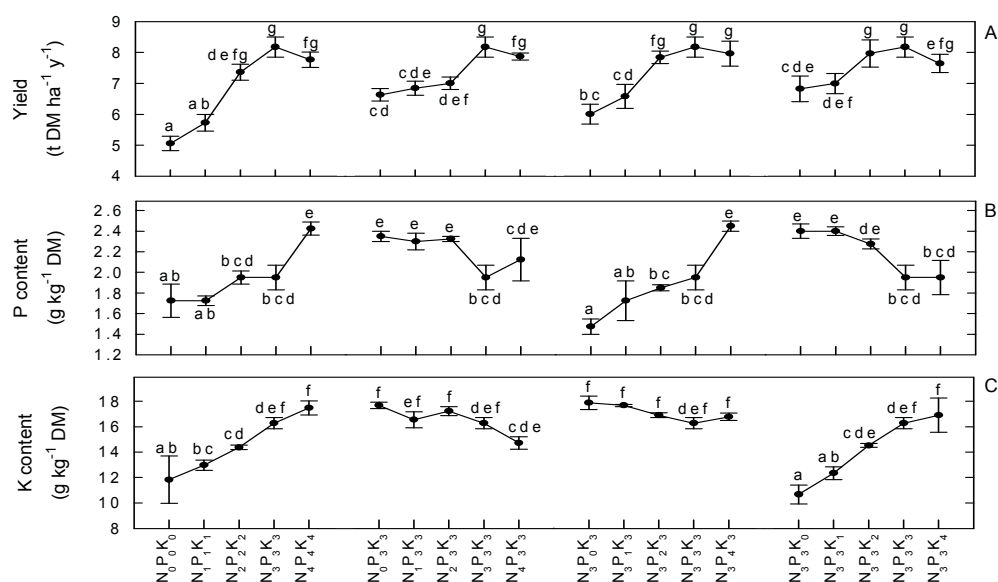


Figure 1. Effect of the fertilisation treatments after 10 experimental years on A) the yield, B) the phosphorus content and C) the potassium content of the herbage. Data are means  $\pm$  SE of 4 replicates. Means labelled with a common letter are not significantly different at 5 % level by DMRT.

The P and K contents in the herbage were strongly reduced by the input reduction of the corresponding nutrient, either alone or in combination with a reduction of the two other nutrients (Figure 1B and 1C). The CP content was rather higher without N fertilisation and was generally lower in the treatments with higher yield. Nevertheless, significant differences could only be found between  $N_0P_0K_0$  and  $N_4P_4K_4$ , and

between  $N_3P_3K_0$  and  $N_3P_3K_3$  or  $N_3P_3K_4$ . No difference in the NEL content was found (Results not shown). The number of species found on  $10\text{ m}^2$  ranged from 19 to 28, but no significant difference was found between the treatments. The species composition was similar in all treatments. However, the fertilisation affected the proportion of the different species in the sward. The proportion of legumes was high in the  $N_0P_3K_3$  and  $N_1P_3K_3$  treatments and lowest in  $N_3P_0K_3$  and  $N_3P_3K_0$  (Figure 2). Some forbs, especially *Galium album* Miller, seemed to be favoured by low K availability combined with N and P fertilisation, whereas *Ajuga reptans* L. was favoured in  $N_0P_0K_0$ . Among the grass species, only *Festuca rubra* L. and *Trisetum flavescens* (L.) P. Beauv. showed significant difference in proportion between the treatments. The yield proportion of *Festuca rubra* was lowest in  $N_0P_3K_3$  (6 %) but highest in  $N_0P_0K_0$  and  $N_4P_4K_4$  (13% in both treatments). The yield proportion of *Trisetum flavescens* was lower in the  $N_0P_0K_0$  and the  $N_1P_1K_1$  treatments with around 2 %, than in the  $N_3P_3K_3$  treatment (7 %).

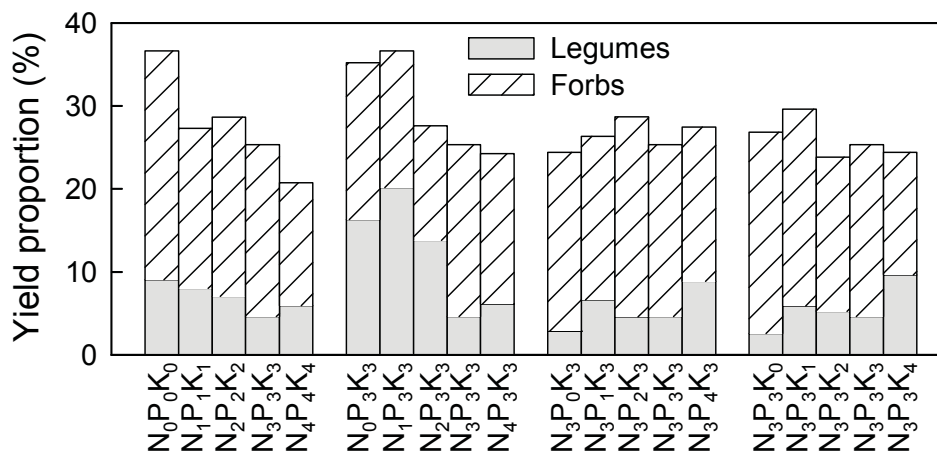


Figure 2. Effect of the fertilisation treatments on the yield proportion of the legumes and the forbs species in the swards, after 10 experimental years.

This study shows that at low levels of fertilisation, a further reduction or the cessation of the fertilisation does not guarantee an increase in plant species richness of permanent meadows. This has been observed in other studies as reviewed by Marriott *et al.* (2004). The cessation of only the P or the K fertilisation strongly reduced the proportion of legumes and did not enhance species richness. Also, the very low P content found in the herbage of the  $N_3P_0K_3$  treatment would complicate the integration of the forage in animal production. The yield and the herbage quality in  $N_0P_3K_3$  was as targeted for this type of meadows, but this treatment was of no special interest for the species richness and assemblage and could not be achieved with manure. We conclude that balanced low inputs of N, P and K in the range of the applications in  $N_2P_2K_2$  to  $N_3P_3K_3$  were appropriate to maintain the species richness of the studied *Arrhenatherion* sward and its agronomic quality.

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# Can a natural protease inhibitor decrease protein degradation of perennial ryegrass in the rumen?

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## Abstract

Recently breeders identified two populations of perennial ryegrass (*Lolium perenne* L.) that were thought to be different in protease activity. One population was bred to contain an increased concentration of the protease inhibitor (cystatin) and one population was bred to have a normal protease inhibitor concentration (control). The two populations were tested in three experiments. In the enzyme assay no significant difference could be found between the cystatin and the control population. It was surmised that the protease inhibitor was inactivated by the freeze drying process. The gas production run was done with fresh material. A small difference ( $P < 0.01$ ) between the cystatin and the control population was found in the final ammonia concentration in the rumen fluid. Therefore it was concluded that the natural protease inhibitor in the cystatin population might decrease proteolysis in ruminants. The *in vivo* experiment showed no reducing effect on the ammonia concentration in the rumen of sheep grazing the cystatin population. It is concluded that either populations were not different, or the effect of the examined protease inhibitor on proteolysis of perennial ryegrass protein in the rumen is limited.

Keywords: Plant proteases, cystatin, rumen kinetics, sheep.

## Introduction

Although large efforts have been made in the last two decades, losses of nitrogen to the environment are still a major problem in livestock production. The efficiency of nitrogen utilization by ruminants is low (16%). The rate of protein breakdown in the rumen frequently exceeds the level at which the released amino acids can be incorporated by the microorganisms. This is especially a problem when ruminants are fed on fresh grass, because grass consists of a large amount of rapidly degradable nitrogen. One possibility to improve nitrogen utilization by ruminants is to reduce the rate of protein degradation in the rumen. Very recently breeders identified, with genetic markers, the gene for cystatin (a protease inhibitor). Two different grass populations of perennial ryegrass (*Lolium perenne* L.) were created. One population was bred to contain an increased concentration of the protease inhibitor (cystatin) and was thought to slowdown the protein degradation another population was bred to have a normal protease inhibitor concentration (control) and was thought to have a faster protein degradation. These two populations were used in an enzyme assay test (exp. 1), a gas production run (exp. 2) and an *in vivo* (exp. 3) experiment to test the effect on the extent of protein degradation of perennial ryegrass in the rumen.

## Materials and Methods

Experiment 1: The two populations were sown in pots and grown in the greenhouse. After two harvests, and 21 growing days the grass was harvested using scissors. The samples were immediately freeze dried, and milled through a 1mm sieve. An enzyme assay test for protein degradation as described by Mathis (2001) was performed. Triplicate forage samples that contained 15 mg N ( $\approx 1$  g dry material) were weighed in 80 mL centrifuge tubes, and incubated in 60 mL of a borate-phosphate buffer solution (pH 8) for 1 hour at 39 °C. Subsequently, 15 mL of separate enzyme solution (*Streptomyces griseus*) was added to the borate-phosphate buffer plus forage and incubated for 0, 4, 24 and 48 hours. Following the incubation, the samples were vacuum filtered through filter floccs. The residue was washed with

distilled water. Flocks and residue were quantitatively brought into a Kjehldahl receiver and analyzed for total nitrogen.

Experiment 2: Grass samples were taken from two established pastures, which were sown with seed from the two contrasting populations, at 28 days of regrowth. The grass was cut fresh at 4 cm, and cut into pieces of 4 cm lengths using a paper cutter. Approximately 4 g of fresh grass were weighed into 140 ml bottles and incubated in triplicate with 81 ml of N-free medium, 5 ml of rumen fluid and 1 ml of reducing agent. Rumen fluid was obtained from three Holstein-Friesian bullocks offered a standard diet, consisting of 80% good quality hay and 20% concentrates. Samples were incubated in the solution at 39 °C for 0, 1, 2, 3, 4, 6, 8, 10, 12 and 24 hours. Two bottles at each time point containing no substrate were included as a blank. Following the incubation, samples (5 mL) were taken and 5 mL of trichloroacetic (TCA) was added. NH<sub>3</sub> was analyzed by the indophenol method (Searle, 1984).

Experiment 3: Four grass fed sheep were equipped with rumen fistulas. Two grazed the cystatin and two grazed the control population. An experimental period lasted for 3 days. The first day was used for adaptation. On day 2 and 3 in the experimental period rumen liquid samples were taken during the photoactive period, at 7:00, 9:00, 12:00, 15:00, 18:00, 20:00 h. and 5 ml of TCA was added. After three days, sheep changed to the other population. In a third period the sheep changed back to the initial population. The rumen liquid samples were analyzed for NH<sub>3</sub>.

Statistical analysis were done on all experiments, using the repeated measures design, with time as the within subjects factor and the population (cystatin or control) as between subjects factor.

## Results and discussion

Experiment 1: During the incubation period, protein concentration was already decreased rapidly (from 17.0 g kg<sup>-1</sup> DM to 10.0 g kg<sup>-1</sup> DM). Due to grinding of the samples (1mm), the soluble protein could easily be released. The residual protein was efficiently degraded within the first 4 hours of incubation in a buffer solution with an enzyme assay. After 4 hours of incubation no further degradation took place. No significant differences could be found between the two grass populations. It was concluded that natural breakdown inside the plant cells was not inhibited by the cystatin. But it might be that the protease inhibitor was inactivated by the drying process. Therefore, an experiment with fresh grass might give different results.

Experiment 2: Figure 1 shows the NH<sub>3</sub> concentration (mg L<sup>-1</sup>) in the bottles in the period after incubation of the grass samples. The mean NH<sub>3</sub> concentration was lower ( $P<0.01$ ) in the cystatin population than in the control population, especially at 8, 10 and 24 hours after incubation when the difference was largest. This could be related to the reduced protease activity due to the protease inhibitor, but could also be due to the DM content and CP concentration, which were lower in the cystatin compared to the control population. When the curves were expressed as ammonia concentration per mg N<sub>input</sub>, no significant differences were found between the two populations. However, the NH<sub>3</sub> concentration at the end of the gas production run was lower ( $P<0.05$ ) in the cystatin population. This might be related to a slightly higher WSC concentration in this population, but could also be an effect of the cystatin protease inhibitor.

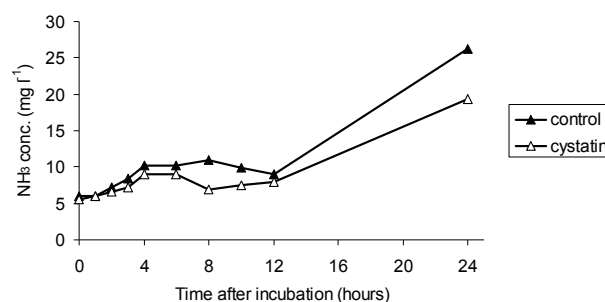


Figure 1. NH<sub>3</sub> concentration (mg L<sup>-1</sup>) of the buffer solution with rumen liquids incubated either with control (closed symbols) or cystatin population (open symbols).

Experiment 3: Figure 2 shows the NH<sub>3</sub> concentration in the rumen liquid of four sheep grazing either the control or the cystatin population during the day. The NH<sub>3</sub> concentration in the rumen was at most time points lower ( $P<0.05$ ) in the control than in the cystatin population, which was against expectation. It was only at the end of the day, at 20:00 h, when the NH<sub>3</sub> concentration was increasing that the NH<sub>3</sub> concentration of sheep grazing the cystatin population was higher than those grazing the control; however this was not significant ( $P=0.06$ ). The animal variation was high and more animals should be used in further experiments.

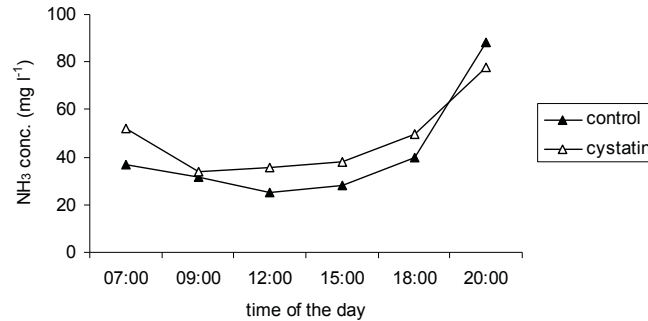


Figure 2. NH<sub>3</sub> concentration (mg L<sup>-1</sup>) of rumen liquid samples of sheep grazing either the control (closed symbols) or the cystatin population (open symbols).

### Conclusions

It is concluded that either the populations were not different, or the effect of the examined protease inhibitor (cystatin) on proteolysis of perennial ryegrass protein in the rumen is limited. In experiments with protease inhibitors, fresh material should be used, because the protease inhibitor could be deactivated by the drying process.

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## The use of NIRS to predict gas production of forage maize in different incubation intervals

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### Abstract

The *in vitro* gas production is a common technique for characterising the nutritive value of feeds for ruminants. Attempts to predict modelled kinetics of gas production in forage crops by near infrared reflectance spectroscopy (NIRS) have demonstrated only moderate ability so far. The aim of the present study therefore was to analyse the potential of NIRS to predict gas production of forage maize recorded in different incubation intervals, which may be related to the fermentation of different feed fractions. Data on gas production were obtained from maize stover and ear samples collected in a 3-year field experiment, which was conducted in Northern Germany. Statistics of NIRS calibration and validation demonstrated only limited ability to predict gas production of intervals 3-5 h, 5-16 h, and 16-72 h. While  $r^2$  values exceeded 0.87 for calibration, validation statistics were poor with  $r^2$  values below 0.70.

Keywords: forage maize, gas production, near infrared reflectance spectroscopy.

### Introduction

The *in vitro* gas production technique has proven promising for quantifying both the extent and the rate of the ruminal degradation of forage crops. This method, however, is quite time-consuming and labour-intensive, and therefore various attempts have been undertaken to use the near infrared reflectance spectroscopy as a fast and inexpensive alternative for predicting gas production kinetics. Commonly, an appropriate model is fitted to the measured gas production curves, and the model parameters are estimated by NIRS. This approach has shown only moderate success so far (Herrero *et al.*, 1997; Lovett *et al.*, 2004). The objective of the current work therefore was to explore the potential of NIRS to predict the gas production of forage maize measured in different incubation intervals, which may be related to the degradation of different feed fractions.

### Materials and methods

The study was based on data collected in a 3-year (2001-03) field experiment conducted in Kiel/Northern Germany, where eight maize varieties, covering a wide maturity range (early, mid-early, mid-late) and different maturation types (dry down, normal, stay-green), were investigated. Growth and quality change of the maize crop was recorded at six times (1 pre- and 5 post-silking) throughout the vegetation period. At each sampling date, 10 adjacent plants were harvested per plot by handclipping, separated into ear and stover, chopped and subsequently freeze-dried. In total, 480 samples of ears and 640 samples of stover were available. Samples were ground in a Cyclotec mill (Tecator) to pass a 1 mm screen and were scanned with NIR-System 5000 (FOSS, Germany) without replicates over a wavelength range of 1100-2498 nm. The software (ISI) for scanning, mathematical processing, calibration and statistical analysis was supplied with the spectrophotometer by Infrasoft International® (ISI, USA). The *in vitro* gas production was analysed according to Menke and Steingass (1988) and the volumes (in ml) of gas produced were recorded after 1, 3, 5, 7, 12, 16, 24, 48, and 72 h of incubation. Gas production of intervals 3-5 h, 3-7 h, 5-12 h, 7-12 h, 5-16 h, 7-16 h, 12-16 h, 12-24 h, 24-72 h, and 16-72 h was calculated, and NIRS calibration and validation was performed. The relationships between laboratory-determined and NIRS-predicted values were quantified using Sigma Plot 8.0.



## Results and discussion

A total of 88 (40) representative samples of ear and 210 (40) samples of stover were originally selected for the calibration (validation) set, respectively. Due to missing gas production values and outliers, the number of samples eventually included into the analysis, decreased by about 20 percent. We restrict the presentation of results to intervals 3-5 h, 5-16 h, and 16-72 h, which gave the highest prediction accuracy. Gas production of the ear samples covered a wide range of 17, 36, and 44 ml 200 mg<sup>-1</sup> DM for intervals 3-5 h, 5-16 h, and 16-72 h, respectively, while for the stover gas production showed a smaller variation (Figure 1).

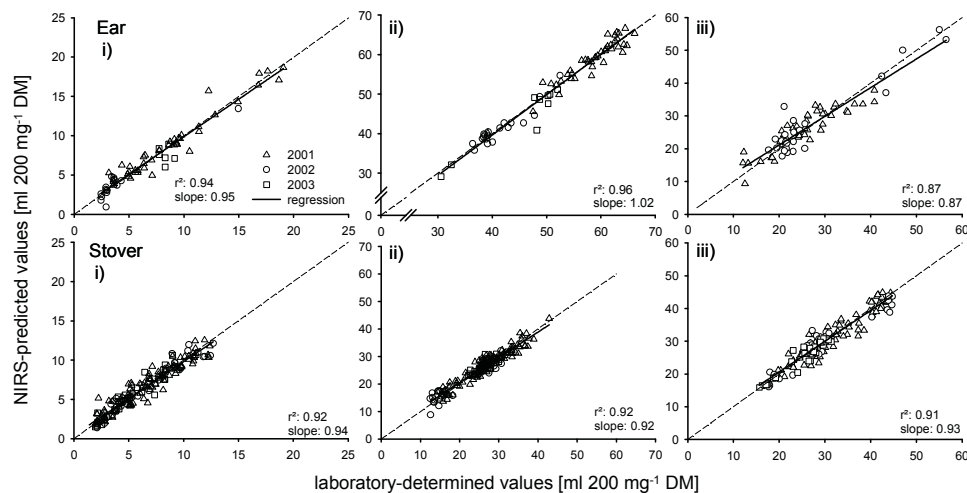


Figure 1. Relationship between laboratory- and NIRS-determined gas production (ml 200 mg<sup>-1</sup> DM) for incubation interval (i) 3-5 h, (ii) 5-16 h, and (iii) 16-72 h; calibration samples of ear and stover.

The calibration statistics generally showed high  $r^2$  values of 0.92 to 0.96 for interval 3-5 h and 5-16 h, with SEC between 0.87 and 1.82, see Table 1. The accuracy of estimate was somewhat lower for the 16-72 h interval, with  $r^2$  values of 0.87 and 0.91, and SEC of 2.20 and 3.17. In contrast to the calibration results, the evaluation of the calibration equations against the validation subsets showed a less satisfactory performance (Figure 2), with significantly higher standard error of validation (Table 1). Apparently, the agreement between observed and predicted values was slightly better for stover ( $r^2 = 0.46$ - $0.69$ ) compared to ear ( $r^2 = 0.35$ - $0.62$ ).

Table 1. Calibration statistics of the gas production (ml 200 mg<sup>-1</sup> DM) in different incubation intervals.

Incubation Interval (h)	N <sup>(1)</sup>		mean		S.D. <sup>(2)</sup>		SEC <sup>(3)</sup>		r <sup>2</sup> <sup>(4)</sup>		SEV/SEP <sup>(5)</sup>	
	ear	stover	ear	stover	ear	stover	ear	stover	ear	stover	ear	stover
3-5	66	164	7.46	6.46	4.29	3.05	1.07	0.87	0.94	0.92	3.66	1.55
5-16	69	169	52.24	25.73	9.19	6.29	1.81	1.82	0.96	0.92	6.06	2.67
16-72	71	161	26.29	29.95	8.88	7.43	3.17	2.20	0.87	0.91	5.92	4.07

<sup>(1)</sup>Number of samples; <sup>(2)</sup>Standard deviation; <sup>(3)</sup>Standard error of calibration; <sup>(4)</sup>Coefficient of determination; <sup>(5)</sup>Standard error of validation/prediction.

Lovett *et al.* (2004) suggested that the nutritional composition of maize silage, particularly the combination of grain and fibrous components, may be the cause for the difficulties to predict its gas production. We analysed ear and stover separately in order to avoid this difficulty. In addition, we differentiated into three incubation intervals, each with a specific primary degradation component (3-5 h: water-soluble carbohydrates, 5-16 h: starch and easily fermentable cell wall, 16-72 h: less degradable cell wall). Still the prediction performance was not satisfactory.

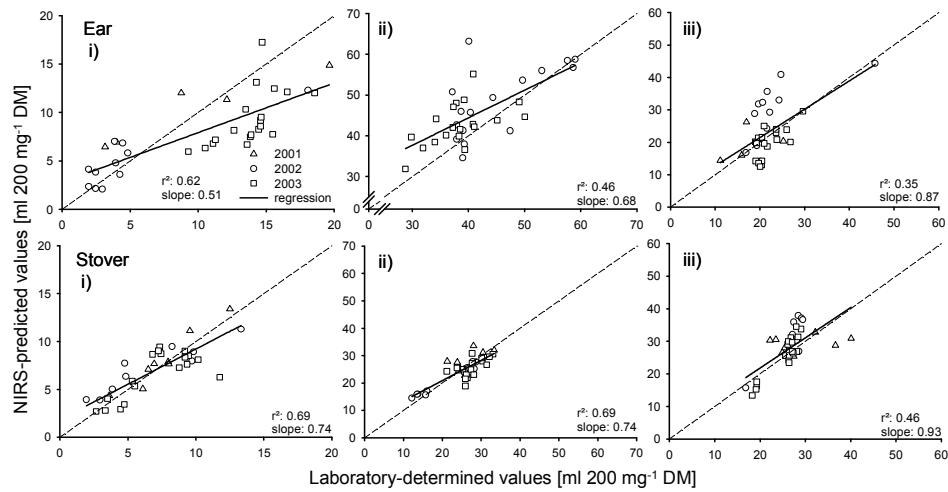


Figure 2. Relationship between laboratory- and NIRS-determined gas production (ml 200 mg<sup>-1</sup> DM) for incubation interval (i) 3-5 h, (ii) 5-16 h and (iii) 16-72 h; validation samples of ear and stover.

### Conclusions

The results indicate that NIRS prediction of gas production for specific intervals needs further improvement. Possibly a different selection of incubation intervals and an extended data base may help to obtain better prediction results.

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# Harvest time prediction of silage maize – model calibration, validation, and implementation in practical agriculture

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## Abstract

Silage maize maturation, particularly the genotype dependent differences in maturation between ear and stover, are extensively discussed in Germany currently. Points of particular interest are the determination of the optimum harvest date and the possibilities to predict maturation. The aim of a current project therefore was to develop a tool for predicting contents of dry matter (DM) and starch. For this purpose the suitability of three models, two growing-degree day (GDD) approaches and the mechanistic MAISPROG model, was tested. Model calibration and validation, which was based on a multi-year, multi-site experiment, showed a generally satisfactory model fit. The consideration of radiation and soil water availability in MAISPROG improved the prediction accuracy substantially compared to the GDD models. In 2005, MAISPROG was implemented nationwide as a regional harvest time prognosis tool for practical use in agriculture.

Keywords: silage maize, modelling, dry matter content, whole crop, ear.

## Introduction

The optimal harvest time for forage crops is of vital concern for ruminant nutrition since crop maturity at harvest affects both roughage yield and quality. Environmental conditions, particularly temperature and soil water supply play a key role in the dynamics of growth and quality. The maturation of silage maize, particularly the genotype dependent differences in maturation between ear and stover, are extensively discussed in Germany currently. Points of particular interest with respect to the optimisation of feed value are the determination of the optimum harvest date and the possibilities to predict maturation. Therefore a project was initiated aiming at the development of a tool for regional harvest time prediction. Performance of the following three models, which forecast dry matter and starch content of the whole crop and ear dry matter content, was compared: (i) a growing degree-day concept (GDD-6) with a 6 °C base temperature (Bloc *et al.*, 1983, AGPM, 2000), (ii) a GDD concept with a 8 °C base temperature (GDD-8), and (iii) the mechanistic MAISPROG model originally developed for forage grasses (Kornher *et al.*, 1991).

## Materials and methods

Model calibration was based on data collected in a 4-year experiment (2000-2003) on more than 20 sites throughout Germany. Data for model validation was acquired in 2004 on 9 sites, with the cultivar assortment being modified (Table 2). Biomass accumulation and quality change, i.e. contents of dry matter and starch (data not presented), of 8 hybrids covering a wide maturity range and different maturation types (normal, dry-down and stay-green), was recorded at 4 to 7 dates throughout the vegetation period. Maize was sown between mid of April and early May in rows 0.75 m apart. The planting density and the amount of nitrogen fertilizer applied was adjusted to local conditions in order to allow maximum production. Nitrogen fertilization, however, was limited to 150 kg N ha<sup>-1</sup> maximum. MAISPROG is one of few models that not only predicts biomass but also provides a comprehensive simulation of various forage quality parameters (Herrmann *et al.*, 2005). It consists of two dynamically

interacting sub-models for dry matter production and quality development driven by plant and soil characteristics, weather data, and soil water availability. In the two GDD models, DM contents of the whole crop and the ear were calculated as a three parameter exponential and polynomial function of GDD units, respectively. Calculations started at sowing for the whole crop and at silking for the ear. The goodness of the model predictions was assessed by the root mean square error (RMSE) and the coefficient of determination ( $R^2$ ).

## Results and discussion

The adaptation of MAISPROG from grassland to forage maize required no modifications with respect to the model algorithms. For the DM content of the whole crop and the ear model calibration resulted in a generally good agreement for all models, see Table 1. The consideration of radiation and plant available soil water in MAISPROG, however, allowed an improved prediction accuracy, reducing the error on average by 9% for whole crop DM content and 6% for ear DM content compared to the GDD-8 model. Especially the 2003 data contributed valuable information with respect to the impact of soil water availability on DM content changes, because growing conditions were characterized by severe drought on various sites. Under such drought conditions MAISPROG was able to reduce the prediction error by up to 71 % (data not presented). A comparison of the two GDD models showed a better agreement for whole crop DM content when using 8 °C base temperature, while for ear DM content GDD-6 resulted in lower RMSE values.

Table 1. Results of model calibration for DM content ( $\text{g kg}^{-1}$  FM) of the whole crop and ear, given as number of observations, coefficient of determination and RMSE for each hybrid.

	Hybrid	n	MAISPROG		GDD-6		GDD-8	
			$R^2$	RMSE	$R^2$	RMSE	$R^2$	RMSE
Whole crop	Arsenal	391	0.90	36.2	0.87	40.8	0.87	40.7
	Oldham	391	0.90	34.0	0.87	39.3	0.87	38.3
	Symphony	391	0.92	30.7	0.87	37.8	0.88	36.4
	Probat	391	0.91	31.9	0.88	36.6	0.88	35.8
	Attribut	391	0.91	30.4	0.88	35.4	0.89	33.2
	Fuego	391	0.90	30.6	0.87	34.4	0.88	33.4
	Clarica	280	0.88	33.3	0.85	36.9	0.87	33.7
	Benicia	281	0.88	31.2	0.86	34.1	0.87	32.0
Ear	Arsenal	267	0.94	32.2	0.94	32.4	0.93	33.4
	Oldham	267	0.92	35.7	0.91	39.4	0.91	39.0
	Symphony	267	0.91	36.4	0.90	38.5	0.89	39.0
	Probat	267	0.95	31.9	0.92	39.2	0.91	40.2
	Attribut	266	0.95	32.2	0.94	32.8	0.94	33.7
	Fuego	266	0.92	37.5	0.92	35.8	0.92	37.6
	Clarica	198	0.93	36.4	0.93	36.9	0.94	35.9
	Benicia	198	0.94	36.0	0.93	36.1	0.93	36.7

Model validation resulted in prediction errors of 1.8 to 3.0% for the whole crop and 2.3 to 3.0% for the ear, with only a slight superiority of the MAISPROG model. The minor differences in model fit may be attributed mainly to the lack of drought conditions in 2004. In contrast to calibration, the GDD-8 model showed a higher prediction accuracy compared to the GDD-6 model for both, whole crop and ear.

Table 2. Results of model validation for DM content ( $\text{g kg}^{-1}$  FM) of the whole crop and ear, given as number of observations, coefficient of determination and RMSE for each hybrid.

	Hybrid	n	MAISPROG		GDD-6		GDD-8	
			R <sup>2</sup>	RMSE	R <sup>2</sup>	RMSE	R <sup>2</sup>	RMSE
Whole crop	Arsenal & Justina	43	0.91	19.4	0.84	25.2	0.89	20.4
	Oldham	36	0.85	23.7	0.78	30.3	0.82	23.9
	Probat & LG3226	39	0.85	19.6	0.78	26.6	0.82	21.8
	Benicia	33	0.88	17.8	0.79	22.0	0.87	18.0
Ear	Arsenal & Justina	40	0.92	29.6	0.92	30.5	0.93	26.9
	Oldham	33	0.93	25.6	0.92	27.5	0.93	26.2
	Probat & LG3226	36	0.96	22.4	0.93	28.5	0.94	27.8
	Benicia	33	0.94	29.2	0.94	31.2	0.96	30.8

In 2005, the MAISPROG model was implemented nationwide as regional harvest time prognosis tool for practical use in agriculture. Forecasts of dry matter content are available free of charge at [www.maisprog.de](http://www.maisprog.de). After logging in, the user receives an individual prognosis by specifying the hybrid grown, the sowing date, and the soil type. Furthermore, the amount of precipitation can be adjusted to local conditions. To obtain more stable predictions, a maximum of eight weather stations closest to the site are selected automatically according to the zip code, predictions are calculated separately for each station, and are then combined to a weighted average using proper distance weights.

### Conclusions

The MAISPROG model proved as a useful tool to determine the optimum harvest time span of forage maize according to the type of maturation, and to allow the production of high quality maize silage. Further model development is in progress and among other modifications will include the impact of photoperiod on quality change, which in the current version is only available for long-day plants.

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# Influence of different silage making technologies on fermentation characteristics and nutritive value of legume-grass silage

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## Abstract

A first-cut 10-12 h wilted red clover-grass (60 % - red clover (*Trifolium pratense* L. cv. Arimaiciai), 25 % - timothy (*Phleum pratense* L. cv. Gintaras), 10 % - fescue (*Festuca pratensis* Huds. cv. Kaita) and 5 % - others) sward was ensiled into a ferro-concrete trench or in big bales. Method of conservation had no effect on the chemical composition and fermentation characteristics of the silages produced. Therefore, both silages were consumed readily and there were no differences among technologies. There were no significant differences in liveweight gain and control slaughtering data between trench or bale silages.

Keywords: trench, big bale, fermentation, fattening bulls, slaughtering.

## Introduction

Grasses are a very important component of feed rations for cattle in Lithuania, because they account for 70-75% of the total feed consumed by cattle. Effective integration of grazing and grass conservation enables conservation of grass as silage and providing forage for winter feeding of livestock. Silage quality and nutritional value are influenced by numerous biological and technological factors, including: the crop species, stage of maturity and dry matter (DM) content at harvest, chop length, type of silo, rate of filling, forage density after packing, sealing technique, feedout rate, weather conditions at harvest and feedout and additive use (Pozdišek *et al.*, 2003). The fermentation processes during ensiling alter the chemical composition that can affect both the nutritive value and the intake of forage (Cushnahan and Gordon, 1995). Clamping is generally the most cost-effective method of producing silage, however if bales are prepared the ensiling process is quicker, resulting in more efficient use of available substrates (Fychan *et al.*, 2002). However, big bales technology does not use chopped material and this means that the fermentation is not same as for clamp silage. One of the differences of these ensiling methods is that grass for trench silage is precision chopped while that for big bale silage is not chopped. Forages that are finely chopped are rapidly consumed and fermented in the rumen, which reduces the animals chewing time, rumen fluid pH and the acetate-to-propionate ratio, and decreases the number and activity of fiber-degrading bacteria (Muck and O'Kiely, 2002). The objective of this trial was to examine the difference in silage fermentation characteristics when herbage is ensiled in big bales or in clamps and to investigate the nutritive value of silages for fattening bulls.

## Materials and methods

The silages were made from a first-cut 10-12 h wilted red clover-grass (60 % - red clover (*Trifolium pratense* L. cv. Arimaiciai), 25 % - timothy (*Phleum pratense* L. cv. Gintaras), 10 % - fescue (*Festuca pratensis* Huds. cv. Kaita) and 5 % - others) sward during its second season after sowing. The sward was cut at the flowering stage of red clover on 21 June, 2004 with a mower conditioner (Kverneland 347). The legume-grass mixture from half of the field was chopped with a chop harvester (E-281) and ensiled in a ferro-concrete trench. The other half of the legume-grass was ensiled using a baler (Greenland RF 130). Samples of fresh forage were collected from the sward to determine its chemical composition. Herbage samples were chopped and subsampled prior to analysis. Five control bags of 1 kg weight each were put into the trench silage to determine dry matter (silage fermentation) losses. Five big bales of silage were weighed after wrapping and again after 90 days of storage to measure dry

matter losses. After withdrawal and weighing of the control bags from the trench silage and after weighing the bales and sampling silages from bales, the chemical composition, fermentation quality and dry matter losses of silages were determined. Aerobic stability was measured by changes in silage temperature following exposure to air for 8 days. Each silage was offered *ad libitum* to Lithuanian Black- and- White fattening bulls (8 bulls per treatment) during the 143-day-trial following a 20 day acclimatisation period. Concentrates (1.85 kg DM per bull per day) were offered at the same level to the both groups of bulls. Silage intake was recorded once per week and the bulls were weighed each month. After the feeding period, three bulls from each group were slaughtered and carcass weight, dressing percentage, and the yield of meat and bone in the left side of the carcass were determined. The data were analysed by one-way ANOVA, and a mean comparison by Fisher'PLSD.

## Results and discussions

When compared to trench silage, silage made in big bales had a significantly higher ( $P<0.05$ ) dry matter content, and therefore the pH value was higher (4.65 vs 4.52) and the content of fermentation acids were lower ( $36.2 \text{ g kg}^{-1}\text{DM}$  vs  $39.7 \text{ g kg}^{-1}\text{DM}$ ) than that of chopped trench silage (Table 1). Ammonia-N concentration and dry matter losses were significantly lower ( $P<0.05$ ) in big bale silage compared with trench silage.

Table 1. Chemical composition of the wilted herbage and legume-grass silage ensiled using different methods.

	Herbage	Silage made in trench (T)	Silage made in big bales (B)	LSD <sub>0.05</sub>	LSD <sub>0.01</sub>	$S_{\bar{x}}$
Dry matter (DM; $\text{g kg}^{-1}$ )	314.6	303.6	308.5*	4.302	7.134	0.358
In DM ( $\text{g kg}^{-1}$ DM):						
crude protein	128.0	139.8	143.3	10.998	18.238	1.979
crude fibre	288.2	313.7	311.8	6.549	10.86	0.533
WSC	92.0	17.5	21.1	4.542	7.532	5.988
NDF	519.0	614.3	615.3	2.903	4.813	0.12
ADF	303.4	362.4	359.2**	0.907	1.503	0.064
D-value		692.5	695.1	7.14	11.84	0.262
Total fermentation acid		39.7	36.2	10.682	17.714	7.161
Lactic acid		22.1	21.7	10.50	17.412	12.216
Acetic acid		17.5	14.4	3.558	5.90	5.645
Butyric acid		0.02	0.00	0.054	0.09	125.967
Ammonia N ( $\text{g kg}^{-1}$ N)		50.8	45.6*	5.049	8.373	2.668
pH		4.52	4.65	0.185	0.306	1.025
ME ( $\text{MJ kg}^{-1}$ DM)		8.89	8.94	0.107	0.177	0.305
DM losses ( $\text{g kg}^{-1}$ DM)		103.5	80.9*	22.023	36.52	6.085

WSC: Watersoluble carbohydrates; NDF: Neutral detergent fiber; ADF: Acid detergent fiber; D-value: Digestibility; ME: Metabolic energy;  $S_{\bar{x}}$  : accuracy of trial; \* and \*\* denotes significant at level 0.05 and 0.01 respectively.

The aerobic stability as observed from the temperature profiles of both silages was similar (Figure 1).

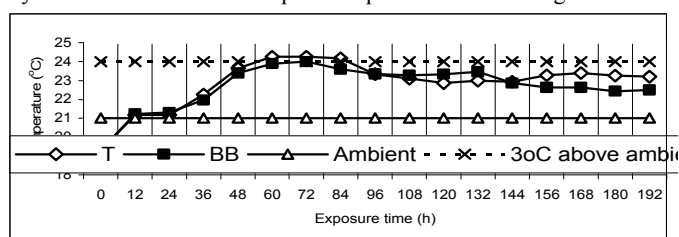


Figure 1. Changes of temperature in trench (T) and big bale silages (BB).

Means for silage DM intake are shown in Table 2. Both silages were consumed readily, and there were no differences among different technologies, though Andrieu *et al.*, (1999) found that silage making in big bales reduces organic matter digestibility and often also voluntary intake. There were no significant differences in liveweight gain or in data between cattle offered trench or bale silages over the whole experiment.

Table 2. Feed intake, animal performance and control slaughtering data.

	Intake (kgd <sup>-1</sup> )			ME (MJ d <sup>-1</sup> )	Body Weight gain			Control slaughtering data		
	Silage DM	Total DM	FU		(kg d <sup>-1</sup> )	Total (kg)	Weight (kg)	Killing out (%)	Carcass and abdominal fat yield (%)	Muscling score (unit)
Trench	7.19	9.04	8.39	88.93	1.115	159.4	551.7	51.76	53.58	4.22
Big bale	7.26	9.11	8.42	89.82	1.143	163.5	547.7	52.05	53.87	4.26
LSD <sub>0.05</sub>	0.205	0.20	0.18	2.106	0.088	12.7	47.912	3.123	3.202	0.323
LSD <sub>0.01</sub>	0.321	0.32	0.29	3.303	0.139	19.92	110.52	7.203	7.386	0.746
S <sub>x</sub>	0.779	0.62	0.61	0.648	2.155	2.164	1.433	0.989	0.979	1.253

FU: Feed units.

### Conclusions

The fermentation quality of either trench or big bale silages was good and they had a high nutritive value. Method of conservation did not influence voluntary intake or the animal performance. Therefore both baling and clamping are suitable methods for ensiling legume-grass, and the choice of which to use can be based on the availability of equipment and facilities.

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## Modelling of fibre fractions in forage maize

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### Abstract

Over the last decades, maize has become a key to high production on many livestock farms, which is mainly based on its proven ability to produce high quality silage. Apart from genotype, which is a major determinant of silage maize quality, environmental conditions play a decisive role in yield and quality formation. The objectives of the current study were (i) to analyse the genotypic variation and the impact of weather conditions on the content of fibre fractions over the vegetation period, and (ii) to investigate whether the influence of environmental conditions can be explained using the weather-based model FOPROQ (Kornher *et al.*, 1991; Herrmann *et al.*, 2005), originally developed for forage grasses. Data for model calibration and validation were collected in a 3-year field experiment conducted in Northern Germany. Eight varieties, covering a wide range of maturity and different maturation types were harvested during each vegetation period six times and separated into ear and stover. A comparison of model output with observed data showed a satisfactory performance indicating the potential of the FOPROQ model to predict fibre fraction content not only for grass but also for forage maize.

Keywords: forage maize, fibre fractions, modelling, variety.

### Introduction

The determination of harvest time in forage maize is a prerequisite for minimising losses during silage storage and the feedout phase, and for exploiting the yield and forage quality potential of hybrids. To achieve this, a Germany-wide project was initiated, aiming to develop a model for prediction of harvest time in forage maize. In addition to dry matter (DM) content other meaningful parameters with respect to fodder quality were analysed such as the content of neutral detergent fibre (NDF) and acid detergent fibre (ADF). The contents of fibre fractions throughout the vegetation period are influenced by genotype and environmental conditions. Increasing maturation of stover is reflected in increasing NDF and ADF contents, while declining contents in ear occur because of increasing starch accumulation. The objectives of this study are to analyse the impact of variety and stage of maturity on the NDF and ADF content of ear and stover and to investigate whether the influence of environmental conditions, e.g. air temperature, radiation and plant-available soil water can be quantified for the whole crop by using the weather-based FOPROQ model.

### Materials and methods

The study was based on data collected in a 3-year (2001-03) field experiment conducted at the experimental farm 'Hohenschulen' of the Christian-Albrechts-University of Kiel on a sandy loam soil with daily mean air temperature in the vegetation periods 2001-03 of 14.8, 16.4 and 16.7 °C, precipitation of 436, 445 and 210 mm, and radiation of 1658, 1651 and 1732 J cm<sup>-2</sup>. A one-factorial block design was used for the field trial, where 8 maize varieties, covering a wide maturity range (early, mid-early, mid-late) and different maturation types (dry down, normal, stay-green), were investigated. Growth and quality change of the maize crop was recorded at 6 times (1 pre-, 5 post-silking) throughout the vegetation period. Ten adjacent plants were harvested per plot by handclipping, separated into ear and stover, chopped and subsequently freeze-dried. The content of NDF and ADF of the fractions was estimated by NIRS with calibration and validation being based on Goering and van Soest (1970, cited in: Naumann and Basler, 1976). Statistical analysis was conducted using Proc Mixed (SAS 8.2), by assuming a heterogeneous, auto-regressive covariance structure for repeated measurements. Comparison of means was performed by t-test with a Bonferroni-Holm adjustment. The contents of the whole crop

were calculated from the corresponding values of ear and stover. Post-silking values served as input for the FOPROQ model, where quality changes depend on genetical, management and environmental input. Environmental factors (temperature, radiation and plant-available soil water) are converted into corresponding change rates based on proper functions, added up and related to the relevant quality parameter. Model parameters were optimised for each cultivar separately, and model fit was assessed by root mean square error (RMSE) and coefficient of determination ( $r^2$ ).

## Results and discussion

Statistical analysis showed significant interactions of variety (within maturity group) and harvest date in both ADF and NDF content (Table 1).

Table 1. Statistical analysis of the stover and ear NDF- and ADF-content.

Effect	Num DF		F Value				Pr>F			
	NDF&ADF		NDF		ADF		NDF		ADF	
	Stover	Ear	Stover	Ear	Stover	Ear	Stover	Ear	Stover	Ear
year	2	2	56.16	15.70	19.50	46.26	<.0001	<.0001	<.0001	<.0001
maturity	2	2	63.02	29.63	27.47	42.38	<.0001	<.0001	<.0001	<.0001
variety (maturity)	5	5	7.20	3.34	10.67	3.08	<.0001	0.0110	<.0001	0.0170
harvest date	5	4	1050.73	276.05	1337.79	316.60	<.0001	<.0001	<.0001	<.0001
block	1	1	7.78	0.59	7.87	0.66	0.0074	0.4491	0.0067	0.4242
maturity*harvest date	10	8	11.71	4.78	6.71	6.22	<.0001	<.0001	<.0001	<.0001
variety (mat.)*har. date	25	20	2.21	2.10	2.27	2.11	0.0068	0.0116	0.0059	0.0109
year*maturity	4	4	2.30	2.74	1.41	7.97	0.0711	0.0435	0.2422	0.0001
year*variety (mat.)	10	10	2.13	2.19	2.27	1.58	0.0388	0.0419	0.0244	0.1600
year*harvest date	10	8	41.44	16.03	38.14	18.92	<.0001	<.0001	<.0001	<.0001

In stover, the contents differed significantly at harvest dates 2 and 3 within the early and at dates 4 and 5 within the mid-early maturity group. Furthermore, significant differences were detected in ear ADF content of the mid-early group at harvest date 5. At silage maturity, differences within the groups were not significant. We therefore displayed in Figure 1 only the means of the maturity groups. In the stover NDF and ADF contents increase from 475 to 730 g kg<sup>-1</sup> DM and 261 to 439 g kg<sup>-1</sup> DM, respectively, whereas contents decline in the ear (401-180 g kg<sup>-1</sup> DM and 193-75 g kg<sup>-1</sup> DM) because of starch accumulation.

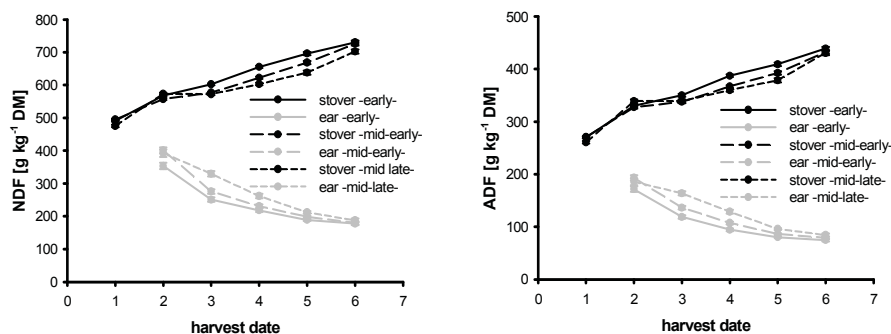


Figure 1. NDF and ADF content of stover and of ear for the early, mid-early and mid-late maturity group; means of three years (I: standard error).

The model was applied in order to examine whether the environmental conditions, i.e. temperature, radiation, plant available soil water are sufficient to explain the observed significant year effects.

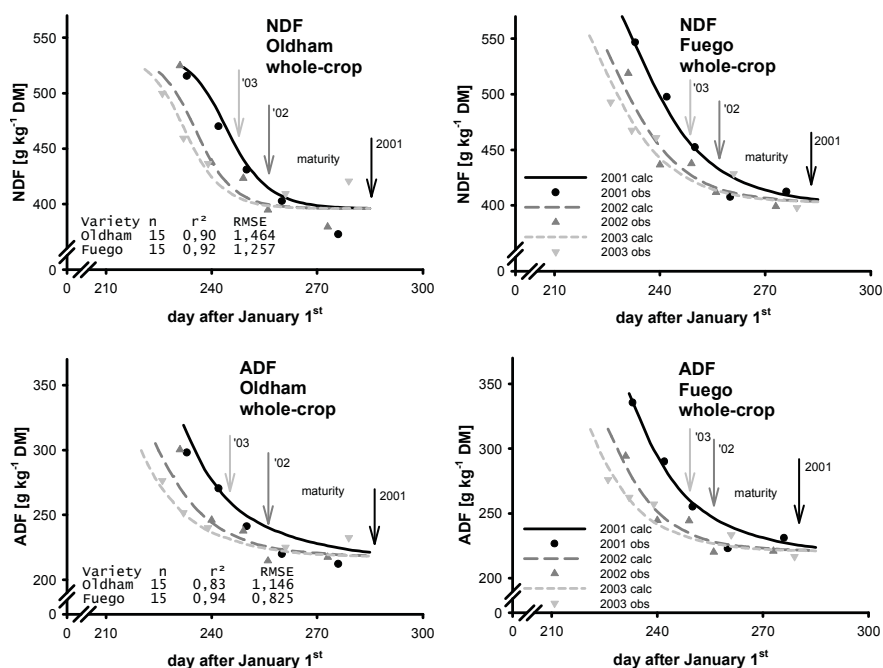


Figure 2. Observed (symbols) and calculated (lines) NDF and ADF contents of two selected cultivars in the years 2001-2003, arrows indicating silage maturity in corresponding years.

Figure 2 presents the observed and calculated NDF and ADF contents of the whole-crop for Oldham (early maturity group; synchronous maturation of ear and stover) and Fuego (mid-early group; stay green type). Overall, the model performs well, as indicated by coefficients of determination between 0.83 and 0.94, and root mean square errors below 14.64. Slight deviations, however, became apparent for NDF content of Oldham at the end of the vegetation period. At silage maturity differences in fibre content among years caused by environmental conditions were approximately 30 g ADF kg<sup>-1</sup> DM and 50 g NDF kg<sup>-1</sup> DM. This was mainly attributed to lower temperature in 2001, whereas the impact of radiation and precipitation (about 230 mm lower in 2003 compared to 2001 and 2002) was not such pronounced.

In conclusion, our results demonstrate that the FOPROQ model allowed to explain the significant year effects on fibre content of forage maize by environmental impact. Further modelling work will include model validation using a fourth experimental year.

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# Prediction of protein in fresh leaves of alfalfa by NIRS with an interactance-reflectance probe

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## Abstract

The objective of this work was to estimate the protein content of fresh leaves of alfalfa (*Medicago sativa* L.), using near infrared reflectance spectroscopy (NIRS) technology. The sample set included 33 varieties grown under irrigation in the province of Salamanca (Spain). Two cutting dates were considered, and samples of leaves were taken from three plant positions (apical, middle and basal). A total of 190 samples were obtained. The protein content ranged between 9.92-45.32 % of dry matter. NIRS calibrations were developed by two regression methods (multiple linear regression-MLR and partial least squares regression-PLSR) and three mathematical treatments (log 1/R, first and second derivatives). The best regression model reached a coefficient of multiple determination ( $r^2$ ) of 0.68 and a standard error of prediction (SEP) of 3.27 % in validation. This study found that NIRS calibrations based on spectra of fresh leaves have potential for the rapid screening of crude protein in forage breeding programs.

Keywords: protein, NIRS, fresh alfalfa leaves.

## Introduction

Forage quality of alfalfa (*Medicago sativa* L.) is a prime consideration in the development of a profitable harvest management program. Nutritional parameters, such as protein content, are important to decide when to harvest, for the selection of varieties, or to detect quality changes between harvests. On the other hand, in plant physiology studies, the diagnosis of nutrient status is based on the chemical analysis of plant material, mainly leaves. Depending on the degree of the nutrient migration, younger or older leaves should be used for analysis. Near infrared reflectance spectroscopy (NIRS) has emerged in the last 30 years as a rapid method for testing the quality, and characterise the composition of forages. Typically, forages analysed using this technique are dried and ground prior to scanning. However, relatively few studies have been carried out to evaluate the potential of NIRS in undried forages (Park *et al.*, 1999). The aim of this study was the development of NIR calibrations for protein content estimation using a spectral reading system based on a reflectance probe and fresh leaves. Fibre-optic offers considerable opportunities to work directly with intact material as a first step toward the non-destructive and rapid evaluation of plant samples.

## Material and methods

A total of 190 samples of fresh leaves of alfalfa were obtained from 33 varieties grown under irrigation in the province of Salamanca (western Spain). In two different harvests done at anthesis (August and September, 2003), leaves were sampled from three positions: basal, medium, and apical. The NIR spectra of the central leaflets were recorded using a 1.5 m optical fibre probe connected to a FT-NIR InfraProver II (Bran+Luebbe, Norderstedt, Germany). The reflectance probe is a bi-directional optical fibre bundle for diffuse reflectance measurements from 1100 to 2200 nm. Protein content (% DM) was estimated by the reference Kjeldahl method. NIR calibrations were developed by two methods: multiple linear regression (MLR), and partial least squares regression (PLSR), using in both cases three data transformations: log 1/R, first and second derivative (1D, 2D). Other details of the experiments and calibration development can be read in Petisco *et al.* (2004).

## Results and discussion

The statistical data of the samples included in the calibration and validation sets, determined by the reference method, are summarized in Table 1. A wide range of variation was obtained as a result of sampling leaves of 33 different varieties in three plant positions, and including two cutting dates.

Table 1. Chemical analysis of alfalfa leaves (% DM).

Component	Set	N <sup>(1)</sup>	Range	Mean	SD <sup>(2)</sup>
Protein (%)	Calibration	120	9.92-45.32	26.88	9.86
	Validation	70	13.44-43.85	28.69	8.24

<sup>(1)</sup> Number of samples; <sup>(2)</sup> Standard deviation.

NIR spectra of central leaflets of three fresh leaves taken from different plant positions (apical, middle and basal) are shown in Figure 1. The hydrogen bonds in water absorb significant amounts of NIR radiation and result in broad peaks that obscure spectral information derived from other compounds (Abrams *et al.*, 1988). Thus, the spectra in Figure 1 are dominated by two IR absorption maximums, peaking around 1450 and 1940 nm and due to the water content.

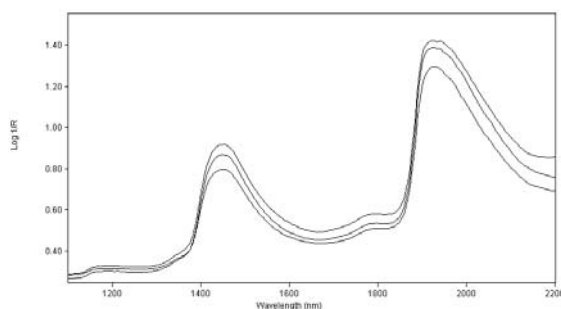


Figure 1. Near-infrared spectra of three alfalfa leaf samples.

Table 2. Calibration and validation statistics using multiple linear regression (MLR) and partial least squares regression (PLSR) methods.

	MLR			PLSR			
	Log 1/R	1D	2D	Log 1/R	1D	2D	
<b>Protein (%)</b>							
<i>Calibration set</i>							
No. terms	7	7	7	No. factors	13	6	7
R <sup>2</sup> <sup>(1)</sup>	0.80	0.84	0.78	R <sup>2</sup> <sup>(1)</sup>	0.94	0.91	0.96
SEC <sup>(2)</sup>	4.43	4.08	4.59	SEC <sup>(2)</sup>	2.45	3.10	2.01
				SECV <sup>(3)</sup>	4.81	4.48	5.42
<i>Validation set</i>							
r <sup>2</sup> <sup>(4)</sup>	0.59	0.62	0.67	r <sup>2</sup> <sup>(4)</sup>	0.66	0.68	0.57
SEP <sup>(5)</sup>	3.13	3.68	3.39	SEP <sup>(5)</sup>	3.63	3.27	4.57

<sup>(1)</sup> Coefficient of multiple determination; <sup>(2)</sup> Standard error of calibration; <sup>(3)</sup> Standard error of cross validation; <sup>(4)</sup> Coefficient of determination; <sup>(5)</sup> Standard error of prediction

Statistics reported for NIRS calibrations using MLR and PLSR are listed in Table 2. Figure 2 shows the relationship between NIRS and reference data for protein content in the external validation set. The

accuracy of the statistics of calibration was better with PLSR, however, there were no large differences between the statistics of external validation obtained with both regression methods. Thus, we obtained a coefficient of determination ( $r^2$ ) and a standard error of prediction (SEP) of 0.67 and 3.39% (MLR, 2D) and 0.68 and 3.27% (PLSR, 1D), respectively. Coefficients of multiple determination obtained were similar to those reported by Dardenne *et al.* (1996) and Gatius *et al.* (2003) for fresh alfalfa; however SEP values were higher than those achieved by the aforementioned authors. These results could be due to the different approach of our experiment, using a reflectance probe, and central leaflets. According to Reeves and Van Kessel (2000) fibre-optic bundles are at least partially responsible for the reduced accuracy found with fibre-optic systems when compared to other methods of sample presentation. However, this study was designed to anticipate agronomic requirements and to this end spectra were collected using a fibre optic interactance probe. Further efforts are needed for developing accurate and robust calibrations for chemical constituents using fibre-optic based spectrometers. NIRS calibrations based on spectra of fresh leaves may have potential for the rapid screening of crude protein in forage breeding programs and agronomical studies, once sampling presentation and calibration and data set size and structure are optimised.

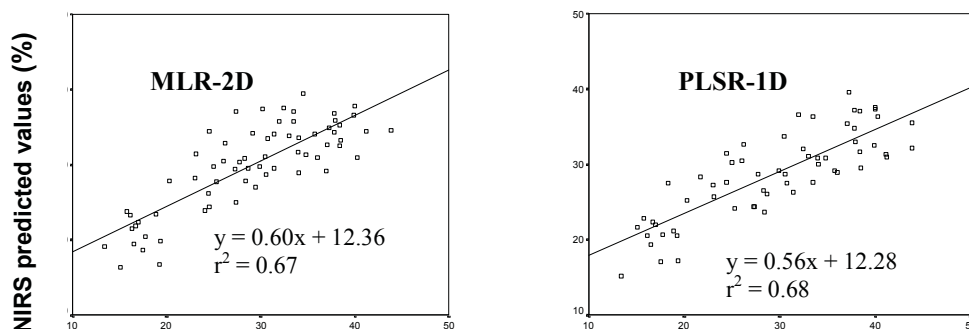


Figure 2. Relationship between protein content determined by Kjeldahl and NIRS methods in the external validation set.

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# NIRS as a tool to predict nutritive quality of raw Total Mixed Rations with silages incorporated

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## Abstract

Traditional wet chemical analysis of animal feeds has been used to characterise their nutritive composition, but these procedures are costly, time-consuming and sometimes hazardous. In recent years, Near Infrared Reflectance Spectroscopy (NIRS) has emerged as a robust and rapid alternative methodology for testing the quality and characterising the composition of animal feeds. Typically feeds analysed using this technique have been dried and milled prior to scanning. The objective of this work was to develop NIR calibrations for a wide range of nutritive parameters based on scanning Total Mixed Rations (TMR) containing all ingredients as raw materials, even undried maize and grass silages. This study has been developed with one hundred and twenty three (n=123) TMR samples, from different dairy farms across Spain. Two different charges of each sample were scanned as duplicated replicates by using a Foss NIRSystem 6500 monochromator and the average spectrum was calculated. Samples were analysed using a traditional wet chemical analysis in order to quantify dry matter, crude protein, crude fat, acid detergent fiber and starch. Calibration equations developed confirm that NIR could accurately predict the nutritive parameters studied.

Keywords: NIR, raw materials, silage, Total Mixed Ration, nutritive value.

## Introduction

Nowadays, different studies have reported that higher milk production is observed when cows consume nutritionally balanced total mixed rations (TMR) (Marbán, 2003); among the potential advantages of TMR (containing undried or prewilted silage) include the provision of a more uniform ration throughout the year and the grazing season, besides milk yield, fat and true protein contents increasing. NIR spectroscopy has proved to be a useful and valuable, non destructive technique for rapid analysis of feed as tool for quality control or composition. However, traditionally NIRS analysis has been performed by scanning dry-milled samples. The use of NIR for ungrounded ingredients (Martínez *et al.*, 2004; Pérez-Marín *et al.*, 2004) has proved to be advantageous, allowing a substantial reduction on sample pretreatment. This is really important in the TMR system feed due to the necessary control feed quality on a daily basis.

## Materials and methods

*Samples.* This study involved one hundred and twenty three (n=123) TMR samples, collected from different farms across Spain during years 2002 to 2004. The same portion of the sample used to record spectrum was dried and milled to 1 mm for chemical determination of the analytical parameters. Chemical analysis was performed using traditional analytical methodologies: dry matter content (DM) using dry-forced oven (60°C for 24 hours), ash and fat (AOAC, 1984), crude protein (CP) as nitrogen ( $N_{\text{Kjeldahl}} \times 6.25$ ), starch (ST) after gelatinisation and hydrolysis to glucose as described by Soldado *et al.* (2003) and acid detergent fiber (ADF) by the procedure described by Van Soest *et al.* (1991).

*Instrumentation and Spectral measurements.* NIR spectra of raw TMR were acquired with a Foss NIRSystem 6500 scanning monochromator (Foss NIRSystem, Silver Spring, MD, USA) provided with a transport module in the scanning range of 400 to 2500 nm at 2 nm interval and the spectral data were recorded as reflectance mode ( $\log 1/R$ ). All samples were scanned as raw materials using natural cells and spectra were recorded with WINISI 2 software v.1.05 (Infrasoft International Inc., Port Matilda, PA,

USA). Two different charges of each sample were scanned as duplicated replicates, and the resulting spectra averaged.

*Data Analysis.* Different chemometric strategies were evaluated. The Standard Normal Variate and Detrending (Barnes, 1989) transformations to remove multiplicative interferences of scatter and then Modified Partial Least Squares (MPLS) algorithm in order to build regression equations between the spectral data and the nutritive parameters. The cross validation was used to avoid overfitting of the equations and to select the optimum number of PLS factors to the final model prediction. The statistics used to select the best equations were the coefficient of determination (1-VR) and the standard error of cross validation (SECV) (ISI, 2000).

## Results and discussion

The main difficulty in measuring NIR spectrum in unmilled TMR is the high heterogeneity in sample presentation (these rations have wide variability of ingredients), and thus considerable variation in reproducibility between collected NIR spectra.

Table 1 shows descriptive statistics (mean, range and standard deviation) for all the nutritive parameters. As can be observed a wide scatter in all the parameters occurred.

Table 1. Statistics for the reference properties in the calibration set.

	Mean	Max	Min	SD
DM (%)	46.34	73.10	28.30	7.673
Ash (%)	7.59	11.15	5.65	1.084
CP (%)	13.87	18.60	9.97	1.171
Fat (%)	4.50	6.67	2.87	0.680
Starch (%)	14.63	22.89	0.55	3.496
ADF (%)	22.46	32.50	18.42	2.380
ADF* (%)	21.21	31.35	17.17	2.368

\* ash free

Table 2 shows the calibration and cross validation statistics for intact TMR samples applying the second derivative. All equations showed correlations higher than 0.5 for all the parameters, with relevant results obtained for DM with a determination coefficient of 0.98. In order to evaluate the performance of calibration models, the RER (relationship between the population range and the standard error of cross validation) was calculated (Williams and Sobering, 1996). Based on this statistic parameter, the results in this work could be classified as acceptable for all the parameters and excellent for DM, and of course, confirm the viability of NIRS to analyse unmilled TMR. However, better results could be obtained increasing the calibration set by addition of more variability in TMR composition, nevertheless the SECV are similar to those obtained in milled samples (de la Roza-Delgado *et al.*, 2003).

Table 2.- Calibration and cross validation statistics for chemical parameters.

	SEC	RSQ	SECV	1-VR	RER
DM	0.832	0.988	1.055	0.981	42.46
Ash	0.599	0.695	0.710	0.575	7.75
CP	0.530	0.795	0.745	0.594	11.58
Fat	0.411	0.634	0.460	0.545	8.26
Starch	2.224	0.595	2.524	0.477	8.85
ADF	1.618	0.538	1.812	0.417	7.77
ADF*	1.337	0.681	1.541	0.574	9.20

SEC: standard error of calibration; RSQ: coefficients of determination for calibration; SECV: standard error of cross validation; 1-VR: coefficients of determination for cross validation; RER: Range/SECV.

\*ash free



## Conclusions

The results of this work suggest that the calibration and cross validation statistics show the potential of NIRS technology as a powerful tool for quality control on unmilled TMR. Further work must be carried out in order to increase the calibration set to develop a new equation able to cope with more variability.

## Acknowledgements

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## Automatic detection of *Rumex obtusifolius* by digital imaging

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### Abstract

*Rumex obtusifolius* L. (*R.o.*) is a major weed in the grassland of Europe, reducing yield and quality mainly in grazed grassland. Automatic mapping of the spatial distribution of *Rumex* in grassland plots would be a major step towards site-specific weed control. The objective was to automatically detect *R.o.* in a grassland sward by means of digital image processing. The analysis was based on digital RGB images taken with a commercial CCD camera at different phenological stages from young seedlings to mature individuals. In the digital images *R.o.* and other grassland plants have been separated from image background. By its specific shape, colour and texture features *R.o.* has been detected and discriminated from other species. The respective classification algorithm shall be implemented in an automatic system to map *R.o.* for site-specific weed control.

Keywords: *Rumex obtusifolius*, image processing, weed control.

### Introduction

In agriculture the potential of weed detection by means of image analysis is well known. Automatic systems for mapping of different weeds are developed and introduced to practice (Gerhards *et al.*, 2002; Oebel *et al.*, 2004). In grassland farming these techniques have rarely been investigated so far. Reasons for that are the complex phenology of grassland as compared to arable crops. Only few research work deals with image processing in grassland (Schut and Ketelaars, 2003; Bonesmo *et al.*, 2004; Lock *et al.*, 2004). The automatic detection of *R.o.* by digital image processing was introduced recently (Dürr *et al.*, 2005), who used the Local Binary Pattern (LBP) (Ojala *et al.*, 2000) as texture feature for *R.o.* with plant detection rates ranging from 87% to 97%. The objective of the presented research work is to automatically map *R.o.* and to discriminate the remaining species in the grassland sward. *Taraxacum officinale* (*T.o.*), and *Plantago major* (*P.m.*), exhibiting similar morphology like *R.o.*, have been observed at the same time.

### Materials and methods

Field experiments have been established on the grassland research station Rengen of the University of Bonn. *R.o.*, *T.o.*, and *P.m.* have been planted in small (2,5\*2,0m<sup>2</sup>) grassland plots. For every plot images have been taken automatically. A commercial RGB-CCD camera with 8 million pixels resolution, fixed in a position of 1,6m above ground has been used. Established plots were cut at 11th of July and images were taken on July 19<sup>th</sup>, 22<sup>nd</sup>, and 28<sup>th</sup>. A total of 36 images (one per plot) per date were recorded and subdivided in two groups of 18 images each. Hence, a total of 6 datasets was analysed. To avoid light reflectance and shadows induced by direct sunlight, images were taken only during late afternoon hours. The first sequence in the image processing procedure was the separation of the image background from the objects of interest (image segmentation). RGB images have been transformed to greyscale intensities using standard formula:  $I=0.2989*R+0.5870*G+0.1140*B$ . Since the texture of leafs of *R.o.* are homogenous as compared to grass, a measure of homogeneity was used to separate it from the background. The 'Local Homogeneity' (Cheng and Sun, 2000) of the intensity image was derived using:  $H=1-(S/Smax*D/Dmax)$ , where  $S/Smax$  and  $D/Dmax$  are the maximum normalised standard deviation and gradient images, both derived from  $I$ . Binary images were derived by applying a threshold of 0.97 to  $H$ , which has been derived empirically. Finally 'morphological opening' (Gonzales and Woods, 1992) was performed on the binary image in order to tear apart connected objects. Objects with

an area smaller than 5000 Pixels have been eliminated since the leaves of *R.o.* and some other weeds are of bigger size so that grass and clover could be eliminated. Remaining objects were described by area, perimeter, eccentricity, circularity and shape factor as geometric parameters. Colour features were represented by means of the intensity (*I*), the red (*R*), and the green (*G*) object pixels. The mean of the gradient was included as a measure of texture. As mentioned above, the 'Local Homogeneity' used for segmentation is a measure of texture as well. That means a pre-classification of objects already took place. The classification was done using a Maximum-Likelihood Estimation (MLE). The respective distribution parameters were derived from features of the segmented objects of the first 8 images from July, 19<sup>th</sup> as training samples. Five classes were built representing *R.o.* (45 samples), *T.o.* (29 samples), *P.m.* (50 samples), 22 soil samples (e.g. canopy gaps) and a residue class with remaining objects (63). Hence, the total number of training samples was 209. The classification was done for all remaining images of the six datasets.

## Results and discussion

The total classification rate ranged from 65% to 80% (Table1). Reducing the number of classes to two (*Rumex* and residue) increased the accuracy to 91% (Table1). The overall rate of correctly detected *R.o.* varied from 70% to 92%. Finally, all datasets were merged (3861 objects) and classified using the same distribution parameters, leading to a total detection rate of 89.4% for the 2 class classification (Table1). Misclassification of *R.o.* in most cases were due to similarity to *P.m.* (Table 2).

Table 1. Results of the MLE Classification for 5 classes and 2 classes separately.

Dataset	Total number		5 classes			2 classes		
			Detection rate (%)		Total <i>R.o.</i>	Detection rate (%)		Total <i>R.o.</i>
	Objects	<i>R.o.</i>	Overall	<i>R.o.</i>		Overall	<i>R.o.</i>	
19.07. - 1	372	82	80.7	80.5	66	90.6	91.5	75
19.07. - 2	635	128	76.4	86.7	111	90.9	92.2	118
22.07. - 1	694	142	71.8	75.3	107	90.6	85.9	122
22.07. - 2	780	146	70.5	82.9	121	87.4	87.7	128
28.07. - 1	671	157	65.3	73.9	116	88.1	74.5	117
28.07. - 2	709	127	66.4	70.9	90	89.7	77.9	99
All dates	3861	782	71.0	78.1	611	89.4	84.3	659

Table 2. Confusion Matrix for the classification for all 3,861 samples. Most errors occur due to misclassification of *R.o.* to *P.m.* (125 plants were assigned to *P.m.*).

	Residue	<i>R.o.</i>	<i>T.o.</i>	<i>P.m.</i>	Soil
Residue	1,238	10	88	25	168
<i>R.o.</i>	39	611	19	113	8
<i>T.o.</i>	122	36	357	86	1
<i>P.m.</i>	109	125	128	450	2
Soil	39	0	1	0	86

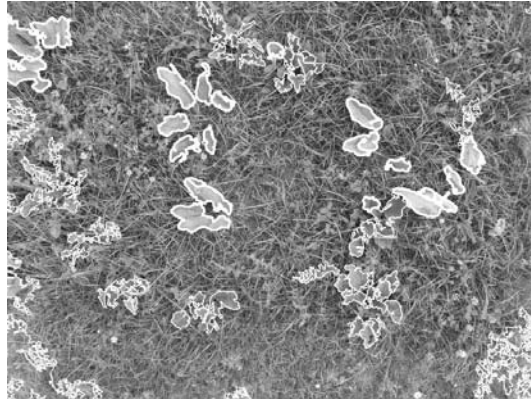


Figure 1. Example of a 5 class classification result. The thick white lines represent detected *Rumex* plants.

### Conclusions

The results show a good detection rate of *R.o.* and a proper discrimination to other classes (Table 2, Figure 1). The recognition rate found decreases with plant growth and extension (Table 1), which is clearly understandable because the distribution parameters for the MLE were derived from training samples of the first recording date only. Further investigations will focus on the derivation of specific distribution parameters, like for critical phenological stages. In addition we will have to increase the amount of training samples. Besides the derivation of the spatial distribution maps, the geometry of the plants might be useful for estimating the rootstock position of the weed which is most important for mechanical weeding.

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# Quality characteristics of five temperate grasses during primary growth

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## Abstract

A study was conducted to determine *in vitro* dry matter digestibility (IVTD) and crude protein (CP), neutral detergent fibre (NDF) and acid detergent fibre (ADF) in five native grasses during primary growth. The field trial in split-plot design with four replications was set up in the pre-Alpine region of Slovenia in 2000. Treatment factors were grass species, i) meadow foxtail, ii) tall oatgrass, iii) cocksfoot, iv) perennial ryegrass, v) Yorkshire fog as the main plots, and eight sampling dates with a one week interval from 23 April to 11 June 2002 as sub-plots. All measured parameters were significantly affected ( $p < 0.001$ ) by the species and sampling dates. There were also significant species  $\times$  sampling date interactions. The IVTD differences between species were higher during the second part of the experimental period while the CP content differences were higher during the first part. During the most of the experimental period perennial ryegrass was superior to the other species regarding the IVTD, and the NDF and ADF contents. Contrary to this, CP content was especially higher in cocksfoot and meadow foxtail. The measured values were in the range: 600-920 g IVTD, 66.7-265.3 g CP, 353.0-562.9 g NDF and 244.1-409.7 g ADF per kg of dry matter.

Keywords: temperate grasses, quality characteristics, primary growth.

## Introduction

Herbage quality of grasses changes with growth advance thus influencing utilisation efficiency in animal nutrition. For temperate grasses this phenomenon, pronounced during primary growth, is associated with their stem elongation and inflorescence development. Such development reduces leaf to stem ratio, enhances proportion of lignified tissues in herbage and impairs its quality mainly through reduction of digestibility and reduction of crude protein and non-structural carbohydrates content. The herbage quality of grasses during primary growth has been subjected to many studies (Pritchard *et al.*, 1963; Cherney *et al.*, 1993; Daccord *et al.*, 2001; Jeangros *et al.*, 2001; Shubiger *et al.*, 2001). However, the data of herbage quality of some grass species are limited to date. The objectives of this study were (i) to identify the patterns of *in vitro* true dry matter digestibility (IVTD), crude protein content (CP), neutral detergent fibre (NDF) and acid detergent fibre (ADF) content in five grass species during primary growth, (ii) to look for differences between the species regarding these patterns and (iii) to determine relationships between the measured parameters. Selected species are of importance for forage production in semi-natural grasslands in Central Europe.

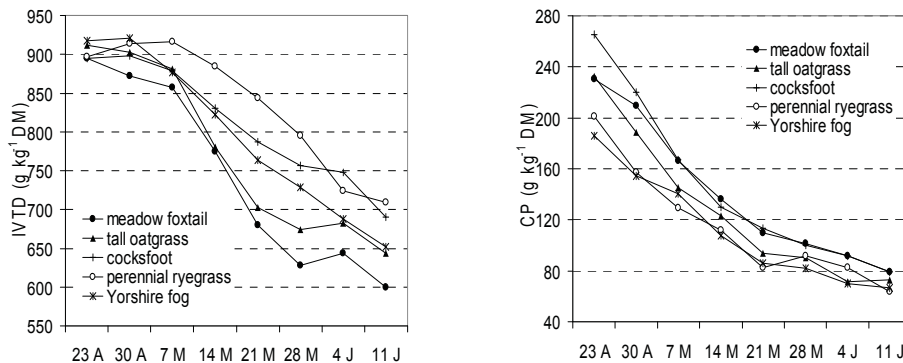
## Materials and methods

A field trial in split-plot design with four replicates was established in the pre-Alpine region of Slovenia (lat. 46°03'N, long. 14°28'E, alt. 312 m) in 2000. Five grass species, meadow foxtail (*Alopecurus pratensis* L.) 'Slovenian ecotype', tall oatgrass (*Arrhenatherum elatius* (L.) Beauv. ex J. & C. Presl 'Sora', cocksfoot (*Dactylis glomerata* L.) 'Kopa', perennial ryegrass (*Lolium perenne* L.) 'Hirka', Yorkshire fog (*Holcus lanatus* L.) 'Slovenian ecotype', were allocated as the main plots, while eight sampling dates with a one week interval from 23 April to 11 June 2002 were allocated as subplots. The subplot size was 1 by 0.6 m. In March 2002 equal fertilizer rate was applied to each grass species: 60 kg N ha<sup>-1</sup>, 35 kg P ha<sup>-1</sup> and 200 kg K ha<sup>-1</sup>. Herbage samples were hand clipped at 4 cm stubble height from 0.2 m<sup>2</sup>. The samples were oven-dried at 55 °C for 48 h and then grind to pass 1 mm sieve on Wiley mill. IVTD was determined according to the Ankom procedure directions with the exception of rumen fluid collection (ANKOM Corp., Macedon, NY). It was collected from a fistulated ram, transported to the

laboratory in a pre-heated thermos flask and filtrated through three layers of cheesecloth into a graduated cylinder. Then, the filtrate was poured immediately into a jar containing samples and buffer solution. No rumen mat was added. Aliquots of 0.25 g were incubated for 48 h using the Daisy<sup>II</sup> incubator. Digested residues were subject to NDF analysis to remove microbial matters. CP content was determined by Kjeldahl procedure (ISO 5983:1997). NDF and ADF contents were determined using an Ankom fiber analyser. A split-plot analysis of variance was used to test statistical significance of treatment effects and interactions using Genstat software, 7<sup>th</sup> edition (Lawes Agricultural Trust). Relationships between the measured parameters were tested by linear regression analysis. The soil at the trial site was classified as a sandy loam with a moderately glazed layer between 25 cm to 70 cm below surface. In the upper 6 cm soil layer pH was 7.0 and the contents of P and K (ammonium lactate extraction) were 4.6 mg and 8.5 mg per 100 g of dry soil respectively. The weather condition during the trial period (March to June 2002) at the trial site differed moderately from a 30-year average (1961-1990). The average air temperature (13.1 °C) was 1.2 °C higher and the sum of precipitation (420 mm) was 65 mm lower than the referenced values.

## Results and discussion

Sampling date (D) and grass species (G) affected IVTD, CP, NDF and ADF significantly ( $p < 0.001$ ). There was also significant  $D \times G$  interaction within each measured quality parameter ( $p = < 0.001$ ). On 23 April IVTD of all grasses were similar and very high (900 g kg<sup>-1</sup> DM in average) and persisted close to that level until 7 May (Figure 1). From that time on IVTD started decreasing markedly and had high species affected response. The highest difference occurred between perennial ryegrass and meadow foxtail on 28 May (167 g kg<sup>-1</sup> DM). The IVTD relationships among perennial ryegrass, cocksfoot and meadow foxtail were in accordance to data from Swiss (Shubiger *et al.*, 2001) where organic matter digestibility was measured using Tilley and Terry (1963) two stage procedure. Regarding the content of CP differences between the species the situation was opposite to that of IVTD. The species differed markedly in the CP content during early spring growth while small differences occurred from mid May onward. As for time course of CP content cocksfoot performed the best and Yorkshire fog the worst. Interestingly, CP content in the herbage of perennial ryegrass was similarly low as that of Yorkshire fog during the whole primary growth what likely resulted from generally high non-structural carbohydrates content in that species. The range of CP content caused by growth period was as high as 200 g kg<sup>-1</sup> DM and was similar to that reported by Jeangros *et al.* (2001). NDF and ADF contents, although different in absolute values, had similar curve patterns for all included grass species. Regarding those two parameters of quality perennial ryegrass was superior to all other species for the major period of primary growth. Meadow foxtail and tall oatgrass showed very similar and the highest NDF and ADF contents during the whole primary growth period. The calculated best fitted linear regressions made for 160 pairs of data were as follows:  $IVTD = 130.97 - 0.15 * ADF$  ( $R^2 = 0.83$ ),  $CP = 373.25 - 0.73 * ADF$  ( $R^2 = 0.66$ ) and  $NDF = 83.6 + 1.16 * ADF$  ( $R^2 = 0.87$ ).



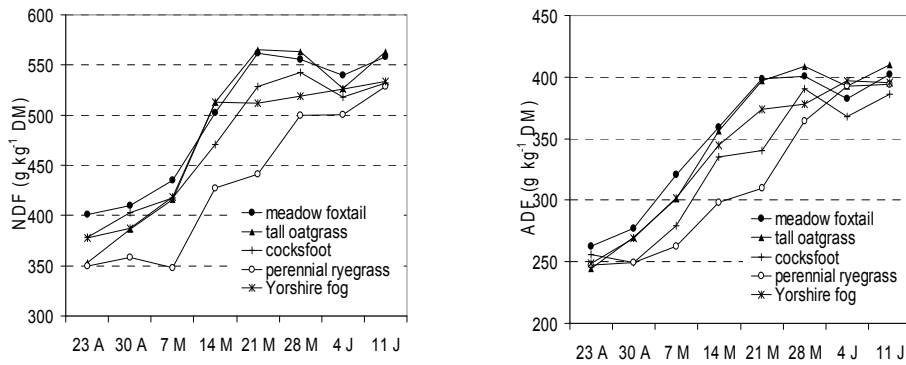


Figure 1. The pattern of IVTD, and CP, NDF and ADF contents in herbage of five grasses during primary growth (23 April to 11 June) in the pre-Alpine region of Slovenia in 2002.

### Conclusions

Included grass species differed markedly in CP content during the first part and in IVTD during the second part of the primary growth period. With exception of perennial ryegrass all other species showed similar time course in NDF and ADF contents. Depending on grass species that prevail in grassland located in similar environmental conditions to the experimental one, the line between higher and lower herbage quality can be placed in the time period from early to mid May.

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# The energetic evaluation of different grasslands management technologies

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## Abstract

The aim of this work was to evaluate energy inputs and outputs of the grasslands with the different utilization intensity from the viewpoint of the energy balance, in the years 2003 – 2004. The utilization intensity: 1. Extensive – 2 cuts per year, 2. Low intensive - 2 cuts per year, 3. Medium intensive - 3 cuts per year and 4. Intensive - 4 cuts per year. Nutrition and fertilization: PK fertilization 30:60 kg ha<sup>-1</sup>, NPK fertilization 90:30:60 kg ha<sup>-1</sup> and 180:30:60 kg ha<sup>-1</sup>. The energy output was calculated on the basis of the values about yield and chemical composition by means of the formula: BE = 0,0239\*gCP + 0,0397\*gF + 0,020\*gCF + 0,0174\*gNFE. In case of the energy inputs we were observing these data: the used industry and fertilizers in the clean nutrients NPK in kg ha<sup>-1</sup>, the energy in engines GJ ha<sup>-1</sup>, the fuel consumption on 1 ha<sup>-1</sup> and the amount of the human work expressed in h ha<sup>-1</sup>. The energy outputs constitutes: the phytomass yield in t ha<sup>-1</sup>. The values of the energy inputs moved from 4.94 GJ ha<sup>-1</sup> to 22.70 GJ ha<sup>-1</sup>. The highest average energy gain was observed by the medium intensive and the lowest by the extensive utilization.

Keywords: energy benefit, fertilization, pratotechnology, production of phytomass.

## Introduction

The energy analysis of farms provides a global view on the efficiency of farming process through the production of various criteria. The issues are environmental, particularly for the control of greenhouse gas emissions and also for the evaluation of the use of scarce non-renewable resources. They show also socio-economic aspects, as fossil energy may be replaced in some cases by human resources. Energy analysis can therefore play a significant role in the evaluation of sustainability of agricultural process (Boisvert and Holec, 1993). Production system is affected by various factors such as weather conditions, nutrition and fertilization, physical and agrochemical characteristics of the soil or natural characteristics of grown plants. It is evaluated fertilizer's effectiveness, efficiency of pesticides and various ways of soil tilling or energetic influence of preceding crops and various agro-ecological conditions. Risoud and Bochu (2002). The aim of this study was to analyse and to quantify energy-material's entrances and outputs in production process of permanent grassland on the basis of various methods of its utilization and fertilization and subsequently to carry out balance from the viewpoint of additional energy sources for their optimal level assessment.

## Materials and methods

We have carried out the observation in 2003–2004 on RICB Rapotín. The locality is situated in 390–402 m above sea level. The soil is sandy-loam, type cambisol. The mean precipitations of year are 700 mm and mean year temperature is 6.2 °C. On the grassland are dominant these species: *Taraxacum sp.* 27%; *Dactylis glomerata* L. 7%, *Lolium perenne* L. 5%, *Poa pratensis* L. 27%, *Trifolium repens* L. 13% and rest 21%. Intensity of utilization: 1. Intensive – 4 cuts per year (1<sup>st</sup> cut on May 15<sup>th</sup> at the latest, next after 45 days); 2. Medium intensive – 3 cuts per year (1<sup>st</sup> cut on May 31<sup>st</sup> at the latest, next after 60 days); 3. Low intensive - 2 cuts per year (1<sup>st</sup> cut on June 15<sup>th</sup> at the latest, 2<sup>nd</sup> cut after 90 days) and 4. Extensive – 2 cuts per year (1<sup>st</sup> cut on June 30<sup>th</sup> at the latest, 2<sup>nd</sup> cut after 90 days). Nutrition and fertilization: A – no fertilization; B – P:K 30:60 kg ha<sup>-1</sup>; C – N:P:K 90:30:60 kg ha<sup>-1</sup>; D – N:P:K 180:30:60 kg ha<sup>-1</sup>. It was used ammonium nitrate with pulverized limestone as nitrogen fertilizer,



superphosphate as phosphorus fertilizer and potassium chloride as potassium fertilizer. The energy output was calculated on the basis of the values about yield and chemical composition by means of the formula:  $BE = 0,0239 * gCP + 0,0397 * gF + 0,020 * gCF + 0,0174 * gNFE$ . The energy contribution quantification, the used energy equivalents and methods of the calculations were realized according to the Preininger's method (Preininger, 1987). For the energy balance evaluation there were included these factors to the additional energy entrances: 1. used industrial and organic fertilizers in pure nutrients NPK, 2. energy in machines, 3. fuel consumption, 4. the human labour. On the basis of these energy values it was counted: 1. energy benefit:  $(GJ ha^{-1}) = \text{phytomass energy} - \text{entrances of the additional energy}$ ; 2. demand of power for 1 t yield:  $(GJ t^{-1}) = \text{inputs of the additional energy} / \text{yield of phytomass}$ ; 3. coefficient of the energy efficiency:  $= \text{energy of phytomass} / \text{inputs of the additional energy}$ .

## Results and discussion

Table 1 shows the values of the the phytomass yield and bruttoenergy outputs. Phytomass and energy production had increased by the fertilization. In graph I there are mentioned the energy entrances according to the particular types of utilisation and fertilisation of the permanent grasslands. The most demanding on energy entrances was the intensive grassland utilization, less demanding was the medium intensive and the least demanding was the low intensive and the extensive grassland utilization. The energy effectiveness has decreased by using of the higher doses of the industrial fertilizers. By the intensive and medium intensive type of grassland utilisation energy benefit increased along with fertilization intensity. By the low intensive and the extensive type of utilization energy benefit increased up to dose of the nitrogen fertilizer  $90 kg ha^{-1}$ , but it was lower by dose of  $180 kg ha^{-1}$ . Comparing with our results and with results of Majernik *et al.* (2002) we can conclude that production of feedstuffs is more energy demanding on the arable land, assuming that nitrogen fertiliser's doses will not be higher than 100 kg. The most participate in energy entrances and in energy benefit are industrial fertilizers. Machinery have indispensable share on the energy entrances. Its extent of contribution is equal as energy additional in fuel form. Energy production increased by the fertilization with industrial fertilizers, but coefficient of energy efficiency obviously decreased. We have noticed the highest energy benefit by the low intensive (3.) utilization and the lowest was by the intensive grassland utilization (4.) These differences were statistically significant. There were not marked any statistically significant differences between the extensive and intensive grassland utilization and between low intensive and medium intensive grassland utilization.

Table 1. Markers of the energy balance (average of 2003 – 2004).

Utilization	Fertilization	FM <sup>1)</sup> (t ha <sup>-1</sup> )	BE <sup>2)</sup> (GJ ha <sup>-1</sup> )	EE <sup>3)</sup> (GJ ha <sup>-1</sup> )	EZ <sup>4)</sup> (GJ ha <sup>-1</sup> )	EEF <sup>5)</sup>	PE <sup>6)</sup> (GJ t <sup>-1</sup> )
1 Intensive	A	5.72	104.24	4.34	99.90	24.02	0.76
	B	5.69	102.85	7.1	95.75	14.49	1.25
	C	7.41	136.09	14.9	121.19	9.13	2.01
	D	8.08	148.31	22.7	125.61	6.53	2.81
2 Medium intensive	A	6.05	110.56	3.25	107.31	34.02	0.54
	B	6.71	121.79	6.02	115.77	20.23	0.90
	C	8.00	146.16	13.82	132.34	10.58	1.73
	D	9.24	169.30	21.24	148.06	7.97	2.30
3 Low intensive	A	6.41	118.01	2.17	115.84	54.38	0.34
	B	6.98	128.76	4.94	123.82	26.06	0.71
	C	8.25	151.31	12.73	138.58	11.89	1.54
	D	8.18	151.75	20.16	131.59	7.53	2.47
4 Extensive	A	5.72	106.12	2.17	103.95	48.90	0.38
	B	6.03	110.57	4.94	105.63	22.38	0.82
	C	7.35	136.34	12.73	123.61	10.71	1.73
	D	7.59	141.76	20.16	121.60	7.03	2.66

Table 2. Analysis of variance (F - ratio)(<sup>++</sup>  $\alpha = 0.01$ ; <sup>+</sup>  $\alpha = 0.05$ ).

Indicator	Utilization	Fertilization	Class	Residual
	d.f. 3	d.f. 3	d.f. 1	
BE <sup>2)</sup>	25.18 <sup>++</sup>	8.1 <sup>++</sup>	54.36 <sup>++</sup>	250.01
EZ <sup>3)</sup>	25.93 <sup>++</sup>	9.31 <sup>++</sup>	20.92 <sup>++</sup>	248.18
EFF <sup>4)</sup>	8.11 <sup>++</sup>	19.97 <sup>++</sup>	178.39 <sup>++</sup>	39.74
PE <sup>5)</sup>	5.87 <sup>+</sup>	34.75 <sup>++</sup>	798.67 <sup>++</sup>	0.04

<sup>1)</sup>Phytomass; <sup>2)</sup>Brutto energy; <sup>3)</sup>Energy inputs; <sup>4)</sup>Energy profit; <sup>5)</sup>Energetic efficiency; <sup>6)</sup>Need of energy.

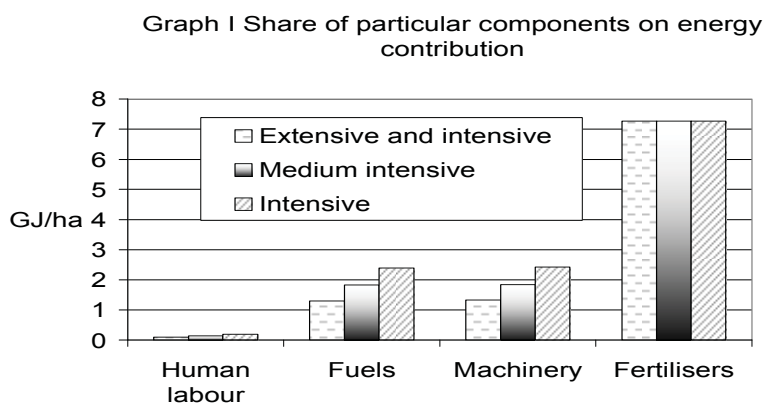


Figure 1.- Graph I Share of particular components on energy contribution.

## Conclusion

On the basis of our results we can conclude for regional climatic conditions and mentioned soil type: Energy outputs increased in this way: 1. (intensive) < 4. (extensive) < 2. (medium intensive) < 3. (low intensive). From the viewpoint of energy benefit and of intensiveness on energy entrances it appears the most available grassland utilization by low or medium intensive. Growing of the feedstuffs on the arable land is more energy intensiveness than by means of permanent grasslands. Decrease of entrances in form of the additional energy is necessary to search in decrease of fuel consumption and in rational nitrogen nutrition.

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## Acorn quality depending on pruning, botanic variety and harvest date

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### Abstract

This paper examines the effects on the chemical composition of holm oak acorns (*Quercus ilex* L. subsp. *ballota* (Desf.) Samp) of three factors: maintenance thinnings of the trees, the particular botanical varieties producing the acorns, and the course of the 2003-04 “montanera” period. Based on the results, the nutritional quality of the acorns differs between botanical varieties, and also as a function of harvest date, but not of thinning time. Botanic variety affects fat, protein and moisture contents. As well, there were found differences in all chemical contents between acorns fruits at the beginning of “montanera” and any later time, as a result of the increasing fruit ripeness.

Keywords: acorn, holm oak, quality, pruning, dehesa system.

### Introduction

Acorn crops can vary widely in output and quality. Thus, López-Carrasco *et al.* (2005) found it to differ between geographical areas, years, etc. A deeper knowledge of these changes could therefore facilitate more efficient use of the fruit by Iberian pigs and expose the potential influence of specific chemical properties on the establishment of holm oaks. In this work, we studied the effect on the chemical composition of acorns of three different factors, namely: botanical varieties, harvest date and tree thinning date.

### Materials and methods

Experimental work was conducted in the district of Cardeña (Córdoba, southern Spain) during 2003-04 “montanera” period. To study the effects of thinning, samples of acorn fruit were collected in trees that were thinned in different years (*viz.* 1994, 1996, 1998, 1999 and 2000) (table 3). To evaluate effects of ripening period on chemical components, the test material was collected fortnightly from 1<sup>st</sup> November 2003 to 15<sup>th</sup> January 2004 (Table 4). Effects of botanic variety in chemical composition were studied with four different varieties, namely: *rotundifolia*, *crassicupulata*, *macrocarpa* and *microcarpa* (Vazquez *et al.*, 1992) (Table 2). There was a different number of samples per treatment because of the large variety of “trees behaviour”, impossible to predict beforehand: for example, there were trees that threw harvest at the beginning of “montanera” and another did it at the end, there were botanic varieties more common than others, etc. Samples were ground and analyzed by means NIRS techniques. They were used to record NIR spectra over the wavelength range 400-2500 nm on a Foss NIR Systems 6500 spectrophotometer of the University of Córdoba. The moisture, fat, protein and total sugar contents in the fruits were predicted by using appropriate calibration equations (Table 1) that were derived from data of holm oak and cork oak acorns. These four parameters were chosen because they have a lot of influence in the quality of Iberian pig products, although principal components of acorn fruit are starch and other reserve-polysaccharides. Composition of the acorn flour was analysed in terms of harvest date, botanical variety and thinning date, using a one-way ANOVA. When a significant F was found, it was used Scheffé-test to know which means were different. Normality of distributions and homogeneity of variances are guaranteed.

Table 1. NIRS Calibration Equation for chemical components of acorn.

	Mean	Maximum	Minimum	Rank	SD	N <sup>o</sup> samples	SECV	R <sup>2</sup>
Moisture (g kg <sup>-1</sup> )	51.676	64.375	34.500	28.57	8.935	155	3.165	0.875
Crude protein (g kg <sup>-1</sup> d.m.)	3.270	4.700	1.988	2.71	0.524	163	0.176	0.888
Crude fat (g kg <sup>-1</sup> d.m.)	5.435	8.800	1.000	7.7	1.993	164	0.61	0.907
Total sugars (g kg <sup>-1</sup> d.m.)	3.748	10.552	0.100	10.45	3.2	138	1.14	0.873

SECV: Standard error of cross validation, estimate of true prediction error.

R<sup>2</sup>: Fraction of explained variance by model.

### Results and discussion

The specific botanical variety of acorns exerts a strong influence on their nutritional composition. The moisture content of *macrocarpa* acorns (577 g kg<sup>-1</sup>) exceeded those of the other varieties. The greater size of the former may in fact result in increased water retention. The proportion of crude protein was higher in *microcarpa* acorns than in the other varieties. On the other hand, the pulp fat content was substantially higher in *microcarpa* (8.0 g kg<sup>-1</sup>) than in *macrocarpa* and *crassicupulata*. Finally, *rotundifolia* had fat and protein contents similar to the mean values (Table 2).

Table 2. Acorn composition (g kg<sup>-1</sup>) depending on botanic variety, means within a row followed by different letters indicate significant differences in the composition at p<0.05, values are mean ±SE.

Chemical composition	Botanic variety			
	<i>Rotundifolia</i>	<i>Macrocarpa</i>	<i>Microcarpa</i>	<i>Crassicupulata</i>
Moisture (g kg <sup>-1</sup> )	49.9 ±0.80 <sup>a</sup>	57.7 ±0.27 <sup>b</sup>	45.6 ±1.15 <sup>a</sup>	50.0 ±2.04 <sup>a</sup>
Crude protein (g kg <sup>-1</sup> d.m.)	3.4 ±0.08 <sup>ab</sup>	2.7 ±0.09 <sup>a</sup>	3.9 ±0.14 <sup>b</sup>	3.6 ±0.20 <sup>ab</sup>
Crude fat (g kg <sup>-1</sup> d.m.)	6.3 ±0.19 <sup>ab</sup>	5.1 ±0.13 <sup>a</sup>	8.0 ±0.32 <sup>b</sup>	5.6 ±0.51 <sup>a</sup>
Total sugars (g kg <sup>-1</sup> d.m.)	6.0 ±0.20 <sup>a</sup>	5.3 ±1.48 <sup>a</sup>	5.9 ±0.43 <sup>a</sup>	5.7 ±0.69 <sup>a</sup>
n	18	7	7	6

As regards the factor thinning date, differences between plots were insignificant in all cases. In fact, light thinning such as that applied to the studied trees had little effect on the amount of acorns obtained or their quality (Table 3).

Table 3. Acorn composition (g kg<sup>-1</sup>) depending on thinning year, means within a row followed by different letters indicate significant differences in the composition at p<0.05, values are mean ±SE.

Chemical composition	Thinning year				
	1994	1996	1998	1999	2000
Moisture (g kg <sup>-1</sup> )	50.4 ±1.90 <sup>a</sup>	50.9 ±1.26 <sup>a</sup>	48.2 ±0.73 <sup>a</sup>	45.6 ±1.20 <sup>a</sup>	51.6 ±1.86 <sup>a</sup>
Crude protein (g kg <sup>-1</sup> d.m.)	3.5 ±0.15 <sup>a</sup>	3.4 ±0.13 <sup>a</sup>	3.4 ±0.12 <sup>a</sup>	3.8 ±0.22 <sup>a</sup>	3.4 ±0.10 <sup>a</sup>
Crude fat (g kg <sup>-1</sup> d.m.)	6.5 ±0.27 <sup>a</sup>	5.9 ±0.45 <sup>a</sup>	7.0 ±0.36 <sup>a</sup>	6.8 ±0.31 <sup>a</sup>	6.2 ±0.33 <sup>a</sup>
Total sugars (g kg <sup>-1</sup> d.m.)	5.4 ±0.46 <sup>a</sup>	6.1 ±0.29 <sup>a</sup>	6.1 ±0.24 <sup>a</sup>	6.5 ±0.40 <sup>a</sup>	5.3 ±0.68 <sup>a</sup>
n	5	5	7	8	7

Acorn production is known to vary in output and quality during the montanera period. In fact, the moisture content in the fruits was found to decrease as the montanera progressed. According to Vázquez *et al.* (2001), changes in this parameter are strongly dependent on the ambient humidity; this is consistent with our results as rainfall decreased from October to January. The increased moisture content of the fruits at the beginning may have been further raised by an increased incidence of predators facilitating moisture absorption via the holes left in the fruit. Consistent with previous results, the proportion of crude protein increased through the montanera (López-Carrasco *et al.*, 2005), from 30 g kg<sup>-1</sup> in late October to 39 g kg<sup>-1</sup> in late December; this can be ascribed to the fruits ripening over that period. Fat contents were somewhat lower than, but changed similarly to, those reported by other authors such as Almeida *et al.* (1992) and López-Carrasco *et al.* (2005); the initial content, 43 g kg<sup>-1</sup>, rose to levels in the region of 70 g kg<sup>-1</sup>. Consistent with previous results of Almeida *et al.* (1992), the total sugar content increased in a gradual manner (from 38 g kg<sup>-1</sup> to 69 g kg<sup>-1</sup>) (Table 4).

Table 4. Acorn composition (g kg<sup>-1</sup>) depending on harvest date, means within a row followed by different letters indicate significant differences in the composition at p<0.05, values are Mean ±SE

Chemical composition	Harvest date					TOTAL
	I	II	III	IV	V	
Moisture (g kg <sup>-1</sup> )	58.3 ±0.92 <sup>c</sup>	52.7 ±1.66 <sup>b</sup>	48.8 ±0.68 <sup>ab</sup>	48.0 ±0.69 <sup>ab</sup>	45.2 ±2.03 <sup>a</sup>	496
Crude protein (g kg <sup>-1</sup> d.m.)	3.0 ±0.18 <sup>a</sup>	3.2 ±0.18 <sup>ab</sup>	3.2 ±0.08 <sup>ab</sup>	3.5 ±0.14 <sup>ab</sup>	3.9 ±0.19 <sup>b</sup>	35
Crude fat (g kg <sup>-1</sup> d.m.)	4.3 ±0.64 <sup>a</sup>	6.9 ±0.58 <sup>b</sup>	6.5 ±0.21 <sup>b</sup>	6.9 ±0.22 <sup>b</sup>	7.1 ±0.36 <sup>b</sup>	64
Total sugars (g kg <sup>-1</sup> d.m.)	3.8 ±0.60 <sup>a</sup>	5.4 ±0.43 <sup>ab</sup>	6.2 ±0.16 <sup>b</sup>	6.0 ±0.25 <sup>b</sup>	6.9 ±0.55 <sup>b</sup>	59
n	6	5	20	13	7	

## Conclusions

The specific botanical variety of acorns influences their chemical composition. The harvest date also influences acorn quality; in fact, the moisture content decreases, whereas those in crude fat, crude protein and total sugars increase, as the “montanera” progresses. This is a result of the increasing fruit ripeness: the largest differences being those between fruits at the beginning of the season and any later time. Finally, the tree thinning date appears to have no appreciable influence on acorn output or quality.

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# Effect of hydrolyzable tannin extract on bovine milk production and composition

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## Abstract

A herd of twenty dairy cows was divided into two equal groups according to their initial milk production and their stage of lactation. At the start of experiment (1<sup>st</sup> sampling, June 20<sup>th</sup>, 2004) both groups received the same diet, based on pasture, which was supplemented with compound feed. After that date one group received 4 kg of compound feed (control group, CON), while other group received the same amount of compound feed mixed with 120 g of chestnut extract (treatment group, TRE). Milk production records and milk composition (fat, protein and urea) were obtained every 14 days from June 20<sup>th</sup> to October 25<sup>th</sup>, 2004 (9 sampling days). No significant differences ( $P > 0.05$ ) between treatments in milk production and milk fat, milk protein and milk urea content were found within any of sampling days. However milk urea nitrogen was somewhat lowered with the diet containing chestnut tannins, which could indicate a decreased rumen protein degradability, resulting in a lowered ammonia concentration in the rumen.

Keywords: cattle, tannins, milk production, milk composition.

## Introduction

The result of excessive rumen degradability of plant proteins are large amounts of ammonia, which have to be eliminated from the body in form of urea. The transformation of ammonia into the urea is energetically wasteful and this could be the reason why in the summer months milk production decrease. However, large amounts of ammonia can cause also the rumen alkalosis, which results in lower nutrient, especially fiber, degradation and lower microbial protein synthesis. The overall results are lower fat and protein contents in the milk.

Excessive degradability of proteins could be reduced by the use of heat and various chemicals. The use of heat is very expensive, thus most often the chemicals, such as formaldehyde, are used. However, the formaldehyde is carcinogenic and, in addition, its incorrect use can lower the absorption of amino acids from intestine (Ashes *et al.*, 1984). Another way to protect proteins against excessive degradation in the rumen is the use of tannins, which form reversible complexes with proteins. These complexes are not degraded at pH values present in rumen (Mangan, 1988, Butter *et al.*, 1999), but they disintegrate at pH values of the abomasum and small intestine (Jones and Mangan, 1977).

The aim of present work is to establish if the supplementation of dairy cows with hydrolysable tannins improves milk yield and milk composition.

## Material and methods

A herd of twenty dairy cows was divided into two equal groups according to their initial milk production and their stage of lactation. At the start of experiment (1<sup>st</sup> sampling day, June 20<sup>th</sup>, 2004) both groups received the same diet, where the main component was pasture, supplemented with 4 kg of compound feed. From that day on one group of cows received the same diet as at the start of experiment (control group, CON), while second group received the same quantity of compound feed containing 30 g/kg of chestnut tannin extract (treatment group, TAN). Milk production records and

milk composition (fat, protein and urea) were obtained every 14 days from June 20<sup>th</sup> to October 24<sup>th</sup>, 2004, when the grazing season ended.

## Results and discussion

The results of the experiment are presented in Table 1. The highest milk yield was obtained for CON group at 3<sup>rd</sup> sampling day (23.0 kg d<sup>-1</sup>), while the lowest milk yield was recorded at the last, 9<sup>th</sup> sampling day for TRE group (16.9 kg d<sup>-1</sup>). The differences in milk yield between CON and TRE within sampling days were not significant ( $P>0.05$ ). These results are in accordance with the results of Orešnik (1996), who also used chestnut tannins, and Maasdorp *et al.* (1999), who used leaves of *Calliandra calothyrsus*, containing condensed tannins. On contrary, Maasdorp *et al.* (1999) found, that feeding the leaves of *Leucaena leucocephala* in *Acacia boliviana* (both containing condensed tannins) increased milk yield for 1.83 and 0.58 kg/day, respectively. Woodward *et al.* (1998) also found that condensed tannins of *Lotus corniculatus* increased milk yield of dairy cows for 35 % comparing with the control group receiving *Lolium sp.*

Table 1: Production and composition of milk in cows receiving diets with (TRE) or without (CON) tannin extract at different sampling days.

Sampling day	Milk yield (kg d <sup>-1</sup> )		Milk fat (%)		Milk protein (%)		Milk urea (mg100 mL <sup>-1</sup> )	
	CON <sup>a</sup>	TRE <sup>a</sup>	CON	TRE	CON	TRE	CON	TRE
1	20.0	19.4	3.58	3.74	3.28	3.36	-	-
2	22.2	22.2	3.68	4.03	3.24	3.41	40.2	38.2
3	23.0	20.2	3.72	4.15	3.22	3.38	42.2	41.4
4	20.2	19.0	3.53	3.67	3.20	3.33	49.7	47.2
5	21.9	18.9	4.19	3.80	3.36	3.49	46.9	47.5
6	19.0	19.5	4.04	3.86	3.37	3.42	49.6	49.1
7	20.7	22.1	3.89	3.97	3.59	3.56	46.6	45.7
8	19.2	18.8	3.84	4.10	3.49	3.65	48.9	45.7
9	18.7	16.9	3.82	4.05	3.54	3.67	49.7	48.1

<sup>a</sup> CON = control group of animals; TRE = animals receiving tannin extract in their diet.

Milk composition (milk fat and milk protein) did not differ between two groups within each sampling day (Table 1). The greatest difference in milk fat contents between Con and TRE group was obtained at the 3<sup>rd</sup> sampling day (3.15 and 4.15 % for CON and TRE group, respectively), while that in milk protein contents at the 2<sup>nd</sup> sampling day (3.24 and 3.41 % for CON and TRE group, respectively). Generally TRE group had slightly higher content of milk fat and milk protein, but differences were never statistically significant ( $P>0.05$ ). On contrary, Orešnik (1996) stated that supplementation of the diet with tannins could influence degradability of proteins in the rumen, which should affect the milk composition. In his experiment Orešnik (1996) proved that the supplementation of the diet with chestnut tannins increased milk protein content for 0.15 %. This is in accordance with the results of Bhatta *et al.* (1999), who demonstrated that a supplementation of the diet with condensed tannins containing seed husks of *Tamarindus indica*, improved milk protein content by 0.04 % (from 3.49 % to 3.53 %;  $P<0.07$ ). In the milk produced with cows fed on *Lotus corniculatus* (containing condensed tannins) the concentration of milk protein was 0.30 % higher than in control group. Woodward *et al.* (1998) also noted that condensed tannins from *Lotus corniculatus* did not have any effects on milk fat content, which agrees with our results (Table 1). If tannins decrease the degradability of proteins, which could be very high in pasture based diets, this should be reflected on the milk urea content. We found that milk urea content was always lower in the milk of TRE group, except at 5<sup>th</sup> sampling day (Table 1, Figure 1). Even if these differences between TRE and CON group within a sampling day were not statistically significant ( $P>0.05$ ) we are convinced that chestnut tannins affect digestion and metabolism of proteins.

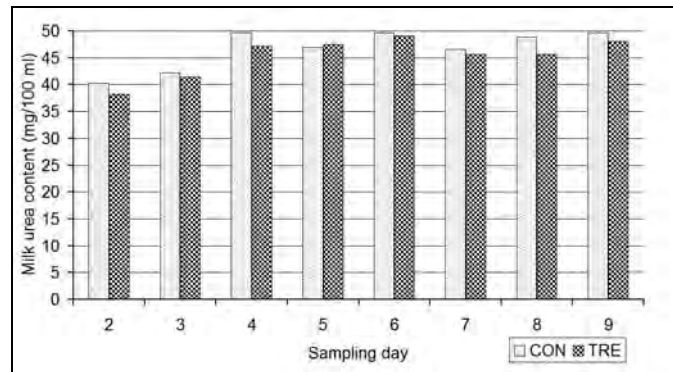


Figure 1: Urea content (mg/100 ml) in milk of groups of dairy cows receiving diets with (TRE) or without (CON) tannin extract at different sampling days.

### Conclusions

There are several reasons why tannins in the diet of dairy cows did not have any pronounced effects on milk yield and composition. The first possible reason is the concentration of tannins in the diet. It is well known that concentrations up to 5 % of diet dry matter may have beneficial effects on animal performances, while in our experiment the concentration of tannin extract was only about 0,7 % (about 0,5 % tannins, determined by the coagulation with skin powder), indicating that the concentration may be too low. Other important effects on milk production and composition are stage of lactation and parity. In such a small herd as we used (20 lactating animals) it is very difficult to select animals which had initially the same milk production and were contemporarily in the same stage of lactation and the same parity.

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# Comparison of the variability in quality of the LIA-bred perennial grasses, relationships between quality parameters

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## Abstract

Comparison of the quality of the LIA-bred perennial grasses was performed. Quality indicators of perennial grass species *Lolium perenne* L., *Festuca pratensis* Huds., *Poa pratensis* L., *Phleum pratense* L., *Dactylis glomerata* L. at heading stage varied in relation to the growing year's weather conditions, grass species and its genetic diversity. During the experimental period the highest average crude protein (CP) concentration was determined for smooth stalked meadow grass, it varied within 123-176 g kg<sup>-1</sup> range. According to average protein concentration in dry matter of herbage mass *D. glomerata* was similar to *F. pratensis*. The highest contents of fibre (NDF) were determined for *D. glomerata* (557-588 g kg<sup>-1</sup>) and *Ph. pratense* (566-632 g kg<sup>-1</sup>), whereas perennial ryegrass was characterised by the lowest contents of NDF, the highest contents of water soluble carbohydrates (WSC) and by the best dry matter digestibility (DMD). The coefficients of linear correlation between values of grass quality components were calculated.

Keywords: grasses, species, quality, NIRS prediction.

## Introduction

Traditional grassland mixtures in Lithuania include grass species *Ph. pratense*, *F. pratensis*, *D. glomerata*, *P. pratensis*. Although perennial ryegrass contributes rapid establishment and forage quality, the species was not popular due to its low overwinter survival. With the introduction of novel varieties, the interest in *L. perenne* has notably increased. Establishment and renovation of swards require introducing species and varieties not only persistent, high yielding, adapted to soil properties, local relief, a range of managements, but also with an adequate quality. The quality of perennial grasses can vary depending on many factors, including genotypic peculiarities of entries, climatic conditions and others (Belanger and McQueen, 1996; Thorvaldsson, 1992). The aim of this study was to compare the quality of the LIA-bred grasses and variability in quality parameters among and within grass species, cut at the heading stage.

## Materials and methods

Overall 491 samples of varieties and breeding lines of *L. perenne* (151), *F. pratensis* (49), *Ph. pratense* (74), *D. glomerata* (175) and *P. pratensis* (42) were assessed for quality over the period 2001-2004 on a sod gleyic, medium heavy, drained loam soil. The plough layer is 25-30 cm, with a pH value of 7.2 -7.3, humus content 1.9-2.2 %, total nitrogen 0.14-0.16%, available phosphorus 201-250, and potassium 108-171 mg kg<sup>-1</sup> of soil. The grasses were sown by a narrow-row method in 10 m<sup>2</sup> plots with 3-4 replications. Fertilisers at rates N<sub>150</sub>P<sub>60</sub>K<sub>90</sub> were applied annually. Composite samples for chemical analyses were formed at grass heading stage of the first cut. Samples, dried and ground by a mill with 1 mm sieve, were analysed by NIR spectroscopy, quality components were predicted by equations developed at the LIA (Butkute *et al.*, 2003).

## Results and discussion

Data of nutritive quality components of herbage mass of grass species and varieties are presented in Table 1 as statistical mean over four years and coefficient of variation (CV%).

Table 1. Mean (g kg<sup>-1</sup>) and coefficient of variation of quality characteristics of LIA-bred grasses.

Grass species	2001		2002		2003		2004		2001-2004	
	mean	CV%	mean	CV%	mean	CV%	mean	CV%	mean	CV%
Crude protein (CP)										
All samples	113	14.00	113	20.85	125	15.56	113	12.94	116	16.61
<i>L. perenne</i>	103	11.18	107	10.34	110	15.73	105	7.93	105	10.29
<i>F. pratensis</i>	not investigated		117	5.35	127	7.21	103	6.10	114	11.50
<i>P. pratensis</i>	139	7.19	176	9.10	153	9.94	123	8.09	149	15.64
<i>Ph. pratense</i>	103	7.11	109	14.09	123	6.39	116	10.54	112	11.57
<i>D. glomerata</i>	119	10.08	106	13.01	137	5.53	120	15.48	118	14.37
Neutral detergent fibre (NDF)										
All samples	511	20.56	526	13.47	540	15.99	517	17.47	520	16.90
<i>L. perenne</i>	376	10.43	448	6.29	434	10.53	364	9.16	409	12.50
<i>F. pratensis</i>	not investigated		527	4.32	555	4.21	529	4.62	538	4.97
<i>P. pratensis</i>	564	8.18	522	6.49	628	4.86	552	7.68	563	9.46
<i>Ph. pratense</i>	566	5.33	632	4.38	571	3.54	579	5.26	587	6.27
<i>D. glomerata</i>	588	6.66	568	6.07	571	10.01	557	11.32	573	8.36
Water soluble carbohydrates (WSC)										
All samples	192	54.68	214	29.65	180	37.90	212	39.53	201	41.56
<i>L. perenne</i>	329	15.69	290	7.68	268	11.05	355	7.05	308	15.20
<i>F. pratensis</i>	not investigated		196	9.03	188	10.84	228	6.49	208	12.32
<i>P. pratensis</i>	131	24.33	148	19.18	83	10.00	177	15.81	135	30.34
<i>Ph. pratense</i>	165	13.11	141	19.22	137	14.91	136	12.46	144	16.46
<i>D. glomerata</i>	108	23.22	184	15.42	134	15.22	178	23.71	148	29.83
Dry matter digestibility (DMD)										
All samples	666	19.67	663	12.82	635	15.51	644	18.72	654	16.86
<i>L. perenne</i>	838	3.35	751	5.29	753	8.12	847	4.59	795	7.72
<i>F. pratensis</i>	not investigated		674	4.38	650	4.69	636	5.52	648	5.37
<i>P. pratensis</i>	587	7.27	680	6.30	535	9.45	609	10.01	607	11.7
<i>Ph. pratense</i>	595	7.07	543	6.75	574	7.27	567	6.57	574	7.34
<i>D. glomerata</i>	568	8.07	610	7.99	581	7.93	578	14.71	585	9.98

During the experimental period the herbage quality varied with a year, among and within tested species. Entries of *P. pratensis* were annually noted for the highest average protein content and in individual samples it ranged from 113 to 214 g kg<sup>-1</sup> DM. According to average data of CP concentration the other species were ranked in the following order: *D. glomerata* > *F. pratensis* > *Ph. pratense* > *L. perenne*. According to 13-years average yield of the first cut the species of LIA-bred grasses (except for *L. perenne*) were ranked in a similar but inverse order: *Ph. pratense* (7.42 t ha<sup>-1</sup>), *F. pratensis* (5.58 t ha<sup>-1</sup>), *D. glomerata* (5.42 t ha<sup>-1</sup>), *L. perenne* (5.20 t ha<sup>-1</sup>) and *P. pratensis* (4.19 t ha<sup>-1</sup>) (Lemežienė *et al.*, 2004). N or CP dilution in DM yield has been discussed by several authors, but in our opinion, other factors play an important role too, firstly, the differences in nitrogen use efficiency among grass genotypes (Zemenchik and Albrecht, 2002). *L. perenne* undoubtedly surpassed the other grass species in terms of the rest of the feeding value characteristics tested, annually and according to averaged data

from all experimental years. According to the highest averaged data of fibre and the lowest DMD, the worst feeding value at the heading stage was characteristic of *D. glomerata* and *Ph. pratense*, and in some years of *P. pratensis*. WSC concentration was found to be the most variable parameter among the tested herbage quality parameters: subject to growing year and species variation coefficient CV was as high as 6.49- 24.33 % for individual species and 29.65-54.68 % for all samples. Mean values, range of variation of quality of each grass species were different each year. That fact could be related with composition of tested entries, their age and conditions of the year. The averaged data of protein and fibre for most species were higher in 2003, WSC in 2004. WSC levels are usually the highest in plants grown in low temperatures and high light intensity. Cell wall materials deposited at lower temperatures are less lignified, which results in a higher digestibility of herbage, than at higher temperatures (Thorvaldsson, 1992). *Ph. pratense* and *F.pratensis* were the most stable species according to coefficient of variation and mean of annual values of quality data of most of the components. For all five species tested the concentrations of fibre (CF, NDF) and WSC, DMD, like concentrations of CP and any of carbohydrate-based constituents (CF, NDF, WSC) with the exception of *F. pratensis* were inversely related (Table 2). Strong positive correlations (at P 0.01) between values of concentrations of fibre fractions (CF and NDF) were found for all species. Concentrations of digestible DM were positively correlated with CP concentrations (at P 0.01 or 0.05, except *L. perenne*) and WSC (at P 0.01, except *F. pratensis*). Similar trends of relationships between forage quality components are well known.

Table 2. Coefficients of linear correlation between values of different grass quality components.

Grass species	CP x CF	CP x NDF	CP x WSC	CP x DMD	CF x NDF	CF x WSC	CF x DMD	NDFx WSC	NDFx DMD	WSCx DMD
<i>L. perenne</i>	-0.225 <sup>a</sup>	-0.07	-0.267 <sup>a</sup>	0.069	0.917 <sup>a</sup>	-0.667 <sup>a</sup>	-0.900 <sup>a</sup>	-0.711 <sup>a</sup>	-0.933 <sup>a</sup>	0.690 <sup>a</sup>
<i>F. pratensis</i>	0.03	0.195	-0.705 <sup>a</sup>	0.299 <sup>b</sup>	0.875 <sup>a</sup>	-0.622 <sup>a</sup>	-0.704 <sup>a</sup>	-0.680 <sup>a</sup>	-0.658 <sup>a</sup>	0.178
<i>P. pratensis</i>	-0.336 <sup>b</sup>	-0.375 <sup>b</sup>	-0.185	0.505 <sup>a</sup>	0.950 <sup>a</sup>	-0.779 <sup>a</sup>	-0.909 <sup>a</sup>	-0.791 <sup>a</sup>	-0.909 <sup>a</sup>	0.680 <sup>a</sup>
<i>Ph. pratense</i>	-0.245 <sup>b</sup>	-0.345 <sup>a</sup>	-0.500 <sup>a</sup>	0.322 <sup>a</sup>	0.913 <sup>a</sup>	-0.527 <sup>a</sup>	-0.833 <sup>a</sup>	-0.391 <sup>a</sup>	-0.799 <sup>a</sup>	0.427 <sup>a</sup>
<i>D. glomerata</i>	-0.310 <sup>a</sup>	-0.380 <sup>a</sup>	-0.170 <sup>b</sup>	0.210 <sup>a</sup>	0.936 <sup>a</sup>	-0.750 <sup>a</sup>	-0.703 <sup>a</sup>	-0.663 <sup>a</sup>	-0.739 <sup>a</sup>	0.606 <sup>a</sup>

<sup>a, b</sup> Significant at the 0.01 and 0.05 probability levels, respectively.

## Conclusions

Large variation in forage quality was found for grass species tested. *L. perenne* should be regarded as a high quality species with high content of WSC, DM digestibility and low content of fibre. The *Ph. pratense* and *F. pratensis* were most stable species in terms of quality. Concentrations of CP and any of carbohydrate-based constituents (CF, NDF, WSC) with some exception were inversely related, like concentrations of fibre (CF, NDF) and WSC, DMD.

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# Herbage yield and quality as influenced by cutting frequency of natural grassland of Inner Mongolia

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## Abstract

Overgrazing is a serious problem in the Inner Mongolian steppe. A cutting-frequency-experiment was conducted to simulate different grazing intensities and examine the reactions of the sward. Results from this experiment are presented, including DM- and N-yield, botanical composition and the quality parameters CP- and NDF-content. The 3 treatments to which the native grassland was subjected were: cut once a year (T-I), cut every 6 (T-II) and every 3 weeks (T-III). The yearly DM-yield increased significantly from 217 g m<sup>-2</sup> in T-I to 291 and 297 g m<sup>-2</sup> in T-II and T-III, respectively. There was no change in botanical composition for the dominant species *Stipa grandis* and *Leymus chinensis* due to cutting frequency. The mean CP-content over all species increased from 77 to 135 and 150 g kg<sup>-1</sup> for T-I, T-II and T-III. For the N-yield this meant an increase of factor 2.3 and 2.7 for T-II and T-III in comparison to T-I. In T-I the NDF-content of *S. grandis* and *L. chinensis* reached almost 750 g kg<sup>-1</sup>, but decreased significantly in T-II and T-III to 680 and 620 g kg<sup>-1</sup> during the growing season. Cutting caused the DM-yield, N-yield and quality to increase, but a further increase in cutting-frequency (T-III) did not result in further significant improvement.

Keywords: DM-yield, herbage quality, *Stipa grandis*, *Leymus chinensis*.

## Introduction

The general objective of the multi-disciplinary project MAGIM (DFG research group 536) is to measure the consequences resulting from overgrazing in the Inner Mongolian native grassland. The research site in the Xilin River catchment is situated on the Mongolian Plateau 1000 to 1200 m a.s.l. The average rainfall is 320 mm per year and the average yearly temperature is 1.7°C creating a semi-arid temperate steppe. Grassland ecosystems in semi-arid regions are more susceptible to overgrazing in the process of land use intensification as a result of mismanagement. In Inner Mongolia farmers traditionally make one hay harvest in the end of August and small ruminants graze intensively from June to September in adjacent areas, with winter grazing up to the end of March. The working-group Grassland and Forage Science/Organic Agriculture, University of Kiel, Germany has established a grazing experiment together with the Institute of Animal Nutrition and Physiology, University of Kiel to find a sustainable stocking rate adapted for the regional circumstances. This experiment started in June 2005 and is working on different topics related to grassland and animal productivity, as well as herbage quality in order to find indicators for overgrazing using a functional approach. During the growing season in 2004 a preliminary experiment was carried out. The objective was to simulate different grazing intensities and to investigate the dynamics of herbage yield and quality of the native grassland. One-year results from the preliminary experiment are presented in this paper.

## Material and methods

An area of 0.7 ha was chosen and 3 treatments differing in cutting frequency were tested on a randomised block-design with 4 replicates: treatment I (T I) was cut once in the end of the growing season, T II was cut every 6 weeks (simulated, moderate grazing intensity) and T III was cut every 3 weeks (simulated, high grazing intensity). The swards were cut to 2.5 cm stubble height. In order to

observe the DM-increment and quality development between cutting dates, samples were taken every 2 weeks for T I and T II. T III was sampled every 3 weeks just before cutting. The sampling period started 5<sup>th</sup> of June and the 1<sup>st</sup> cut was done 2<sup>nd</sup> of July. Sampling and cutting were finished around 10<sup>th</sup> September for all treatments. The above-ground biomass was divided in four groups: the 2 dominant species *S. grandis* and *L. chinensis* (both about 30 %), the necrotic material and the other remaining species (4 quadrates per plot, each sized 0.25 m<sup>2</sup>). Samples were dried for 24 h at 60°C, and ground to 1 mm. In Kiel all samples were scanned by a Near-Infrared-Spectrometer (NIRS) to estimate quality parameters. Samples were analysed for its chemical components. In this paper the total N-content (C/N-Analyzer) and the NDF-content (ANKOM-apparatus) are presented. Data were subjected to analysis of variance. Due to significant interaction effects of species group and treatment for herbage quality parameters, treatment means within species were compared using Student's t-test, with probabilities corrected with the Bonferroni-Holm test.

## Results and discussion

By increasing the cutting frequency the DM-yield increased significantly from about 2.2 t ha<sup>-1</sup> to almost 3 t ha<sup>-1</sup>. The increase in cutting frequency between T II and T III did not result in a significantly higher DM-yield (Table 1).

Table 1. DM-Yield (g m<sup>-2</sup>) and Botanical Composition (% of DM) in 2004.

Treatment	I	II	III	SE
Yield (g DM m <sup>-2</sup> ) 2004				
	217.4 <sup>b</sup>	291.1 <sup>a</sup>	296.9 <sup>a</sup>	5.88
Botanical Composition in September 2004 (% of DM)				
<i>S. grandis</i>	32 <sup>a</sup>	27 <sup>a</sup>	25 <sup>a</sup>	1.51
<i>L. chinensis</i>	28 <sup>a</sup>	22 <sup>a</sup>	28 <sup>a</sup>	1.03
Remaining species	28 <sup>b</sup>	51 <sup>a</sup>	47 <sup>a</sup>	2.22
Necrotic material	12	0	0	0.48

Means with different superscripts within a row differ ( $P < 0.05$ )

There was no change in botanical composition for the dominant species *S. grandis* and *L. chinensis* as a result of cutting frequency, but there was a significant increase in the proportion of remaining species. Species especially growing close to the ground increased, which may have been due to more space and light being available for them after cutting.

Table 2. Mean Crude-Protein-Content (g kg<sup>-1</sup> DM) and N-Yield (kg ha<sup>-1</sup>) in 2004.

	CP-content (g kg <sup>-1</sup> DM)			N-yield (kg ha <sup>-1</sup> )		
	T I	T II	T III	T I	T II	T III
<i>S. grandis</i>	79.3 <sup>c</sup>	132.6 <sup>b</sup>	149.0 <sup>a</sup>	9.34 <sup>b</sup>	17.61 <sup>a</sup>	18.47 <sup>a</sup>
<i>L. chinensis</i>	75.2 <sup>b</sup>	149.6 <sup>a</sup>	159.9 <sup>a</sup>	6.58 <sup>b</sup>	14.01 <sup>a</sup>	17.37 <sup>a</sup>
Remaining species	75.8 <sup>c</sup>	121.6 <sup>b</sup>	142.4 <sup>a</sup>	4.97 <sup>c</sup>	15.81 <sup>b</sup>	20.42 <sup>a</sup>
Necrotic material	46.8 <sup>a</sup>	55.8 <sup>a</sup>	56.2 <sup>a</sup>	1.28 <sup>a</sup>	2.10 <sup>a</sup>	1.83 <sup>a</sup>

Means with different superscript within a row differ ( $P < 0.05$ ); SE<sub>CP</sub>: 0.44; SE<sub>N-yield</sub>: 1.55.

The mean CP-content increased with increasing cutting frequency. For *S. grandis* and the remaining species the more frequent cut increased CP-content, whereas for *L. chinensis* no further increase between T II and T III was observed. The maturing process of plants was interrupted due to cutting and less cell wall constituents as well as more CP-content in the regrowth was observed. This is also

demonstrated in N-yield, where an increase for T II and T III of factor 2.3 and 2.7 compared to T I was found (Table 2). Figure 1 shows the dynamics of the NDF-content of *S. grandis* and *L. chinensis* for T I and T III. To keep the figure clearer T II was not included, as no significant differences between T II and T III have been found.

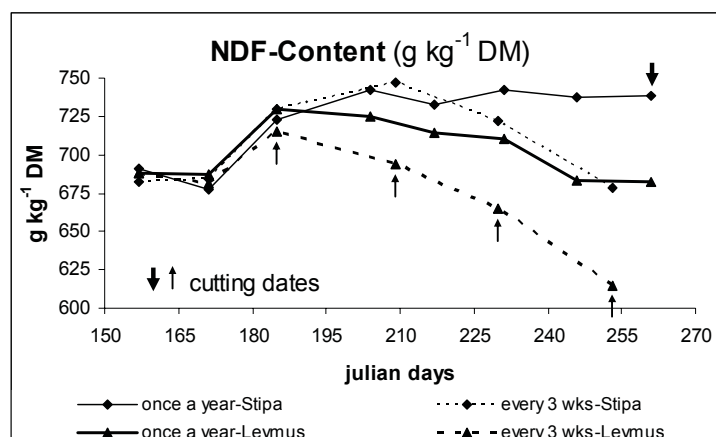


Figure 1. NDF-Content of *S. grandis* and *L. chinensis* (g kg<sup>-1</sup> DM).

With increasing maturity the NDF-content in both species increased up to almost 750 g kg<sup>-1</sup> (T I). Both species reached their highest NDF-content after starting stem elongation (around day 215 for *S. grandis* and day 180 for *L. chinensis*). *S. grandis* kept this level, whereas *L. chinensis* showed a slight, but steady decline over time. In *L. chinensis* the NDF-content decreased after the first cut (T III), which coincided with reaching the reproductive stage. In the end of the growing season the NDF-content was 620 g kg<sup>-1</sup>, which meant a decline of about 10 % points from the 1<sup>st</sup> to the 4<sup>th</sup> cut within T III. In T III *S. grandis* showed similar results as there was a decline in NDF-content after cutting, but it started only after the 2<sup>nd</sup> cut. An explanation for this could be, that after the 1<sup>st</sup> cut there were still living and growing reproductive tillers left. T I showed that there is a close relationship between the NDF-content and the reproductive stage, and it seems that the appearance of reproductive tillers was not interrupted by cutting.

## Conclusions

Increasing the intensity of utilisation beyond one cut per year up to simulated grazing intensities resulted in a significant increase in herbage yield and herbage quality of the native grassland in Inner Mongolia. The N yield increased substantially with cutting frequency. Although the proportion of dominant grasses did not change between treatments, a conclusion is restricted by the short-term observation period.

## Variation in the non-protein nitrogen content (fraction A) of several forages during the growing period

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### Abstract

Fraction A represents the non-protein nitrogen (NPN) of feeds. In high yielding animals high proportions of fraction A can be related to low N use efficiency. In plants these compounds are involved in transport and storage of N. Different contents of fraction A are expected for silage maize and forage legumes. The objective of this project was to test the hypothesis that differences in N metabolism between N<sub>2</sub>-fixation vs. non-N<sub>2</sub>-fixation plants has an impact on the level and changes of fraction A during the growing period. The data basis of this study included several forage legumes and different cultivars of silage maize (*Zea mays* L.). Increasing contents in forage legumes and decreasing contents of fraction A in silage maize were observed, confirming the different N metabolism of N<sub>2</sub>-fixing and non-N<sub>2</sub>-fixing plants. Differences within legume species were observed for red clover (*Trifolium pratense* L.) and birdfoot trefoil (*Lotus corniculatus* L.) in comparison to white clover (*Trifolium repens* L.) at several sampling dates. For silage maize differences in the content of fraction A were observed at the earing stage, but not in stover.

Keywords: non-protein nitrogen, fraction A, legume, maize.

### Introduction

The fractionation of feed protein according to Licitra *et al.* (1996) is well accepted for the characterization of protein quality. The fraction A consists of non-protein nitrogen (NPN) compounds, like amino acids and nitrate. For the animal a high content of fraction A in the diet together with low content of fermentable organic matter are considered disadvantageous. In such situation the efficiency of N utilization by the animal is reduced, increasing the risk of N pollution in dairy farming systems. In plants the fraction A represents important N compounds, which are involved in the transport and storage of N. Differences in the content of fraction A may appear between N<sub>2</sub>-fixing and non-fixing plants, as well as during the growing period due to the different N metabolism. The determination of fraction A of forages and the changes which may occur during the growing period are important information a) to define feeding and/or grazing strategies, b) to increase the N use efficiency of high yielding animals, c) to decrease the N losses in the production system and d) for modelling purposes. This study aimed to determine the fraction A content of several forage legume species as well as of silage maize cultivars during the growing period.

### Material and Methods

Forage legumes were collected in a trial, which was carried out in 2003 and included white clover (*Trifolium repens* L.), red clover (*Trifolium pratense* L.), birdfoot trefoil (*Lotus corniculatus* L.), lucerne (*Medicago sativa* L.), and kura clover (*Trifolium ambiguum* M. Bieb.). All legumes were sown in binary mixtures with perennial ryegrass (*Lolium perenne* L.). Samples were taken from April to July and separated in three components: legume, grass and herbs. Samples were dried at 60°C, milled in a Cyclotech mill (1 mm) and scanned by NIRS for laboratory analysis on selected samples. The N content was determined by a rapid combustion (850°C), conversion of all N products to N<sub>2</sub>, and subsequent measurement by thermoconductivity cell (C/N Analyzer). Fraction A of legumes was determined with a 10% tungstic acid solution (Licitra *et al.*, 1996) and N measured in the residue after filtration. The fraction A was calculated as difference between total N content and N content in residue. The

experiment was a complete randomised block design with 3 replicates. Data was submitted to analysis of variance and means were compared to white clover using LSD. Probabilities were adjusted using Bonferroni-Holm test. Silage maize samples (*Zea mays* L.) were collected in an experiment carried out from 2001 to 2003 (randomised block design, two replicates). Eight cultivars were included, involving early, mid-early and mid-late maturity group, as well as different maturation types (stay green, normal, and dry down). In total 150 kg N ha<sup>-1</sup> was applied in three different occasions, before the 6/8 leaf stage was achieved. At six sampling dates (one before and five after silking) ten plants from each plot were harvested, separated in ear and stover stages, chopped, freeze-dried and milled to pass a 1 mm sieve. The content of fraction A in silage maize was estimated by NIRS. In selected samples, the N content (C/N Analyzer) in the residual remaining in the filter was subtracted from the total N content to obtain the amount of fraction A (Gierus *et al.*, 2005). Means were compared using Student's t-test, with probabilities corrected with the Bonferroni-Holm test.

## Results and Discussion

An increase of fraction A content over all legume species was observed from the stage of grazing maturity (2<sup>nd</sup> sampling date, vegetative) up to the stage of silage maturity (4<sup>th</sup> sampling date, vegetative/budding) (Figure 1). Later development of forage legumes did not show any further increase in the content of fraction A. The analysis of variance for forage legumes revealed a significant interaction between sampling date and legume species. Differences were observed for birdfoot trefoil in comparison to white clover from the 3<sup>rd</sup> sampling date onwards as well as for red clover from the 4<sup>th</sup> sampling date onwards. The lower contents of fraction A for red clover and birdfoot trefoil may be related to the activity of polyphenoloxidase and subsequent formation of quinones, and the presence of tannins, respectively. Both substances form complexes with proteins, decreasing their availability for proteases of plants and rumen micro-organisms. In contrast to forage legumes, decreasing contents of fraction A were observed for silage maize. For the whole crop, contents of fraction A decreased from 310-360 g kg<sup>-1</sup> of total N before flowering to 160-210 g kg<sup>-1</sup> at the last sampling date (Figure 1). For the ear contents of fraction A decreased from 520 to 200 g kg<sup>-1</sup> in the mid-late cultivars, from 480 to 130 g kg<sup>-1</sup> in the mid-early cultivars and from 400 to 140 g kg<sup>-1</sup> in the early cultivars (Figure 2). In stover contents of fraction A varied between 170 and 360 g kg<sup>-1</sup> without significant differences between cultivars. At silage maturity (5<sup>th</sup>/6<sup>th</sup> sampling dates) maize had a slightly higher fraction A content compared to red clover and birdfoot trefoil, and a lower content compared to white clover, lucerne, and kura clover. The analysis of variance for silage maize showed a significant interaction of 'cultivar within maturation group \* sampling date' for the ear only, where cultivar differences were observed at the 2<sup>nd</sup> and 4<sup>th</sup> sampling dates for the mid-early, and at the 2<sup>nd</sup> sampling date for the early and mid-late cultivars. The largest differences were observed for the mid-early cultivars. The mid-early cultivar *Attribut* differed significantly from all other cultivars within its maturation group (Figure 2), showing similar contents as the mid-late cultivars *Clarica* and *Benicia*.

The results support the hypothesis that the N metabolism influences the protein quality of forage crops. Increasing fraction A contents observed for forage legumes may be attributed to an intensified N fixation, which is strongly dependent on environmental conditions, particularly temperature and soil moisture. Silage maize, in contrast, takes up large part of the N before silking. After silking N uptake slows down and N is allocated from the vegetative parts to the grain. Because of the fast transfer of NPN compounds to the grain, a high enzymatic activity and an intensive starch deposition in grain, an accumulation of fraction A was not observed in maize during the growing period.



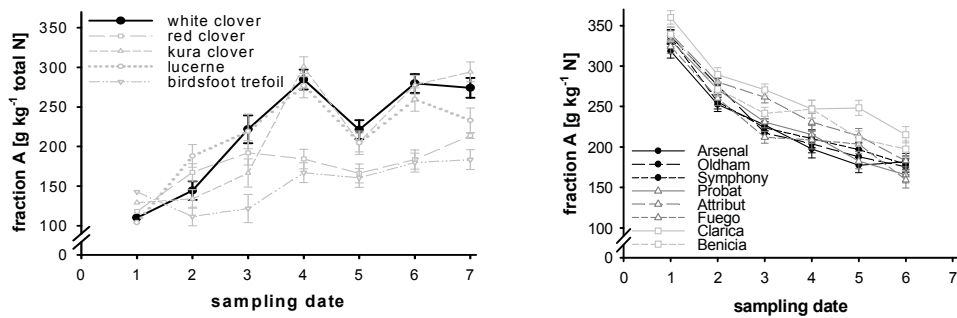


Figure 1. Content of fraction A in  $\text{g kg}^{-1}$  of total N from several forage legumes as well as in selected cultivars of maize whole crop.

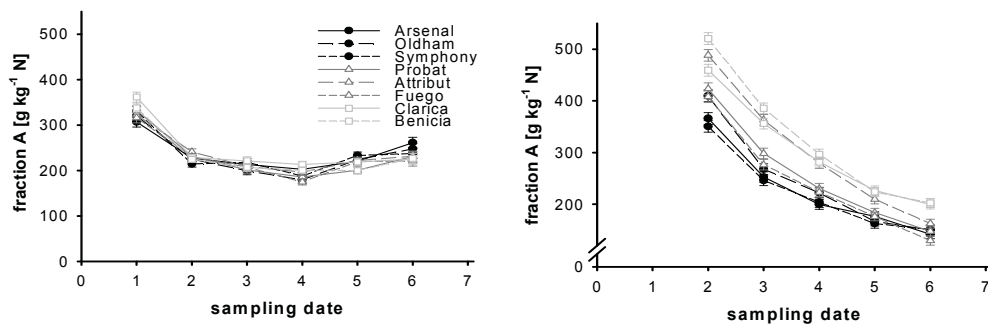


Figure 2. Content of fraction A in  $\text{g kg}^{-1}$  of total N in maize stover (left) and ear (right) from selected cultivars.

## Conclusion

The comparison of fraction A in silage maize and forage legumes showed a different content during the growing period as a consequence of differences in N metabolism between N-fixing and non-fixing plants. Differences for maize cultivars at silage maturity are less contrasting for the different maturation groups and types. The lower content of fraction A observed for red clover and birdfoot trefoil may positively influence N use efficiency by ruminants.

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# NIRS calibration equations to determine species contribution in grassland swards

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## Abstract

NIRS equations were developed to determine contribution of different grass and legume species to biomass in grassland swards. Equations were developed calibrated on artificial mixtures and validated on true mixed swards. A total of 1336 artificial two or three species mixtures were made with *Dactylis glomerata* L. (*Dg*), *Lolium perenne* L. (*Lp*), *Festuca arundinacea* Schreb. (*Fa*) and *Trifolium repens* L. (*Tr*) taken from pure stand crops. The spectral data of each artificial mixture sample was recorded in a Foss NIRSystems 6500 and the NIRS data were correlated with the percentage of each species expressed in % of total dry biomass. The equations obtained were later validated on 216 natural mixed swards harvested in 2004 and 2005. The results of the validation show that NIRS predicted with a variable degree of accuracy the percentage of *Tr* ( $R_v^2 = 0.97$  and SEP = 2.3), *Dg* ( $R_v^2 = 0.82$  and SEP = 10.1), *Fa* ( $R_v^2 = 0.78$  and SEP = 14.6) and *Lp* ( $R_v^2 = 0.85$  and SEP = 15.5). Reasons of the poor performance on *Lp* are not yet identified. It may reveal a large phenotypic plasticity of plant chemical composition of this specie.

Keywords: grasslands, species composition, NIRS, prediction.

## Introduction

When analysing grasslands swards, assessment of species contribution to total biomass is essential. It is one of the key factors explaining variations in forage production and quality. Species composition may be assessed visually but precise measurement of species contribution to biomass requires hand separation of every species and this method is tedious and time consuming. Visual score is accurate for species occurrence but too imprecise for estimation of contribution to sward biomass. Since the pioneering paper of Shenk *et al.* (1979), several researchers have demonstrated the possibility to rapidly estimate contribution of species in mixtures using NIRS technology. Coleman *et al.* (1985) and Petersen *et al.* (1987) suggested as strategy to develop equations on artificial mixtures and their validation on true mixtures. The main goal of the present work is to evaluate the strategy proposed by Coleman to develop equations for the prediction of the contribution of four species, three grasses and one legume, in different grassland mixtures.

## Materials and Methods

The samples used to develop the calibration set of artificial mixtures were taken from a forage yield trial where species were grown in pure stands. This forage trial was sown in spring 2003 at INRA, Lusignan, France. To prepare the artificial mixtures, plant material was harvested at different periods on plots where each species was grown under combinations of two levels of nitrogen fertilisation and two defoliation frequencies. Six types of artificial mixtures were prepared using four species, *Lolium perenne* (*Lp*), *Dactylis glomerata* (*Dg*), *Festuca arundinacea* (*Fa*) and *Trifolium repens* (*Tr*). The artificial mixtures were prepared either using two species, *Lp/Dg* (M1); *Lp/Fa* (M2); *Dg/Fa* (M3); *Lp/Tr* (M5) or three species *Lp/Dg/Fa* (M4); *Lp/Tr/Dg* (M6). Harvested biomass was dried in an oven during 72 hours at 60°C and ground to pass a 1 mm sieve. 1336 artificial mixtures were prepared with 208 for M1, M2, M3 and M5 mixtures (128 in 2004 and 80 in 2005) and 252 for M4 and M6 mixtures (160 in 2004 and 92 in 2005). Artificial mixtures combined N levels, defoliation frequencies and harvest dates to provide the largest possible spectrum database. Contribution of species to mixtures varied from 0 to 100% with 5% increments.

216 natural mixed samples were harvested in spring and autumn 2004 and 2005 on swards obtained with six different initial botanical compositions grown under the four combinations of agronomic practices. Species composition was manually measured. Samples were dried and ground as reported above. Spectra were collected from 400 to 2500 nm with 2 nm step using a Foss NIRSystems 6500 instruments. Samples were presented to the instrument in a standard ring cup and reflectance (log 1/R) data between 1100 nm and 2500 nm were obtained from 32 scans.

Partial Least Square regression was used to develop equations using the calibration data set of 1336 artificial mixture samples even though it only corresponds to 24 true observations for each species. The equations were validated with the 216 natural mixtures.

## Results and Discussion

Calibration with artificial mixtures showed that the best fit was obtained for *Tr* percentage with a Standard Error of Cross-Validation (SECV) of 1.8 and a  $R_c^2$  value of 1. Proportion of *Lp* is the least accurately predicted with a SECV of 6.8 and a  $R_c^2$  value of 0.94 (Table 1).

Table 1. Calibration statistics for predicting the percentage of individual species as ingredients in artificial mixtures.

Species	n <sup>(1)</sup>	Mean	SD <sup>(2)</sup>	SEC <sup>(3)</sup>	$R_c^2$	SECV <sup>(4)</sup>	n T <sup>(5)</sup>
<i>Lolium perenne</i>	1321	41.67	27.65	6.50	0.94	6.78	16
<i>Dactylis glomerata</i>	1076	40.68	27.50	4.14	0.98	4.32	16
<i>Festuca arundinacea</i>	886	44.54	28.09	4.87	0.97	5.08	16
<i>Trifolium repens</i>	646	44.46	28.39	1.72	1.00	1.84	14

<sup>(1)</sup> Number of samples; <sup>(2)</sup> Standard deviation; <sup>(3)</sup> Standard error of calibration; <sup>(4)</sup> Standard error of cross validation; <sup>(5)</sup> Number of terms.

Among the 216 true mixtures used for validation, hand assessment showed that contribution of species ranged from 10.8 to 89.3% for *Lp*, 3.3 to 79% for *Dg*, 9.1 to 52.4% for *Fa* and 40 to 80.7% for *Tr*.

Table 2. Validation statistics for predicting the percentage of individual species in hand separated mixtures.

Species	n	H.S. mean <sup>(1)</sup>	NIRS mean	$R_v^2$	SD	SEP <sup>(2)</sup>	SEPC <sup>(3)</sup>	RPD <sup>(4)</sup>
<i>Lolium perenne</i>	180	52.18	42.68	0.85	23.82	15.51	12.29	1.5
<i>Dactylis glomerata</i>	144	34.96	33.22	0.82	22.88	10.13	10.02	2.3
<i>Festuca arundinacea</i>	108	23.66	36.06	0.78	13.21	14.63	7.79	0.9
<i>Trifolium repens</i>	72	64.13	63.71	0.97	13.58	2.29	2.27	5.9

<sup>(1)</sup> Hand separated mean; <sup>(2)</sup> Standard error of performance; <sup>(3)</sup> Standard error of performance corrected for mean bias; <sup>(4)</sup> Ratio to the SD values to the standard error of performance.

Results of validation on true mixtures are shown in table 2. The best prediction was achieved for *Tr* with  $\pm 2.2$  points, no bias and a high RPD value (5.9). This may be explained by the peculiar biochemical composition of harvested plant organs compared to grass species (low dry matter content, high N content, low soluble sugars, presence of secondary metabolites) and, consequently a typical spectral signature. *Dg* and *Fa* are predicted with a confidence interval of  $\pm 10$  and  $\pm 7.8$  points, with a large bias and a medium to low RPD of 2.3 and 0.9 respectively. With a SEPC of  $\pm 12.3$  points and a RPD value of 1.5, prediction of *Lp* was the least accurate. Analysis of spectra on the first axes of a principal component analysis indicated that data points corresponding to grasses investigated in true mixtures of grasses were located at the border of the clouds (data not shown). This suggests that the spectral

signature of a grass species may vary when grown in pure stands or in mixtures with other grass species. As a consequence, the SEPC were high for all grasses and differed among species. A possible explanation of this discrepancy may reside in a possibly higher proportion of senescing leaves in grasses, the mean proportion of senescent material being 3% in both springs. These senescing leaves were discarded in the true mixtures as they were not attributable to any species while they were maintained in the artificial mixtures.

Table 3. Calibration and validation statistics for predicting the percentage of individual species in hand separated samples.

Species	Calibration				Validation							
	n	SD	R <sub>c</sub> <sup>2</sup>	SEC	n	H.S. mean	NIRS mean	R <sub>v</sub> <sup>2</sup>	SD	SEP	SEPC	RPD
<i>Lolium p.</i>	1628	27.32	0.92	7.50	180	52.18	49.54	0.87	23.82	9.16	8.80	2.6
<i>Dactylis g.</i>	1322	26.81	0.97	4.78	144	34.96	34.33	0.93	22.88	6.00	5.99	3.8
<i>Festuca a.</i>	1069	27.34	0.96	5.74	108	23.66	27.42	0.78	13.21	7.63	6.67	1.7
<i>Trifolium r.</i>	769	27.39	1.00	1.78	72	64.13	64.06	0.98	13.58	1.96	1.97	6.9

Broadening calibration spectrum database with addition of true mixtures (Table 3) greatly improve prediction for all three grass species, but the validation was not anymore performed with independent datasets. SEP were respectively 6.0, 7.6 and 9.1 for *Dg*, *Fa* and *Lp*, respectively, these values getting close to the SEC values, with RPD values of 3.8, 1.7 and 2.6 respectively. In the present study, SEP obtained for *Tr* were better than those reported in most studies. Locher *et al.* (2005), in grass-legume mixtures, reported standard errors of prediction for forage legumes (*Trifolium repens*, *Trifolium pratense* and *Medicago sativa*) between 2.9 and 5.4 depending on calibration strategy with higher RPD values. In mixtures with *Festuca arundinacea*, Petersen *et al.* (1987) reported a SEPC of 3.1 points while Coleman *et al.* (1990), using calibration equations based upon spectra of pure species, reported SEP for legumes between 4.7 and 5.3 depending on harvests.

### Conclusions

Predictions of proportion of the three grass species were satisfactory given their close morphological and biochemical traits. However, spectrum database will be enlarged with more artificial and true mixtures in autumn 2005 and 2006 to increase equations robustness.

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# Aerial biomass assessment of *Stipa tenacissima* in forest ecosystems (a case study)

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## Abstract

The results of a survey on the aerial biomass of *Stipa tenacissima* L. allows a better understanding of the potential of the various vegetation groups of the forest ecosystems in Debdou zone. The aerial biomass of this species varies according to the altitude and the slope, and to human pressure (overgrazing, deforestation, vegetation clearing, etc.). The biomass varies from 411 to 1417 Kg DM ha<sup>-1</sup>, with a mean value of 869 Kg DM ha<sup>-1</sup>. The results show that the morphological parameters (height, diameter) and the aerial biomass of the *Stipa tenacissima* increase with altitude. The altitude influences the species development more than the slope. *Stipa tenacissima* appears at 900 m in scattered populations becoming dense formations above 1100 m. At high altitude sites (above 1100 m) *Stipa tenacissima* formations are in a good development compared to those of the fringes. In addition to these parameters, the overgrazing in this region contributes to reducing *Stipa tenacissima* aerial biomass, notably in areas most accessible to livestock. Therefore, if the pressure is maintained in the present way, the deterioration of the ecosystems will be intensified, and the desertification process needs to be considered according to the dry ecological conditions.

Keywords: aerial biomass, *Stipa tenacissima*, arid bioclimat, morphological parameters.

## Introduction

*Stipa tenacissima* constitutes an evolutionary stage of forest ecosystem degradation in the Debdou zone (Qarro and Chablou, 1996). However, this species is not utilised due to the lack of knowledge concerning its potential biomass. Gaining this knowledge should contribute to the socio-economic development of the zone.

Indeed, *Stipa tenacissima* participates especially in providing green forage to livestock when herbaceous vegetation is dry. It is also a species that is the basis of numerous uses by the residents and can provide a primary material for better quality paper or be used as a source of energy. However, the quantification of the aerial biomass of this forest species requires evaluation of its leaf and stem biomass. The assessment of the instantaneous aerial biomass (phytomass) may permit a rationalization of its utilisation by implementing appropriate management methodologies.

## Materials and methods

To undertake an assessment of aerial biomass, some measurements were collected during the winter season. The sites where measurements were taken were located in different vegetation types (facies) that contain *Stipa tenacissima* among other dominant species.

The main vegetation types are:

Facies 1: *Stipa tenacissima* and *Rosmarinus officinalis*.

Facies 2: *Tetraclinis articulata*, *Stipa tenacissima* and *Rosmarinus officinalis*.

Facies 3: *Quercus rotundifolia*, *Stipa tenacissima* and *Rosmarinus officinalis*

Facies 4: *Juniperus oxycedrus* and *Stipa tenacissima*.

Facies 5: *Quercus rotundifolia*, *Tetraclinis articulata*, *Stipa tenacissima* and *Rosmarinus officinalis* .

Facies 6: *Juniperus oxycedrus*, *Stipa tenacissima* and *Rosmarinus officinalis*.

Facies 7: *Stipa tenacissima*.

For each vegetation type the minimum land area was determined using the method of the curves areas – species (Gounot, 1969). An area of 256 m<sup>2</sup> was calculated for Facies 1, 2, 3, 6 and 7, and 512 m<sup>2</sup> for Facies 4 and 5. The data was collected inside land parcels with a surface equal to the minimum area and defined in every vegetation type. The number of the land parcels sampled in each Facies varied according to the characteristics of the sites and the importance of each vegetation type. Simple random sampling was undertaken. The data collected for each species included, vegetation cover, density, morphological parameters (crossed diameter, height) and aerial biomass. Tree volume classes were distinguished and the individual samples for biomass measurement were determined randomly according to the main morphological parameters.

The vegetation cover (R%) was estimated by the linear interception method and the density parameter by the method of land parcels (named method of the quadrats). These methods consist of the numbering of individual species inside every parcel sample in a surface equal to the minimal area. The vegetation type mean density was estimated on the basis of the average density of the individuals inventoried in all parcels and the volume class.

## Results and discussions

The correlation analysis between aerial biomass and all parameters was determined by the “multiple regression method”. The main results obtained are presented below.

The retained model giving the aerial biomass (AB), according to morphological parameters (Table 1), is:  $AB = 67.299 + 1280.46 \times MD^2$ . Define what it is MD (mean plant diameter) and MH (mean plant height).

The variability of the edible biomass (EB) is explained by the same variable at the level of 79.2%.  $EB = 31.552 + 624.685 \times MD^2$ .

The same variable explains 82.9% of the non edible biomass (NEB) by the model:

$$NEB = 35.747 + 655.777 \times MD^2.$$

Table 1. Aerial biomass according to morphological parameters.

	Diameter (m)			Mean plant height (m)	Mean density (np ha <sup>-1</sup> )	Vegetation cover (%)	Aerial biomass kg DM/ha		
	Big section	Small section	Average (MD)				Total	Edible biomass	Non edible biomass
Average	0.66	54	0.6	0.58	2006	22.5	868.71	430.57	438.14
Standard - Error	0.16	0.11	0.13	0.1	787	8.11	459.23	228.82	223.65
VC %	24	20	22	17	39	36	53	53	51
Minimum	0.3	0.3	0.3	0.3	858	9	191.27	102.43	88.84
Maximum	1.15	0.85	0.92	0.9	3984	41	2081.1	1054.12	1026.98

np: number of plants, MD: mean plant diameter.

The models driving the variability of *Stipa tenacissima* aerial biomass (highly significant at 5% level) using site altitude (Al) and height (MH) parameters are shown below:

$$AB = 328.565 + 434.684 \times MH + 0.100 \times Al + 1077.556 \times MD^2 \quad R^2 = 0.86$$

$$CB = - 175.625 + 238.115 \times MH + 0.056 \times Al + 479.925 \times MD^2 \quad R^2 = 0.83$$

$$NCB = - 152.940 + 196.568 \times MH + 0.044 \times Al + 537.630 \times MD^2 \quad R^2 = 0.85$$

The aerial biomass of the *Stipa tenacissima* varied from 411 to 1417 kg DM ha<sup>-1</sup> with a mean value of 869 kg DM ha<sup>-1</sup>. Aerial biomass is well expressed using only mean diameter. The altitude parameter influenced growth of *Stipa tenacissima* more than the slope parameter (Tables 2 and Table 3).

Table 2. Aerial biomass according to morphological and altitude parameters.

Altitude classes (m)	Aerial biomass (kg DM/ha)			MD (m)	MH (m)	Density (nb ha <sup>-1</sup> )	Vegetation cover (%)
	Total	CB	NCB				
800 – 1100	523.53	252	271.83	0.54	0.54	1805	19
S	217.42	100.65	117.77				
1100 - 1400	1079.14	529.98	549.16	0.65	0.61	2124	23
S	385.83	191.41	179.53				
1400 - 1700	1345.31	659.99	685.32	0.7	0.64	2160	32
S	475.32	251.72	224.43				

np: number of plants, S: Standard error, MD: mean plant diameter, MH: mean plant height.

Table 3. Aerial biomass according to morphological and slope parameters.

Slope classes (d°)	Aerial biomass (kg DM ha <sup>-1</sup> )			MD (m)	HM (m)	Density (np ha <sup>-1</sup> )	Vegetation cover (%)
	Total	CB	NCB				
5 to 15	725.82	347.89	377.92	0.59	0.55	2041	22.94
S	420.63	196.44	225.2				
15 to 25	927.68	464.15	463.54	0.61	0.58	1966	23.71
S	516.08	275.72	246.45				
25 to 35	755.29	369.16	402.21	0.6	0.58	1893	21
S	366.65	180.6	186.68				

np: number of plants, S: Standard error, MD: mean plant diameter, MH: mean plant height.

The preliminary results show a relative correlation between the total aerial biomass and the different morphological parameters (diameters and height), whereas it is correlated neither with the density nor with the vegetation cover (El Mouttaqui, 1988).

The “multiple regression method” shows that the mean diameter square (MD<sup>2</sup>) explains 82.3% of the variability of the total aerial biomass (AB). The height parameter alone or in combination with the altitude parameter does not better explain this variability.

The results of regression analysis show that the different components of the aerial biomass can be estimated significantly by using only the mean diameter.

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# Shrub quality evaluation in different areas of North West of Spain

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## Abstract

Galicia holds the sixteen percent of fired area of Europe. Shrub grazing under the trees is one of the most adequate methods for Galicia fire prevention. It allows controlling vegetal fuel, reducing clearing costs. On the other hand, protein is one of the most important quality variables for feeding domestic animals. Therefore, knowledge of protein content and its seasonal distribution of most important shrub species in different areas will allow us to know their quality as feed. This study aims to evaluate the protein concentration of most common shrubby species in Galicia comparing two different areas from a climatic point of view: Taboada (Mediterranean-Atlantic transitional area) and A Fonsagrada (Mountain Atlantic region). A seasonal analysis of main nutrients of *Cytisus striatus* Rothm., *Erica arborea* L., *Erica cinerea* L., *Ulex europaeus* L. and *Ulex minor* Roth as well as a comparison between two areas is shown. Protein high quality was associated to shrub legume species and to those temperature and precipitation conditions which enhances vegetative growth.

Keywords: *Cytisus*, *Erica*, *Ulex*.

## Introduction

Forest grazing is an adequate land management system for Galicia as it reduces fire risk, improves silviculture practices, increases land rent and promotes rural population stabilisation. Forest understory mainly consisted of shrubs. Knowledge about shrub quality, i.e. crude protein, will allow us to manage silvopastoral systems in a more adequate way. Most important shrub species in Galicia are those belonging to *Ulex* sp. genus, representing around 73.47% (Conselleria de Medio Ambiente. Xunta de Galicia, 2001) following by species of genus *Erica* (*Erica cinerea* 29.98% and *Erica arborea* 16.43%) and *Cytisus* sp. which accounts around 18.28% of shrubby vegetation of Galicia.

## Materials and methods

The experiment was conducted in Taboada and A Fonsagrada (Galicia, NW Spain) at an altitude of 625 m and 800 m above sea level, respectively. Mean temperature during winter and spring was below 11°C and mean annual precipitation was around 913 mm in the first area, and 7°C and 1400 mm in the second. Five shrub species were chosen based on the high importance for the Galician shrublands. The analysed species were: *Cytisus striatus* (Hill) Rothm and *Erica arborea* L. *Erica cinerea* L., *Ulex europaeus* L. and *Ulex minor* Roth. Samples were randomly and monthly taken, by using pruning shears, and phenology was assessed from flowering bud appearance to flower disappearance in the plant. Shrub branches were cut at 0.5 cm diameter, as animals use to eat parts of the branches with diameters lower than this size. Main site soil characteristics (pH and organic matter) can be seen in Table 1. Soils were very acid which reduces herbaceous pasture development, enhancing shrub encroachment in grasslands. Organic matter soil content was high as it is usually in forest and shrubland soils of Galicia.

Table 1. pH and organic matter (OM in two study areas).

	pH	OM (%)
Taboada	4.66	22.89
A Fonsagrada	5.01	12.72



Once harvested, samples were transported to laboratory, dried (48 hours at 40 °C) and milled and N was analysed after microkjeldahl digestion (Castro *et al.*, 1990). Statistical analysis was made with an ANOVA and means were separated by Duncan test.

### Results and discussion

Phenology: The periods of flowering of both studied can be seen in Figure 1. Flowering periods were quite similar in both localities, varying the period between the different species. *Cytisus striatus* flowers were only present in the plants during the summer (May to August in Taboada and April to July in A Fonsagrada). By the contrary, *Erica cinerea* flowered during all year in the two areas and *Erica arborea* flowers were continuously observed in A Fonsagrada and Taboada, with the exception of the summer period in both cases and February in the last case. *Ulex europaeus* did not have flowers during the summer in both areas, this no flowering period was increased during the autumn in Taboada. *Ulex minor* flowered from July to January in Taboada and from August to January in Fonsagrada

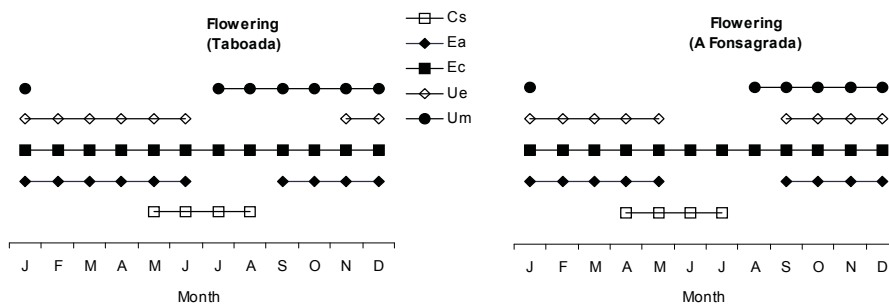


Figure 1. Flowering period to *Cytisus striatus* (Cs), *Erica arborea* (Ea), *Erica cinerea* (Ec), *Ulex europaeus* (Ue) and *Ulex minor* (Um).

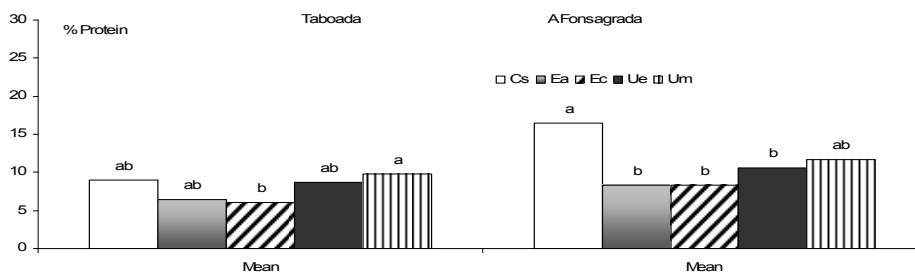


Figure 2. Crude protein, annual mean, of *Cytisus striatus* (Cs), *Erica arborea* (Ea), *Erica cinerea* (Ec), *Ulex europaeus* (Ue) and *Ulex minor* (Um) in both study areas (Taboada and A Fonsagrada). Different letters indicate significant differences between species.

N: Protein percentage of species in Taboada and A Fonsagrada, can be seen in Figure 2. Protein annual mean value was higher in A Fonsagrada for all species. Protein range were 8.9% for *Cytisus* sp, 6% for *Erica* sp and 9% for *Ulex* sp in Taboada and, 16%, 8%, 11% for these species in A Fonsagrada. These range were similar than those found in the other areas in Galicia with very acid pH (Rigueiro-Rodríguez *et al.*, 2002).

Lower temperatures and higher annual rainfall found in A Fonsagrada could reduce the destruction of the tissues and therefore the reduction of the senescent material in the plant, which enhanced shrub protein quality. Protein seasonal variation of these species can be seen in the Figure 3. All species had higher protein values in the autumn in Taboada. In A Fonsagrada, higher protein concentrations of all species were found in the spring with the exception of *Cytisus striatus* and *Ulex minor* which reached the higher protein concentration during the autumn. In both areas, lower protein percentage was associated to the winter due to the low temperatures of this period in both areas. Shrub protein content are high enough for goat maintenance, around 6% (Arbiza-Aguirre, 1986), during all the year, with the exception of the winter period in Taboada.

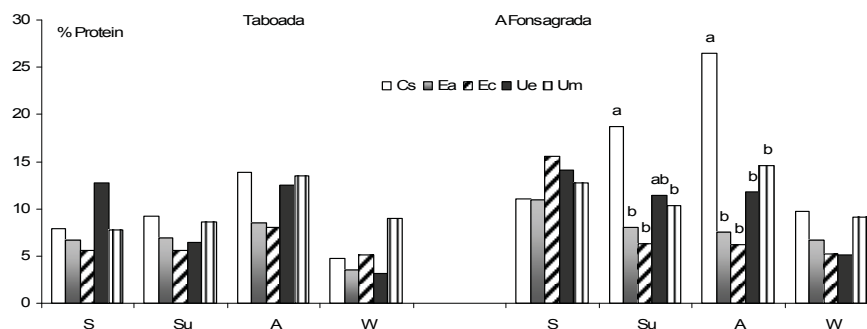


Figure 3. Crude protein, estacional mean, of *Cytisus striatus* (Cs), *Erica arborea* (Ea), *Erica cinerea* (Ec), *Ulex europaeus* (Ue) and *Ulex minor* (Ue) in both areas (Taboada and Fonsagrada). Different letters indicate significant differences between species in each locality.

### Conclusions

Higher protein shrub concentration was associated to those climatic conditions which enhances vegetative growth. Protein concentration values would allow maintaining goats during all the year.

### Acknowledgements

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## Nutritive quality of pasture in mountainous land afforested with *Pinus radiata*

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### Abstract

The experiment is based on randomized blocks design applying 13 different treatments of fertilization under a young *Pinus radiata* D. Don. plantation in Lugo (Galicia, Spain). The soil presents a low water pH (around 4) and it has not been cultivated in the last 25 years. The nutritive quality of the sown pasture has been studied for a year after its establishment. The treatments consisted of adding lime or not, in combination with two different doses of sewage sludge (meaning 50 or 100 kg total N ha<sup>-1</sup>) applied on three different periods before harvesting. Total pasture production has not been significantly different among treatments and it has been rather low compared with pasture production in agricultural land. Crude protein concentration, phosphorus, potassium and calcium of the pasture presented lower levels than what is common in sown pastures in Galician grasslands mainly due to soil acidity. The concentration of micronutrients and heavy metals was higher than in pastures due to the low pH, high proportion of shrubs and senescent material.

Keywords: nutrients, wooded-pasture.

### Introduction

Silvopastoral systems constitute an alternative land management system in order to increase the profit of the forestland in Galician region. Feeding based on fodder in stable farming accounts for more than 50% of the production costs, which can be lowered if using fresh fodder from own natural resources. The European Union subsidies for afforestation are indirectly enhancing abandonment of agricultural land. Nevertheless understory growing in the afforested sites could be used for feeding animals, as a way of clearing the undergrowth. It is feasible to combine both tree and pasture production. Fertilization should enhance the pasture and tree growth. One alternative for such fertilization is using sewage sludge contributing to reducing waste landfill and reusing its nutrients. The objective of this study was to assess the effect of applying lime treatment and two different doses of sewage sludge in different periods on the nutritive quality of pasture.

### Material and methods

The study was carried out in a collective private forest in Lugo, north-western Spain at 550 m above sea level. The experiment was based on randomized blocks design with three replicates applying 13 different treatments of fertilization under a *Pinus radiata* plantation newly established in 1999. The soil presented an initial low water pH (around 4) and it had not been cultivated for the last 25 years. The nutritive quality of the sown pasture has been studied for one year after its establishment. The sown mixture consisted of 25 kg ha<sup>-1</sup> of *Lolium perenne* L., 4 kg ha<sup>-1</sup> *Trifolium repens* L. and 10 kg ha<sup>-1</sup> *Dactylis glomerata* L. The treatments consisted of adding lime or not, in combination with two different doses of sewage sludge (50 or 100 kg total N ha<sup>-1</sup>) applied on three different periods (1<sup>st</sup> February, 3<sup>rd</sup> February and 31<sup>st</sup> March 2000). The used sewage sludge derived from the sewage plant of Lugo (Gestagua, S.A.) and met the requirements to be applied into agricultural and forest land according to the legislation (RD 1310/1990). In order to analyse the effect on the pasture, two harvests were performed, in July and November 2000. Four 1x1 m<sup>2</sup> samples were taken randomly in each plot. Samples were transported to laboratory and micro Kjeldahl digestion was performed to determine N and

P (TRAACS- 800+ autoanalyser) and Ca, K and Cu (VARIAN FS espectrophotometer) pasture concentrations. ANOVA was used for data analysis and Duncan test for mean separation.

## Results and discussion

Treatments have lead to significant differences on botanical composition but not on total dry matter (DM) production, which ranged from 1.8 to 2.7 t ha<sup>-1</sup> (Mosquera-Losada *et al.*, 2002). These are lower figures than what it has been observed in more fertile agricultural land (6 to 12 t ha<sup>-1</sup>) (Rigueiro *et al.*, 1999) but in the range of other pastures established in forest areas.

The crude protein concentration ranged from 6.41% to 8.14% for the first harvest and between 7.87% and 10.6% for the second harvest. They are rather low values that could be explained by the high percentage of existing shrubs and senescent material. The protein percentage increase in the second harvest related to the seasonal evolution found by Brea (1993) and Mosquera *et al.* (1999).

Values of P were also low but there have been significant differences with treatment\*harvest interaction. The highest values were obtained with the high dose of sewage when no lime had been applied. In some cases 1<sup>st</sup> and 3<sup>rd</sup> application date caused the highest concentration of P in the pasture. The treatments offered significant differences also in the concentration of K (at a 10% level), which in any case showed low values due to the high quantity of shrubs. As expected K concentration reached lowest levels with lime application (due to the antagonism with Ca) and the highest with the high doses of sewage sludge (due to the input made with the sludge fertilization). In the Figure 1 it is observed that for the high doses of sewage after applying lime, the highest value of K concentration is achieved for the first date of sewage application.

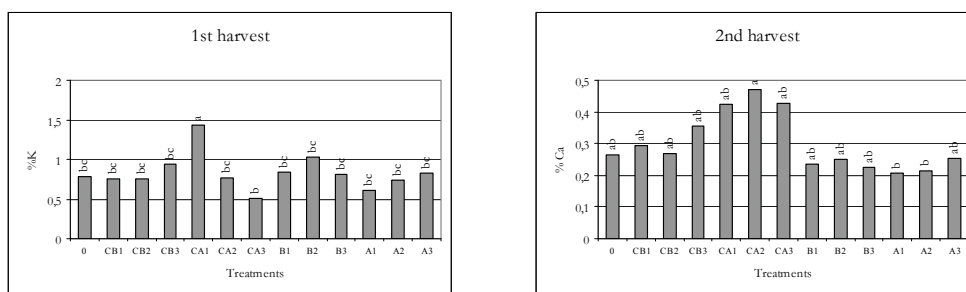


Figure 1. Percentage of K in the first harvest and percentage of Ca in the second harvest by treatments (0: control; C: lime; B: low doses of sewage sludge; A: high doses of sewage sludge; 1, 2 & 3: first, second and third sludge application date. Different letters mean significant differences between treatments.

Values of Ca were low also due to the high proportion of shrubs and the low availability of this element in acid soils. As expected liming leads to a higher Ca concentration but it is not significantly different to other treatments (Figure 1). Ca concentration will also vary with the existing vegetation, more shrubs were developed with low doses of sewage sludge and these have less Ca percentage. Therefore at the time of the second harvest the highest dose of sewage seems to lead also to higher concentration of Ca. The second application date gave normally the lowest levels of Ca content.

Cu is more available in acid soils (Mosquera *et al.*, 2001). Late application of low doses of sewage sludge reduced nutrients availability and caused a higher percentage of shrubs and therefore more Cu concentration (Figure 2).

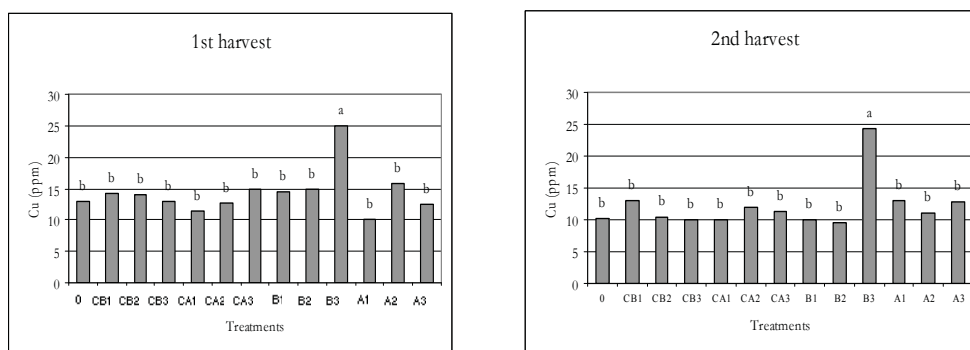


Figure 2. Percentage of Cu by treatments in the first and second harvests (0: control; C: lime; B: low doses of sewage sludge; A: high doses of sewage sludge; 1, 2 & 3: first, second and third sludge application date. Different letters mean significant differences between treatments.

### Conclusions

Despite of clearing the understory before the plantation and the sowing and of the effect of the treatments increasing the herbaceous component, there has been a high percentage of shrubs and dry material, which offer lower nutritive value than other pasture species due to the low soil fertility and the slow effect of liming and organic fertilizer (sewage sludge) applications. However, liming and sewage contribute to a better development of the sown species. The content on micronutrients and heavy metals is higher than in pastures due to the high proportion of shrubs and senescent material and the high acidity of the soil, which increased notably the bioavailability where the bioavailability increases notably. Some of the nutrients meet the maintenance needs of the animals (e.g. K, Cu for cows, goat or horses) but some others suggest the need to compensate its low level with complementary feeding (e.g. Crude protein, Ca and P for cows, goat or horses). Several years results should be analyzed in order to study possible residual and accumulative trends.

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# Residual effect of organic fertilization and liming on soil phosphorus and pasture in a silvopastoral system

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## Abstract

The aim of this study was to evaluate the residual effect of liming and sewage sludge application on soil and pasture phosphorus levels in a silvopastoral systems established in Lugo (NW Spain) under five years old *Pinus radiata* D. Don. stand with a density of 1667 trees per hectare. The plots were established in 1997 in an acid soil, liming half of the plots, and applying sewage sludge during three consecutive years at a rate of 0, 160, 320, 480 kg total N ha<sup>-1</sup>. Control treatment was also established which consisted of mineral compound application (500 kg of 8:24:16 ha<sup>-1</sup>) in no limed plots. In 2001, four subplots of 1 m<sup>2</sup> were established in each experimental plot which were not fertilized, receiving the rest mineral fertilization of 250 kg of 8:24:16 ha<sup>-1</sup>. Liming and organic fertilization increased phosphorus soil and pasture content due to the direct phosphorus application with the sewage sludge and indirect effect on soil pH of both liming and organic fertilization. Inorganic fertilization in 2001 increased phosphorous concentration in pasture.

Keywords: sewage sludge, pH, pasture.

## Introduction

Nowadays the European Union has an important wood demand which is causing a change in the land use in Galicia, making an important increase of forest land. In front of this point of view, several farmers are abandoning their activity because they have small and no competitive farms, so that they decide reforest their lands. The use of silvopastoral systems could be an alternative to the abandon of the farm activity as it allows to connect forestry production and livestock production to obtain benefits from both of them. Generally, soils of forest sites in Galicia are acid and with low fertility (López-Mosquera, 1995), which makes necessary apply lime and a fertilizer. On the other hand, UE normative about the treatment or wastes has caused important increase in the production of sewage sludge. This residue has good biodegradability and an important potential value as fertiliser and for this reason, UE promotes its use in agriculture. Different experiences in Galicia has shown the enhancement of pasture production (López Díaz *et al.*, 2005) and quality (Rigueiro-Rodríguez, 2000) when sewage sludge is applied as a fertilizer.

## Material and methods

The experiment was carried out in Lugo (NW Spain) following a randomised block design with three replicates in autumn 1997. After soil preparation, pasture mixture was sown (25 kg ha<sup>-1</sup> *Lolium perenne* L. cv 'Brigantia', 10 kg ha<sup>-1</sup> *Dactylis glomerata* L. cv 'Artabro', 4 kg ha<sup>-1</sup> *Trifolium repens* L. cv 'Huia') under a five year old *Pinus radiata* plantation (1667 stems ha<sup>-1</sup>). Every plot consisted of 25 trees distributed in a square of 5x5 trees. Fertilization treatments consisted of three sewage sludge doses (LB: 160 kg N total ha<sup>-1</sup>, LM: 320 kg N total ha<sup>-1</sup>, LA: 480 kg N total ha<sup>-1</sup>), no fertilization (NF), all of them with and without lime (2.5 t CO<sub>3</sub>Ca ha<sup>-1</sup>), and a mineral fertilization (MIN: 500 kg 8:24:16 ha<sup>-1</sup>). Fertilization treatments were applied in spring 1998, 1999, 2000. In spring 2001, mineral fertilization (250 kg 8:24:16 ha<sup>-1</sup>) was applied in the plots with the exception of four subplots of 1m<sup>2</sup> installed in each main plot. Sampling consisted of harvesting four squares of 0.09 m<sup>2</sup> in each experimental unit at a height of 2.5 cm above soil level. Samples were transported to laboratory, where were dried at 60°C

during 48 h and they after were grind. Phosphorous concentration was determined after microkjeldal digestion (Castro *et al.*, 1990) using Bran Luebbe's (1979) method. Treatments were evaluated with the ANOVA using the statistic program SAS (1985).

## Results and discussion

Phosphorous levels in the pasture were between 0.1-0.6% (Figure 1), which are into the range described by Mosquera and Gonzalez (2003), and into the normal levels at Galicia forest sites (Mosquera *et al.*, 2002).

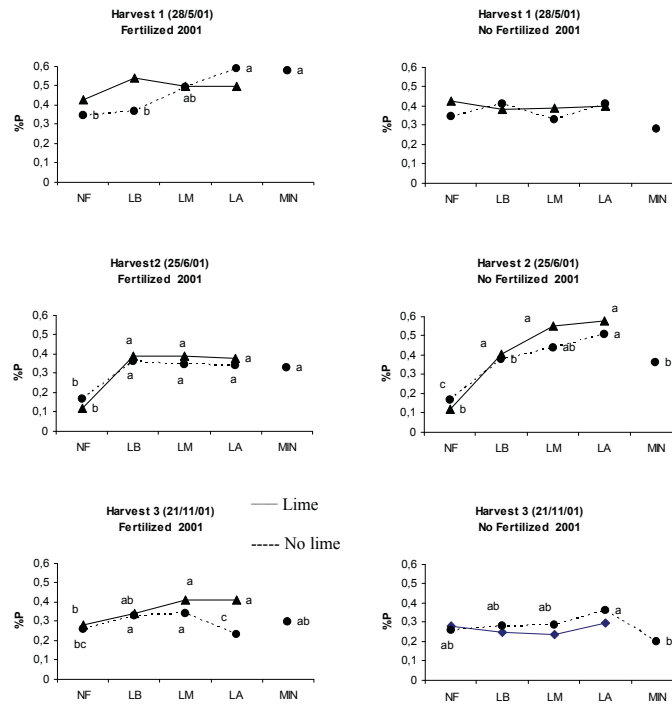


Figure 1. Percentage of phosphorous (%) in the pasture in the different harvests carry out along 2001 according to the treatment applied during the previous tree years of 2001. The fertilizer treatments applied during the previous tree years of 2001 are: Limed ( $2.5 \text{ t CO}_3\text{Ca ha}^{-1}$ ), No limed, three sewage sludge doses (LB:  $160 \text{ kg N total ha}^{-1}$ , LM:  $320 \text{ kg N total ha}^{-1}$ , LA:  $480 \text{ kg N total ha}^{-1}$ ), no fertilization (NF) and mineral fertilization (MIN:  $500 \text{ kg 8:24:16 ha}^{-1}$ ). Different letters show significant differences between treatments of fertilization for lime doses.

The P evolution along 2001 shows a maximum concentration in spring and the lower levels of P in pasture at the end of the year. This may be caused by the important percentage of senescent tissues in pasture in the last harvest. In general, the presence of P in the soil (Figure 2) and pasture was high in the limed plots due to the pH increase caused by the application of  $\text{CO}_3\text{Ca}$  and the direct phosphorus input made by sewage sludge application. The experiment was installed in an acid soil (pH 4.5), where an increase in the pH produces an aluminium levels reduction into the exchangeable complex, increasing phosphorous availability.

Inorganic fertilization in 2001 equalled phosphorous concentration in those plots with previous inorganic and sewage sludge applications. However, when no inorganic fertilization was applied in

2001 (subplots), the P levels were similar to those obtained in the NF treatment, which is indicative of the low availability of P in the acid soil of the experiment.

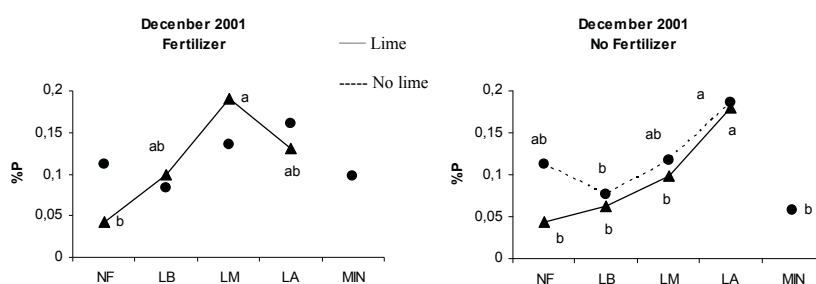


Figure 2. Percentage of phosphorous (%) in soil (5.5 cm) according to the treatment applied during the previous tree years of 2001. The fertilizer treatments applied during the previous tree years of 2001 are: Limed (2.5 t CO<sub>3</sub>Ca ha<sup>-1</sup>), No limed, three sewage sludge doses (LB: 160 kg N total ha<sup>-1</sup>, LM: 320 kg N total ha<sup>-1</sup>, LA: 480 kg N total ha<sup>-1</sup>), no fertilization (NF) and mineral fertilization (MIN: 500 kg 8:24:16 ha<sup>-1</sup>). Different letters show significant differences between treatments of fertilization for lime doses.

## Conclusions

Liming and organic fertilization increased phosphorus soil and pasture concentrations due to the direct phosphorus application with the sewage sludge and indirect effect on soil pH of both liming and organic fertilization. No inorganic fertilization in 2001, reduced phosphorous concentration in pasture.

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# Session 4

## Changes in animal production systems to meet CAP reforms



# Impacts of CAP reforms on animal production systems

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## Abstract

The aim of this paper is to describe the main factors, which have determined the evolution of the Common Agricultural Policy (CAP) over the last forty years and to outline the implications for the future of grazing animal production systems, of the latest set of major reforms adopted in 2003. The gradual transition from the initial policy of price support to a policy of coupled (production-linked) in the 1990s through to the decoupled regime introduced by the 2003 CAP reform is set against the changes in key indicators over the same period of the size and structure of the animal production systems in the enlarging European Community. The specific contribution of the animal sector in that policy evolution is also outlined. The paper closes with an analysis in which the main determinants for the future of animal production systems, in the context of the reformed CAP, are identified and an attempt is made to relate these possible developments to research needs in animal production science in the coming decade.

Keywords: CAP, reform, animal production systems

## The Animal Sector and the Common Agricultural Policy

### Introduction

Agriculture policy is unique within the EU. Since the laying down of Articles 38 to 47 of the Treaty of Rome in 1957, agriculture has, unlike any other economic sector, been the object of a pan-European policy, the common agricultural policy or CAP. Furthermore, the policy objectives of the original CAP have continued to apply: increasing productivity and promoting technical progress; ensuring a fair standard of living for the agricultural community; stabilising markets and assuring availability of food supplies; and ensuring supplies reach consumers at reasonable prices.

Since the establishment of the EU by the founding Member States (Germany, France, Italy, Belgium, Luxembourg and the Netherlands), though the transformation that has taken place in EU agriculture cannot be minimised, assessing the role the CAP in that evolution has been the subject of frequent and ongoing debate. There are two key features in the history of the CAP, which go some way to explaining the difficulty in determining the effect of that policy. While the objectives of the CAP may have remained unaltered, the size and composition of agriculture sector of the Community has continually, and sometimes abruptly, changed with successive enlargements. Although fundamental changes in the agriculture may be observed in each of the Member States, analysing common trends is obscured by the difficulty of taking the EU itself as a baseline for comparison. Furthermore, the policy tools employed to attain the CAP's objectives have changed significantly with time.

With these constraints in mind, it clearly cannot be the pretension of this paper to make an exhaustive, scientific evaluation of the impact of the CAP on the development of grazing animal production systems over the last forty years. Instead, major changes in both the policy and the sector are sketched, highlighting those key factors, which have lead up to most recent reform decisions, and which will condition the future of the sector over the next decade. The paper closes with a description of research needs in animal production science in the coming decade.

### *The first years of the CAP (1962-1973)*

The CAP of the 1960s was heavily conditioned by the state in which European society found itself in the years following the end of the Second World War. Firstly, food supply had been heavily disrupted and Europe's overall rate of self-sufficiency was still no higher than 87 % in the period 1956-1960 (Clerc, 1964). Secondly, farm structures, which had been weakened by years of conflict and economic depression, revealed gross deficiencies in terms of size of holding and technical level.

Under these circumstances, the joint-objectives of increasing farm productivity, stabilising markets and ensuring food supplies spawned direct, market policy tools: price support mechanisms, including public intervention buying-in and export refunds, in the context of a common market, were established for many EU farm commodities. Furthermore, after a protracted period of elaboration, the Community adopted in 1968 the first of a series of reforms of structural measures, namely the "Mansholt Plan", aimed at improving labour productivity, farm size and marketing of farm products.

### *The years of excessive plenty (1973-1986)*

Such was the policy framework into which the first enlargement of the Community, involving the admission of Denmark, Ireland and the United Kingdom, took place in 1973 and which marked a turning point with regard to several animal products in the Community. As a direct consequence of the almost 50% increase in beef and dairy cattle numbers in 1973 (Figures 1 and 2) in a production-oriented policy framework, the rate of self-sufficiency for beef rose from around 85% to excess levels by 1975. For dairy products, self-sufficiency in butter rose from around 100% to 148% by 1983 while sheep meat self-sufficiency, as low as 55% in 1972, grew to 88% by 1993.

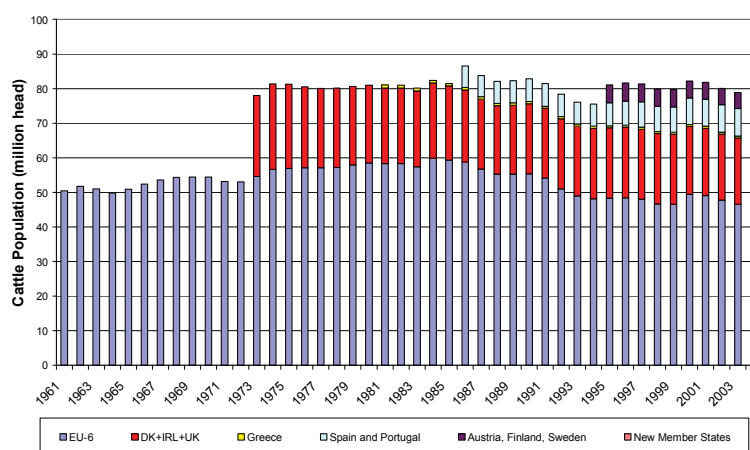


Figure 1. Evolution of Beef Cattle population in the EU.

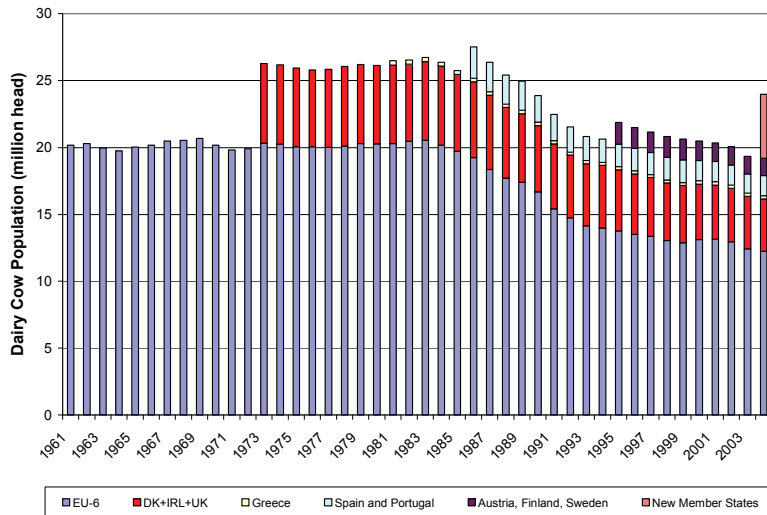


Figure 2. Evolution of Dairy Cattle population in the EU.

This dramatic growth in animal production brought increasing pressure in terms of market imbalance, international friction from selling the surplus on world market and exploding CAP expenditure. With the CAP fast gaining a reputation as a builder of 'butter and beef mountains', this period saw the first introduction of strict budgetary measures, in the form of stabilisers and monetary instruments, the co-responsibility levy for the co-financing of the common market organisations, and supply control tools, such as milk quotas in 1984.

Finally, an even more spectacular growth took place in the sheep and goat population with the entry of Greece into the EU in 1981. Figure 3 illustrates how, from a population of just over 20 million head in 1973, the Community had trebled this number by 1981 and with the entry off Spain and Portugal in 1986 was to reach over 100 million by 1990.

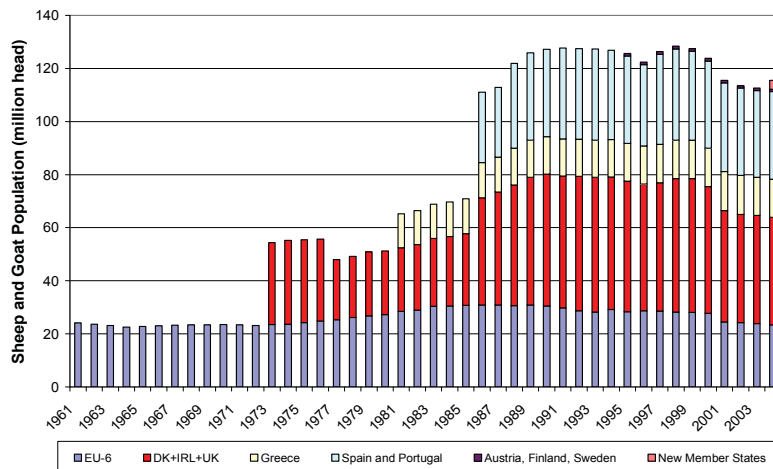


Figure 3. Evolution of sheep and goat population in the EU.

### *The years of reform (1986-1995)*

The late 1980's were thus a time of major reflection on the future of the CAP. In 1985, the Commission white paper entitled 'Perspectives for the Common Agricultural Policy' (European Commission, 1985) brought attention to new areas of policy: product quality, development of the food processing industry and support for agricultural activity, with a view to maintaining social balance and protecting the environment and the landscape. Three years later, the Commission made a major review of rural development policy, entitled 'The Future of Rural Society' (European Commission, 1988), which was drawn up in preparation for two further important milestones in the history of the EU - the Single Market, adopted in 1989, and the re-unification of Germany in 1990.

But without doubt the most important re-appraisal of the time was the outline for 'The Development and Future of the CAP' (European Commission, 1991), which ultimately lead to the 1992 CAP reform. In those proposals, the Commission set the reformed CAP five major objectives: an improvement in the competitiveness of EU agriculture; an increase in market stability and a control on surpluses and spending; diversification in rural areas; and linking the productive function of the farmer to the protection of the environment.

To boost competitiveness, substantial reductions in institutional support prices were agreed. Compensation to farmers for the resulting revenue loss was made in the form of coupled (i.e. production-linked) per hectare payments for arable crops and headage payments for cattle and sheep. To control spending, limits in terms of national ceilings for headage payments reinforced the existing supply control measures (e.g. milk quotas).

The impact of these measures, in terms of the relative decline in livestock numbers since 1992, can be seen in Figure 4. In the sheep and goat sector, the 1992 Reform introduced a strong brake on population growth and stabilised beef cattle numbers. In relation to the dairy sector, the effect of the introduction of quotas in 1984 can be seen not only in the absolute but also the relative decline in cow numbers in Figure 4.

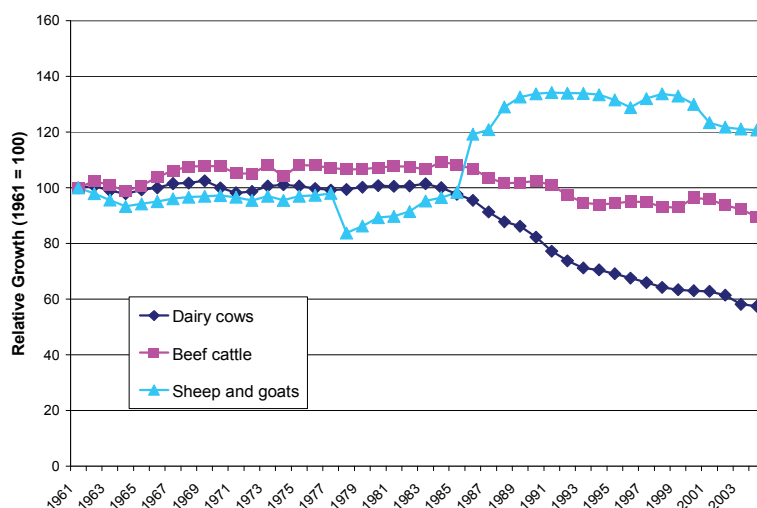


Figure 4. Relative growth of the EU beef cattle, dairy cow, and sheep and goat populations.

During the period, the round of political discussions, which had been triggered by the 1985 paper, lead to the adoption of two other sets of legislation, each with its own particular relevance for the animal sector. Firstly, the 'Accompanying Measures' of the 1992 CAP reform introduced greater incentives for early retirement and marked a starting point for today's agri-environmental measures. Secondly,

legislation was adopted dealing specifically with product quality through the rules governing organic production and those covering 'Protected Denominations of Origin' (PDO), 'Protected Geographical Indications' (PGI) and 'Traditional Speciality Guaranteed' (TSG).

### ***Preparation for enlargement (1995-2003)***

Between 1995 and 2003, the development of the CAP was marked by two major events, related to the enlargement of the EU. With the entry of Austria, Finland and Sweden, 1995 saw expansion to EU-15, and the introduction of a "Nordic" dimension to EU agriculture. The year 1997 came with the publication of the Commission's framework document entitled "Agenda 2000: For a Stronger and Wider Union" (European Commission, 1997), which represented the EU's proposals for building the concept of sustainability into EU policy and preparing the EU-15 for the forthcoming enlargement to EU-25 through adhesion of central and east European candidate countries (CEECs).

In agricultural policy terms, the Agenda 2000 package, adopted in 1999, did not suppose a notable shift from the direction taken in the 1992 reform: additional price cuts, with compensation in the form of coupled payments, were agreed in sectors such as beef, milk and cereals, in the interest of further improving the competitiveness of EU agriculture.

Nevertheless, a number of other significant policy elements were integrated into the Agenda 2000 package. The previously dispersed 'structural' measures were brought together under a separate rural development pillar and the Berlin European Council approved the Agenda 2000 for the CAP with an explicit budget ceiling for the first time.

Indeed, the sustainability concept introduced by Agenda 2000 was taken further in 2001 with the adoption by the Göteborg Council of the European Sustainable Development Strategy (SDS), which, in agricultural terms, served to sharpen the policy focus on the economic, social and environmental aspects of agriculture.

However, a review of this period in the history of the CAP, in particular as it relates to the animal sector, would not be complete without reference to an event, which would have a significant influence on public perception of the CAP in future years. In 1996, the announcement of the possible link between bovine spongiform encephalopathy (BSE) and disease in humans brought the EU into its first 'mad cow crisis'. The widespread public concern over food safety, and the significant market disruption it created, was relived in 2000, with the detection of more cases in continental Europe. These events, which had far-reaching influence at a European level in terms of food safety policy, risk management and consumer attitudes, are an integral part of today's CAP.

### **The 2003 CAP Reform**

In 1999, at the time the Agenda 2000 package for the period 2000-2006 was adopted, the Council agreed a Mid-term Review of the policy. This was taken by the Commission, in 2002, as 'the opportunity to examine EU agricultural policies and ensure that they better meet the objectives set in Agenda 2000 and Göteborg' (European Commission, 2002), which were defined as: competitiveness in the agricultural sector; environmentally friendly production of quality products that the public wants; a fair standard of living and income stability for the agricultural community; diversity in agriculture and supporting rural communities; simplicity in agricultural policy; and linking support to providing public services that farmers are expected to provide.

Such an analysis revealed one underlying trend: since 1992, the CAP had been immersed in a fundamental reform process, aimed at moving away from a policy of price and production support to a more comprehensive policy of farmer income support. Consequently, with the decision reached at the Luxembourg Council in June 2003, the last step in this process was achieved with the incorporation of the arable area and livestock headage payments (i.e. dairy, beef, sheep and goats) into the decoupled, single farm payment scheme.

The subsequent decisions in 2004 to integrate the olive oil, cotton and tobacco sectors into the 2003 CAP Reform framework, and the political agreement on sugar reform of November 2005, see European

agriculture provided with a long-term policy perspective, which will enhance competitiveness, promote stronger market-orientation and the develop the entrepreneurial role of farmers. Farmer incomes will be stabilised by giving priority to producer income and not product support through the transfer of a significant part of the former production-linked direct payments to the single farm payment scheme. Subjecting those payments to the respect of the statutory EU environmental and food safety standards, through cross-compliance, and rules of good agricultural and environmental condition, will improve the environment. Expenditure will be better controlled through the introduction of a financial discipline mechanism, within the new framework for agricultural expenditure agreed at the Brussels European Council in October 2002. Finally, a better balance of expenditure will be achieved with the transfer of more funds to rural development measures through the modulation mechanism.

### **Implications of the 2003 CAP Reform for Animal Production Systems**

As a first consideration of the implications of the 2003 CAP Reform process on the EU agriculture sector, it should be noted that the Commission's projections for EU-25 agricultural markets and income (European Commission, 2005), for the period 2005-2012, indicate that real farm income has a moderately positive outlook, with particularly high income gains in the new Member States.

In terms of the competitiveness of the EU animal sector over the same period, the medium-term perspectives are projected to be positive for the EU dairy sector, due to an increase in domestic demand for cheese and other value-added dairy products and a decrease in the production of bulk products, such as butter and skimmed milk powder. Milk quotas will constrain EU milk production while growing milk yields will reduce cow numbers. Revenue earned by dairy producers from selling culled animals are expected to decline while an increase in specialised beef production, as more forage and grazing area becomes available, is possible. However, increasing the intensity of dairy production by more use of concentrated feed and a reduction of the area used for grazing animals could increase environmental pressures in some localities.

Prospects are somewhat more negative for EU beef sector, where beef production, which was lower than consumption in 2003 for the first time in twenty years, is expected to remain so, falling from 8.2 to 7.9 million tonnes. This is due to the declining cattle numbers in the dairy sector, the effects of decoupling of direct payments and the residual impact of market disruptions following the BSE crisis, though the size of this deficit will be determined by the return of Over Thirty Month beef to the UK market from November 2005. Given the continued strength in domestic consumption and the absence of intervention stocks, beef prices are at present recording high prices at retail level.

Finally, prospects in the EU sheep and goat sector are characterised by a limited recovery (i.e. production stable at 1.1 million tonnes), after the 2001 Foot-and-Mouth outbreak reduced supplies and caused high market prices. This is a sector, which also suffers from slack demand, compared to the stronger trends in pork and poultry.

However, the more localised, dynamic effects of the reform are harder to be translated to individual regions. The work of Swinbank and Tranter (2004) has even suggested that policy reform will be unlikely to prompt farmers to make any changes to the main farming activities in the UK, Germany and Portugal. Since it will be possible to grant additional payments, up to 10% of the component of national ceilings for specific types of farming and quality production under the new common rules for direct support schemes, many of the local impacts will depend on Member State decisions. Anders *et al.* (2004) have indicated that transfers targeted in terms of absolute support under the CAP funding may induce an arbitrary interregional distribution of producer support in relation to farm revenues. However, Chatellier and Jacquerie (2004) highlighted that the amount of the single payment per hectare will be different between dairy production areas, according to intensification level and fodder system, and that payments may have direct effects on production, competitiveness, land use intensity and farm management. On the other hand, milk quotas have in some areas locked-in the regional distribution of production and thus contributed to the maintenance of farming in less economically competitive areas.

In the past, many farmers have focussed too heavily on how to increase income from EU subsidies, which has diverted attention away from consumer-oriented activities. Though income supported, EU



farmers will undoubtedly become more receptive to market indicators and the development of better marketing strategies, based on private initiative or within an institutional framework (i.e. PDO, PGI and organic production) will be necessary. One key to ensuring market penetration could be to raise the quality status of animal products by development of Quality Assurance Schemes that address management issues from 'farm to fork'. Nevertheless, there are many regional products in the EU, which can earn a market premium because of their quality and reputation. There are many excellent innovative and market orientated practices in farming that can become market signals about what customers want to get through to many farmers and diversification will be an increasingly important earner for farmers. Therefore, to maintain profitability, the future of farming under the new CAP will also depend on farmers reconnecting with consumers through their supply chains.

In relation to extensification, one of the main economic benefits of decoupling comes through the so-called 'freedom to farm', which allows changes in stocking rates, for the purpose of reducing their costs, without loss of subsidy income. As land is increasingly a limited factor in developed countries, the management of natural resources and the environment will remain an important issue. While the inclusion of grassland in the new support system is especially important (Gay *et al.*, 2005), improvements in efficiency in animal performance of extensive livestock farming based on grazing will still be related to adequate grass quantity and quality required under a sustainable stocking rate. On the other hand, in recent years, though the seasonality of disposals has changed in some countries (Denmark, Germany, Ireland and Sweden), with direct payments gone, some producers could move towards more intensive, all-year round finishing systems producing cattle at younger ages.

In relation to the dairy sector, the reform might provide incentives for increased intensification of production. Lower milk prices could speed up structural adjustment, thereby exacerbating the underlying trend of recent decades to smaller numbers of more highly productive cows, and put more pressure on cost reduction in terms of purchased feeds, power, machinery, labour, herd depreciation, veterinary and medicine costs.

Decoupling also removes one of the main obstacles to the implementation of agri-environmental programmes and, in relation to the new requirements for cross-compliance and good agriculture practice, the impact on the EU animal sector will be significant. Overgrazing or loss of traditional extensive livestock grazing systems can damage vulnerable environments. Major environmental issues such as soil erosion and pollution from livestock holdings will have to be addressed. Improvements in farm size on holdings with cross-compliance programmes and conservation of the pasture may favour viability of smaller and less profitable Mediterranean farms more prone to erosion damage (Varela-Ortega and Calatrava, 2004), though it is difficult to assess the extent to which environmental services has had impacts on farming, since implementation of such measures is still at a relatively early stage (OECD, 2004). Moreover, the contribution of livestock to gaseous emissions is noteworthy: 94% of total EU ammonia emissions originate from housed animals and 49% of total methane emissions arise from animal husbandry (EEA, 2002). Furthermore, high livestock population densities are associated with excessive concentration of manure that can lead to an increased risk of water pollution. In this context, Oenema (2004) identified nitrogen loading and slurry storage capacity as priorities to be dealt with in future animal production systems.

Cross-compliance will also have implications for the application of animal welfare standards and the future of animal health policy. To Swinbank and Tranter (2004), this translates into an increase in welfare for the farming. Higher costs incurred by European farmers, due to required compliance with regulations covering environment, food safety and animal welfare, could be used as an opportunity to increase market share by building greater consumer confidence in both the product and farm practice. Haskings (2002), referring to the Finnish experience, recorded instances where local farmers have managed to increase their share of the domestic market in face of increasing farm standards.

## Possible developments of research needs

This review of the evolution of the CAP, in relation to grazing animal production systems, has illustrated the gradual transition, which has taken place, from a commodity-based policy, to one oriented more to market demand and the provision of public goods.

In meeting the challenges of the dynamic changes, which are likely to take place in the EU agricultural sector in the coming decade, as a consequence of the 2003 CAP Reform process, and in light of the overarching objectives for the competitiveness of the EU economy, laid down in the Lisbon Strategy (European Commission, 2005), the EU farming sector is looking with renewed interest at the solutions the research community can provide.

In this context, the Commission has initiated a reassessment of the role of agricultural research, within the 7th Framework Programme for Research and Technological Development (European Commission, 2005) and has identified three main areas of research: developing of sustainable EU agricultural production systems; strengthening the competitiveness and sustainability of the EU agri-food sector; and achieving a knowledge-based EU agricultural economy.

The continued emphasis on the development of sustainable agricultural production systems will focus firstly on enhancing competitiveness through the search for efficient farming practices and animal production systems, improved biotechnology and breeding, better integration of technological developments into farming and diversification, including non-food bio-materials. Actions are also foreseen in supporting certified production schemes, including organic production, quality product labels, and improving the authenticity and safety of agricultural products and promoting the environmental aspects of sustainability, including methodologies for the agri-environmental and “cross-compliance” policy areas.

In relation to the development of a sustainable EU agri-food sector, attention will be paid to technological advances aimed at developing an efficient agri-food sector, reflecting consumer demands and the needs of society. Improved food processing and product quality, by supporting a “total quality” concept for new or improved high quality foods, organic produce, as well as small scale production processed on the farm will include attention to food safety and developing new, high added value products, while improving the awareness of the role of nutrition and diet in promoting and sustaining health.

With regard to building a knowledge-based agricultural economy, the success of EU policies for agriculture and rural development is not simply a question of innovation and improving performance at production level. It is necessary that economic actors and policy makers take the right decisions and establish an appropriate regulatory framework for EU agriculture in the context of a highly competitive global economy. In order to succeed in this task, research can play a key role by providing the right monitoring and assessment tools for guiding CAP reform, analysing the role of the institutional framework and improving the analysis of trade issues.

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# Towards sustainable intensive dairy farming in Europe

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## Abstract

Increased production from grassland has been achieved at considerable environmental costs, including the loss of biodiversity, many wildlife habitats and landscapes. Since the 1980s policy makers in many European countries require that elements of a sustainable forage production be addressed by researchers. In many European countries intensive dairy farms are facing difficulties in achieving these goals, with nitrogen surpluses and losses being a major topic in large parts of northern continental Europe, where sandy soils predominate. Results from two comprehensive studies (experimental farms Karkendamm and De Marke) show the current nitrogen status of forage-based dairy production in this area and identify shortcomings in the production systems which should be improved in the future. Simulations with the worldwide leading modelling system IFSM (Integrated Farming System Model) verify the long-term effects of technological changes and point at potential solutions for the actual dilemma. Among many agronomical factors determining feed production for dairy cows grassland cultivation and renovation is a quite important issue. A literature review indicates that little knowledge exists in many aspects but also shows that the application of good farming practices can reduce the environmental risks for dairy dominated regions.

Keywords: grassland management, dairy farming, sustainability, nitrogen losses, energy efficiency.

## Introduction

Sustainable production systems aim at the integration of social, production and environmental goals, including improved nutrient management and reduced environmental pollution. A suitable balance must be made between minimizing nutrient losses and production costs to provide sustainable production systems. Limited information exists on the management and nutrient flows in grassland agriculture at the whole-farm or systems scale. Most of this type of research has focused on arable cropping systems. In this paper we focus on two themes which are relevant for grassland production in areas with intensive dairy farming where a major environmental issue in the future will be the improvement of nutrient efficiency: 1. *Nitrogen and energy use efficiency in forage production systems* based on data from two recent experimental research efforts which focused on nutrient management in whole-farm systems and 2. *Efficient grassland cultivation and renovation* as a tool for improvement of feed supply and herbage quality in dairy farms and which also has an impact on environmental issues. The latter is based on a comprehensive review of literature.

## Focus Theme 1: Nitrogen and energy use efficiency in forage production systems

### *Karkendamm Experimental Farm*

Multiple interactions influencing nitrogen fluxes in the soil-plant-animal system were studied at the Karkendamm experimental farm in northern Germany (Taube and Wachendorf, 2001). Experiments were established on permanent grassland (Trott *et al.*, 2004; Wachendorf *et al.*, 2004; Lampe *et al.*, 2004) including cutting (i.e., mechanical harvest for hay or silage), grazing, and two mixed systems of cutting and grazing with mineral fertilizer input (0, 100, 200, 300, and 400 kg N ha<sup>-1</sup>) and slurry

application (0 or 20 m<sup>3</sup> ha<sup>-1</sup> at 2.4 kg N m<sup>-3</sup>). The N surplus across all treatments was linearly related to total N supplied (Figure 1; Trott *et al.*, 2004). The increase in N surplus per kg N applied was 2.5 times higher in GO than in CO. Thus, to reduce N surpluses in rotational stocking systems, less N must be applied. The inclusion of a silage harvest with rotational stocking systems reduced N surplus and cutting-only systems allowed N application rates beyond 300 kg ha<sup>-1</sup>. Nitrate leaching losses were strongly affected by the type of defoliation, with nitrate concentrations in the leachate in the CO and SG systems generally below the EU threshold (Wachendorf *et al.*, 2004). Highest concentrations were measured in the grazed-only treatment and intermediate nitrate losses occurred in the mixed systems. The regression in Figure 1 implies that N surpluses of not more than 30 kg ha<sup>-1</sup> are acceptable to meet the EU standard for drinking water. Total N<sub>2</sub>O emissions measured from the soil surface over an 11-month period ranged from 1.7 to 4.9 kg N ha<sup>-1</sup>. The lowest N<sub>2</sub>O emissions occurred with an application of 100 kg mineral N ha<sup>-1</sup> and the highest emissions occurred when a combined N supply of slurry (74 kg N ha<sup>-1</sup>) and mineral fertilizer (100 kg N ha<sup>-1</sup>) was applied (Lampe *et al.*, 2004). Emissions in winter, primarily during freezing and thawing cycles, made a considerable contribution to the total annual N<sub>2</sub>O loss.

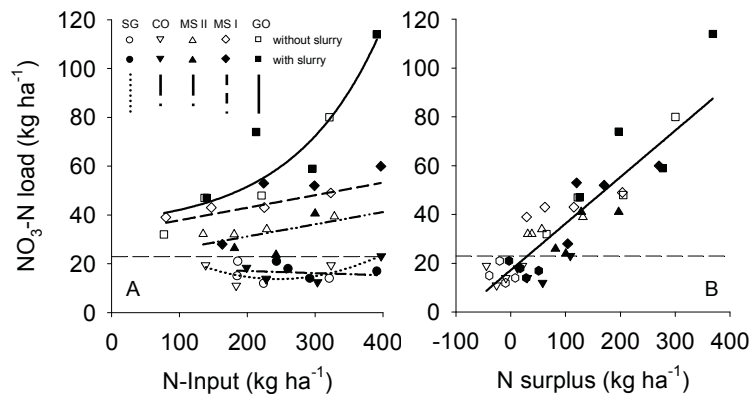


Figure 1. Relationship between annual N leaching losses and (A) N-Input (including N from slurry, mineral fertilizer, biological fixation and atmospheric deposition ) and (B) annual N surplus on grassland (GO=pasture; MSI/II=mixed system with one cut and two cuts in spring resp.; CO=cutting; SG=simulated grazing) (Wachendorf *et al.*, 2004).

The efficiency of fossil energy use was determined as the feed net energy yield per unit of fossil energy input in production activities. Energy inputs included both direct (diesel use for field operations) and indirect (fossil energy input in the manufacture and distribution of fertilizers, pesticides, machinery, seeds, etc.) inputs (Kelm *et al.*, 2004). Energy efficiency declined with increasing mineral N fertilizer input and was most pronounced on pastures. A given net energy yield was produced most energy-efficiently in a mixed system (MS1) where additional yield compensated for the higher energy input from increased machinery activities. With increasing grazing intensity, CO<sub>2</sub> emission was reduced whereas N loads increased continuously. The CO<sub>2</sub> emissions were lowest in unfertilised pastures, but increased again with increased input of mineral N fertiliser. The benefit of reduced nitrate leaching loss from cutting systems must be considered along with the significantly lower energy efficiency and higher CO<sub>2</sub> emissions of these systems compared with grazing-only systems (Figure 2). The selection of the optimal or best production strategy is dependent upon the relative value of each of these factors as determined by the demands of society.

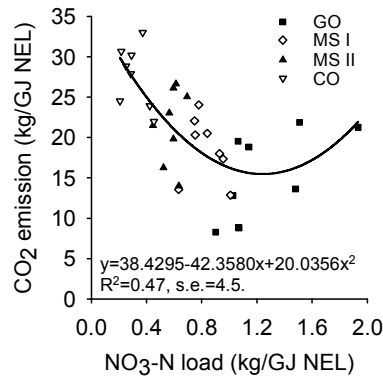


Figure 2. Relationship between CO<sub>2</sub> emission and nitrate-N load in the drainage water from grassland (GO=grazing only; MS I/II=mixed systems with one cut and two cuts in spring, respectively, and subsequent grazing; CO=cutting only; NEL=net energy lactation) (source: Wachendorf and Taube, 2005).

### *The De Marke Experimental Farm*

The De Marke farming system (located near Hengelo in the province of Gelderland, The Netherlands, on a sandy soil) was designed to minimize external inputs of feed and fertilizer and to maximize the use of homegrown feeds and manure. The goal was a high milk production per cow with a minimum number of calves and replacement heifers to reduce feed requirements per unit of milk produced. The farm area consists of 11 ha of permanent grassland and 44 ha of rotated grass and maize. Fields are in grass for 3 years followed by 3 to 5 years of maize. Grass and maize are rotated to stimulate maize growth and to avoid build-up of high organic matter contents with the associated risk of nitrate leaching during decomposition of the organic matter(OM) In the first year after grass, maize is not fertilized. Nitrogen fertilization levels at De Marke, including N from slurry, clover, and the residue of ploughed-under Italian ryegrass and grass sod, are about 40% lower than those on conventional farms. About 75% of the slurry produced is applied to grassland by shallow injection in two to three splits depending on grassland management. Additional inorganic N fertilizers are applied on grassland at a rate of 107 kg N ha<sup>-1</sup>. Total surplus N of the farm (Table 1) includes NH<sub>3</sub> volatilization, denitrification loss, accumulation in soil organic matter, runoff, and leaching. Average annual surplus from 1993 to 2002 was 146 kg N ha<sup>-1</sup> and 3 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>. The design of the farming system was modified in 2000 (shorter grazing periods and reduced fertilization), which reduced the N surplus to 117 kg N ha<sup>-1</sup> by 2002. A comparison of the nutrient balance of De Marke to that of an average current farm (on sandy soil in the mid 1990's with a similar milk quota) shows that high nutrient use efficiencies in animal nutrition and crop cultivation allow similar milk production with a lower level of nutrient input.

Table 1. Nitrogen balances of the De Marke experimental farm compared to the balances of the average Dutch farm in the middle of the 1990s (source: Hilhorst *et al.*, 2001).

kg N ha <sup>-1</sup>	De Marke		Average Farm
	1993-2002	2002	
<b>Input</b>			
Concentrates	86	87	125
Roughage	8	0	20
Chemical fertilizer	64	35	242
Organic manure	0	0	50
Biological N fixation	11	27	0
Animals	0	0	0
Deposition	49	49	49
Miscellaneous	5	5	0
Total	223	203	486
<b>Output</b>			
Milk	66	64	64
Animals	9	8	14
Roughage	1	0	0
Organic manure	1	0	0
Total	77	72	78
<b>Balance</b>	<b>146</b>	<b>131</b>	<b>408</b>

#### *Computer simulation as a tool for long-term evaluation of farming systems*

With computer simulation, many variants of the production system can be easily evaluated, including different climatic regions, soil types, and farm management scenarios. As one of the few system-oriented models the Integrated Farm System Model (IFSM) integrates the many biological and physical processes on dairy and beef farms (Rotz *et al.*, 1999; Rotz and Coiner, 2003; Rotz *et al.*, 2005). Confidence in the simulation results can best be gained by a combination of experimental and modelling evaluations with measured data and information from actual production systems. As an example how the farm model can be applied to extrapolate the experimental results of the Karkendamm study to whole farm systems, a representative dairy farm with the characteristics of farms in this region was simulated using weather data from Kiel, Germany (Table 2). Rotational grazing of the dairy herd had little effect on N import and export from the farm, but N losses were greater. To determine the long-term benefits of the management strategies and technologies used at De Marke, three production systems (previous technology, current technology, and the De Marke technology used for nutrient conservation; details in Table 3) were compared for a representative dairy farm of this region (Reijneveld *et al.*, 2000; Aarts *et al.*, 1999). The production system under previous technology reflected inefficient use and cycling of N. Excessive amounts of N, primarily in the form of mineral fertilizer, were imported, causing high losses to the environment. A comparison of current and previous technologies indicates both positive and negative environmental impacts for recent changes in the Dutch dairy industry. A large reduction in N volatilization loss was obtained using the enclosed manure storage and manure injection; however, this caused substantial increases in nitrate leaching and soil denitrification. This occurred because the reduction in volatile loss led to higher levels of soil N, even with a reduction in the use of N fertilizer. Only by fully implementing the practices of De Marke were substantial improvements in N use efficiency achieved through a large reduction in the import of N in fertilizer and feed. Nitrogen volatilization losses were greatly reduced along with reduced leaching and denitrification losses.

Table 2. Annual feed production and nitrogen flows of a simulated dairy farm\* in northern Germany (source: Rotz *et al.*, 2005).

Production parameter	No grazing <sup>§</sup>	Grazed <sup>¶</sup>
Feed production (Mg DM)		
Grass silage production	288	144
Grazed forage consumed	0	137
Forage purchased	57	87
Supplemental feed purchased	127	111
Nitrogen flows (kg ha <sup>-1</sup> )		
Nitrogen imported	310	314
Nitrogen exported	82	82
Nitrogen lost by volatilization	80	94
Nitrogen lost by leaching	43	55
Nitrogen lost by denitrification	29	30

\* 55 cows (8,000 kg milk cow<sup>-1</sup>) and 48 replacement heifers on 34 ha of loamy sand soil simulated over weather years 1980 to 2002 for Kiel, Germany.

§ Entire grass crop is harvested, conserved and fed as silage.

¶ About 50% of the annual grass forage consumption is fed through grazing.

Table 3. Effect of using technologies for nitrogen conservation on annual feed production and nitrogen balances of a simulated dairy farm in the Netherlands\* (source: Rotz *et al.*, 2005).

Production parameter	Previous technology <sup>†</sup>	Current technology <sup>‡</sup>	De Marke technology <sup>§</sup>
Milk production (kg cow <sup>-1</sup> )			9,000
(kg ha <sup>-1</sup> )			
Nitrogen imported <sup>¶</sup>			228
Nitrogen exported <sup>‡</sup>			75
Nitrogen surplus <sup>§</sup>			153
Nitrogen lost by volatilization			27
Nitrogen lost by leaching			55
Nitrogen lost by denitrification			27

\* 55 cows and 48 replacement heifers on 34 ha (26 ha of grass and 8 ha of maize or 17 ha of maize with De Marke technology) of loamy sand soil simulated over weather years 1977 to 2001 for Wageningen, The Netherlands.

† Includes standard barn floor, bottom loaded six-month manure storage, broadcast application, and full-day grazing.

‡ Includes standard barn floor, enclosed six-month manure storage, injection application, and half-day grazing.

§ Includes 9 ha more maize land harvested as high-moisture ear maize and stover, low fertilizer use, a grass catch crop following maize, low emission barn floor with feces and urine separation, an enclosed six-month manure storage, and manure application by injection.

¶ Via fertilizer, feedstuff and animals

‡ Via slurry, feedstuff and animals

§ Nitrogen surplus= Nitrogen imported- Nitrogen exported



Though permanent grassland has a high potential for biodiversity and nature conservation in lowland areas, it was found difficult to achieve these goals in swards managed for high producing dairy cows under the given economic conditions. The prevailing nutrient level and the frequent defoliation favour productivity and forage quality through the dominance of few productive and valuable plant species but usually do not enable rare or endangered plant species to persist in the swards. Results from an extensive survey on 275 German grassland sites confirm, that likewise in organic grassland such low levels of management intensity necessary for high biodiversity rarely occur (Mahn, 1993). Data from another survey on organic farms suggest that biodiversity decreased with increasing clover content in the sward (Taube et al., 1997). Thus, a significant reduction in grassland management (e.g. through cessation of fertilization, reduction of stocking rates and defoliation frequencies, abandonment of sward improvement measures) like in extensive grazing systems with suckler cows is a prerequisite for an increase in biodiversity.

### ***Conclusions from Focus Theme 1***

Intensified forage production systems result in more productive pastures, but also increase the risk of nutrient losses with negative environmental impact. Perennial grasslands, however, have inherent capacities to reduce adverse environmental effects, and appropriate dietary supplementation of livestock and grazing management, tactical fertilizer application, injection of manure, and other means can be used to improve nutrient management in grassland systems. Computer simulation supported by field studies provides a powerful and cost-effective tool for developing, evaluating, and promoting more sustainable grassland systems for commercial livestock production.

### **Focus Theme 2: Efficient grassland cultivation and renovation**

In sustainable forage production systems the grassland cultivation (resowing of permanent grassland after ploughing and establishment of temporary grassland in rotation with arable crops) and renovation (different methods of permanent sward improvement) play an important role. The demand for large quantities of high-quality forages occurs primarily in dairy farms because one of the prerequisites to maintain profitability in milk production and to utilise the genetic potentials of cows is adequate herbage characterized by excellent quality. Unfortunately, in many regions of European countries with unfavourable weather (e.g. droughts, flooding, frosts and other negative effects of winter) and/or unfavourable soil conditions (e.g. compaction), degradation processes regularly occur on grasslands. One of the indicators of the degradation of permanent meadows and pastures is the decline in the sward of the proportion of valuable forage grasses and legumes and the invasion of species unwelcome from the nutritional point of view (weeds) as well as the deterioration of the sod quality. Decreasing herbage yields and disappointing animal performance are the main reasons to consider grassland renovation. The renovation of grasslands should aim at developing a permanent botanical composition of the sward which becomes fine-tuned to the site yield potential. Many authors have reported that ploughing and resowing is the fastest method of re-establishment of degraded grasslands. Possible alternatives to ploughing are: shallow cultivation and oversowing, or overdrilling syn. direct drilling (Goliński, 2003). Results of numerous investigations indicate that grassland renovation had a positive impact on the herbage quality rather than the yield. Goliński and Kozłowski (2000) came to the conclusion that overdrilling increased yield and the chemical composition of the herbage, but no significant differences were observed in the case of production cost of 1 kg DM. The herbage with higher protein and energy concentration used to feed dairy cows allowed a reduction in concentrate rations in individual farms by 200-289 kg head<sup>-1</sup> annually. However, the employed renovation methods and techniques can affect the environment considerably and, therefore, it is essential to carry them out in accordance with good farming practices. Particularly, ploughing and reseedling of grassland has become increasingly questioned with regard to environmental aspects such as nutrient loss and soil fertility. It is necessary to develop management rules for grassland management, e.g. renovation, which are acceptable for both farmers and society (Kemp and Michalk, 2005).

This was the main reason why, during the 19<sup>th</sup> EGF General Meeting in La Rochelle, France, a Working Group on Grassland Resowing and Grass-arable Rotations was officially launched (Taube and Conijn, 2004). Within the framework of this Group, two workshops have already been held, one in Wageningen and

the other in Kiel, and two reports from these meetings have been published (Conijn *et al.*, 2002; Conijn and Taube, 2003). The basic working hypothesis of the Group is that grassland productivity declines with increasing age of the sward and that yield decreases in the year of grassland renewal, but increases during the first production years after cultivation. Nitrogen losses are also likely to increase due to increased mineralisation after ploughing. A very important aspect in the hypothesis is the different response of grassland to resowing in different parts of Europe due to different soil-climate conditions and farm management (Taube and Conijn, 2004). Knowledge about these interactions lead to specific strategies in grassland renovation, e.g. traditional methods based on ploughing are applied mainly on mineral soils, whereas on organic soils the application of non-selective herbicides and direct drilling is preferred in order to prevent a boost in mineralisation, and to reduce nitrate leaching and weed ingress.

#### ***Nutrient cycling, including emissions to the environment***

Most of the total soil nitrogen is organic N and its rate of accumulation under grassland is approximately linear in the early years of a sown ley (Tyson *et al.*, 1990). Sward type, intensity of management and environmental factors affect the rate of soil N accumulation following resowing. All these factors also affect both the development of soil biota and the utilization of soil nitrate which in turn has implications for the rate of N cycling and its efficiency in terms of sward productivity (Tilman *et al.*, 1996). In established leys and permanent swards, N mineralisation proceeds at rates influenced by agricultural management, e.g. lime applications, changes to drainage status, inputs of manures and other nutrients as well as soil type, soil biota, sward composition, available water capacity and climate (Hatch *et al.*, 2003). After grassland resowing, increased N mineralisation occurs due to exposure of organic matter to microbial decomposition and increased aeration. Ploughing short term leys released 100-250 kg N ha<sup>-1</sup>, according to the age of the ley and the soil type (Johnston *et al.*, 1994). Okruszko (1991) reported, that in the case of peat soil under conditions of a grass/arable crop rotation and low moisture content, 357 kg N ha<sup>-1</sup> was released annually from a field of temporary grassland, while on permanent grasslands, but with higher moisture content, N mineralisation amounted to 138 kg ha<sup>-1</sup>. This nitrogen becomes mineralized and is available for plant uptake by the succeeding resown sward, but a proportion will be lost from the soil N pool through gaseous emissions and leaching. These have negative consequences for sward performance and the wider environment (Hatch *et al.*, 2003). The huge N-pool in grassland mineralized with cultivation presents a potential environmental hazard but when using good management practices such as spring ploughing and catch crops, the release of N can be controlled and nitrate concentrations in leachates may be kept below the EU Drinking Water Directive upper limit of 50 mg l<sup>-1</sup>. Results of a study by Davies *et al.* (2001) showed that ploughing of grassland increased N losses via leaching, N<sub>2</sub>O emission and denitrification when the soil was left fallow. Ploughing followed by reseeded considerably decreased N losses, and especially from denitrification. After cultivation of grassland, growing arable crops with a high N uptake capacity will decrease the risk of N losses to the environment. Research in Belgium shows that very low, or even no N fertilizer should be applied when maize, fodder beet or arable crops are grown in the first year after grassland. In agricultural systems the choice of the crop following grassland is normally based on the economic value and does not usually include an assessment of the risks of N loss. Decreasing the age of grassland in ley-arable systems to less than 3-years is uneconomic (Hatch *et al.*, 2003).

Organic matter mineralisation in grassland renovation depends on the techniques used for cultivation and management of the subsequent sward. Six *et al.* (2002) confirmed the beneficial effect of minimal tillage operations compared with conventional ploughing in terms of decreasing soil N and C mineralisation and hence the likelihood of reducing nitrate-N leaching losses to surface and ground waters and CO<sub>2</sub> emissions to the atmosphere. The results of Loiseau *et al.* (1992) showed that the decrease of OM was stronger after rotavation than after direct drilling. After mechanical preparation, part of the OM evolved to the fine OM particle size fraction below 200 µm. carbon loss as CO<sub>2</sub> was also higher in intensive soil preparation: 11 (control), 43 (direct drilling) and 52 t C ha<sup>-1</sup> (rotavator). Studies by Goliński and Kozłowski (2000) on the effects of renovation method on soil chemical composition showed only a slight difference in the content of mineral nitrogen between the overdrilled area and the control. Increases in the content of mineral nitrogen (N-NO<sub>3</sub> + N-NH<sub>4</sub>) occurred in the remaining surfaces ranged from 4.2 to 8.7 kg ha<sup>-1</sup>. This can indicate minimal damage of the sod by working parts of the seed drill in the process of overdrilling and reduced oxygen availability in soil which prevents accelerated mineralisation of OM.

Timing of grassland reseedling plays an important role in sustainable forage production. Spring, rather than autumn, cultivation is suggested as a means of decreasing nitrate leaching. Shepherd *et al.* (2001) reported that the effect of spring ploughing for reseedling was relatively short-term and did not cause increased leaching in the following autumn, whereas autumn ploughing and reseedling leached 60-350 kg N ha<sup>-1</sup>. Timing of grassland cultivation can also have environmental implications other than N leaching, including effects on gaseous emissions, habitats for wildlife, carbon sequestration and soil erosion (Hatch *et al.*, 2003). In The Netherlands ploughing of grassland might contribute significantly to the emission of N<sub>2</sub>O. Measurements on clay soil indicate, that compared to permanent grassland, N<sub>2</sub>O emissions were 7 and 113 times higher after ploughing in spring or autumn, respectively (Schils *et al.*, 2002). Phosphorus dynamics in the context of grassland cultivations have received relatively little attention. However there is evidence that tilled grassland results in a short and long term increase in soluble P in the soil profile, presumably related to increases in mineralisation through altered patterns of wetting/drying and increased soil aeration (Turner and Haygarth, 2001).

### ***Soil quality and water balance***

The effects of grassland renovation on soil quality and the subsequent effects on other factors are variable and usually hard to quantify. Grassland renovation of permanent grassland has positive effect on the soil quality of sandy (Schils *et al.*, 2002) and clay soils and a negative effect on the soil quality of peat soils. Grassland ploughing influenced soil OM dynamics, the rooting capacity, the water holding capacity, the bearing capacity and the susceptibility to soil compaction. On peat soils, the physical qualities of the soil, such as soil aggregate stability and bearing capacity are generally negatively affected at grassland renovation. Ploughing up grassland for reseedling or conversion to arable has a significant impact on soil biodiversity, e.g. cultivation reduces the number of macrofauna such as earthworms in soil (Pérès *et al.*, 2002). Conversion of grassland to arable land usually causes a strong release of soil C. Eriksen and Jensen (2001) measured losses of 2.6 t C ha<sup>-1</sup> during the 3 months following cultivation of 3-year-old grassland, compared to 1.4 t C ha<sup>-1</sup> in the undisturbed control. In agreement with other long observations, C accumulation is half as slow as the C release occurring after the grassland is ploughed (Vertès *et al.*, 2003). Roots are recognized to be an important factor of soil quality and also play a major role after ploughing. Grass roots are more resistant to degradation than roots of annual crops because of higher contents of lignin and phenolic compounds. Grass roots are also well protected from degradation within the undisturbed soil matrix, where high microbial biomass activity promotes aggregation and soil structural stability (Six *et al.*, 2002). Because the C mineralisation for roots is significantly lower in comparison with the aerial organ tissues for several different plant species, the soil OM coming from roots would be found in significant proportions in the soil and has a particular dynamic, which would deeply affect physical, chemical and biological characteristics of the soil during the months following reseedling (Vertès *et al.*, 2003).

### ***Conclusions from Focus Theme 2***

Ploughing of grassland followed by reseedling is a common strategy to maintain productive grass swards. However, grassland cultivation is being more and more challenged by increasing demands of legislation and society in terms of reduction of nutrient losses, conservation of biotic diversity, protection against erosion and carbon sink. Science is challenged to provide sustainable solutions but few systematic experimental studies into the effects of grassland renovation on environmental and agronomic parameters currently exist in Europe. Identification of farmers' motives for grassland renovation would help to develop scientifically sound and practically applicable criteria to support farmers in decisions on grassland renovation. There is a particular need for a quantification of the effects of grassland renovation on nutrient cycling in the plant-soil system and on DM yield, herbage quality and animal performance, with respect to soil type, renovation strategy and sward management. The goal of the research efforts should thus be the development of optimised management strategies which ensure minimum agronomic and environmental risks during grassland renovation.

## Perspectives of sustainable forage production in Europe

The perspectives of sustainable forage production are tightly interlinked with European agricultural policy. The 2003 reform of the CAP with its new support system, including decoupling, modulation and cross-compliance, aims at enhancing the sustainability of agro-ecosystems. Regarding the possible consequences for grassland production, it is necessary to differentiate between high- and low-input production systems. It is most likely that areas with high-yielding grassland nowadays will be managed with orientation towards production in the future as well. The main environmental issue here will be the improvement of nutrient efficiency. An increased knowledge in this field will be necessary and all measures available have to be utilised to achieve this goal. The fact that payment will be decoupled from production may ensure that landscapes in low-forage yielding areas (mainly mountains) will remain open, but the required minimum management will promote very extensive farming systems or even may fail to maintain a satisfactory forage production and use due to low quality of the herbage. More research should be directed towards alternative uses of grassland (e.g. biomass for energy production) which is becoming of increased interest.

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## Extensive Iberian pig production grazing systems

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### Abstract

The Iberian native pig breed from the south west of the Iberian Peninsula is characterized by its full use of the natural meadowland resources of pastures and acorns. Its evolution has developed to make full adaptation to an extensive management system under unfavourable climatic conditions. Feeding in the late fattening phase, a long productive cycle under extensive management (with slaughter at over 15 months old), and the lipid characteristics of its meat have combined to enable the production of a high quality meat, especially for cured meat products: hams, fillet and shoulder of pork. The Iberian pig is a unique animal of the *dehesa* that has capacity for the transformation of feed to high value fat and muscle. The objective valuation of this quality and its consequences for commercial transactions between breeders and meat processors, and the application of a specific quality designation for these meat products, are two of the main challenges for this breed and its fundamental link with meadowland. At present, the Iberian pig is the most important agrarian production of the *dehesa* and it is exceptional in having recently shown increased prices for producers at a time when the profitability of cattle and sheep production has fallen.

Key words: *montanera*, quality, *dehesa*, meat regulation, fat.

### Origin and distribution

In many respects the Iberian pig breed can be regarded as probably the most successful of the native pigs whose breeding is restricted to particular geographic areas, not only in terms of the size of its population, but also for commercial or economic reasons. This breed, the result of *Sus scrofa mediterraneus* evolution, which has an historical antecedent of African origin, is widespread in the whole southwest Iberian Peninsula, in the Spanish autonomous regions such as Extremadura (Cáceres and Badajoz), the west of Andalucía (Sevilla, Córdoba, Huelva, with important herds of pigs in Cádiz and Málaga), the south west of Castilla and León (mainly in Salamanca, with extensions in Zamora, Valladolid or Ávila), and Castilla - La Mancha (Toledo and Ciudad Real). In addition, its Portuguese version, known as *Porco alentejano*, whose origin and characteristics are similar to the Spanish one, is abundant in the whole Alentejo region, from Algarve (in the south) to Beira (in the north).

For readers familiar with the environmental resources of the Iberian Peninsula, the peculiarities and distribution of fauna and flora in the different ecosystems provides the first clue to understanding the reasons for the success of this breed: the ecological area of the Iberian pig coincides with the large areas of meadowland of Spain and Portugal. Nevertheless, although the breed's traditional geographical distribution was valid until the middle of the 1990s, it is now undergoing serious changes. As a consequence, in areas where the Iberian pig was previously almost negligible (existing only thanks to some breeders who wanted to keep them for sentimental reasons), the numbers of Iberian pigs have now greatly increased. In areas where people raise mainly early maturing white pigs such as Barcelona, Lérida (both in Cataluña), Murcia, provinces of Castilla and León like Segovia, purchase of Iberian sows is now increasing as breeders want to participate in the production of Iberian pigs, while retaining their normal intensive production systems. This trend, instead of stopping, is still tending to increase, and now includes some other Spanish regions such as Galicia and Aragón.

## Census and evolution

When people talk about Iberian pigs, it is necessary to explain the changing circumstances that this breed has suffered, because the production systems which are really favourable have been underestimated. However, work and perseverance by some breeders, industrialists and authorities, have allowed, to a certain extent, restoration of the situation.

Various historical references exist showing the connection between the Iberian pig and its environment, such as its presence in Montánchez and Trujillo's charters in XVI century, the regulations of Carlos V, etc., which confirm the old Iberian pig breeding and its use of environmental resources. The following paragraphs consider the events of the last 50-60 years. At the beginning of the twentieth century, the status of the Iberian pig was considered as being protected because its breeding and consumption were basic components of human diets across a large area of the peninsula. Then, at the beginning of the 1970s, several factors plunged the Iberian pig breed into a deep crisis. As a result, there was a drastic reduction in its numbers, which put the breed's survival in danger. This disaster in the population led to a big loss in the previously abundant genetic variability. It is of interest to mention some of the economic, social and animal health factors that converged to cause the crisis.

One aspect was the demand for other kinds of animal products, which caused a decrease in consumption and a reduction in the traditional family production of pigs for home slaughter. Moreover, at that time, having overcome the enormous nutritional deficiencies caused by the conflicts of the 1930s and 40s, there began several national and international campaigns, sometimes unfounded, against the consumption of meat with high fat content. The growing worry about health caused a change in meat quality objectives and the main objective at that time was reducing the amount of fat in meat products. This second aspect provoked a high selection process towards white breeds for intensive production. The proposed objective of decreasing the fat in Iberian pigs, and also increasing the growth rate and muscle yield, caused the beginning of crossbreeding out with foreign breeds, which had a leaner constitution; all of which contributed to an important fall in the amount of pure Iberian breeding stock. With this crossbreeding, people want to shorten the long productive cycle associated with the meadowland farming, thereby increasing their low economic profitability. From all of this crossbreeding that was tried out during these years, we keep one, made with the *Duroc* breed, even though more controlled and being aware of the current quality requirements.

The animal health aspects related to the Iberian pig population crisis deserve a special mention. The most unfortunate chapter of this story occurred in 1960, when there was an outbreak of African Swine Fever (ASF) in Badajoz, a border town with Portugal. The later virus infection, and its rapid extension to the rest of the pig population, resulted in the total destruction of many herds and also in a gradual change in breeders' customs, with very little exchange of breeding stock. This reduction stressed the population structure further and led to genetic isolation of some herds of pigs.

Two further aspects contributed to the decline in Iberian pig breeding. One was the exodus of rural people during the 1950s-1970s, which affected the Andalusian, Castilian and Extremeños populations, with movements from their towns to the big urban Spanish centres that had important industrial developments (Madrid, Barcelona, País Vasco), as well as emigration to other European countries where there was a need for labour (Switzerland, Germany, France), and to Latin American countries with emerging economies (Argentina, Venezuela). The other aspect, and probably the most unfortunate one, was the transformation of thousands hectares of botanically diverse meadowland on farms to crops that were more profitable at that time (sown grass, vines, etc.) or for tree planting of species with a rapid growth rate (e.g. pine, eucalyptus). Thus the native trees, *Quercus rotundifolia* and *Quercus suber*, were extracted, the meadowlands were lost, and with them everything related to the traditional land-use systems, including the Iberian pigs.

Authors who have given figures for this population fall have indicated that the numbers of female Iberian pigs decreased from more than half a million in the 1950s to 53,000 by 1982. From 1985 we have seen a re-emergence of the Iberian pig herds as a result of five basic effects. First, there was the important work done by breeders, veterinarians and animal health authorities from the various state and autonomous organizations, resulting in the gradual decrease of the effects of ASF. By 1995, this disease had been totally eradicated. Secondly, the greatly improved socio-economic conditions in Spain during the last 30 years have increased the demand for quality food products, in spite of the higher market



price of such products. Iberian acorn ham is the one of the best examples of this trend. The third factor is related to the environmental concerns by some sections of the population, and the recognition that the meadowland is a unique ecosystem in the world. The meadowland is an example of how it is possible for an interaction between man and the environment, and provision of economic resources for the populations that settle in it being associated with an enormous biodiversity. Another element that has increased the Iberian pig value, and of its products, has been the recent accreditation of the beneficial dietary effects of unsaturated fats, compared with saturated fats. Olive oil and Iberian pig meat consumption have benefited from the return to a Mediterranean diet. As has been noted, many pig producers are replacing part of their female pigs by Iberian female pigs, or going directly into Iberian pig production and in its curing products. This has been in response to the progressive deterioration in the price for early maturing pig carcasses.

Table 1 summarize the census changes over recent years (MAPA, 2003), figures that largely coincide with those for Iberian pigs, while Table 2 shows changes in the number of specimens registered in the Genealogic Breed Book.

Table 1. Extensive pig census evolution.

Year	Total	Breeding stock of weight >50 kg		
		Boars	Females	
			Total	Covered
1994	1,144,792	10,639	106,908	3,203
1995	867,058	10,449	97,858	42,479
1996	990,085	7,635	75,638	32,408
1997	945,887	7,026	70,354	39,028
1998	1,295,318	12,898	134,357	69,164
1999	1,660,181	14,887	169,406	97,000
2000	1,878,260	19,653	183,782	87,792
2001	2,098,026	21,898	203,853	117,859
2002	1,753,363	20,273	193,167	102,793

Source: MAPA (2003)

Table 2. Changes in numbers of extensive pigs from census returns 1988-2004.

Year	Boars	Females	Year	Boars	Females
1988	158	3,043	1997	409	1,977
1989	475	3,856	1998	421	2,609
1990	294	1,411	1999	791	8,148
1991	185	451	2000	865	9,157
1992	403	1,646	2001	1,579	14,860
1993	514	1,636	2002	1,776	24,673
1994	817	2,263	2003	2,416	42,570
1995	276	3,020	2004	1,339	26,079
1996	381	3,897			

Source: AECERIBER, 2004

Nowadays, the facts indicate about 300,000 female Iberian breeding stock (10.5% of the total herd of pig) with 2.9-3.3 million pigs slaughtered (7%) from which 400,000-500,000 (15-20%) Iberian pigs would be fattened in the productive system of the *montanera* and the rest would be fattened with fodder.

### The *dehesa*

The most important feature of the Iberian pig lies in its farming system, based on utilization of the natural products of the meadowland in an extensive system. Meadowland is here defined as a forest for grazing, whose origin was the result of human action in the Mediterranean forest over thousands of years, in order to adapt large spaces of it for utilization, and then to practice breeding of pigs. The meadowland is characterized by the presence of arboreal species of *Quercus* (*Quercus rotundifolia* and *Quercus suber*) and herbaceous species, mainly grasses and legumes. The shrub species and other less profitable herbaceous species have been eliminated by human actions in order to make better use of the resources. Meadowlands are situated on hard and acid substrates, with rock of silica, granite or slate, which results in shallow soils that have low organic matter content, and deficient in phosphorus, nitrogen and potassium. The climate is Mediterranean and semi-arid, with average precipitation of between 300 and 800 mm per annum and a mean temperature of 14°C in winter and 25°C in summer. The climatic conditions determine the level of herbage productivity, which is characterized by two peak periods, one in spring and the other in autumn.

Table 3: Geographical distribution of the meadowland areas in Spain.<sup>A</sup>

Autonomous regions and provinces	Thousands of hectares	% of the total
Badajoz	585	18.6
Cáceres	541	17.2
Extremadura	1,126	35.7
Ávila	41	1.3
Salamanca	244	7.7
Zamora	156	4.9
Castilla and León	441	14.0
Ciudad Real	254	8.1
Toledo	124	3.9
Castilla La Mancha	378	12.0
Cádiz	150	4.8
Córdoba	305	9.7
Granada	62	2.0
Huelva	329	10.4
Jaen	92	2.9
Málaga	57	1.8
Sevilla	211	6.7
Andalucía	1,206	38.3
TOTAL	3,151	

(Source: Rueda, 2004)

This domesticated fauna include not only the Iberian pig, but other species represented by native bovine breeds (retinto, avileño, morucho), native sheep breeds (merino) or native caprine breed (retinta), although the last two occupy areas with smaller arboreal density. Among the wild fauna of the Mediterranean forest are the usual mammals which inhabit, use or visit these spacious areas (wild boar, deer, roebuck, rabbit, genet, fox) and a large number of scavengers and other birds of prey (black and imperial vultures, eagles, owls, sparrow hawk, kites). Crane and storks, both black and white, occur. The meadowland also supports other small mammals and reptiles, and amphibians in damp areas. It thus represents a source of richness both in terms of its agricultural productivity and its high biodiversity.

The holm oak and cork oak forest in Spain occupies almost 3 millions hectares (Table 3) with densities of 30-100 trees ha<sup>-1</sup>, the average being 40-50 trees ha<sup>-1</sup>. Apart from producing acorns, these trees provide cork, firewood, charcoal and food for other animal species. The reported values for acorn productivity are very variable, due not only to the enormous annual variations, but also to variation between different areas and different trees, e.g. 300-1000 kg ha<sup>-1</sup> and 7-18 kg tree<sup>-1</sup>. A pig has to consume approximately 13 kg of acorns to increase its weight by 1 kg. With this daily gain, and set stocking at 0.5 pigs ha<sup>-1</sup>, it would be fattened a maximum of a million and a half, supposing that the whole meadowland arboreal area was prepared for an Iberian pig production system in *montanera*. Thus, several authors' attempts to give breeders and engineers objective tools to evaluate the *montaneras*, in order to adapt the set stocking to the acorn production in fixed areas and seasons, are very admirable.

### Productive cycle and farming management

The variations in breed differences, including effects of crossbreeding, as well as in feeding systems (extensive, semi-extensive or intensive systems), mean that the different Iberian pig production systems cannot easily be summarized. The focus will therefore be on the most representative type of production, the acorn pig production of the *montanera*.

The Iberian female pig begins puberty at an average age of 207 days, and commences mating at 8-12 months. The minimum mating period must be at least 25 days in order to include at least one oestrus cycle. Mating is carried out in a traditional way, with a natural mount in the management corrals. There are several boars per female group, and a highly variable number of animals with between 6-10 females boar<sup>-1</sup>. Except on farms where people work according to classical animal breeding methods, there is no control over paternity. Although this system is likely to be retained on small and extensively managed farms, artificial insemination will inevitably replace traditional breeding. During gestation, female pigs are subjected to extensive management, using the available natural resources in the course of the year. Therefore, they will be supplemented to a greater or lesser extent (from 1 to 2.5 kg day<sup>-1</sup>), depending on the period of the year and their productive state.

Parturition (farrowing) occurs in groups having similar stages of development. During the lactation period the sows are kept, whenever possible, in corrals adjoining the farrowing house, where they continue making use for the available pastures, or they stay in the camping area if that is the chosen system.

One characteristic of the Iberian female pig is its low prolificacy. Table 4 shows the accumulated details for two herds of pigs from public property: Valdesequera, property of Junta de Extremadura and Dehesón del Encinar, which belongs to Junta de Castilla - La Mancha.

Table 4. Total numbers of piglets born, and total live-piglet births, in two public herds.

VALDESEQUERA			DEHESÓN DEL ENCINAR		
Pig litter size	Total born	Total alive born	Pig litter size	Total born	Total alive born
1	7.97	7.26	1	7.25	7.03
2	8.33	7.72	2	7.91	7.68
3	8.41	7.52	3	8.65	8.31
4	8.75	7.79	>3	9.01	8.49
5	8.87	7.63			
>5	8.95	7.74			

When the management is totally extensive, the animals are kept loose in the field in areas adjacent to livestock accommodations for the transition from piglet to shearling (103.5-115 kg), ready to go into the *montanera* stage). This arrangement enables the piglets to make better use of natural pastures. The main

farrowing periods are in the months of November-December (piglets then called navideños or yerbizos), May-June (agostones) or February-March (marceños). Moreover, due to the higher commercial value of the pigs fattened in a whole *montanera* and in relation to the wooded area of each farm and the acorn yield in each season, the farrowing season is timed to produce animals that can take part in this type of fattening system, with an age of 12-18 months and an initial weight of 90-100 kg.

The breeding season includes the phase from birth to weaning, and a second phase, from weaning to 23-25 kg. In the most extensive systems, weaning can last up to 8 weeks, but in the most intensive ones it is reduced to 3-4 weeks. The 15-20-day-old piglets begin to take increasing amounts of protein concentrate supplement, from 100 g day<sup>-1</sup> initially, increasing to 1 kg day<sup>-1</sup> by the end of this weaning period. Castration of both males and females is carried out in the early ages. The rearing stage lasts until weight reaches 100-105 kg. In the most extensive systems there are two phases: the first until 60-70 kg at 8-10 months old (tuskers), and the second until about 100-105 kg (shearlings). This last phase is crucial to get the animal ready for the *montanera*. It is such an important stage that some authors consider it as a different phase of the rearing stage, referred to as the *premontanera*, for which the objective is to attain an optimum stage of development which lets the pigs manage themselves correctly in the *montanera* and acquire enough weight before slaughter. The basic feeding consists of concentrate feeds, combined with making good use of available natural resources including pastures, sown fields, stubbles and fallow lands.

The final, and most characteristic stage of Iberian pig production is the *montanera*. In the *montanera* the pigs feed on acorns on the ground below the oak trees, both *Quercus rotundifolia* and *Q. suber*. This phase usually begins in October but its most important period is between November and January. The pig begins the *montanera* at 90-120 kg live weight and three months later it finishes this period at 150-158 kg live weight. Acorn consumption per animal is related to its weight, the average being 6-10 kg of acorns per animal per day, with an additional intake of at least 3 kg of grass. It is usually necessary to consume 10-15 kg of acorns for each 1 kg gain in live weight, and the daily weight gain is typically 0.5-1 kg day<sup>-1</sup>. This weight increase is appropriate for animals that enter the *montanera* stage when more than one year old. The *montanera* is conditioned by numerous factors including:

- Acorn abundance.
- Acorn maturity.
- Quality and quantity of fresh grass as protein and vitamin supplement.
- Flat areas or areas with relatively smooth differences in height, because hilly areas can result in exercise involving energy expenditure and wasted fruit.
- Existence of drinking areas and appropriate shelters.

On most of the farms where pigs were formerly put on to the *montanera* for a period of 90-100 days, this is reduced nowadays to 60-70 days, with a weight increase of 45-50 kg. This allows for an increase in set stocking. Although it is arguable whether this management adversely affects the final quality of the meat products, the higher pressure on the meadowland can cause damage in the long term. Thus, the excessive numbers of pigs results in it being no longer possible to make use of acorns in ways that enable natural regeneration, needed for the long-term management of meadowland and the conservation of the wooded areas. Under these circumstances the forest trees are growing old without any possibility of recovery. For this reason subsidies are now becoming available for the meadowland areas to encourage reforestation and regeneration programmes.

The acorn is the basic food of the *montanera*. The pig does not take the whole acorn; it rejects fibrous material that is unusable and this represents approximately 27% of the acorn weight. The moisture content of the pulp is about 40%. Expressed as a proportion of dry matter, the nutrient content of acorn pulp is of low protein concentration (about 5-6%), with a high amylaceous substances content (more than 50 % in starch) and 9.1 % of fat; all of this makes the acorn a very high energy food.

The amounts of acorns available are, in many cases, insufficient to obtain commercial weights in the herd of pigs. In these cases a system for finishing is used known as *recebo*, which consists of giving the pig a supply of an additional specific feed. This is provided either as a small daily amount during the whole *montanera* (called 'dessert', at 1 or 2 kg day<sup>-1</sup> per animal) or concentrating the full supply at the end of the period (at 4-5 kg day<sup>-1</sup> per animal). The second system is more appropriate than the first one,

and it is the real *recebo*, whereas the first one has as an objective to increase the set stocking without losing the acorn guarantee for the products, as described in the previous section on quality regulation. Features and variations in the farming system, including the time of year when the piglets are born, the season when fattening takes place, or because the amount of acorn is minimal, mean that it is not always possible to provide acorns in sufficient quantity for the system to be classed at least, as *recebo*. On those occasions, the fattening takes place on a real extensive system, with a maximum number of pigs per hectare, making good use of the resources available at that time (pastures, stubbles, etc) with a daily supply of appropriate commercial feeds. Moreover, attempts are made to ensure the animals obtain some exercise by putting the drinkers far away from the feeding area. As a result of this kind of management it is usual to describe the pig as a 'countryside' or 'extensive' pig.

### **The quality evaluation system**

The high and recognized quality of the Iberian pig curing products is the result of a combination of factors, all working together. If we take into account only the raw material (excluding the later curing process), the breed, the age, the feeding and the exercise are four of these quality factors. Having a pure Iberian pig breed rather than a crossbreed, a pig raised in *montanera* as opposed to a pig raised in an intensive system, pigs aged 18 months old at the time of slaughter as opposed to ones only 10 months, and pigs that are able to exercise (free range) as compared with pigs that do not move, there is the conviction that the more pure the breed is, the higher the daily weight gain in *montanera* to consume acorns and grass is, the older the age at slaughter, and the more exercise the pigs do, then the more tasty and aromatic the curing products will be.

However, there are difficulties in describing this quality objectively, especially for one of the basic factors - the *montanera* daily gain. While there are DNA analyses that enable the identification of crossed animals or products produced by them, there is no test to determine precisely whether pigs have consumed fodder or not during the *montanera*, or if they have gained 103.5 or 115 kg in pure *montanera* using only the meadowland natural resources. The total amount of acorns consumed and the supply, or not, of fodder are basic elements required to classify the final quality of the product. There is a now a large problem which directly affects the dealing arrangements between breeder and the meat industry, and indirectly the quality-price relationship, and which is one over which the consumer must decide.

For more than 10 years, the standard method used to determine the feeding type in the final fattening phase is the composition of fatty acids in subcutaneous fat. This is measured with gas chromatography, taken from samples obtained from a representative percentage of pigs from group at slaughter. Acorn fat has a fatty acid composition that is closely related to the excellent quality of the curing products of the Iberian pig. Its concentration in oleic acid is very high (60% of the total), being low in linoleic acid and in other saturated fatty acids. Being a monogastric animal, which is incapable of changing the fats, the Iberian pig incorporates them into its body tissue in the same form in which they are ingested. So the fat of the Iberian pig whose feeding was made with acorns in the *montanera*, is characterized by having a higher oleic acid content (about 55%) and relative low concentrations of linoleic and palmitic acids (around 8% and 20%, respectively). These figures are different not only from those found in the meat of white pigs raised in intensive systems, but also from meat of Iberian pigs fattened under the more usual intensive conditions.

Each season from the Interprofessional Association of the Iberian pig (ASICI) a fatty acid profile, based on maximum and minimum values for oleic, linoleic, palmitic and steric acid, is proposed to the Ministry of Agriculture, Fishing and Feeding (MAPA) with the aim to classify the feeding of pigs slaughtered that year. This profile is used in every dealing activity, and so it is a critical element in the commercial exchanges between producers and processors and the meat industry. The main reasons for using this technical solution instead of any other are, its simplicity in terms of having samples, the speed of obtaining the results, and the economy of the whole process. Moreover, previous studies have demonstrated a higher guarantee of obtaining correct details than from previous methods based on physical fat features, Iodine index or melting point.

However, this system is enormously controversial and it leads to numerous errors. Two examples of these errors are, that the addition of determined fatty components to commercial fodder (work has been carried out by people in the feed industry in this field), allows acorn profiles to be obtained even when

animals are really from *recebo*, even from intensive fattening. This produces false positives, which means that there are animals which obtain the high quality denomination, even when they really do not have it, because the products have shown the same sensory quality as the products of the *montanera*. This has been proven when a consumer test or a volatile component analysis (in order to find the most aromatic ones) has been carried out. On the other hand, pigs which have been fed in *montanera* without any additional supply of fodder, can show a *recebo* profile for many different reasons (sometimes unknown), such as a very disproportionate lipidic profile at the moment of going into the *montanera* (these established parameters are difficult to change), excessive grass consumption, unusual fat composition in the acorns, age or breed which work together, etc. In these cases, which can be damaging for the breeders, we talk about false negatives, since pigs with the highest quality go to the market with the measured characteristics of a *recebo* one, and it means a lower price.

These problems, linked with the necessary regulation of this sector and the difficulties of controlling one product - the fattened pig which stays in the farming system for long period of time, under extensive management on a large area of land - have resulted in the Ministry of Agriculture publishing the Quality Regulation for Iberian pig products. Given the current importance of regulation, the problems which are being generated by it, and the changes that is causing working procedures for breeders, meat processors, sellers, etc., the following section attempts to summarize these aspects.

### Quality regulation

On 15 October 2001, the Ministry of Agriculture published the Royal Decree 1083/2001. As a result, an important customs change was officially introduced for all sectors relating to the Iberian pig and its high quality curing products, such as ham, shoulder of pig and loin. The Royal Decree states in its preambles that ... *in a first stage by means of a regulation that secures at least 50% of Iberian blood, in animals that will contribute to raw material for the products referred in this regulation (insisting on the fact that the dam have to be pure Iberian pig), one market organization is intended to be start...*

A fast critical-point revision of this regulation could be the following one:

- The racial purity of female breeding stock (the boar can be Iberian, *Duroc* or the result of their crossbreeding) is compulsory.
- It classifies pigs for slaughter as pure Iberian pig or Iberian (crossed).
- A weight increase of at least 46 kg and a single age of 10 months to go to the *montanera* and obtain acorns or *montanera* denomination is compulsory.
- It gives the *recebo* denomination only for pigs which have go into the *montanera* at 10 months and have increased in weight by at least 28.7 kg, without any feed supplementation.
- It establishes one age of slaughter, at least 10 months for fattened pigs (fed with fodder) which are named *de cebo*.
- It forces the traceability of the whole process, from birth to point of sale, either in the field or in the industry, establishing the concept of self-regulation as fundamental.
- It establishes that field control of the classification in relation to feeding regime, is confirmed by the fatty analysis in subcutaneous fat (by annual agreement with ASICI).
- It allows field control and the breeders' obligations and requirements to fall into the hands of Inspection Entities (regulation EN 45.004).
- It confers the control in the industries upon the Certification Entities (regulation EN 45.011).
- It forces laboratories involved in the fatty acid profile analysis to become accredited under the UNE-EN ISO/IEC 17025:2000 regulation.
- The National Entity of Accreditation (ENAC) is to become the guarantee for the operation of correct control entities (accreditation and laboratories).

Nowadays, this quality regulation is accepted by almost everyone, although there are some hesitations and discussions, Ministry of Agriculture and Autonomous region's rectifications, defects in control entities interventions and even ENAC inexperience in this complex sector. However, it is true that this decree contains some polemical regulations such as gaps and incongruities which make starting-up more difficult. As example of this problem we can say:

- One product which is crossed is denominated as Iberian and the one which is fattened with fodder as *de cebo*.
  - The fact that both exist in one delimited area of Spanish meadowland without any reference to Portuguese *montados*.
  - It does not establish a minimum period of time that the pig has to stay in *montanera*.
  - The absence of objective guidelines that allows estimation of the *montanera*'s capacity, applying the same procedures for all every inspection entities.
  - The absence of a different feeding category called *extensive fattening*, the high quality of which could be recognized when compared with intensive fattening (it would enable a higher protection of the meadowland).
  - One *recebo* meaning which doesn't include the traditional 'dessert' system, so usual for the breeders.
  - The lipidic profile references as a system which confirms the field controls, but without fixing when and how one is considered more important than another.
  - The higher clarity in the minimum curing periods of these products (mainly hams and shoulder of pigs) related to their weight.
- At present, breeders and processors, represented both in ASICI in their respective organizations, are debating some changes in the quality regulation, with the aim of getting an agreement that can be transferred to the Ministry of Agriculture and the Autonomous regions' governments.

### **Complementary methods in the quality valuation**

The intense debates about the possible analytical methodologies for characterizing the feeding regime, has for a long time driven many research objectives towards developing alternatives to fatty acid profiling, either replacing it or complementing it. Some research groups are working on this alternative, deepening the knowledge of it and of the flavour and sensory quality of the meat products. Some of these techniques are scientifically validated. Some of the most interesting ones are summarized in the following paragraphs:

- Statistical methods with different variants for the fatty acid analysis, including every fatty acid in the model, not only the most important ones (oleic, linoleic, palmitic and stearic).
- The percentage of some triglycerides differs with the feeding, and is detected in the raw material or in cured meat products.
- In the unsaponifiable fractions of the fat, there are components which indicate the type of feeding: tocopherols, branched hydrocarbon, phenolic components as terpenic alcohols, differences between total phenols, etc.
- Instrumental methods related to carbon and nitrogen isotopes or to measures in the infrared spectrum.

Most of these alternatives are not as simple, cheap or fast as fatty acid gas chromatography, but they can become decisive methods when there were unresolved disputes between breeders and meat industry processors. They also could become methods of curing-product control or of control methods for products that are still in the process of development. These methods could be put at the disposal of any organizations which require them, such as accreditation entities, ENAC, Ministry of Agriculture, Autonomous regions' government, etc.

However, according to Ventanas (2005), any alternative technique can not make the existing techniques redundant; it has to be validated scientifically and to be based on the three feeding categories (acorn, *montanera*, fodder). It would be advisable that this technique should be applied to both raw material and manufactured material, and also that it was difficult to copy and finally, that this new technique makes differences between not only the feeding type, but also the components that are related to the quality.

Every effort to make measurement of the final quality of the animals and their products both objective and clear, and to provide one tool that is independent of commercial considerations, as well as assuring the consumer in terms of the real characteristics of the acquired product, will be welcome by all sectors involved.

## Future prospects

The evolution of the sector is strongly influenced by the success of the quality regulations, and also by the commercial tendencies in sectors that are competitive with the Iberian pig system, mainly the white early maturing pig production systems. After that, these are the key issues:

- There is consolidation of the population explosion experienced in recent years. Moreover, it has kept the interest of the white pig producers in the Iberian pig sector.
- Preference of pure the Iberian breed as a quality reference and guarantee, particularly in *montanera* fattening productions.
- Tendency to use hybrids of 50%, instead of using crossed 75% (with 25% *Duroc*).
- Movements towards non-traditional areas.
- The extensive breeding recovery to produce pigs for acorn feeding.
- Uncertainty about quality valuation of pigs whose feeding is based on fodder in an extensive system and, in breeding raise and fattening productions with fodder which are intensifying their system.
- Increase of the average size of the farms.
- Essential advancement in Iberian pig feeding and knowledge of nutrition.
- Making the relation between the different fattening qualities and the Iberian pig fat composition, and making a connection with the market value.
- Giving higher commercial importance to particular cuts of meat consumed fresh (loins, *secretos*, *plumas...*), in clear distinction from those of white pigs.
- In spite of this, a large number of small farms and industries, having important and traditional components, will continue to exist and they need to consider quality as being of vital importance in order to become competitive.
- An increase in the amount of vertical integration within the Iberian pig sector, with processing and supply industries supplying their own pigs from their own farming operations.
- An increase of the amount of enterprises related to the product accreditation, in the care of new market developments.

In spite of the problems and unknown situations set out in this list of predictions, there is no any option to be optimistic, in the context of any breeding type production, respect on Iberian pig future, at least, in the middle or short term. The enormous European production of meats whose origin is an early maturing pig breed, and the decrease of their quality caused by selection for improvements related to economic profitability (growth rate, lean carcass composition); do not suggest a serious competition for the Iberian pig. Rather the contrary, the Iberian pig sector is increasing because of the white pig producers are taking part in this market. Moreover, the quality of its products, its traditional extensive system of production (making good use of natural resources), the relationship between management and meadowland preservation, its easy adaptation to animal welfare requirements etc; all of these reasons point to the maintenance of its social and commercial prestige. Even though national and autonomous authorities seem to be aware of these circumstances, attracting the attention of the EU officials has been problematic, and until now have been uninformed about the economic, social and environmental profits of this special production system.

Nowadays, the profitability of extensive farming (of caprine, sheep and bovine production) is in doubt due to the revisions of the Common Agricultural Policy (CAP) and reduction of subsidies for breeding animals. We think that there is now only one way to be competitive in the characteristic meadowland environment: convincing consumers of the higher quality of production from these environments and of the environmental benefits that are associated with this kind of management. Iberian pigs fattened in meadowlands provides a clear example of these two concepts, and it is the only animal in the European Union which doesn't receive any economic support from the CAP.



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# Effects of livestock breed and grazing intensity on biodiversity and production in sustainable grazing systems

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## Abstract

Effects of moderate vs. lenient stocking rates and ‘commercial’ vs. ‘traditional’ breeds on foraging behaviour, production, botanical, structural and invertebrate biodiversity, and economic outcomes were measured in semi-natural grazing systems in France, Germany, Italy and UK, and within a heather/gorse community in Spain. Treatment did not affect plant species number. Lenient stocking rates increased competitive grasses, particularly on grass-dominant sites. Diet selection reduced legumes cover, particularly with lenient grazing. Vegetation patch size was greater for lenient grazing on grass dominated sites. Lenient grazing generally improved invertebrate diversity. Farmers seem willing to adjust stocking rate provided there are appropriate agri-environment schemes. Net margins without subsidy were low or negative in all systems, whatever the stocking rate. In UK, F, D and I breed affected biodiversity little. In Spain local goats controlled shrubs better than Cashmeres, despite poorer performance. Economic performance of traditional breeds was generally poorer often due to marketing difficulties. Farmers were loath to use traditional breeds even in agri-environment schemes.

Keywords: biodiversity, grazing, stocking rate, breed, economics.

## Introduction

Natural and semi-natural grazed grasslands, their associated fauna and the landscapes of which they form a part, are important biodiversity and production resources in Europe and are subject to numerous agri-environmental schemes. Management of these grasslands should be both ecologically and economically sustainable, enhancing environment and landscape while maintaining farm incomes and sustaining rural economies. Unfortunately, the management of many schemes has to date been based on anecdotal evidence or on empirical studies with limited applicability. We report results of an integrated study at 5 European sites (EU FP5 project QLK5-2001-00130 FORBIOBEN) on the effects of stocking rate and breed of grazing animal in natural and semi-natural grassland systems on foraging behaviour, agricultural production, botanical, structural and invertebrate biodiversity, and economic outcomes.

## Materials and Methods

Each site had 3 replicate paddocks of 3 treatments (T1-3) for 3 summer grazing seasons. T1 and T2 were designed to optimise livestock production or biodiversity respectively, using a commercial breed. T3 was designed to optimise biodiversity but using a traditional breed. Treatments and systems at each site were tailored to local conditions. Sites were in Devon, UK (50°N 3°W, altitude 100m, lowland mesotrophic grassland, continuous variable stocking with growing cattle, commercial breed – Charolais x Holstein-Friesian, traditional breed – North Devon); Auvergne, France (F) (45°N 3°E, altitude 1100m, natural upland grassland, continuous fixed stocking with growing cattle, commercial breed - Charolais,

traditional breed - Salers); Solling Uplands, Germany (D) (51°N 9°E, altitude 500m, mesotrophic hill grassland, continuous variable stocking with growing cattle, commercial breed – Limousin x Friesian, traditional breed – German Angus); Pordenone, Italy (I) (46°N 12°E, altitude 400m, natural grassland, rotational stocking with sheep, commercial breed - Finnish, traditional breed - Karst); Asturias, Spain (E) (43°N 5°W, altitude 1000m, natural heather/gorse vegetation, continuous stocking with goats, commercial breed – Cashmere, traditional breed – local). At UK, F, D and I sites, lower stocking rates were used for T2 and T3 as these areas have lost biodiversity due to intensive grazing. T1, T2 and T3 were designated MC (moderate stocking, commercial breed), LC (lenient stocking, commercial breed) and LT (lenient stocking, traditional breed) respectively. At the E site, higher stocking rates were used for T2 and T3 as the heather-gorse community in this area has been undergrazed and this has reduced biodiversity. T1, T2 and T3 were designated LC (Cashmere goats, lenient stocking rate), HC (Cashmere goats, high stocking rate), HL (local goats, high stocking rate). Output per animal and per ha, net margin per ha, farmer attitudes to the systems (from survey), vegetation type selected by animals, cover estimates in fixed 1m<sup>2</sup> quadrats, structural heterogeneity (from sward surface height (SSH) measurements along fixed transects), and butterflies and grasshoppers abundance and diversity (used as indicator groups) were measured.

## Results

At UK, F, D and I sites, animals selected short vegetation and non-grass species. Selection did not differ between treatments. Individual animal performance was similar across treatments but higher stocking rates on MC gave greater output ha<sup>-1</sup>. Output ha<sup>-1</sup> did not differ between breeds. As expected, number of plant species m<sup>-2</sup> did not change during this short-term experiment. Overall, grass cover increased and legume cover decreased across the 3 years of study. The effect was greater at lenient stocking rate and at sites (D, UK) with high initial grass cover. Treatment did not affect patch size of different vegetation types except at the grass dominant UK site where patches were generally larger and MC had smaller patch size. Species richness and abundance of butterflies and grasshoppers were higher at lenient stocking rate. This was true for both long and short grassland specialists. Breed had little effect on invertebrate diversity. Farmers identified lower costs and suitability for part time farming as benefits of reduced stocking rate but reduced outputs and weed problems as drawbacks. Ease of handling and better grazing ability were seen as benefits of traditional breeds but poor carcass quality and marketing opportunities as drawbacks. Some of these perceptions are not supported by objective studies but may nevertheless affect uptake of agri-environment schemes. Most farmers would reduce stocking rate with suitable agri-environmental payments but would not use traditional breeds. Without subsidies all systems had small or negative net margin. These were less affected by breed than by stocking rate, dependent on market conditions in each country. At the E site local goats lost more weight than Cashmeres (-30g vs -1g). The effect increased through the experiment as selective browsing by the local goats on tall heaths (*Erica arborea* L., *Erica australis* L.) reduced availability of these preferred species (-30 vs -66 percentage frequency units over 3 years). Stocking rate did not affect individual live weight change of Cashmeres but there was better shrubs control at higher stocking rate (-9 vs -30 percentage frequency units over 3 years). Neither stocking rate or breed affected invertebrate diversity

Table 1. Site results from UK, F, D and I sites.

	UK			F			D			I		
	MC	LC	LT	MC	LC	LT	MC	LC	LT	MC	LC	LT
Live weight gain kg ha <sup>-1</sup> d <sup>-1</sup>	0.98	0.97	0.83	0.88	0.92	0.77	0.58	0.87	0.87	0.07	0.06	0.07
Live weight gain kg <sup>-1</sup> ha <sup>-1</sup> grazing season <sup>-1</sup>	407	274	210	225	144	116	265	218	241	24	13	15
Change in no. plant species m <sup>-2</sup> over 3 years	0.3	-0.2	-0.5	2.5	2.0	2.3	0.9	-0.2	0.3	-0.5	-2.0	-2.9
Change in cover of grass as % total cover over 3 years	4	0	6	2	8	-3	6	11	17	2	8	10
Change in cover of legume as % total cover over 3 years	1	-5	-12	-13	-20	-15	-6	-11	-17	2	-8	-3
Vegetation patch length cm	44	43	48	45	41	59	59	71	93	123	425	294

Table 2. Combined results from UK, F, D and I sites.

	MC	LC	LT
Percentage of potential bites in sward containing non-grass species	24	17	19
Percentage of actual bites taken containing non-grass species	53	43	44
Percentage of potential bites in sward containing short vegetation	27	16	16
Percentage of actual bites taken containing short vegetation	46	26	27
Mean no. species of butterflies and grasshoppers per paddock	10.3	13.4	13.1
Net margin without subsidy €/ha	-395	-278	-359

## Conclusions

The study only lasted 3 years, a short time in ecological terms. However, at least in the short-term, lenient stocking rates led to an increase in competitive grasses, particularly on very grass-dominant sites. Animals selected for legumes whatever the stocking rate. Grazing thus reduced legume cover, particularly under lenient grazing where grass competitiveness is increased. Patch size was greater under lenient grazing on highly grass dominated sites. Lenient grazing generally improved invertebrate diversity. Choice of stocking rate in agri-environment schemes therefore needs to target the specific biodiversity objectives of the scheme. Farmers appear willing to adjust stocking rate provided there are appropriate agri-environment payments. The need for payments is demonstrated by low or negative net margins in the absence of subsidy in all the systems studied, whatever the stocking rate.

At UK, F, D and I sites breed had little effect on biodiversity. At the E site local goats controlled shrubs better than Cashmeres, despite poorer performance over the season. Economic performance of traditional breeds was generally poorer often due to lack of appropriate markets. Farmers were reluctant to use traditional breeds even with agri-environment payments. The lack of 'breed' effects requires care as rearing experience may have masked genetic differences.

## Profitable savings on nitrogen inputs of intensive dairy farms

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### Abstract

In the project 'Cows & Opportunities' 17 Dutch dairy farmers agreed to lower N inputs and hence, reduce nitrogen (N) surpluses to levels that are in accordance with standards for environmental quality. These pilot farms represent the national range of conditions for dairy farming. During the period 1998 – 2002 milk production per ha increased by 5% and surpluses decreased from 266 to 176 kg N ha<sup>-1</sup> (-34%). Main savings were mineral fertilizers (-53%) and concentrates (-11%). Crop yields were not reduced as organic manures were better utilised as fertilizer (improved timing, methods and rates of application) and by less grazing. Milk production per cow increased. On average farmers' income increased by 3,500 euro yr<sup>-1</sup> as a consequence of improved nutrient management.

Keywords: dairy farming, nitrogen, profits, intensive, management, environment.

### Introduction

A large part of total milk production within the European Union (EU) is produced in regions with large and specialised dairy farms (Bos *et al.*, 2005). The Netherlands is one of these regions. The use of fertilizers and feeds has strongly increased over time, enabling high stocking rates of high-yielding cows. The increase in external N inputs led to high nitrogen surpluses, which contribute to environmental pollution by emissions of ammonia and nitrous oxide to the air, and nitrate to the water. In addition to pollution, N losses represent a waste of energy and money. Losses can be reduced by improved management. The objective of this paper is to present the results of the pilot commercial farmers in the project 'Cows & Opportunities'. These farmers agreed to reduce surpluses to levels that are in accordance with environmental quality standards, by improving farm management. In return, farmers got access to the knowledge from research and advisory organisations (Oenema *et al.*, 2001).

### Materials and methods

The pilot farms were selected to represent the range of conditions for rather intensive dairy farms in The Netherlands. For that purpose, Dutch dairy farming was analysed in terms of structural characteristics (farm size, crops, grazing system, etc.) and environmental conditions (soil type, hydrology, etc.). Results were used to identify selection criteria. Advertisements and articles in agricultural magazines were used for publicity and for recruitment of potential participants. After a first screening of over one hundred applicants, potential participants were visited and evaluated in terms of motivation and communication ability. Finally, 17 farms were selected, with emphasis on dry sandy soil (11) and farms with an above average milk production per ha (to make it more difficult to realise environmental goals). After collecting farm data, each participating farm was thoroughly analysed to identify its strengths and weaknesses and to analyse the opportunities to realize environmental and economic targets. Outlines for farm development were formulated in close cooperation between farmer and scientists; a farm plan was made, discussed and implemented. Farmers had to monitor all incoming and outgoing products and to register a detailed account of farm activities. Furthermore, a substantial amount of data were collected from industry (milk factories, financial accountancy offices, Dutch Herd Book, etc.) and by research organisations (flows of nutrients, soil quality, manure production, crop yields, etc.). Data were analysed to gain an understanding of the fate of nutrients and to evaluate fertilisation and crop production, animal nutrition and animal health, the emission of greenhouse gasses and economics.

In fact, these farmers were testing and demonstrating the practical feasibility of prototype dairy farming systems, developed by research for Dutch farming conditions. Farmer-to-farmer interaction is regarded

as the best way to transfer knowledge from research to practice. Therefore, pilot farmers present their results at meetings of farmers and host visits to their pilot farms. During these meetings, all relevant data (including the financial information) are presented and compared with data of conventional Dutch farmers in order to stimulate discussion.

## Results and discussion

Between the starting year (1998) and 2003, milk quota and farm area increased by 27 and 22 %, respectively (Table 1). Because milk quota per farm increased faster than farm area, milk production per ha was intensified by 5%, on average. The main reasons for this are the high price of land and the high costs of producing feed, compared to the costs of purchased feed. Milk production per cow was increased and the number of young stock reduced, to lower feed needs per unit milk. Development of the pilot farms was faster than average rate of development in Dutch dairy farming.

Table 1. Averages of the main characteristics of the pilot farms at the start of the project (1998) and in 2003.

	1998	2003	difference
Milk quota (kg)	554,500	703,000	148,500
Farm area (ha)	41	50	9
Milk production intensity (kg ha <sup>-1</sup> )	14,300	15,000	700
Cows	69	80	11
Milk cow <sup>-1</sup> (kg)	8,000	8,700	700
Young stock per 10 cows	8	7	-1

Despite the increase in milk production per ha, farm output of N decreased (-10%) between 1998 and 2002 (Table 2). The main reason was the strongly reduced export of organic manure. However, inputs decreased far more than outputs. Main savings were purchased mineral fertilizers (-53%) and concentrates (-11%). As a result, farm N surplus decreased strongly from 266 to 176 kg ha<sup>-1</sup> (-34%). Grazing was restricted and more of the excrements were collected indoors. As a result, excretion during grazing decreased from 73 to 40 kg N ha<sup>-1</sup>. Despite the lower export of organic manure, the increased milk production per ha and reduced grazing, the quantity of applied slurry was rather constant (204 and 209 kg N ha<sup>-1</sup>). The reason was a lower level of protein in the diet of the cattle as a consequence of lower N content in purchased concentrates and lower fertilisation levels of home grown forages. As a result, intake of N in feed by cattle was lower and a higher proportion was transformed into milk and meat (from 21 to 24%; Table 3) resulting in lower N excretion.

Table 2. Average N farm balances (kg ha<sup>-1</sup>).

	1998	2002	difference
Input:	383	280	-103
Animals	1	0	-1
Organic manure	9	9	0
Mineral fertilizers	178	84	-94
Clover	4	8	4
Deposition	43	44	1
Concentrates	123	109	-14
Forage	26	26	0
Output:	117	105	-12
Milk	78	77	-1
Animals	14	14	0
Organic manure	23	9	-14
Forage	2	4	2
Surplus	266	176	-90

Table 3. Inputs and outputs of the farm components cattle and soil/crop (kg N ha<sup>-1</sup>).

	1998	2002	difference
Component cattle:			
Input (feed)	425	378	-47
Output (milk and meat)	91	92	1
Surplus (input – output = excrements)	334	287	-47
Efficiency (output/input)	21%	24%	3%
Component soil/crop:			
Input (fertilisers, deposition, clover)	515	424	-91
Output (crop)	306	286	-20
Surplus (input – output)	209	138	-71
Efficiency (output/input)	59%	67%	8%

Despite the rather constant application level of slurry and the strongly reduced fertilisation with mineral fertilizers, dry matter yield and energetic value of the crops did not decrease. Main reason was the improved application of fertilizers (in terms of timing, methods and rates of application). However, forage N content was slightly reduced. As a result, a higher proportion of the (reduced) input was recovered by the crops and, hence, soil surplus decreased. Savings on purchases of feeds and fertilizers increased income with 0.005 € per kg milk (annually 3,500 € for a farm producing 700,000 kg milk).

### Conclusions

This research has shown that, in many cases, intensive dairy farms can comply with environmental quality standards without a reduction in milk production per ha. This can be achieved by improving nutrient management. Improvements can be profitable due to savings on purchases of fertilizers and concentrates and better performance of cattle. However, farmers have to spend more time planing and controlling fertilization and feeding, keeping records and analyzing farm data and increasing their knowledge of these topics. A higher degree of management skills is needed.

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# Impact of the CAP reform on small-structured grassland regions in Southern Bavaria

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## Abstract

The objective of this research is to investigate the effects of the German implementation of the reform of the common agricultural policy (CAP) of 2003 on the land use in Bavarian grassland regions. To assess the impacts of the reform two municipalities in Southern Bavaria are selected. They differ significantly with respect to the natural productivity of the grassland sites and the current farm structure. The farmers in both municipalities are interviewed and the impact of the CAP reform on the respective farms is modelled using an integrated farm-sample approach. The different single farm models of each respective community are embedded in an equilibrium land market model. The model results show that the land use in the more intensively used region will be more severely affected by the reform. In contrast the positive impact on the land rent will be stronger in the less intensively used region. Further, the results show that farming will even continue in regions which are currently dominated by low profit dairy cattle farming.

Keywords: CAP-reform, cattle farming, integrated farm-sample-model.

## Introduction

The CAP reform of 2003 is expected to have far-reaching consequences for future land use. Germany was one of the few countries to opt for full decoupling with a regional hectare premium BMVEL (2004). Especially, the land use in regions characterised by a high share of grassland and dairy farming is expected to change dramatically. This expectation is based on the probable decline of the milk price. Currently, most of the grassland in Southern Bavaria is still consumed by dairy cattle.

## Materials and methods

In order to assess the consequences of the CAP reform on the land use, an integrated farm sample approach was used. The future land use in two study areas located in Southern Bavaria is simulated. In both regions the majority of the farmers were interviewed. Furthermore, the calculations are based on the data of the integrated accounting and control system (IACS).

Both regions have several features in common. Firstly, 100% of the agricultural land is grassland which is exclusively used by cattle (Table 1). Secondly, significant parts of the landscape are rough pastures which are managed co-operatively. In the first region, which is located in the Bavarian Alps (UBA), only part-time farmers are still active. Even private pastures and meadows are managed rather extensively. In the second area which is located in the pre-alpine moraine belt (UA) dairy farming is the clearly dominant farm type.

The modelled farms try to optimize their respective utility function using linear programming. The various modelled farms of each study area interact on an equilibrium land market. Consequently, the land rent is the result of demand and supply on this market. For further information on details on layout of the model see Kantelhardt *et al.* (2005).



Table 1. Essential agronomic characteristics of the Bavarian study areas.

	Upper Bavarian Alps (UBA)	Upper Allgäu (UA)
Grassland area (ha)	820	930
Thereof of co-operative rough pasture	600	200
N° of farms: full-time / part-time	0 / 20	15 / 10
Farm type: Dairy cattle / Suckler cows / Heifer breeding / Mixed	7 / 2 / 3 / 8	23 / 1 / 1 / 0
Avg. Utilization intensity of privately managed grassland	2 cuts	4 cuts

In order to assess the impact of the reform three simulation runs were conducted. The first one (I) represents the situation before the reform (Table 2). Both scenarios represent a full decoupling approach. In scenario I (S1) all market prices remain constant except for milk. The decline in the milk price is reflecting the milk premium. In a second scenario (S2) the milk price drops to world market level and the prices for beef products are assumed to be at a comparable high level. Local peculiarities like yield levels or special premiums for endangered breeds are taken into account. The rough pastures are not included in the calculations of the land use. These areas only provide an opportunity to feed heifers during the vegetation period free of charge.

Table 2. Key economic figures of the different calculation runs.

	Initial Situation (I)	Scenario I (S1)	Scenario II (S2)
Price per kg of milk	35 cent	31 cent	25.5 cent
Price per kg of milk quota	60 cent	55 cent	40 cent
Price level for other agricultural products	Long term average market prices	Long term average market prices	Market prices of spring 2005
Area payment	0 EUR ha <sup>-1</sup>	300 EUR ha <sup>-1</sup>	300 EUR ha <sup>-1</sup>
Direct payments	Coupled	Decoupled	Decoupled
Cultural landscape program	Up to 200 EUR ha <sup>-1</sup>	100 EUR ha <sup>-1</sup>	100 EUR ha <sup>-1</sup>
Compensatory allowance	Locally defined	Locally defined	Locally defined

## Results and discussion

The land use in the pre alpine study area (UA) will change significantly (Figure 1). Nearly a quarter of the municipality will be mulched due to a modest decline of milk prices and decoupling (S1). A significantly further declining milk prices and high beef prices in addition to the reform will lead to lower intensity of land use. In the alpine study area (UBA) the effects of the reform on the land use are less pronounced. In scenario one mulching sets the tenure fee since the average land rent of approximately 250 Euro per hectare reflects the area payment minus the costs of mulching.

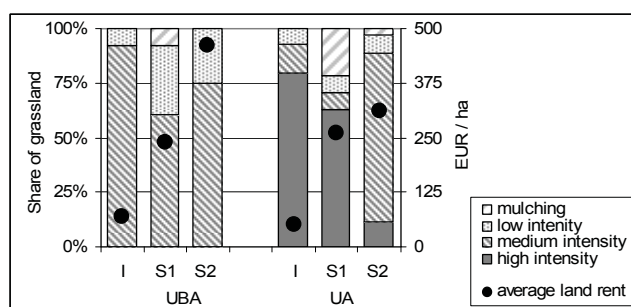


Figure 1. Land use and average land rent in the model run for the respective study areas.

With increasing beef prices more fattening heifers are kept (Figure 2). With high beef prices heifers, kept at a low intensity, replace mulching as the most efficient way of maintaining the landscape. In contrast to the pre-alpine region where the stocking density drops by nearly 50% the stocking in the alpine region does not change. The reduced stocking in UA is mainly a consequence of the changing number of dairy cattle.

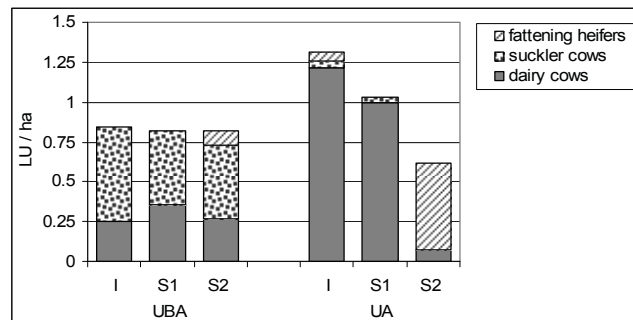


Figure 2. Stocking densities per ha of privately managed farm land in the two study areas.

### Conclusions

The CAP reform leads to increasing land rents in marginal grassland regions. However, the question remains open how this higher land rent will be distributed between farmers and land owners. In intensively managed grassland regions the land use might change dramatically since the pressure on dairy farming will increase. The growth potential for the remaining farms is fairly limited since the high competitiveness of mulching will result in comparable high land rents. As consequence of the reform the abandonment with an encroachment of woody vegetation is quite unlikely. Whether mulching is a desirable option for entire landscape must be questioned, especially, if one takes into account its negative impacts on flora and fauna (Spatz, 1994).

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# Structural analysis of cattle farming and its development in the Entlebuch UNESCO Biosphere Reserve

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## Abstract

As Switzerland is not a member of the European Community, it pursues its own agricultural policy, which imposes a higher competitiveness while maintaining multi-functionality and a stepwise approach to European conditions. State subsidies to support agriculture are above the European average. Contributions are primarily linked to grassland farming, animal welfare and nature protection. The Entlebuch UNESCO Biosphere Reserve (UBE) is a mountain grassland region with particularly unfavourable topographical and climatic conditions. 76.5% of the farms operate on a full-time basis. The average agricultural area per farm is only 14.0 ha. Dairy farms have a milk quota of 64,500 kg. Local identification and characteristics favour the production of specialities. The demand for site-dependent effects and achievements (environment, landscape) is high. Alternative cattle farm models (dairy, meat and cattle rearing farms) were defined and their stocking rate, agricultural work unit, gross margin and productivity were calculated.

Keywords: Swiss agriculture, mountain region, livestock systems, sustainable development.

## Introduction

As Switzerland is not a member of the EU, it pursues its own agricultural policy (Agricultural report 2004). The agricultural act imposes a higher competitiveness and a stepwise approach to EU conditions. State subsidies supporting agriculture are above the average of the EU. Contributions are primarily linked to grassland farming (acreage) and are coupled with the ecological performance certificate (EPC). Ecological compensation areas must make up at least 7% of cultivated farm area and animal welfare has to be respected. In addition, a number of protective tariffs on feedstuffs and meat are still in force, resulting in a high degree of self-sufficiency. According to WTO negotiations, Switzerland is obliged to cut back tariffs, reduce product bound internal measures of support and on a medium and long-term basis, phase out any form of export aid. These measures will substantially decrease the farm income as Swiss agriculture is characterised by small-scale structures in a high-cost context. High priority is given to increasing productivity and efficiency as well as to improving production systems.

## Material and methods

Data on agricultural structures and zones, livestock, farming and production methods and agricultural work units (AWU, man-year) were taken from BFS (2004) and AfS (2004). Five alternative farming models operating under the same environmental conditions (Eggimann, 2003; Hausheer and Meier, 2003) were defined. The typical full-time farm is a grassland dairy farm situated, in a mountain area zone 2, and cultivating an area of 15.25 ha, with a milk quota of 64,500 kg ha<sup>-1</sup> (ZMP, 2004). The livestock consists of 13 cows and 10 heifers and pigs. As all farms are managed according to nutrient balance e.g. Swiss balance (Walther *et al.*, 2001), livestock systems and not farming methods (e.g. organic farming) were compared. The labour requirement, the amount of subsidies, the gross margin and from this the agricultural output were calculated (BETVOR and DBKAT, 2004). The dairy farms produce non-silage milk. The other model farms are supposed to make silage. The high structural and labour costs of these small structured farms were not considered.

## Results and discussion

76.1% of the farms in the UBE are managed as a main-earning holding. The average agricultural area (14 ha) is 25% lower than in other Swiss mountain regions. AWU, milk quota (64,500 kg a<sup>-1</sup>) and number of cows per farm are similar to the Swiss average. Extensively used meadows and pastures make up 7 % of the agricultural area. In addition, the farms manage 7.7 ha of forest. 60% of the agricultural areas are steep slopes reaching an inclination of 18- 35% which involves a high working intensity. Most farms (94%) have breeding cattle, 48% of them have pigs, 24% have sheep and 18% have goats. Most of the cows are dairy cows and only 10% are suckling cows.

Alternative 1: A dairy farm with pig production requires an AWU of 1.43. This farm type results in a high stocking rate of 1.7 LU ha<sup>-1</sup>. In order to maintain the nutrient balance, 140 kg of phosphorous must be exported. The part of total income (gross margin + subsidies) stemming from animal production can be as much as 64 % which is higher than alpine pastures dairy farms (A2, Table 1). Total income per livestock unit (LU) is the lowest of all the livestock farm types. Milk productivity (kg ha<sup>-1</sup>) is low compared to average Swiss figures (Gazzarin *et al.*, 2004).

Table 1. Income and productivity for a model dairy-farm in comparison to alternatives (A 2-5) in a Swiss mountain region (Entlebuch UNESCO Biosphere Reserve).

Production system	A 1 Dairy farm	A 2 Dairy farm	A 3 Beef farm	A 4 Cattle rearing farm	A 5 Veal farm
Gross margin (€)	43,588	30,348	14,263	15,947	23,909
Percentage of total income (%)	64	50	30	33	41
Acreage subsidies (€)	15,635	15,635	15,635	15,635	15,635
Subsidies linked to animal production and welfare (€)	9,033	14,393	18,219	16,748	18,578
Income AWU <sup>-1</sup> (€)	47,731	35,725	74,020	63,311	40,084
Income LU <sup>-1</sup> (€)	2,610	2,614	2,872	3,059	3,142
Milk productivity (kg ha <sup>-1</sup> )	4,230	2,544	378	279	479
Meat productivity (kg ha <sup>-1</sup> )					

Alternative 2: Dairy farms with alpine pastures can graze their cattle on alpine pastures along with some boarder animals from lowland farms for almost 100 days which entitles them to additional subsidies. The stocking rate of 1.0 LU ha<sup>-1</sup> drops to the lowest level of all alternatives. As labour input is higher, the AWU of 1.69 surpasses the other alternatives. 50% of total income comes from animal products. Income per work unit (AWU) and productivity are the lowest. This type of farm is linked with additional costs for infrastructure (e.g. alpine stables, roads).

Alternative 3: A beef farm with suckler cows coincides with a low stocking rate (1.1 LU ha<sup>-1</sup>). This model requires the least labour input (AWU 0.65) and results in the lowest total income, but when relating it to AWU, the beef farm has the highest income with 30% of total income being derived from animal products. Thus, this production system strongly depends on subsidies.

Alternative 4: Cattle farms with rearing cattle need less AWU (0.74) than dairy farms and the stocking rate of 1.04 LU ha<sup>-1</sup> is also lower. The contribution of animal production to total income is comparable to A3 and the income per work unit is quite high.

Alternative 5: A veal farm fattens the calves using cows milk (1,000 kg) and milk replacer (50 kg). The AWU is slightly higher than in a dairy farm. Animal production makes up 40% of total income. A high income per LU is attained. This farm type reaches the highest meat productivity (weight gain ha<sup>-1</sup>).

## Conclusions

Dairy farms generate a high total income, 50 % to 65% stemming from animal production. The required AWU is high compared to A3 and A4. Grassland farming with suckling cows and rearing cattle have a low stocking rate which is ecologically advantageous. These alternatives farming types generate a very

high income per hour. However, total income strongly depends on subsidies. As the AWU is low, a subsidiary income can be maintained.

Co-operation, particularly with respect to farm mechanisation, or a change from conventional grassland farming to organic farming becomes very important. The efficiency of all alternatives mentioned above must improve due to rising competition.

Sheep and goat farming is still of minor importance. Nevertheless, sheep and suckling cows are increasingly brought to alpine pastures as less rearing cattle is available from lowland farms.

It is important that possibilities for subsidiary income, for instance from winter and summer tourism supported by regional development policies, exist in regions like the UBE.

In 2009, the milk quota regulation will be abrogated and subsidies for cheese production will decrease. This will favour milk production in regions with more favourable climatic and topographic conditions. The production of local specialities and labels - dairy and meat products – and selling them on Swiss or neighbouring markets with the brand “Origin from Entlebuch Biosphere Reserve” is a promising prospect for mountain regions like UBE. Labelled products have a higher added value and obtain a higher price. Dairies and farmers will thus obtain additional income from their products.

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# Protecting Biodiversity in the Harz Region by introducing new Marketing Policies

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## Abstract

The use of species diverse mountainous meadows in the Harz (Lower Saxony) by old domestic cattle breeds – like the Harzer Rotvieh - is in danger of being abandoned by farmers as the income support by local authorities may be cancelled in the future.

One alternative to replace this vanishing income support is to sell the beef to consumers at a higher price in order to pay for the additional costs generated by old landraces and the protection of species rich meadows. Using the discrete choice method, 150 consumers in St. Andreasberg were asked if they preferred ordering beef in restaurants from endangered landraces or from conventional farming systems. The data was analysed with the help of the conditional logit model. The results have shown that consumers (tourists as well as inhabitants) in the Harz region significantly prefer beef production based on extensified systems with a strong link to the Harzer Rotvieh and the mountainous meadows. Overall it can be concluded that, apart from subsidies, introducing a specific marketing program can ensure the protection and restoration of species rich meadows as well as endangered cattle.

Keywords: agrobiodiversity, meadows, beef, land races, income support.

## Introduction

The use of species diverse mountainous meadows in the Harz (Lower Saxony) by old domestic cattle breeds – like the Harzer Rotvieh - is in danger of being abandoned by farmers as the income support by regional or national authorities may be cancelled in the future. Currently the protection and use of meadows in this region is supported by two different measures under the regulation (EU) 1257/1999: Less favoured areas and areas subject to environmental constraints and the agri-environment. As scarcity on the European level increases, it appears obvious that in the coming years the financial support on behalf of these measures could diminish. One alternative to replace this vanishing income support is to sell the beef to consumers at a higher price in order to ensure the profitability of the system. To evaluate the opportunities of such an alternative, the consumers of beef were asked, if they would like to order beef of local or conventional origin in restaurants. The main question was: What factors significantly influence the choice of a regional environmentally sound beef production system.

## Methods and Material

In order to examine this question the discrete choice method for eliciting preferences was used. The procedure is comparable to the conjoint analysis. Different goods, described by characteristics and each described by levels, are presented to the participants who have to make according choices. Methodologically the discrete choice analysis is based on a conditional logit model (McFadden, 1973; Urban, 1993). In general the responses are modelled in a utility function and provide information about the choice and the importance of the attributes. The underlying suggested probability function has been modelled according to Profeta and Enneking (2004) in the following form:

$P(\text{buy of Harzer Rotvieh beef})_i = \alpha_i + \beta_i X_i$  ; With  $X_i$  = own price; cross price; production system; socio-demographics and tourist attitudes.

The initial objective of a discrete choice analysis is to develop an experimental set, which closely reproduces the reality of consumer choices in purchasing situations (Profeta and Enneking, 2003). Three different attributes have been varied systematically. The real price for a beef dish including side orders in a restaurant in St. Andreasberg was 12 €. The experimental attributes were: the origin of the beef (Harzer Rotvieh versus conventional beef); the production system (outdoor keeping versus all season stable keeping, and three price levels (12 €, 15 €, 18 €). Harzer Rotvieh has never been kept in stables all season, so that neither this option was tested in the framework of the study, nor the option that conventional beef is sold at a price of 18 € per serving size as this seemed to be unrealistically high. Thus a total of 8 options was tested. 150 Tourists in St. Andreasberg were interviewed during two local events on the June 11 and 12, 2005 using a standardized questionnaire (for advantages and disadvantages of this approach see Bergmann (2003). The examined sample can be characterised in the following way: lieu of questioning (N = 150) St. Andreasberg; gender distribution 52% female, 48% male; average age of 58 years.

## Results and discussion

The data was analysed with the help of the multinomial Logit-Analysis in SPSS 12.0.

Table 1. Estimated model and significance of variables.

Choice of meat originated from		$\beta$	Standard deviation
	Absolute term	2.85211145**	1.02132738
	Own price	-0.29099846***	0.06283738
	Cross price	0.10989824*	0.04884764
	Length of stay	0.02884617**	0.01102535
	Daily expenses	0.29516815**	0.10243751
Harzer Rotvieh	Factor Attractions	0.1967281	0.14891011
	Factor promenading	0.43736549***	0.11179146
	Age	-0.00960028	0.00680885
	Intensity of Production	-0.14332074	0.27057066
	Nature pretensions	-0.49868189*	0.23826381
	Touristic pretensions	0.54517323*	0.22389627

The category of reference is "no Choice" - Cox und Snell  $r^2 = 0.216$  - Nagelkerke  $r^2 = 0.256$  - McFadden  $r^2 = 0.127$ .

The results of the model (Table 1) show that most of the chosen variables have a significant influence on the choices made. The  $r^2$  in conditional Logit-Analysis between 0.256 and 0.127 is a sign of good estimation (Urban, 1993 or Backhaus *et al.*, 2002). As expected, the prices had a significant influence. Surprisingly the daily expenses had a significant and positive influence on the choice of Harzer Rotvieh beef, while the monthly income had no significant influence. Furthermore, the length of the stay in St. Andreasberg had a significant influence. As the age distribution of the tourists is rather homogenous, it was predictable that this variable would not have a significant influence. However, it remained only slightly below the required level. The estimation of the specific choice functions showed additional information. The most interesting being that people who strongly like the landscape environment tend to dislike buying the regional beef, while people who prefer active holidays had a much higher preference for local beef. However, because the results of a multinomial logit analysis are always difficult to interpret, a more common interpretation and use of such data is to calculate the elasticities and the possible market share of Harzer Rotvieh beef. The market shares were calculated using the general formulation of multinomial logit models:

$$P_w = \frac{e^{j_{wr}}}{1 + \sum_{j=1}^J (e^{j_{jr}})} \quad j \neq r \text{ formula (1)}$$

with:  $P_w$  – Possibility of choices;  
 $V_{wr}$  – individual equation  $a + \sum(\beta * x)$

The market share was calculated using the individual probabilities and dividing them by the sample population. The own price elasticity of the Harzer Rotvieh is – 1.146. This results in the following calculated market shares (see Table 2):

Table 2. Market share ( % ) of Harzer Rotvieh beef depending on own price.

Price in Euro	Market share
12	49.28
15	35.15
18	22.56
21	13.10

It can be seen, that a marginal change in prices results in above average changes in demand. For this reason the price for beef in restaurants must be chosen cautiously. However, the table also shows that tourists express a willingness to pay for this regional product and that, with a price two times higher than that of the conventional product, there will be a market share of at least 10%.

Overall it can be concluded that, in addition to paying subsidies, introducing a specific new product based on extensive nature protected grasslands would gain from an increasing market share between 10 and 50%. Therefore it can be concluded, that apart from policy changes, the protection and restoration of species rich meadows as well as of endangered cattle can be ensured by the market rather than solely through financial public support in the coming years when using intelligent marketing instruments.

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# Selected agricultural and economic problems of organic meadow farms in Poland

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## Abstract

The study was based on data collected in 39 organic farms in 9 Polish regions. The mean share of grasslands was 47.3%, which is more than twice of the country average (21%). These organic farms were classified according to the EU system of farm classification (MAFF, 1992). Among 5 general agricultural types (out of 9 general types defined by the EU), the best economic results were found in type 4 farms (specialized in breeding grazing cattle) and the lowest were found in the type 1 farms (specialized in field crops). Among 9 basic agricultural types (out of 17 basic types defined by the EU) the best economic results were found in type 43 farms (cattle) and the lowest were found in type 14 farms (other field crops excluding cereals, oil and legume plants). Among 14 detailed agricultural types (out of the 50 types defined by the EU), the highest gross margin was achieved by type 412 farms (milk production and dairy cattle breeding) and the lowest gross margin was achieved by type 712 farms (various animals with the dominance of grazers, excluding dairy cattle). It was found that the costs of production in the organic farms were not always satisfactorily compensated by the incomes.

Keywords: organic farming, permanent grassland, animal production, gross margin, types of farms.

## Introduction

Organic farming has raised a big interest among farmers in Poland, as the integration into the EU enlarges the market for organic products to other countries (Austria and Switzerland). Organic farms specialized in animal production are characterized with a significantly higher use of permanent grasslands than conventional farms.

## Materials and methods

Thirty nine organic farms in 9 provinces were studied with the questionnaire method in 2004 (Zastawny, Jankowska-Huflejt, 2005). It was assumed that organic farms can produce healthy food which is naturally allowable, socially acceptable and economically effective. Studied farms covered 1501 ha. Surface area of farms varied from 3.13 to 319.42 ha (average 38.49 ha).

Table 1. Land use and cropland structure in studied farms

Farm groups (ha)	Number of farms	Farm area		Share in the cropland structure		Forests and other (ha)	Employment per 100 ha of croplands	Gross margin (€) per	
		Total (ha)	crops (%)	Arable lands	Permanent grasslands			1 ha	capita
1.0-10.0	6	7.63	85.5	49.7	50.3	0.88	2.43	291.00	871.50
10.1-20.0	12	15.71	83.5	63.7	36.3	2.10	1.91	404.25	3433.15
20.1-50.0	15	33.29	90.4	36.7	63.3	3.17	1.72	358.25	4931.25
> 50.0	6	127.3	94.7	60.4	39.6	4.31	3.04	149.75	4386.25
Mean	39	45.98	88.5	52.7	47.3	2.62	2.31	287.00	3524.50

Grasslands contributed by 47.3 % to the total farm area (at an average rate of 21 %). Productive and economic results of the studied farms are compared with their natural, agricultural and economic

characteristics in Table 1. Gross margin was adopted as the main criterion for economic evaluation of the farms. It was calculated per ha of croplands and per capita (farmer). Farms were classified into general, basic and detailed types according to the farm typology used in the EU (MAFF, 1992). European Size Unit (ESU) equal to 1200 € was used to determine the economic size of farms and to classify them into 9 classes according to their economic size.

## Results and discussion

The farms studied obtained average or low incomes from agricultural production, and particularly low incomes (10 % of the total farm income) from plant production. Animal production, mostly dairy cattle breeding, accounted for 90% of the total farm income. Mean gross margin was 287 € (between 150 and 404 € ha<sup>-1</sup> croplands) (Table 1). Per capita mean income was 3524 € and varied between 871 € in 1-10 ha farms to 4931 € in 20-50 ha farms. Its value per ha of croplands was higher in 10-20 ha farms and decreased in the largest farms while per capita income increased in 10-30 ha farms and then slightly decreased.

Based on calculated mean ESU, each type of farms was classified into 9 classes of production size. Share of gross margin (SGM) from particular types of production in total values SGM was in turn the basis for determining the agricultural type (general, basic or detailed) of a farm. Five general types (out of 9 defined by the EU), 9 basic types (out of 17 defined by the EU) and 14 detailed types (out of 50 defined by the EU) were used (Table 2).

From among 5 set general types of farms (Table 2) type 4(specialized in breeding grazing cattle) obtained the best economic results (14618 € gross margin and 12.18 ESU); type 1 (specialized in field crops) obtained the worst results (2724 gross margin and 2.26 ESU). The highest economic results (11712 € of gross margin and ESU 9.75) among the basic types were obtained by type 43 (cattle) and the lowest were obtained by basic type 14 (other field crops excluding cereals, oil and legumes) (2724 € gross margin and ESU 2.26).

Among detailed types (Table 2), the highest mean gross margin (18605 € and ESU = 15.50) was obtained by type 412 (milk production and dairy cattle breeding) and the lowest was obtained by type 712 (various animals with the dominance of grazers, excluding dairy cattle) (1801 € and ESU = 1.50).

Costs of production of organic systems in the studied farms are not always satisfactorily compensated by the incomes. Organic farms income might, however, rise with increasing wealth of the population of our country and with the improvements in the market of organic products, particularly in western regions due to possible export to Western Europe. Legally provided subsidies to organic agriculture have already increased the farmers' interest in such a type of production. Integration of Poland into the EU enlarged the agrifood market which might be competitive both in quality and price.

## Conclusions

The best 4 organic grassland farms (specialized in breeding grazing cattle), and the worst economic results were obtained by general type 1 (specialized in field crops). Among basic types the highest economic result were obtained by type 43 (cattle) and type 44 (sheep, goats and other animals in the grazing system), and type 412 (milk production and dairy cattle breeding in total) obtained best economic results among detailed types. Gross margin per ha of croplands was the highest in 10-20 ha farms followed by 20-50 ha farms.

Table 2. General, basic and detail agricultural types of studied farms acc. to the EU principles.

Type	Description	N. of farms	Gross margin* (€)	Mean economic size of a farm* EU		
				in ESU	Class	Nomenclature
General agricultural type						
1	Specialized in field crops	2	2724	2.26	II	very small
2	Specialized in gardening	2	5298	4.41	III	small
4	Specialized in breeding grazing cattle	28	14618	12.18	VI	moderately small
5	Specialized in breeding cattle fed with concentrates	3	5825	4.85	III	small
7	Various animals in total	4	5930	4.94	III	small
Basic type						
14	Other field crops excluding cereals, oil and legumes	2	2724	2.26	II	very small
20	Garden crops	2	5298	4.41	III	small
41	Dairy cattle	17	6763	5.63	III	small
42	Cattle	2	4209	3.50	II	very small
43	Cattle in total	2	11712	9.75	V	moderately small
44	Sheep, goats and other animals in grazing systems	7	8740	7.28	IV	small
50	Animals fed with concentrates	3	5825	4.85	III	small
71	Other animals with the dominance of those fed in the grazing system	2	4651	3.84	II	very small
72	Other animals with the dominance of those fed with concentrates	2	7209	6.00	III	small
Detail agricultural types						
144	Field crops excluding tuber crops, cereals and vegetables	2	2724	9.07	V	moderately small
201	Vegetables and strawberries	2	5298	2.26	II	very small
412	Milk production and dairy cattle breeding in total	17	18605	15.50	VI	moderately small
421	Cattle, mainly growing cattle	1	4243	3.53	II	very small
422	Cattle, mainly slaughter cattle	1	4243	3.53	II	very small
431	Cattle, mainly dairy cattle	2	11712	9.75	V	moderately small
441	Sheep	2	12596	10.49	V	moderately small
442	Sheep and cattle in total	4	6044	5.03	III	small
443	Goats	1	11826	9.83	V	moderately small
502	Poultry	3	5807	4.83	III	small
711	Various animals with the dominance of dairy cattle	1	7500	6.25	IV	small
712	Various animals with the dominance of grazers (excluding dairy cattle)	1	1801	1.50	I	very small
721	Various animals fed with concentrates and dairy cattle in total	1	10905.00	9.08	V	moderately small
722	Various animals fed with concentrates and in the grazing system in total	1	3512.50	2.92	II	very small

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# Gaseous emissions from soil fertilized with slurry of pigs fed with manipulated diets

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## Abstract

The manures were originated from pigs fed with manipulated diets in order to reduce negative environmental impacts: The study was carried out in an Aluvisol, where different experimental types and levels of fertilization were used (mineral and organic with the different manures), respecting the impositions of the Nitrate Directive (maximum of 170 kg ha<sup>-1</sup> of organic N). Sorghum was used as plant test. A set of 6 measurements were made at different levels of slurry application: Analyzing all data, we can conclude that manure from pigs fed with the manipulated diet (diet N) has the lowest environmental impact when applied to the soil as fertilizer.

Keywords: diet manipulation, gaseous emissions, nitrogen, pig manure, soil fertilization.

## Introduction

Livestock manures are suitable sources of nutrients to the soil-plant systems, their use as fertilizers can be a pathway for a biological hazard into the soil and the food chain. In addition, animal wastes may contribute to agricultural source of pollution and contaminate streams and ground water, via runoff and leaching from agricultural areas, if their management is inadequate. Moreover, the gaseous emissions to the air are of increasing concern, and factors such as temperature, rainfall, soil moisture conditions, and solar radiation, potentially contribute to the greenhouse effect through gas emissions as methane (CH<sub>4</sub>), ammonia (NH<sub>3</sub>) and carbon dioxide (CO<sub>2</sub>) (Hatch *et al.*, 2004). Based on the option that provides the least damage to the environment and choosing the diet manipulation as a mean of manure management strategy, the objective of this study was to evaluate the effect of soil application of pig manures on gaseous emissions, through their measurement after spreading the manures.

## Materials and Methods

The manures were originated from pigs fed with a standard diet and two manipulated diets: diet T (standard diet with 18% crude protein), diet N (with 15% crude protein and balanced in essential amino acids), and diet P (where bicalcique phosphate was replaced by monocalcique phosphate). The study was carried out in an Aluvisol, in a field with an area of 2500 m<sup>2</sup>, subdivided into experimental unities, and located at the farm of Fonte Boa, of Estação Zootécnica Nacional (Portugal). The experimental treatments were as follows: one control (0 kg ha<sup>-1</sup> of organic N) and, for each diet, three levels of organic nitrogen were applied (42.5, 85, and 170 kg ha<sup>-1</sup> of N). After soil mobilization, the experimental treatments were allocated to the experimental unities. Slurry applications were made by spreading followed by soil incorporation. Slurry compositions were determined by the methods of AOAC (1990). Sorghum was used as plant test. The crop was seeded after manure incorporation into the soil. A set of 6 measurements were made at the maximum level of slurry application: before spreading (initial situation), 15 minutes and 4 hours after spreading (situation that reflects the time of the day with the highest temperature), and 12 hours after the scattering (situation reflecting the emissions during the night period), 15 minutes and 4 hours after incorporation in the soil (situation reflecting the emissions

at the highest temperature). Three PVC structures of conical shape, with 2 m of diameter and 1 m of height, were constructed to allow the confinement of the emitted gases from soil after spreading and incorporation of the slurry. Gas emissions measurements were performed using a portable analyzer (GAS DATE LMSxi G4.18).

## Results and Discussion

The physical-chemical composition of the slurries obtained from the pigs fed with the experimental diets (Table 1), show that slurry from diet N presented the lowest levels of dry and organic matter, nitrogen, calcium, phosphorous, magnesium, copper, and zinc. Although high electric conductivity values were observed, probably indicating high levels of dissolved salts, they were lower in the slurries from the manipulated diets (N and P).

Table 1. Physical-chemical composition of slurries of three diets (results reported to original matter)\*.

Parameters	T	N	P
Dry matter (%)	4.2	2.5	4.7
pH	8.1	8.1	8.0
Electric conductivity (mS cm <sup>-1</sup> )	34.7	30.5	30.4
Organic matter (%)	2.9	1.6	3.6
Total Nitrogen (N, %)	0.50	0.37	0.45
Total Phosphorous (P <sub>2</sub> O <sub>5</sub> , %)	0.24	0.10	0.20
Potassium (K <sub>2</sub> O, %)	0.27	0.25	0.26
Calcium (CaO, %)	0.16	0.08	0.10
Magnesium (MgO, %)	0.04	0.02	0.03
Copper (mg kg <sup>-1</sup> )	43	21	36
Zinc (mg kg <sup>-1</sup> )	42	19	36
Nickel (mg kg <sup>-1</sup> )	<0.25	<0.25	<0.25
Chromium (mg kg <sup>-1</sup> )	1.0	1.2	1.3
Cadmium (mg kg <sup>-1</sup> )	<0.1	<0.1	<0.1
Lead (mg kg <sup>-1</sup> )	<1.0	<1.0	<1.0

\* Mean of 2 samples taken, before spreading, at the first and last 1000 l of slurries.

Figure 1 shows the patterns of the controlled gaseous emissions during the experimental period. Considering the type of slurry, the same pattern was observed for all of them and the controlled gases (exception for CO<sub>2</sub> with slurry from T diet), although at different levels for some of them.

As expected, slurry from pigs fed with diet N lead to the lowest losses of N (63 ppm) due to volatilization, compared with P (103 ppm) and T (139 ppm) diets. Of importance is the low dry matter content of the slurries, namely that from pigs on N diet. This parameter is related with gaseous emissions, and a linear relationship was reported between ammonia emissions and slurry dry matter content (Smith *et al.*, 2000).

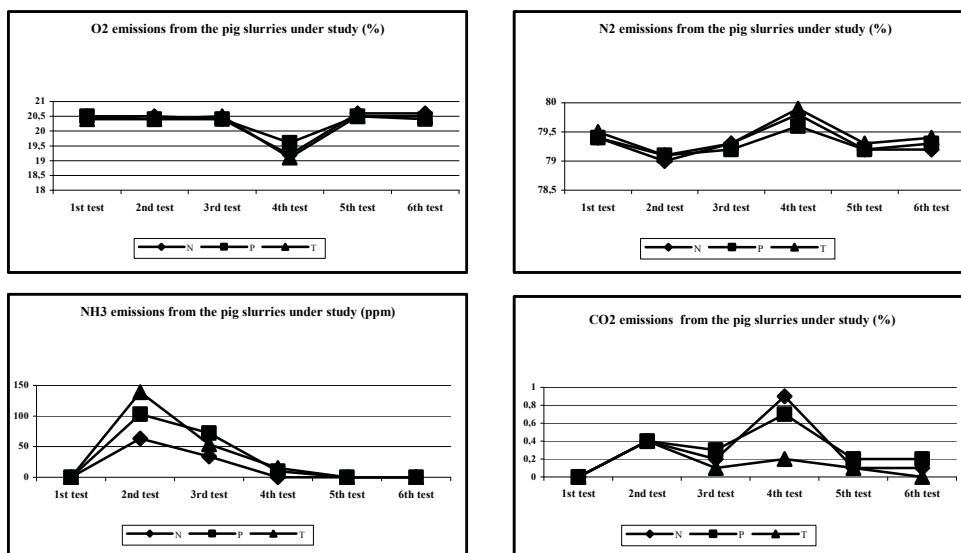


Figure 1. Patterns of controlled gas emissions following pig slurry application.

### Conclusions

In terms of the environmental impact from slurry application to the soil, it was shown the benefic effect of the manipulation of pig diets, particularly in what concerns nitrogen and, particularly, ammonia emissions. Looking at the quantified patterns of gaseous emissions, nitrogen manipulated diets would be a contribution to the reduction of environmental pollution. This study will be performed in a second year, for the validation of the obtained results.

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# CAP goals and consumer demands for multifunctional grassland management

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## Abstract

CAP reform focuses on cross-compliance and the externalities associated with agriculture. The concept of multifunctional agriculture guides agricultural policy, particularly in mountainous areas where tourism is important. Consumer and farmer understanding of multifunctionality, together with CAP guidelines, determine the room for manoeuvre afforded regional policymakers. A survey of 1020 consumers was carried out in the Province of Bolzano in the summer of 2005 in order to analyse the demands they place on mountain farming and their preferences regarding different functions of mountain grassland. The results show preferences for specific externalities arising from grassland management, including (1) landscape management, (2) water management, (3) protection from natural disasters, (4) maintenance of traditional culture, and (5) nature conservation. A further 353 interviews with farmers focused on their self-perception regarding the tasks they perform for society. A comparison of consumer demands, CAP goals and farmer self-assessments identifies a need for changes in grassland management. Consumers and EU agricultural policy rank nature conservation and environmental protection much higher than do grassland farmers in South Tyrol. These farmers should continue to give priority to water protection and landscape management, but also give more attention to environmental protection, nature conservation, and prevention of natural disasters.

Keywords: consumer demands, multifunctionality, landscape conservation, nature conservation, environmental protection.

## Introduction

The keyword "agricultural multifunctionality" appears increasingly often as a guiding principle in both international and European agricultural policy discussions (Durand and Van Huylenbroeck, 2003). The more widespread the concept of multifunctionality becomes in the minds of consumers and farmers, the more grassland management will need to take account of the idea of combining the production of raw fodder with the creation of those particular non-commodity outputs desired by society.

This paper examines the range of functions performed by grassland management, as perceived by consumers and farmers in the mountain regions of South Tyrol. It also examines how the expectations of the two groups match or differ. The likely adaptations required of grassland management in South Tyrol are drawn out from the comparison between the two perspectives (within the context of policy requirements). The analyses of the specific demands for multifunctionality also serve to address an important knowledge gap that has been identified by much of the recent literature on the subject (Caird *et al.*, 2005).

## Materials and methods

Two parallel surveys were carried out in the summer of 2005 to investigate different perspectives regarding the multifunctionality of mountain grassland. These surveys took place within the context of a broader research project concerning the future of mountain agriculture, which was commissioned by the regional government in South Tyrol. The survey was designed so as to cover the entire province and, together with standardised questionnaires, allow the results to be as representative as possible.

Street interviews were carried out with 1020 local individuals in towns and villages. The aim was to identify the range of opinions regarding the multifunctionality of agriculture, using 14 (mainly closed) questions. The respondents were asked, for example, in a half-open question to articulate their associations with, and demands of, (grassland) agriculture in the mountains. They were also shown a list of (grassland) functions and asked to rate the importance of each. Various socio-demographic characteristics of the respondents were also recorded.

A separate survey was carried out to capture the attitudes of those who actually provide this multifunctionality. Using a state farm database, 500 grassland farmers were selected at random and suitable interviews were obtained face-to-face on the farm with 353 of them. Each interview covered 30 questions, whereby some questions contained several parts. Some questions were also identical to those used in the consumer survey; this paper focuses on a comparative evaluation of responses in both surveys to those identical questions, using an SPSS analysis of the coded answers.

## **Results and discussion**

A set of relevant non-commodity outputs from mountain grassland formed the basis of the function list presented to both the local population and farmers in the question where respondents were asked to rate the importance of each function. This set was drawn out of a review of both specialist literature and documents relating to local and EU agricultural policy (see first column in Table 1). More than 80% of the general population rated nearly all the individual functions as either very important or of some importance. The exceptions were "social services" (54 %), "services performed on behalf of the local authorities" (71 %), and "provision of areas for recreation and leisure" (70 %). In general, the responses of the farmers followed the same pattern, whereby the percentage numbers rating each function as either very important or of some importance tended to be higher. This however was not the case for the functions "nature conservation and environmental protection" (62 %) and "provision of areas for recreation and leisure" (just 52 %) (see Table 1). This noticeable difference is presumed due to the somewhat strained relationship between a considerable proportion of South Tyrolean farmers and nature conservation per se, and farmer reservations regarding the possibility of grassland areas turning into quasi-play areas for recreational activities.

It is also worth noting that the percentage of farmers rating a function as either very important or of some importance is much higher than the percentage who declared that the relevant function was actually fulfilled on their farm. This was particularly so in the case of social and communal service functions. Relatively few farmers saw themselves as actually fulfilling the functions of "nature conservation and environmental protection" and "provision of areas for recreation and leisure". Regarding these last two functions, there is a clear need for farmers to make appropriate changes, not least because consumers regard both functions as important and because EU policy actively encourages the fulfilment of these tasks (see, for example, Natura 2000 sites). The revealed dissonance in South Tyrol between farmer attitudes to mountain grassland's role in nature conservation, environmental protection, leisure and recreation, and the demand for exactly these functions raised in the broader discussion of agricultural multifunctionality, is partly reflected in similar results from Austrian studies (Wytrzens and Neuwirth, 2004).

## **Conclusions**

In South Tyrol, there is a discrepancy between consumer and farmer perceptions of multifunctionality with regard to the use of grassland for nature conservation, environmental protection and leisure and recreation. Educational and training initiatives directed at farmers, as well as targeted regional-specific support measures, could help improve the awareness and recognition of multifunctionality. They could also offer those managing mountain grassland areas new job and income opportunities. Farmers need to address the new demands placed on their grassland, particularly given that there still seems to be a generally positive attitude towards mountain agriculture among consumers. After all, 93% of surveyed consumers answered "yes" to the question of whether South Tyrol needs its mountain farmers. Additionally, around half were very much in favour of giving additional public money to mountain



farmers as compensation for the tasks they carry out on behalf of society (only a third were decidedly against this).

Table 1. The multifunctionality of (grassland) agriculture in mountainous regions from the perspective of both the non-agricultural population and farmers in South Tyrol (each figure refers to the percentage of respondents - respectively consumers and farmers - giving the relevant answer).

Function	Consumers (n=1020)				Farmers (n=353)			
	Very important	Of some importance	Currently performed	Not performed	Very important	Of some importance	Currently performed	Not performed
Landscape management	70.5	25.7			94.9	5.1	98.9	1.1
Water management	57.4	31.4	18.7	14.4	63.3	31.8	58.2	41.8
Protection from natural disasters	51.9	32.3	31.2	10.6	68.9	23.9	53.1	46.9
Maintenance of traditional culture	46.5	35.3	50.0	7.0	71.4	22.7	84.4	15.6
Nature conservation and environmental protection	46.6	34.3	28.3	16.0	22.3	40.5	25.4	74.6
Construction and maintenance of access routes	43.2	34.0	40.2	12.2	66.1	24.5	71.4	28.6
Services performed on behalf of the local authorities	35.9	35.2	24.4	14.6	52.8	29.5	16.8	83.2
Recreation and leisure	33.5	36.7	26.8	16.6	17.1	35.0	22.7	77.3
Social services	24.3	33.0	10.9	20.7	28.0	38.0	7.8	92.2

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## **Adaptation of livestock farming systems to the CAP changes: case of the PDO label cheese production**

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### **Abstract**

To maintain cheese production in less favoured regions, farmers must develop high quality cheese production. The specifications of the PDO and PGI quality labels require many changes, particularly for the feeding system. These changes must be compatible with the consistency of the farm system functioning. To build and to assess scenarios of farming systems well fitted to specific contexts, a better knowledge of the farmer's scopes is needed. We study how farmers have designed their farming systems, identifying their main characteristics. The analysis was based on comparative analysis of farmers' livestock and forage management practices. It was highlighted considering the inside and between-farms diversity of the grassland use, and the local milk production context. The 24-studied farms are localized in three wet upland areas of the South-Massif-Central. In each area, a local dairy cooperative collects milk to produce PDO cheese. To perpetuate the farm viability, farmers have implemented different livestock and forage managements depending on soil and climate conditions: increasing the sown pastures area, developing the hay-barn-drying, or changing the calving dates. Different patterns could be identified and the price of the milk has appeared like an important criterion to favour some technical changes.

Keywords: livestock farming systems, farm management, technical changes, PDO labels, cheese production.

### **Introduction**

The Massif Central region (Center of France) is a low mountain area where ten-cheeses with PDO labels are produced. The adoption of European PDO (Protected Designation of Origin) and PGI (Protected Geographical Indication) quality labels is generally essential to the local development (Sylvander, 1994; Barjolle *et al.*, 2002; Barham, 2003). The traditional milk production was based on the grass (cutting and grazing) and a milk production during the spring and summer season. But many transformations in the farming systems have conduced to a large diversity of the production patterns (Brunschwig, 2000). Some studies have shown that to maintain dairy production in less favoured regions, the value of the milk produced must be increased and the recognition of a quality cheese production could accomplish this (Brunschwig, 2000; Chatellier and Delattre, 2003). Also, the label specifications required many changes, particularly in the feeding system, to develop high quality cheese production and to give more guarantees to the consumers. Changes in production rules can produce changes in the farmers' land use strategy (Quetier *et al.*, 2005). Faced with changes in the CAP, there is uncertainty as to how dairy systems should evolve. Stakes of these changes in Massif-Central region concern: the farms survey, the cheese quality production, the biodiversity preservation. In this paper, (i) we present the livestock and forage system managements which we have identified in 3 areas of the south of this region (ii) we propose a description of patterns which could explain the diversity of livestock farming systems (iii) we identify few points about their probably evolution to adapt them faced with changes in the CAP.

*A methodology based on the consistency of the livestock farming system:* To build and to assess scenarios of farming systems evolutions well fitted to specific contexts, a better knowledge of the farmer's purpose is needed. We have shown that the technical changes in the farm management must be compatible with farmers' strategies (Thénard *et al.*, 2005). To assess evolution of the farming systems we have chosen to study their current management practices (Osty *et al.*, 1998). Our objectives are to

produce formalized descriptions of livestock farming systems based on the consistency analysis of the farmer production systems like an interaction between the forage system and herd management (Thénard et al., 2004). This work was based on comparative analysis of farmers' livestock and forage management practices. The 24-studied farms are localized in three areas of the South-Massif-Central. In each area, a local dairy cooperative collects milk to produce PDO cheese. Livestock data have been analysed with a graphical method (Bertin, 1977) to identify synthetic criteria of livestock practices. These criteria have been computed with a Multiple Correspondence Analysis (MCA of ADE4 software) to distribute farms in categories of livestock management (Girard, 2004). Technical decision of the grassland use has been translated with the degree-day method (Theau et al., 2004). These data have been computed with a Principal Components Analysis (PCA of ADE4 software) to describe categories of forage managements.

We have combined these different categories to describe the patterns of livestock farming systems. It is a mean to explain the inside and between-farms diversity in management practices, linking categories to the grassland use, and the local milk production context.

*Three livestock farming patterns and their probable evolutions:* The MCA of the livestock practices has could show 5 livestock managements (Figure 1a). They have been described with the milk production period, the winter ration and the herd replacement rate. The PCA of the forage data has described 4 grassland managements (Figure 1b). They have been characterized by the precocity of the grass use (sweet or palatable grass for early grazing or cutting, tough or low palatable grass for late grazing or cutting).

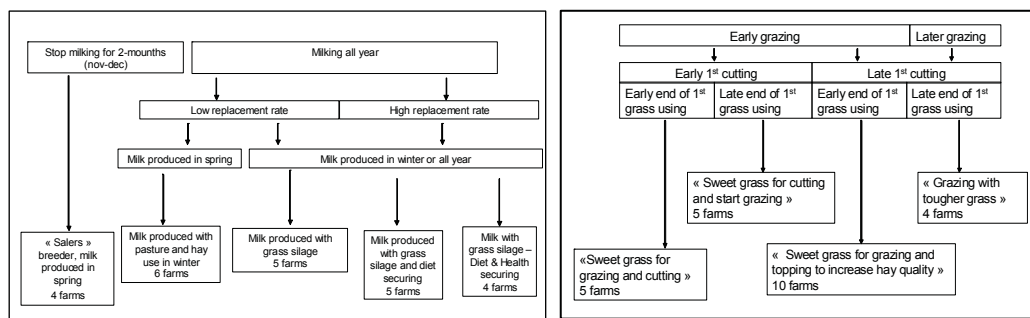


Figure 1. Distinguishing practices and different (a) livestock management (b) grassland management.

The combination of these results has permitted to define 3 different patterns of livestock farming system:

-The first pattern: “producing milk with a traditional management for cheese production”; it is characterised by a milk production in spring, dietary requirement and fodder resources are based on pasture and hay use, soil and climate conditions are favourable at the grass growth; cutting is not early because farms need a large hay quantity during long winters. The farmers could maintain their system if the milk price keep its actual bonus (Delattre et al., 2005).

-The second pattern: “Size increasing and diversifying production to increase income”. It is characterised by dairy and meat production, grass (or maize) silage secures the forage resources; grassland management uses complementarities of requirements between dairy and suckler cows. The farmers could be tempted to switch milk production to meat production (Perrot, 2005), in particular if workforce will be reducing.

-The third pattern: “Intensifying the milk and grassland production in the case of cheese production without renown”. It is characterised by a milk production in winter with diets based on grass silage and concentrates; during grazing period hay supply is using; sown grassland use permits early grazing and cutting management. Faced with the changes in the CAP (milk price in fall), these farms will be fragile; also few actions to increase the cheese recognition could be a chance to maintain the milk price (Espinasse et al., 2005).

## Conclusions

This study has shown that the methodology based on the consistency of the livestock farming system has permitted to explain a diversity of livestock farming systems. This diversity has appeared linked with the different types of grassland use, livestock practices and the local milk production context. In particular, the price of the milk has appeared like an important criterion to favour some technical changes. So this work could be an interesting start to study the diversity of the livestock farming systems and their agri-environmental impacts.

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# Effects of grazing on grasslands diversity along altitude gradient

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## Abstract

Greek grasslands are considered rich in species number and these natural ecosystems constitute precious reservoirs of biodiversity. For this reason their management must combine the forage production and biodiversity maintainance. The effects of grazing and abiotic factors on grassland diversity along altitude gradient were the objective of this study.

Keywords: diversity, grazing, altitude gradient, animal species

## Introduction

Biodiversity has played an important role in natural ecosystems and is principally a mechanism which generates community stability (Mc Naughton, 1967 1968). In recent years biodiversity maintainance is one of the main goals worldwide which correlate with the enviroment protection (Brown *et al.*, 2001). Greek grasslands occupy an area of about 1.7 milion hectares which corresponds to one third of the total rangelands of the country. The majority of them are succesional and only a small proportion of them are climax communities (Ganiatsas, 1964). Their productivity varies widely depending on the particular climatic and soil conditions. Several studies have shown that moderate grazing is the key factor in maintaining their productivity, biodiversity and environmental value (Papanastasis and Koukoura, 1992, Tsiouvaras *et al.*, 1998).

## Materials and Methods

The research was conducted on Othrys Mountain in central Greece. Three grasslands were selected along altitude gradient, at altitude ranges between 800 – 1000 m, 1000 – 1200 m and 1200 – 1500 m (alpine zone). All grasslands were under grazing conditions by three animal species (cattle, sheep and goat) for a long time but with different stocking rates. Grasslands of the alpine zone were grazed at a stocking rate of 1.7 cattle ha<sup>-1</sup> for 4 months while those of the other altitude ranges of 0.63 cattle ha<sup>-1</sup> for 7 months.

Three experimental areas 50 x 50m were selected randomly in each grassland in which vegetation composition was measured using the line point method (Cook and Stubbendieck, 1986). Also species richness was measured with 0.5 x 0.5m sampling quadrates. From these data Shannon- Weiner (H'), species richness (N), species abundance (d) and equitability (J) index were calculated according to the formulas:

$$1. H' = - \sum_{i=1}^S P_i \cdot \ln P_i ,$$

where H' = Shannon- Weiner index  
Pi is the proportion of the individuals

2. Species richness (N) = number of species m<sup>-2</sup>
3. Species abundance (d) = number of individuals m<sup>-2</sup>

$$4. J = \frac{H'}{H \max} = \frac{H'}{\ln S}$$

where Equitability (J) is the ratio of the observed diversity which could possibly occur  
 $H_{\max} = \ln S$  (case where all species are equally abundant).

One way ANOVA was used to compare diversity indexes of the three grasslands. Further differences were evaluated with LSD posthoc test, at level 5% of significance according to Sokal and Rolf (1996). Statistical analysis were performed using SPSS rel. 7.5 for Windows (©C SPSS Inc., 1986-96).

### Results and discussion

Shannon – Weiner indexes ( $H'$ ) were increased significantly ( $P < 0.05$ ) along altitude gradient, due to significant increase of species number and species abundance (Table 1). On the contrary equitability was not significant altered along that gradient.

Table 1. Means of diversity index, species richness, species abundance and equitability along altitude gradient in three grasslands.

Diversity parameters	Altitude		
	800- 1000 m	1000 – 1200 m	> 1200 m
Species richness (N)	7a	9a	12b
Species abundance (d)	32.5a	35.2a	43.6b
Shannon – Weiner ( $H'$ )	2.44a	2.72a	3.07b
Equitability (J)	0.81a	0.88a	0.91a

Letters indicate differences at 0.05 significant level using LSD posthoc test

SE for Species richness, Species abundance, Shannon-Weiner and Equitability were 2.04, 4.86, 0.104 and 0.024 respectively.

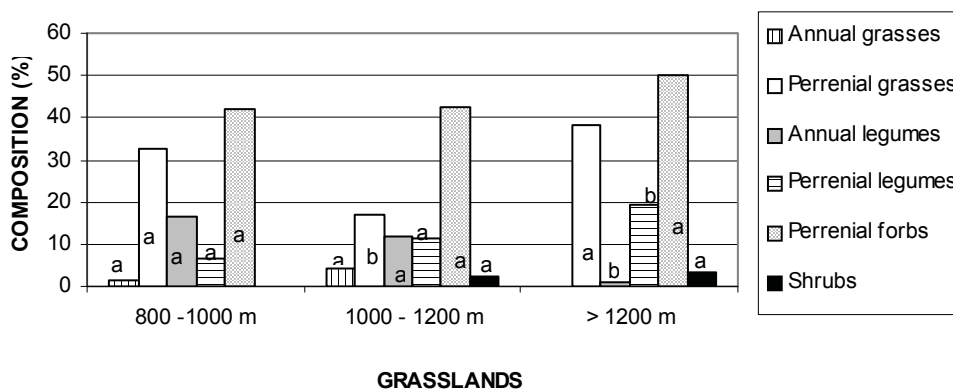


Figure 1. Means species groups composition (%) of grasslands.

According to Koukoura *et al.* (1998) the increase of perennial grasses number could be used as index of the best adapted species to the climate and soil conditions as well as to the grazing and that contribute to the ecosystem stability. So the grasslands with > 1200 m altitude are more stable ecosystems than those of the lower altitudes. The higher participation of the annual grasses and legumes to the vegetation of grasslands with 1000-1200m altitude could be due to the influence of the heavy grazing intensity. According to Noy-Meir *et al.* (1989) annual grasses and legumes participation increase in relation to the grazing intensity.

### Conclusions

The results showed that the plant diversity is related mainly with topography (altitude - inclination). Also the grazing intensity by three animal species in combination with the effects of ecological factors contributed to the increase of diversity from the lower to the higher altitudes.

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# Constraints to grassland production and their impact on livestock husbandry in a semi-arid environment

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## Abstract

Grassland production in semi-arid environments of northern Greece is relatively high but greatly variable due to certain climatic, physical and socioeconomic limitations, thus affecting its spatial and temporal availability to grazing animals. The aim of this study was to investigate the way farmers cope with such constraints to grassland production in order to meet animals' needs. The study was carried out in Lagadas county, northern Greece, while the methodology included the collection of diachronic population and livestock data as well as information concerning livestock production systems in the area. It was found that livestock production, in order to cope with feed gaps created during the critical periods of the year, is based on a complementarity of forage resources including grasslands, artificial pastures and cereal stubble fields. However farmers also extensively utilize complementary feedstuffs, especially concentrates, in order to improve animal performance resulting in semi-intensive production systems based to a large extent on subsidies from the European Union.

Keywords: animal production systems, small ruminants, feedstuffs, Mediterranean grasslands.

## Introduction

Grasslands in Greece are multifunctional areas but their main use is grazing by livestock. Their productivity is relatively high but prone to temporal variability. This variability results in the creation of two feed gaps, a short gap during winter (about two months) and a longer one (about four months) during the summer period (Papanastasis, 1982). Additional physical constraints to grassland production are topography and altitude. Herbage production changes widely from one site to the next, due to differences in soil characteristics, especially soil depth (Papanastasis, 1994). Furthermore, there are considerable differences in herbage production as altitude changes (Papanastasis, 1982; Papanastasis *et al.* 2003).

Over the last decades, however, new constraints have been imposed related to changes in socio-economic conditions. They include reduction of grassland area due to its conversion to arable lands or pine plantations, or to shrublands through shrub encroachment (Chouvardas *et al.*, 2004). The increase in woody plant cover results in a significant reduction of herbage production available to the grazing animals (Platis and Papanastasis, 2003; Zarovali *et al.*, 2004). The aim of this study was to assess the impact that such limitations in grassland production might have on the way livestock husbandry is practiced in a typical semi-arid Mediterranean environment of northern Greece.

## Materials and methods

The study was conducted in Lagadas county, northern Greece (ca. 40° 47' N, 23° 12' E). The climate is semi-arid Mediterranean with cold winters and a long hot and dry summer period of at least four months. Soils are acid and are derived mainly from metamorphic rocks. Grasslands are composed of both annual and perennial species and are communally grazed, mainly by sheep and goats. Diachronic population and livestock data were collected for eight municipal districts and settlements of the county, situated at three different altitude zones, namely low (<200m), middle (200-600m) and high (>600m)



over a period of 40 years (1961-2001). In addition, livestock husbandry systems were investigated during 2002 with the help of questionnaires that were personally addressed to all farmers in the area.

## Results and Discussion

The evolution of human population and livestock over the 40 year-period 1961-2001 is presented in Table 1. Human population decreased after 1961 mainly due to rural emigration, whereas it started increasing again later and mostly during the last decade. Sheep and goats also decreased by almost 31.7% in 40 years, indicating a gradual abandonment of animal husbandry by farmers. The number of goats, however, although reduced in the 60's, started to pick up again in the 70's to reach in 2001 almost the same level as in 1961.

Table 1. Diachronic data of human population and livestock numbers during the period 1961-2001.

Year	Population				Livestock numbers							
	Low zone	Midle zone	High zone	Total	Low zone		Middle zone		High zone		Total	
					Sheep	Goats	Sheep	Goats	Sheep	Goats	Sheep	Goats
1961	1977	1608	3177	6762	7175	2849	7287	7100	6881	7461	21343	17410
1971	1666	1140	2396	5202	5001	2500	5209	5788	5425	3332	15635	11620
1991	1824	1219	2317	5360	3526	3765	4096	3161	5803	8025	13425	14951
2001	1959	1902	2356	6217	2638	4025	3200	4150	6700	8400	9900	16575

The increase of goats in the study area probably reflects the policy of subsidising livestock numbers since 1981, when Greece became a member of the European Union, in combination with the reduction of grasslands in favour of shrublands as a result of socio-economic changes (Chouvardas *et al.*, 2004). It is well known that this kind of animal is well adapted to utilizing the highly fibrous low protein woody resources (Silanikove, 2000). However, the utilization of this resource by goats is usually restricted when shrubs become too high or too dense to be reached or penetrated by the animals. Maybe this is one of the reasons that woody foliage was not the predominant component of goats' diet in the study area throughout the year (less than 42%; Yiakoulaki, unpublished data).

Results obtained from the questionnaires suggest that the majority of the animals stay in the study area throughout the year, whereas only 4.7% of them move out during summer, practicing transhumance. Animals graze in grasslands but also utilize alternative resources, including artificial pastures with barley or wheat grown in private lands that are used in late winter-early spring, as well as cereal stubble fields after crop harvest during summer-early autumn (Table 2).

Table 2. Grazing characteristics and alternative to grassland production resources used by sheep and goats.

Elevation zones	Grazing period	Grazing time (h d <sup>-1</sup> )		Other sources of pasture	Period of feedstuff supply	Farmers using feedstuffs (%)	
Low (<200m)	All year round	Winter	5-6	a. Stubble (June-October)	All year round	Winter	100
		Spring	5-6			Spring	90
		Summer	6.8	b. Artificial pastures (spring)		Summer	70
		Autumn	8			Autumn	75
Middle (200-600m)	February - November	Winter	5-6	a. Stubble (June-October)	All year round	Winter	100
		Spring	5-6			Spring	97
		Summer	11.5	b. Artificial pastures (spring)		Summer	65
		Autumn	7.8			Autumn	66
High (>600m)	April - October	Winter	-	a. Stubble (July-September)	All year round	Winter	100
		Spring	5-6			Spring	98
		Summer	12.3	b. Artificial pastures (spring)		Summer	58
		Autumn	10.1			Autumn	60

The latter provide a satisfactory diet to both sheep and goats (Yiakoulaki and Papanastasis, 2003). On the other hand, farmers extensively utilize complementary feedstuffs for their animals, in order to increase milk production. Feedstuffs, mainly roughage and concentrates are used during the entire period of the year, thus making animal production systems semi-intensive. This means that farmers mainly use the EU subsidies to buy feedstuffs in the market.

### Conclusions

Grassland production in semi-arid environments of northern Greece is constrained by both physical and socio-economic factors. To cope with the feed gaps, livestock production is based not only on grasslands but also on artificial pastures and cereal stubble fields, as well as on the extensive use of concentrates. Concentrates are essential in meeting the animal needs during the critical periods of the year and in increasing milk yields, but they also result in semi-intensive production systems based to a large extent on EU subsidies.

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# Location as a factor determining recent changes in the vegetation of alpine pastures

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## Abstract

Changing socio-economic circumstances is leading to changes in the traditional management of Swiss alpine pastures. To evaluate possible effects on the vegetation, we investigated how the botanical composition of alpine pastures in Grindelwald has changed over the past two decades. To do so, we repeated 44 botanical relevés first recorded in 1980/81. Although there were statistically significant changes in species composition, these explained only minor proportions of the total variance in floristic data, indicating that the initial characteristics of swards had been mostly maintained. However, there was some evidence for directional changes that could be explained by alterations in grazing management. At sites far from cattle sheds, swards of initially intermediate forage quality showed evidence of nutrient enrichment, whereas poor quality swards tended to become encroached by shrub vegetation. These trends suggest a shift in grazing pressure towards more favourable areas. In contrast, initially high forage quality of pastures close to cattle sheds tended to decrease over time, perhaps as a result of a decline in nutrient availability and high disturbance.

Keywords: vegetation change, alpine pastures, shrub encroachment, nutrient indicator, relevés.

## Introduction

The beauty of the alpine landscape is an important attraction for tourists, with high-altitude pastures being a significant element of this landscape. Alpine pastures were created and maintained over centuries by traditional grazing during summer. However, as a result of socio-economic changes, increasing alterations in management can be expected which could negatively affect these diverse yet sensitive agro-ecosystems.

The aim of this study was to evaluate what changes, if any, have occurred in the botanical composition of alpine pastures over the last two decades, and to determine whether they reflect a response to changes in grazing management.

## Study area and methods

Changes in the botanical composition of alpine pastures were studied in Grindelwald (Switzerland) by repeating 44 botanical relevés originally recorded in 25 m<sup>2</sup> plots in 1980/81 (Pfister, 1984). The plots were classified into five vegetation groups (VG's A1 to A5) according to differences in nutrient level, soil acidity and shrub encroachment. The distance "as the crow flies" between each plot and the building where the cattle were housed was also measured. The fraction of total variance in species data explained by temporal changes was analysed by Redundancy Analysis and significance of changes was tested by Monte-Carlo permutation test (ter Braak and Šmilauer, 2002). Mean indicator values for nutrients (Landolt, 1977) and forage quality (Briemle *et al.*, 2002) per relevé were weighted by species' abundance.

## Results and discussion

*Spatial distribution of the vegetation:* There was considerable variation in species composition in 1980/81 which could be partly related to distance from cattle sheds (Table 1). The two vegetation

groups common at more distant sites (i.e. VG's A1 and A2) included species indicating low to intermediate forage quality and nutrient-poor site conditions. The main difference between these two VG's was the much higher cover of dwarf shrubs (e.g. *Vaccinium myrtillus* and *Calluna vulgaris*) in A1 (37.5%) than in A2 (1.2%). In contrast, the species composition of the VG's occurring closer to cattle sheds (VG's A3-A5) indicated high forage quality and more nutrient-rich conditions (Table 2). Mean nutrient indicator values (N-value) ranged between 2.00 and 3.76 and were correlated with distance to a cattle shed ( $r = -0.58$ ,  $p < 0.001$ ) and with inclination ( $r = -0.62$ ,  $p < 0.001$ ). Mean indicator value for forage quality (FQ-value) ranged between 3.00 and 6.14.

Table 1. Characteristics of the five vegetation groups (A1 to A5). Values are means  $\pm$  SD.

Vegetation group	A1	A2	A3	A4	A5
Number of relevés	10	7	10	7	10
Distance to cattle shed (m)	386 $\pm$ 231	468 $\pm$ 261	271 $\pm$ 178	256 $\pm$ 163	99 $\pm$ 57
Altitude (m a.s.l.)	1909 $\pm$ 108	1979 $\pm$ 172	1909 $\pm$ 198	1544 $\pm$ 146	1763 $\pm$ 224
Inclination (%)	54 $\pm$ 15	35 $\pm$ 11	34 $\pm$ 12	49 $\pm$ 19	16 $\pm$ 7
pH (CaCl <sub>2</sub> )	3.70 $\pm$ 0.33	4.0 $\pm$ 0.27	4.27 $\pm$ 0.29	6.07 $\pm$ 0.80	4.78 $\pm$ 0.59
Common species	<i>N. stricta</i>	<i>N. stricta</i>	<i>Poa alpina</i>	<i>A. vulgaris</i>	<i>A. vulgaris</i>
	<i>V. myrtillus</i>	<i>L. helveticus</i>	<i>N. stricta</i>	<i>C. sempervirens</i>	<i>Poa supina</i>
	<i>Ca. vulgaris</i>	<i>Pl. alpina</i>	<i>A. vulgaris</i>	<i>F. rubra</i>	<i>F. pratensis</i>
	<i>V. uliginosum</i>	<i>Pot. erecta</i>	<i>Cr. aurea</i>	<i>T. pratense</i>	<i>R. alpinus</i>
	<i>L. helveticus</i>	<i>F. rubra</i>	<i>Pl. alpina</i>	<i>Cy. cristatus</i>	<i>Poa alpina</i>

A: *Alchemilla*, C: *Carex*, Ca: *Calluna*, Cr: *Crepis*, Cy: *Cynosurus*, F: *Festuca*, L: *Leontodon*, N: *Nardus*, Ph: *Phleum*, Pl: *Plantago*, Pot: *Potentilla*, T: *Trifolium*, V: *Vaccinium*.

*Temporal changes in the vegetation:* Species composition of all VG's changed significantly between the two surveys ( $p < 0.05$ ; Table 2), with these temporal effects accounting for between 4.0% (A1) and 8.8% (A2) of the total variance in the data. These changes were of a similar magnitude to those detected in alpine pastures in another region of the Swiss Alps (Peter *et al.*, 2004).

Species richness increased in all VG's, with means of 2.3 to 8.3 more species per plot and totals of 8 to 27 more species per VG. Shannon equitability also increased in all VG's except A1 where it remained constant.

The character of the changes in species composition depended upon location and also the former condition of the vegetation. At sites far from a cattle shed there was a clear increase in the cover of dwarf shrubs (+13.9%), indicating either reduction or giving up of grazing. This was especially the case at sites where shrubs were already abundant in the initial survey (Table 2) and where initial forage quality (FQ) was poor (A1). In contrast, in swards equally far away from the cattle sheds but with an initially intermediate FQ (A2) there was usually no shrub encroachment and N-value increased by 0.21 units, indicating increased grazing pressure. The appearance of nitrophilous species (e.g. *Alchemilla vulgaris*, *Phleum rhaeticum* and *Ligusticum mutellina*) increased at the expense of nitrophobic species like *Nardus stricta* and *Leontodon helveticus*. This indicates an increasing concentration of grazing in areas with favourable conditions. At sites close to the cattle sheds (VG's A3-A5), there was a general tendency for forage quality to decrease. One likely reason for this change was a decrease in nutrient availability (N-value, Table 2), probably as a result of improved distribution of manure. This could account for the decline in nitrophilous species of high forage quality such as *Poa alpina*, *Poa supina*, *Cynosurus cristatus*, *Crepis aurea*, *Alchemilla vulgaris* and *Carum carvi*. On the other hand, the increase in undesirable species characteristic associated with disturbed conditions (e.g. *Ranunculus*

*acris*, *R. aconitifolius*, *R. repens*, *Rumex obtusifolius* and *Veronica chamaedrys*) could have been due to inappropriate grazing management and/or reduced weeding.

Table 2. Extent of temporal changes of swards (fraction of temporal variance in species data), of the development of diversity indices (species number and Shannon's equitability) and of temporal changes in the sward characteristics (indicator values for nutrients (N) and forage quality (FQ) and proportions of grasses and dwarf shrubs). Values are means of initial relevés and changes over time ( $\Delta$ ). Significance refers to Wilcoxon's signed rank test. (\*)  $p < 0.10$ , \*  $p < 0.05$ , \*\*  $p < 0.01$ , ns = not significant.

Vegetation group	A1		A2		A3		A4		A5	
	initial	$\Delta$	initial	$\Delta$	initial	$\Delta$	initial	$\Delta$	initial	$\Delta$
Expl. var. (%)		4.0 *		8.8 *		5.3 **		5.4 *		4.9 **
# species per VG	119	+12	84	+20	101	+27	133	+20	83	+8
# species per plot	38.7	+5.0 (*)	33.0	+8.1 *	35.8	+8.3 *	59.1	+6.0 ns	25.5	+2.3 ns
Equitability	0.70	-0.02 ns	0.63	+0.10 ns	0.74	+0.07 **	0.74	+0.07 (*)	0.64	+0.12 *
N-value	2.00	-0.09 ns	2.23	+0.21 (*)	3.11	-0.14 ns	2.91	-0.07 ns	3.76	-0.13 ns
FQ-value	3.00	-0.11 ns	4.21	-0.01 ns	5.67	-0.29 (*)	4.99	-0.33 (*)	6.14	-0.57 *
% grasses	35.7	-14.3 **	53.3	+2.6 ns	36.7	-0.3 ns	34.1	+1.6 ns	41.8	-0.2 ns
% dwarf shrubs	37.5	+13.9 *	1.2	+0.8 ns	0.3	+0.4 ns	0.2	+0.9 ns	0.0	0.0 -

## Conclusions

Both the species diversity and the typical characteristics of alpine pastures in Grindelwald have been maintained over the last two decades. However, there have been some changes in the vegetation during this period that reflects alterations in grazing management. At sites distant from cattle sheds grazing has focused increasingly on favourable swards, while closer to cattle sheds forage quality has tended to decline.

## Acknowledgements

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## A decision support tool to design rangeland sustainable grazing systems

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### Abstract

Mountain rangelands of northern Spain are valuable ecological and economical communal resources. Cattle and horse summer grazing have historically shaped these landscapes and continue to be their main revenue. In the last decades temporal and spatial disequilibriums in grazing intensity are becoming serious threats to the ecological sustainability of these rangelands through localised pasture degradation. The high ecological value of these ecosystems and their associated grazing utilisation, are explicitly supported by the Common Agricultural Policy through its agri-environmental schemes. However, vegetation complexity and high number of heterogeneous ungulate herds result in decision making difficulties for rangeland managers and policy makers. A grazing simulation model based on vegetation mapping and spatial and temporal distribution of livestock herds is presented. The model predicts pasture utilisation and livestock performance at patch and livestock herd levels for time steps of 10 days. The model is evaluated for the mountain rangeland of Sejos (Cantabria) under present grazing management. Its results highlight current pasture degradation. Formulation of different grazing scenarios using this model can be a valuable tool both in rangeland management and design of governmental subsidies schemes.

Keywords: simulation model, forage utilisation, pasture degradation, cattle, horses.

### Introduction

European Union grants for livestock production systems are increasingly focused towards the protection and enhancement of the environment. Although traditionally beneficial, current farming systems in the mountainous areas of Northern Spain are nowadays causing several negative environmental externalities. A main spatial and temporal resource used in these systems is the Cordillera Cantabrica summer grazing rangelands: “*puertos*”. These communal areas have been historically grazed by cattle and mares from local farmers. They are characterised by their high ecological value, with most of their land classified as of high conservation interest (EU Habitat Directive 92/43). Changes in livestock management have produced a negative impact in vegetation and landscapes (Busqué *et al.*, 2005).

Management of agri-environmental EU premiums in these areas should focus on the positive effects of grazing in the maintainance and restoration of the ecosystems. This objective should be addressed considering sound scientific knowledge, which in many cases is fragmented, dispersed and thus of difficult access to the manager or policy maker.

The aim of this work is to present a computer based tool to calculate livestock vegetation utilisation and performance in the communal rangelands of the Cordillera Cantabrica.

### Materials and Methods

The model integrates explicit geographic information on the distribution of vegetation and livestock, databases on plant communities forage values and livestock energy requirements, and processes algorithms concerning livestock vegetation selection and energy balances at 10-days time units (Figure 1).

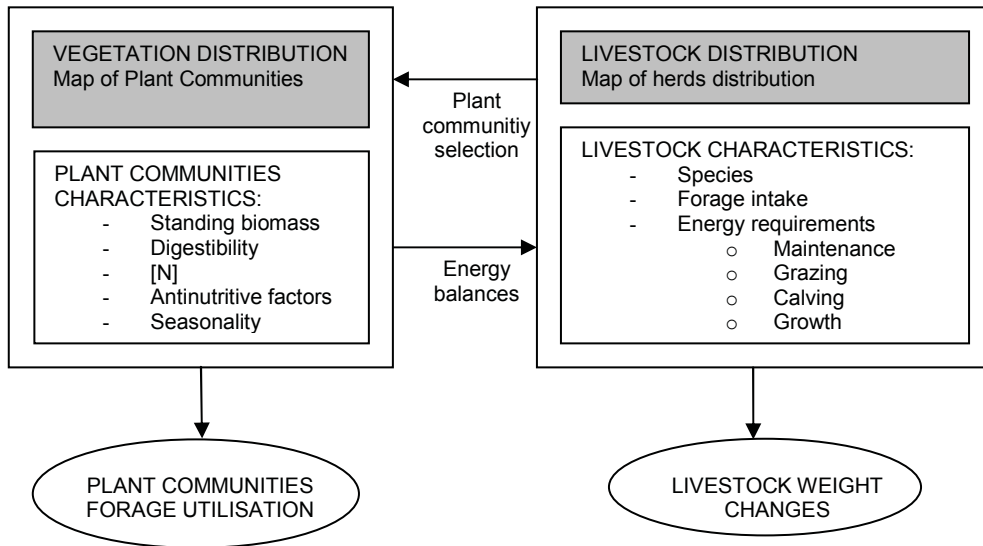


Figure 1. Structure of the 'puertos' grazing model. Grey boxes correspond to the information to be collected. White boxes are the databases of the model. Horizontal arrows refer to the processes simulated.

Each homogeneous patch of the vegetation map is defined by its mean altitude, slope, and the proportion of its area covered by different vegetation communities. Information on spatio-temporal livestock distribution is obtained directly from the farmers, and assigned to pastoral units defined by geomorphological factors.

Each vegetation community is characterised by a mean annual productivity, growth phenology, digestibility, nitrogen concentration and a correction factor for antinutritive compounds. Mean annual productivity is corrected by altitude and slope. Livestock energy requirements are estimated for the different species, breeds and physiological states.

Forage selection of a plant community  $i$ , from a vegetation patch  $f$  by a livestock herd  $h$  in the time unit  $t$  is simulated by three consecutive algorithms:

$$1. \text{ DAYS}_{hft} = \frac{PV_{ift}}{\sum_{if} PV_{ift}} \quad ; \quad 2. \text{ GP}_{ift} = \frac{\sum_h (IMax_{ht} \times \text{DAYS}_{hft})}{\sum_h \text{DAYS}_{hft}} \quad ; \quad 3. \text{ RIC}_{hft} = \frac{\text{DAYS}_{hft} \times PV_{ift}}{\text{GP}_{ift}} \div \sum_{if} \left( \frac{\text{DAYS}_{hft} \times PV_{ift}}{\text{GP}_{ift}} \right)$$

where  $PV_{ift}$  (pastoral value) is the edible digestible nitrogen existing in the  $if$  vegetation unit at the start of the time unit  $t$ ; and  $IMax_{ht}$  is the maximum forage intake of the herd  $h$  in the time unit  $t$  (assumed values of 2.6 kg DM/d/100kg LW for cattle (Oliván *et al.*, 1994) and 3.0 kg DM/d/100kg LW for horses (Fleurance *et al.*, 2001).

Equation 1 assigns, for every herd, a grazing time in each  $if$  vegetation within the defined herd distribution, as if no other herds would exist. Equation 2 quantifies the potential grazing pressure ( $GP$ ) on each  $if$  vegetation unit through the integration of all the existing herds. Equation 3 ( $RIC$ : relative intake coefficient) considers pastoral values and grazing pressures to estimate the definite proportion of forage intake of herd  $h$  in time  $t$  on the  $if$  vegetation unit.

Forage intake of herd  $h$  in time  $t$  is defined as:  $I_{ht} = IMax_{ht} \times C_{GrassOnOffer}$ , where  $C_{GrassOnOffer}$  is a correction index which takes the following values:

Cattle

- $C_{GrassOnOffer} = 1$  if mean grassland biomass  $\geq 1200$  kg DM ha<sup>-1</sup>
- $C_{GrassOnOffer} = (-1,56 + 0,0035 \text{ mean biomass}) / 2.6$  if mean grassland biomass  $< 1200$  kg DM ha<sup>-1</sup>
- $C_{GrassOnOffer} = 0$  if mean grassland biomass  $< 446$  kg DM ha<sup>-1</sup>

Horses

- $C_{GrassOnOffer} = 1$  if mean grassland biomass  $\geq 900$  kg DM ha<sup>-1</sup>
- $C_{GrassOnOffer} = (-1,26 + 0,0047 \text{ mean biomass}) / 3.0$  if mean grassland biomass  $< 900$  kg DM ha<sup>-1</sup>
- $C_{GrassOnOffer} = 0$  if mean grassland biomass  $< 268$  kg DM ha<sup>-1</sup>

## Results and discussion

The model has been applied to the 'puerto' of Sejos (Cantabria), of approximately 2.000 hectares and at a mean altitude of 1.600 m.a.s.l. The 'puerto' was grazed by a maximum of 2.600 cows and 420 mares from mid-june to mid-october in 2003, corresponding to 61 and 29 herds respectively. Nine herbaceous and six shrub pastoral vegetation communities were identified and mapped. Table 1 shows the plant communities forage utilisations as simulated by the model. Values estimated for Festuca-Agrostis rich and poor grasslands agree with data collected in the field (Busqué *et al.*, 2005), and may surpass critical forage utilisation values leading to pasture degradation processes (Rickert, 1996).

Table 1. Proportion of the area covered and simulated forage utilisation<sup>1</sup> of the main pastoral plant communities of the 'Puertos' of Sejos (Cantabria).

Plant community	Proportion of total area	Simulated Forage Utilisation <sup>1</sup>
Poor <i>Festuca-Agrostis</i> grassland	0.28	0.79
<i>Calluna</i> heathland	0.18	0.13
<i>Genista florida</i> shrub (low herbaceous strata)	0.06	0.77
<i>Nardus</i> grassland	0.04	0.73
<i>E. vagans</i> heathland	0.04	0.17
<i>E. arborea</i> heathland (low herbaceous strata)	0.04	0.47
<i>E. arborea</i> heathland (shrub)		0.09
<i>Agrostis curtisii</i> grassland	0.02	0.60
Rich <i>Festuca-Agrostis</i> grassland	0.02	0.89

<sup>1</sup> Forage utilisation: proportion of forage growth that is consumed.

## Conclusions

The use of the 'puertos' simulation model at its initial version is helpful in detecting grazing driven disequilibriums. The implementation of different type of scenarios in the model will provide assistance when planning livestock and conservation management projects.

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## Effect of seed rates of Italian ryegrass in organic production

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### Abstract

The objective of this research was to evaluate the effect of seed rates between 20 and 40 kg ha<sup>-1</sup> of non alternative Italian ryegrass (*Lolium multiflorum* Lam.), on the production and nutritional value, during a period of one year and a half and following the legislation and rules of organic agriculture. The experiment was conducted in rainfed conditions, in a certified organic farm located in Navarra (in the north of Spain). The results of the two year-trial, show no statistical differences between ryegrass seed rates in organic matter production, net energy value or crude protein contents (CP), although the lowest seed rate (20 kg ha<sup>-1</sup>), gave a somewhat lower values. The two-year total average of organic matter yields, net energy and CP were 9,881 kg ha<sup>-1</sup>, 12,743 UFL ha<sup>-1</sup>, and 1,552 kg ha<sup>-1</sup>, respectively.

Key words: organic production, sowing rate, Italian ryegrass non alternative, nutritive value.

### Introduction

The demand for organic products is increasing among the EU consumers, worried about food safety and quality, and also concerned about environment protection. This is the reason why several research projects are being conducted in the Atlantic area of Spain. This projects have the objective of developing crop rotations based on forage in order to optimise the use of these resources by the livestock and recycling the nutrient cycle by fertilising the land. In this trial we present the results of the influence of Italian ryegrass non alternative sowing rate in a field under organic regulations during a period of one year and six months. Italian ryegrass is one of the three proposed seeds (Mangado, 2004) for the Atlantic region of Spain.

### Materials and methods

The trial was established in Oskotz (Navarra), belonging to the Cantabrian-Atlantic biogeographical province, although in the Mediterranean slope. The soil was of clay type, slightly acid and with high levels of organic matter that can oxidize P and K. After applying 60 t ha<sup>-1</sup> of manure increasing seed rates of Italian ryegrass (*Lolium multiflorum* Lam.) *non alternativum* variety CABALLO, with a thousand grain weight of 3.2g were sown in autumn. Seed rates went from 20 to 40 kg ha<sup>-1</sup>, in intervals of 5 kg ha<sup>-1</sup>. In each case 5 kg ha<sup>-1</sup> of red clover (*Trifolium pratense* L.) variety VERDI, with a thousand grain weight of 1.8g, were also sown. The statistical design was a randomized block design with three replications. The elemental plot size was 5 x 1.3 m<sup>2</sup>. In 2004, the plots were harvested 4 times (26/04, 17/06, 11/08, 01/12) and in 2005 only once (26/05). Each time the amount of green forage was weighted and a sample was sent to the laboratory. The quality parameters analysed by the Laboratorio Agrario de Villava were; dry matter, ash, crude protein, crude fibre (CF) and neutral detergent fibre (NDF). The INRA *Prév Alim* (INRA, 1999) method was used to determine forage quality, estimating the digestibility of the organic matter and the energy measured in UFL per kg of dry matter. The parameters of nutritional value calculated for each harvest are the production of digestible organic matter (DOMY), the production of net energy (NEY) expressed in UFL and the production of crude protein (CPY). The results were analysed using the Duncan's test as a multiple comparison system, with the statistics package SPSS 8.0.

## Results and discussion

The nutritional values are presented in Table 1 (production of digestible organic matter), Table 2 (production of net energy) and Table 3 (production of crude protein). There are no significant differences between seed rates in any harvest in the whole period.

**Table 1. Digestible organic matter (kg ha<sup>-1</sup>)**

Seed rate (kg ha <sup>-1</sup> )	Harvest date						
	26/04/04	17/06/04	11/08/04	01/12/04	2004	26/05/05	2004 + 2005
20 RG + 5 RC	3064	1942	619	200	5825	3997	9822
25 RG + 5 RC	3099	2251	672	228	6250	3745	9995
30 RG + 5 RC	3458	2337	745	279	6819	3190	10009
35 RG + 5 RC	3594	2116	618	253	6580	3342	9923
40 RG + 5 RC	3284	2034	607	242	6168	3489	9657
Significance 0.05 %	NS	NS	NS	NS	NS	NS	NS

RG= Italian ryegrass. RC= red clover

**Table 2. Net energy (UFL ha<sup>-1</sup>)**

Seed rate (kg ha <sup>-1</sup> )	Harvest date						
	26/04/04	17/06/04	11/08/04	01/12/04	2004	26/05/05	2004 + 2005
20 RG + 5 RC	4129	2453	789	292	7664	5024	12688
25 RG + 5 RC	4162	2799	855	317	8133	4738	12871
30 RG + 5 RC	4826	2908	943	414	8871	3994	12866
35 RG + 5 RC	4606	2656	789	362	8633	4214	12847
40 RG + 5 RC	4426	2530	772	364	8092	4354	12446
Significance 0.05 %	NS	NS	NS	NS	NS	NS	NS

RG= Italian ryegrass. RC= red clover

**Table 3. Crude protein (kg ha<sup>-1</sup>)**

Sowing dosage (kg ha <sup>-1</sup> )	Harvest date						
	26/04/04	17/06/04	11/08/04	01/12/04	2004	26/05/05	2004 + 2005
20 RG + 5 RC	425	226	108	44	803	756	1559
25 RG + 5 RC	446	260	119	53	878	705	1584
30 RG + 5 RC	503	238	133	66	940	663	1603
35 RG + 5 RC	458	234	106	58	856	705	1561
40 RG + 5 RC	436	218	106	60	821	635	1456
Significance 0.05 %	NS	NS	NS	NS	NS	NS	NS

RG= Italian ryegrass. RC= red clover

In the first year of production there were no significant differences between treatments, but the rate of 30 kg ha<sup>-1</sup> seems to give higher DOMY, NEY, and CPY values.

In the harvest of the second year, the seed rate of 20 kg ha<sup>-1</sup> seems to give higher DOMY values, possibly because of the less ryegrass density in this second year.

The lack of chemical fertilisers (nitrogen mainly) in organic production could also explain that difference in the second year. However, above all, the 25 to 30 kg ha<sup>-1</sup> seed rates seems the most appropriate.

### **Conclusions**

Although no significant differences were observed, seed rates of 25 to 30 kg ha<sup>-1</sup> with 5 kg ha<sup>-1</sup> of clover, seem the most appropriate for the production of forage of Italian ryegrass in organic farming systems.

### **Acknowledgements**

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## Variable application of fertiliser in permanent pastures

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### Abstract

Alto Alentejo is a southern province of Portugal with over 200,000 ha under permanent pasture. This paper describes the major steps required to accomplish the objective of demonstrating new technology for fertiliser application in permanent pasture. A description of the equipment gathering, methodology followed as well as the results of the first year will be presented.

Keywords: permanent pasture, precision farming, fertiliser spreading.

### Introduction

Precision farming is the term given to a method of crop management by which areas of land or crop within a field area managed by adjusting application rates of agricultural inputs according to local needs (Godwing *et al.*, 2003). This study is the first project carried out in Portugal to demonstrate precision agriculture techniques and technology. In the Alto Alentejo region, a high proportion of soils place limitations for farming. A strategy aiming the recuperation of this land over the medium- and long-term, based on the proven aptness of the region for the raising of native breeds of cattle and sheep in an extensive farming regime, will be the establishment and maintenance of permanent pastureland as an integral part of the *montado* ecosystem. Pastureland managers are aware of the variability of productive capacity present even within individual plots of their farmland and will be responsible for taking decisions, based on their experience and technical knowledge, regarding the differentiated application of fertilisers, amendments and seeds. What is not normally available to managers are the means for responding to the pattern of variability occurring on the ground and solutions are uniformly applied to the field without regard for precision management techniques. The advent of precision agriculture technology has produced two advances: the first is the ability to precisely identify and map small-scale variability and, the second is the development of variable rate fertiliser application technology (Gillingham, 2001). The advantage of transferring state-of-the-art technology, traditionally used for cereal crops, to pastureland may be an incentive for extensive livestock farming and the maintenance of *montado* pastureland (cork-tree groves and forage pasture).

### Material and methods

In this study, the following equipment was used for carrying out field work (Fig. 1): a Massey-Ferguson, 6130 Datatronic 2 agricultural tractor (63kW); a FieldStar precision farming system; a Garmin GPS/DGPS 16 receiver, mounted on the tractor; a Vicon-RS-EDW fertiliser spreader with its Ferticontrol standard controller; a four-wheeled buggy; a GPS Trimble 4700, RTK, station with two rovers.

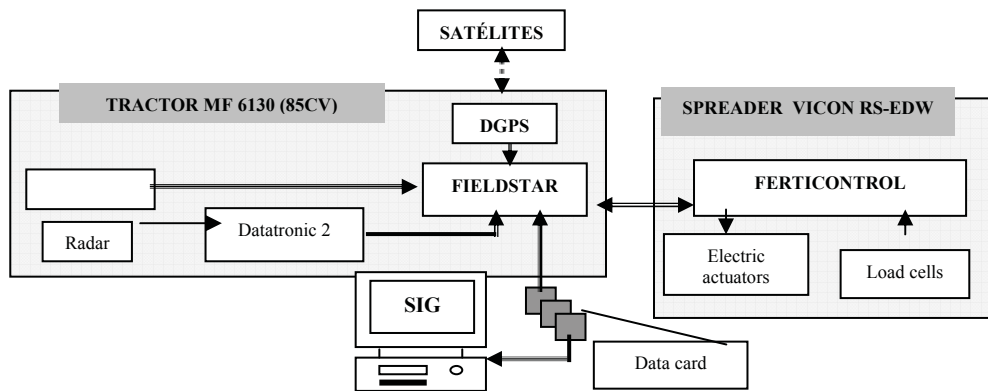


Figure 1. Technology for variable application of fertiliser.

The following five major steps were required to accomplish the objective: a) Fertiliser spreader evaluation. b) GPS evaluation. c) Soil and pasture evaluation. Quantifying the degree of variability inherent in the natural environment is essential for taking decisions to managing a given plot differently (Zwaenepoel and Bars, 1997). The FieldStar package was used to draw the field map, to draw a sampling plan and to guide the operator to the actual sample location. Geo-referenced samples were therefore collected for the analysis of the physical (texture) and chemical composition of the soil (nitrogen, phosphorus, potassium, pH and organic matter). As the assessment of production is indisputably the only method for proving the heterogeneity of plots which takes into account the final production figure for the crop (Berducat and Boffety, 2000), the collection of the pastureland samples was also carried out with a view to the analysis of floristic composition and the evaluation of dry matter. d) Establishment of maps of soil properties and pasture variability. e) Definition of recommendation plan.

The application plan will be transferred to the tractor terminal and spreading will be done accordingly in the autumn of 2004. A new set of analysis to the soil and to the composition of the pasture will be held later in the spring of 2005, as a starting point for a second cycle for fertiliser application planning to that field.

### Results and discussion

All the data layers are analysed in ARCVIEW, geographical information system (GIS). The distribution of soil phosphorus and dry matter on the field are shown in Figure 2 (left and centre). The information on the map of the plot and the densities of fertilisers to be applied in each plot zone was processed by using the FieldStar software on the laptop computer and transferred onto a memory card at the tractor terminal. The maps were presented to agronomists for interpretation and a treatment plan was tailored to suit the pasture. Figure 2 (right) also show the first set of fertiliser recommendations to be applied in each field location in November 2004.

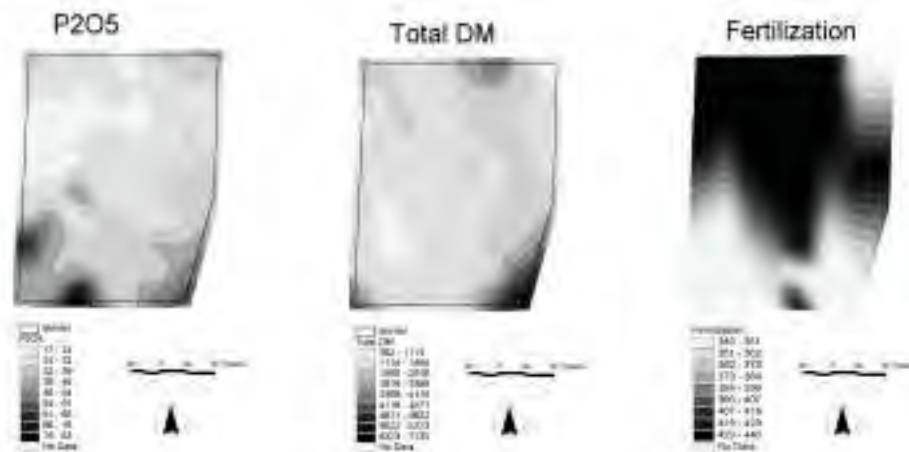


Figure 2. Phosphorus soil distributions map (mg kg<sup>-1</sup>) (left); pasture dry matter map (kg ha<sup>-1</sup>) (centre); fertiliser recommendations map (18% Super phosphate fertiliser, in kg ha<sup>-1</sup>) (right).

### Conclusions

Now that the initial euphoria has died down and the advantages and inputs clearer, the technology of precision farming has become established as a valuable attribute to further development on farms. An important requirement for successful work with the mapping system is the integration of practical farm management experience into the application maps. The results obtained in this paper confirm the advantages of implementing precision farming systems in connection with pastureland management. However, in Portugal, the lack of training of those who market the technology on the ground, who are thus unable to provide solid support for farmers at the different stages of system installation and equipment calibration, constitutes a serious obstacle to the introduction of such systems. At the same time, there is an urgent need for companies providing decision-making support systems and efficient soil- and pastureland-sampling systems in order to ensure the investment required by farmers to introduce such systems and ensure their success is made profitable.

### Acknowledgements

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## Using sewage sludge in pastures without environmental risks

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### Abstract

Sewage sludge (SS) application to agricultural soils can have economic and environmental benefits, but the occurrence of heavy metals in SS may involve some contamination risks. Dry matter yield, copper (Cu) concentration in the biomass and in the surface runoff water (SRW), and some soil indicators were evaluated in three successive years of a field experiment with a sown pasture mixture and three SS application rates ( $L_0 = 0$ ,  $L_1 = 12$ , and  $L_2 = 24 \text{ t ha}^{-1}$ ) as treatments. For the three growing seasons, the SS application increased total dry matter yield, much probably due to the increase in topsoil available phosphorus (P). At the highest SS rate, soil aqua regia extractable Cu (0-10 cm depth) exceeded the maximum Portuguese legislated limit ( $100 \text{ mg kg}^{-1}$ ) in the 1<sup>st</sup> and 2<sup>nd</sup> years, but substantially decreased in the 3<sup>rd</sup> year. Plant biomass Cu concentrations were adequate both for the normal plant growing and for the small ruminant's diet. Also, Cu concentration in SRW was below the regulated emission limit value ( $1.0 \text{ mg L}^{-1}$ ) for the wastewater discharge. The  $L_1$  SS rate would simultaneously preserve pasture, soil, and water quality and would increase biomass production.

Keywords: sewage sludge, copper, pasture, yield, animal nutrition.

### Introduction

The SS application to soils reserved for pastures, still scarcely used in the country, often contributes to improve organic matter and some plant nutrient contents (Smith, 1996) and to reduce the erosion risk, by increasing the soil vegetation cover. Also, SS can replace mineral fertilisers containing nitrogen (N), P or calcium at a much lower price. However, the occurrence in SS of high levels of heavy metals, among other factors, restricts the SS rate to apply and requires their environmental control. Aiming to evaluate the feasibility of the application of a high Cu SS to a poor and sloppy soil of South Portugal, some soil, biomass plant, and SRW indicators were followed in three successive years of a field experiment with a sown pasture mixture cropped for successive exploitations.

### Material and methods

The field experiment was established in October 2001 on a Haplic Luvisol of the Alentejo region. It was a randomised block design, with three SS application rates ( $L_0 = 0$ ,  $L_1 = 12$  and  $L_2 = 24 \text{ t ha}^{-1}$ ) and two replicates of each treatment. Erosion devices ( $4 \text{ m}^2$ ) were installed in each plot ( $48 \text{ m}^2$ ) to evaluate the SRW volumes and the respective Cu concentrations. A biologically treated SS was applied once, in 2001. It had a high aqua regia extractable Cu concentration ( $2042 \text{ mg kg}^{-1}$ ) determined by ISO 11466 (1995). Soil available P and extractable aqua regia Cu concentrations were  $9.0 \text{ mg kg}^{-1}$  and  $11.5 \text{ mg kg}^{-1}$  at 0-10 cm depth, respectively. The sown mixture consisted of italian ryegrass (*Lolium multiflorum* Lam.), cocksfoot (*Dactylis glomerata* L.), five clover species (*Trifolium subterraneum* L., *Trifolium michelianum* Savi., *Trifolium resupinatum* L., *Trifolium vesiculosum* Savi., and *Trifolium incarnatum* L.), serradella (*Ornithopus sativus* Brot.), and biserrula (*Biserrula pelecinus* L.). In each growing season, dry matter yield was evaluated over two exploitations. The Cu concentration in the plant biomass was determined by wet digestion using nitric and perchloric acids (Ulrich *et al.*, 1959). The Cu concentration in foliar extracts and SRW samples was determined by flame atomic absorption. Soil samples were collected on all plots, at 0-10 and 10-20 cm depths, before the SS application and at the

end of each growing season. They were analysed for pH in water, organic matter, total N, extractable Egnér-Riehm P and potassium, and exchangeable cations. Aqua regia extractable Cu was also determined (ISO 11466, 1995). SRW samples were collected in 2002 (7 dates), 2003 (5 dates), and 2004 (2 dates). The data for the soil and plant parameters were statistically analysed using analysis of variance and the L.S.D. test ( $P \leq 0.05$ ).

## Results and discussion

For the three cropping cycles, the SS application significantly increased ( $P \leq 0.05$ ) total dry matter yield (Table 1). In the 1<sup>st</sup> year, the L<sub>2</sub> rate produced a higher dry matter yield increase (close to 3170 kg ha<sup>-1</sup>) than the L<sub>1</sub> rate (about 1360 kg ha<sup>-1</sup>) when compared to the L<sub>0</sub> rate, but the increases were similar at the L<sub>1</sub> and L<sub>2</sub> rates in 2003 and 2004.

Table 1. Total plant biomass yield (kg ha<sup>-1</sup>) for three successive years of the field experiment.

Date	SS rate		
	L <sub>0</sub>	L <sub>1</sub>	L <sub>2</sub>
2002	2713c	4071b	5887a
2003	2790c	4050b	4554b
2004	2717c	4613b	4216b

For each column and row, values followed by the same letter do not differ ( $P \leq 0.05$ ).

Among the selected soil indicators, only extractable P (0-10 cm) increased ( $P \leq 0.001$ ) when the residue was applied, in each sampling date following the SS application. In fact, at the L<sub>1</sub> and L<sub>2</sub> rates, 68 and 136 kg P ha<sup>-1</sup> had been added in 2001 to the superficial layer of this low available P soil. Therefore, the improvement in the biomass production induced by the SS application can be mainly imputed to the increase in available soil P.

The Cu concentration in the plant biomass (Figure 1) significantly increased ( $P \leq 0.05$ ) at the L<sub>1</sub> and L<sub>2</sub> rates (2002) and at the L<sub>1</sub> rate (2003). However, in 2004, the SS positive effect vanished, as there were no significant differences between the SS treatments.

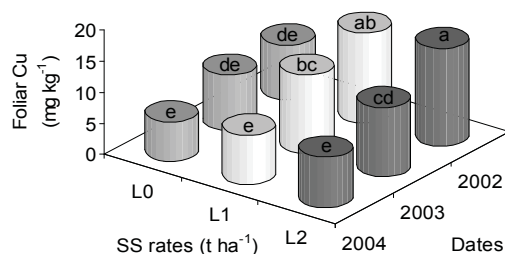


Figure 1. Copper concentration (mg kg<sup>-1</sup>) in the plant biomass evaluated in three successive years (columns with the same letter do not differ significantly at  $P \leq 0.05$ ).

The plant biomass Cu concentrations (6.3 – 15.7 mg kg<sup>-1</sup>) were below the lower Cu threshold (20 mg kg<sup>-1</sup>) of excessive contents in whole plant shoots, suggested by Pendias and Pendias (1992). Moreover, all the Cu concentration values were below the maximum tolerable level (25 mg kg<sup>-1</sup>) of NRC (1985) for the small ruminant's diet. Soil aqua regia extractable Cu increased with the SS



application (Table 2), namely at the highest SS rate and the 0-10 cm depth. This rate raised soil aqua regia extractable Cu concentration to values exceeding the maximum Portuguese permissible level (100 mg kg<sup>-1</sup>) (Portaria 176, 1996) in the first two experimental years (2002 and 2003). However, at the last sampling date (2004), soil Cu concentration (83 mg kg<sup>-1</sup>) was already lower than the legislated value.

Table 2. Soil aqua regia extractable Cu content (mg kg<sup>-1</sup>) at 0-10 cm and 10-20 cm depths.

SS rate	Sampling date							
	2001		2002		2003		2004	
	0-10 cm	10-20 cm	0-10 cm	10-20 cm	0-10 cm	10-20 cm	0-10 cm	10-20 cm
L <sub>0</sub>	10.9f	11.6f	13.8f	12.4f	12.8f	13.2f	11.1f	11.4f
L <sub>1</sub>	13.2f	12.4f	73.2b	19.2ef	65.0bc	37.8de	47.9cd	18.2f
L <sub>2</sub>	10.5f	12.7f	133.5a	25.7ef	133.1a	23.7ef	82.9b	20.3ef

For each column and row, values followed by the same letter do not differ ( $P \leq 0.05$ ).

At all the SS rates, the Cu concentration in the SRW samples, ranging between 0.00 and 0.04 mg L<sup>-1</sup>, was below the regulated emission limit value (1.0 mg L<sup>-1</sup>) for wastewater discharge and for the irrigation water (0.2 mg L<sup>-1</sup>), according to the values established by the Portuguese legislation for water quality (D.L. 236, 1998).

### Conclusions

Besides much higher biomass production, induced likely by higher soil available phosphorus concentration, the L<sub>1</sub> sewage sludge rate (12 t ha<sup>-1</sup>) was not harmful as regard to the copper concentration in the plant biomass, soil, and surface runoff water. Therefore, this rate would be recommended for fertilizer purposes.

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# Effect of grazing in areas of high conservation value in Central Norway

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## Abstract

The semi-natural salt marshes in Norway have probably been used as pastures for cattle, horses and sheep, as long as there have been domestic animals in Norway. Due to regular flooding, the salt marshes are nutrient rich and high production is maintained without fertilizing. Today, the biodiversity of the salt marshes is threatened due to land drainage, cultivation, development, and pollution, or due to cessation of grazing and overgrowth. The results of this study clearly show that the semi-natural vegetation types found in 1974 and in 1985, and the wading bird population found in 1975/1976, are drastically reduced due to cessation of grazing. To re-establish and maintain some of the salt marshes and the biodiversity, restoration and management is necessary

Key words: biodiversity, management, vegetation type, salt marshes.

## Introduction

Norwegian agriculture has created cultural landscapes maintained during centuries by traditional production of food, fodder and goods (Norderhaug *et al.*, 1999). This traditional production is still focused, but at the same time the production of biodiversity, landscape and nature conservation values have become more important (Olsson, 2003). The agricultural improvement during the last decades has caused impoverishment of European cultural landscapes. Large semi-natural habitats have been transformed to cultivated land or been abandoned and reforested (Burel, 1995). In Norway, as well as in many other European countries, semi-natural salt marshes represent high nature conservation values with many important habitats regarding both flora and fauna, especially (wading) birds. The semi-natural salt marshes in Norway have probably been used as pastures for cattle, horses and sheep, as long as there have been domestic animals in Norway (Norderhaug *et al.*, 1999). Due to regular flooding, the salt marshes are nutrient rich and high production is maintained without fertilizing. Today, the biodiversity of the salt marshes is threatened due to land drainage, cultivation, development, and pollution, or due to cessation of grazing and overgrowth. This paper focuses on the change in vegetation types and wading birds diversity due to abandonment.

## Materials and methods

The investigated salt marshes Rinnleiret and Dekkerhus are both situated in the County of Nord-Trøndelag, Central Norway. Grazing at Rinnleiret ceased in 1977 and at Dekkerhus in the 1960ies. Both salt-marshes were characterized as valuable with regard to nature conservation, due to high plant and wading bird diversity in the mid 1980s (Kristiansen, 1988a; Kristiansen, 1988b).

The salt marshes at Rinnleiret were recorded by field investigations and the vegetation types were mapped in 1974 (Eklo, 1980). The vegetation types in the salt marshes at Dekkerhus were recorded by a 1-3 scale (1= rare, 2= common, 3= dominating) by field investigations in 1985 (Kristiansen, 1988b). In the summer of 2003 the vegetation types at Rinnleiret and Dekkerhus were reinvestigated. The plant species were recorded and the vegetation types classified according to Fremstad (1997). The results were mapped by use of GPS and GIS and compared to the records from 1974 at Rinnleiret. The results from Dekkerhus were converted to a 1-3 scale and compared to the results from 1985.

At Rinnleiret the total breeding populations of waders were counted in 1975 and 1976. In 2003, a total survey of the present breeding wader fauna was carried out at Rinnleiret again. In Dekkerhus there are no historical data, but an ornithological survey where all individuals being present at the location were counted in the breeding season in 2003.

## Results and discussion

At Rinnleiret several vegetation types decreased in % cover of the total area from 1974-2003: the lower salt marshes (U4) (from 17.4% to 12.6%), the upper salt marshes (U5) (from 37.2% to 20.6%), the brackish water grassland (U7) (from 21.7% to 2.4%) and the heathlands (W2) (from 17.9% to 11.1%). Forest and bushes have encroached most of these areas from 1974 to 2003: Alnus-forest (C3) (from 6.5% in 1974 to 41.6%) and bushes of sea-buckthorn (*Hippophæ rhamnoides*, Q3) (from 0% to 7.2%). This vegetation changes give dramatically decreases in the breeding population of wading birds at Rinnleiret from 1975/1976 to 2003: *Venellus vanellus* (93% decrease), *Tringa tetanus* (92% decrease), *Hamematopus ostralegus* (89% decrease), *Charadrius hiaticula* (50% decrease), *Gallinago gallinago* (50% decrease) and *Numenius phaeopus* (50% decrease). Some birds were not found in 2003; *Philomachus pugnax*, *Calidris alpina* and *Calidris temmincki*. Two species had no change from 1975/1976 to 2003: *Numenius arquata* and *Tringa nebularia*. For supplementary results see Bele *et al.* (2005).

In 2003, Filipendula grassland (U9a) (from cover 1 in 1985 to cover 3 in 2003) dominated at Dekkerhus and bushes and trees had started to establish. Upper salt marshes (U5) (from cover 3 in 1985 to cover 1 in 2003) and brackish water grassland (U7) (from cover 3 in 1985 to cover 2 in 2003) were frequent and dominated parts of the area in 1985, but had declined in 2003. There were few indications of breeding wading birds at Dekkerhus in 2003, but *Hamematopus ostralegus*, *Charadrius hiaticula*, *Gallinago gallinago* and *Numenius arquata* were observed in the area. For supplementary results see Bele *et al.* (2005).

Upper and lower salt marshes and brackish water grassland have declined in both areas in 2003. These vegetation types are favoured by moderately grazing pressure (Norderhaug *et al.*, 1999), and are quickly replaced by different overgrowing-phases after abandonment. Alnus-forest has expanded at Rinnleiret, and this vegetation type is a typically overgrowing-phase in Central Norway (Frivold, 1998; Rosef, 2004). Also sea-buckthorn (*Hippophæ rhamnoides*) has expanded at Rinnleiret, this species is a typically overgrowing-phase in this area. When *Alnus incana* and *Hippophæ rhamnoides* first establish in an area after cessation of grazing, they are quickly encroaching new areas due to root offshoots. Filipendula grassland is an early overgrowing-phase and also common along the coastline of Norway (Norderhaug *et al.*, 1999), and the first step to Alnus-forest.

Nesting wading birds at Rinnleiret have become rarer, and at Dekkerhus, there were few indications of breeding wader birds. This may be because most of the wader species are known to prefer grazed areas with short vegetation (Johansson *et al.*, 1986), and will accordingly disappear if the areas become too overgrown (Ekstam and Forshed, 2000).

These results clearly show that the semi-natural vegetation types found in 1974 and in 1985, and the wading bird population found in 1975/1976, are drastically reduced due to cessation of grazing. The high nature conservation value due to both high plant and wading bird diversity indicated in mid 1980s are quickly disappearing. To secure the high nature conservation value and to re-establish and maintain the high plant and wading bird diversity, restoration and management is necessary (e.g. Hobbs and Harris, 2001). In order to achieve the desired effect of conservation and restoration, the right types of management have to be chosen based on knowledge of the area, of the effect of different management regimes and of semi-natural ecosystems (Rosef *et al.*, 2004a; Rosef *et al.*, 2004b). Such extensive and specific management is often more time consuming than today's farming practices, and economical compensation must be given to farmers or others to do this.

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## Modelling the nitrogen excretion of dairy cows

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### Abstract

The Austrian data for the N excretion of dairy and suckler cows have been recalculated following the guidelines of the European Commission. It was assumed that the feeding of the dairy cows is mainly practiced considering the actual requirements for energy and protein (GfE, 2001). The relatively low protein content of forage from mountainous meadows and pastures as a consequence of extensive grassland management is finally the main reason for the low N input via feed stuff and therefore the low N excretion level of livestock in Austria.

Keywords: N excretion, crude protein, forage quality, forage intake, milk urea content.

### Introduction

On most of the Austrian grassland and dairy farms both home-grown forage from grassland and farm manure are the main natural nutrient resources. On the other hand the use of external inputs like concentrates and mineral fertilisers is very low compared to intensive production areas in Europe (Taube and Poetsch, 2001). Discussing the nutrient excretion of livestock, these specific circumstances have to be taken into consideration.

### Materials and methods

Principally, the calculation of nitrogen excretion of dairy and suckler cows in Austria follows the guidelines of the European Commission (2002). It was assumed that the feeding of the dairy cows in Austria is mainly practiced according to requirements. This seems justified since there is an extensive advisory service established. As feeding standards, the "Recommendations for the Supply of Energy and Nutrients of Cows and Heifers" of the German Society of Nutrition Physiology were used (GfE, 2001). The DMI has been calculated using the feed intake prediction equation of Gruber *et al.* (2001). Forage quality data from different and representative grassland types in Austria were taken into account for the excretion calculations (Poetsch, 2005).

### Results and discussion

To reach realistic results when modelling in milk production, it is necessary to account for the stage of lactation and the dry period since nutrient requirements and therefore feed intake are changing during lactation and dry period as a consequence of variable nutrient outputs (milk and foetus). As a consequence of these facts, in the present model the calculations were performed for every week of lactation and dry period. The results presented in Table 1 and 2 are therefore means of 52 weeks each. In Figure 1 one example is given to illustrate how feed intake, concentrate level and the respective protein content of the ration are reduced during progress of lactation.

Table 1. Ration composition, feed intake of the cows, energy and protein concentration of the total ration (average between winter and summer feeding period).

Yield per lactation	Forage composition				Feed intake (per day)			Concentration	
	Fresh grass (% DM)	Grass silage (% DM)	Hay (% DM)	Maize silage (% DM)	Forage (kg DMI)	Concentrate (kg DMI)	Total (kg DMI)	NEL content (MJ/kg DM)	CP content (% DM)
3,000 <sup>1,3</sup>	50.0	35.0	15.0	0.0	13.87	0.42	14.29	5.62	11.9
4,000 <sup>1,3</sup>	50.0	35.0	15.0	0.0	14.04	0.92	14.95	5.70	12.0
5,000 <sup>1</sup>	45.0	30.0	15.0	10.0	13.83	1.77	15.60	5.88	12.3
6,000 <sup>1</sup>	43.8	30.0	15.0	11.3	13.77	2.78	16.55	6.03	12.7
7,000 <sup>2</sup>	37.5	35.0	15.0	12.5	14.33	3.34	17.67	6.10	12.9
8,000 <sup>2</sup>	31.3	40.0	15.0	13.8	14.22	4.42	18.64	6.25	13.2
9,000 <sup>2</sup>	25.0	45.0	15.0	15.0	14.13	5.49	19.61	6.39	13.6
10,000 <sup>2</sup>	18.8	50.0	15.0	16.3	14.03	6.54	20.57	6.51	13.9

<sup>1</sup> Milk yield: 3000, 4000, 5000, 6000 kg: Simmental, 700 kg LW, 4.18 % milk fat, 3.44 % milk protein.

<sup>2</sup> Milk yield: 7000, 8000, 9000, 10000 kg: Holstein, 640 kg LW, 4.15 % milk fat, 3.28 % milk protein.

<sup>3</sup> 3000 and 4000 kg milk yield represent suckler and nurse cows, respectively (ZAR, 2003).

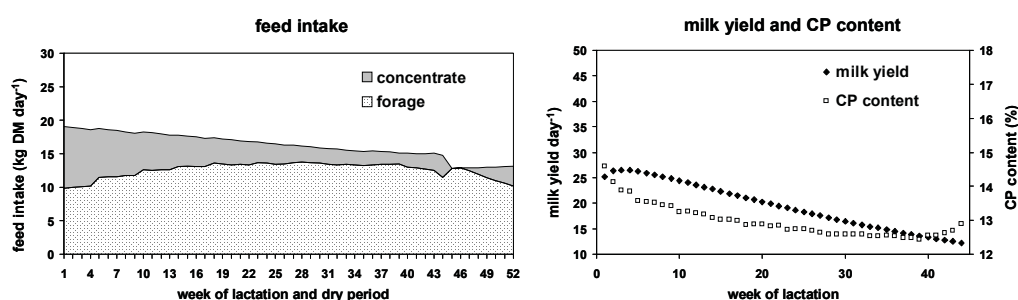


Figure 1. Production data for Simmental cows (6,000 kg milk yield).

Table 2. Calculation of N excretion of the cows (kg per year).

Yield per lactation	DM intake	N diet	N milk	N calf	N weight gain cow	N products	N excretion	N gaseous losses	N manure
3,000 <sup>1,3</sup>	5,216	98.9	16.2	0.9	1.1	18.2	80.8	8.1	72.7
4,000 <sup>1,3</sup>	5,457	104.4	21.5	0.9	1.1	23.5	80.8	8.1	72.7
5,000 <sup>1</sup>	5,694	112.5	26.9	0.9	1.1	28.9	83.6	8.4	75.2
6,000 <sup>1</sup>	6,039	123.1	32.3	0.9	1.1	34.3	88.8	8.9	80.0
7,000 <sup>2</sup>	6,448	133.0	35.9	0.9	1.0	37.8	95.2	9.5	85.7
8,000 <sup>2</sup>	6,804	143.6	41.1	0.9	1.0	43.0	100.7	10.1	90.6
9,000 <sup>2</sup>	7,158	155.7	46.2	0.9	1.0	48.1	107.6	10.8	96.8
10,000 <sup>2</sup>	7,506	167.5	51.3	0.9	1.0	53.2	114.3	11.4	102.8

<sup>1</sup> Milk yield: 3000, 4000, 5000, 6000 kg: Simmental, 700 kg LW, 4.18 % milk fat, 3.44 % milk protein.

<sup>2</sup> Milk yield: 7000, 8000, 9000, 10000 kg: Holstein, 640 kg LW, 4.15 % milk fat, 3.28 % milk protein.

<sup>3</sup> 3000 and 4000 kg milk yield represent suckler and nurse cows, respectively (ZAR, 2003).

Based on these assumptions the mean calculated N excretions of cows per year are presented in Table 2, dependent on milk yield. The N intake with diet increases from 99 to 168 kg per year and the corresponding N output with products (mainly milk) rises from 18 to 53 kg. This results in N excretions of 81 to 114 kg and – when gaseous losses from buildings, manure storage and grazing of 10 % are considered – in values of N in manure of 73 to 103 kg per cow and year. These excretion data are lower than assumed in several EC member states. One main reason is the low protein content of grassland forage ranging from 12 to 14 % due to the relatively extensive grassland management, as shown by the data of Poetsch (2005).

The other very important reason for low N excretion is the low milk yield level of Austrian dairy cows. The mean milk production in Austria is 5,432 kg per cow and year which is much lower compared to the milk production in Scandinavian and Western European countries. As described in Table 1, the protein content and hence the N excretion is considerably low at this level of milk production.

The milk urea content can be used as an indicator of the ruminal N balance and hence protein content of the diet (Kirchgessner *et al.*, 1986; Verite *et al.*, 1995; Steinwider and Gruber, 2000). The value of 20.8 mg milk urea has turned out to correspond to an optimal CP content of the ration, i.e. a ruminal N balance of zero (Steinwider and Gruber, 2000). The statistical evaluation of the official milk recording and breeding organisation in Austria (ZAR, 2004) indicates that the average milk urea content is around 20 – 22 mg/100 ml in the relevant milk yield classes (3,000 – 7,000 kg milk).

## Conclusions

Up to now the N excretion of dairy cows in Austria was calculated on the basis of 4,500 kg milk per year, which is about 1,000 kg lower than the actual average amount. In future different production levels, ranging from 3,000 kg (suckler cows) to 10,000 kg milk per cow and year, will be taken into account. This will strongly improve an environmental friendly use of farm manure, following the rules of good agricultural practice. Aiming at the reduction of N excretion to avoid negative impact on the environment, the compliance of actual energy and protein requirements has to be seen as a key point.

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## Forage production in sown meadows under several organic fertilization strategies

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### Abstract

The effects of three organic fertilization strategies (based either in cattle slurry, dairy sludge or dried broiler litter application) on meadows sown with ryegrass and white clover have been studied in comparison with a mineral fertilization management for three years. Fertilization with dairy sludge and especially with broiler litter resulted in increased forage production respect to other fertilizing strategies in the last two growing seasons, suggesting a residual effect of both organic residues on soil fertility. The presence of clover in forage was generally favoured by the slurry and broiler litter treatments, although it was greatly influenced by temperature and precipitation, respect to the mineral fertilization.

Keywords: cattle slurry, dairy sludge, dried broiler litter, organic residues.

### Introduction

Protection of environment involves the development of forage management systems that decrease the negative ecological impacts of intensive agriculture. Organic farming systems based on the recycling of organic animal and agroindustrial residues as fertilizers can be a sustainable alternative to conventionally managed production systems. In the present work, the effects of three organic fertilization strategies (based either in cattle slurry, dairy sludge or dried pelletized broiler litter application) on sown meadows have been studied in comparison with a mineral fertilization management for three years. The dairy sludge was an effluent of milk residues and cleaning waters from a dairy processing and packaging plant that had underwent a biological treatment. Broiler litter was obtained from broiler faeces and rice hull as bedding material that had been dried and pelletized. Dairy sludge has significant amounts of organic matter content and mineral nutrients (mainly N and P), except for low K concentration. Dried broiler litter is also rich in organic matter and provides N, P and K. The C/N ratio of both residues is low, indicating that they are easily mineralizable.

### Material and methods

A field study was carried out at Goiriz-Lugo, NW Spain. The soil was a humic Umbrisol, which maintained a natural vegetation of *Pinus pinaster*, *Betula* sp., *Castanea sativa*, *Ulex* sp. and *Pteridium aquilinum*. The soil was tilled and limed with 3 t ha<sup>-1</sup> of CaCO<sub>3</sub> (60% OCa). Then plots of 3 x 1.3 m<sup>2</sup> were established to randomly apply five fertilizing treatments before seeding (four plots per treatment): *control*, which received a low annual dose of a NPK mineral fertilizer in order to ease establishment and competitiveness of seeded species against natural vegetation; *mineral NPK*; *cattle slurry*; *dairy sludge*; *dried pelletized broiler litter (BIOF)*. Main characteristics of cattle slurry, dairy sludge and BIOF are shown in Table 1.

A seed mixture of *Lolium perenne* L. cv Tove, *Lolium hybridum* Hausskn. cv Texy and *Trifolium repens* L. cv. Huia was sown in autumn 2001. Dates and doses of fertilizers in each treatment during the first three production years are shown in Table 2.



Table 1. Main characteristics of cattle slurry, dairy sludge and broiler litter (BIOF).

	Dry weight (g l <sup>-1</sup> )	pH	E.C. (dS m <sup>-1</sup> )	C (%)	N (%)	P (%)	K (%)	Na (%)	Ca (%)	Mg (%)
Cattle slurry	18.2	7.1	4.0	40.0	5.1	2.0	9.6	2.4	0.8	0.7
Dairy sludge	20.0	7.1	3.4	35.6	6.2	2.1	1.1	3.2	2.2	0.4
Broiler litter	89.1*	7.9	11.1	36.8	4.0	1.6	2.8	1.6	1.9	0.7

\* % dry matter.

Table 2. Dates and fertilization treatments.

Treatment	Application dates	Doses of fertilizers
Control	Oct-01	300 kg ha <sup>-1</sup> NPK 5-15-13
	Mar-03	30 kg N ha <sup>-1</sup> , 45 kg P <sub>2</sub> O <sub>5</sub> ha <sup>-1</sup>
	May-04	30 kg N ha <sup>-1</sup> , 45 kg P <sub>2</sub> O <sub>5</sub> ha <sup>-1</sup>
Mineral	Oct-01	600 kg/ha NPK 5-15-13
	Mar-02, May-02	60 kg N ha <sup>-1</sup>
	Mar-03	60 kg N ha <sup>-1</sup> , 90 kg P <sub>2</sub> O <sub>5</sub> ha <sup>-1</sup>
	May-03	30 kg N ha <sup>-1</sup>
	Mar-04, May-04	60 kg N ha <sup>-1</sup> , 90 kg P <sub>2</sub> O <sub>5</sub> ha <sup>-1</sup> 30 kg N ha <sup>-1</sup>
Cattle slurry	Oct-01	20 m <sup>3</sup> ha <sup>-1</sup> , 78 kg P <sub>2</sub> O <sub>5</sub> ha <sup>-1</sup>
	Mar-02	20 m <sup>3</sup> ha <sup>-1</sup>
	Mar-03	40 m <sup>3</sup> ha <sup>-1</sup> , 65 kg P <sub>2</sub> O <sub>5</sub> ha <sup>-1</sup>
	May-03	10 m <sup>3</sup> ha <sup>-1</sup>
	Mar-04	40 m <sup>3</sup> ha <sup>-1</sup>
Dairy sludge	Oct-01	80 m <sup>3</sup> ha <sup>-1</sup> , 62 kg K <sub>2</sub> O ha <sup>-1</sup>
	Mar-02	40 m <sup>3</sup> ha <sup>-1</sup>
	Mar-03	80 m <sup>3</sup> ha <sup>-1</sup> , 50 kg P <sub>2</sub> O <sub>5</sub> ha <sup>-1</sup>
	May-03	40 m <sup>3</sup> ha <sup>-1</sup>
	Mar-04	80 m <sup>3</sup> ha <sup>-1</sup>
Broiler litter	Oct-01	600 kg ha <sup>-1</sup> NPK 5-15-13
	Mar-02, Mar-03, Mar-04	4500 kg ha <sup>-1</sup>

A uniform, low-trajectory application of the sludge or the cattle slurry was achieved by using a tank which was tractor-drawn. Mineral fertilizers and pellets of broiler litter were surface-applied. Only one annual application of dairy sludge (supplemented with K due to its low content of this element) was done, as advised by López-Mosquera *et al.* (2001). Similarly, only one annual dose of BIOF was applied, the dose being chosen considering that about 60 %N of this organic fertilizer would be available for forage uptake during the growing season.

Environmental conditions allowed a silage cut in may and a simulated grazing cut in july in the three years of study. However, but in the first year, no forage production was obtained in autumn due to scarce monthly precipitation and high mean temperature up to september.

Forage was mown in each plot to a height of 5 cm above ground level. Forage samples of 500-1000 g were taken for dry matter determination and to assess botanical composition.

## Results and discussion

Organic treatments were always as effective or more than the mineral treatment to enhance forage dry matter (DM) yield respect to the control (Table 3). In the last two years, fertilization with dairy sludge and especially with broiler litter resulted in increased annual DM production respect to other fertilizing strategies, suggesting a residual effect of both organic residues on soil fertility. As shown by López-Mosquera *et al.* (2001) and Matos-Moreira *et al.* (2005), the improved forage yield in the dairy sludge plots was most probably due to N and P supplied by the residue, but also to the water it provided, since

a period of drought was observed from July to September in both years. With time, the application of dried broiler litter resulted in higher soil availability of P and K respect to the control (Matos-Moreira *et al.*, 2005), which probably accounted for the effects on forage production.

Table 3. Forage DM yield responses to mineral, slurry, dairy sludge and BIOF treatments in 2002, 2003 and 2004 growing seasons. For each harvest date and the annual total, values followed by different letter are significantly different at 0.05 % level.

	Forage DM yield (t ha <sup>-1</sup> )									
	May-02	Jul-02	Nov-02	Total 2002	May-03	Jul-03	Total 2003	May-04	Jul-04	Total 2004
Control	3.0 a	1.6 a	1.7 a	6.3 a	3.6 a	1.4 a	5.0 a	2.2 a	1.1 a	3.3 a
Mineral	7.7 c	2.1 a	1.3 a	11.1 c	5.4 b	2.1 b	7.5 b	3.8 b	2.2 b	6.0 b
Cattle slurry	5.9 b	3.4 b	1.6 a	10.9 c	5.2 b	2.0 b	7.2 b	3.8 b	1.7 b	5.5 b
Dairy sludge	3.4 a	3.6 b	1.8 a	8.8 b	5.3 b	3.2 c	8.5 bc	5.6 c	2.1 b	7.7 c
Broiler litter	6.9 bc	1.6 a	2.5 b	11.0 c	6.4 c	3.0 c	9.4 c	5.0 c	2.8 c	7.8 c
Significance	*	*	*	*	*	*	*	*	*	*

As expected, in silage cuts, forage was mainly ryegrass (data not shown). In the first growing season, climatic conditions led to a very high proportion of clover in summer forage (more than 40%), but in the two following seasons the presence of white clover in forage was progressively decreasing in favour of grass in all treatments. Although greatly influenced by temperature and precipitation, respect to the mineral fertilization plots, the presence of clover in forage was generally favoured by the slurry and broiler litter treatments.

### Conclusions

The use of cattle slurry, dairy sludge and dried broiler litter as fertilizers is a good alternative to the conventional mineral fertilization to achieve good forage DM yield in sown meadows. Results of forage production in plots fertilized with dairy sludge and broiler litter suggest a residual effect of both organic residues on soil fertility.

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## Metal concentration in soil and forage in meadows fertilized with dairy sludge

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### Abstract

Dairy industry sludges can be applied as fertilizer to agricultural land. The fertilizer use of these wastes is governed by Directive 86/278/EEC, which establishes limits of metal concentrations in the sludge and the receiving soil. Although dairy sludges have low metal content, the possibility of metal accumulation in soil with regular use of these residues can not be discarded. A field trial was performed to study soil and plant metal levels in a ryegrass-white clover pasture under three fertilizing treatments (control, mineral fertilization, and application of dairy sludge) over a two-year period. Each year, metal concentrations in soil and ryegrass samples from a silage cut made in spring were analyzed. Results showed that application of dairy sludge did not increase either soil or plant concentrations of Cd, Cr, Cu, Ni, Pb or Zn respect to the control in the short term, what can be explained by the low metal content of this agroindustrial residue.

Keywords: mineral fertilization, organic residues, ryegrass

### Introduction

Dairy industries generate large quantities of wastewater which, if directly discharged to watercourses, would cause severe pollution, in view of their high biological oxygen demand. The application of physical, chemical, and biological treatments yields a characteristic sludge with properties different from sewage sludge (López-Mosquera *et al.*, 2005). Current European legislation does not deal specifically with the fertilizer use of wastes of this type. Such use is governed by Directive 86/278/EEC relating to the fertilizer use of sewage sludge (European Communities, 1986). The principal determinant of acceptability is metal concentration in the sludge and the receiving soil. The legislation specifies acceptable metal concentrations for different soil pH values, with lower concentrations for more acidic soils. Dairy sludges have low metal contents (López-Mosquera *et al.*, 2005). However, we cannot rule out the possibility that regular application might lead to metal accumulation in soil and the trophic chain. In this work, soil and plant metal levels were monitored in a ryegrass/white clover pasture that was fertilized with dairy sludge over a two-year period.

### Material and methods

A field trial was carried out in Goiriz (Vilalba, NW Spain) over a 2-year period. The study plot had natural vegetation, dominated by english oak and gorse species. The soil was a humic Umbrisol with the following characteristics: texture sandy loam, pH 5.0, organic matter content 25.5%, Olsen-P 15.4 mg kg<sup>-1</sup>, and effective exchange capacity 8.4 cmol(+) kg<sup>-1</sup>. After tillage (October 1998), the plot received 3 t ha<sup>-1</sup> of dolomite (CaMg(CO<sub>3</sub>)<sub>2</sub>). One month later, 12 subplots (1.3 x 3.0 m) were marked out, separated by 1.65 m walkways. The following treatments were then randomly assigned to these subplots: T0, an unfertilized control; T1, fertilization with 500 kg ha<sup>-1</sup> of 8:24:16 NPK; T2, fertilization with 80 m<sup>3</sup> ha<sup>-1</sup> of dairy sludge (with total contents of 200 kg N ha<sup>-1</sup>, 160 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> and 26 kg K<sub>2</sub>O ha<sup>-1</sup>), supplemented with 435 kg ha<sup>-1</sup> of 18% superphosphate and 120 kg ha<sup>-1</sup> of 50% potassium sulfate. Metal contents in the dairy sludge are shown in Table 1. No attempt was made to equalize nutrient levels in the different treatments, as the aim was to compare organic and mineral fertilization.

Table 1. Metal contents (mg kg<sup>-1</sup>) of the dairy sludge applied over the study period.

	Metal content (mg kg <sup>-1</sup> )		EU limit <sup>1</sup> (mg kg <sup>-1</sup> )
	Nov 1998	Mar 2000	
Cd	0.6	0.9	20
Cr	8.2	14.3	1000
Cu	19.2	69.7	1000
Ni	16.3	12.5	300
Pb	12.5	18.8	750
Zn	234.0	513.7	2500

<sup>1</sup>Directive 86/278/EEC (European Communities, 1986)

Subplots were seeded with a mixture of 20 kg ha<sup>-1</sup> of perennial ryegrass (*Lolium perenne* L. var. Nui), 10 kg ha<sup>-1</sup> of hybrid ryegrass (*Lolium hybridum* Hausknn. var. Balto) and 3 kg ha<sup>-1</sup> of white clover (*Trifolium repens* L. var. Huia). In the next two growing seasons, a silage cut was made in May and one or two further cuts in the summer-autumn period. Forage was mown in each plot to a height of 5 cm above ground level. To favour grass growth, the mineral fertilization treatment received fractionated doses of N as ammonium calcium nitrate. In March of the second production year, 180 kg ha<sup>-1</sup> of 33% ammonium nitrate were applied in the mineral treatment, whereas the dairy sludge subplots received another 80 m<sup>3</sup> ha<sup>-1</sup> dose.

For the metal study, 500-g samples from the silage cut made in spring were taken, since this was the first cut after fertilization, and the largest cut in terms of dry matter throughout the year. The 1999 silage cut was more than 90% ryegrass, so the metal analyses were performed with ryegrass in both years. One g of dried milled ryegrass was digested in 65% nitric acid in Teflon bombs in a microwave oven. The concentrations of Cd, Cr, Cu, Ni, Pb and Zn were then determined by atomic absorption spectrometry (AAS). Coinciding with the silage cuts, soil samples, each 15 cm deep, were taken in each subplot to determine total Cd, Cr, Cu, Ni, Pb and Zn by AAS.

## Results and discussion

Table 2 shows total metal contents in soil at the spring silage cuts of 1999 and 2000. Cd levels were in all cases below the detection limit (0.02 mg kg<sup>-1</sup>). In both years, Cr, Cu, Ni, Pb and Zn levels did not show any significant changes with respect to control after application of either dairy sludge or chemical fertilizers. These results show that the addition of dairy sludge to soil does not lead to significant accumulation of metals in the short term.

Table 2. Metal content in soil (mg kg<sup>-1</sup>) in each treatment over the study period.

Treatments	Cr (mg kg <sup>-1</sup> )		Cu (mg kg <sup>-1</sup> )		Ni (mg kg <sup>-1</sup> )		Pb (mg kg <sup>-1</sup> )		Zn (mg kg <sup>-1</sup> )	
	1999	2000	1999	2000	1999	2000	1999	2000	1999	2000
Control	42.6	38.2	20.2	17.4	20.2	19.7	22.3	17.6	45.5	37.3
Mineral	34.8	53.6	21.2	16.0	20.2	20.5	20.5	17.2	37.0	35.9
Dairy sludge	40.2	47.1	20.7	19.2	20.4	22.7	22.0	18.0	39.2	39.1
Significance	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

Both the dairy sludge and the mineral fertilizer significantly enhanced forage yield (Table 3). In the first year of forage production, the silage cut was 8.8 t DM ha<sup>-1</sup> in the plots receiving dairy sludge, 4.7 t ha<sup>-1</sup> in the plots receiving mineral fertilizer, and 0.1 t ha<sup>-1</sup> in the control plots. In the second year (with poor climatic conditions for forage production), the silage cut was 2.7 t ha<sup>-1</sup> in dairy sludge plots, 0.9 t ha<sup>-1</sup> in the mineral plots, and 0.2 t ha<sup>-1</sup> in the control plots. Metal levels in all ryegrass samples were below the critical levels for plant development defined by Kabata-Pendias and Pendias (1992).

Table 3. Metal concentrations in forage (mg kg<sup>-1</sup> dry matter) in each treatment over the study period.

Treatments	Cd (mg kg <sup>-1</sup> )		Cr (mg kg <sup>-1</sup> )		Cu (mg kg <sup>-1</sup> )		Ni (mg kg <sup>-1</sup> )		Pb (mg kg <sup>-1</sup> )		Zn (mg kg <sup>-1</sup> )	
	1999	2000	1999	2000	1999	2000	1999	2000	1999	2000	1999	2000
Control	1.1	0,8	23.9	22.1	13.4	5.9	13.5	11.7	33.7	15.1	23.0	26.2
Mineral	1.2	1.0	24.1	23.8	13.3	8.8	13.8	14.7	32.5	15.8	21.9	41.1
Dairy sludge	1.0	0.8	24.9	22.3	13.4	7.0	13.7	16.1	31.8	18.9	23.4	29.8
Significance	NS	NS	NS	NS	NS	*	NS	*	NS	*	NS	*

\* Significant at 0.05 % level.

No significant between-treatment differences in metal content in samples of the first-year silage cut were observed; however in samples of the second-year silage cut, Cu, Zn and Ni contents were all slightly higher in the mineral- and sludge-fertilized plots than in the control plot. Cu and Zn contents were higher in the mineral-fertilized plots than in the sludge-fertilized plots. In any case, all metal contents are similar to those reported by other authors for ryegrass cultivated under diverse conditions of soil and climate and with diverse types of fertilizer (Antoniadis and Alloway, 2001; Kiss *et al.*, 2002).

### Conclusions

Application of dairy sludge, at a dose of 80 m<sup>3</sup> ha<sup>-1</sup>, at the beginning of the growing season, did not increase either soil or plant concentrations of Cd, Cr, Cu, Ni, Pb or Zn respect to the control in the short term, what can be explained by the low metal content of this agroindustrial residue.

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## Forage heavy metal concentrations during the withholding period in sown meadows fertilized with dairy sludge

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### Abstract

In NW Spain, an agro-industrial sludge produced by a dairy plant has been regularly applied to sown meadows as fertilizer over the last 15 years. To protect soil and plants from metals, current European legislation indicates that sewage sludges can be used as fertilizers in pastures and forage crops, provided that a grazing/harvesting withholding period of three weeks after application is observed. In the present work, metal content in forage was monitored weekly for up to four weeks in sown meadows after application of four fertilizer treatments: unfertilized control; 675 kg ha<sup>-1</sup> of a 15:15:15 NPK fertilizer; 160 m<sup>3</sup> ha<sup>-1</sup> of dairy sludge; and 160 m<sup>3</sup> ha<sup>-1</sup> of dairy sludge supplemented with 140 kg ha<sup>-1</sup> of K<sub>2</sub>SO<sub>4</sub>. In all treatments, metal content in forage did not reach toxic levels for animals. However, concentrations of Cu, Pb, and Zn were higher in dairy sludge than in the control and mineral-fertilized plots. To prevent any risk of accumulation of metals in animal tissues compared with sown meadows with mineral fertilizers, it is suggested that forage in dairy sludge fertilized plots should not be grazed unless leaves have been washed free of residues by rainfall.

Keywords: animal health, environmental protection, grazing, mineral fertilization, organic residues.

### Introduction

Recycling of sewage sludges through land application provides the soil with organic matter and nutrients, but also may add heavy metals. In forage systems, surface-applied sludges may adhere to herbage, what may elevate the risk of direct uptake of potentially toxic metals by grazing animals (Hillman *et al.*, 2003). In NW Spain, an agro-industrial sludge produced by a dairy plant has been regularly applied to sown meadows as fertilizer in the last 15 years. The initial waste product is an effluent of milk, water, sodium hydroxide, and nitric acid, that undergoes a biological treatment that converts it into a semi-liquid sludge, and has low metal content (López-Mosquera *et al.*, 2005). There are no specific rules controlling the disposal of agro-industrial sludge in the European Union. By default, the use of these sludges as fertilizers is governed by Directive 86/278/EEC (European Communities, 1986), which was originally directed at sewage sludges. This Directive states that sewage sludges can be used as fertilizers in pastures and forage crops if their application does not increase metal concentration in soil above certain limits, and provided a grazing/harvesting withholding period of three weeks after application is observed. The CAP reform notes that farmers receiving agricultural support have important responsibilities towards the protection of the environment, and animal health and welfare. Therefore those using sludges in their farms will have to meet EU legislation. The aim of the present work was to study the effect of dairy sludge application on forage metal content in sown meadows during the withholding period.

### Material and methods

A field study was carried out at Goiriz-Lugo, NW Spain. The soil was a humic Umbrisol, which maintained a meadow sown with *Lolium perenne* L. cv Barbestra and *Trifolium repens* L. cv Huia, and had been fertilized with NPK and N mineral fertilizers for two years. Forage management consisted of a silage cut in spring and rotational grazing of beef cattle in summer and autumn. At the beginning of the third growing season, sixteen plots of 400 m<sup>2</sup> were established in order to apply randomly four fertilizer treatments: an unfertilized control; 160 m<sup>3</sup> ha<sup>-1</sup> of dairy sludge; 160 m<sup>3</sup> ha<sup>-1</sup> of dairy sludge +

140 kg ha<sup>-1</sup> of K<sub>2</sub>SO<sub>4</sub>; and a mineral treatment consisting of 675 kg ha<sup>-1</sup> of a 15:15:15 NPK fertilizer. The sludge provided the following amounts of metals (in g ha<sup>-1</sup>): Cd 2.0, Cr 0.1, Cu 56.6, Ni 37.7, Pb 37.7, Zn 880.6.

For a one month period, and at seven day intervals, after treatment application (which was done on March 15<sup>th</sup>), four 33 x 33 cm<sup>2</sup> quadrats were randomly harvested from each plot. Forage in each quadrat consisted mainly of ryegrass, and was cut with electric shears to a height of 5 cm, then dried at 60 °C and digested in 65% nitric acid in Teflon bombs in a microwave oven. The concentrations of Cd, Cr, Cu, Ni, Pb and Zn were then determined by flame atomic absorption spectrometry.

### Results and discussion

Concentrations of Cd, Cr, Cu, Ni, Pb and Zn in forage during the first four weeks after treatment application are shown in Figure 1. In all forage samplings, as expected from sludge metal content, Cd and Cr concentrations were similar in control, mineral and dairy sludge fertilized plots. However, the concentrations of Cu, Pb and Zn were significantly higher ( $P < 0.05$ ) when dairy sludge, amended or not with K, was used, compared with the mineral and control treatments. Ni concentration was higher in forage of dairy sludge plots than in mineral and control plots for the first two weeks, but these differences disappeared by the third week. Concentrations of all metals in any treatment were much lower than those considered to present risk for animal health and the trophic chain (Kabata-Pendias and Pendias, 1992). However results showed that Cu, Pb and Zn from dairy sludge remained on leaf surfaces for longer than the mandatory 3-week no-grazing/harvesting period. This can be explained by the lack of precipitation during the four weeks of the study, and also by different initial adherence of metals to herbage and interaction with grass growth rate. Aitken (1997) found that, when applying liquid sewage sludge on a grass sward, the hazard to livestock (in terms of metal adhesion) remained until extensive regrowth or rainfall occurred, especially if sludge solids had dried on grass leaves even for only one day. Different adhesion indices have been found for metals after sewage sludge application. As recently reviewed by Hillman *et al.* (2003), in general Cu and Zn are retained on plant lamina at elevated concentrations for 21 d after application of sewage sludge, which agrees with our results, but state that there is a degree of uncertainty regarding Cd, Ni and Pb. Forage was cut for silage in June, and no differences in yield and metal concentration were found between mineral and dairy sludge treatments (data not shown), suggesting that the additional Cu, Pb and Zn contents in dairy sludge plots during the withholding period was related to temporal adhesion of metals to herbage and not to crop uptake. Despite dairy sludges having low metal content, further research is needed to know the fate of adhered metals to forage after sludge application, in relation to grass growth rate, and the amount and distribution of rainfall.

### Conclusions

The application of dairy sludge as fertilizer in sown meadows did not increase forage metal concentration above levels considered to present risk for animal health, but led to higher Cu, Pb and Zn concentrations than in control and mineral-fertilized plots for longer than the mandatory 3-week no grazing/harvesting period. To prevent any risk of accumulation of metals in animals compared with sown meadows grown with mineral fertilizers, forage in dairy sludge fertilized plots should not be grazed unless rainfall had completely washed plant leaves.

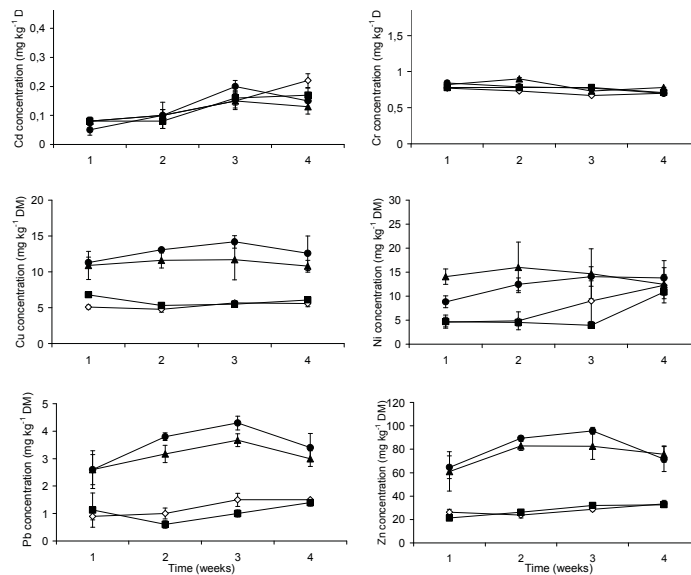


Figure 1. Forage Cd, Cr, Cu, Ni, Pb and Zn concentrations in control (◇), mineral (■), dairy sludge (▲) and dairy sludge + K (●) plots. Error bars represent standard errors.

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# Effect of *Festuca rubra* cultivars and nitrogen fertilisation on selected mycotoxins in winter pasture

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## Abstract

The importance of winter pastures in beef production in Poland has been growing steadily. In the western part of our country there are farms which are utilising pasture sward during the late autumn and winter. The major problem, however, is the quality of forage ingested by animals. During 2002-2004, an experiment was carried out on extensive pasture for the purpose of investigating the influence of *Festuca rubra* L. cultivars (Areta, Atra, Barma, Kos, Reda) and nitrogen fertilisation (0 and 60 kg ha<sup>-1</sup> per year) on the concentration of ochratoxin A and zearalenone in the sward. Forage from each plot was harvested in January and analysed for the concentration of mycotoxins using Vicam tests kits and HPLC. The highest concentration of ochratoxin A was 1.95 ng g<sup>-1</sup> DM in sward dominated by the Barma cultivar and zearalenone – 43.78 ng g<sup>-1</sup> DM in sward dominated by the Atra cultivar. The fertilisation of extensive pasture sward dominated by *Festuca rubra* with 60 kg ha<sup>-1</sup> of nitrogen during the vegetative season decreased the accumulation of ochratoxin A and increased the accumulation of zearalenone.

Keywords: extensification, mycotoxins, winter pasture.

## Introduction

*Festuca rubra* is a grass of considerable agronomical significance on winter pastures. On the one hand, it constitutes an important sward component of pastures utilised for feeding beef cattle. On the other hand, red fescue is highly regarded for its turf-forming capability. Therefore, it is a species which makes the turf of winter pastures dense, strong and not easily destroyed by trampling, a trait very important when heavy cattle are grazed on these pastures, which is the case of suckler cows of meat breeds. One of the biological nutritionally disadvantageous characteristics of red fescue is that its older shoots have a tendency to die out and considerable amounts of dead shoots appear during the vegetation season in the sward (Goliński and Kozłowski, 1998). In addition, dead shoots encourage the development of fungi which stimulate the decomposition processes of the biomass. This, in turn, leads to the development of mycotoxins which are both harmful to animals and reduce meat quality (Laser *et al.*, 2004).

The aim of this study was to assess the effect of the *Festuca rubra* cultivar and the level of nitrogen fertilisation on the accumulation of the ochratoxin A and zearalenone in the sward of winter pastures.

## Materials and methods

During 2002-2004, in Brody (52° 26' N, 16° 18' E) near the Poznań Experimental Station of August Cieszkowski Agricultural University studies were carried out to evaluate the accumulation of ochratoxin A and zearalenone in a pure stand sward of *Festuca rubra* cultivars harvested in January. The experiment was set up in a random block design, on plots in three replications. The factors in the experiment over three consecutive years were Polish cultivars of red fescue (Areta, Atra, Barma, Kos, Reda) and nitrogen fertilisation (0 and 60 kg ha<sup>-1</sup> per year). Nitrogen was applied to plots in early spring (30 kg ha<sup>-1</sup>) and after pre-utilisation in July (30 kg ha<sup>-1</sup>). The soil was a Luvisols with a (results of 2002

year analyses): pH<sub>KCl</sub> of 6.5, total nitrogen of 0.61% and 36.0, 11.2 and 3.0 mg of P<sub>2</sub>O<sub>5</sub>, K<sub>2</sub>O and Mg per 100 g of soil, respectively. The weather conditions during the winter pasture regrowth in the experiment are presented in Table 1.

Table 1. Weather conditions influencing the winter pasture regrowth during the experiment.

Item	2001/2002				2002/2003				2003/2004			
	X	XI	XII	I	X	XI	XII	I	X	XI	XII	I
Mean air temperature (°C)	12.3	3.4	-1.4	0.7	7.5	3.7	-3.4	-1.6	6.1	5.3	2.0	-3.5
Minimum air temperature (°C)	9.2	0.7	-3.5	-2.2	4.4	1.2	-6.0	-4.3	2.4	2.1	-0.8	-6.4
Maximum air temperature (°C)	16.2	6.1	1.1	3.6	11.2	6.5	-0.8	1.1	9.6	8.3	4.4	-1.1
Soil temperature at 5 cm (°C)	12.2	3.5	0.1	0.5	8.0	3.8	-1.3	-1.2	6.7	4.6	1.0	-1.2
Rainfall totals (mm)	28.0	18.4	41.5	43.4	127.0	69.7	13.0	60.2	39.9	27.6	41.9	73.2
No. of days with snow cover	-	-	13	21	-	-	5	11	-	-	5	11

Each year the plots (10 m<sup>2</sup>) were harvested at a standardised stubble height of 5 cm, plant materials were carefully mixed and representative, homogenised samples of herbage were collected. The forage samples were dried at 60 °C and ground to pass through a 1-mm screen. Forage from each plot (30 samples) was analysed for ochratoxin A (OA) and zearalenone (ZEA) using the Vicam tests kits and HPLC, respectively.

The method for OA analysis was described in an earlier paper (Goliński *et al.*, 2004). ZEA was analysed by extracting forage samples (10 g) with an addition of 1 g KCl overnight with 25 ml of acetonitrile : water (90 : 10, v/v), filtering through Whatman # 1 filter paper and the filtrate (1 ml) was diluted to 50 ml with de-ionised water and again filtered using a Vicam microfibre filter (catalogue number 31955). The final filtrate (10 ml) was applied to the top of a ZaeralaTest™ column with a rate of 1-2 drops sec.<sup>-1</sup>, then the column was dried with air (pressure) and washed with 10 ml of de-ionised water at 1-2 drops sec.<sup>-1</sup>. ZEA was eluted with 1.5 ml of HPLC grade methanol (1-2 drops sec.<sup>-1</sup>), the solvent was evaporated at 40 °C under a gentle stream of nitrogen, the residue was reconstituted in 250 µl of water : methanol : acetonitrile (70 : 20 : 10, v/v/v) and a 100 µl was injected onto a C<sub>18</sub> Nova Pak Waters column (3.9 × 150 mm) and analysed by HPLC using a Waters 2695 Separation Module with a Waters 2475 Multi λ Fluorescence Detector (λ<sub>EX</sub> 274, λ<sub>EM</sub> 440 nm) and a Waters 2996 Photodiode Array Detector (λ<sub>max</sub> 235 nm) with a mobile phase of acetonitrile : water : methanol (46 : 46 : 8, v/v/v) with a flow rate of 1 ml min.<sup>-1</sup>. The detection limit was 1.5 ng g<sup>-1</sup>.

## Results and discussion

On the basis of mean values for the three year studies, the highest concentration of OA was 1.05 ng g<sup>-1</sup> DM in sward dominated by the Kos cultivar (Table 2). Contamination levels of OA in red fescue cultivar sward changed over the years of the investigation, being significantly higher in 2002. This was probably associated with higher mean air temperature and air humidity in the 2001/2002 autumn-winter period as well as a longer period when pastures were snow covered (Table 1). The maximal concentration of OA in all years of investigations was 1.95 ng g<sup>-1</sup> DM in the sward of Barma cultivar.

In the case of ZEA, the highest concentration was 43.78 ng g<sup>-1</sup> DM in sward dominated by Atra cultivar in 2003. On the basis of mean values for the three years of studies, the highest concentration of ZEA was also determined in sward of Atra cultivar (Table 2). According to German literature data, dietary concentrations of ZEA in fodder higher than 500 ng g<sup>-1</sup> DM may have adverse effects on heifers, dairy and suckler cows. The results of our investigations show that the ZEA accumulation in the sward of *Festuca rubra* cultivars does not pose a serious hazard to animals. However, the prediction of health hazards for particular swards or locations is extremely difficult because of external effects, e.g. weather patterns (Laser *et al.*, 2004).

Table 2. Concentration of selected mycotoxins in winter pasture sward depending on *Festuca rubra* cultivars and nitrogen fertilisation – means 2002-2004.

<i>Festuca rubra</i> cultivar	Ochratoxin A (ng g <sup>-1</sup> DM)		Zearalenone (ng g <sup>-1</sup> DM)	
	Nitrogen fertilisation (kg ha <sup>-1</sup> ) per year			
	0	60	0	60
Areta	0.84	0.57	8.23	4.5
Atra	0.91	0.64	6.52	17.16
Barma	0.95	0.42	3.57	6.55
Kos	1.05	0.43	0.58	5.92
Reda	0.80	0.37	3.21	8.32

The fertilisation of extensive pasture sward dominated by *Festuca rubra* with 60 kg ha<sup>-1</sup> of nitrogen during the vegetative season decreased the accumulation of OA by 30% to 59% depending on the cultivar. In the case of ZEA, nitrogen fertilisation, as a rule, increased the accumulation of this mycotoxin in the sward of the red fescue cultivars, especially in the case of cv. Atra. A reverse trend was observed only in the case of the Areta cultivar.

### Conclusions

Cultivars of *Festuca rubra*, despite their tendencies to produce considerable quantities of dead shoots in the sward, failed to accumulate significant amounts of the mycotoxins, ochratoxin A and zearalenone, in the sward harvested in January. That is why these cultivars are suitable for use on winter pastures, especially for the improvement of the density of their swards and as a source of fodder for animals. The fertilisation of extensive pasture sward dominated by *Festuca rubra* with 60 kg ha<sup>-1</sup> of nitrogen during the vegetative season decreased the accumulation of ochratoxin A while increasing the accumulation of zearalenone. This phenomenon was confirmed in all (OA) and in the majority (ZEA) of the analysed cultivars of *Festuca rubra*.

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## Slurry quality affects the soil food web

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### Abstract

The organic matter in slurry is an important substrate for the soil organisms in grassland and hence its composition may influence the soil food web. The soil food web has many direct and indirect relations with grass production and nutrient efficiency. This paper describes effects of slurry quality on the abundance and functional groups of nematodes. Large differences in slurry organic matter composition could be expected, because of the huge variation of rations within dairy farming. This has been assessed in a database of 420 slurry analyses. Furthermore, eight slurries were analysed intensively and used in a pot experiment. The soil was analysed for nematodes. Nematode faunal analysis is accepted as method of assessing the structure of the soil food web (Ferris *et al.*, 2001). The pot experiment yielded useful relationships between slurry quality and abundance of different types of nematodes. We conclude that slurry quality affects the soil food web.

Keywords: slurry, organic matter, soil food web, nematodes.

### Introduction

Slurry quality is often defined in terms of plant available N. However, it is not possible to predict exactly when and in what amount N will become available for the plant. The effective N in cattle slurry may vary between 35 and 80% of total N (Bruinenberg and Van Middelkoop, 2004). This paper proposes a different concept of slurry quality. We hypothesise that the quality of slurry relates to the composition of the organic matter. Large differences in slurry organic matter composition could be expected, because of the huge variation of rations within dairy farming. Furthermore, we hypothesise that the organic matter in slurry is an important substrate for the soil organisms in grassland. Hence its composition influences the structure of the soil food web and in particular the abundance of nematodes. The soil food web on its turn has many direct and indirect relations with grass production and nutrient efficiency, depending on soil type (Van Eekeren *et al.*, 2003).

### Materials and methods

Slurry characteristics were explored using a database of 420 slurries obtained from both conventional and organic dairy farms in the Netherlands in the period 1996-2003. Sixteen slurries were analysed intensively. A principal component analysis was conducted to define the most important components to characterise the slurries. Three important characteristics for slurry quality were found: (1) the soluble nitrogen (NH<sub>4</sub>) content, N<sub>m</sub>, g kg<sup>-1</sup> DM; (2) the non-ammonia-nitrogen content, N<sub>org</sub>, g kg<sup>-1</sup> DM and (3) the neutral detergent fibre content of the organic matter, NDF<sub>OM</sub>, g kg<sup>-1</sup> organic matter. Eight of the slurries, originating from organic farms, were used in a pot experiment with ryegrass on sandy soil. There were two additional treatments: one with artificial fertiliser and a reference with unfertilised soil. All slurry and artificial fertiliser treatments were fertilised to a level of 170 kg total N ha<sup>-1</sup>. The applied effective N was calculated as  $W_m * N_m + W_{org} * N_{org}$  using standard values for W<sub>m</sub> and W<sub>org</sub>. Typical W<sub>m</sub> and W<sub>org</sub> for the first cut are 0.56 and 0.04, respectively. The experiment lasted one year after which the abundance and functional groups of nematodes were measured. Categories of nematodes were: plant feeders (plant pathogenic and plant associated), bacterial feeders, omnivores and carnivores (Yeates *et al.*, 1993).

## Results and discussion

The dataset of 420 slurry analyses showed that variation in  $N_m$  and  $N_{org}$  was considerable. Furthermore, a high  $N_m$  was not always related to a high  $N_{org}$  and vice versa (Table 1).

Table 1. Ratio between  $N_m$  (g kg<sup>-1</sup> DM) and  $N_{org}$  (g kg<sup>-1</sup> DM) in a dataset of 420 slurry analyses (% of total number of samples).

	Low, $N_{org} < 2.2$	Average, $N_{org} 2.2-2.5$	High, $N_{org} > 2.5$
Low, $N_m < 2.0$	9	11	3
Average, $N_m 2.0-2.8$	18	25	10
High, $N_m > 2.8$	6	11	8

With regard to the three variables  $N_m$ ,  $N_{org}$  and  $NDF_{OM}$ , the eight slurries of the pot experiment could be divided into three groups (Table 2). The groups related to the abundance and functional groups of nematodes. In general, fertilisation stimulated the abundance of nematodes (Table 3). Soil fertilised with slurry from group 1 contained a high number of nematodes and a relatively large number of carnivores. Both are indicators of a high level and stable soil food web (Ferris *et al.*, 2001). Soil fertilised with artificial fertiliser contained a relatively large number of plant feeding nematodes.

Table 2. Characteristics of slurries used in a pot experiment.

Group number	Number of slurries included	$N_m$ , g kg <sup>-1</sup> DM	$N_{org}$ , g kg <sup>-1</sup> DM	$NDF_{OM}$ , g kg <sup>-1</sup> organic matter
1	2	1.3	2.1	490
2	4	2.2	2.0	440
3	2	2.2	1.8	520

Table 3. Number of nematodes in unfertilised soil, soil fertilised with the three groups of slurry and soil fertilised with artificial fertiliser.

	Unfertilised	Slurry, group 1	Slurry, group 2	Slurry, group 3	Artificial fertiliser
Total number (per 100 g dry soil)	1611	2758	2593	2118	2687
Plant feeders (% of total)	29	26	27	39	54
Bacterial feeders (% of total)	47	49	55	44	35
Omnivores (% of total)	18	18	13	11	10
Carnivores (% of total)	1	4	2	2	0

Other examples of the effect of slurry quality on nematodes are the negative relation between applied effective N and predacious nematodes (carnivores plus omnivores) (Figure 1) and a positive relation between  $N_{org}$  and bacterial feeders (not shown). Effective N is essential for yield, but Figure 1 shows that less effective N corresponds to a larger number of predacious nematodes, i.e. to a better-developed soil food web (Ferris *et al.*, 2001), which in turn may lead to a higher natural fertility of the soil (Van Eekeren *et al.*, 2003).

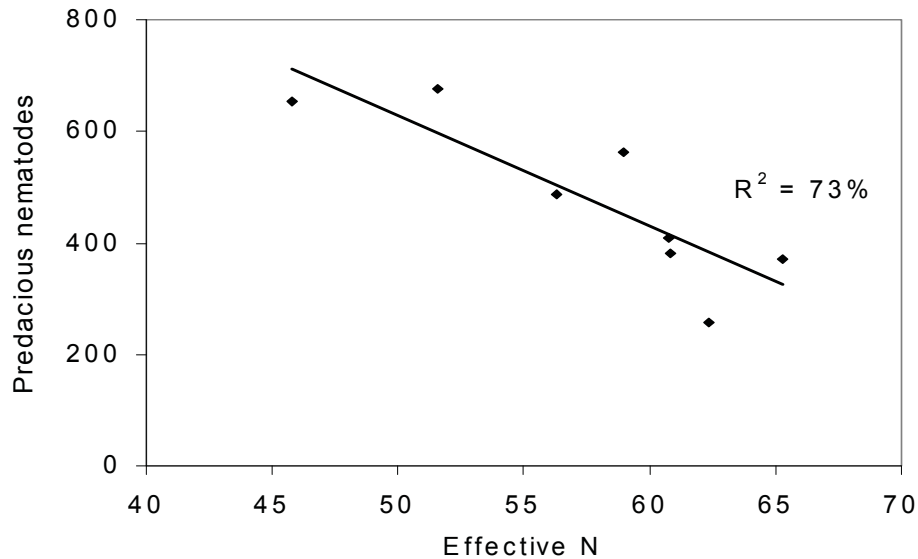


Figure 1. Relation between applied effective N (kg ha<sup>-1</sup>) and predacious nematodes (number per 100 g dry soil).

Slurry quality may be an instrument for farmers to optimise the soil-plant-animal-manure-system depending on soil type. Animal rations and manure processing can influence the quality of slurry. Additional research is needed to assess the interaction between slurry quality, soil food web, grass production and environment in relation to soil type.

### Conclusions

Large differences in slurry organic matter composition were found. The abundance and functional groups of nematodes in the soil appeared to be related to the composition of the slurry applied. We conclude that slurry quality affects the soil food web.

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## Nutrient management on Galician dairy farms

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### Abstract

Soil fertility was studied in 687 plots of 103 intensive dairy farms from Galicia (Northwest Spain). The average characteristics of these farms are 78.8 LSU (Live Stock Units), 8,198 L of milk by cow and year and an stocking rate of 2.8 LSU ha<sup>-1</sup>. Average soil test values of P and K were very high with of 50.9 mg kg<sup>-1</sup> and 184.6 mg kg<sup>-1</sup> for phosphorus and potassium, respectively. The average pH (water) was low (5.6), and the percent of soil acidity (Al<sup>3+</sup> + H<sup>+</sup>) was high (20.3 % of the CEC). In 41% of the plots, the application of P was unnecessary, and in 29% of the plots it was unnecessary the application of K. The percent of soil acidity was higher than 10 in the 63% of plots. Picked out the 427 plots that had P and K soil test higher that maintenance level, in the 55 % of these plots, the percent of soil acidity was higher than 10, showing that regardless of intensive use of mineral fertilisers, the use of lime is poor. A good technical advising on nutrient management would be necessary.

Keywords: soil analysis, phosphorus, potassium, lime, slurry, fertilizer recommendations.

### Introduction

Dairy farming is the first source of jobs and incomes in many rural areas in Galicia (INE, 2005). There were 16308 dairy farms in October of 2005, that represent 53% of the total Spanish dairy farms and that have 40,5 % of the total Spain milk quota (FEGA, 2005).

The change from grazing systems to zero grazing, with high stoking rates and high levels of milk production was necessary to increase their farm income due to the scarcity and the high price of the land and the market competitiveness. In these types of farms many nutrients come into the farm as purchase feeds, an only part of them are exported as milk, with about 75 % of nitrogen and phosphorus remaining in the dairy slurry (Novoa *et al.*, 2005). Fertilizer recommendations generally don't take into account organic fertilization, and thus mineral fertilizer is normally applied in excess increasing the levels of soil phosphorus and potassium.

### Materials and methods

Technical advisers of PROGANDO S.L (Galician animal nutrition company), selected 103 farms and supplied soil samples to CIAM, as well as farm and plot information. Records of mineral and organic fertiliser applied were registered for every plot. Some characteristics of this selected 03 dairy farms, like total LSU (Livestock Units) and SAU (total agricultural area), annual milk yield per cow, and kg of concentrates by litre of milk were registered. (Table 1). Soil pH (water), percent of soil acidity expressed as ((Al<sup>3+</sup>+ H<sup>+</sup>) \*CEC<sup>-1</sup> \* 100) (Mombiela and Mateo, 1984), phosphorus (Olsen) and potassium (ammonium nitrate) levels, were determined in 687 plots of the 103 intensive dairy farms. Records of mineral and organic fertilizer applied the last year were registered for every plot.

Table 1. Some characteristics of the dairy farms

	Minimum	Maximum	Mean
Agricultural area (ha)	5	140	27.7
Livestock units (LSU)	15	350	78.8
Milk yield (L cow <sup>-1</sup> yr <sup>-1</sup> )	5950	10000	8197
Stocking rate (LSU ha <sup>-1</sup> )	1.5	5	2.8
Concentrate (kg L <sup>-1</sup> )	0.3	0.5	0.4

## Results and discussion

*Phosphorus and potassium:* Soil tests show that soil P and K, levels are very high (Table 2), with average values of 50.9 and 184.6 mg kg<sup>-1</sup>, respectively. Considering that recommended maintenance values in soils are 25 and 100 mg kg<sup>-1</sup> for P and K, respectively, 74 and 80 % of plots had soil test values higher than the maintenance levels for P and K respectively, and 69 % of plots had higher values than the maintenance simultaneously for P as for K.

Table 2. Soil analysis and fertilisation of dairy plots.

	N	Minimum	Maximum	Mean	Std. Deviation
Soil pH	687	4.3	7.2	5.6	0.4
Soil acidity (%)	667	0	88	20,3	17
Soil P (ppm)	687	2	274	51	36
Soil K (ppm)	687	31	1044	185	104
P <sub>2</sub> O <sub>5</sub> (kg ha <sup>-1</sup> yr <sup>-1</sup> )	539	0	144	70,4	32
K <sub>2</sub> O (kg ha <sup>-1</sup> yr <sup>-1</sup> )	539	0	150	49	38
Slurry (m <sup>3</sup> ha <sup>-1</sup> )	253	20	100	41	12

We suggest, as good management practise, that plots with soil fertility levels higher than the recommended, the slurry produced by 2.5 LSU ha<sup>-1</sup> is enough to maintain soil test levels and obtain the optimum forage yields and in those cases, do not need any mineral fertilizer applied. In the plots that we had complete information about soil analysis, farm stocking rate and mineral fertilizer applied (458 plots of 687), it was found that in the 41% (Figure 1) of those mineral P was applied unnecessarily. In 29% of those plots, mineral K, was also applied unnecessarily, because the soil test values were higher than maintenance levels.

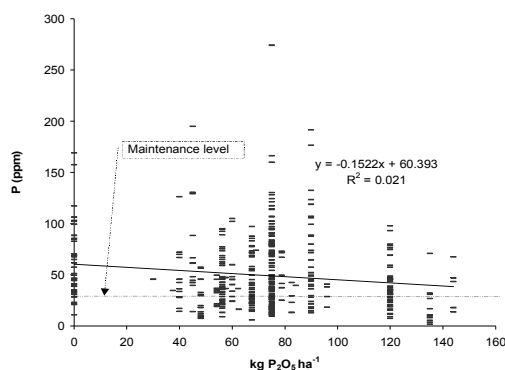


Figure 1. Soil P and annual mineral fertilizer (P<sub>2</sub>O<sub>5</sub>) applied in 458 plots.



In these cases the purchase of mineral fertiliser was unnecessary, showing a deficient nutrient management that leads to accumulation of P and K in soil with risks of leaching (Heckrath *et al.*, 1995). *Soil acidity*: Soil tests (Table 1) show that average pH is low (5.6), and that average percent of soil acidity is high (20.3). Soil acidity may be a limiting factor for forage production because  $Al^{+++}$  is toxic for plants (Kamprath, 1970). In Galician soils, a maximum of 10 % soil acidity is recommended to obtain optimum yields (Mombiela and Mateo, 1984).

Percentage of soil acidity was over 10 in 435 of 687 plots (63%) and in 427 of those plots, soil P and K levels were higher than those recommended for maintenance. In 55 % of those plots, the percentage of soil acidity was over 10, showing that regardless the intensive use of mineral fertiliser in these farms, the use of lime is poor. High level of aluminium in soil causes poor root growth (Adams and Moore, 1983) and therefore, even if soil fertility is high, the assimilation of nutrients can be deficient.

### Acknowledgements

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### Conclusions

Intensive dairy farms in Galicia show accumulation of P and K in the soil. Nevertheless, P and K fertilization were applied unnecessarily in 41% and 29 % of plots, respectively. Farmers need to improve their management and be confident of the slurry fertilizing value, because the high stoking rate (2.8 LSU ha<sup>-1</sup>) and the high use of concentrates (0.4 kg L<sup>-1</sup> of milk), allow enough P and K to be recycled on farm forage crops and obtain good yields. Soil acidity was high in 63% of plots probably due to a deficient liming, therefore farmers should increase the use of lime and reduce the applications of mineral P and K. A good nutrient management would allow saving money while reducing the risk of pollution.

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# Calcium and magnesium concentrations in the leachate from permanent meadow soils

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## Abstract

The concentrations of  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  in leachate and their relations with soil pH were calculated and presented in the paper. The investigation was carried out in permanent meadow in Western Lithuania. Research was conducted on acid and limed loamy *Haplic-Luvisols (LVh)* in 2003-2004. Samples of lysimetric water were collected separately according to the season of the year: in spring and autumn. As soils in Western Lithuania are under high precipitation amount (767.8 and 904.1 mm per 2003 and 2004 respectively), the risk of nutrient leaching occurs. Concentrations of calcium and magnesium in the leachate increased where ground limestone was used. Though, much less magnesium was leached out comparing with calcium. The concentrations of  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  in leachate differed from 139.67 to 239.33  $\text{mg L}^{-1}$  and from 8.13 to 25.00  $\text{mg L}^{-1}$  respectively. Soil pH had a strong influence on  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  concentrations in the leachate. Correlation coefficient ( $r$ ) differed from 0.825 to 0.996.

Keywords: lysimeter,  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  concentrations, liming, permanent meadow.

## Introduction

According to the data of Agrochemical Research Centre of Lithuanian Institute of Agriculture about 30 % of soils in Western Lithuania are acid ( $\text{pH} \leq 5.5$ ) (Mažvila *et al.*, 2004). Soil pH is one of the factors influencing pasture productivity and sustainability (Daugėlienė 1995, Marcinkonis and Tripolskaja, 2001, Grewal and Williams, 2003). Therefore, agricultural soils are limed, to adjusting pH. Most often the soils become acidic in Western Lithuania, where there was the greatest amount of high and medium acidity soils before liming and where intensive leaching processes are still taking place (Mažvila *et al.*, 2004). Half of calcium and magnesium compounds are mobile in acid soils, therefore they leach easily. This process is more rapid when organic matter decomposes into hydrocarbonates, nitrates, chlorides and other ions of soluble salts (Švedas, 2000). The amount of calcium leached out in comparison to the other cations is most likely greater because calcium is a readily leached cation, easily exchanged for hydrogen ions (Stinner *et al.*, 1984). Migration and leaching of incorporated and other soil elements increased under the influence of liming and fertilisation. Leaching of  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  increases by 20 %, when soil acidifies by one pH unit (Čiuberkienė and Ežerinskas, 2000). As soils in Western Lithuania are under high precipitation amount (767.8 and 904.1 mm per 2003 and 2004 respectively), the risk of nutrient leaching increases. To determine the concentrations of  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  in leachate, they were measured and their relations with soil pH were calculated and presented in the paper.

## Materials and Methods

The investigation was carried out in permanent meadow in Western Lithuania. The research was executed on acid and limed loamy *Haplic-Luvisols (LVh)* (FAO-Unesco, 1997) from 1991. 2003-2004 data are presented in the paper. The main liming treatment was done in spring 1991 and established 4 different  $\text{pH}_{\text{KCl}}$  levels (5.0-5.5, 5.6-6.0, 6.1-6.5, 6.6-7.0). Ground limestone (calcium carbonate) was introduced into the top 10 cm depth of the soil before sward sowing. Limestone rate was calculated according to the titration curves (Remezov method), neutralizing the soil with 0.033 N  $\text{CaCl}_2$  solution. Ecological P (bonemeal) and K (potassium magnesium) fertilisers at a rate 60 kg  $\text{P}_2\text{O}_5$  and 60 kg  $\text{K}_2\text{O}$  per ha were applied early in spring before grass growth resumed after winter. The applied amount of water soluble MgO was 24,6 kg  $\text{ha}^{-1}$ . Lysimeters (Shilova type) were put in to 40 cm depth. Samples of

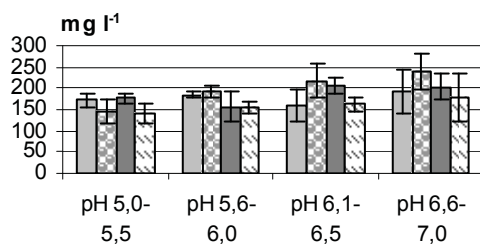
lysimetric water were collected separately according to the season of the year: in spring (01.04.2003 and 30.03.2004) and autumn (27.10.2003 and 14.10.2004).

## Results and Discussion

Calcium output in soil leaching was high compared to the other elements leached. The use of a lime material such as  $\text{CaCO}_3$  increased concentrations of  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  in the leachate. The highest  $\text{Ca}^{2+}$  concentrations ( $239.33 \text{ mg L}^{-1}$ ) were determined in the autumn of 2003, after grass vegetation ended, when soil pH was 6.6-7.0 (Figure 1).  $\text{Mg}^{2+}$  leaching partly (correlation coefficient  $r = 0.47$ ) was related to  $\text{Ca}^{2+}$  leaching. It could be because Ca and Mg are held and released under similar chemical and biological conditions (Brady, 1974). The highest  $\text{Mg}^{2+}$  concentrations ( $25.00 \text{ mg L}^{-1}$ ) were determined in the autumn of 2003, after grass vegetation ended, when soil pH was 5.6-6.0.

Any EU directive does not present any requirement for the Ca and Mg levels or water hardness (apart from the lower limit for  $\text{pH} \geq 6.5$  which requires indirectly a certain level of dissolved solids) in drinking and all the more in ground water.

### $\text{Ca}^{2+}$ concentrations



### $\text{Mg}^{2+}$ concentrations

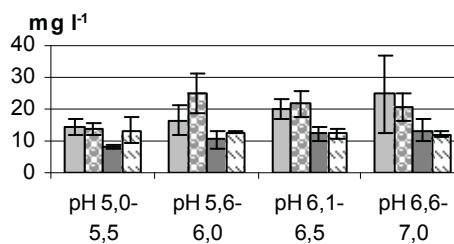
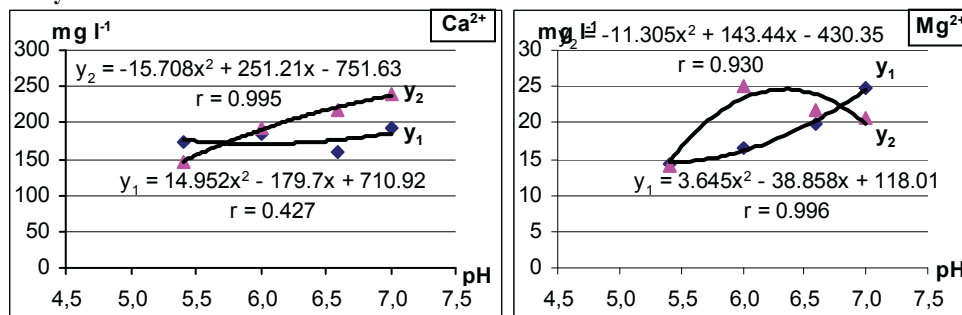


Figure 1.  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  concentrations in the leachate in different season of the year, where - spring 2003, - autumn 2003, - spring 2004 and - autumn 2004. The bars show standard error.

Correlations between soil pH and  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  concentrations in the leachate are shown in Figure 2. Soil pH had a strong influence on  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  concentrations in the leachate. The correlation coefficient ( $r$ ) varied from 0.825 to 0.996. Only in one case, the spring of 2003, correlation between soil pH and  $\text{Ca}^{2+}$  concentrations was weak ( $r = 0.427$ ). When soil pH increased from 5.4 to 7.0 in 2003 and from 4.9 to 6.6 in 2004, both  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  concentrations in the leachate in spring also increased. In the samples, which were collected in the autumn,  $\text{Ca}^{2+}$  concentrations increased both in 2003 and 2004. However,  $\text{Mg}^{2+}$  concentrations dependence on soil pH differed. In 2003 autumn when soil pH increased from 5.4 to 6.0, the  $\text{Mg}^{2+}$  concentrations also increased. Then, increasing soil pH from 6.0 to 7.0,  $\text{Mg}^{2+}$  concentrations decreased. And in 2004 autumn –  $\text{Mg}^{2+}$  concentrations in the leachate slightly decreased.

### 2003 year



2004 year

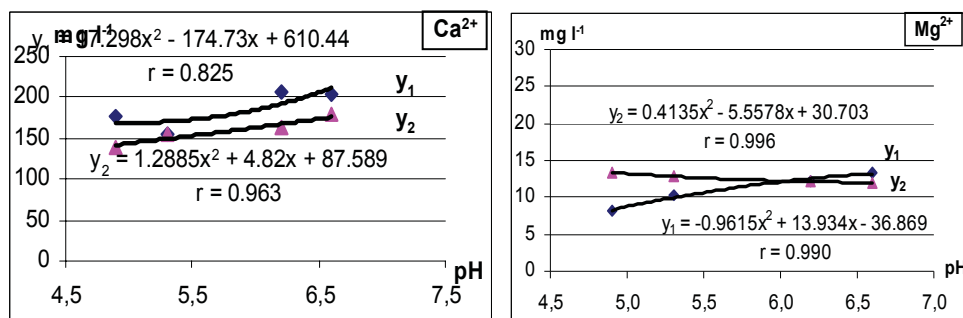


Figure 2. Soil pH effect on the Ca<sup>2+</sup> and Mg<sup>2+</sup> concentrations in the leachate, where y<sub>1</sub> – spring, y<sub>2</sub> – autumn.

### Conclusions

The research showed that concentrations of Ca<sup>2+</sup> and Mg<sup>2+</sup> in leachate differed from 139.67 to 239.33 mg L<sup>-1</sup> and from 8.13 to 25.00 mg L<sup>-1</sup> respectively. The highest Ca<sup>2+</sup> concentrations (239.33 mg L<sup>-1</sup>) were determined when soil pH was 6.6-7.0 and Mg<sup>2+</sup> (25.00 mg L<sup>-1</sup>) - when soil pH was 5.6-6.0 in autumn of 2003, after grass vegetation ended. Soil pH appears to have a strong influence on Ca<sup>2+</sup> and Mg<sup>2+</sup> concentrations in the leachate. Correlation coefficient (r) differed from 0.825 to 0.996, with one exception.

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# The effect of nitrogen fertilization on the yield and quality of field pea (*Pisum sativum*)

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## Abstract

A three-year study examined the effects of increasing fertilizer N rates (0, 20, 40, 60 N kg ha<sup>-1</sup> and equal P and K rates) and utilization stage on the yield and quality of pea (*Pisum sativum* L. Cv Junior) fodder and grain, with the aim of determining optimal N fertilizer rates and the most favourable stage for exploitation. The highest dry matter yield at the stage of flowering and at the milk-waxy maturity stage was obtained in the first study year in the treatment receiving 60 kg ha<sup>-1</sup> N. This was a result of optimal temperatures and adequate moisture supply during the period of vegetative growth. However, the grain yield was highest in the 40 kg N ha<sup>-1</sup> treatment, having been caused by the vegetative growth stimulation and crop lodging at the end of the developmental cycle. The crude protein yield varied in accordance with the dry matter yields.

Keywords: fodder pea, nitrogen fertilization, yield, quality.

## Introduction

Although mineral N present in the soil inhibits both nodule formation and nitrogenase activity, increased mineral N absorption by the root is complementary to symbiotic N fixation with respect to supplying necessary plant N demands (Sagan *et al.*, 1993). Considering the relatively poor development of pea roots and its reduced activity at the end of the development cycle, N uptake limitations in this period may occur due to reduced N availability in the soil. The amount of N accumulated in the grain can be increased by prolonging symbiotic N fixation, by more efficient assimilation of the remaining mineral N from the soil or by optimizing the two processes, since remobilization is directly correlated with photosynthesis, which decreases during the seed filling stage (Salon *et al.*, 2001).

Under field conditions, plants grown in the presence of mineral N establish a balance between symbiotic N fixation and mineral N absorption. The possibility of providing an optimal level of N by symbiotic N fixation or by mineral N uptake from the soil, being complementary processes, gives rise to a question of what is the most effective use of N fertilizer.

## Materials and methods

The trial was set up at the farm of the Faculty of Agronomy in Cacak over three years (1996-98), using a randomized block design with four replications. The fodder pea variety selected for sowing was Junior. The following rates of mineral N (in the form of NH<sub>4</sub><sup>+</sup>) were applied in the trial: 0, 20, 40 and 60 kg N ha<sup>-1</sup>, with equal amounts of P and K (75 kg ha<sup>-1</sup> each).

Samplings were performed at three phenological stages: the stage of flowering and onset of pea pod formation (I), the milk-waxy maturity stage (II) and the full grain maturity stage (III). The following parameters were examined: dry matter (DM) yield, grain yield, crude protein content and protein yield. The trial was conducted on a smonitza type soil, characterized by an acid reaction (pH 5.01 in 1M KCl), low N content (1.34 mg g<sup>-1</sup> of soil), good K supply (0.264 mg g<sup>-1</sup>) and a lack of readily available P (0.178 mg g<sup>-1</sup>). Mean air temperatures in the growing season were close to the long-term average, whereas the most favourable distribution of precipitation was recorded in the first research year.

## Results and discussion

The use of N fertilizer significantly increased DM yields at the I and II utilization stage in all the treatments when compared to the control (Table 1). The highest yield was recorded in the treatment with 40 kg N ha<sup>-1</sup>, whereas the use of the 60 kg N ha<sup>-1</sup> rate resulted in an insignificant yield decrease compared to the former rate at both growth stages, with the exception of the first study year.

Table 1. The dry matter and grain yields of fodder pea (t ha<sup>-1</sup>).

Stages		I stage			II stage			Grain		
Years (B)		1996	1997	1998	1996	1997	1998	1996	1997	1998
Fertilization (A)	0	4.94	4.19	4.28	6.53	5.70	6.13	3.31	2.94	2.89
	20	5.91	5.93	5.25	7.44	7.18	7.20	4.05	3.25	3.00
	40	6.48	6.97	6.18	8.02	8.25	8.08	4.68	3.33	3.15
	60	6.59	6.53	6.19	8.28	7.84	7.61	4.32	3.27	3.03
Average		5.98	5.91	5.48	7.57	7.24	7.26	4.09	3.20	3.02
Lsd		A	B	AB	A	B	AB	A	B	AB
0.05		0.36	0.33	0.64	0.45	0.35	0.85	0.44	0.31	0.76
0.01		0.49	0.35	0.85	0.66	0.46	1.14	0.59	0.41	1.01

The grain yield was significantly higher in the treatments receiving 40 and 60 kg N ha<sup>-1</sup> compared to the control (Table 1). The highest grain yield was produced in the treatment with 40 kg N ha<sup>-1</sup>. Using relatively high mineral N rates (0, 100, 200 and 400 kg N ha<sup>-1</sup>) Voisin *et al.* (2002) recorded no significant differences in grain yield between the treatments considered, at which the highest grain was attained in the treatment with 200 kg N ha<sup>-1</sup>.

Compared to 1997 and 1998, a considerably higher grain yield was reached in all treatments in 1996, which appeared to be the result of higher rainfall and a favourable distribution during the pea growing season. Making comparisons of the grain DM yields of pea with respect to different mineral N rates, Jensen (1996) observed low grain yield stability over the years, with the highest yields produced in the years with adequate precipitation in June (critical moisture stage). In his analysis of pea grain yields in South Australia, McDonald (1995) determined a positive correlation between the amount of precipitation in the growing season and grain yield. However, this explained less than 50% of the yield variation observed.

The decreases of grain yield and N concentration in the grain and of protein content with the highest N rate (60 kg ha<sup>-1</sup>) resulted from N incorporation in the vegetative organs, which led to excessive luxuriance and crop lodging at the end of the development cycle (Table 2). The prevention of N remobilization from the vegetative parts as well as the impossibility of using mineral N caused lower N and protein contents in the grain, being in accordance with the results of Voisin *et al.* (2002). Owing to relatively small differences in protein content, protein yields varied in a similar way to the DM yields of fodder and grain (Table 2).

Table 2. The protein content ( $\text{g kg}^{-1}$ ) and protein yield ( $\text{t ha}^{-1}$ ) in the dry matter of field pea fodder and grain (a three-year average).

Stage	I stage		II stage		III (grain)		
	$\text{g kg}^{-1}$	$\text{t ha}^{-1}$	$\text{g kg}^{-1}$	$\text{t ha}^{-1}$	$\text{g kg}^{-1}$	$\text{t ha}^{-1}$	
Fertilization	0	166.2	0.70	144.2	0.82	252.1	0.64
	20	168.9	1.00	150.1	1.08	254.0	0.71
	40	178.9	1.25	132.3	1.09	267.0	0.76
	60	165.4	1.08	135.9	1.07	253.0	0.71
	Average	169.8	1.01	140.6	1.02	256.5	0.71
CV %	4.89	19.8	4.52	11.4	2.62	6.5	

A significant difference in the content of crude protein at the first two growth stages was presumably due to a high proportion of leaf material contributing to yield and quality at the flowering stage, in accordance with the results of Čupina (1997).

### Conclusions

The application of increasing mineral N rates brought about a significant increase in DM and grain yield. The highest yields of DM and of grain were achieved in the treatment with  $40 \text{ kg N ha}^{-1}$ , with the exception of the fodder DM yield in the first and in the second research year, respectively. Considerably higher fodder and grain yields were achieved in the first study year, which was a result of optimal precipitation amount and distribution in the growing season. No significant differences were registered in crude protein content between the treatments, whereas the crude protein yield varied in accordance with the fodder and grain DM yields.

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# Nitrogen mineralization in soils receiving different rates of cattle-slurry and cropped with forage maize

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## Abstract

The quantification and the time-course of organic nitrogen release from slurry applied at sowing of maize (*Zea mays*, L.) grown for silage is of major importance to increase the efficient use of slurry-N and to reduce environmental impacts. The objective of this work was to quantify the net N mineralization measured in the 0-30 cm soil layer during the forage maize growth period, when cattle slurry was applied at sowing to supply 340 (T1), 190 (T2) and 0 (T3) kg total N ha<sup>-1</sup>. The experiment was carried out during 1999 in the NW region of Portugal. Net mineralization rates were significantly different and reached high values, being a relevant source of N for the plants. During the forage maize crop net mineralization represented 401, 281 and 174 Kg N ha<sup>-1</sup> respectively on treatments T1, T2 and T3, values that correspond to average net N mineralization rates of 1.3, 0.9 and 0.6 mg N kg<sup>-1</sup> soil d<sup>-1</sup>. The forage maize dry matter yields on these treatments reached 22, 19 and 13 t ha<sup>-1</sup>, respectively for T1, T2 and T3.

Keywords: cattle-slurry, N mineralization, maize forage.

## Introduction

In highly intensive dairy systems based on silage maize, cattle-slurry is often applied to this crop at considerable rates. Moreover, additional N fertilisers are generally applied to compensate for the low slurry N use efficiency and to ensure optimal maize yields. Due to the difficulty in predicting the slurry N availability, the amounts of fertiliser N applied frequently exceed the crop requirements leading to the accumulation of high levels of nitrate-N in the soil after harvest (Trindade *et al.*, 1997). The ability to predict the amount of N that is released from the organic matter in the soil and is available for the nutrition of plants is extremely important for agriculture as a sustainable activity. The objective of our work was to quantify the net N mineralization measured on the 0-30 cm soil layer during the forage maize growth period, when different rates of cattle slurry were applied at sowing.

## Materials and methods

The experiment was carried out at Vila do Conde (near Porto) in the NW region of Portugal between May and October 1999 on a highly intensive dairy farming area. The soil was a deep well-drained sandy loam derived from granite and classified as RGuo soils, with a pH value of 5.8, organic matter (OM) content of 2.5%, available P<sub>2</sub>O<sub>5</sub> and K<sub>2</sub>O, of 117 and 157 mg kg<sup>-1</sup> respectively. The experiment was a randomised block factorial design with three replications and three treatments. The dimension of the plots was 160 m<sup>2</sup> (20 x 8). Cattle slurry was applied just before the sowing of forage maize at rates to reach 340 (T1), 190 (T2) and 0 (T3) kg total N ha<sup>-1</sup>. About 50% of the slurry-N was in the form of ammonium. No other fertiliser was applied to the soil in the year previous to this experiment and the soil was under a double crop system with maize and Italian ryegrass. Maize cv. Nubia (600 FAO) was sown on June 2 and harvested on September 29. During the growth period net N mineralization was measured at the soil top layer (30 cm) using the field soil core incubation with acetylene method (Ryden *et al.*, 1987), on all experimental treatments between June 4 and October 1. Net mineralization rates were determined using the method described by Hatch *et al.* (1990; 1991). For each incubation period and in each plot, five soil cores of 3 cm diameter and 30 cm depth were collected and placed in sealed jars. To facilitate the collection and management of the soil cores for incubation, PVC pipes were used



with appropriate dimensions and perforated sidelong to accelerate the diffusion of the acetylene into the soil. Each lid had two septum seal stoppers through which acetylene ( $C_2H_2$ ) was injected to give a concentration of 2% (vol/vol) in the headspace of the jar to inhibit nitrification. The cores in the jars were incubated in holes in the ground adjacent to the study area for ca. 14 days. At the same time and near the place where the cores were picked, soil samples for analysis were collected to the same depth using a probe. Soil samples were homogenised and the humidity, mineral N content ( $NH_4^+$ -N and  $NO_3^-$ -N) and bulk density determined by standard lab methods. In the end of the forage maize crop the production was measured and plant samples were dried for determination of dry matter content. Results were analysed by ANOVA and LSD.

## Results and discussion

Figure 1 shows the time-course of net N mineralization rates by treatment observed in the soil plough layer (0-30 cm) during the forage maize growth period.

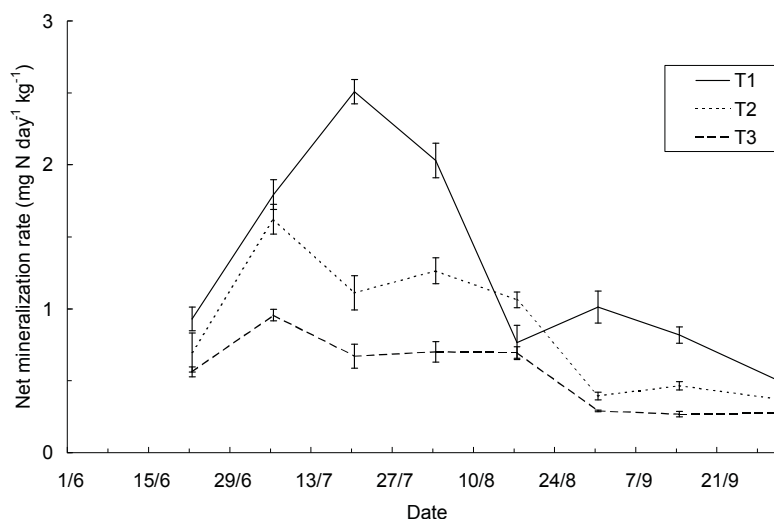


Figure 1. Net N mineralization rates at the soil plough layer (30 cm deep) by treatment during forage maize growth period. The vertical bars represent SEM (df = 4).

Net N mineralization rates were significantly ( $p < 0.05$ ) affected by slurry treatments. Measured mean daily net mineralization rates on treatments T1, T2 and T3 were 1.3, 0.9 and 0.6 mg N kg<sup>-1</sup> respectively. Total N released during the forage maize crop reached 401, 281 and 174 kg N ha<sup>-1</sup> respectively on T1, T2 and T3. Assuming that the amount of N mineralised on the treatment not fertilised (T3) was the contribution of soil organic matter and subtracting this amount to the total N mineralised on T1 and T2, the N mineralised on these two treatments was proportional to organic-N applied throughout the slurry. The high amounts of N released on T1 and T2 suggest that slurry organic pools are mainly composed by labile and easily degradable compounds, as was shown by Matus and Rodriguez (1994). The cattle slurry degradation kinetics during the maize growth period showed a pattern similar to that of the crop N-uptake requirements.

Maize DM forage yields differed significantly ( $P < 0.001$ ) among treatments and were proportional to the values of mineralized N. Values of DM yield on treatments T2 and T3 were respectively 19.0 and 13.0 t ha<sup>-1</sup> and, on these treatments, soil residual  $NO_3^-$ -N content at the end of the forage maize crop was insignificant. Meanwhile, on treatment T1, DM yield was 22.2 t ha<sup>-1</sup>, but the soil residual  $NO_3^-$ -N

content was about 20 mg kg<sup>-1</sup>. Consequently, it was considered that the rate of N applied by this treatment exceeded the crop requirements, leading to the accumulation of nitrate-N after harvest.

### **Conclusions**

Net mineralization rates from treatments receiving different rates of cattle slurry varied significantly and reached high values, being a relevant source of N for the plants. During the maize crop growth period, an important fraction of the slurry organic N was mineralised and the release of N showed a pattern very similar to the maize crop N uptake requirements.

### **Acknowledgements**

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# Modern transfer of grassland knowledge via WorldWideWeb using the German grassland platform *gruenland-online.de*®

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## Abstract

The decline in governmental support to Agriculture necessitates the use of new methods for the transfer of grassland management knowledge to farmers and advisers. Using the internet as an ideal platform, it was necessary to develop webfiles with information for grassland production on farm level. This information has to be well structured, extensive, updated and with a particular reference to different ecological features of several grassland sites. The web-page [www.gruenland-online.de](http://www.gruenland-online.de) is especially designed for the use of German speaking grassland farmers and consultants with a huge amount of information on miscellaneous topics like fertilization, sward renovation, weeds control, forage quality and the overall pasture management. The direct assignment to different grassland locations is possible within the view and botanical composition of grasslands, whereas the determination of grass species can be done by using a botanical guide of grass species via internet. Web site *gruenland-online.de*® is well decorated with an award for agricultural innovation in Germany.

Keywords: grassland consulting, knowledge transfer, ecological grassland production, expert system.

## Introduction

The current changes in EU - agricultural policies lead to large scale changes in grassland farming systems and the need to support farmers through the current structural variations. In the same time, the decreasing financial capacity of government changes drastically the systems of rural extension services in Germany. The efforts for the farmers in order to stay in the agriculture are very intense therefore advisory systems have to be developed and adapted to the new demands. Information about grassland production systems is one thing, the presentation and availability for farmers and advisers is the other. Therefore the aim of a collaboration work between the applied science of Education- and Science Centre for cattle, grassland, deer and fishery in Aulendorf and the University Hohenheim, Institute of grassland and crop science, was to develop a new extension service system, where the farmers have the ability to search practical solutions by using the World Wide Web. The special structure of “gruenland-online®” with its tools of easy use even for persons without special EDV knowledge, gives farmers the chance to use the programme for their specific problems.

## Structure of the *gruenland-online.de*® site

Using *gruenland-online* you will start with a visual taxation of grassland swards. The botanical composition of grassland is mainly influenced by the factors: fertilization, utilisation frequency and ecological conditions of the grassland site. Therefore, five typical grassland swards for favourable and also for unfavourable conditions are pictured in order to give the mainstream for specific advices. Where are we? What is our specific problem? Grassland types are further described and the causes for unfavourable grassland swards with low forage quality are given and typed in a classification system (Figure 1).

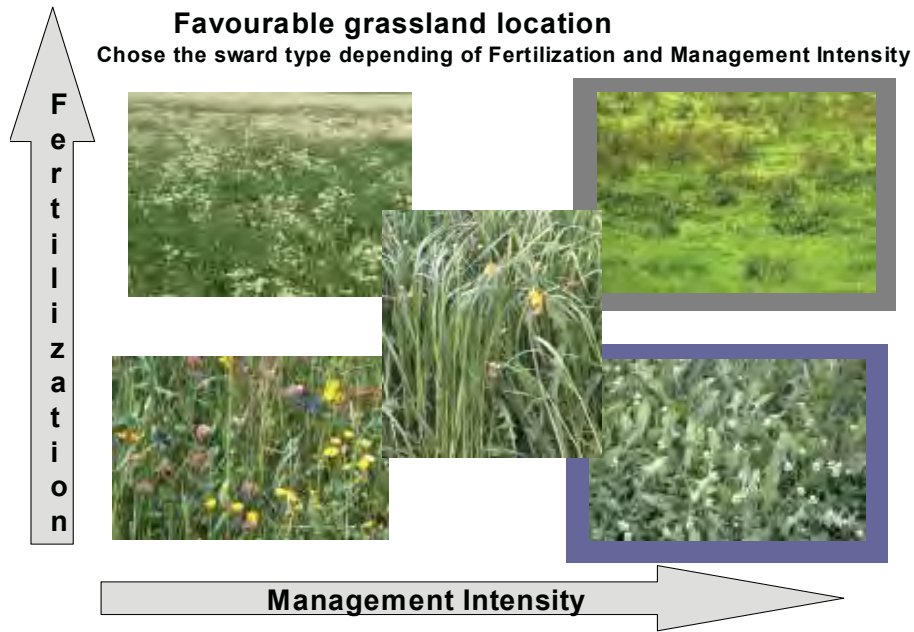


Figure 1: Initial page of the gruenland-online.de site presenting different types of grassland swards

	I	II	III	IV	V	VI	VII
<b>Nutzungstadium</b>	im Schraam	vor Abos/ Kappenschoben	Beginn Abos/ Kappenschoben	Ende Abos/ Kappenschoben	in der Blüte	nach der Blüte	Beginn Streuweide
<b>Löwenzahn</b>	Blütengras, 1/4 oder Pflanzen voll geblüht	alle Pflanzen unvollständig 1/2 verblüht	alle Pflanzen voll geblüht, 1/4 im Sommerschub	alle Pflanzen voll Sommerschub	mit noch meisten Blütenständen	Blütenstände vor über 100% verblüht	
<b>Kneulgras</b>	oberster Holm- knoten 10 cm über Erdoberde	Rest der Blüte- stängel 1/2 Holm- länge	erste Kappens- spitzen treten aus der Blätterhaube	Kappe voll geblüht oben, volle Holm- streckung noch nicht erreicht	volle Holm- streckung erreicht	abgestorbene Holm- stängel noch grün	Holm voll geblüht, wenn von Spätsommer beim Schlagen auf Heud

Figure 2: Stage of growth of main species as basic information for the assessment of grassland forage quality (Nussbaum et al., 1999).

The next step provides further information to the users, through the possibility of determining grassland species by using the built-in grass-species taxation book. Possibilities for previous estimation of forage quality are given by the system of the DLG "Grundfutterbewertungssystem" (Nussbaum et al., 1999; system for the evaluation of hay and silage quality through their sensory properties) (Figure 2).

Clear instructions are given in order to judge the grassland swards - botanical composition and gaps of the swards are to estimate. Weed and grass control methods are described for more than twenty five plant species. All known methods for the grassland renovation with the specifications of different grassland reseeding machinery are also given, as well as the advantages of systems for grazing different kind of animals.

*gruenland-online.de*® gives specific information about grassland fertilization, nutrient balances and new ordains of the law for fertilizers. It is described, how to use in environmentally friendly manner solid and liquid manure and also the specific effects of different kinds of fertilizers can be found. Additionally in a special chapter instructions are included to estimate damage caused by wild boars with a tool for compensation payment.

This webpage does not contain results of particular grassland experiments, but it is linked with *Infodienst*, the homepage of agricultural administration of Baden-Wuerttemberg and to the University of Hohenheim, where this type of information can be found.

### **Conclusions**

Changing conditions in milk and meat production systems, according to the new EU policies, lead to a demand of specific information. The *gruenland-online.de* ® is a large database especially designed for use by farmers and advisers with a special focus to the botanical composition of different grassland swards, their advantages and problems. This site presents updated information on grazing systems, weed plants, fertilization, grassland improvement and grassland renovation. The *gruenland-online.de* ® was the winner of the innovation award in agrar-computer days in Augsburg for the year 2005.

### **Acknowledgements**

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# **Session 5**

## **Grassland and climate change**





# Climate change and grasslands through the ages - an overview

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## Abstract

Climate changes from cool to warm and vice versa have occurred throughout geological times. During the Jurassic and Cretaceous periods (206 – 65 million years ago, mya) the climate was more uniformly warm and moist than at present and tropical rainforests were wide spread. Grasses evolved during the Jurassic and they expanded greatly as the climate differentiated with reduced rainfall and temperatures. C<sub>4</sub> grasses probably arose during the Oligocene (24-35 mya). During the Miocene (23.8 – 5.3 mya) grasslands expanded into huge areas (e.g. prairies in the USA, steppe in Eurasia, pampas and llanos in South America).

During the Quaternary (1,8 mya till now) some 22 different ice ages with periodicities of about 100,000 years occurred. North Western Europe had a polar climate with tundra vegetation 18,000 years ago and the Mediterranean region was covered by steppe. During that time Amazonia was so dry that it was covered in extensive areas of savanna and the Sahara expanded rapidly. Only in the last 10,000 years has a closed rainforest covered the Amazonian region again.

However, 9000 years ago a brief period of global warming caused excessive rains, which caused the sea- and river-levels to rise in North Western Europe with tremendous loss of life. The present period of extreme dryness in the Sahara only started some 5000 years ago and then the desert expanded rapidly into the Sahel. Before that the Sahara was covered by steppe. Global warming took place between about A.D. 900 and about 1200 or 1300 just before the Little Ice Age (1550 – 1700 AD).

The paper concludes with temperature and vegetation changes that are occurring in Europe at present. It is predicted that C<sub>4</sub> grasses, which are already present in southern Europe, will further expand, but that in the short term land abandonment will have much more deleterious effects than temperature change due to increased wild fires, loss of biodiversity and desertification.

## Introduction

Climate change is a hot issue on which much research effort and money are being spent. Global warming is taking place, but it is not certain what causes it, although politicians and the Intergovernmental Panel on Climate Change (IPCC) attribute this to human activities—primarily burning of fossil fuels and changes in land cover. However, according to Friis-Christensen and Lassen (1991), and more recent publications by others (e.g. Krivova and Solanki, 2004), solar activity, which also causes global warming, has increased at the same pace as global warming. Whatever the cause may be, the effects are the same and the purpose of this paper is to review the effects on grasslands from earliest geological times till the present.

Nevertheless, uncertainty was raised again by Bryden *et al.* (2005), who reported that the Atlantic Ocean currents (Gulf Stream) that carry heat from the tropics to high latitudes, have substantially weakened over the past 50 years because more fresh water flowing into the ocean from rivers, rain and melting ice, could ultimately lead to substantial cooling of the North Atlantic.

## Historic review

During the Cretaceous, 144 – 66 million years ago (mya), some 4,000 million years after the oldest geological time period, when dinosaurs dominated the earth, the first grasses and dicotyledonous plants appeared.

During Jurassic times (206 –144 mya) and at least until the close of the Cretaceous (144 –65 mya) the climate was far more uniformly warm and moist than at present and the tropical rainforests, as we know them now, were widespread. With increasing climatic differentiation, with the advent of pronounced dry

seasons and a general lowering of temperatures, the humid tropical region became more and more contracted and grasses came to great expansion some 20 mya during the Miocene. This is not only apparent from the fossil flora, but also from the adaptation of the teeth of mammals of that time. The earliest grasses were markedly adapted to moist and damp environments and occurred at the margins of forests in the tropics. Those growing in primitive habitats (forests, forest margins and damp moist environments) remained primitive, but as the climate differentiated and the forests contracted further the grasses also developed more advanced species that became associated with sclerophyllous regions and moorland habitats (Bews, 1929). Enormous areas of grasslands developed in Eurasia (steppes), Eastern Europe (pusta), southern Africa (veld) South America (pampas and llanos), Australia (downs) and north America (prairies) on black soils (chernozems) and vertisols (Bews, 1929; Kroonenberg, 1983).

Following the Tertiary (65 to 1.8 mya) the earth became cooler and drier, which was characterised by an increase in grasslands. Eventually the icecap on Antarctica began to develop, but it was only 2.5 mya that the cooling extended globally: the subtropical vegetation on the European continent was replaced by more temperate species (Kroonenberg, 1983).

The Quaternary (1,8 mya till now) was the period of the ice ages. Some 22 different ice ages with a periodicity of about 100,000 years have been recognised. During the last ice age (18,000 years ago) North Western Europe was partly covered by ice. In Western Europe there was a polar climate with tundra vegetation and the Mediterranean region was covered by steppe. Only in the Balkan there were areas in which conifers and broad-leaved deciduous trees could survive.

The variability of Northern Hemisphere temperatures is shown from ice core data to a depth of about 3000 m obtained between 1990 and 1992 in Greenland. Ice cores contain well-defined layers that indicate levels of precipitation during the last several hundred thousand years. This has allowed a detailed reconstruction of the climate. Results of this project were reported in a special issue of the *Journal of Geophysical Research* (Vol. 102, No. C12, pages 26315-26886, 1997). Data are also available on <ftp://ftp.ngdc.noaa.gov/paleo/icecore>.

As is happening at present, drastic melting of snow also took place 9000 years ago, due to global warming. Even though this period of warming didn't last more than twenty years, glaciers melted, which caused excessive rains, which in turn caused the sea- and river-levels to rise with tremendous loss of life.

During the time that ice covered the northern areas the climate in Amazonia was so dry that extensive areas of savanna, with scattered trees dominated the region. Only in the last 10,000 years has a closed forest covered the Amazonian region again.

The present period of extreme dryness in the Sahara only started some 5000 years ago and then the desert expanded rapidly into the Sahel. Before that the Sahara was covered by steppe.

There was a Little Ice Age from A.D. 1550 till 1700, that was preceded by a period of several hundred years between about A.D. 900 or 1000 and about 1200 or 1300 with temperatures that were generally warmer than average. The cultivation of grapes for wine making was extensive throughout the southern portion of England from about 1100 to around 1300 (Lamb, 1965). This represents a northward latitude extension of about 500 km from where grapes are presently grown in France and Germany. Grapes were also grown in the north of France and Germany at this time, areas that until recently did not sustain commercial vineyards. As the Little Ice Age approached, the weather continually degenerated. There was an increase in the amount of Arctic sea ice. Once-productive Icelandic farms were covered by advancing glaciers. So serious was the climatic change experienced by Icelanders that the parent country Denmark considered evacuating all the islanders and re-settling them in Europe. In Greenland, harvests failed, farms were abandoned as the permafrost level rose and glaciers spread south. During the Little Ice Age the global average temperature dropped between 1 and 2 °C for several hundred years. The Baltic Sea and the Thames River froze regularly. Crops failed; famine and disease were rife in Europe. (Tkachuck, 1983).

In contrast to the above mentioned variability in the temperature of the earth Mann *et al.* (1998) presented a picture of stable temperatures in the Northern Hemisphere when they reconstructed the climate of the last 1000 years on the basis of annual tree rings, ice nuclei, coral and silt samples. On the basis of their reconstructed data they found about 900 years of approximately flat global temperature and a sudden rise after about 1900 (the hockey stick).

However, McIntyre and McKittrick (2002) claimed that the proxy data<sup>1</sup> of Mann *et al.* (1998) to create their temperature reconstruction for the years 1400 to 1980 AD contained "collation errors, unjustifiable truncation or extrapolation of source data, obsolete data, geographical location errors, incorrect calculation of principal components and other quality control defects". They found in fact that global average temperatures peaked in the fifteenth century, and not in the twentieth.

Von Storch *et al.* (2004) worked around the problem of data unavailability using a climate model that is able to reasonably simulate the 20<sup>th</sup> century temperature record. They concluded that Mann's pseudo-proxies greatly underestimated the amount of long-term variability.

### **The present**

Nevertheless, it is generally accepted that the climate is warming up with significant ecological consequences (Walther *et al.*, 2002, Thuiller *et al.*, 2005). Evidence is the melting of polar ice and glaciers. For example, the glaciers in nearly all parts of Europe have decreased more than 50% in volume during the 20<sup>th</sup> century. The sea level has risen 0.8–3 mm per year in the past century, depending on the region. Recently, central and northern Europe have received more rain than in the past. In contrast, southern and southeastern Europe have become drier.

There have also been changes in the flora. During the last thirty years the number of plant species has decreased in parts of Europe, which is attributed to loss of habitat and climate change. However, in other parts of Western Europe, amongst others The Netherlands, the number of plant species has increased because of an increase in warm-temperate species, whilst cool-temperate species have slowly decreased. On the other hand the number of plant species in Europe will probably decrease again during the 21<sup>st</sup> century because temperate to cool-temperate species that cannot adapt to the higher temperatures will become extinct. The length of the growing season for plants in The Netherlands has increased by an average of 10 – 14 days during the last thirty years. (RIVM, 2004; [www.rivm.nl](http://www.rivm.nl)).

RIVM (2004) predicted that extreme cold winters will no longer occur in the Northern Hemisphere towards the end of the present century, whilst extreme warm summers and heavy showers will occur more frequently. The rate of the sea level increase will probably accelerate and may continue to do so for centuries. Agriculture in Europe will profit with increased yields as a result of rises in temperature and atmospheric CO<sub>2</sub> and by expansion in a northerly direction and in central Europe. However, at the same time in parts of Southern Europe harvests will become more insecure because of water shortages due to reduced summer rainfall by about 20%. The intensity of rain showers is likely to increase and may become more tropical in nature. The higher temperatures will lead to increased evaporation that together with reduced precipitation will lead to more frequent droughts.

There are different predictions for temperature rises in Europe that range from 0.1 – 0.4 °C for every 10 years, compared to the world temperature rise of 0.95 °C during the last 100 years. The mean surface air temperature is predicted to increase by 1.5 to 5.8 °C by the end of the present century. The temperature in the northern latitudes is expected to rise more than elsewhere (IPPC, 2001).

### **Climate change in the Mediterranean region**

Temperatures have increased in the Iberian Peninsular in the last 100 years by 1.5 °C, somewhat above world (0.95 °C) and European average rises (0.6 °C). This rise has been particularly noted during winter. Rainfall has also been lower in winter, especially in the south. It has been projected (Tin, 2005) what a 2 °C world average temperature increase might mean for the Mediterranean region. The climate of the Mediterranean region would become hotter, drier and more variable. Annual mean temperature would increase by 1-2 °C over present conditions, but at inland locations, such as in Turkey, northern Italy and in Morocco, Algeria and Tunisia, at a distance from the sea, maximum temperatures could rise by up to

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<sup>1</sup> Climate reconstruction incorporates concurrent measurements from modern periods during which both instrumental and proxy indicators exist. Their relationship during the modern era (typically derived using statistical regression techniques) then is applied to proxy measurements from the past (when there were not instruments) to 'hindcast' climate.

5 °C. Annual precipitation would likely decrease by up to one-fifth over the southern Mediterranean, while reduction in summer rainfall over the northern Mediterranean could exceed 30%. Drought periods would be expected to shift in time and last longer.

With 2 °C global warming, the whole southern part of the Mediterranean would likely be at risk from forest fires all year round. In other parts of the region, the period of fire risk would be expected to increase by up to six weeks. Extreme fire risk would lengthen by over a month in the Iberian Peninsula, northern Italy and the Balkans.

The hotter and drier climate would lead to lower agricultural yields, particularly in summer crops that are not irrigated. Beans, soy beans and lentils are among the most affected crops in the region, with a reduction of up to 40% in yield depending on locations. The reduction in crop yields would be more severe in the southern than in the northern Mediterranean. A drier climate, accompanied by reduced precipitation and surface runoff, and increasing demand from the agricultural sector, would exacerbate the already high level of water stress in the region. It has been reported that a mere 1 °C warming is likely to lead to a reduction of 5-14% in water yields in Spain.

Latest studies show that global warming of more than 2 °C could lead to a loss of over 50% of plant species in the northern Mediterranean region, with losses exceeding 80% in north-central Spain and in the mountains, especially in France. An increase in forest fires would encourage the spread of invasive grass species, which in turn would fuel even more frequent and more intense fires.

Temperatures in Spain are forecast to rise steadily by 0.4 °C a decade in winter and by 0.7° C in summer. By 2100 temperatures in the hinterland of the Iberian Peninsula would be 5-7 °C higher in summer and 3-4 °C in winter. Temperatures on the coast would rise by some 2 °C less than the hinterland. The number of days with extreme high temperatures would rise. Rainfall prediction is less reliable. However, all world models indicate a significant fall in total annual precipitation. This will be noticed most in spring in all areas. Rainfall would increase in the already wet Northeast in winter and decrease in the already dry Southwest. In summer, rains will be less everywhere. Water resources would fall by 22% (Rodríguez, 2005). In the Atlantic North-eastern part of Spain global warming may increase productivity. The Mediterranean (most of Iberia) is characterised by summer drought and productivity may fall. Such changes would upset for some time the ecological balance and favour invasive and pest species ([www.iberianature.com](http://www.iberianature.com)).

#### **Possible changes in landscape and grassland composition**

Climate change expresses itself in the grassland-forest equilibrium. Long periods of low rainfall lead to fewer trees and more grassland. An example is the Great Plains in North America, which receives less than 400 - 600 mm of rain a year. However, this was not always so. During a cold period, some 12,000-14,000 years ago, the area was covered in spruce and deciduous forest. The trees retreated northward as the ice front receded, and the Great Plains has been a treeless grassland for the last 8,000-10,000 years (Trimble, 1980).

Lüscher *et al.* (2005) reported research results from Switzerland and New Zealand indicating that legumes and other dicotyledenous species showed a greater response to increased atmospheric CO<sub>2</sub> than grasses. In relation to global warming, the existence of C<sub>3</sub> and C<sub>4</sub> type grasses is very important, because C<sub>4</sub> grasses grow at higher temperatures (optimum 30-35°C), absorb CO<sub>2</sub> more efficiently and have greater water-use efficiency than C<sub>3</sub> grasses. The C<sub>4</sub> pathway has been recorded in some 3000 species of 18 families of flowering plants. C<sub>4</sub> photosynthesis first arose in grass subfamilies Chloridoideae (syn. Eragrostoideae) and Panicoideae, probably during the Oligocene epoch (24-35 mya). The Chloridoideae prefer hot, dry habitats; they increase in dominance with increased grazing pressure and N availability and the Panicoideae usually prefer humid, wet environments and generally decline in importance with increasing grazing pressure and increased soil N levels (Johnston, 1996). C<sub>4</sub> plants are thought to have evolved under conditions of high temperatures and lower CO<sub>2</sub> concentrations, in the early stages of plant evolution (Sage, 2004). The very big increase in C<sub>4</sub> species is clearly documented as having occurred in the late Miocene, 4-7 mya. Increasing seasonality has been widely suggested as an important climatic stimulus for this C<sub>4</sub> expansion (Pagani *et al.*, 1999). Rising temperatures would give a competitive advantage to C<sub>4</sub> plants. C<sub>4</sub> grasses have a greater DM production capacity than C<sub>3</sub> grasses, but the feeding value of C<sub>4</sub> grasses is lower than that of C<sub>3</sub> grasses, because

higher temperatures lead to lower digestibility and because C<sub>4</sub> grasses have a greater proportion of less digestible tissues (Hill *et al.*, 1989). In regions that are transitional between warm and cool climates grasslands contain both C<sub>3</sub> and C<sub>4</sub> grasses, e.g. in northern New Zealand (Campbell and Mitchell, 1996), Australia, (Johnston, 1996), South Africa (Franz-Odenaal *et al.*, 2002), Greece (Papanastasis, 1998), Spain and Portugal. With increasing temperatures and drier weather a shift towards more C<sub>4</sub> species is likely. This will have negative effects on feed quality from these grasslands. For example, *Chrysopogon gryllus* and *Dichanthium ischaemum*, which are widely distributed in more humid parts of central and southern Europe, including southern France, are low quality stemmy invasive grasses. *Cynodon dactylon* is found in overgrazed grasslands and *Hyparrhenia hirta* in the warmer parts of the northern Mediterranean (Papanastasis, 1998).

The following C<sub>4</sub> grasses, many of which are weeds, are known to occur in the European Mediterranean region:

<i>Andropogon ischaemum</i> , <i>A. hirtum</i>	<i>Hyparrhenia hirta</i>
<i>Chrysopogon gryllus</i> .	<i>Imperata arundinacea</i>
<i>Cynodon dactylon</i>	<i>Panicum sanguinale</i> , <i>P. lineare</i> , <i>P. debile</i>
<i>Dichanthium ischaemum</i>	<i>Paspalum dilatatum</i> , <i>P. vaginatum</i> , <i>P. distichum</i>
<i>Digitaria spp.</i>	<i>Setaria verticillata</i> , <i>S. italica</i> , <i>S. glauca</i> , <i>S. viridis</i>
<i>Echinochloa crus-galli</i>	<i>Sorghum halepense</i>
<i>Eleusine coracana</i>	<i>Sporobolus pungens</i>
<i>Eragrostis pilosa</i> , <i>E. megastacya</i> ,	<i>Stipa spp</i>
<i>E.poacoides</i>	

Changes in botanical composition of European alpine grasslands has also been reported (Stanisci *et al.*, 2005). Sub alpine species are progressing to higher levels up the mountains, particularly on east-facing slopes, whilst north-facing slopes will be less affected by invasive species.

An excellent recent global review on climate change and grasslands, particularly in relation to physiological changes and productivity was published by Morgan (2005).

## Conclusion

The global climate change is likely to have big long term effects on grassland composition, quality and biodiversity in southern Europe. However, in the short term, land abandonment in extensively farmed areas, which is often linked to the economic uncertainty and fragility of extensive farming systems, will have more severe consequences with the danger of increased wild fires, loss of biodiversity and desertification.

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## Temperate grasslands and global atmospheric change

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### Abstract

Recent reviews confirm and extend previous observations that elevated CO<sub>2</sub> concentrations stimulate photosynthesis, leading to increased plant productivity. Elevated CO<sub>2</sub> tends to reduce the sensitivity to low precipitations of grassland ecosystems, but induces a progressive N limitation which can be alleviated by supplying a significant external input of N in the form of mineral fertiliser or through the increased development of N fixing legumes. Other nutrients, such as phosphorus, may act as the main limiting factor restricting the growth response to [CO<sub>2</sub>] of legumes. The botanical composition of temperate grasslands will be affected by the rise in atmospheric CO<sub>2</sub> concentration, possibly through a decline in the relative abundance of grasses. Elevated CO<sub>2</sub> will also alter food quality to grazers both in terms of fine-scale (protein content, C/N ratio) and coarse-scale (legumes vs. grasses and C<sub>3</sub> versus C<sub>4</sub> species) changes. The management guidelines of grasslands will need to be adapted to global atmospheric and climatic changes and to increased climate variability.

Keywords: CO<sub>2</sub>, climate change, carbon sequestration, legume, productivity, forage quality.

### Introduction

Grassland covers about 70% of the world's agricultural area. Rising atmospheric CO<sub>2</sub> concentrations will likely affect several aspects of importance for grassland such as: the quantity and quality of the forage produced, plant species composition, soil fertility, and the potential to sequester carbon in the soil in order to mitigate the rise of CO<sub>2</sub>. Pastures and livestock production systems are extremely diverse. They occur over a large variation in climate and soil conditions and range from very extensive pastoral systems where domestic herbivores graze and browse rangelands to intensive systems based on forage and grain crops, where animals are mostly kept indoors.

### Atmospheric CO<sub>2</sub> rise and C sequestration by grassland ecosystems

Anthropogenic combustion of fossil fuels has caused mean concentrations of CO<sub>2</sub> in the atmosphere to reach and exceed 380 μmol mol<sup>-1</sup>, a level that is about 32 % greater than in pre-industrial times (Keeling and Whorf, 2005). Because CO<sub>2</sub> absorbs long-wave energy, it warms the Earth's atmosphere. Predictions of future atmospheric [CO<sub>2</sub>] in the year 2100 range between 540 μmol mol<sup>-1</sup> and 970 μmol mol<sup>-1</sup> (Houghton *et al.*, 2001). Additional inputs of carbon to the atmosphere will produce further warming (Houghton *et al.*, 2001) and may contribute to the occurrence of more-intense spells of heat (Meehl and Tebaldi, 2004).

The current increase in atmospheric CO<sub>2</sub> levels has, however, proved to be less marked than was previously anticipated from CO<sub>2</sub> emission records and oceanic uptake, which has led to postulate the existence of a carbon 'sink' in continental ecosystems. The demonstration of this sink has made it possible to envisage its use and development to sequester carbon and thus slow down the current rise in the greenhouse effect. The Marrakesh Accords allow biospheric carbon sinks and sources to be included in attempts to meet emission reduction targets for the first commitment period of the Kyoto protocol. Signatory Annex I countries may take into account any sequestration of carbon induced by 'additional human activities'. These activities principally target the storage of carbon in biomass and soil.

Carbon accumulation in grassland ecosystems occurs mostly below ground and changes in soil organic carbon stocks may result both of land use changes (e.g. conversion of arable land to grassland) and of

grassland management (Soussana *et al.*, 2004). The soil organic C sequestration potential is estimated to be 0.01 to 0.3 Gt C yr<sup>-1</sup> on 3.7 billion ha of permanent pasture worldwide (Lal, 2004). Thus soil organic C sequestration by the world's permanent pastures could potentially offset up to 4% of the global greenhouse gas emissions. In Europe, Vleeshouwers and Verhagen [2002], further quoted by Janssens *et al.* [2003] applied a semi-empirical model of land use induced soil carbon disturbances to the European continent, and inferred a carbon sink of 101 Gt C yr<sup>-1</sup> over grasslands (0.52 t C ha<sup>-1</sup> yr<sup>-1</sup>) with uncertainties greater than the mean. Using the eddy covariance method, the average net C storage was estimated at -0.13 kg C m<sup>-2</sup> yr<sup>-1</sup> (range -0.40 to +0.11) on average of nine European grassland sites. However, part of this carbon sink was offset by the emission of non CO<sub>2</sub> greenhouse gases (Soussana *et al.*, 2005).

### **Impacts of elevated CO<sub>2</sub> on photosynthesis and growth in grasslands**

Recent reviews confirm and extend previous observations that elevated CO<sub>2</sub> concentrations stimulate photosynthesis, leading to increased plant productivity and modified water and nutrient cycles (e.g., Nowak *et al.*, 2004; Kimball *et al.*, 2002). Experiments under optimal conditions show that doubled CO<sub>2</sub> increases leaf photosynthesis by 30-50% in C<sub>3</sub> plant species and by 10-25% in C<sub>4</sub> species, despite a small but significant down-regulation of leaf photosynthesis by elevated CO<sub>2</sub> at some sites (Ainsworth and Long, 2005; Ellsworth *et al.*, 2004). The canopy photosynthesis is also increased by 30 % (Casella and Soussana, 1997, Aeschlimann *et al.*, 2005). The stimulatory effect of elevated CO<sub>2</sub> on grassland ecosystem production above-ground averages about +17% (Campbell *et al.*, 2000, Ainsworth and Long, 2005) in ecosystem studies, although responses for particular systems and seasonal conditions can vary widely. This figure is about half that expected from consideration of the photosynthetic response to CO<sub>2</sub> alone. Under Free Air CO<sub>2</sub> fumigation grassland production was increased by 15-20% (e.g., Nowak *et al.*, 2004; Hebeisen *et al.*, 1997; Ainsworth *et al.*, 2003). However, the long-term response to elevated CO<sub>2</sub> may differ substantially from the short-term response. In the Swiss FACE experiment, the yield response of *L. perenne* to elevated [CO<sub>2</sub>] increased from 7 to 32% over the years under high N fertilisation. This increase was probably due to a decreased N limitation of plant growth (Lüscher and Aeschlimann, 2006). These results demonstrate that the immediate response of an ecosystem to a step increase in [CO<sub>2</sub>] at the start of the experiment may not represent an appropriate base to predict the response of the ecosystem to the ongoing slow increase in [CO<sub>2</sub>] in the atmosphere.

### **Interactions of elevated CO<sub>2</sub> with abiotic factors.**

As temperature increase enhances photorespiration in C<sub>3</sub> species (Long, 1991), the positive effects of CO<sub>2</sub> enrichment on photosynthetic productivity are usually greater when temperature rises. The long-term ratio of shoot dark respiration to photosynthesis was found to be approximately constant with respect to air temperature and CO<sub>2</sub> concentration (Gifford, 1995; Casella and Soussana, 1997). Although the stomatal conductance is decreased under elevated CO<sub>2</sub>, the ratio of the intercellular to the ambient CO<sub>2</sub> concentration is usually not modified and stomata do not appear to limit photosynthesis more in elevated compared to ambient CO<sub>2</sub> (Drake *et al.*, 1997). The relative enhancement of growth owing to CO<sub>2</sub> enrichment might be greater under drought conditions than in wet soil because photosynthesis would be operating in a more CO<sub>2</sub>-sensitive region of the CO<sub>2</sub> response curve. In the absence of water deficit, the C<sub>4</sub> photosynthesis is believed to be CO<sub>2</sub> saturated at present atmospheric CO<sub>2</sub> concentration. However, as a result of stomatal closure, it can become CO<sub>2</sub> limited under drought. An increased productivity from increased water-use efficiency is the major response to elevated CO<sub>2</sub> in a C<sub>3</sub> or C<sub>4</sub> crop that is exposed frequently to water stress (Casella *et al.*, 1997; Drake *et al.*, 1997; Aranjuelo *et al.*, 2005). Moreover, elevated CO<sub>2</sub> can reduce soil water depletion in different native and semi-native temperate and Mediterranean grassland (Morgan *et al.*, 2004). These results support a general view that elevated CO<sub>2</sub> reduces the sensitivity to low precipitations of grassland ecosystems (Volk and Niklaus, 2000; Morgan *et al.*, 2004).



## Interactions of elevated CO<sub>2</sub> with soil nutrients

Reviews of the available data indicate that, on average, plants grown at high nutrients supply respond more strongly to elevated CO<sub>2</sub> than nutrient-stressed plants (Poorter, 1998). FACE experiments confirm that high N levels increase relative response to elevated CO<sub>2</sub> (Nowak *et al.*, 2004). With *Lolium perenne*, in the Swiss FACE experiment, the above-ground DM yield response to elevated [CO<sub>2</sub>] increased from not significant to +17% (range +7 to +32%) when the N fertilisation was raised from 14 to 56 g N m<sup>-2</sup> yr<sup>-1</sup> (Schneider *et al.*, 2004; Lüscher and Aeschlimann, 2006). Under elevated [CO<sub>2</sub>], *L. perenne* showed a significant reduction in the shoot N concentration (Soussana *et al.*, 1996; Zanetti *et al.*, 1997). With a non-limiting N fertilizer supply, the leaf N concentration (% N) declined with the shoot dry-matter (DM) according to highly significant power models in ambient (% N = 4.9 DM<sup>-0.38</sup>) and in elevated (% N = 5.3 DM<sup>-0.52</sup>) CO<sub>2</sub>. The difference between both regressions was significant and indicated a lower critical leaf N concentration in elevated than in ambient CO<sub>2</sub> for high, but not for low values of shoot biomass. With the sub-optimal N fertilizer supplies, the nitrogen nutrition index of the grass sward, calculated as the ratio of the actual to the critical leaf N concentration, was significantly lowered in elevated CO<sub>2</sub> (Soussana *et al.*, 1996; Zanetti *et al.*, 1997). This indicated a lower inorganic N availability for the grass plants in elevated CO<sub>2</sub>, which was also apparent from the significant declines in the annual nitrogen yield of the grass sward and in the nitrate leaching during winter (Soussana *et al.*, 1996).

Over the 10 years of fumigation in the Swiss FACE, the most important change was the increasing response to elevated [CO<sub>2</sub>] of the annual N yield (from -13 to +29%) of *L. perenne* monocultures in the high N treatment but not in the low N treatment (Daapp *et al.*, 2000; Schneider *et al.*, 2004). Changes observed in the highly N-fertilised swards of *L. perenne* may be summarised as decreasing N limitation (Lüscher *et al.*, 2006) in reference to the concept of progressive N limitation in natural systems (Luo *et al.*, 2004). The CO<sub>2</sub> induced N limitation was alleviated in the high N treatment only, by supplying a significant external input of N in the form of mineral fertiliser. These results confirm that nitrogen is a major limiting factor for the response of grasslands to elevated CO<sub>2</sub>.

When other nutrients are not strongly limiting, a decline in N availability may be prevented by an increase in biological N<sub>2</sub>-fixation under elevated CO<sub>2</sub> (Gifford, 1994). Indeed, in fertile grasslands, legumes benefit more from elevated CO<sub>2</sub> than non-fixing species (Hebeisen *et al.*, 1997; Lüscher *et al.*, 1998) resulting in significant increases in symbiotic N<sub>2</sub> fixation (Soussana and Hartwig, 1996; Zanetti *et al.*, 1997). An experiment with N<sub>2</sub>-fixing and non-fixing alfalfa (*M. sativa*) showed that the uptake of mineral N from the soil did not increase under elevated [CO<sub>2</sub>] (Lüscher *et al.*, 2000). All the additionally harvested N under high [CO<sub>2</sub>] derived solely from increased activity of symbiotic N<sub>2</sub> fixation. This is in line with the results of *T. repens* in the Swiss FACE experiment (Zanetti *et al.*, 1996, 1997) and from an experiment conducted with micro-swards (Soussana and Hartwig, 1996). Nevertheless, other nutrients, such as phosphorus, may act as the main limiting factor restricting growth and yield response to [CO<sub>2</sub>] of legumes. This has been demonstrated both in calcareous grasslands (Stöcklin *et al.*, 1998) and under controlled environmental conditions (Almeida *et al.*, 2000). N<sub>2</sub>-fixation does, however, not limit the growth of clover experiencing P deficiency (Hogh-Jensen *et al.*, 2002), since a low P status (below 0.27%) induces changes in the relative growth of roots, nodules, and shoots rather than changes in the resource uptake rates per unit mass or area of these organs.

## Elevated CO<sub>2</sub> induced changes in C and N cycling below-ground

Plants grown under elevated CO<sub>2</sub> generally increase the partitioning of photosynthates to roots which increases the capacity and/or activity of below-ground carbon sinks. In *L. perenne* monocultures under elevated CO<sub>2</sub>, the imbalance between a strongly increased C uptake in the shoot zone and a relatively reduced N uptake from the soil leads to an increased partitioning (up to 108 % increase) of growth to the root system (Soussana *et al.*, 1996; Hebeisen *et al.*, 1997; Suter *et al.*, 2002). This was demonstrated from the strong relationship between the root fraction and the N concentration in shoots (Soussana *et al.*, 1996; Daapp *et al.*, 2001). The ratio between leaf area index and total plant (root and shoot) biomass varied with the nitrogen supply, the CO<sub>2</sub> concentration and the temperature (Calvet and Soussana, 2001).

Soil organic carbon stocks result from the balance between inputs and decomposition of soil organic matter. An increased allocation below-ground may lead to an increment in soil organic carbon content. In a mesocosm experiment with *L. perenne* monocultures, continuous CO<sub>2</sub> exchange measurements indicated an increased C storage below-ground (Casella and Soussana, 1997). The same trend was obtained in the Swiss FACE experiment, but the corresponding difference was not statistically significant (Aeschlimann *et al.*, 2005; Xie *et al.*, 2005). The supplemental C input at elevated [CO<sub>2</sub>] was mainly to the less protected soil particles >50 µm in size (Loiseau and Soussana, 1999a; Allard *et al.*, 2004; Xie *et al.*, 2005). This may imply a greater sink capacity for atmospheric CO<sub>2</sub>, but could also be accompanied by a more rapid turnover of the older, finer and more recalcitrant pools (Cardon *et al.*, 2001). The increase in C storage in the particulate soil organic matter with CO<sub>2</sub> concentration was found to be non linear and declining at above ambient CO<sub>2</sub> concentrations, which may indicate that the soil C sink in grasslands will become saturated in a high CO<sub>2</sub> world (Gill *et al.*, 2002). Due to a large variability of soil C, significant differences in total soil organic carbon content are very hard to detect in individual studies and are usually not significant (Loiseau and Soussana, 1999a; Gill *et al.*, 2002). Several studies (Newton *et al.*, 1996; Cardon *et al.*, 2001) have suggested a higher carbon turnover rather than a substantial net increase in soil carbon under elevated CO<sub>2</sub>. In a <sup>13</sup>C labelling experiment, the <sup>13</sup>C isotopic mass balance method was used to calculate, the carbon turnover in the stubble and roots and in the soil particulate organic matter above 200 µ. With *L. perenne* monocultures, elevated [CO<sub>2</sub>] stimulated the turnover of organic carbon in the roots, stubble and particulate OM fractions at high but not at low N supply (Loiseau and Soussana, 1999b). In the same study, the soil nitrogen cycle was investigated using <sup>15</sup>N-labelling. Elevated [CO<sub>2</sub>] reduced to a greater extent the harvested N derived from soil than that derived from fertilizer and significantly increased the recovery of fertilizer N in the roots and in the soil particulate organic matter fractions (Loiseau and Soussana, 1999b). The increase of fertilizer N immobilization in the soil fractions was associated with a decline of fertilizer N uptake by the grass sward, which supported the hypothesis of a negative feedback of elevated [CO<sub>2</sub>] on the sward N yield and uptake. Atmospheric CO<sub>2</sub> elevation had little effect on nitrifying and denitrifying enzyme activity in four European grasslands (Barnard *et al.*, 2004). Nevertheless, an increased greenhouse gas emissions of N<sub>2</sub>O was found in response to elevated CO<sub>2</sub> (Baggs *et al.*, 2003) in the Swiss FACE, which may exacerbate the forcing effect of elevated CO<sub>2</sub> on global climate. As a result of these interactions with soil processes, experiments which impose sudden changes in temperature or [CO<sub>2</sub>] and which last only few years, are unlikely to predict the magnitude of the long-term responses in crop productivity, soil nutrients (Thornley and Cannell, 2000) and carbon sequestration. This may imply that the actual impact of elevated CO<sub>2</sub> on yields in farmer's fields, could be less than in earlier estimates which did not take into account limitations by nutrients availability and plant-soil interactions.

### **Plant species dynamics and diversity**

Much of the world's grasslands are characterised by swards that are botanically diverse. In a field experiment at varying levels of plant species diversity, the enhanced biomass accumulation in response to elevated levels of CO<sub>2</sub> was greater in species-rich than in species-poor assemblages (Reich *et al.*, 2001). In some studies grassland communities grown in elevated CO<sub>2</sub> displayed a higher plant species diversity than controls under ambient CO<sub>2</sub> (Teyssonneyre *et al.*, 2002a), but this was not confirmed by Zavaleta *et al.* (2003).

The relative growth response to elevated CO<sub>2</sub> obtained for isolated plants cannot be used to predict the response in multispecies mixtures, but the CO<sub>2</sub> response of monocultures may be a better predictor (Poorter and Navas, 2003). In the Swiss FACE experiment, the interspecific differences in the response to elevated [CO<sub>2</sub>], particularly in the mixture, resulted in a consistent and significant increase in the proportion of *Trifolium repens* in the binary mixtures at both levels of N supply (Lüscher *et al.* 2005; Lüscher and Aeschlimann, 2006). Similarly, in more complex mixtures containing other grass, legume and non-legume dicot species, the proportion of legumes was significantly higher at elevated [CO<sub>2</sub>] (Lüscher *et al.*, 1996). This effect was also observed in diverse permanent plant communities in FACE and mini-FACE experiments (Ross *et al.* 2004; Teyssonneyre *et al.*, 2002a; Harmens *et al.*, 2004). In a mini-FACE experiment, elevated CO<sub>2</sub> significantly increased the proportion of dicotyledons

(forbs+legumes) and reduced that of the monocotyledons (grasses). Management differentiated this response as elevated CO<sub>2</sub> increased the proportion of forbs when infrequently and of legumes when frequently defoliated (Teyssonneyre *et al.*, 2002a).

However, not all grass species responded negatively to high CO<sub>2</sub>. In subsequent studies of between species competition among three grasses, it was observed that grasses that capture relatively more light per unit leaf area in mixtures than their competitors become increasingly dominant under elevated CO<sub>2</sub> (Teyssonneyre *et al.*, 2002b). Moreover, a high nitrogen use efficiency would confer a competitive advantage under elevated CO<sub>2</sub> to mixed grasses (Soussana *et al.*, 2005). Such experiments show that the diversity and botanical composition of temperate grasslands is likely to be affected by the current rise in the atmospheric CO<sub>2</sub> concentration, and that grassland management guidelines will need to be adapted to a future high CO<sub>2</sub> world.

Species composition change is also an important mechanism altering production and its value for grazing livestock in drier rangelands with woody shrub invasion and in warm humid climates with C<sub>4</sub> invasion. Woody plant proliferation in grasslands and savannas in recent history has been widely reported around the world. The causes for this shift in vegetation are controversial and center around changes in livestock grazing, fire, climate, and atmospheric CO<sub>2</sub> (Hibbard *et al.*, 2001). Increased CO<sub>2</sub> levels is predicted to increase C<sub>3</sub> plants over C<sub>4</sub> but the projected increase in temperature will favour the C<sub>4</sub> plants. Results from White *et al.* (2001) indicate that competition is highly important in limiting the invasion of C<sub>3</sub> grasslands by C<sub>4</sub> species.

### **Forage quality**

Animal requirements for crude proteins from pasture range from 7 to 8% for animals at maintenance up to 24 % for the highest producing dairy cows. In conditions of very low N status the reduction in crude proteins under elevated CO<sub>2</sub> may put a system into a sub-maintenance level for animal performance. C<sub>4</sub> grasses are a less nutritious food resource than C<sub>3</sub> grasses both in terms of reduced protein content and increased C/N ratios. Elevated carbon dioxide levels will likely alter food quality to grazers both in terms of fine-scale (protein content, C/N ratio) and coarse-scale (C<sub>3</sub> versus C<sub>4</sub>) changes (Ehleringer *et al.*, 2002). However, when legume development is not restricted by adverse factors (such as low phosphorus and low soil water), an increase in the legume content of swards may compensate for the decline in the protein content of the non-fixing plant species (Hartwig *et al.*, 2000; Allard *et al.*, 2003; Picon-Cochard *et al.*, 2004).

### **Interactions between elevated CO<sub>2</sub> and climate change**

Experiments with elevated CO<sub>2</sub> and increases in temperature and precipitation showed increased net primary production with strong multifactor interactions, including changes in species distribution and litter composition (e.g. Henry *et al.*, 2005; Zavaleta *et al.*, 2003; Shaw *et al.*, 2003). In a Mediterranean annual grassland, the largest increase in plant species diversity was found in response to the combination of warming, elevated CO<sub>2</sub> and increased precipitation (Zavaleta *et al.*, 2003). Future CO<sub>2</sub> levels may favor C<sub>3</sub> plants over C<sub>4</sub>; yet associated temperature increases may again favor C<sub>4</sub> species. Increases in climatic extremes may suppress C<sub>3</sub> dominance and promote C<sub>4</sub> species, including weeds, due to faster migration rates, greater production of seeds, better ability to colonize many habitats and quick maturity (White *et al.*, 2001). Combined to elevated CO<sub>2</sub>, climate change is therefore likely to cause profound changes in the diversity, productivity and stability of grassland ecosystems.

### **Conclusions**

There are three critical issues for the fate of temperate grasslands in a high CO<sub>2</sub> world with an altered climate:

First, the production and the quality of forages will be affected by the climatic variation and by the frequency of extreme climatic events (Porter and Semenov, 2005). To some extent, elevated CO<sub>2</sub> will reduce the vulnerability of pasture and forage production to climatic variation and climatic change. However, for a number of sub-optimal environmental and soil conditions, the increased resource use

efficiency of plant growth under high CO<sub>2</sub> will not allow to prevent a decline in the productivity and quality.

Second, global environmental change - e.g., rising temperature, changing precipitation, rising atmospheric [CO<sub>2</sub>] - will become a major driver of plant diversity change and loss in the 21<sup>st</sup> century. A recent modeling study of 1350 European plant species predicted that half of these species will become classified as 'vulnerable' or 'endangered' by the year 2080 due to rising temperature and changes in precipitation (Thuiller *et al.*, 2005). Although such model predictions are highly uncertain, experiments do support the concept of fast changes in species composition and diversity under elevated CO<sub>2</sub>, with complex interactions with other global change drivers.

Third, the net carbon exchange by terrestrial ecosystems will be affected by projected global warming, and by the increased incidence of extreme climatic events such as heat waves and droughts (Ciais *et al.*, 2005). The conservation of grassland carbon stocks and the role of grasslands as carbon sinks will become increasingly difficult to preserve in an altered climate with a high temporal variability and under high atmospheric CO<sub>2</sub> concentrations which may saturate the C sink in soils.

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# Implications of climate change for grassland: impacts, adaptations and mitigation options

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## Abstract

Climate change associated with greenhouse gas (GHG) emissions has important implications for Europe's grasslands. Projected scenarios indicate that increased temperatures and CO<sub>2</sub> concentrations have potential to increase herbage growth and to favour legumes more than grasses, but changes in seasonal precipitation would reduce these benefits particularly in areas with low summer rainfall. Further problems may arise from increased frequency of droughts, storms and other extreme events. Farm-scale adaptive responses to climate change are identified. Grassland agriculture also contributes to GHG emissions, particularly CH<sub>4</sub> and N<sub>2</sub>O, and agricultural land management affects net carbon balances and C sequestration. Management options are identified for mitigating grassland agriculture's contribution to GHG emissions which need to be developed further in a holistic way that considers other pressures.

Key words: climate, grassland, Europe, carbon dioxide, methane, nitrous oxide

## Introduction

There is increasing evidence that the world's climate is changing and that the rate of change since the onset of the industrial revolution is greater than would be expected from natural variability alone. The Third Assessment Report (TAR) of the Intergovernmental Panel on Climate Change (IPCC, 2001a) details the extent of recent global change. Key indicators of change (increased mean temperatures, changes in patterns of precipitation, increased cloud cover, more frequent floods and other extreme events) are widely accepted to be due, at least in part, to the radiative forcing effects of increased concentrations of atmospheric greenhouse gases (GHGs), exacerbated by land-use changes. Carbon dioxide (CO<sub>2</sub>) is the principal GHG by virtue of its high atmospheric concentration (at the present time about 365 ppmv, having increased by >30% since *ca.* 1750). Methane, nitrous oxide and halogens are the other main GHGs and have also increased over the same period.

Changes in climate have important implications for agriculture generally, including grassland-based livestock production and the wider objectives of grassland management. While the agricultural industry is well adapted to some seasonal variability, departures from average weather conditions have, in recent years, resulted in a range of management problems including issues of heat stress and animal welfare, storage and applications of manures, and coping with storms, floods and droughts. As these issues increase in frequency and severity they present increasing challenges for farmers, researchers and policy makers. Although the major causes of increased GHG emissions are due to population growth and industrialization, agriculture contributes to emissions of CO<sub>2</sub> through its use of fossil fuels during cultivations, and indirectly through energy-intensive inputs, such as fertilizers. Of the other GHGs, methane has increased by over 150% and nitrous oxide by about 17% since *ca.* 1750. Grassland agriculture is a significant contributor to increased emissions of these gases.

Table 1. Some key features of climate scenarios for Europe.

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<p><i>Temperature</i></p> <ul style="list-style-type: none"><li>• Annual temperatures increase by 0.1-0.4°C per decade. Effect greatest over southern Europe (Spain, Italy, Greece) and northeast Europe (Finland, western Russia) and least along the Atlantic coastline.</li><li>• Rapid winter warming (0.15–0.6°C per decade) of continental interior of eastern Europe. In summer, southern Europe warming 0.2-0.6°C per decade and northern Europe warming 0.08-0.3°C per decade.</li><li>• Less frequent cold winters (i.e. those that occurred 1 year in 10 during 1961–1990) and disappearing almost entirely by the 2080s. Hot summers become much more frequent. Under the 2080s scenario, nearly every summer is hotter than the 1-in-10 hot summer as defined under the present climate.</li><li>• All model simulations show warming in the future across the whole of Europe and in all seasons.</li><li>• Alternative scenario, at present considered low-risk (but high potential impact) of regional cooling due to shutdown of Atlantic thermohaline.</li></ul> <p><i>Precipitation</i></p> <ul style="list-style-type: none"><li>• Increased annual precipitation in northern Europe (+1-2% per decade), small annual decreases across southern Europe (maximum –1% per decade), and small or ambiguous changes in central Europe.</li><li>• Marked contrast between winter and summer patterns of precipitation change. Most of Europe gets wetter in the winter season (+1-4% per decade), except in Turkey and Balkan region. Pronounced summer north-south gradient: northern Europe increases by 2% per decade, and southern Europe decreases (up to -5% per decade).</li><li>• Only for the A2-high GHG emissions scenario are there substantial areas (Fennoscandia and north-west Europe) where precipitation changes by the 2020s are larger than might occur within natural climate variability. Even for this scenario with rapid global warming, not all regions in Europe have well-defined precipitation signals from GHG-induced climate change by the 2080s.</li></ul> <p><i>Weather Extremes</i></p> <ul style="list-style-type: none"><li>• It is considered likely that frequencies and intensities of summer heat waves will increase throughout Europe; that intense precipitation events will increase in frequency, especially in winter, and that summer drought risk will increase in central and southern Europe; and possible that gale frequencies will increase.</li></ul> <p><i>Sea Level</i></p> <ul style="list-style-type: none"><li>• Although global-mean sea level may rise by up to 70 cm during 21st century, due to thermal expansion and glacial melt, regional effects in Europe will vary because of post-glacial tectonic movements.</li><li>• Potential interactions of sea-level rise with increased storms and tidal surges.</li></ul>
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Sources: Kundzewicz and Parry (2001), Hulme *et al.* (2002), Wood *et al.* (2005)

As these have a much greater radiative forcing potential than CO<sub>2</sub>, there is now increasing pressure to curb their emissions.

In this paper we review the key features of future climate change in the context of grassland in Europe, and consider the possible impacts and adaptations and the need for integrated management options for reducing the emissions of GHGs from grassland.

## Impacts and adaptations of anticipated future climate change for grassland in Europe

The TAR Report (IPCC, 2001a) estimated that by 2100 global temperatures could increase by between 1.4 and 5.8°C, and that CO<sub>2</sub> concentrations could reach 500-900 ppmv. Table 1 summarizes the key features of 21st century climate change for Europe and highlights some of the geographical variation in anticipated effects. In general terms vulnerability is greatest in Mediterranean and southern Europe (Schroter *et al.*, 2005), due to summer heat and drought, and also at the highest latitudes and elevations, where natural ecosystems such as wetlands, tundra and periglacial vegetation are threatened, and changes in snowfall affect summer water availability. The adaptation potential for natural systems is low, even though these systems contribute to a range of ecosystem services. In contrast, the adaptive capacity of many managed land-use systems in Europe is relatively high (IPCC, 2001b). Climate-change induced effects such as coastal and river flooding, droughts, and threats to some vulnerable habitats are likely to be felt locally, whereas the effects of higher temperatures and enhanced CO<sub>2</sub> in situations where water is not the limiting resource, e.g. in northern and north-west Europe, could be generally positive for agricultural production. Increased concentrations of CO<sub>2</sub> may modify the responses of grassland to the effects of changes in temperature and in water availability.

A recent major UK study involving sward growth experiments under elevated CO<sub>2</sub> and its interaction with temperature, linked to regional and livestock enterprise scenarios applied to the LEGSIL grassland model, has revealed the likelihood of several impacts and highlighted possible farmer responses (Topp and Doyle, 1996; Hopkins *et al.*, 2003; Harmens *et al.*, 2004; Hopkins, 2004; Scholefield *et al.*, 2005). These include:

- Increased herbage growth potential: compared with ambient CO<sub>2</sub> and temperature (ACAT) DM yield for swards cut frequently was enhanced by elevated temperature (ET), elevated CO<sub>2</sub> (EC) and elevated CO<sub>2</sub> + temperature (ECET) by 30%, 46% and 56%, respectively.
- Changes favour legumes, particularly red clover and lucerne, more than grasses, but reduced opportunities for grazing and harvesting on wetter soils.
- Changes in herbage quality with higher content of water-soluble carbohydrate and lower N content at a given yield.
- Greater incidence of summer drought (which at its most serious would off-set the advantages that may arise).
- Leaching may be increased due to increased winter rainfall, but some potential for improved N use by the growing sward and the animal.
- Farm-scale adaptive responses could include greater reliance on conserved food for housed livestock, increased use of maize, greater use of forage legumes in place of N-fertilized grass, increased need for manure storage and improved applications, increased demand for irrigation (although with greater pressure on water resources this option may be unavailable in many areas). Feed budgeting for dry seasons in northern Europe could require alternative forage species / mixtures adapted to drought and managed accordingly.
- Adaptive responses have further implications for N<sub>2</sub>O and CH<sub>4</sub> emissions.

Aside from grassland agriculture, there are also important challenges for the management of grassland and related habitats that contribute to amenity and ecosystem services, including their role in soil and water conservation, and in nature and biodiversity protection. There are potential threats to many species and habitats; extinctions are linked to the frequency of extreme weather events (Easterling *et al.*, 2000). Species with bounded distributions are under high risk, as are those with poor dispersal capability and restricted range (Green *et al.*, 2003).

## Grassland management to mitigate greenhouse gas emissions

Grassland-based agricultural systems contribute to the biosphere-atmosphere exchange of radiative-forcing gases, with fluxes intimately linked to management practices (Soussana *et al.*, 2004). Carbon dioxide is exchanged with the soil and vegetation, N<sub>2</sub>O is emitted by soils and manures and CH<sub>4</sub> is emitted by livestock and manure.

In addition to the GHG emissions generated naturally from these activities, mechanical operations on farms consume fossil fuel, and there is an indirect consumption of fuel, and therefore to CO<sub>2</sub> emissions, from the manufacture and transport of farm inputs, particularly fertilizers. On the other hand, the growing of crops and pasture, the maintenance of other farmland vegetation (hedges, trees, scrub etc.) and the accumulation of carbon as organic matter in soils can all contribute to the temporary removal, and in some cases to the long-term sequestration, of CO<sub>2</sub> from the atmosphere. There is the potential to increase the sequestration of CO<sub>2</sub> (Freibauer *et al.*, 2004) and also to reduce the emissions of methane and nitrous oxides from changes in agricultural and other land management.

However, due to the multiple interactions, these systems are quite complex. Hence, they require an integrated approach as most mitigation options to reduce emissions involve important trade-offs. As much as one-half of the climate mitigation potential of some C sequestration options could be lost when increased emissions of other greenhouse gases (N<sub>2</sub>O and CH<sub>4</sub>) were included (Smith *et al.*, 2000, 2001). Opportunities to mitigate CH<sub>4</sub>, N<sub>2</sub>O and CO<sub>2</sub> emissions from agriculture therefore need to be developed.

### Methane

Enteric fermentation in livestock is the main agricultural source of methane in Europe, with emissions from livestock manures accounting for most of the rest. Methane is produced as a by-product of digestion of structural carbohydrates, principally cellulose, due the action of microbes (bacteria, fungi and protozoa) in the rumen. During this digestion, mono-saccharides are fermented to H<sub>2</sub>, CO<sub>2</sub> and volatile fatty acids (VFAs) such as acetate, propionate or butyrate. As part of this stage of ruminant digestion some of the microbes (called methanogens) produce CH<sub>4</sub> from and CO<sub>2</sub>.

Several studies have formulated abatement strategies to mitigate CH<sub>4</sub> emissions. Mitigations can generally be split into two groups, (i) those aimed at enteric fermentation and (ii) those targeting manure management.

#### *Enteric fermentation strategies*

These may be addressed at three different levels (Jarvis, 2001): dietary changes, direct rumen manipulation and systematic changes. The dietary changes suggested so far involve measures which enhance the efficiency of feed energy use. Even assuming a constant percentage of methane loss, this strategy will decrease methane loss per unit of product and probably decrease CH<sub>4</sub> emissions in the long term (Johnson and Johnson, 1995). The most natural way to depress CH<sub>4</sub> production would be to manipulate the diet to give high rates of fermentation and/or passage through the rumen, which might increase the molar percentage of propionate and decrease that of acetate in the rumen VFAs. These changes in VFA proportions have been associated with a decrease in the fibre content of the diet (i.e. switching to high starch concentrates or maize silage). Ingestion of organic acids (aspartate, malate and fumarate) and yeast culture has been associated with reduced emissions in total CH<sub>4</sub> per cow and also with beneficial increases in animal product (i.e. milk yield). Their reactions in the rumen produce propionate and butyrate, which behave as electron metabolic sinks competing for the H<sub>2</sub> and thus minimising the chances of methanogens to produce CH<sub>4</sub> from H<sub>2</sub>.

The use of some plant extracts (i.e. tannins, saponins) has also been associated with CH<sub>4</sub> reduction (Sliwinski *et al.*, 2002; Hess *et al.*, 2003; Carulla *et al.*, 2005; Hu *et al.*, 2005; Puchala *et al.*, 2005). As yet there is no consensus on its efficacy (Newbold and Rode, 2005).

There are some drawbacks to using these dietary supplements. The organic acids are not commonly used as yet, and they may also trigger pH problems in the rumen. Wallace (2005) showed that the pH problem may be overcome by the encapsulation of the organic acid (i.e. fumarate). Plant extracts may also have anti-nutritional effects and even be toxic (Teferedegne, 2000). For instance, in a study by Hess

(2005), extracted tannins had a positive effect on feed rates and hence a possible reduction of CH<sub>4</sub> per kg product, whereas the use of shrub legumes rich in tannins resulted in decreased feed rates. Yeast culture, on the other hand, although variable, may be promising as a successful mitigation option as it is already in common use.

There have been several attempts to reduce enteric CH<sub>4</sub> production through direct rumen manipulation; for instance, defaunation of protozoa may decrease the number of methanogenic bacteria as an important proportion of rumen methanogenic bacteria are parasitic to protozoa (Takahashi, 2005). The main drawbacks though are that protozoa defaunation may trigger some metabolic diseases.

The ingestion of ionophores acts as propionate enhancers and hence increases the ratio of propionate:acetate. Their use is very limited as they are antibiotics (i.e. monensin) and their main drawback is that they may enhance bacterial resistance to antibiotics.

Some changes in the dietary fat contents of the ration have been described to reduce CH<sub>4</sub> emissions from ruminants (Dong *et al.*, 1997; Machmüller *et al.*, 1998; Johnson *et al.*, 2002; Giger-Reverdin *et al.*, 2003) as some fats alter the ruminal microbial ecosystem and, in particular, the competition for metabolic H<sub>2</sub> between the CH<sub>4</sub> and propionate production pathways (Czerkawski, 1972).

Systematic changes may involve identifying animal breeds which result in a reduction of CH<sub>4</sub> output per animal. However, and so far, no clear evidence has been found (Münger and Kreuzer, 2005). Waghorn *et al.* (2005), for instance, found that variability within animals is greater than between animals, and it is therefore difficult to come to any conclusions. Increasing productivity per head (i.e. milk yield per cow), or increasing the number of lactations for which the average cow remains economically productive, would decrease CH<sub>4</sub> production per unit of milk, and within the framework of production quotas would decrease total CH<sub>4</sub> emissions. Although more intensive forms of animal production tend to decrease total CH<sub>4</sub> output, they might not be compatible with other issues for water, atmosphere, soil, biodiversity, landscape or animal welfare.

### ***Manure management***

Opportunities to decrease total CH<sub>4</sub> outputs from farming systems are limited to either increasing the O<sub>2</sub> supply to restrict methanogenesis, minimizing the release of CH<sub>4</sub> to the environment (e.g. covered lagoons) or using anaerobic digesters to produce more CH<sub>4</sub> in a controlled environment and hence use this CH<sub>4</sub> as a source of energy. This last technique could represent a future sustainable option, but currently has the drawback of high capital cost.

### **Nitrous oxide**

Nitrous oxide is formed in the soil through nitrification and denitrification and is controlled by a number of soil factors, including moisture content (del Prado *et al.*, *in press*), temperature (Hatch *et al.*, 2005), fertiliser additions (del Prado *et al.*, *in press*), pH (Merino *et al.*, 2000), organic matter content (Smith *et al.*, 1997; Chadwick *et al.*, 1998; Estavillo *et al.*, 2002), nitrate (NO<sub>3</sub><sup>-</sup>) and ammonium (NH<sub>4</sub><sup>+</sup>) (Tiedje, 1988; Granli and Bockman, 1994).

Nitrate and NH<sub>4</sub><sup>+</sup> in the soil are subject to the following process dynamics. Nitrate may: (1) undergo denitrification to gaseous oxides of N and to N<sub>2</sub>; (2) be taken up by organisms (assimilatory reduction); (3) be used by micro-organisms as an electron acceptor and become reduced to NH<sub>4</sub><sup>+</sup> (dissimilatory reduction); (4) be leached or removed in run off; or (5) accumulate in the soil. Ammonium may: (1) be taken up by plants; (2) be immobilised in microbial biomass; (3) nitrify to NO<sub>3</sub><sup>-</sup> and be partially lost as gaseous oxides of N (4); be leached; (5) accumulate in the soil (Paul and Clark, 1996); or (6) be volatilised as ammonia (NH<sub>3</sub>).

The regulation of trace N-gas production via nitrification and denitrification has been described by the 'hole-in-the-pipe' conceptual model (Firestone and Davidson, 1989). The rate of the processes (denitrification and nitrification) and the relative proportions of end products are controlled at two different levels. First-level factors control the movement of N through the 'pipe'. Second-level factors control the partitioning of the reacting N species to N<sub>2</sub>, N<sub>2</sub>O, NO, etc. and therefore control the size of the 'holes' in the pipe through which the gases 'leak'.

In general, N<sub>2</sub>O emissions can be reduced by implementing practices aimed at enhancing the ability of the crop to compete with processes that lead to the escape of N from the soil-plant system (Freney, 1997). For instance, there are several methods for increasing the efficiency of the crop to remove mineral N from the soil. These include improving fertilizer efficiency (Brown *et al.*, 2005), optimising methods and timing of applications (Dosch and Gutser, 1996), using ammonium-based fertilisers rather than nitrate-based ones (Eichner, 1990; Dobbie and Smith, 2003) and employing nitrification chemical inhibitors (Dittert *et al.*, 2001; Merino *et al.*, 2002; Macadam *et al.*, 2003).

Increasing the soil aeration may significantly reduce N<sub>2</sub>O emissions. Improving drainage would be particularly beneficial on grazed grassland (Monteny *et al. in press*). Hence, avoiding compaction by traffic, tillage (Pinto *et al.*, 2004) and grazing livestock may help to reduce N<sub>2</sub>O emissions. Housing system and management will also influence N<sub>2</sub>O emissions, e.g. straw-based manures result in greater N<sub>2</sub>O emissions than slurry-based ones (Groenestein and Van Faassen, 1996). Minimizing the grazing period is likely to reduce N<sub>2</sub>O emissions as long as the slurry produced during the housing period is uniformly spread.

### **Carbon dioxide**

Carbon dioxide is formed naturally in grassland systems through respiration (below-ground soil, shoot plants and herbivores) and is fixed into carbohydrates via photosynthesis. Grasslands are generally regarded as potential sinks for CO<sub>2</sub> although soil management factors such as the frequency of ploughing and reseeding might alter the potential for carbon sequestration. Other management factors may also affect on the whole C balance of a farm, including emissions from farm machinery, and indirect CO<sub>2</sub> emissions associated with fertiliser manufacturing, transport of feed and other inputs to the farm. There is also the issue of the C balance of the whole farming and food supply business, including the fuel consumption issues of food chains and connectivity between the farm and the consumer (Jones, 2001).

There are a number of possible C sequestration measures that may be applied on grasslands and some present management schemes have probably helped to maintain soil-C stocks (Smith, 2004; Freibauer *et al.*, 2004). These include improving efficiency of animal manure and crop residue use, reducing soil disturbance, maximising the C returns in manure, use of deeper rooting species, application of sewage sludge or compost to land, irrigation, extensification and improved management to reduce wind and water erosion. The increase in soil C content after a shift from arable to grassland is partly explained by a greater supply of C to the soil under grass, mainly from the roots, but also from the shoot litter, partly by the increased residence time of C due to the absence of tillage (Soussana *et al.*, 2004). Whereas this rate of increase of soil C after conversion to grassland is slow, the rate of C disappearance from soil after returning grassland to arable is rapid. A grassland sward aged more than 20 years no longer acts as a C sink (Frank, 2002). Converting grassland to forest can lead to an accumulation or to a release of soil C, depending on the conditions (Post and Kwon, 2000). Introducing short duration leys tends to have an intermediate C sequestering potential between crops and permanent grasslands (Soussana *et al.*, 2004).

In this context we can also include the growing of biofuel and other industrial crops on agricultural land, with the potential for non-food crops to further mitigate CO<sub>2</sub> emissions by displacement of fossil fuels.

### **The need for integration**

There is need to develop useful tools in order to explore agricultural systems in a holistic way. Climate change, and consequently greenhouse gases, unfortunately, account for only a part of the whole challenge in grassland systems. Agriculture is also subject to other pressures, including environmental (i.e. eutrophication, erosion, and acidification), socio-economic and sustainability issues. Identification of win-win strategies requires development of appropriate modelling systems together with the acquisition of field and farm data (Scholefield *et al.*, 2005).

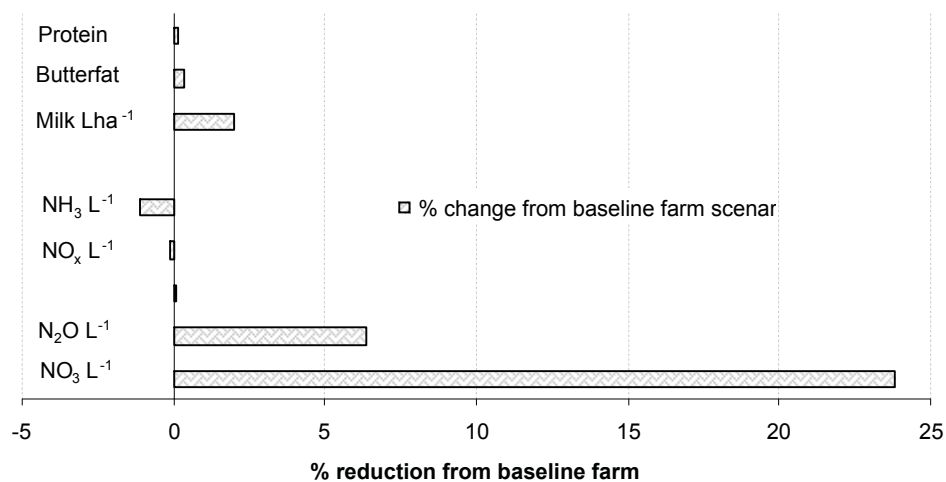


Figure 1. Percentage reduction from baseline farm scenario in environmental losses per L of milk ( $\text{NO}_3^-$  leaching,  $\text{N}_2\text{O}$ ,  $\text{CH}_4$ ,  $\text{NO}_x$  and  $\text{NH}_3$ ) and in milk yield and properties.

So far, some modelling studies have been developed to (i) assess the effects of different dietary strategies on the sustainability of a grassland system (del Prado *et al.*, 2006), (ii) evaluate the economic cost for implementing mitigation strategies for GHGs (Jarvis, 2001), (iii) evaluate the impact of  $\text{NO}_3^-$  leaching abatement measures on  $\text{N}_2\text{O}$ ,  $\text{NH}_3$  and  $\text{CH}_4$  emissions (Brink *et al.*, 2005; del Prado *et al.* 2005), (iv) assess successful mitigation strategies for GHGs (Schils *et al.* 2005), and (v) evaluate not only environment ( $\text{N}_2\text{O}$ ,  $\text{CH}_4$ ,  $\text{NH}_3$ ,  $\text{NO}_3^-$  and P leaching) and economics, but other attributes which define the sustainability of a farm (e.g.  $\text{SIMS}_{\text{DAIRY}}$  model: del Prado *et al.*, *this congress*).

For instance, using the  $\text{SIMS}_{\text{DAIRY}}$  model we compared environmental losses, milk yield and milk properties in two typical dairy farms in the UK ( $2 \text{ LU ha}^{-1}$ , 30.5 ha grazed grass, 16.5 ha cut grass and 3 ha maize) which differ only in terms of their past grassland management. Whereas the baseline farm had a history of long-term grassland with old swards (>11 years), the second farm had a history of short-term grass leys with new swards (2-3 years). As indicated in the previous sections, the baseline farm's past history would offer more opportunities for C sequestration than would the history of the second farm. However, if we compare the predicted environmental losses (Figure 1) of the  $\text{SIMS}_{\text{DAIRY}}$  model, we find that  $\text{NO}_3^-$  leaching (24%) and  $\text{N}_2\text{O}$  (6%) per L of milk were significantly lower from the second farm than from the baseline farm. Milk yield and milk properties (butterfat and protein) were also slightly improved, which may have a modest impact on the economy of the dairy farm.

## Conclusions

Climate change presents a number of challenges for the future management of grasslands in Europe, including effects on biodiversity and ecosystem functions, but particularly in terms of agricultural adaptations to ensure food production. This needs to be considered against a background of global population growth and climate change impacts on agriculture in other continents which could have profound impacts on world food supplies. There remain uncertainties about the effects and timescales of climate change, and regional variations, and possible outcomes such as a shutdown of the Atlantic thermohaline circulation, though at present a high impact, low probability event (Wood *et al.*, 2005) underline the potential dangerous consequences of failing to adapt in the short term, and to develop long-term mitigation responses to reduce the causes of climate change for the longer term.

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# A whole farm approach for mitigation of greenhouse gas emissions from dairy systems

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## Abstract

Dairy systems are a significant source of greenhouse gases. We propose the development of mitigation strategies in a whole farm approach, ensuring that the interactions between carbon and nitrogen cycles within the system are taken into account. It reveals the trade-offs between the different greenhouse gases. Furthermore, an integrated system approach shows the effect of greenhouse gas mitigation strategies on other environmentally relevant emissions like ammonia volatilisation and nitrate leaching. The whole farm approach was applied in a study to assess the effect of changes in nitrogen management on greenhouse gas emissions. Farm data were collected from the 'cows & opportunities' project, a research network of 17 dairy farms in the Netherlands. These farms are primarily committed to reduce nitrogen losses. Over a period of five years they have reduced the fertiliser inputs, improved manure management and optimised their feeding strategies. The farm gate nitrogen surplus was reduced from 270 to 176 kg ha<sup>-1</sup> yr<sup>-1</sup>. The improved nitrogen management reduced the nitrous oxide emissions from 1.03 to 0.85 g N (kg milk)<sup>-1</sup>. The methane emission decreased from 38.7 to 38.1 g CH<sub>4</sub> (kg milk)<sup>-1</sup>. Overall, the emission of greenhouse gases was reduced from 1.59 to 1.46 kg CO<sub>2</sub>-equivalents (kg milk)<sup>-1</sup>.

Keywords: nitrous oxide, methane, carbon dioxide, farming system, nitrogen cycle.

## Introduction

In the European Union, the agricultural sector contributes approximately 10% to the total greenhouse gas (GHG) emissions. Until now, research has mainly focussed on identifying the sources, and reviewing individual mitigation options. Most studies focus on a single gas, and the mitigation options are viewed as isolated activities. Effective mitigation strategies can only be developed within a whole farm approach. It ensures that interactions between the carbon and nitrogen cycles are taken into account, and reveals the trade-offs between emissions of the different greenhouse gases. Also, the possible effect of greenhouse gas mitigation strategies on other environmentally relevant emissions, like ammonia and nitrate, can be assessed better in a whole farm approach. Moreover, farmers will more readily adopt mitigation strategies if these are tailored to their specific farming system.

The objective of this paper is to present a farm level method for mitigation of greenhouse gas emissions for ruminant livestock systems. The whole farm approach is illustrated with results from 17 dairy farms participating in the 'cows & opportunities' project.

## Methods

In this paper, we consider greenhouse gas emissions at the farm level. A ruminant livestock farm is defined as an enterprise that transforms external resources, as feed and mineral fertiliser, into milk and meat (Figure 1). Inputs and outputs are designated to or derived from the relevant pools, respectively. The inner nutrient cycle (animal-soil-crop) represents the grazing cycle, with direct herbage intake by animals and direct faeces and urine excretion to the soil, whereas the outer nutrient cycle (animal-manure-soil-crop-feed) represents the housing system. In the full accounting approach, we include both direct and indirect emissions of greenhouse gases methane, nitrous oxide and carbon dioxide. A more detailed account of the methodology is given by Schils *et al.* (2005).

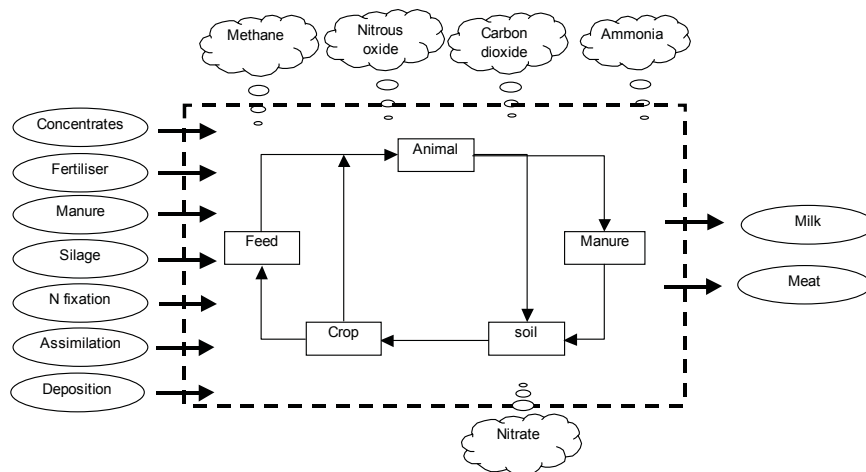


Figure 1. Flow diagram of a ruminant livestock system.

Data were used from the 17 dairy farms participating in the 'cows & opportunities' network (Oenema *et al.*, 2001; Schils *et al.*, 2006). These farms are primarily committed to reduce nitrogen losses. They are located on sand (11), clay (4) and peat (2). The data collection was carried out by the farmers themselves, by suppliers of fertilisers, feed and services, by milk and meat buyers, and by participating research organisations. For the baseline, we used data from the year preceding the start of the project (1997). Hence, no specific management changes had yet been adopted by the farmers. Between 1997 and 2002, the participating farmers reduced the fertiliser inputs, improved manure management and optimised their feeding strategies. The farm gate nitrogen surplus was reduced from 270 to 176 kg ha<sup>-1</sup> yr<sup>-1</sup>.

### Results and discussion

Between 1997 and 2002, the greenhouse gas emissions per kg milk have been reduced from 1.59 to 1.46 kg CO<sub>2</sub>-equivalents, a reduction of 8% (Figure 2). The largest reduction was observed on farms on mineral soil types.

The reduction of nitrous oxide emissions accounts for approximately 65% of the total reduction. A lower fertiliser application and less grazing are the driving forces for the lower emission. On the other hand, the emissions from manure application have increased slightly.

The methane emission per kg milk from enteric fermentation was reduced from 27.7 kg in 1997 to 26.7 kg in 2002. Considering the 17 farms in 'the cows & opportunities project', the change in methane emission per kg milk varied from -8.6 to +3.2 kg. Reduction of the feed intake per kg milk was not only brought about by efficient feeding of the dairy cattle itself, but also by a reduction of the proportion of young-stock. The reduction in methane emission from enteric fermentation was partially offset by the increase in methane emission from manure storage, which is related to the reduction of the grazing time of dairy cattle.

The reduction in carbon dioxide emissions accounts for approximately 20% of the total reduction, predominantly through the change in indirect emissions. The reduction in indirect energy use is mainly related to the reduced inputs of concentrate and fertiliser.

The emission of greenhouse gases per kg milk was reduced at an average ratio of 29 g CO<sub>2</sub>-equivalents per g nitrogen surplus. We suggest that the use of farm N surplus as an indicator for greenhouse gas emissions is a reasonably quick and robust method. Data on farm nitrogen surpluses are becoming more available throughout most countries of the European Union.

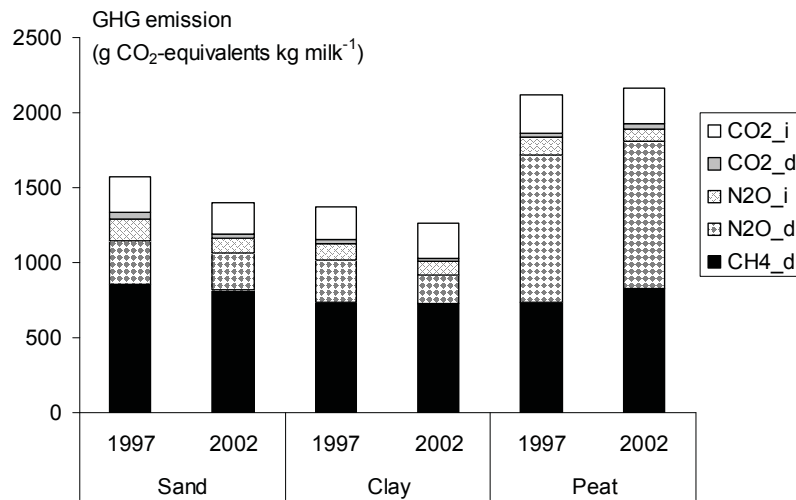


Figure 2. Direct (d) and indirect (i) emissions of carbon dioxide (CO<sub>2</sub>), nitrous oxide (N<sub>2</sub>O) and methane (CH<sub>4</sub>) from dairy farms participating in the 'cows & opportunities' project, in 1997 and 2002.

### Conclusions

The whole farm approach is a powerful tool to develop greenhouse gas mitigation strategies for farming systems, taking into account the transfer of effects to other environmental issues. Efficient nitrogen management in dairy systems reduces the greenhouse gas emissions from dairy farming.

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# Carbon sequestration by Polish grassland biomass

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## Abstract

CO<sub>2</sub> is one of the most important gases responsible for the greenhouse effect. Total CO<sub>2</sub> emission in Poland is above 300 millions tons per year. Different strategies could be used for reduction of greenhouse effect and special attention has been paid recently to the grassland ecosystems. The goal of studies carried out in the years 2000 -2002 was to measure in field conditions CO<sub>2</sub> accumulation in grassland biomass (both underground and aboveground) and on the base of field trials to estimate the global carbon accumulation in Polish grassland. Two trials were established in two sites (moderate dry and moderate wet) on the black earth soil. C content in plants and soil was determined by spectrophotometer method. It has been proved that carbon accumulation in aboveground plant biomass was higher on the most intensive grassland management but C content and accumulation in root system was higher in rather less intensive grassland management. It is concluded that total CO<sub>2</sub> accumulation in grassland ecosystems in Poland could be about 122 millions tons (31.5 mtC), but net carbon sequestrations is 14 millions CO<sub>2</sub> ton (3.6 mt C).

Keywords: carbon contents, carbon sequestration, grassland, greenhouse effect.

## Introduction

The natural greenhouse effect has become harmful for the earth climate and environment as a consequence of anthropogenic activities. The accelerated greenhouse effect or global warming could be caused by emission of greenhouse gases (GHGs) into the atmosphere. The main anthropogenic activities leading to emission of GHGs (CO<sub>2</sub>, CH<sub>4</sub>, N<sub>2</sub>O) are fossil fuel combustion, land use change, deforestation, biomass burning and soil ploughing. Different strategies could be used for reduction of greenhouse effect and special attention has been paid recently to the grassland ecosystems. It is known that one of the methods to decrease CO<sub>2</sub> emission is carbon sequestration in soils, seas, oceans and plant biomass. The grasslands in the world store about 15 % of all global organic carbon (Körner, 2002). Grassland could be a very important carbon sink although carbon accumulation in grassland aboveground biomass is relatively small in comparison with forest. However more than two thirds of annual grassland biomass is allocated to below ground (Körner, 2002). Theoretically grassland root production could add more carbon to soil humus, so the surplus of CO<sub>2</sub> could be accumulated not only in grassland aboveground biomass but in roots, stolons and top soil layer. Grassland are treated by many authors as good example of positive response to CO<sub>2</sub> higher concentration in atmosphere (Lüscher *et al.*, 1998, Jones and Carter 1992) and experimental CO<sub>2</sub> research (Körner, 2002) focused on grassland plants or whole grassland communities but still more research is needed for more precocious estimation of carbon balance in grassland, especially at different level of management intensity (Sapek, 2000).

The main goal of our study was to measure C accumulation in grassland biomass (both underground and aboveground) and based of this data to calculate the total carbon sequestration in semi-natural grassland in Poland. We also wanted to prove that the recently observed switch of grassland into arable land could be harmful for environment.

## Material and methods

Our study has been carried out in two steps. First two field trials were established on permanent grassland sward in Experimental Station Jaktorów on the black earth soil (Typical haplaqiols acc. to FAO). During three consecutive years (2000 – 2002) grassland carbon content (both in aboveground and underground dry matter biomass) was measured by spectrophotometer MATT 5500. Grassland

yield and underground biomass (in two layers 0-10 and 11-20 below the ground surface) were estimated at three levels of management intensity (Stypiński and Mastalerczuk, 2005b) in two different sites: moderate dry, water table about 150 cm and moderate wet, water table about 60 cm. On the base of the results of field experiments the evaluation of total carbon accumulation in Polish grassland was estimated

## Results and discussion

Total emission of CO<sub>2</sub> is calculated in Poland as 308 mln tons (Statistical Yearbook, 2004) and probably part of this amount could be stored by forest and grassland. Unfortunately area of grassland in Poland decreased in last decade as a result of change in land use (Table 1).

Table 1. Grasslands land area (in thousand ha) and DM yields (t ha<sup>-1</sup>) in the years 1980-2003 in Poland (Statistical Yearbook 2004).

Years	Land area			DM yields
	Meadows	Pastures	Total	
1980	2503	1543	4046	45.9
1985	2518	1551	4069	54.0
1990	2475	1585	4060	52.3
1995	2272	1498	3770	44.6
2000	2503	1369	3872	36.0
2003	2341	928	3269	35.3

On the other hand intensification of grassland management also caused the decrease of carbon accumulation in comparison with less intensive management (Stypiński and Mastalerczuk, 2005b). Nevertheless permanent grassland is still important in carbon sequestration because content of C in grass yield and grass root is rather high as it is shown in table 2. Less intensive use caused the increase of C content in underground biomass, also the production of root biomass was better under less intensive management (Stypiński and Mastalerczuk, 2005a).

On the base of field results it is possible to calculate that average carbon accumulation from two tested sites is about 9.65 tons per ha per year (Table 2).

Total carbon storage in Polish grassland is estimated and result are presented in table 3. Carbon sequestration by grassland biomass seems to be very high but it must be corrected for loses via respiration, digestion and soil mineralisation. According to the method described by Lal *et al.* (2001) total CO<sub>2</sub> biological emission from Polish grasslands estimated as about 27 thousand tonnes. Nevertheless net carbon sequestration is calculated as 14 million CO<sub>2</sub> tones (3.6 mtC per year).



Table 2. Mean carbon contents (g C kg<sup>-1</sup> DM) and carbon accumulation (C t ha<sup>-1</sup>) of meadow plants regarding to management intensity and soil moisture.

Management intensity	Carbon content		Carbon accumulation		
	Underground mass	Aboveground mass	Underground mass	Aboveground mass	Total biomass
Moderate dry site					
Intensive	397.4a	540.8a	5.2	4.2	9.5
Moderate	401.3b	555.0b	5.8	3.8	9.6
Extensive	404.7b	555.6b	6.4	3.3	9.6
Mean	401.1	550.5	5.8	3.7	9.5
Moderate wet site					
Intensive	396.2a	550.7a	5	4.6	9.6
Moderate	421.5b	552.5b	5.4	4.1	9.5
Extensive	435.0c	552.3b	6.1	4.2	10.3
Mean	417.5	551.9	5.5	4.3	9.8

Values in column followed by the same letters did not differ significantly at the probability level of P=0,05 according to Newman – Keuls q test.

Table 3. Carbon accumulation (C thousand t) on permanent Polish grassland in 2003.

	Meadows	Pastures	Total	Emission	Net C accumulation
Underground mass	13,226.65	5,243.20	18,469.85		
Aboveground mass	9,346.00	3,712.00	13,058.00		
Total biomass	22,590.65	8,955.20	31,527.85	27,875.2	3,623.00

## Conclusions

On the base of field trials it is possible to estimate that total carbon accumulation on Polish grassland is about 31.5 mlt of C per year (122 mlt CO<sub>2</sub>), and net carbon sequestration (after losses for biological emission) by grassland biomass (mostly by root system) is about 34.6 millions tonnes of organic C (14 millions tones of CO<sub>2</sub>). The less intensive grassland seems to be better in carbon sequestration than intensive one. Grassland conversion into arable land could be a reason of increasing carbon emission grassland into arable land can be a reason of strong carbon emission. Grassland seems to be important in total carbon balance and in reduction of greenhouse effect but more studies is needed for more precocious data.

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# A modelling framework to identify new integrated dairy production systems

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## Abstract

This study describes the development of a new modelling framework (SIMS-Dairy) comprising nitrogen (N), phosphorus (P) and methane (CH<sub>4</sub>) models connected to 'score matrices' for measuring attributes of biodiversity, landscape, product quality, soil quality and animal welfare in a dairy farm. The aim of the framework is to identify a range of site conditional specifications for systems that can comply with the criteria of sustainability.

Keywords: model, sustainability, dairy.

## Introduction

UK dairy farming has evolved mainly in response to economic drivers but additionally is now being given environmental goals. Ways to reconcile these economic and environmental pressures are needed in the form of more sustainable systems. Although linking these concepts to practical actions and decisions is not easy, mathematical models offer this capability. Different models have been produced to analyse economic and ecological sustainability on dairy farms (i.e. van Calster *et al.*, 2004), but there is still a need for modelling tools which can integrate all the relevant attributes that define the whole dairy farm sustainability. The objective of this study is to describe a new modelling framework which is capable of: a) exploring combinations of farm attributes and nutrient managements that lead us to a sustainable farm and b) indicating ways/impacts to overcome lack of sustainability.

## Description of the model

The model integrates existing models for N (NGAUGE: Brown *et al.*, 2005; NARSES: Webb and Misselbrook, 2004) and P (PSYCHIC: Defra, 2005), equations to predict CH<sub>4</sub> losses (Chadwick and Pain, 1997; Giger-Reverdin *et al.*, 2003) and cows' nutrient requirements (Thomas, 2004), 'score matrices' for measuring attributes of biodiversity, landscape, product quality, soil quality and animal welfare and an economic model. Fig 1 gives a schematic representation of how the model works. The model consists of a generator of management options to be optimised, a simulator that simulates each option and an evaluator that assesses which option meets the user-weighted multiple criteria. At the start of a model simulation the main farm parameters (ie. diet, manure, fertiliser, crops or animal types), site characteristics, management options to optimise, environmental goals (ie. 11.3 mg NO<sub>3</sub>-N l<sup>-1</sup> in the leachate) and sustainability matrices (SM) are entered. One value (j index) from a management option (i index) is selected from the 'management options to optimise' generator matrix (Man<sub>i,j</sub>). Every time the model runs through this stage, either a new farm scenario (through changes in management) is generated or the model stops (if all the management options have already been evaluated).

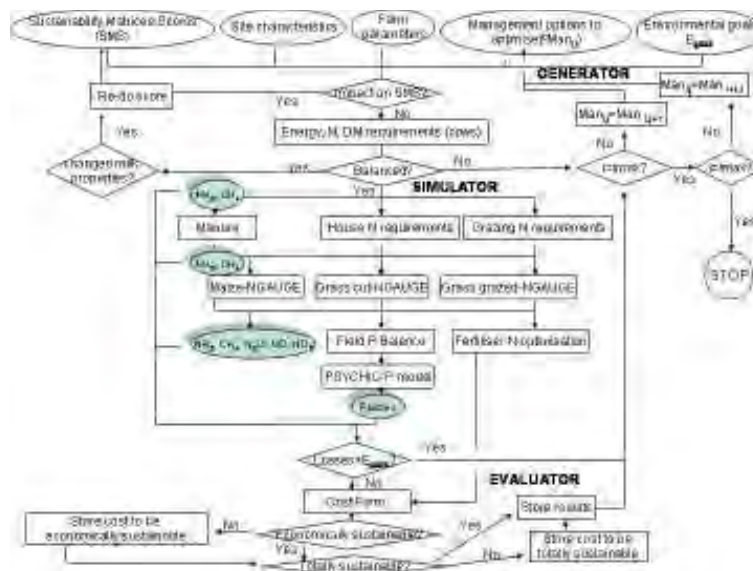


Figure 1. Flow diagram for the SIMS-Dairy modelling framework.

Subsequently, the model calculates any effect that the main farm parameters may have on the SM and scores them. Then it calculates animal energy, dry matter and protein requirements as a function of the diet profile and milk yield targets. The model then checks whether proteins on offer from the diet meet the predicted true protein requirements; if not, the model starts the calculations again with different indices for the matrix  $Man_{i,j}$ ; if yes, the impacts of different diets on fat (Dewhurst *et al.*, 2003), protein and total milk yield are assessed and the score for the product (milk) quality SM is re-assessed. Total N animal requirements are split into requirements for 2 periods (during housing and grazing) and manure N during housing is calculated by subtracting N in the milk from the predicted ingested N. Ammonia and  $CH_4$  losses in the house are calculated and the remaining N in the manure is used then as an input to the field-scale NGAUGE submodels of N cycling in grasslands (cut and cut&grazed) and maize. Grass and maize silage ingested during the housing period is grown within the farm. The silage ( $kg\ N\ ha_{field}^{-1}$ ) needed for the housed animal is area-normalised into  $kg\ N\ ha_{field}^{-1}$  of grass and maize. The model then predicts the best monthly fertiliser  $kg\ N\ ha_{field}^{-1}$  distribution and amount according to NGAUGE optimisation criteria (maximise N in herbage: N in loss) and using harvested  $kg\ N\ ha_{field}^{-1}$  grass and maize as the target. Grazed grass N flow calculations are also made using the same approach as with grass and maize silage and N fertiliser distribution is optimised for an area-normalised  $kg\ N\ ha_{field}^{-1}$  grass grazed required as the target. Nitrogen ( $NO_3$  leaching,  $N_2O$ ,  $N_2$  and  $NH_3$ ) and  $CH_4$  field losses are calculated at this stage.

Metabolisable energy (ME) and P in the harvestable part of cut and grazed grass, maize and clover are estimated from standard values of  $ME\ DM^{-1}$  (Thomas, 2004).

Annual P fluxes in the soils are calculated by using a simple mass balance approach by which P from mineral fertiliser, atmospheric deposition, manure (indirectly from milk P, Aikman, *pers commun.*), dung, plant roots and stubbles are added to a starting total soil P and converted into Olsen P. Phosphorus in the harvestable part of cut and grazed grass and maize is estimated from standard values of  $ME\ DM^{-1}$  and should the Olsen P level in the soil be limiting for plant growth, predicted plant dry matter (NGAUGE) is reduced and hence, P in the plant is reduced too. Using some of these inputs through the PSYCHIC submodel,  $kg\ P\ ha_{field}^{-1}$  losses are calculated as losses from fertiliser, manure and soil (dissolved and particulate P).

Environmental goals may be used as constraining targets. The model checks at this point if any of the minimum environmental goals are not achieved. If they are not, the model returns to the beginning of

the sequence of calculations and generates a new management option (or value) to be evaluated through the generator matrix (Man  $i,j$ ) and starts simulating the new farm scenario. Calculations, otherwise, enters the economic submodel and costs are evaluated. Should the farm be economically viable, the model, together with SM scores, evaluates if it can be totally sustainable, it stores the results and determines the level of sustainability. Should it not be economically viable, the model estimates the cost to be viable, determines the level of sustainability and stores the results. Subsequently, the model returns to the generator matrix and starts simulating the new farm scenario.

### Examples of possible trade-offs

Polyunsaturated fatty acids (PUFA<sub>s</sub>) levels in the diet are a good example of how parameters may well have different effects on different sustainability controls of a dairy system. High level of PUFA<sub>s</sub> in the diet may have a butterfat depressing effect in the milk, which could impose penalties on the farm profits. On the positive side, the ingestion of high levels of some PUFA<sub>s</sub> have been reported to reduce CH<sub>4</sub> emissions from the rumen, have a positive effect on fertility and increase the level of PUFA<sub>s</sub> in the milk, which could also have a positive effect on the health of consumers.

### Acknowledgements

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## Changes in the carbon and nitrogen cycles with land use in grasslands

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### Abstract

One of the key issues in climate change research is the assessment of the effect of land use on C storage and the role of management on the C cycle. Recent studies suggest that grasslands could be a sink for C, but information is scarce on the impact that grassland management could have on this sink. In order to address these questions, a regional survey on the soil organic C and N was made in grassland in the Pyrenees. In addition to climate and topography, the survey included management variables, such as livestock type. C and N of faeces were investigated in the same context. An experiment was set in 2 locations in the Eastern Pyrenees and 3 treatments applied: ungrazed, moderately and intensively grazed. Results from the survey suggest that cattle and mixed grazing favour C storage in the soil. In the experiments, ungrazed areas showed lower pools of active soil C than grazed areas, but results were dependent on location and climate of the year.

Keywords: climate change, SOC, active C, livestock, management.

### Introduction

Modelling efforts have been applied to understanding the mechanisms that drive soil organic carbon (SOC) dynamics through the analysis of regional SOC patterns (Jobbágy and Jackson 2000). Most regional studies focus on the effect of abiotic factors, such as climate (McCulley *et al.*, 2005), and less is known about management. In the context of the Kyoto protocol, it is of maximum importance to assess the potential for C storage of factors we can manipulate. In Europe, grasslands cover 180 million ha. Meta-analyses suggest that grasslands have the capacity to store much SOC (Conant *et al.*, 2001). We combined data from a regional survey with results from two grazing experiments to understand the role of management on SOC content in grasslands in the Pyrenees.

### Materials and methods

Regional study: We assessed the role of geographic (NE, E, Central and W Pyrenees), climatic (mean annual rainfall and temperature), topographic (altitude, slope, aspect, macro and microtopography), bedrock (calcareous, acidic), biotic (aboveground biomass), and management (sheep, cattle, mixed grazing) factors on SOC in the 0- 20 cm layer through a survey of 139 grassland sites. At each site, we harvested biomass in 4 50 x 50 cm plots and collected soil. We counted faeces on 2 100 x 1 m transects. Grazing experiments: In two locations we carried out an experiment in which 3 grazing treatments were applied to 6 plots for 2 years: ungrazed, moderately (16 cattle ha<sup>-1</sup> per 2 days) and intensively (48 cattle ha<sup>-1</sup> per 2 days) grazed. Soil and faeces C and N were analysed by the autoanalyser and labile C by fumigation.

## Results and discussion

Regional modelling based on abiotic variables (see Table 1) indicated that SOC content in the upper 20 cm decreased with mean annual temperature and altitude. In the Western Pyrenees altitude is lower than in other sectors. Therefore, SOC content was higher but predicted SOC was lower there when all other conditions were held constant. Protected macrotopographies and moist microtopographies favoured SOC accumulation. Calcareous bedrock had lower capacity for SOC storage. Contrary to other regional studies (Jobbágy and Jackson, 2000), texture did not appear in our SOC content models.

Table 1. Results from the two models on SOC content in the upper 20 cm soil layer.

Abiotic model	t	Sig.	Combined model	t	Sig.
Constant	4.327	.000	Constant	5.302	.000
Western	-2.504	.014	Central	2.171	.032
Mean annual temperature (°)	-2.024	.045	Mean annual temperature (°)	-2.502	.014
Altitude (m)	-2.780	.006	Altitude (m)	-3.088	.002
Slope (°)	-2.946	.004	Slope (°)	-2.991	.003
Protected macrotopography	2.829	.005	Protected macrotopography	2.597	.011
Moist microsites	2.415	.017	Moist microsites	1.804	.074
Dry sites	1.758	.081	Bedrock	-3.060	.003
Bedrock	-2.702	.008	Aboveground biomass (g m <sup>-2</sup> )	3.333	.001
			Sheep grazing	-1.711	.090
			Mixed grazing	1.434	.154

Left, abiotic model including biogeographic, climatic and topographic variables. Right, combined model including abiotic, biotic and management variables.  $R^2_{adj}$  abiotic = 27%;  $R^2_{adj}$  combined = 31%.  $P < 0.001$  for both models.

The inclusion of biotic variables such as aboveground biomass and management improved the models (Table 1), although the effect was smaller than that of abiotic variables (Table 1). In this combined model, SOC content was higher in the Central Pyrenees compared to other sectors, suggesting that part of the previous differences in the Western sector could be in part related to different management. In fact, the Western Pyrenees have been traditionally used differently: this area is open to many big transhumant livestock flocks as well as local shepherds, while in the other sectors grassland use is more restricted. The comparison of the number and chemical composition of faeces in two locations in the Central and Eastern Pyrenees (Table 2) indicates that differences in SOC could be related to variations in both quantity and quality of faeces deposited in the grassland. A more detailed analysis of management factors was impossible because of the extreme difficulty of determining local livestock pressure. However, analysis of the effect of livestock type indicates that grassland grazed only by sheep had lower SOC content than mixed and cattle-grazed areas (Table 1). In addition to possible changes in grassland vegetation with management, C input with faeces from large grazers could be responsible for the increased SOC content in these last areas. In fact, in a detailed study in the Western Pyrenees, we found that SOC was related to the amount of cattle and horse faeces ( $P < 0.05$ ).

Table 2. Characteristics of faeces in a sample from two geographic sectors.

Sector	Number faeces per 100 m <sup>2</sup>	Nitrogen (g 100 m <sup>-2</sup> )	Carbon (g 100 m <sup>-2</sup> )
Central Pyrenees	11 ± 5.1	51 ± 4.2	1443 ± 178
Eastern Pyrenees	8 ± 4.3	36 ± 3.1	1040 ± 156

Results from the experiments confirmed that management must play a role in SOC accumulation in grasslands. Ungrazed plots showed lower changes in the pools of active microbial and non-microbial soil C than grazed plots, although results were dependent on site and climate conditions of the particular year (Fig. 1).

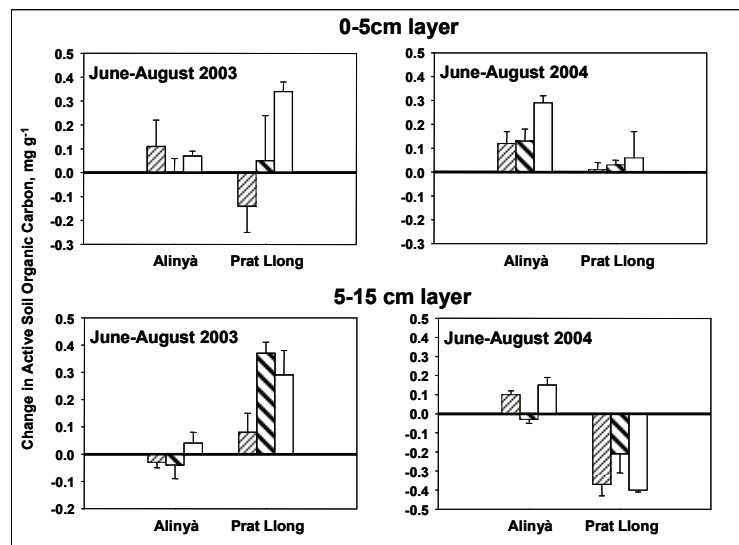


Figure 1. Temporal change in active SOC in an experiment in 2 locations, Prat Llong and Alinyà and 2 years. Thin line, ungrazed; thick line, moderate; open bars, intensive.

## Conclusions

SOC content was successfully modelled by geographical, climatic and topographic factors. Management explained part of the variation in SOC content in both the regional survey and the experiments but the effects were weak and variable, confirming the complexity of the processes and the difficulties of assessing the role of management.

## Acknowledgements

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## Response of *Arrhenatherum elatius* to soil moisture deficiency

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### Abstract

Two greenhouse experiments were performed. In the first one the pots were filled with mineral soil, and in the other with organic soil. Mineral soil moisture was 35% and 70% of field water capacity, and organic soil moisture 40% and 80%, respectively. During the growing season the rate of photosynthesis was measured with a LiCor 6400 gas analyser, and leaf greenness with a SPAD 502 chlorophyll meter. The plants were cut three times.

It was found that water stress significantly reduced photosynthesis rate and plant yield, and increased the chlorophyll concentration in *Arrhenatherum elatius* L. leaves. The type of soil applied in the study affected photosynthesis rate, plant yield and chlorophyll concentration expressed as leaf greenness (SPAD index).

Keywords: *Arrhenatherum elatius*, chlorophyll, photosynthesis, water stress.

### Introduction

The threat of climate stepping makes researchers investigate the effects of water stress on plants, paying particular attention to the species that tolerate such conditions. *Arrhenatherum elatius* L. occupies an important position in the group of grasses found in moisture deficient habitats (Swędrzyński, 1995; Gaff *et al.*, 1990). The aim of the present study was to determine the effects of soil moisture deficiency on the photosynthesis rate, leaf greenness (SPAD index) and yield of *Arrhenatherum elatius* grown on mineral and organic soil.

### Materials and Methods

Two duplicate one-factor experiments were established in 2002 at the greenhouse of the University of Warmia and Mazury in Olsztyn. In the first experiment modified Kick-Brockmann pots were filled with 10 kg mineral soil (very fine light loamy sand) containing 1.84% organic matter. The concentrations of available phosphorus, potassium and magnesium were high, and the soil pH<sub>KCL</sub> was 5.6. In the other experiment the pots were filled with 8 kg peat-muck soil containing 25.25% organic matter. The concentrations of available phosphorus, potassium and magnesium were low, and the soil pH<sub>KCL</sub> was 4.9. French ryegrass var. Skrzyszowicki was tested. Two to three seeds were sown at 10 points of each pot. Immediately after emergence poorly developed seedlings were removed, leaving six plants per pot. The plants were tested under conditions of differentiated soil moisture content. Mineral soil moisture was 70% FC (optimum moisture content) and 35% (water stress), and organic soil moisture was 80% and 40% respectively. In order to maintain the appropriate soil moisture, water losses were made up on a regular basis, to achieve a specified weight of the pot with soil. Nitrogen fertilization was applied over the entire experimental period, at a rate of 0.5 g per pot on organic soil and 0.75 g per pot on mineral soil. Phosphorus, potassium and magnesium were applied once only, before sowing. On organic soil the rates were as follows: 0.25 g P, 1.00 g K and 0.25 g Mg per pot. On mineral soil, due to a high abundance of nutrients, the above rates were reduced by half. During the growing season the rate of photosynthesis was determined with a Li-Cor 6400 portable gas analyser, and leaf greenness with a SPAD-502 chlorophyll meter (Minolta). The photosynthesis rate and leaf greenness were measured on the youngest, fully developed leaf of shoots selected randomly of each pot. Four measurements were performed for each regrowth, and readings were taken every week. The results presented in the paper are means of particular regrowths. The plants were defoliated three times over the growing season. The



results were analyzed statistically using STATISTICA software. The significance of difference was verified by the Tukey's test at a significance level  $p = 0.99$ .

## Results and Discussion

The results presented in Table 1 show that water stress significantly reduced the photosynthesis rate in *Arrhenatherum elatius* leaves.

Table 1. Intensity of photosynthesis in leaves.

Soil	Soil moisture	1 <sup>st</sup> cut	2 <sup>nd</sup> cut	3 <sup>rd</sup> cut	Mean	Decrease
	(% FC)	(μmol CO <sub>2</sub> m <sup>-2</sup> s <sup>-1</sup> )				(%)
Mineral soil	70	9.5 b	10.6 b	10.5 b	10.2 b	-
	35	4.6 a	9.4 a	7.4 a	7.1 a	30.4
Organic soil	80	9.7 b	10.6 b	7.3 b	9.2 b	-
	40	7.1 a	7.9 a	3.7 a	6.2 a	32.6

a, b- Homogenous groups.

In plants grown on mineral soil the photosynthesis rate decreased by 11% to 52%, depending on regrowth (on average by approx. 30%). The fastest photosynthesis rate was recorded in the second regrowth. Plants grown on organic soil also responded to water stress by a decrease in the photosynthesis rate, by about 33% compared with the control treatments. Similarly as in plants grown on mineral soil, the rate of this process was less affected in the second regrowth. A slower rate of photosynthesis in response to water deficit was also reported by Huang and Gao (1999). Moisture deficiency in the soil resulted in a significant increase in the chlorophyll content of leaves, expressed as leaf greenness (SPAD index) (Table 2).

Table 2. Leaf greenness index SPAD.

Soil	Soil moisture	1 <sup>st</sup> cut	2 <sup>nd</sup> cut	3 <sup>rd</sup> cut	Mean	Increase
	(% FC)	(SPAD)				(%)
Mineral soil	70	33.7 a	35.3 a	29.6 a	32.9 a	-
	35	34.8 a	36.7 a	34.8 b	35.4 b	7.6
Organic soil	80	31.5 a	32.1 a	31.4 a	31.7 a	-
	40	36.4 b	40.1 b	35.6 b	37.4 b	18.0

a, b- Homogenous groups.

This is consistent with the results of previous own studies (Olszewska, 2003). In plants grown on mineral soil the chlorophyll concentration increased by about 8%, and in plants grown on organic soil by 18%. Regardless of soil type, plants in the second regrowth contained the most chlorophyll. Water deficit significantly reduced the yield of *Arrhenatherum elatius*. The yield decreased by 60% in plants grown on mineral soil with a moisture content of 35% FC, and by about 44% in those grown on organic soil, in comparison with the control treatments (Table 3). Other authors also demonstrated a decrease in the yield of grasses under conditions of water stress (Szozkiewicz *et al.*, 1991). Higher yields were attained in the experiment with organic soil, although plants grown on this type of soil responded to moisture deficiency by a greater decrease in the photosynthesis rate, compared with plants grown on mineral soil. This is probably related to the fact that the first one contained more chlorophyll, which participates in photosynthesis and performs protective functions in the plant.

Table 3. Dry matter yield.

Soil	Soil moisture (% FC)	1 <sup>st</sup> cut	2 <sup>nd</sup> cut (g pot <sup>-1</sup> )	3 <sup>rd</sup> cut	Total	Decrease (%)
Mineral soil	70	4.7 b	7.4 b	6.0 b	18.1b	-
	35	2.8 a	1.9 a	2.5 a	7.2 a	60.2
Organic soil	80	10.5 b	9.6 b	8.3 b	28.4 b	-
	40	6.2 a	3.8 a	6.0 a	16.0 a	43.7

a, b- Homogenous groups.

### Conclusions

It was found that water stress significantly reduced the photosynthesis rate and the yield of *Arrhenatherum elatius*, and increased the chlorophyll concentration expressed as leaf greenness (SPAD index). Plants grown on organic soil were less sensitive to soil moisture deficiency and the decrease in their yield was lower, compared with plants grown on mineral soil. This shows that organic soil is more suitable for growing *Arrhenatherum elatius* plants.

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# Carbon contribution and organic matter decomposition of different herbaceous species in forest systems

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## Abstract

The present paper looks at the role of the most abundant herbaceous species as carbon capturers in four riparian systems (three forested and one deforested areas) in terms of their contribution in biomass. Preliminary data is provided on weight loss of the various species in the decomposition process that is, of the input of organic matter and/or carbon to the soil of the different species in each different riparian zone.

Keywords: riparian system, grass, biomass loss, carbon contain.

## Introduction

Vegetation systems (induced forests and agricultural) are able to capture and sequester carbon. However, the carbon that is captured and stored in pasture and the below-ground part of the terrestrial ecosystems, in contrast to what is captured in the aerial part, is not normally regarded as a sequester mechanism by designers of climate change policies.

According to the Kyoto protocol, pastures, along with forests, play an important role in carbon sequestering. According to the FAO (1993), pasture land covers 3,200 million hectares and stored between 200 and 420 Pg in the overall ecosystem. A large part of this is below the surface and hence in a relatively stable state.

Analysis of the biomass of pastures in natural riparian systems in central Spain, which have been deforested and restored over many years (Martínez *et al.*, 2003, 2005), has shown that the spring biomass production of herbaceous vegetation in restored and non-forested areas is relatively high (4 year average, 4,500 kg ha<sup>-1</sup>). Moreover, this biomass is primarily represented by a series of families, particularly plant species. Consequently, the objective of this paper has been show data of decomposition process and input of organic matter and/or carbon to the soil of the herbaceous species more abundant in four different riparians systems.

## Materials and methods

Experimental area is located in the riparian zone of Henares river in Central Spain, in Alcalá de Henares municipal district. The study zone were a natural gallery forest (zone A), two riparian plantations, one this in 1994 (zone B) and the other in 1999 (zone C) and a riparian deforested area, with herbaceous vegetation. The species study were *Cardaria draba* Desvau, *Cirsium arvense* L. evaluated in the four areas; *Bromus* sp. evaluated in the riparian plantation and deforeted area; *Hordeum murinum* L. evaluated in the riparian plantation, and *Brachypodium phoenicoides* L. y *Melica ciliate* L. that was been evaluated only in zones that representation biomass abundant zones A and D, respectively.

In order to study the decomposition process, pasture samples were collected in August, air-dried and then 10 g of dry plant matter from each species was put in fibreglass bags with 2mm mesh. From 21 samples of each species, three were used as controls with three in each sample per species and zone, applying the procedure developed by Akanil and Middleton (1997). The organic matter content of the samples was defined by calcination, with the carbon content estimated as 50 % of the organic matter (Maithani *et al.*, 1998). The results of this work are taken from the fourth sampling period after 243 days of incubation in the field.

## Results and discussion

In spring 2004, the analysed species formed biomass that fluctuated between 15% and 42.1%, depending on the study area and species. On the other hand, the mean pasture biomass in the restored and deforested zones in the same year was 3,803.7 kg ha<sup>-1</sup>.

Tables 1 summarise the results for weight loss in plant matter in decomposition. No significant differences were found between the study zones in terms of the decomposition process of the respective grassland species, however differences were noted the decomposition of the various plant species.

Table 1. Biomass loss (%) in every study area of each herbaceous species after 243 days of the decomposition process.

Species	Zones				Total
	Zone A	Zone B	Zone C	Zone D	
<i>Cardaria draba</i>	44.20a	57.37a	45.26a	51.58a	49.60
<i>Cirsium arvense</i>	37.66ab	50.01a	37.53ab	44.45a	42.41
<i>Bromus</i> sp.	-	20.33b	23.11b	26.07b	23.17
<i>Hordeum murinum</i>	-	31.46b	38.00ab	-	41.40
<i>Brachypodium phoenicoides</i>	31.34b	-	-	-	31.34
<i>Melica ciliata</i>	-	-	-	25.76b	25.76
Significance	NS	*	NS	*	-

\* Significant at 0.05% level.

Tables 2 also show the carbon content of the pasture samples at the start and after 243 days of the decomposition process. Significant differences can be seen between zones in the dicot species but not in the grass species. The carbon content of the incubated samples of the different plant species did not show any significant differences in the various study areas.

Table 2. Carbon content (%) in every study area of each herbaceous species after 243 days of the decomposition process.

	Species					
	<i>C. draba</i>	<i>C. arvense</i>	<i>Bromus</i> sp.	<i>H. murinum</i>	<i>B. phoenicoides</i>	<i>M. ciliata</i>
Check sample	47.02	44.91	47.16	43.70	45.83	47.12
Zone A	43.16a	44.27a	-	-	44.22	-
Zone B	44.25a	43.07ab	40.98a	40.12a	-	-
Zone C	38.12b	41.15b	42.08ab	41.12a	-	-
Zone D	47.64c	45.30a	45.51b	-	-	46.55
Significance	*	*	NS	NS	-	-

\* Significant at 0.05% level.

## Conclusions

In the same time period, the herbaceous dicot species *Cardaria draba* and *Cirsium arvense* provided an input of more plant matter to the soil than the grasses. In one of the reforested areas, *Cardaria draba* lost more than 57% of its biomass, in contrast to *Bromus* sp. which lost only 20%. In general, the grass

species remained on the soil surface for longer and consequently its organic matter and/or carbon took longer to enter the soil.

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# Short-term effects of grazing abandonment on grassland ecosystem respiration

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## Abstract

Grassland management is considered a key factor in the global carbon cycle dynamics. We aimed to evaluate how ecosystem respiration (ER) of grazed systems is influenced by abandonment and determine to which extent abiotic and biotic factors might explain CO<sub>2</sub> effluxes. In two subalpine grasslands in the eastern Pyrenees, we established two grazing treatments (abandoned and grazed) randomly distributed in four plots. In each plot, we measured ER using a closed PP-System, EGM-4 CO<sub>2</sub> analyzer in four subplots. After each measurement, we recorded soil temperature (T), clipped aboveground biomass and sampled the first 15cm of soil to determine belowground biomass and soil water content (SWC) and to analyze soil organic carbon fractions. In the abandoned plots, CO<sub>2</sub> efflux and SWC were significantly higher than in the grazed plots. The main factors explaining CO<sub>2</sub> efflux variability were T and SWC. Adding management to this model contributes to a better understanding of ER processes. Results of this work suggest that grassland abandonment increases CO<sub>2</sub> efflux from the ecosystem to the atmosphere.

Keywords: CO<sub>2</sub> efflux, carbon fractions, PP-System CO<sub>2</sub> analyzer, grassland management.

## Introduction

In a global change context, the role of grasslands on global CO<sub>2</sub> budget requires additional information on how land use changes may affect ecosystem respiration (ER). Soil temperature (T) and soil water content (SWC) are important driving factors of CO<sub>2</sub> ecosystem fluxes. However, limited information is currently available to further our understanding of the possible mechanisms originated by grassland management (Cao *et al.*, 2004).

As a consequence of socio-economic shifts affecting rural areas, pasture abandonment of less accessible grasslands is envisaged. In addition to many functional services of mountain areas, this abandonment may affect the capacity of grasslands to act as a CO<sub>2</sub> sink. There are inconsistent data relative to the effects of grazing on ecosystem CO<sub>2</sub> emissions. This study aimed to evaluate short-term effects of grazing abandonment on ER rate. Since in the Pyrenees, abandoned grasslands seem to have lower soil active C stocks than grazed ones (Casals *et al.*, 2004), in this study, we hypothesize that pasture abandonment will increase ER.

## Material and methods

The study was carried out in two subalpine grasslands extensively pastured at two locations in the Eastern Pyrenees: Alinyà (42°10' N, 1°28' E; 1848 m a.s.l.) and Prat Llong (42°12' N, 1°31' E; 2140 m a.s.l.). At each location we established one large plot where two treatments were randomly assigned to four 25x25 m<sup>2</sup> plots. The treatments were abandonment (ungrazed) and grazing (three adult cows per plot for two days, equivalent to 48 cows ha<sup>-1</sup> per two days). Grazing treatment was applied in July in 2003 and 2004 until the forage became exhausted in the grazed subplot.

In 2004, we recorded ER one week before the grazing treatment (end of June) and two months after its completion (September). We measured ER (soil and plant respiration under dark conditions) using a

closed *PP-System (EGM-4 CO<sub>2</sub> analyzer)* on 4 plastic collars per plot. Just after ER measurements, we recorded soil temperature (T) at each collar using a thermocouple (0-10 cm soil depth integration). In addition, we harvested aboveground biomass and took two soil samples per collar (15 cm depth). In the laboratory, aboveground biomass was sorted into living and dead biomass and we separated roots from soil by the flotation method. We determined SWC by the gravimetric method and estimated soil C fractions (microbial and active C) through chloroform fumigation- K<sub>2</sub>SO<sub>4</sub> extraction method (Vance *et al.*, 1987). To assess the influence of the grazing treatment on ER we performed a general lineal model with T and SWC as covariables. To analyze the combined effect of SWC, T and management we performed linear and non-linear regressions (SPSS v. 11.0).

## Results and discussion

Table 1. Ecosystem respiration rate ( $\mu\text{molm}^{-2}\text{s}^{-1}$ ; mean  $\pm$ SE) in June and September in grazed and abandoned plots. (Sampling time x management, *p*-value<0.01).

Sampling time	Management			
	<i>Abandoned</i>		<i>Grazed</i>	
June: <i>before treatment</i>	5.01	$\pm$ 0.38	5.39	$\pm$ 0.45
September: <i>after treatment</i>	4.29	$\pm$ 0.46	3.19	$\pm$ 0.31

In September, two months after the grazing treatment, ER was significantly higher in the abandoned than in the grazed plots (Table 1) and SWC and total green biomass was lower in grazed plots than in abandoned plots. As the relationship between T and CO<sub>2</sub> efflux (Q<sub>10</sub>) depends, among other factors, on plant phenology (Yuste *et al.*, 2004), we modelled ER of June and September samplings separately. In both cases, the model that best fit measured soil CO<sub>2</sub> efflux is a multiplicative formulation with power functions (Qi and Xu, 2001) including T and SWC (Table 2; model 1). Including management as a dummy variable increased the precision of our estimates, especially for ER in September (Table 2; model 2), suggesting the relevance on ER of factors affected by the management as for instance above- and belowground biomass or soil microbial activity.

Table 2. ER predicted by T and SWC as a multiplicative power function (model 1) and management as adding factor (model 2)

Sampling	Model		r <sup>2</sup>
<i>Model 1</i>			
June	ER=	$17.477 * T^{-0.037} * SWC^{0.649}$	0.37
September	ER=	$0.069 * T^{2.261} * SWC^{0.786}$	0.47
<i>Model 2</i>			
June	ER=	$0.671 * \text{management}^\dagger + 15.065 * T^{0.049} * SWC^{0.745}$	0.41
September	ER=	$-1.322 * \text{management}^\dagger + 0.050 * T^{2.194} * SWC^{0.786}$	0.60

<sup>†</sup>Management dummy variable: 0=abandoned; 1=grazed

The predicted ER rate was significantly higher in abandoned than in grazed plots in September after the grazing treatment (Figure 1). Residuals derived from model 2 were related to the ratio of microbial C biomass to active C (micC/actC) (Figure 2;  $\text{adj}R^2=0.21$ ,  $p\text{-value} = 0.01$ ) while factors like green aboveground biomass and belowground biomass were not included in the model (step-wise linear regression,  $p\text{-value}>0.05$ ). Considering micC/actC rate as an estimate of microbial activity, our results suggest that large soil microbial activity contributes to explain CO<sub>2</sub> efflux variability, although this index was not significantly different between grazed and abandoned plots.

## Conclusions

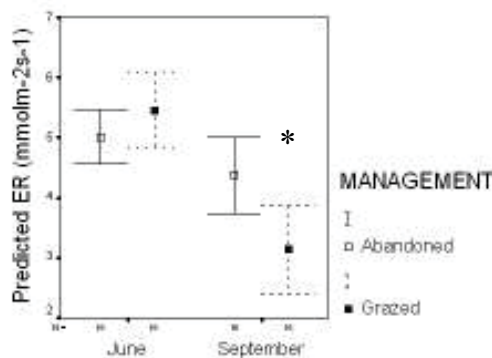


Figure 1. Predicted ER rate from model 2 in abandoned and grazed plots. Vertical bars show confidence intervals (95%). \* indicate significant differences between treatment.

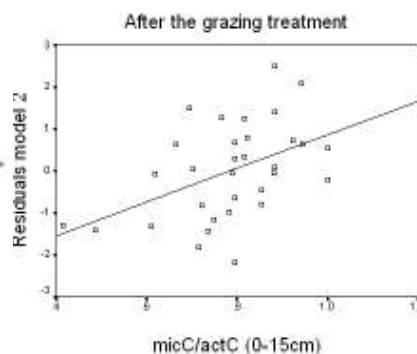


Figure 2. Relationship between microbial C to active C rate with residuals from model 2.

Short-term grazing abandonment enhanced CO<sub>2</sub> efflux from subalpine grasslands to the atmosphere. In addition to soil T and SWC drivers, this study suggests that other factors related to pasture grazing, as green biomass, contribute to explain ER variability. Further analyses are required to discern the role of above- and belowground biomass and soil C fractions in the complex mechanisms controlling ecosystem respiration.

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# The quality of aboveground grass biomass used for direct combustion

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## Abstract

Selected parameters of grass biomass quality were evaluated in the second and third year of vegetation. Biomass quality is one of the most important indicators which can decrease the environmental risks from direct combustion and also influences biomass price. The experiment was established in Prague in 2002. The studied treatments were: two cuts with nitrogen fertilisation ( $100 \text{ kg N ha}^{-1}$ ), two cuts without fertilisation, one cut at the end of growing season without fertilisation and a delayed harvest in spring without fertilisation. The content of ash, N, K, S and Cl was measured in dried samples from each cut of *Bromus inermis* Leyss. and *Bromus marginatus* Nees ex Steud.. Grass species did not significantly affect the measured parameters. The content of N in the dry biomass in these grass species ranged from 6.5 to  $18.5 \text{ g kg}^{-1}$ . The lowest content of K and Cl was in the biomass from one-cut systems. The sulphur content was highest in the second cut of the two-cut management system:  $2.3 \text{ g kg}^{-1}$  in *B. inermis* biomass. One-cut harvest systems provided biomass of better quality for direct combustion than two-cut systems.

Keywords: direct combustion, biomass quality, *B. marginatus*, *B. inermis*.

## Introduction

Perennial grasses display many beneficial attributes as energy crops, and there has been increasing interest in their use in the USA and Europe since the mid 1980s. The characteristics which make perennial grasses attractive for biomass production are their high yield potential, the high contents of lignin and cellulose of their biomass and an environmental impact which is generally considered to be positive (Lewandowski *et al.*, 2003). Perennial, herbaceous energy crops offer a significant opportunity to improve agricultural sustainability through crop diversification, decreased erosion, and improved water quality compared with a traditional annual crop system (Tolbert and Wright, 1998). Primary fuel attributes that determine the suitability for combustion are: total energy content, total moisture content and the chemistry of the ash. Ash content and chemistry are important in the combustion process because they can contribute to the formation of deposits that reduce boiler efficiency and increase costs. The concentration of particular minerals varies, depending on the plant species and the plant parts. Qualitative parameters of harvested biomass are impaired by the presence of weeds (Fuksa *et al.*, 2003). Plant age, or stage of development when harvested and the concentration of other minerals also have a significant influence (Landström *et al.*, 1996). To avoid problems with biomass quality associated with summer harvests, it is recommended to harvest during the winter or spring, following the growing season (Hadders and Olsson, 1997). The objective of our study was to evaluate some quality parameters (concentrations of ash, nitrogen, potassium, sulphur and chlorine) of grass biomass used for direct combustion related to grass species and harvest time. In this paper, we report results from the second and third year of vegetation.

## Materials and methods

Mountain brome (*Bromus marginatus* Nees ex Steud. cv. Tacit) and smooth brome (*Bromus inermis* Leyss. cv. Tabrom) were studied in a plot experiment. The field experiment was carried out at the Czech University of Agriculture in Prague ( $50^{\circ}08' \text{ N}$ ,  $14^{\circ}24' \text{ E}$ ) which is 286 m above sea level. The soil in the experimental area was a deep loamy degraded chernozem with a permeable under layer. The area is classified as having a moderate to warm and mostly dry climate. The average growing period is 172

days with a mean annual temperature of 7.9 °C (30 year mean) and long-term annual average precipitation of 526 mm.

Pure stands of *B. marginatus* and *B. inermis* were sown at a rate of 85 and 40 kg seed ha<sup>-1</sup>, respectively. Seeds were drilled on April 19 in rows 125 mm apart. The experiment comprised of four main treatments (A-D): A, two cuts per year with fertiliser (100 kg N ha<sup>-1</sup> applied in the spring as ammonium nitrate) with the first cut (A1) at seed ripening (July) and a second cut (A2) at the end of the growing season (October); B, two cuts per year without fertiliser, B1 (July) and B2 (October); C, one cut per year at the end of the growing season (October); D, one cut per year - the delayed harvest until the following spring (March). Individual plot size was 10 m<sup>2</sup> and the experimental treatments were established in a randomized block design with four replicates. Herbage samples (250 g) were taken when the experiment was harvested. The samples were dried and analysed for N, K, S, Cl and ash concentration. Two factors were studied: species and treatment (combination of harvest date and N fertilization). An analysis of variance was carried out on the variables for concentrations of ash, N, K, Cl and S.

## Results and discussion

The analyses of elemental contents in dry grass biomass are shown in the Table 1. The average ash content of *B. inermis* biomass was 90.2 g kg<sup>-1</sup> and in *B. marginatus* 73.4 g kg<sup>-1</sup>. Harvest date had little effect on this parameter although nutrients can decrease in later cuts due to re-mobilisation and natural leaching from leaves and stems (Christian *et al.*, 2002). A higher content of ash in biomass from one-cut systems in our experiment can be caused by deposition of soil on plant parts during harvest in bad weather conditions.

The N in the biomass is mainly responsible for the NO<sub>x</sub>-emissions of the combustion system (Lewandovski and Kicherer, 1997). The highest N contents: 18.5 and 10.9 g kg<sup>-1</sup> were found in the biomass from the second cut in two-cut systems, treatments A2 and B2, respectively and the lowest (6.5 g kg<sup>-1</sup>) in treatment B1, the first harvest of the two-cut system without fertilisation. There was no significant effect of N fertilisation on N content in the biomass from the first cut. This can be ascribed to the late timing of the first cut in all treatments.

Most plant materials are low in S, mostly containing between 1 and 2 g kg<sup>-1</sup> (Lewandovski and Kicherer, 1997). S content in the observed grass species ranged from 0.8 g.kg<sup>-1</sup> in *B. marginatus* biomass (treatment A1) to 2.3 g kg<sup>-1</sup> in *B. inermis* biomass (treatment A2). Grass species and treatment had no significant effect on S content in the biomass.

Treatments C and D provided a biomass with a lower K content than treatments A1, A2 or B1 (P=0.0008). Late harvest causes a decrease in K concentration due to leaching from the biomass (Burvall, 1997; Lewandovski and Kicherer, 1997). A significant increase in the K concentration with increasing N fertiliser application was also found in *Miscantus* (Lewandowski and Kahnt, 1994). This relationship was found in our experiment, but differences in K content between fertilised and non fertilised two-cut treatments were not significant.

Chlorine leads to the emission of HCl and under unfavourable conditions can cause emissions of dioxin (Lewandovski and Kicherer, 1997). The critical limit for chlorine (Cl) concentration (2.0 g kg<sup>-1</sup>) was not exceeded in the biomass of the observed grasses. Treatment influenced this parameter significantly. The lowest Cl concentration (0.16 g kg<sup>-1</sup>) was found in the delayed one-cut treatment (D). Treatment A2 provided biomass with a higher Cl content than treatment C or D (P=0.0369).

Table 1. The average content of evaluated elements in dry biomass.

	Ash (g kg <sup>-1</sup> )	N (g kg <sup>-1</sup> )	K (g kg <sup>-1</sup> )	S (g kg <sup>-1</sup> )	Cl (g kg <sup>-1</sup> )
A1	60.73	7.28	12.55	0.99	0.85
A2	85.70	18.52	12.61	2.40	1.07
B1	67.90	6.50	10.36	1.06	0.88
B2	99.70	10.94	6.54	1.86	0.49
C	80.00	7.15	5.19	1.10	0.34
D	96.75	7.35	2.92	1.17	0.16
F-test	2.43	5.97	9.45	1.70	3.44
P-value	0.0967	0.0053	0.0008	0.2100	0.0369
Dmin LSD <sub>0,05</sub>	30.64	5.74	4.08	1.35	0.60

A: two cuts per year with fertilisation, first harvest (A1), second harvest (A2); B: two cuts per year without fertilisation, first harvest (B1), second harvest (B2); C: one cut per year, harvested in autumn; D: one cut per year, harvested in following spring.

### Conclusion

Grass species did not significantly influence the contents of selected elements. Harvest management can improve some quality parameters. Biomass of better quality for combustion was obtained from a one-cut harvest system. However, grass biomass shows higher concentrations of unfavourable elements in comparison with wood or straw.

### Acknowledgements

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# Greenhouse gas emissions from Irish grassland livestock production systems

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## Abstract

Irish dairy, suckler beef and mid-season lamb production systems were defined and emissions quantified using an LCA approach. Emission factors for enteric fermentation, dung management, fertiliser, energy and external feed sources were considered. The total GHG emissions were expressed as CO<sub>2</sub> equivalents and scaled per hectare used for production per year (CO<sub>2</sub> (eq) ha<sup>-1</sup> yr<sup>-1</sup>). GHG emissions per hectare ranged from 6,835 kg CO<sub>2</sub> (eq) ha<sup>-1</sup> yr<sup>-1</sup>; for dairy through 4,859 kg CO<sub>2</sub> (eq) ha<sup>-1</sup> yr<sup>-1</sup> for beef and 4,242 kg CO<sub>2</sub> (eq) ha<sup>-1</sup> yr<sup>-1</sup> for lamb. These emission values include those emissions associated with all land area used for production. Utilising the LCA approach for emission inventory permitted comparisons between systems. The area scaling approach may not be the most appropriate from an LCA perspective but can be used as part of the formulation of an robust national grassland livestock production policy.

Keywords: greenhouse gas emissions, livestock, Ireland, grassland.

## Introduction

GHG emissions from livestock production systems are of importance both within the European Union (EU) and globally. GHG emission reduction strategies should ensure that system attributes such as concentrate feed, inorganic fertilizer, organic waste management and energy are considered as well as emissions from enteric fermentation. To evaluate system emissions holistically a methodology that accounts for all emission sources in a structured framework is required. Life cycle assessment (LCA) allows for holistic system analysis to quantify emissions from agricultural livestock production provided data for system inventory and emission factors can be compiled. Strategies to reduce emissions can be evaluated in terms of whether the emissions are actually reduced or transferred within the system (Casey and Holden, 2005a). The objective of this paper is to present an overview of GHG emissions from typical dairy, beef and sheep production systems in Ireland, and to compare the systems in terms of the emissions per unit area used. The compiled results should be useful for national policy development but are not strictly appropriate within the International Standard Organisation definition of LCA methodology (ISO, 1997).

## Materials and methods

The LCA methodology defined by ISO was used (ISO, 1997) and estimated emissions were scaled in terms of unit area used by a production system. The methodology and detail of calculations can be found in Casey and Holden (2005a, b, c, d, e). The methodology required that: (i) a goal and scope was defined and a system boundary set; (ii) a functional unit (FU) was chosen to scale the emission data, in this case emissions per unit area (Casey and Holden, 2005b,d) (iii) impact was quantified in terms of the global warming potential (GWP) index; (iv) a system inventory was created and used with emission factors (EF) (Table 1) to quantify GHG emissions for the system; and (v) the emissions were analysed scaled relative to the FU. Average or typical Irish grassland milk production (Casey and Holden, 2005a), suckler beef production (Casey and Holden, 2005c) and mid-season lamb production (Casey and Holden, 2005e) systems were defined as the basis for evaluating the GHG emissions from these types of enterprise.

Table 1. Emission factors compiled from peer-reviewed data for the systems analysed.

DAIRY SYSTEM	CH <sub>4</sub>	CO <sub>2</sub>	N <sub>2</sub> O	DAIRY SYSTEM
Cows in milk	100 kg cow <sup>-1</sup>			Cows in milk
Other cattle	50 kg other <sup>-1</sup> stock <sup>-1</sup>			Other cattle
Fertiliser production	0.00174 kg kg <sup>-1</sup>	2.66 kg kg <sup>-1</sup>	0.0134 kg kg <sup>-1</sup>	Fertiliser production
Distance (fertilizer) <sup>^</sup>	0.014 kg	9 kg	0.002 kg	Distance (fertilizer) <sup>^</sup>
Merchant to farm*	0.32 kg kg <sup>-1</sup>	207.8 kg kg <sup>-1</sup>	0.045 kg kg <sup>-1</sup>	Merchant to farm*
Fertilizer applied			1.25 % -N	Fertilizer applied
Diesel used (l)	0.00064 kg	3.56 kg	0.0007 kg	Diesel used (l)
Electricity (kw h)		0.78 kg		Electricity (kwh)
Manure management				Manure management
Storage	0.001 - 0.01 kg m <sup>3</sup> d <sup>-1</sup>		2 g kg <sup>-1</sup> N <sup>-1</sup>	Storage
Dung in field	0.001683 kg cow <sup>-1</sup> d <sup>-1</sup>		2 % of N	Dung in field
Collecting yard	4.3x10 <sup>-7</sup> kg m <sup>-2</sup> h <sup>-1</sup>		7.5x10 <sup>-10</sup> kg m <sup>-2</sup> h <sup>-1</sup>	Collecting yard
Spreading	0.00286 kg t <sup>-1</sup>		0.0083 kg m <sup>-3</sup>	Spreading
Storage	0.001-0.01 kg m <sup>-3</sup> d <sup>-1</sup>		2g kg <sup>-1</sup> N <sup>-1</sup>	Storage
Dung in field	0.003-0.0003 kg cow <sup>-1</sup> d <sup>-1</sup>		2 % of N	Dung in field
Spreading	0.00286 kg t <sup>-1</sup>		0.0083 kg m <sup>-3</sup>	Spreading
BEEF SYSTEM	CH <sub>4</sub>	CO <sub>2</sub>	N <sub>2</sub> O	BEEF SYSTEM
Cows in milk	Tier 2			Cows in milk
Other	Tier 2			Other
Fertiliser production	0.0016 to 0.0018 kg kg <sup>-1</sup>	2.5 to 2.7 kg kg <sup>-1</sup>	0.012 to 0.014 kg kg <sup>-1</sup>	Fertiliser production
Distance input (fert.)	0.013 to 0.014 kg	8.55 to 9.45 kg	0.001 to 0.0021 kg	Distance input (fert.)
Merchant to farm#	0.304 to 0.336 kg kg <sup>-1</sup>	197.4 to 218.1 kg kg <sup>-1</sup>	0.042 to 0.047 kg kg <sup>-1</sup>	Merchant to farm#
Fertilizer applied			0.002 to 0.02 kg N <sup>-1</sup>	Fertilizer applied
Diesel used (kg)	0.00057 to 0.00063 kg	3.38 to 3.73 kg	0.00066 to 0.00073 kg	Diesel used (kg)
Electricity (kw h)		0.7 to 0.81 kg		Electricity (kw h)
Manure management				Manure management
Storage (Slurry)	0.001 to 0.0063 kg m <sup>-3</sup> d <sup>-1</sup>		0.01 to 0.04 kg N <sup>-1</sup>	Storage (Slurry)
Storage (Solid manure)	0.001 to 0.059 kg m <sup>-3</sup> d <sup>-1</sup>		0.01 to 0.04 kg N <sup>-1</sup>	Storage (Solid manure)
Pasture	0.008 to 0.002 kg cow d <sup>-1</sup>		0.01 to 0.04 kg N <sup>-1</sup>	Pasture
Spreading (Slurry)	0.0014 to 0.0042 kg t <sup>-1</sup>		0.0041 to 0.012 kg m <sup>-3</sup>	Spreading (Slurry)
Spreading (Solid manure)	0.003 to 0.009 kg t <sup>-1</sup>		0.007 to 0.02 kg t <sup>-1</sup>	Spreading (Solid manure)
Other cattle				Other cattle
Storage (Slurry)	0.001 to 0.0063 kg m <sup>-3</sup> d <sup>-1</sup>		0.01 to 0.04 kg N <sup>-1</sup>	Storage (Slurry)
Storage (Solid manure)	0.001 to 0.059 kg m <sup>-3</sup> d <sup>-1</sup>		0.01 to 0.04 kg N <sup>-1</sup>	Storage (Solid manure)
Pasture	0.003 to 0.0003 kg cow d <sup>-1</sup>		0.01 to 0.04 kg N <sup>-1</sup>	Pasture

Spreading (Solid manure)	0.003 to 0.009 kg t <sup>-1</sup>		0.007 to 0.02 kg t <sup>-1</sup>	Spreading (Solid manure)
Spreading (Slurry)	0.0014 to 0.0042 kg t <sup>-1</sup>		0.0041 to 0.012 kg m <sup>-3</sup>	Spreading (Slurry)
SHEEP SYSTEM	CH <sub>4</sub>	CO <sub>2</sub>	N <sub>2</sub> O	SHEEP SYSTEM
Ewes	7.59 to 9.27 kg yr <sup>-1</sup>			Ewes
Lambs 0 to 75 days	1.01 to 1.21 kg yr <sup>-1</sup>			Lambs 0 to 75 days
Lambs 76 to 162 days	1.17 to 1.44 kg yr <sup>-1</sup>			Lambs 76 to 162 days
Fertiliser production	0.0016 to 0.0018 kg kg <sup>-1</sup>	2.5 to 2.7 kg kg <sup>-1</sup>	0.012 to 0.014 kg kg <sup>-1</sup>	Fertiliser production
Distance input (fert.)§	0.013 to 0.014 kg	8.55 to 9.45 kg	0.001 to 0.0021 kg	Distance input (fert.)§
Merchant to farm#	0.304 to 0.336 kg kg <sup>-1</sup>	197.4 to 218 kg kg <sup>-1</sup>	0.042 to 0.047 kg kg <sup>-1</sup>	Merchant to farm#
Fertilizer applied			0.002 to 0.02 kg N <sup>-1</sup>	Fertilizer applied
Diesel used (kg)	0.00057 to 0.00063 kg	3.38 to 3.73 kg	0.00066 to 0.00073 kg	Diesel used (kg)
Electricity (kw h)		0.7 to 0.81 kg		Electricity (kwh)
Manure management				Manure management
Storage (Solid manure)	0.001 to 0.059 kg m3 d <sup>-1</sup>		0.01 to 0.04 kg N <sup>-1</sup>	Storage (Solid manure)
Pasture			0.01 to 0.04 kg N <sup>-1</sup>	Pasture
Spreading (Solid manure)	0.003 to 0.009 kg t <sup>-1</sup>		0.007 to 0.02 kg t <sup>-1</sup>	Spreading (Solid manure)

Adapted from Casey and Holden, 2005a,c,e

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## Will feeding high sugar grass reduce methane emission by grazing dairy cows?

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### Abstract

This study aimed at investigating the effect of feeding high sugar grasses to dairy cows on CH<sub>4</sub> emission using modeling approaches. The dynamic mechanistic rumen model of Dijkstra *et al.* (1992) coupled with the CH<sub>4</sub> module of Mills *et al.* (2001) was used. Data from 4 experiments in which high and low sugar grasses were fed to dairy cows was used as an input to the model. The differential magnitude in sugar content between high and low sugar grasses ranged from 24 to 66 g kg<sup>-1</sup> DM. Predicted CH<sub>4</sub> production by dairy cows consuming high and low sugar grasses was not different. A hypothetical situation in which the difference in sugar content between the two grasses was inflated to 100 g kg<sup>-1</sup> DM on the expense of both protein and fiber that were reduced by 50 g kg<sup>-1</sup> DM each, while keeping other input parameters constant was simulated. In this situation CH<sub>4</sub> production was 20.4 MJ d<sup>-1</sup> (1.2 MJ kg<sup>-1</sup> DMI) for the high sugar scenario compared to 18.6 MJ d<sup>-1</sup> (1.1 MJ kg<sup>-1</sup> DMI) for the low sugar scenario. It was concluded that feeding high sugar grass to dairy cows may not reduce methane emission from dairy production systems.

Keywords: methane, green house gases, high sugar grass, modeling.

### Introduction

Emission of methane (CH<sub>4</sub>) from ruminants on grasslands makes a substantial contribution to total agricultural methane emission. The global estimate of 44 Tg of CH<sub>4</sub> yr<sup>-1</sup> from ruminants on grasslands implies that approximately 20 % of all agricultural CH<sub>4</sub> emissions, and between 40 and 55 % of the total ruminant CH<sub>4</sub> emissions, arise from grasslands (Clark *et al.*, 2005). In ruminants, CH<sub>4</sub> is produced as an end product to enteric fermentation of feed in the rumen, and to a lesser extent the large intestine, by specific groups of microbes (methanogens) present in these segments of the digestive tract. Methane produced either in the rumen or the large intestine cannot be absorbed and utilized by the animal and hence is considered a loss of feed energy. In free ranging animals on temperate pastures, between 6.5 and 8 % of the gross energy (GE) consumed is lost as CH<sub>4</sub> (De Ramus *et al.*, 2003). Therefore, reducing CH<sub>4</sub> production by ruminants constitutes not only a reduction in the contribution of ruminant production systems to green house gases emission and hence environmental pollution, but also an improvement in feed-energy utilization. At present practical mitigation options for CH<sub>4</sub> emission that relate to grazing ruminants and grazed pasture are limited, with the best option for significant reductions in CH<sub>4</sub> being the direct manipulation of the rumen ecosystem (Clark *et al.*, 2005). Many strategies to directly manipulate the rumen ecosystem in order to reduce CH<sub>4</sub> emission have been investigated, i.e. the use of halogenated compounds, ionophores, and fatty acids (C<sub>12</sub> and C<sub>14</sub>). However, there are many limitation and restriction for those compounds to be widely used and accepted, i.e. toxicity, legislation and palatability. Changes in feed composition, mainly sugar and starch, have shown to significantly influence the rumen microbial population and ecosystem. Therefore, changing the composition of grass pastures to contain higher concentrations of sugars and starch presents a possibility to manipulate the rumen ecosystem and may be beneficial in reducing CH<sub>4</sub> emission. Recently, grass breeders succeeded in producing grass varieties that have high sugar content. Studies with high sugar grass (Peyraud *et al.*, 1997) showed a shift in rumen fermentation toward higher proportions of propionate and butyrate in rumen fluid. However, these studies did not measure CH<sub>4</sub> production. Measuring CH<sub>4</sub> production is laborious, expensive and requires sophisticated equipment. Mechanistic models have shown to be valuable for predicting CH<sub>4</sub> emission from dairy cows (Benchaar *et al.*, 1998; Mills *et al.*, 2001).

Therefore, the aim of this paper was to investigate the effect of feeding high sugar grass on CH<sub>4</sub> emission from dairy cows using modelling approaches.

## Methodology

The dynamic mechanistic rumen model of Dijkstra *et al.* (1992) coupled with the rumen CH<sub>4</sub> module of Mills *et al.* (2001), was used to simulate the effect of feeding high sugar grass on CH<sub>4</sub> emission by dairy cows. Benchaar *et al.* (1998) evaluated the ability of several dynamic and static models to predict CH<sub>4</sub> production over a large range of diets and concluded that the Dijkstra *et al.* (1992) model was the most reliable one. Mills *et al.* (2001) version of the model contains a post-ruminal digestive element that predicts CH<sub>4</sub> production in the large intestine. However, considering the objective of this paper and the fact that less than 10 % of CH<sub>4</sub> originates from fermentation in the large intestine (Mills *et al.*, 2001), we chose to neglect post-ruminal CH<sub>4</sub> emission. As an input, the model requires a full and accurate description of the ingested feed, amount of ingested feed, degradation and solubility characteristics of starch, protein and fibre, passage rate of the liquid and solid phases, and rumen volume. The data from four experiments in three publications that evaluated the effect of feeding high sugar grass on intake and milk production were used (Peyraud *et al.*, 1997; Miller *et al.*, 2001; Taweel *et al.*, 2005). Most of the input parameters needed for the model to run were reported in those studies (Table 1). When needed input data were not available, data from literature and feeding tables for perennial ryegrass were used. Passage rates for the liquid and solid phases were not reported in any of the studies; therefore the values of 0.15 and 0.03 h<sup>-1</sup>, respectively, were used and kept constant for all diets in all simulations.

## Results and Discussion

The differential magnitude in sugar content between the high and low sugar grasses ranged from 24 to 66 g kg<sup>-1</sup> DM (Table 1).

Table 1. The effect of feeding high sugar (HS) versus low sugar (LS) grass on CH<sub>4</sub> production by dairy cows predicted using the dynamic model of Dijkstra *et al.* and the CH<sub>4</sub> module of Mills *et al.*

	Peyraud <i>et al.</i> , 1997		Miller <i>et al.</i> , 2001		Taweel <i>et al.</i> 2005 <sup>1</sup>		Taweel <i>et al.</i> 2005 <sup>2</sup>	
	HS	LS	HS	LS	HS	LS	HS	LS
WSC (g kg <sup>-1</sup> )	246	180	165	126	181	157	180	149
CP (g kg <sup>-1</sup> )	106	150	92	106	159	162	151	157
S-fraction	0.47	0.50	0.15	0.12	0.07	0.06	0.07	0.07
D-fraction	0.44	0.41	0.77	0.72	0.75	0.77	0.76	0.77
U-fraction	0.09	0.09	0.08	0.16	0.18	0.17	0.17	0.16
kd-Protein	0.15	0.19	0.12	0.06	0.07	0.07	0.07	0.07
NDF (g kg <sup>-1</sup> )	496	528	544	589	415	428	414	430
D-fraction	0.86	0.82	0.85	0.85	0.86	0.87	0.86	0.86
U-fraction	0.14	0.19	0.15	0.15	0.14	0.13	0.14	0.14
kd-NDF	0.04	0.05	0.04	0.04	0.025	0.025	0.025	0.026
DMI (kg d <sup>-1</sup> )	15.1	15.3	11.6	10.7	16.2	16.6	16.2	17.1
Sugar intake (g d <sup>-1</sup> )	3715	2754	1914	1348	2932	2606	2916	2547
CH <sub>4</sub> (MJ d <sup>-1</sup> )	20.9	20.2	13.24	13.18	18.4	18.7	18.1	18.6
CH <sub>4</sub> (MJ kg <sup>-1</sup> DMI)	1.38	1.32	1.14	1.23	1.14	1.13	1.12	1.09

<sup>1</sup> the first Latin square experiment in Taweel *et al.* (2005).

<sup>2</sup> the second Latin square experiment in Taweel *et al.* (2005).



At the level of dry matter intake (DMI) reported in the studies (10.7 to 17.1 kg), a difference of 326 to 960 g in daily sugar intake between the cows on high and low sugar grass was achieved. The predicted rumen CH<sub>4</sub> production by dairy cows in those studies ranged from 13.2 to 20.9 MJ d<sup>-1</sup> and from 1.09 to 1.38 MJ kg<sup>-1</sup> DMI. The range in CH<sub>4</sub> production predicted by the model is within reported ranges in the literature (Benchaar *et al.*, 1998). The predicted CH<sub>4</sub> production by cows on high sugar grass was not different than that of cows on low sugar grass in all of the studies (Table 1). A hypothetical situation in which the difference in sugar content between the two grasses was inflated to 100 g kg<sup>-1</sup> DM at the expense of both protein and fiber that were reduced by 50 g kg<sup>-1</sup> DM each, while keeping the other input parameters constant was simulated. In this situation CH<sub>4</sub> production was 20.4 MJ d<sup>-1</sup> (1.2 MJ kg<sup>-1</sup> DMI) for the high sugar scenario compared to 18.6 MJ d<sup>-1</sup> (1.1 MJ kg<sup>-1</sup> DMI) for the low sugar scenario. From this hypothetical scenario, it appears that dairy cows on high sugar grass pasture may produce slightly higher amounts of CH<sub>4</sub>. In light of those findings, and the reported negligible effect on DMI and milk production (Peyraud *et al.*, 1997; Taweel *et al.*, 2005), it can be expected that higher amounts of CH<sub>4</sub> will be produced per unit of product when cows are offered high sugar grass pastures.

## Conclusion

From the simulated results of CH<sub>4</sub> production, it can be concluded that offering dairy cows a high sugar grass pasture will not reduce CH<sub>4</sub> production and hence green house gases emission from dairy production systems.

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# Mediterranean grasslands: ecological observations related to the climate of the past 55 years

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## Abstract

This evaluation was designed to gain insight into the ecological behaviour of herbaceous plant species growing in Mediterranean grasslands according to temperature and rainfall variations recorded over the past 55 years. Our evaluation included 100 sites in the central Iberian Peninsula differing in terms of land use, but lying on the same substrate in the same climatic region. We report the initial results for species acting as indicators of marked soil aridity and species representing the sites with less rainfall. Also discussed are observations, which together with these data provide knowledge on the responses shown by plant distribution to global warming in a semi-arid Mediterranean setting.

Keywords: precipitation, land use, plant indicators of soil aridity.

## Introduction

The real consequences of rapid climate change, such as those currently taking place, are not well known with regard to localized effects. It is foreseen that these potential effects will be considerable and substantially irreversible and will no doubt represent a threat to the country. The Iberian Peninsula is a transition boundary region separating contrasting climatic domains. Given the scarce and irregular nature of the rainfall, the abundant solar energy and elevated albedo, water and energy cycles show very particular characteristics. Under these conditions vegetation is scarce and provides the soil with little protection or organic matter. The natural habitats looked at – herbaceous formations, stony habitats and sclerophilic Mediterranean woods – are listed in “Natural Habits of Interest within the European Community” and have been the subject of recent conservation studies.

## Materials and methods

Study area: The landscape of the central Iberian Peninsula is mainly formed by a climax vegetation of terminal associations of the *Quercus rotundifolia* mesomediterranean series. Past vegetation only persists in regions where the excessive slope or private interests have impeded reclamation of the land. We are faced with a landscape of agricultural dry lands: fields of cereal interspersed with those left fallow, abandoned crops, vineyards, olive plantations and areas given over to grazing in oak wood clearings (Hernández *et al.* 1994).

Several ecosystems of the Iberian Peninsula's southern submeseta were considered by means of 100 sampling points corresponding to different soil uses following the abandonment of cereal cultivation: 50 livestock rearing and hunting grazing systems stabilized to a greater or lesser degree; 25 fallow systems subjected either to traditional management or intensely automated agriculture; 25 old fields. All these systems are found on the same arkosic substratum and represented most of the main soil types of the study area. These sampling points have their corresponding phytoecological inventories (based on random sampling of 1 m<sup>2</sup> plots) and soil analysis data. Several experimental plots were established to represent the different situations in which the plant communities referred to are found, in terms of soil use and climate variation. These plots were set up in the experimental farm “La Higuera”, Toledo (40° 04' latitude and 450 m altitude). The ecological and climatic characteristics (Table 1) of this farm, representative of the studied territory, are such that it provides, to a large degree, ideal scenarios for the study of relationships occurring between climate, land use and plant.

Table 1. Monthly rainfall values recorded on the experimental farm over the period.

Rainfall 1949-2004	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec	Year
Mean <sup>55 years</sup>	53.7	49.0	41.1	48.0	43.8	26.4	8.2	9.1	31,5	51.5	58.8	64.5	488.7
St.dev.	44.2	40.1	30.5	29.8	30.2	30.0	10.2	12.4	29,0	48.0	48.0	50.0	132.3

The ecological behaviour of 245 species was examined in relation to several environmental factors: 15 climatic and edaphoclimatic, 36 edaphic, 18 biotic and 2 soil management. Next, the 174 most frequently occurring species that also/alternatively showed most balanced ecological behaviour with respect to the environmental descriptors were selected. The methods employed for this analysis included the calculation of “species-environmental factors mutual information” and that of “corrected characters and indices profiles”.

## Results and discussion

Ecological behaviour of the species.- Among the 10 factors of greatest ecological significance for the species were: rainfall, soil moisture and greatest or least insolation received by the plot. Other factors that also showed importance were soil use, type of plant formation, species diversity of the community, soil pH and the proportions of OM and total N.

Table 2. Representative species of dry localities.

Species representing the sites with less rainfall			
Anagallis arvensis	Calendula arvensis	Daucus carota	Lactuca serriola
Andryala integrifolia	Cynosurus echinatus	Desmazeria rigida	Lathyrus angulatus
Atractyllis cancellata	Carduus tenuiflorus	Echium vulgare	Lolium rigidum
Avena sterilis	Centaurea ornata	Vulpia hispánica	Lotus conimbricensis
Bromus diandrus	Convolvulus arvensis	Galium tricornutum	Sonchus tenerrimus
Species acting as indicators of marked soil aridity			
Aegilops geniculata	Astragalus stella	Echinops strigosus	Tolpis barbata
Brachypodium dystachion	Coronilla scorpioides	Hedypnois cretica	Linum strictum
Bromus diandrus	Trifolium angustifolium	Helichrysum stoechas	Myosostis discolor
Bromus scoparius	Trifolium scabrum	Tolpis barbata	Neatostema apulum
Bromus tectorum	Trifolium stellatum	Arenaria leptoclados	Paronychia argentea
Coynephorus fasciculatus	Trigonella polyceratia	Diploaxis catholica	Plantago afra
Lamarckia aurea	Andriala integrifolia	Diploaxis virgata	Plantago albicans
Vulpia ciliata	Atractyllis cancellata	Echium vulgare	Silene colorata
Vulpia hispanica	Centaurea melitensis	Herniaria hirsuta	Silene inaperta
Vulpia myuros	Centaurea ornata	Jasione crispa	Velezia rigida

It was observed (Table 2) that 20 species showed a preference for areas of least precipitation (mean <500 mm per year) and 15 for areas in which greatest precipitation values were recorded (500-700 mm). Sixteen species showed an intermediate preference and the remainder were indifferent to this variable within the range examined. Analysis of the effects of this rainfall on the frequency and abundance of 42 of the most significantly affected species, revealed the following ecological behaviour patterns: 28 species showed similar behaviour in terms of frequency and abundance; in 7 species both these

characteristics were enhanced with increasing rainfall; and in a further 7, they were also enhanced with increasing rainfall but showed a subsequent fall. In another 10 species, frequency and abundance were independent of rainfall and in another 4 species, these characteristics showed a reduction with increasing rainfall. Only in 3 species was this type of behaviour antagonistic; while frequency increased with rainfall, abundance was reduced. Finally, the remaining species showed other types of behaviour with respect to the precipitation factor. The different degree of insolation received by each plot gave rise highly particular responses: 53 species showed a clear preference for “sun and shade” locations, while only 12 and 14 respectively preferred sites with full sunshine or shade.

Loss of definition of plant communities. The start of the rainy season is very sudden and depends on unpredictable meteorological events. In favourable years, the drought usually commences towards the second half of April or even later. However, in dry years, it may start much earlier with the arrival of dry, warm winds. The effects of drought may also be enhanced by spatial variability of the micro relief, the shade of the oaks and bushes and the fact most sp. show great intraspecific flexibility in their phenological behaviour. These changing factors give rise to “patterns”, or mosaics of plant populations in different states of maturity and desiccation. The status of the seed bank of these ecosystems is directly linked to the annual fertility of the adult plants. If seed renewal is interrupted for a year such as may occur during prolonged drought, impoverishment of the seed bank could reach significant proportions and threaten the stability of the population in terms of reproducibility. Most areas occupied by pasture land are suffering a phytoclimatic change for the worse through a process that is fairly regular. For the studied region, meteorological data recorded between the years 1949 and 1968 indicate that there were 9 more humid years, 7 comparable years and only 3 drier years than mean values corresponding to the last 50 years. However, in the 20 consecutive years (1969 to 1988), there were 8 relatively wet years, 6 normal and 5 dry. And over the last decade (1989-1998), there have only been 3 wet years, and 2 normal and 5 dry (Hernández et al., 2005). The situation may be summarized in any case as a generalized increase in the duration and intensity of aridity and a reduction, also generalized, in annual rainfall, with a certain increase nevertheless in summer rainfall. There is consequently, a danger of a stepwise decline or decay of the plant communities of the region. This has already been confirmed in the case of the “majadales”, or *Poa bulbosa* and subclover communities, whose species have been the subject of previous investigations centring on the effects of environmental factors (Pastor and Martín 1984, Pastor et al., 1994). Over the last years these communities have become scarcer and their floristic composition less typical (Hernández et al. 1994) than observed in the recent past (Pastor and Martín, 1984). Further, over the last few years we have observed that increased yearly temperatures negatively affect plants accelerating their development and reducing the time available for seed dispersal.

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# The improvement of the degraded grasslands with the new machine for oversowing MSPD 2.5

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## Abstract

Oversowing is one of the principal methods for the improvement of degraded grasslands and is applied successfully to increase the utilisation period, by correction of the botanical composition. The first part of the paper describes a new direct drilling machine for improving degraded grasslands. This specialized machine works by making rows with each active drilling organ following the topography of sward. The results from performance testing, presented in the second part of the paper, demonstrate the distinct advantages of the new machine in terms of agricultural requirements, functional and fuel economy performance.

Keywords: oversowing, degraded grassland, direct drilling machine.

## Introduction

Oversowing is one of the main techniques for improving degraded grasslands. In comparison with reseeded, oversowing grassland offers the following principle advantages: it can be carried out on grassland where reseeded by total tillage isn't feasible (i.e. due to superficial layers, surface stones, high levels of acidity or alkalinity in the deeper horizons, excess of humidity, eroded ground etc.); there are lower production penalties in the year of work; the sowing rates are lower, and both the energy consumption and costs are reduced.

Consequently, oversowing is applied successfully on permanent grassland and elderly leys to increase the utilisation period through correction of the botanical composition. This paper describes a new direct drilling machine and trial results obtained from oversowing degraded grassland.

## Materials and methods

The direct drilling machine for oversowing degraded grassland, MSPD 2,5 (Figure 1) is a towed machine working on the principle of 'gutter opening'. It is composed of a frame 1 of fused profiles, onto which the gutter opening and sowing equipment are assembled. The gutter opening equipment is made from sections arranged in two rows and assembled on the frame with two shoulder traps. Between the shoulder brace for fixing on the frame and the body of the section, deformed parallelograms are attached, activated by a spring attached behind, and allowing the ground contours to be mirrored and maintenance of a constant angle of attack by the sowing drill. The sowing equipment consists of a seed box with two partitions, one for large seeds (of perennial grasses or other crops) and one for small seeds (perennial legumes), distributors and stirrers for grass seeds, distributors for herbage legume seeds, tubes for the guiding the seeds into the furrows and a mechanism for their burial made from a stellar wheel which follows the soil, a Northon type gear box and transmission with chains between these and the axis of distributors and agitators. The main technical characteristics of the new machine are as follows: a power requirement of 48-59 kW; an operating width of 2.55 m; 17 sections of gutter opening; a minimum distance between adjacent gutters of 15 cm; a continuously adjustable working depth between 0.5 - 4 cm; seed box volume of 300 dm<sup>3</sup> for large seeds and 55 dm<sup>3</sup> for small seeds; a net weight of 1,035 kg.



The new machine was tested under the following conditions, comparing two types of grassland: Grassland in its 6<sup>th</sup> year of exploitation after sowing and natural grassland with *Agrostis tenuis*. The soil types were leaching chemozem and brown mollic; soil humidities in the 0-5 cm depth interval were 26.5% and 24.6%. The botanical composition of the natural grassland was *Agrostis tenuis* (32%), *Dactylis glomerata* (17%), *Festuca arundinacea* (12%), *Phleum pratense* (10%), *Festuca pratensis* (6%), *Achillea millefolium* (6%), *Urtica dioica* (6%), *other species* (11%). The botanical composition of the sown grassland was *Festuca pratensis* (25%), *Dactylis glomerata* (20%), *Lolium perenne* (18%), *Festuca rubra* (15%), *Phleum pratense* (10%), *Achillea millefolium* (3%), *Urtica dioica* (2%), *Other species* (7%). Preparations prior to oversowing consisted of low mowing and gathering up of vegetation. The seed mixture for oversowing the natural grassland was *Lolium perenne* (31%), *Festuca pratensis* (25%), *Phleum pratense* (16%), *Lotus corniculatus* (19%), *Trifolium repens* (9%). The mixture for oversowing the sown grassland was *Lotus corniculatus* (62.5%), *Trifolium repens* (37.5%).

### Results and discussion

Oversowing with the new machine gave average inter row distances of 14.85-15 cm, average depths of seed introduction of 1.78-2.2 cm, working widths of 2.52-2.55 m, working speeds of 6.05-9.5 km h<sup>-1</sup>, work capacity times of 1.05-1.54 ha h<sup>-1</sup>, and specific fuel consumption of 5.6-7.6 l ha<sup>-1</sup>. The quality control indices exceeded the agro-technical requirements associated with oversowing. The machine accommodated ground topography to an accuracy of  $\pm 10$  cm; specific fuel consumption per hectare was more than 2.5 lower than comparable methods for grassland renovation. Finally, the sown mixtures showed good establishment and growth.

### Conclusion

The overseeding of grass with this special seeder permits minimum tillage work with minimal damage of the old sward;

The new machine improves the mechanisation of overseeding for grassland under different uses, in different location and of different sizes;

The MSPD 2.5 type seeder, designed and built by ICDP Brasov, has proved to be a simple machine, easy to operate and does not use the power take off tractor.

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# The influence of mix composition and management on perennial ryegrass and Kentucky bluegrass presence in turf stand

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## Abstract

This work assessed differences between three sward mixtures with different proportions of perennial ryegrass (*Lolium perenne* L.) and Kentucky bluegrass (*Poa pratensis* L.) after four years of intensive or extensive management. The proportion of the two species in the three seed mixtures were 90:10, 75:25, and 60:40% of perennial ryegrass and Kentucky bluegrass, respectively. All plots were cut at a sward height of 30 mm. Intensively managed plots were cut 20 times year<sup>-1</sup> and fertilized with 300g N m<sup>-2</sup> and extensively managed plots were cut 8 times year<sup>-1</sup> and fertilized with 180 g N m<sup>-2</sup>. No significant differences were found between the mixtures in any parameters. The mean proportion in DM weight was 28 and 71% of ryegrass and Kentucky bluegrass, respectively. There were 58% more tillers of ryegrass in intensively managed turf than in extensively managed turf ( $P=0.03$ ) but no difference in number of Kentucky bluegrass tillers. There were no significant differences in DM weight between the two management regimes neither in perennial ryegrass or Kentucky bluegrass.

Under conditions of our experiment, extensive management yielded sufficiently, thereby allowing reducing costs and impact. The final decision about the choice of turfgrass mixtures and management has to take in account all factors.

Keywords: perennial ryegrass, kentucky bluegrass, turfgrass mixtures, fertilisation.

## Introduction

Using mixtures of grass species ensures genetic diversity and higher adaptive potential and increases the tolerance to pests, diseases and other environmental stresses compared with monostands (Beard, 1973). In temperate areas, turfgrass seed mixtures often consist of cultivars of perennial ryegrass (*Lolium perenne* L.) and Kentucky bluegrass (*Poa pratensis* L.) (Larsen and Bibby, 2004). Mixture of turf-types perennial ryegrass and Kentucky bluegrass are desirable for lawn planting because they combine a uniform appearance with genetic diversity (Gibeault *et al.*, 1980). Perennial ryegrass, because of its seedling vigor, helps to protect against establishment failures by stabilizing the soil soon after planting (Blaser *et al.*, 1956). Kentucky bluegrass is among the most wear tolerant turfgrass species, together with perennial ryegrass (Shearman and Beard, 1975; Canaway, 1981), and it is used notably for ability to develop rhizomes (Etter, 1951). However, Kentucky bluegrass germinates and emerges more slowly than perennial ryegrass (Skirde, 1967). When seed mixtures are used to establish polystands, differential germination and seedling growth rates must be considered in making compatible seed mixtures (Henderlong, 1971). The aim of this study was to obtain information on the effect of the applied turfgrass seed mixture on turf structure for mixtures of perennial ryegrass and Kentucky bluegrass, which should aid in the choice of optimum species composition in seed mixtures for football pitches and golf courses.

## Material and Methods

The field trial was carried out at the Breeding Station Větrov (620 m above sea level) from May 2000 to August 2005. Individual plot size was 1 m<sup>2</sup> and the experimental plots were established in a randomized block design with four replications. Plots were sown by hand in May 2000. The prevailing soil type was a loamy sandy brown podzolic soil. The soil reaction is acidic. Mean annual temperature for the past 50



years has been 6.9°C. The long-term annual precipitation is 642 mm. Three different turfgrass mixtures for football pitches were used, comprising 90:10 (mixture 1), 75:25 (mixture 2), and 60:40 (mixture 3) weight percent of 6 perennial ryegrass cultivars and 4 Kentucky bluegrass cultivars, respectively. Each mixture was exposed to intensive and extensive management. Grass stands were mowed 20 times per growing season using mainly a reel mower and 8 times per growing season using rotary mower for intensively and extensively managed plots, respectively, both at a sward height of 30 mm. Intensively managed plots were fertilized 8 times per year, 300g N m<sup>-2</sup>, and extensively managed plots 5 times per year, 180 g N m<sup>-2</sup>. Verticutting was done 3 times and 1 times per year on intensively managed and extensively managed plots, respectively. In 2005, four sample of 100x100 mm was taken from each plot and the above ground biomass was separated into grass species. The number of tillers of each species was counted, and biomass DM weight was determined for each species and for dead biomass after drying at 105°C for 24 hours. The results were analysed by ANOVA.

## Results and discussion

There were no significant differences among the three species mixtures neither in any of the evaluated parameters or their interaction except PR tillers ( $P>0.05$ ) (Table 1). There was a significant effect of management on the number of perennial ryegrass tillers ( $P= 0.03$ ). The number of tillers of ryegrass was 132%, 84% and 33% higher at the intensive management for the mixtures 90:10, 75:25, 60:40 respectively. There was no effect of management on Kentucky bluegrass tillers ( $P=0.61$ ). The total number of ryegrass + bluegrass tillers was higher in intensively managed plots for all three mixtures and was 16% higher as a mean for all mixtures but this increase wasn't significant. The total number of tiller was between 24,225 to 26,350 and 20,925 to 22,100 pieces m<sup>-2</sup> in intensively, extensively managed plots respectively (Table 1). There was no effect of different management on DM weight of neither perennial ryegrass, Kentucky bluegrass nor dead biomass for any of the mixtures ( $P>0.05$ ).

Table 1. Number of tillers and above-ground DM weight of two species in different turfgrass mixtures and at different management four years after establishment.

Mixture	Management	Shoot density (tillers m <sup>-2</sup> )			Weight of DM (g m <sup>-2</sup> )			
		Perennial ryegrass	Kentucky bluegrass	Total	Perennial ryegrass	Kentucky bluegrass	Dead biomass	Total
1 90:10	intensive	8,425	15,800	24,225	67.0	152.4	55.5	281.5
	extensive	3,625	18,400	22,025	36.0	185.2	240.0	462.0
2 75:25	intensive	9,975	16,375	26,350	86.0	164.4	241.0	491.3
	extensive	5,750	16,350	22,100	58.7	170.5	140.9	370.0
3 60:40	intensive	9,000	16,100	25,100	79.2	172.0	212.5	463.6
	extensive	6,750	14,175	20,925	68.5	147.3	230.7	446.5
Average	intensive	9,133	16,092	25,225	77.4	165.5	207.5	450.3
	extensive	5,269	16,308	21,683	54.4	167.6	204.1	426.1

## Conclusions

In conclusion, four years after establishment there were very few differences among the mixtures in the evaluated parameters. After the establishment phase, however, the botanical composition depends on other factors including the weather, soil conditions, diseases, insect, weeds and other organisms (Copeland and McDonald, 2001). Also, the ability of Kentucky bluegrass to develop rhizomes and to develop new shoots from rhizomes is really high when growth conditions favour this species, and the proportion of this species may potentially increase in the established turf even if the proportion in the seed mixture is rather low. The present results confirm that it is very difficult to predict the development of the botanical composition of turfgrass mixtures in a lawn.

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# Influence of sowing time and seed treatment on establishment of *Dactylis glomerata*

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## Abstract

Seeds of *Dactylis glomerata* L. non-coated or coated by the polymer Extender<sup>®</sup> were sown in three dates (December, February-March, April) in Prague in 2004 and 2005. The aim was to compare survival under dormant or frost seeding. The February or March sowing time and use of the coated seed were the most favourable for sward establishment. There was a significantly higher plant number by 8-51% in comparison with the sward established in April. Under the alternative times of sowing the plants from the coated seeds were, on average, higher by 23%, they had longer roots by 25%, higher number of leaves by 32% and the height of tillers by 71% ( $P < 0.01$ ) in comparison with the plants from uncoated ones. The alternative sowing times (December – March) accelerated the plants development significantly. The roots were longer by 60-250% (2004) or by 24-116% (2005), the number of leaves per plant was higher by 137-260% (non-coated seed) or by 254-427% (coated seed), the plants were higher in average by 29-152% in comparison with the swards sown in April. This work was supported by the grant No. MSM6046070901 of Czech Republic.

Keywords: *Dactylis glomerata*, Extender<sup>®</sup>, sowing, seed coating, emerging, plant development

## Introduction

Establishment of forage grasslands sward is influenced by many factors, such as weather conditions, soil moisture content and temperature. Spring (April) or summer is the traditional sowing time of grasses in Czech conditions. Sometimes the economically more profitable crops stands are sown in preference in the more favourable time in spring while sowing of forage crops is shifted in less suitable time. An alternative time of sowing is possible during the cold period - dormant or frost seeding and is used for many crops, such as oilseed rape and others (Johnston *et al.*, 2004; Casler *et al.*, 1999). Sowing in these times enables better distribution of workloads during the spring period and an earlier germination of seeds in good soil moisture conditions. Seed coating is used for the prevention of premature seed germination during winter and spring under the conditions of low temperatures and high moisture (Taylor *et al.*, 1998; Clayton *et al.*, 2004). A seed coat (e.g. Extender<sup>®</sup>) is impermeable to water in cold soil and serves as a physical barrier, which does not allow the entry of oxygen into the seed; it protects seeds against low soil temperature and soil moisture stress, and is based on polymer and organic acids inhibitors. This polymer is widely used to protect canola seeds in Canada. Plant stands established during alternative times of sowing emerge earlier, and then cover more rapidly the soil in spring. The main aim of the experiments was to prove the effects of Extender coating and different sowing time on *Dactylis glomerata* initial development under the Czech conditions.

## Material and methods

The field experiments with *Dactylis glomerata* L. were conducted in Prague (altitude 286 m, 50°08'N, 14°24'E, vegetation period 172 days, average year temperature 7.9°C, mean year precipitation 526 mm). Coated seed (26 kg ha<sup>-1</sup>) and non-coated seeds (24 kg ha<sup>-1</sup>) were sown in rows in three times (late autumn-December, late winter-February or March and spring-April) in 2003-2004 and 2004-2005 (Table 1). The April sowing was used as the check. Sowing depth was 10-20 mm, the rows length 10 metres. Time of emergence, height of plants, and number of plants were recorded. The height of plants and tillers, number of additional tillers, number of leaves, and roots depth, were measured on

50 plants during the vegetation (12<sup>th</sup> and 26<sup>th</sup> May and 30<sup>th</sup> June in 2004, 11<sup>th</sup> and 30<sup>th</sup> May and 27<sup>th</sup> June 2005). The data were evaluated by the analysis of variance using Statgraphics Plus 4.0, (Tukey HSD test,  $\alpha = 0.01$ ).

## Results and discussion

The number of plants (Table 1) from February or March sowing time was significantly higher (on average of coated and non-coated seed) by 8-51% in comparison with the sward established as usual in April, but the seedling emergence and persistence from the December sowing time was lower by 16-64%. The number of plants from the coated seed was always higher (by 3-11%) with the alternative sowing time. The April sowing time showed that the seed coating was not necessary since there was a lower plants number (by 10-14%) in the sward comparing it with the control plots. The beginning and duration of the growth stages were influenced by the time of sowing. Plants from seeds, which had been sown in late autumn and late winter, began to grow one month earlier than the plants from the April sowing time (4<sup>th</sup> April 2004 and 12<sup>th</sup> April 2005).

Table 1. Time of sowing and average number of plants (plants m<sup>-1</sup>).

Year of evaluation	2004		Time of sowing	2005	
	Non-coated	Coated		Non-coated	Coated
1 <sup>st</sup> December 2003	28.9	32.1	8 <sup>th</sup> December 2003	17.9	18.4
17 <sup>th</sup> February 2004	53.1	57.8	24 <sup>th</sup> February 2004	53.5	52.7
22 <sup>nd</sup> April 2004	44.5	38.3	20 <sup>th</sup> April 2004	49.5	44.5

Table 2. Plant characteristics in three samplings in the years 2004 and 2005.

Sowing time:	Year of evaluation 2004						Year of evaluation 2005						
	December 03	February 04	April 04	December 04	March 05	April 05	December 03	February 04	April 04	December 04	March 05	April 05	
Sampling time:	N*	C**	N	C	N	C	N	C	N	C	N	C	
Height of plants (mm)	1st	107a	68c	88ab	96b	38d	34d	61a	66a	32b	44b	0c	0c
	2nd	129a	124ab	119ab	112b	38c	46c	79ab	73abc	55bc	99a	39c	33c
	3rd	333a	314a	302a	331a	176b	178b	127a	155a	114a	138a	142a	117a
Leaves per plant	1st	6.7a	5.6a	6.5a	6.7a	1.5b	1.0b	3.5a	3.9a	1.7b	1.9b	0c	0c
	2nd	9.9a	8.7ab	8.0ab	7.3b	1.9c	1.0c	5.8a	8.4a	4.3a	5.0a	2.0b	1.7b
	3rd	11.3ab	14.0a	14.5a	13.9a	8.1b	7.3b	11.6a	12.9ab	4.9bc	9.3ab	3.7c	3.6c
Tillers per plant	1st	0.7a	0.7a	0.7a	0.7a	0b	0b	0.0b	0.3a	0b	0.03b	0b	0b
	2nd	1.3a	0.8a	1.0a	0.8a	0b	0b	1.0a	1.3a	0.4ab	0.4ab	0b	0b
	3rd	1.8ab	2.1a	2.4a	2.2a	0.7bc	0.5c	3.0a	2.2ab	0.2abc	1.2bc	0c	0c
Height of tillers (mm)	1st	67a	42b	55a	58a	0c	0c	52a	32b	0c	10c	0c	0c
	2nd	101a	73b	79b	75b	0c	0c	46a	68a	59a	61a	0b	0b
	3rd	210a	236a	214a	240a	117ab	156b	83b	115ab	148a	102ab	0c	0c
Leaves per tiller	1st	2.9a	1.3b	2.9a	3.0a	0c	0c	2a	1.7a	0b	1ab	0b	0b
	2nd	3.7a	3.7a	3.0b	3.0b	0c	0c	2.4a	3.3a	3.0a	2.4a	0b	0b
	3rd	3.4a	3.9b	3.6ab	3.5ab	3.3a	3.4ab	3.0b	3.2b	3.5ab	3.5a	0c	0c
Root depth (mm)	1st	82a	58b	77a	80a	26c	21c	47a	39a	18b	21b	0c	0c
	2nd	118a	104a	102a	136a	39b	34b	47a	55a	34b	60a	33b	30b
	3rd	172a	166a	141b	127b	98c	77c	94a	87a	66ab	93a	53b	42b

\* N – plants from non-coated seeds, \*\*C – plants from coated seeds  
*a, b, c* – shows significant differences in rows in each year separately ( $p=0.01$ ).

There was no significant difference between the plants from coated and non-coated seeds in the year 2004 (Table 2). The significant differences were found among the earlier time of sowing and the traditional April time. However there were no significant differences between the sowing in December and in February in all measured attributes. There were differences 70% in plant height and root depth between the sowing in April and the alternative times. In 2005 there were significant differences in all the characteristics, in dependence on the sowing time as well as on the seeds coating. Under the alternative time of sowing the plants from the coated seeds were on average higher by 23% ( $P<0.01$ ), they had longer roots ( $P<0.01$ ) by 25%, higher number of leaves ( $P<0.01$ ) by 32% and the height of tillers by 71% ( $P<0.01$ ) in comparison with the plants from non-coated ones, only the number of leaves per tiller was lower. The alternative sowing time (December – March 2005) significantly accelerated ( $P<0.01$ ) the plants development (due to the earlier emergence). The roots were longer by 60-250% (2004) or by 24-116% (2005), the number of leaves per plant was higher by 137-260% (non-coated seed) or by 254-427% (coated seed), the plants were higher on average by 29-152% in comparison with the sward sown in April.

### Conclusions

The results proved that the late winter sowing time (February – March) is the most favourable for seed germinating and the following development of *D. glomerata* plants. The consequences of the earlier germination and more successful initial development can take effect in the grass yields. Plants from earlier sowing time cover the soil surface faster and they can better withstand a competition from weeds. Sowing the grass in December (with a coated or non-coated seed) can be unsafe under comparable climatic conditions. The protective effect of seed coating by Extender<sup>®</sup> was not statistically proved in this experiment. We can expect a similar reaction of other grass species to alternative sowing time and seed treatment.

### Acknowledgements

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# Utilization of altitudinal gradients for interdisciplinary knowledge on mountain grasslands productivity

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## Abstract

In order to outline the mountain ecological gradients we extracted and processed, on altitude level, a large volume of data existing in our speciality literature about climate, soils, vegetation, biological productivity, outdoors human activity and so on. For these factors there were found out for the first time the special altitude gradients for Romanian Carpathians. Compared to the hill level the achievement of the animal production in the studied farms situated at more than 1400 m a.s.l. is twice lower and the expenses for one product are twice higher. If we consider as base the prices for the animal products obtained at the altitude of 0-600 m, the subsidies would have an increasing with 10% for each 100 m altitude.

Keywords: altitudinal gradients, mountain ecosystems, grassland productivity, subsidies.

## Introduction

Without the knowledge of evolution on altitude of natural, economical and habitat factors in mountainous zone, we cannot determine the current deficiencies and the level of necessary subvention. Utilization of altitude gradients makes possible the synthesis of the results obtained about these factors and a better knowledge of phenomena for the specialists of different activities.

## Materials and methods

There were used the climatic data from meteorological stations, grassland production in experimental fields, animal productions (milk, live weight gain) from the experiments carried out in 1950-2000 period in mountain zone of Romanian Carpathians and so on. After recording of data on squared paper, using different scales in function of analysed factor and altitude quota of meteorological stations and experimental fields in grasslands, have been connected by lines deliberately drawn straight, similarly of mathematical linear functions, estimating the vertical gradients.

## Results and discussion

Climatic conditions of Romanian Carpathians present a decreasing rate of mean annual temperatures of  $-0.5\text{ }^{\circ}\text{C}$  and annual rainfall increase of  $+45\text{ mm}/100\text{ meters altitude}$  (Table 1). The climate has an important influence on soil characteristics and grassland productivity (Table 2). So, the depth of soil level decreased with  $7.5\text{ cm}$ ,  $\text{pH}_{\text{H}_2\text{O}}$  with  $0.15\text{ units}$  and saturation degree of bases with  $3\%$  at every  $100\text{ meters altitude}$ . In these conditions the grassland production of control experimental plots situated at  $600\text{-}800\text{ m altitude}$  was  $3.0\text{ t DM ha}^{-1}$  and decreased to  $0.9\text{ t DM ha}^{-1}$  at  $2000\text{-}2200\text{ m altitude}$ , with a decrease of  $0.15\text{ t DM per }100\text{ m altitude}$ .

Table 1. General data regarding the climatic conditions of mountains in Romanian Carpathians.

Altitude (m)	Mean temperature (°C)			Annual rainfall (mm)	Mean air pressure (mm)	Mean speed of wind (m/s)
	Annual	January	July			
2000-2200	0	-8.7	7.2	1400	600	9
1800-2000	1	-8.1	8.6	1350	615	8
1600-1800	2	-7.5	10.0	1250	630	7
1400-1600	3	-6.9	11.4	1150	645	6
1200-1400	4	-6.3	12.8	1050	660	5
1000-1200	5	-5.7	14.2	950	675	4
800-1000	6	-5.1	15.6	850	690	3
600-800	7	-4.5	17.0	800	705	2
Gradients for 100 m alt	- 0.5	- 0.3	- 0.7	+ 45 mm	- 7.5 mm	+ 0.5 m/s

Table 2. Data regarding the soil characteristics and dry matter yield (DM) of the grazed mountains.

Altitude (m)	Depth layer (cm)	Horizon "A"		DM production (t/ha)		
		pH in H <sub>2</sub> O	V%	Unfertilized	Medium fertilized	Δ (%)
2000-2200	35	3.9	12	0.9	1.6	178
1800-2000	50	4.2	18	1.2	2.3	192
1600-1800	65	4.5	24	1.5	3.0	200
1400-1600	80	4.8	30	1.8	3.7	206
1200-1400	95	5.1	36	2.1	4.4	210
1000-1200	110	5.4	42	2.4	5.1	212
800-1000	125	5.7	48	2.7	5.8	215
600-800	140	6.0	54	3.0	6.5	217
Gradients for 100 m alt	-7.5 cm	- 0.15	- 3 %	- 0.15 t	- 0.35 t	*

Table 3. Efficiency in animal products on improved pastures.

Altitude (m)	Grazing period (days)	DM production (t ha <sup>-1</sup> )	Organic matter digestibility (%)	DM consumption for		Animal production (kg ha <sup>-1</sup> )	
				1 L milk	1 kg live weight gain	Milk	Weigh t gain
2000-2200	55	1.6	60.0	1.5	18	1100	90
1800-2000	70	2.3	61.5	1.4	17	1700	140
1600-1800	85	3.0	63.0	1.3	16	2300	190
1400-1600	100	3.7	64.5	1.2	15	3100	250
1200-1400	115	4.4	66.0	1.1	14	4000	310
1000-1200	130	5.1	67.5	1.0	13	5100	390
800-1000	145	5.8	69.0	0.9	12	6600	490
600-800	160	6.5	70.5	0.8	11	8100	590
Gradients for 100 m alt							
1800-2000						- 300	- 25
1200-1800	- 7.5	- 0.35	- 0.75	+ 0.05	+ 0.5	- 450	- 30
600-1200						- 750	- 50

The animal production is strongly influenced by pasture production, digestibility and grazing period (Table 3). Because of decreasing of these factors with altitude it is estimated that the additional subventions of 10% for every 100 m altitude are necessary to maintain these farmsteads in mountain zone (Table 4).

Table 4. Degree of economical unpropitiousness of the mountain area and necessity of supplementary subventions (% of base altitude).

Altitude limits (m)	Possibilities of outdoor working	Difficulties encountered in unfavourable climate	Extraefforts feeding of animal in sheds	Level of achievement of animal productions	Estimated for the same food product	Supplementary subventions for the agricultural products
1400-1600	75	125	150	50	200	100
1200-1400	80	120	140	60	180	80
1000-1200	85	115	130	70	160	60
800-1000	90	110	120	80	140	40
600-800	95	105	110	90	120	20
0-600 (base)	100	100	100	100	100	0
Evolution for 100 m alt.	- 2.5 %	+ 2.5 %	+ 5.0 %	- 5.0 %	+ 10 %	+ 10 %

### Conclusions

By complex analysis of natural and economical factors in mountain zone using the gradients, it can be established with more accuracy the necessary subventions to sustain the animal breeding in zones where the grasslands represent the principal feeding sources and a good co-operation and exchange of knowledge between different mountains specialists.

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# Estimating floristic richness of pastures using indicator species: a study case in the Dolomitic area

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## Abstract

Floristic richness is a fundamental aspect of specific biodiversity, and its maintenance nowadays is considered a very important factor in the land use planning. The aim of this work was to characterize, on a pasture of the dolomitic area, a set of indicator species of floristic richness, and to evaluate the optimal number of indicators for a reliable prediction. Fifty botanical surveys were performed in 2002. With the use of logistic regression, 31 species were found, significantly more frequent in the species-rich areas than in the species-poor ones. Ten subsets of  $n = 3, 5, 8, 10, 15, 18, 20$  species were randomly drawn from the 31 potential indicators; the predicted mean species richness and the precision of the estimate were measured in each case. On the base of the results it was highlighted a low estimate efficiency in the case of the highest  $n$  values; the precision, instead, was positively correlated with increasing subset size. However, for each  $n$ , the random choice of some species among the 31 indicators did not influence the final accuracy and precision of the estimate.

Keywords : biodiversity, indicator species, floristic richness.

## Introduction

Considering the study and quantification of specific biodiversity in the ecological assessment, a possible solution is given by the realization of exhaustive lists of species. This method, however, is time-consuming, it needs a high economic investment and, moreover, the resulting information may be more detailed than needed for planning (Muller and Gusewell, 2003). A possible alternative is represented by the use of only a few indicator species. Anyway, indicator species are often selected according to ad hoc criteria, such as their legal protection status (Andelman and Fagan, 2000), while this selection is rarely based on convenient statistical principles. The aim of this work was to contribute to the individuation of a possible criterion for the selection of indicator species using just statistical methods.

## Materials and Methods

The study area is represented by a sub-alpine pasture (set from 1677 to 2060 m a.s.l.) located in the municipality of Livinallongo del Col di Lana (Belluno, NE of Italy). In this area, during year 2002, 50 botanical surveys were performed, according to the Braun-Blanquet approach. The plot size was 100 m<sup>2</sup>. Applying binary logistic regression to all the 173 species that were recorded, 31 potential indicators of floristic richness were found statistically more frequent in the species-rich areas than in the species-poor ones (Table 1). Subsequently, some subsets of  $n = 3, 5, 8, 10, 15, 18, 20$  species were randomly selected from the 31 indicators; each of these subsets was represented by 10 replicates (with  $k = 1, 2, \dots, 10$ , indicating the individual subset with same  $n$ ). For each of the 70 subsets, identified by  $n$  and  $k$ , the 50 botanical surveys were divided in  $n + 1$  classes of plots, containing  $i = 0, 1, 2, \dots, n$  indicator species. For each class, defined by a value of  $n, k$  and  $i$ , the mean floristic richness and the relative standard deviation (SD) of the plots included in the class were calculated. Finally, in order to assess the total accuracy of the estimate for a certain  $n$ , the variability of predicted species richness among the 10 replicates (SD of the 10 values obtained for each  $n$  and  $i$ ) and the level of precision of the estimate (mean of the 10 SD for each  $n$  and  $i$ ) were calculated and discussed.

Table 1. Potential indicator species of floristic richness (significant at  $P < 0.01$ ). All species are ordered by decreasing  $\text{Chi}^2$  values of binary logistic regression relating their occurrence to the number of species in 100 m<sup>2</sup> plots. High  $\text{Chi}^2$  values indicate a good regression fit.

Species	$\text{Chi}^2$	Species	$\text{Chi}^2$	Species	$\text{Chi}^2$
<i>Anthoxanthum odoratum</i>	34.0	<i>Trifolium badium</i>	16.3	<i>Nardus stricta</i>	10.89
<i>Cirsium acaule</i>	24.8	<i>Onobrychis viciaefolia</i>	15.7	<i>Nigritella nigra</i>	10.85
<i>Agrostis tenuis</i>	22.0	<i>Phyteuma orbiculare</i>	14.0	<i>Pedicularis elongata</i>	10.66
<i>Campanula rotundifolia</i>	21.4	<i>Carex sempervirens</i>	14.0	<i>Centaurea jacea</i>	10.24
<i>Anthyllis vulneraria</i>	20.4	<i>Rhinanthus aristatus</i>	12.8	<i>Euphrasia rostkoviana</i>	8.92
<i>Lotus corniculatus</i>	20.0	<i>Plantago media</i>	12.3	<i>Gentianella germanica</i>	8.77
<i>Koeleria pyramidata</i>	18.4	<i>Gentiana ciliata</i>	12.2	<i>Poa alpina</i>	8.66
<i>Leucanthemum vulgare</i>	17.2	<i>Festuca ovina</i>	11.8	<i>Phleum alpinum</i>	8.65
<i>Avenula versicolor</i>	16.9	<i>Trifolium montanum</i>	11.8	<i>Trollius europaeus</i>	7.79
<i>Ranunculus montanus</i>	16.7	<i>Carex flacca</i>	11.50		
<i>Erigeron alpinus</i>	16.6	<i>Dactylis glomerata</i>	11.28		

## Results

The number of indicators in each survey was linearly related ( $R^2 = 0.75$ ) to the total number of species (from 26 to 55); anyway, it was not correlated with the number of non-indicator species ( $R^2 = 0.10$ ). The number of indicators, in fact, represented a high fraction of the total number of species, and the increase of the indicators' presence strongly influenced the general variability of species richness. However, even when the subsets of  $n = 3, 5, 8, 10, 15, 18, 20$  indicator species were considered, there was still a linear relation between the number of indicators in each survey and floristic richness (Figure 1).

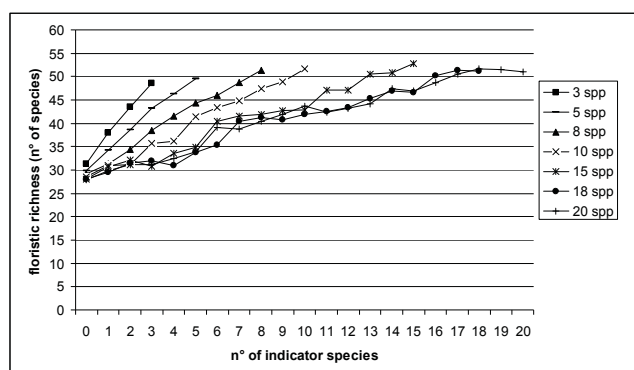


Figure 1. Relation between number of indicator species and mean predicted floristic richness (floristic richness expressed as mean of 10 replicates for each  $n$  and  $i$ ).

The importance of each species in the estimate of floristic richness was inversely related to the subset size: the presence of 5 indicators, for example, estimated a higher number of species if these indicators were included in a subset of 8 species than 10 species. Considering, for each  $n$  and  $i$ , the mean variability (SD) of the floristic richness among the 10 replicates (Table 2a), this value was not related to the subset size and it was included between 1.90 and 2.69. For each value of  $i$ , the differences between

the 10 replicates are nearly all not significant, and if compared with the range of variation of predicted species (from 27 to 52), these values are relatively small, meaning that, for a certain  $n$ , the choice of a particular combination of indicator species among all it is not important for the total accuracy of the estimate. Table 2b, instead, underlined the overall precision of the estimate, considering the mean of the 10 SD of the replicates for each  $n$  and  $i$ . In this case, the precision of the estimate increased with the subset size. Moreover, considering only the cases in which there was the presence of at least 50% of the indicators, it was observed that for  $n = 8, 10, 18$ , there were not significant differences (SD= 4.27, 4.15, 4.11, respectively).

Table 2. **(a)** Variability in predicted species richness among 10 subsets (SD), and **(b)** variability of actual floristic richness among surveys. Decreasing values of SD indicate a higher precision. The presence of \*, in table 2a, indicates that among the 10 replicates some significant differences were found.

	subset size													
	3	5	8	10	15	18	20	3	5	8	10	15	18	20
Indicat. present														
	<b>a) SD of predicted flor. richness among subsets</b>							<b>b) SD of actual flor. richness among surveys</b>						
0	1.70	0.93	0.99	1.01	1.01	1.19	1.65	4.44	3.25	2.91	2.55	2.93	2.87	3.21
1	4.55*	4.10*	2.26	1.28	1.11	1.75	1.12	5.86	4.30	3.93	2.34	2.51	2.80	2.31
2	1.77	3.10	2.49	1.07	1.84	2.34	1.13	5.56	5.22	6.04	2.85	2.41	1.31	1.54
3	1.74	1.50	2.90	2.76	2.23	1.43	2.70	4.71	5.60	5.28	5.78	2.47	2.49	4.39
4		1.29	1.74	5.25	4.69	2.78	2.65		4.50	5.03	4.72	4.95	3.46	6.36
5		1.59	1.39	1.03	5.79	6.92	6.49		4.63	5.09	4.94	4.50	4.95	5.19
6			2.43	1.16	3.97	4.82	3.98			4.59	5.52	4.14	6.60	-
7			1.18	2.21	2.49	2.61	3.99			3.94	4.67	4.94	1.51	2.67
8			2.24	1.35	1.52	2.38	1.66			2.69	3.76	4.61	3.28	3.43
9				2.03	2.91	3.32	2.79				3.39	4.90	5.82	2.68
10				1.72	1.98	2.18	2.92				2.63	5.77	6.72	5.66
>10					1.31	2.23	2.55					2.48	3.57	3.58
mean	2.44	2.08	1.96	1.90	2.26	2.61	2.69	5.14	4.59	4.39	3.92	3.53	3.71	3.67
>50% <sup>a</sup>	1.76	1.46	1.80	1.58	1.62	2.34	2.58	5.13	4.91	4.27	4.15	3.46	4.11	3.79

<sup>a</sup> mean value based on the presence of more than 50% of the indicator species.

## Conclusion

On the base of the results it was highlighted that the use of a high number of indicators is not suitable, because of the high influence of the indicators themselves on the variability of floristic richness. Anyway, when a subset size  $n$  is selected, the choice of some indicators among all did not influence the accuracy of the estimate, especially when at least 50% of the indicators were present. Even if the use of small indicators subsets gave to each species a higher importance in the estimate, for small  $n$  values the estimate resulted more imprecise. A suitable compromise seemed to be  $n=10$  or 15.

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## Wood ash as fertilizer and soil acidity corrector: effects on soil quality and crop yield

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### Abstract

Soil acidity limits soil microbial activity and biodiversity as well as crop yield. In the Basque Country (northern Spain), the forestry industry annually generates great amounts of ash, which is considered a useless waste. In this context, a field assay was established to study the effect of the application of wood ash on the acidity (pH, % Al saturation), fertility (yield, forage quality) and soil biological quality (*e.g.*, soil enzymes, potentially mineralizable nitrogen, respiration, abundance of earthworms, and microbial community metabolic profiles) of a typical acid soil of this region. This effect was also compared with that of lime (CaO), applied at the same rate of Ca. In March 2005, three contiguous areas of *Lolium multiflorum* Lam. received the following treatments, respectively: (1) ash (15.5 t ha<sup>-1</sup> of wood ash and N fertilizer), (2) lime (1.1 t ha<sup>-1</sup> CaO and N-P-K fertilizer, in order to equal the doses of nutrients) and (3) no addition. A contiguous native meadow was also studied as absolute control (treatment 4). Soil and herbage samplings were made in May 2005. Results showed that wood ash can be a useful alternative as an acidity corrector, and is a useful means of utilizing a waste material.

Keywords: soil acidity, microbial activity and biodiversity, yield.

### Introduction

As a consequence of its high annual rainfall, in the Basque Country (northern Spain), soil acidity is a common problem (as the result of leaching of monovalent and divalent cations) which negatively affects the establishment and productivity of grasslands. Under these conditions, the values of % Al saturation frequently reach phytotoxic levels. Many timber companies produce, as a residue, a considerable amount of wood ash which is known to have relatively high values of pH and a high content of carbonates and other frequently limiting nutrients such as P and Mg. Therefore, in the literature, there are reports on the successful utilization of wood ash to correct soil acidity and increase fertility (Santoalla *et al.*, 2004). However, the effect of the addition of wood ash on biological indicators of soil health has been rarely studied. Biological indicators of soil health are becoming increasingly used rather than physicochemical properties because of their sensitivity and capacity to provide information that integrates many environmental factors. The main objective of the current work was to study the short-term effects of the addition of wood ash, as compared to that of lime (traditionally, CaO has been added to the soil to correct soil acidity) on both (i) forage production and quality and (ii) biological indicators of soil health, *i.e.* microbial community metabolic profiles, soil respiration, enzyme activities (dehydrogenase,  $\beta$ -glucosidase and acid phosphatase), mineralizable N and earthworms.

### Materials and methods

The experiment was established in spring 2005 at Derio, Basque Country (northern Spain) in a former grassland that had been seeded in January with Italian ryegrass (*Lolium multiflorum* Lam. cv. Nival) without any fertilization. According to the FAO classification system, the soil was an Epidystric Cambisol, with the following initial characteristics: silty clay loam texture, pH=5.1 (1:2.5 in water), 17.2 g kg<sup>-1</sup> OM, 1.3 g kg<sup>-1</sup> total N, 8.5 mg kg<sup>-1</sup> Olsen P and 125 mg kg<sup>-1</sup> extractable K (NH<sub>4</sub>Ac). Early in March, a homogeneous zone of 2,400 m<sup>2</sup> was divided into three plots receiving the following treatments, respectively: (1) ash (15.5 t ha<sup>-1</sup> of wood ash and 60 kg ha<sup>-1</sup> N), (2) lime (1.1 t ha<sup>-1</sup> CaO and 60-60-190 kg ha<sup>-1</sup> N-P<sub>2</sub>O<sub>5</sub>-K<sub>2</sub>O, in order to equal the doses of Ca, P and K applied by ash) and (3) no

addition. An absolute control (4), consisting of a contiguous native meadow, was also studied. In May, soil sampling was carried out randomly in each of the plots. In plot (1), 27 composite soil samples were taken whereas 4 composite samples were taken in plots (2), (3) and (4). In order to give a composite sample, 15 soil sub-samples were collected at a depth of 0-10 cm and mixed together. Before soil analysis, soils were air-dried at 30°C for 48 h, sieved to <2 mm, and stored at 4°C. Soil physicochemical properties were determined according to MAPA (1994): texture, pH, % Al saturation, OM content, total N, Olsen P and extractable K (NH<sub>4</sub>Ac). Enzyme activities (*i.e.*, dehydrogenase,  $\beta$ -glucosidase, acid phosphatase) were determined according to Dick *et al.* (1996). Potentially mineralizable N (Powers, 1980), soil respiration (Parkin *et al.*, 1996) and the abundance of earthworms were also measured. Finally, microbial community metabolic profiles were obtained using Eco-BIOLOG<sup>TM</sup> plates, according to Crecchio *et al.* (2004). Furthermore, to quantify forage yield and quality, a unique cut of the ryegrass was done and the following properties were analysed: digestibility (D), neutral (NDF) and acid detergent fibre (ADF) and crude protein (CP) according to Zasoski and Burau (1977). One-factor analysis of variance (ANOVA) and the Fisher test were used to determine significance of differences between the treatments ( $p < 0.05$ ).

## Results and discussion

As shown in Table 1, in the native meadow absolute control treatment, soil acidity (pH and % Al saturation) was lower and significantly higher values of biological indicators of soil health were found. The plot treated with lime did not show significant differences when compared with the plot that did not receive any acidity corrector addition, but both had higher values of  $\beta$ -glucosidase and acid phosphatase activities than the ash treated plot. These biological activities control the rates of soil C and P cycling processes, respectively. In contrast, the ash treatment, as well as correcting soil acidity, increased Shannon's diversity index ( $H'$ ) as measured with BIOLOG<sup>TM</sup> plates. This microbial diversity reflects the potential of the culturable portion of the soil microbial community to respond to substrates, and it can be very important where there has been potential external perturbances to the system. Both ash and lime treatments seem to enhance dehydrogenase activity, associated with viable microbial populations (Burns and Dick, 2002), although the differences in comparison with the 'no addition' plots were not statistically significant. Mineralizable N and soil respiration (associated with *in situ* microbial activity) did not show differences between these treatments. Finally, earthworms were not found in the lime plot, perhaps because of a possible irritant effect of the lime, although earthworms were not abundant in the whole zone.

Table 1. Effect of treatments on soil physicochemical (*i.e.*, pH and % Al saturation), Shannon's diversity index ( $H'$ ) and biological properties. Different letters within each column indicate statistically significant differences.

	pH	%Al sat.	Respiration (g CO <sub>2</sub> m <sup>-2</sup> h <sup>-1</sup> )	$\beta$ -Glucosidase (mg 4-NP kg <sup>-1</sup> dry soil h <sup>-1</sup> )	Ac. Phosphat. (mg 4-NP kg <sup>-1</sup> dry soil h <sup>-1</sup> )	Dehydrogenase (mg INTF kg <sup>-1</sup> dry soil h <sup>-1</sup> )	Min. N (mg kg <sup>-1</sup> dry soil)	$H'$	EW (ind. m <sup>-2</sup> )
Control	5.9 <sup>a</sup>	3.4 <sup>a</sup>	0.39 <sup>a</sup>	391.4 <sup>a</sup>	871.0 <sup>a</sup>	10.6 <sup>a</sup>	63.2 <sup>a</sup>	3.1 <sup>ab</sup>	2.0 <sup>a</sup>
Ash	5.4 <sup>b</sup>	11.7 <sup>b</sup>	0.16 <sup>b</sup>	58.1 <sup>b</sup>	250.3 <sup>b</sup>	1.7 <sup>b</sup>	18.5 <sup>b</sup>	3.1 <sup>a</sup>	0.3 <sup>b</sup>
Lime	5.0 <sup>c</sup>	21.3 <sup>c</sup>	0.17 <sup>b</sup>	107.2 <sup>bc</sup>	378.1 <sup>c</sup>	1.7 <sup>b</sup>	16.1 <sup>b</sup>	3.0 <sup>c</sup>	0.0 <sup>c</sup>
No add.	4.9 <sup>c</sup>	18.8 <sup>c</sup>	0.16 <sup>b</sup>	133.3 <sup>c</sup>	319.9 <sup>bc</sup>	0.7 <sup>b</sup>	16.8 <sup>b</sup>	3.0 <sup>bc</sup>	0.3 <sup>b</sup>

The results presented in Table 2 show a higher forage yield in the plot treated with wood ash, but for forage quality characteristics, the proportion of crude protein was smaller and there was a higher acid detergent fibre content. With respect to neutral detergent fibre, ash content and digestibility values, there were no statistically significant differences between treatments.

Table 2. Effect of wood ash and lime treatments on forage yield and quality. Different letters within each column indicate statistically significant differences.

	DM yield (t ha <sup>-1</sup> )	Crude Protein (g kg <sup>-1</sup> DM)	Acid Detergent Fiber (g kg <sup>-1</sup> DM)	Neutral Detergent Fiber (g kg <sup>-1</sup> DM)	Digestibility (g kg <sup>-1</sup> DM)	Ash (g kg <sup>-1</sup> DM)
Ash	5.49 <sup>a</sup>	98.1 <sup>a</sup>	362.7 <sup>a</sup>	542.1 <sup>a</sup>	706.1 <sup>a</sup>	127.3 <sup>a</sup>
Lime	4.81 <sup>b</sup>	110.1 <sup>b</sup>	344.7 <sup>b</sup>	528.2 <sup>a</sup>	711.2 <sup>a</sup>	124.8 <sup>a</sup>

## Conclusions

Wood ash enhanced soil biological quality and fertility in acid soils and can be a valid alternative to the traditional treatment with lime. Biological indicators of soil health might be useful tools for soil assessment, because of their sensitivity and quick response to the treatments.

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## Effect of fertilizer source on N<sub>2</sub>O emissions from grasslands

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### Abstract

A field experiment was carried out to study the effect of organic (slurry; one application) *versus* mineral (three applications) fertilization on N<sub>2</sub>O emissions from a grassland located in the Basque Country (northern Spain). Initially, before the first cut (March), organic (slurry) and mineral (ammonium calcium nitrate) fertilizer was applied at 150 and 60 kg N ha<sup>-1</sup>, respectively. A second mineral application (60 kg N ha<sup>-1</sup>) was carried out after the first cut (April). Finally, a third mineral application (30 kg N ha<sup>-1</sup>) was carried out after the second cut (May). Emissions of N<sub>2</sub>O following fertilizer application were determined using the static chamber method. In addition, soil samples were collected for the determination of mineral N. Cumulative emissions of N<sub>2</sub>O from organically and inorganically fertilized plots were 6.28 and 9.90 kg N-N<sub>2</sub>O ha<sup>-1</sup>, respectively. Our results suggest that, under optimal edaphoclimatic conditions for plant nutrient absorption, soil mineralization, nitrification and denitrification, a single application of organic fertilizer leads to levels of N<sub>2</sub>O emissions similar to those produced by a splitted mineral fertilization procedure.

Keywords: fertilization, grassland, N<sub>2</sub>O emissions, split application.

### Introduction

Nowadays, the cattle farms of the Basque Country (northern Spain) are facing a difficult situation due to, among other factors, the accumulation of considerable amounts of farm wastes derived from an intensive farm production. One possibility to alleviate this problem of waste accumulation is the utilization of organic (animal) waste for fertilization. Usually, in fields located next to the farm, the slurry is applied to the soil in a liquid form, whereas in those fields far away from the farm, the slurry is composted before its transportation in order to reduce its volume and weight. It is a well-known fact that excessive N fertilization has many negative consequences for the environment. In this respect, emissions of nitrous oxide (N<sub>2</sub>O) to the atmosphere are of great importance. The amount of gases emitted to the atmosphere depends on complex interactions between soil physicochemical properties, climatic factors, and agricultural practices (Granli and Bockman, 1994). The objective of the present study was to quantify the emissions of N<sub>2</sub>O from a grassland subjected to different N fertilization procedures.

### Materials and Methods

A field experiment was carried out in a grassland established on a silty-clay-loam soil, and located in Derio (Basque Country, northern Spain). The grassland was established in September using a mixture of *Lolium perenne* L. cv 'Fanda' (15 kg ha<sup>-1</sup>), *L. hybridum* Hausskn. cv 'Butter' (10 kg ha<sup>-1</sup>), *L. multiflorum* Lam. cv 'Nival' (5 kg ha<sup>-1</sup>), *Trifolium repens* L. cv 'Milk' (1 kg ha<sup>-1</sup>) and *T. arvense* L. cv 'King' (1 kg ha<sup>-1</sup>). The climate in this region is Atlantic. During the experimental period, mean temperature was 13.6 °C and annual mean precipitation was 1,350 mm. The effect of organic (slurry; one application) *versus* mineral (three applications) fertilization on N<sub>2</sub>O emissions was studied. The organic fertilizer consisted of bovine (50%), pig (30%) and poultry (20%) slurry (the slurry was previously dried and then pelleted) with a N-P<sub>2</sub>O<sub>5</sub>-K<sub>2</sub>O ratio of 8.2-2.7-2.0. The first application was made in March, before the first cut, at the rate of 150 kg N ha<sup>-1</sup> for organically fertilized plots and of 60 kg N ha<sup>-1</sup> for inorganically fertilized plots. The second application of mineral fertilizer took place after the first cut in April, at the rate of 60 kg N ha<sup>-1</sup>. The third application of mineral fertilizer was carried out after the

second cut in May, at the rate of 30 kg N ha<sup>-1</sup>. Emissions of N<sub>2</sub>O to the atmosphere were measured about one month after the applications of fertilizers, using the static chamber method (two chambers per plot were used). After these measures, samples of soil were taken at a depth of 0-10 cm for the determination of water filled pore space (WFPS). After each cut, and before fertilizer application, soil mineral N was also determined at a depth of 0-50 cm.

## Results and discussion

N<sub>2</sub>O emissions were lower and more uniform in organically fertilized plots as compared to inorganically fertilized plots. The emissions of N<sub>2</sub>O for organically fertilized plots ranged from 10.39 (±2.50) to 518.48 (±462.84) g N<sub>2</sub>O-N ha<sup>-1</sup>d<sup>-1</sup>, with a mean value of 87.83 (±30.37) g N<sub>2</sub>O-N ha<sup>-1</sup>d<sup>-1</sup>. The highest value of N<sub>2</sub>O emission was observed 30 days after the application of the organic fertilizer, most likely due to the mineralization of the OM. Thirty-four days after organic fertilization, the soil presented a mineral N content of 253.40 kg N-NH<sub>4</sub><sup>+</sup> ha<sup>-1</sup> (table 1).

Table 1. Quantity of nitrate-N and ammonium-N (kg ha<sup>-1</sup>) in soil (0-50 cm) before fertilization with organic (S) and mineral (M) fertilizer.

	S		M	
	kg N-NO <sub>3</sub> <sup>-</sup> ha <sup>-1</sup>	Kg N-NH <sub>4</sub> <sup>+</sup> ha <sup>-1</sup>	Kg N-NO <sub>3</sub> <sup>-</sup> ha <sup>-1</sup>	kg N-NH <sub>4</sub> <sup>+</sup> ha <sup>-1</sup>
Before 1 <sup>st</sup> fertilization (24 <sup>th</sup> March)	22.2	32.1	26.4	30.2
Before 2 <sup>nd</sup> mineral fertilization (8 <sup>th</sup> April)	45.1	253.4	35.6	61.2
Before 3 <sup>rd</sup> mineral fertilization (26 <sup>th</sup> May)	26.5	106.0	41.4	17.4

In inorganically fertilized plots, N<sub>2</sub>O emissions ranged from 11.22 (±4.62) to 1984 (±927.78) g N<sub>2</sub>O-N ha<sup>-1</sup>d<sup>-1</sup>, with a mean value of 182.38 (±113.58) g N<sub>2</sub>O-N ha<sup>-1</sup>d<sup>-1</sup>. In this case, the highest value was found on the second day after the first mineral fertilizer application. The greater availability of NO<sub>3</sub>-N in the inorganically fertilized plots, as compared to those organically fertilized, leads to higher values of N losses, either as gaseous emissions (Estavillo, 1994) or through leaching (Estavillo *et al.*, 1996). Consequently, to avoid these problems, the application of mineral fertilizers is usually carried out in a splitted form. In the organically fertilized plots, 17.60% of measurements showed high rates of N<sub>2</sub>O emissions (> 0.1 kg N<sub>2</sub>O-N ha<sup>-1</sup>d<sup>-1</sup>) (Jordan, 1989), whereas in plots subjected to mineral fertilization this proportion was 29.40%.

Cumulative losses in the period of maximum growth of grassland were 7.57 (±4.46) and 9.64 (±2.21) kg N<sub>2</sub>O-N ha<sup>-1</sup> for organically and inorganically fertilized plots, respectively (differences were not significant). Nitrogen losses accounted for 5.04 and 6.42% of the amount of applied fertilizer, respectively.



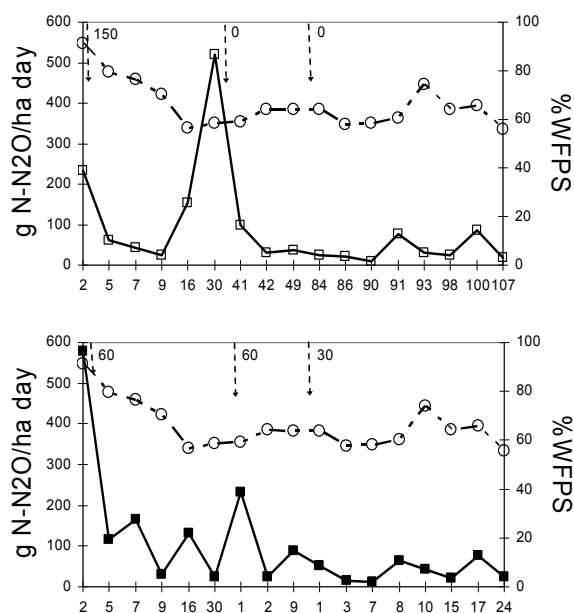


Figure 1. Emissions of N<sub>2</sub>O (squares) and WFPS values (circles) after organic (S; slurry) and mineral (M) fertilization.

### Conclusion

In conclusion, our results suggest that, under optima edaphoclimatic conditions for plant nutrient absorption, soil mineralization, nitrification and denitrification, a single application of organic fertilizer leads to levels of N<sub>2</sub>O emissions similar to those produced by a splitted mineral fertilization procedure.

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## Effect of fertilizer source on N<sub>2</sub>O emissions from winter forage crops

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### Abstract

Experimental trials to determine nitrous oxide (N<sub>2</sub>O) emissions were conducted in a soil cropped to different winter forages: ryegrass (*Lolium multiflorum* Lam.) (R), triticale (*xTriticosecale* Wittmack.) (T), triticale/pea (*Pisum sativum* L.) (TP) and oat (*Avena sativa* L.)/vetch (*Vicia sativa* L.) (OV). Organic fertilizer was supplied in a single application, whilst mineral fertilization was split between two applications. The first application for both types of fertilizers was made in November 2003, after the maize harvest. Organic fertilizer was applied at 150 kg N ha<sup>-1</sup> for R and T and at 90 kg N ha<sup>-1</sup> for TP and OV, while mineral fertilizer was applied at 40 kg N ha<sup>-1</sup> for all winter crops. The second application for mineral fertilization took place in March 2004, at a rate of 110 kg N ha<sup>-1</sup> for R and T and at 50 kg N ha<sup>-1</sup> for TP and OV. N<sub>2</sub>O measurements in both fertilizer types were made during the month following fertilizer applications. No significant differences in cumulative N<sub>2</sub>O emissions were found amongst crops when organic fertilizer was applied, while OV showed lower N<sub>2</sub>O emissions if mineral fertilizer was used. There were no differences between fertilizer type on the different forages, except in the OV treatment with higher losses from organic than from mineral fertilization.

Keywords: Forage rotations, legume, N<sub>2</sub>O emissions, split fertilisation.

### Introduction

The market offers a great variety of fertilizers, with different characteristics and properties. When choosing an appropriate fertilizer, there are different agronomic aspects to consider: nutrient content, crop requirements, solubility, chemical formulation, availability, price etc. as well as environmental considerations: e.g. losses of N by leaching and gaseous emissions. The excessive use or poorly timed application of N fertilizer can have damaging consequences for the environment. In terms of the overall losses of N, nitrous oxide emissions are important in the particular climatic conditions of the Basque Country (northern Spain). The amount of gases emitted depends on interactions produced between the physico-chemical properties of the soil, climatic factors and agricultural practices (Granli and Bockman, 1994). The objective of the present study was to quantify the emissions of N<sub>2</sub>O from several winter forage crops fertilized with different N sources.

### Materials and Methods

A trial was carried out in 2003 after the maize crop harvest on a silty-clay-loam soil in Derio in the North of Spain. Sowing (19<sup>th</sup> November) was with the following varieties: ryegrass cv 'Nival' (35 kg ha<sup>-1</sup>), triticale cv 'Senatrit' (400 seeds m<sup>-2</sup>), and mixtures of: triticale-pea cv 'Senatrit'-'Pursan' and oat/vetch cv 'Aintrée'-'Albina' (both 300 seeds m<sup>-2</sup>) in proportions of 60% cereal to 40% clover. The climate is Atlantic with an average temperature of 9.9 °C and an annual precipitation of 1002 mm in the period October 2003-April 2004 (at the Derio weather station). Different sources of N were applied: organic (S) and mineral (M); with split applications in the case of the mineral fertilizer. The incorporated organic fertilizer was in pellet form made from bovine (50%), pig (30%) and poultry (20%) slurries, that had been dried before being pelleted, and with a N:P<sub>2</sub>O<sub>5</sub>:K<sub>2</sub>O ratio of 8.2:2.7:2.0.

The first application for both fertilizers was made in November 2003, after the maize harvest. Organic fertilizer was applied at 150 kg N ha<sup>-1</sup> for R and T and at 90 kg N ha<sup>-1</sup> for TP and OV, while mineral fertilizer was applied at 40 kg N ha<sup>-1</sup> for all winter crops. The second application of mineral fertilizer was made in March 2004, at a rate of 110 kg N ha<sup>-1</sup> for R and T and at 50 kg N ha<sup>-1</sup> for TP and OV.

Emissions of N<sub>2</sub>O were determined about one month after the application of fertilizers by the static chamber method. After measurements of N<sub>2</sub>O, soil samples were taken to a depth of 0-10 cm for the determination of water filled pore space (WFPS).

### Results and Discussion

N<sub>2</sub>O emissions were slightly lower and more uniform with M than with S. The emissions of N<sub>2</sub>O for M varied from 1.0 (±1.4) to 741.0 (±292.8) g N<sub>2</sub>O-N ha<sup>-1</sup> d<sup>-1</sup> with an average of 72.0 (±13.9) g N<sub>2</sub>O-N ha<sup>-1</sup> d<sup>-1</sup>. The highest flux was obtained 16 days after mineral fertilization in TP. If S was applied, gaseous emissions varied from 0.0 to 571.0 (±310.9) g N<sub>2</sub>O-N ha<sup>-1</sup> day<sup>-1</sup> with an average of 89.1 (±16.5) g N<sub>2</sub>O-N ha<sup>-1</sup> d<sup>-1</sup>. The highest flux was shown between 16 and 30 days after organic fertilization, possibly due to net mineralization of the organic matter which, together with a high WFPS (Figure 1), increased the flux of N<sub>2</sub>O. In M, 19.6% of measurements showed high rates of N<sub>2</sub>O emissions (> 0.1 kg N<sub>2</sub>O-N ha<sup>-1</sup> d<sup>-1</sup>) (Jordan, 1989), whereas in S this proportion was 25%. When comparing split (M) and single (S) applications after the first application for both fertilizers, the only differences were observed in OV (p=0.0289), being higher in S than in M (4.97±0.84 and 1.77±0.34 kg N<sub>2</sub>O-N ha<sup>-1</sup>, respectively). After the second application of mineral fertilizer, only differences were shown in T (p=0.0291), being higher in M than in S, with 2.55±0.46 and 0.16±0.04 kg N<sub>2</sub>O-N ha<sup>-1</sup>, respectively.

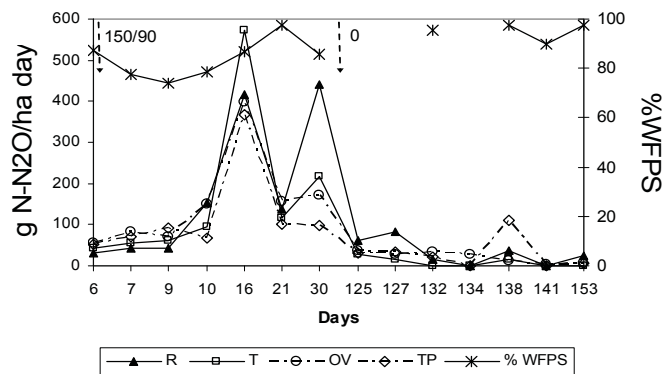


Figure 1. N<sub>2</sub>O flux and WFPS after fertilizer applications in (S) during winter forage crops.

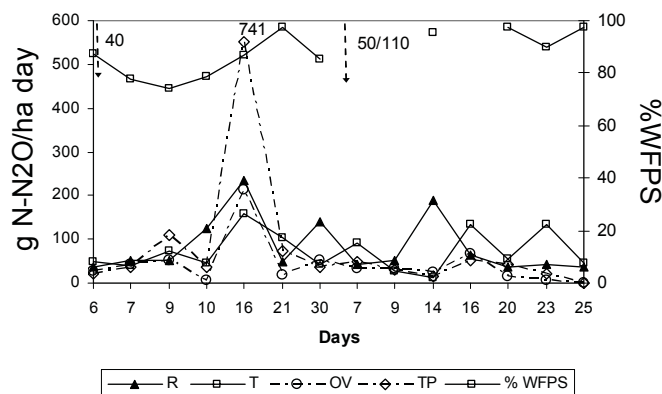


Figure 2. N<sub>2</sub>O flux and WFPS after fertilizer applications in (M) during winter forage crops.

There were no differences between cumulative N<sub>2</sub>O emissions in the winter crops in plots fertilized with organic fertilizer (mean 5.57 (±0.53) kg N<sub>2</sub>O-N ha<sup>-1</sup>). This represents losses of 6.2% of the total N applied in TP and OV, and 3.7% in T and R. On the other hand, OV plots fertilized with mineral fertilizer gave slightly lower emissions than the other crops: 2.36 (±1.17) kg N<sub>2</sub>O-N ha<sup>-1</sup> for OV and from 4.77 (±0.53) to 5.90 (±1.61) for R, T and TP, respectively. These values represent 3.2% of the total N applied in R and T, 2.6% in OV and 6.5% in TP.

N<sub>2</sub>O emissions from the same crops differ depending on fertilizer management. Thus, from an environmental point of view, split mineral fertilization is preferred in contrast to a single organic (pelleted slurry) application, which probably varies according to the rate of mineralization of organic matter. Nevertheless, when cumulative N<sub>2</sub>O emissions are compared, around 5 kg N<sub>2</sub>O-N ha<sup>-1</sup> are lost from both pelleted slurry and mineral fertilizer (except for OV with only 2.36 kg N<sub>2</sub>O-N ha<sup>-1</sup>).

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## Ecophysiological mechanisms involved in competition in C3/C4 mixed stands

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### Abstract

We analyze the role of structural and physiological differences between a C3 (*Lolium perenne* L.) and a C4 grass (*Paspalum dilatatum* Poir.) in determining the carbon economy of individuals growing in mixtures. Further, we assess to what extent such differences are intrinsic or size-mediated (allometric). C3/C4 mixtures were grown at 15°C and 25°C, to produce C3- and C4-dominated stands, respectively. Daily whole-plant carbon gain was estimated using steady-state <sup>13</sup>C-labelling. Light capture was estimated from profiles of incident light and leaf area measured down the canopies. The dominant species –*Lolium* at 15°C, *Paspalum* at 25°C– had higher relative photosynthesis rates and proportionally lower respiratory losses than their subordinate neighbours. Therefore, at both temperatures, the mixtures were becoming more dominated by the dominant species. At 15°C, subordinate C4 plants were as efficient as their dominant C3-neighbours in capturing light, but had a lower light use-efficiency. At 25°C, subordinate C3 plants had similar light use-efficiency but captured less light than dominant C4-neighbours. Hence, changes in the C3/C4 balance were related to physiological determinants at 15°C, but to structural characteristics at 25°C.

Keywords: coexistence, light, carbon, use-efficiency, allometric analysis.

### Introduction

C3 and C4 species co-occur in many temperate and subtropical grasslands. However, an understanding of the mechanisms determining the balance between C3 and C4 species is only emerging, with models able to estimate light capture and photosynthesis of individual plants being used to assess the relationship between structure/productivity of individuals and the coexistence of species (Anten and Hirose, 2003). Here we analyzed the consequences of differences in structural and physiological characteristics between a C3 (*Lolium perenne* L.) and a C4 grass (*Paspalum dilatatum* Poir.) for the carbon economy of individuals growing in mixed stands. Instead of modelling C gain, we directly measured it with a novel labelling approach. Since structural parameters are often correlated with plant size (Niklas, 1994), we assessed to what extent differences were intrinsic or allometric.

### Materials and methods

Mixed C3/C4 stands grew undisturbed at 15°C or 25°C for ~2 months, to produce C3- and C4-dominated canopies, respectively. Daily whole-plant C gain was estimated using steady-state <sup>13</sup>CO<sub>2</sub> labelling (Schnyder *et al.*, 2003). Simultaneously, light capture by each species was estimated from the profiles of incident irradiance ( $I$ ) and leaf area ( $F$ ) measured down the canopies. Extinction coefficients ( $K$ ) were estimated as the slope of  $\log(I/I_0) = K F$  (0.49 at 15°C, 0.57 at 25°C), where  $I_0$  is the irradiance at the top of the canopy ( $550 \mu\text{mol m}^{-2} \text{s}^{-1}$ ). Light capture by each species was a direct function of its contribution to  $F$  at each canopy layer. Finally, light capture per unit mass ( $\Phi_{\text{mass}}$ ) and per unit area ( $\Phi_{\text{area}}$ ), and light use-efficiency ( $P_{\text{light}}$ , C fixed per unit captured light) were calculated. Allocation between leaf parts (sheath/blade), and the relationship between blade mass and area (*i.e.* specific leaf area, SLA) were analyzed allometrically (Niklas, 1994). Briefly, the scaling exponent ( $\alpha$ ) and the scaling coefficient ( $\beta$ ) in  $Y_1 = \beta (Y_2)^\alpha$  were obtained from reduced major axis regression of  $\log(Y_1) = \log b + \alpha \log(Y_2)$ .

## Results and Discussion

The dominant species –*Lolium* at 15°C, *Paspalum* at 25°C– had higher relative photosynthesis rates and proportionally lower respiratory losses than their subordinate neighbours. Therefore, at both temperatures, the dominant species had higher relative growth rate (RGR, Table 1). This implies that the mixtures were not at equilibrium, but were becoming more dominated by one of the species. The reasons for these displacements of the C3/C4 balance differed between the C3- and C4-dominated stands.

Table 1. Relative rates of photosynthesis (RPR), respiration (RRR) and growth (RGR); proportion (BWR), specific area (SLA) and average N content ( $n_L$ ) of blades; and average irradiance ( $I_L$ ), light capture per unit area ( $\Phi_{\text{area}}$ ) and mass ( $\Phi_{\text{mass}}$ ) and light use-efficiency ( $P_{\text{light}}$ ) of C3 (*Lolium*) and C4 (*Paspalum*) individuals growing in mixtures at 15°C or 25°C.

	25°C		15°C	
	<i>Lolium</i>	<i>Paspalum</i>	<i>Lolium</i>	<i>Paspalum</i>
RPR, d <sup>-1</sup>	0.046 ±0.015	0.116 ±0.008	0.086 ±0.022	0.047 ±0.012
RGR, d <sup>-1</sup>	0.026 ±0.010	0.097 ±0.010	0.065 ±0.021	0.011 ±0.008
RRR, d <sup>-1</sup>	0.020 ±0.005	0.019 ±0.002	0.021 ±0.001	0.036 ±0.004
BWR, kg C (kg C) <sup>-1</sup>	0.72 ±0.00	0.64 ±0.01	0.59 ±0.07	0.75 ±0.03
SLA, m <sup>2</sup> (kg C blade) <sup>-1</sup>	73 ±3.4	89 ±2.0	55 ±0.3	112 ±2.8
$n_L$ , mmol m <sup>-2</sup>	96 ±0.6	69 ±2.9	80 ±0.3	75 ±6.5
$I_L$ , μmol (m blade) <sup>-2</sup> s <sup>-1</sup>	52 ±7.8	132 ±7.9	125 ±5.7	73 ±5.4
$\Phi_{\text{area}}$ , mol quanta m <sup>-2</sup>	1.9 ±0.34	4.8 ±0.29	4.4 ±0.31	2.8 ±0.27
$\Phi_{\text{mass}}$ , mol quanta (kg C) <sup>-1</sup>	86 ±13	186 ±1	153 ±24	144 ±2
$P_{\text{light}}$ , mmol CO <sub>2</sub> (mol quanta) <sup>-1</sup>	42 ±7.5	50 ±3.7	44 ±4.5	26 ±6.3
Mean ±SD (n = 2 growth chambers, 4 to 10 plants)				

(i) The disproportionately greater respiratory burden of subordinated individuals contributed substantially to differences in RGR at 15°C, but less so at 25°C. If subordinate plants would have had the same proportional respiratory losses of their dominant-neighbours, differences in RGR would decrease by 40% at 15°C, but only 13% at 25°C. Insufficient down-regulation of respiration as plants become shaded seems to stem from maintenance requirements (Lötscher *et al.*, 2004) (ii) Differences in relative photosynthesis rates were associated with differences in  $P_{\text{light}}$  at 15°C, but not at 25°C (Table 1). The low  $P_{\text{light}}$  of the C4 at 15°C was related to a failure of the CO<sub>2</sub> concentrating mechanism, as suggested by higher <sup>13</sup>C discrimination: 2.1‰ at 25°C vs. 4.8‰ at 15°C. Whether this was a low temperature- or shade-related effect is not clear. In any case,  $n_L$  did not limit C gain in these plants (Table 1). At 25°C,  $P_{\text{light}}$  was similar in both species, as also found in a C4 dominated grassland (Anten and Hirose, 2003). Therefore, these results only partially support the role of  $P_{\text{light}}$  at low irradiances in determining the C3/C4 balance proposed by Ehleringer (1978). (iii) Differences in relative photosynthesis rates were related to differences in  $\Phi_{\text{mass}}$  at 25°C, but not at 15°C (Table 1). Subordinate C4 plants were as efficient as their dominant *Lolium*-neighbours in capturing light per unit mass because higher BWR and SLA compensated for a lesser and less advantageously disposed leaf area (*i.e.* lower  $\Phi_{\text{area}}$ ). Conversely, at 25°C, subordinate C3 plants were not able to develop this response to the same extent and had lower  $\Phi_{\text{area}}$  and  $\Phi_{\text{mass}}$  than dominant C4-neighbours. Therefore, competition for light was asymmetric in this case, as bigger plants captured disproportionately greater amounts of light. Adjustments in BWR and SLA are common responses of subordinate plants in dense stands, which can be adaptive or size-mediated (Schwinning and Weiner, 1998; Anten and Hirose, 2003). Scaling exponents of blade/sheath and area/mass relationships were always lower than unity (Fig. 1). This means that, as plants got bigger, (proportionally) less C was allocated to blades, and less area was

produced per unit C invested in blade tissue. This often-described trade-off is believed to arise from greater requirement of support tissue in taller plants (Schwinning and Weiner, 1998). In this study, higher BWR and SLA in subordinate plants were mainly allometric effects, *i.e.* they had higher BWR and SLA because they were smaller than dominant plants. In fact, *Paspalum* had lower scaling coefficients ( $p < 0.05$ , Fig. 1A), and thus intrinsically lower amounts of blade per unit sheath, both at 15°C and 25°C. In both species, scaling exponents of the area vs. mass relationship were higher (close to 1) in subordinate plants ( $p < 0.05$ , Fig 1B). Thus, shading did trigger a morphological response, allowing these plants to become (relatively) taller with minor changes in SLA.

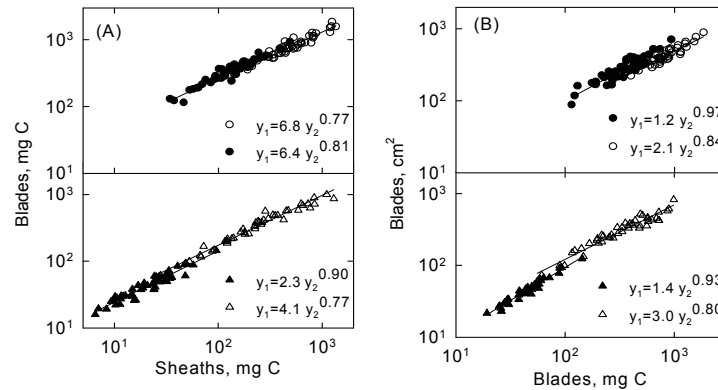


Figure 1. Allometric analysis of allocation of C to sheaths vs. blades (A), and between blades mass vs. area (B) in C3 (circles) and C4 (triangles) individuals growing in mixed stands at 15°C or 25°C, and thus in dominant (open symbols) and subordinate (closed symbols) hierarchical positions. Equations are:  $Y_1 = \beta Y_2^\alpha$ .

Morphological responses in subordinate *Paspalum* at 25°C were sufficient to reach a similar  $\Phi_{\text{mass}}$  than taller and greater *Lolium*-neighbours. Why were they not in *Lolium* at 15°C? One possibility is that Kranz anatomy of C4 leaves allows thinner/lower density blades, and thus maximum attainable SLA is inherently lower in C3 than in C4 species. In conclusion, different structural and physiological parameters were responsible for the displacement of the C3/C4 balance at low and high temperature. C3 dominance was based on high  $P_{\text{light}}$  and proportionally low respiration of *Lolium*. But C4 dominance was based on high efficiency in capturing light of *Paspalum*.

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## Nitrogen loading and the C3/C4 balance in grazed grasslands

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### Abstract

Studies in North American prairies demonstrated that nitrogen loading can drastically reduce the contribution of C4 species in mixed C3/C4 communities. To what extent this is a general response is uncertain, and whether grazing may alter it is unknown. Here we report on the consequences of loading 0 (control) or 10 g N m<sup>-2</sup> yr<sup>-1</sup> on the C3/C4 balance of grasslands in temperate/subtropical South America. Swards were kept at defined target canopy height by continuous grazing and frequent adjustments of stocking rate. Further, the effect of closure –i.e. removal of grazing animals– was analysed on fertilized plots. Control plots showed a typical seasonal C3/C4 pattern: <10% C4 biomass in winter increasing to 40% in summer. Nitrogen loading slightly increased the spring-summer contribution of C3 grasses in grazed plots. This was not at the expense of C4 species nor forbs. Instead, legume contents decreased. Conversely, in closures, nitrogen loading was associated with drastic decreases in C4 contribution (to <5%). This confirms previously reported responses of the C3/C4 balance to nitrogen loading, but indicates that grazing can modulate this response. Such an interaction between grazing and nitrogen loading suggests the mechanism underlying changes in C3/C4 balance in response to nitrogen loading is related to competition for light.

Keywords: coexistence, fertilization, functional groups, grazing, species composition.

### Introduction

C3 and C4 species coexist in many temperate and subtropical grasslands. In these ecosystems, the C3/C4 balance exerts a strong influence on biogeochemical cycles, and environmental and/or managerial changes affecting this balance hold important consequences for ecosystem functions. One of these changes is the increase in nitrogen (N) loading rates most grasslands nowadays experience, either because of atmospheric deposition and/or fertilization (Matson *et al.*, 2002). Evidence from undisturbed North American prairies indicates N loading can reduce the contribution of C4 species (Suding *et al.*, 2005), which then leads to lower N retention and less efficient carbon storage (Wedin and Tilman, 1996). Since these have been the only ecosystems studied thus far, it is not known to what extent this is a general response, nor whether grazing may alter it. Here we report on the interactive effects of N loading and grazing upon the C3/C4 composition of grazed South American grasslands.

### Materials and methods

Grazing experiments were set up in temperate Argentina pampas (37°S, 58°W) and subtropical Uruguay campos (31°S, 58°W). At both sites, 0 (control) and 10 g N m<sup>-2</sup> yr<sup>-1</sup> were applied in autumn to two replicate plots (2 ha each). Swards were kept at a standing biomass of 150 ±25 g m<sup>-2</sup>, which corresponded to a leaf area index of ~1.5, by continuous grazing by cattle and frequent (once to twice a week) adjustments of stocking rate based on variation of swards surface height. In the temperate site (*i.e.* Argentina), a closure was set up in late spring on parts of the fertilized plots to assess the impact of exclusion of grazing animals. Eight quadrats (0.04 m<sup>2</sup>) per replicate were sampled either every season



(Argentina) or at the end of autumn (Uruguay), and separated into four species functional groups: N<sub>2</sub>-fixing legumes, C3 grasses, C4 grasses, and forbs (which were all C3).

## Results

N loading effects upon the balance between C3 and C4 species were minor in grazed plots. Eight to ten months after N application, the contribution of C3 grasses to standing biomass was slightly greater in fertilized than in control plots, in both the temperate (Argentina) and subtropical (Uruguay) sites (Fig 1). But this was not at the expense of C4 species, instead dicots were reduced. Conversely, a drastic decrease in the contribution of C4 grasses was observed in response to N loading and exclusion of grazing (Fig 1A).

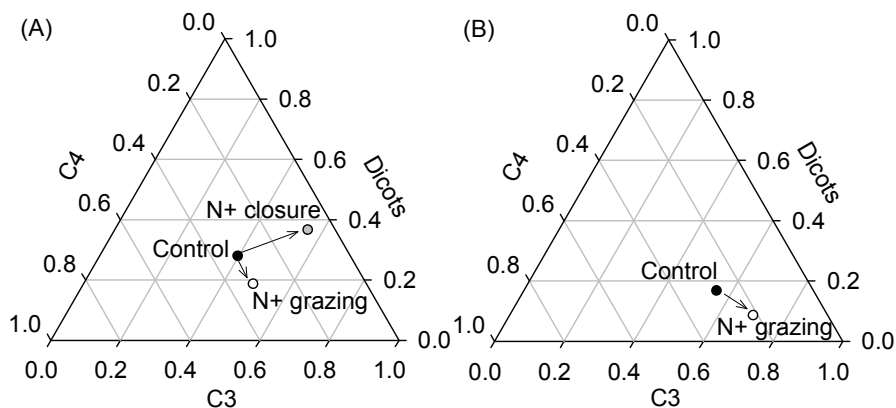


Figure 1. The effect of N loading and exclusion of grazing (*i.e.* closure) on the contribution of functional groups to the standing biomass of (A) temperate (Argentina, summer sampling) and (B) subtropical (Uruguay, end of autumn sampling) grasslands.  $LSD_{0.05} = 0.12$  (10 *d.f.*). Arrows indicate the change observed from the control treatment.

Detailed sampling on the temperate site revealed only minor transient effects of N loading on the seasonal contribution of C4 species in grazed swards (Fig. 2). Likewise, forbs contributed a roughly constant 20 to 30% to the standing biomass. Hence, the N-enhanced contribution of C3 grasses was mostly associated with a decrease of N<sub>2</sub>-fixing legumes (Fig. 2).

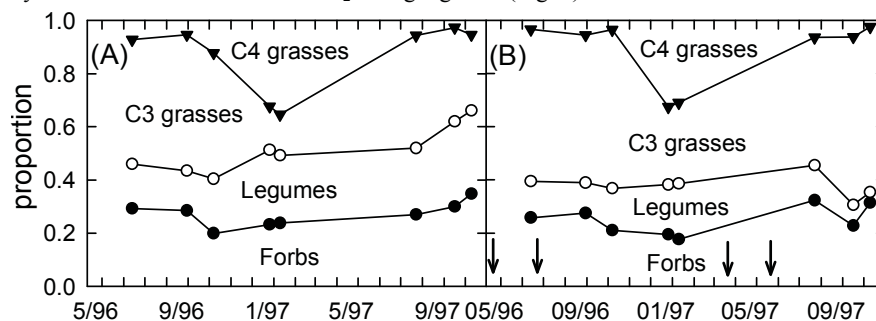


Figure 2. Changes throughout time in the proportional contribution of four functional groups to herbage mass of grazed grasslands in Argentina receiving 0 (A) or 10 g N m<sup>-2</sup> y<sup>-1</sup> (B).  $LSD_{0.05} = 0.08$  (31 *d.f.*). Arrows indicate N applications.

## Discussion

These results confirm, in native grasslands of South America, the displacement towards C3 vegetation in response to N loading under undisturbed conditions observed elsewhere (Wedin and Tilman, 1996; Suding *et al.*, 2005). But, importantly, the results also indicate that such a response can be substantially attenuated by grazing regimes which minimize aboveground interactions. This suggests light competition as the mechanism driving changes in C3/C4 balance in response to N loading in non-grazed plots.

Changes in legume/grass ratios in response to fertilization are widely suspected to be due to a N-based competitive trade-off (Schwinning and Parsons, 1996), with legumes and grasses having the advantage in low- and high-N scenarios, respectively. In principle, our results agree with this hypothesis.

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## NBPT effect on NO emissions from grassland after cattle slurry and urea application

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### Abstract

The urease activity inhibitor *N*-(*n*-butyl) thiophosphoric triamide (NBPT) was applied in a mixed clover/ryegrass sward in the Basque Country together with urea or cattle slurry at a rate of 70 kg N ha<sup>-1</sup> as urea and 70 kg NH<sub>4</sub><sup>+</sup>-N as slurry. NBPT reduced NO emissions from 1.36 to 0.88 kg NO-N ha<sup>-1</sup> (35%) after urea application and from 0.78 to 0.65 kg NO-N ha<sup>-1</sup> (17%) after application of slurry but it had no effect on herbage yield or N uptake.

Keywords: nitrogen losses, urease activity inhibitor.

### Introduction

Nitric oxide emissions are an important N loss mechanism from agricultural soils, coming from both denitrification and nitrification processes. Agricultural soils are seen as important sources of nitric oxide (NO). In the edaphoclimatic conditions of the Basque Country they have been shown to be a significant pathway for N losses from cut grassland (Pinto *et al.*, 2004). From an ecological point of view, using a urease activity inhibitor with urea based fertilisers may be a potential management strategy to lower NO emissions, thus decreasing its undesirable effects. The aim of this work was to test the effect of *N*-(*n*-butyl) thiophosphoric triamide (NBPT) on NO emissions from grassland after cattle slurry and urea application. Soil urease activity, herbage yield and herbage N concentration were also determined in order to assess the efficiency of the inhibitor and its co-lateral effects on yield.

### Materials and Methods

This work was conducted on cut grassland of the Basque Country (northern Spain) during the spring 2004. The experiment was established on a poorly drained clay loam soil on a typical permanent pasture in October 2003. Sowing density was 40 kg seeds ha<sup>-1</sup> (*Lolium perenne* L.var. Herbus, 60%; *Lolium hybridum* L. var. Texi, 32%; *Trifolium repens* L.var. Huia, 8%).

A randomized complete block design with four replicates was established, each experimental plot covering an area of 12m<sup>2</sup> (4x3m). Two kinds of fertilizers were applied on 7<sup>th</sup> June 2004 at a rate of 70 kg N ha<sup>-1</sup> as urea and 70 kg NH<sub>4</sub><sup>+</sup>-N as slurry. Characteristics of the slurry were: total N= 0.34% (w/w); NH<sub>4</sub><sup>+</sup>-N= 0.18% (w/w) and C:N ratio= 11.2. NBPT was applied or not with both types of fertilizer. The slurry was obtained from a concrete storage pit on a dairy farm. A treatment receiving no N application was included as a control.

Nitric oxide emissions were measured using an open chamber technique as described by Harrison *et al.* (1995) with a chemiluminescence analyzer (AC31M Environment S.A.).

Urease activity was determined by measuring NH<sub>4</sub><sup>+</sup> production as described by Kandeler and Gerber (1988). Herbage yield was assessed on 27<sup>th</sup> July on one randomly chosen area of 3.6m<sup>2</sup> per plot. Grass was oven-dried at 70°C for at least 48 hours, weighed and then ground. Nitrogen concentrations were determined on dried and ground herbage by the Macro Kjeldahl method.

## Results and discussion

NO emissions, water content (0-10 cm depth) (expressed as water filled pore space, WFPS) and soil temperature (10 cm depth) are presented in Figure 1.

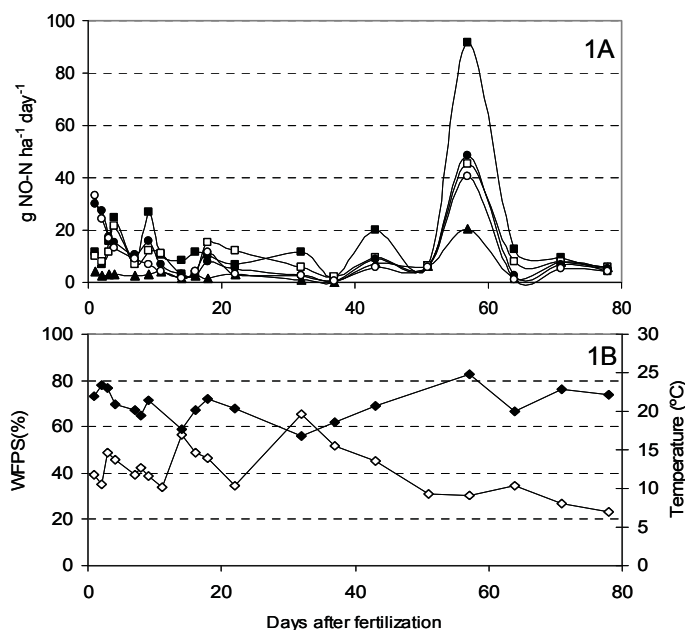


Figure 1. NO emission rates (1A), soil WFPS and soil temperature (1B). ▲ = control, ■ = urea, □ = urea with NBPT, ● = slurry, ○ = slurry with NBPT, ◆ = soil temperature, ◇ = WFPS.

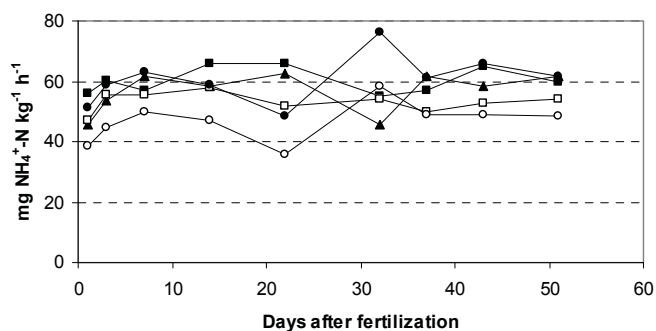


Figure 2. Urease activity during the experiment. ▲ = control, ■ = urea, □ = urea with NBPT, ● = slurry, ○ = slurry with NBPT.

During the first 20 days after fertilizer application the emission rates in all the treatments were higher than in control treatment, and no apparent clear difference between fertilized treatments could be observed. The highest rates of NO emissions were observed in the urea treatment on day fifty-seven after fertilizer application, with rates of  $91 \text{ g NO-N ha}^{-1} \text{ day}^{-1}$  (Figure 1A). Probably this peak of NO emission was due to the low WFPS and the increase in soil temperature that took place during those

days. Cumulative NO losses up to 78 days after fertilizer application showed that NBPT reduced the emissions from urea by 35% and those from the slurry by 17% (Table 1). Urease activity ranged between 36 mg NH<sub>4</sub><sup>+</sup>-N kg<sup>-1</sup> h<sup>-1</sup> in the slurry with NBPT treatment and 76 mg NH<sub>4</sub><sup>+</sup>-N kg<sup>-1</sup> h<sup>-1</sup> in the slurry treatment (Figure 2). The addition of fertilizer did not increase statistically urease activity with respect to the control. Although Dick (1984) described that the accumulation of soil organic matter in surface layer increases urease activity, slurry application did not have this impact on urease activity in this experiment. NBPT reduced the urease activity around a 21-24% for both urea and cattle slurry. Neither herbage yield nor N concentration in herbage were affected by the application of NBPT (Table 1).

Table 1. Cumulative NO-N emissions, herbage yield and herbage N content.

Treatment	kg NO-N ha <sup>-1</sup> (78 days)	Yield (DM t ha <sup>-1</sup> )	N g kg <sup>-1</sup>
Control	0.40 e	1.55 b	0.28 a
Urea	1.36 a	2.55 a	0.29 a
Urea+ NBPT	0.88 b	2.46 a	0.32 a
Slurry	0.78 c	2.43 a	0.29 a
Slurry+ NBPT	0.65 d	2.34 a	0.28 a

Different letters within a column indicate significant differences (P<0.05, n=4).

### Conclusions

The use of NBPT lowered NO emissions after slurry and urea application and it has no effect on herbage yield or N concentration in herbage.

### Acknowledgements

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# Effects of climate and grazing on leaf attributes at the community-level

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## Abstract

Predicting how environmental factors affect plant traits at the community-level can be a way forward in generalizing consequences of global change on ecosystem functioning. Thus, we studied leaf traits in communities under different sheep grazing regimes (high-, low-intensity, abandonment) and in different climatic conditions (from semi-desert to subalpine pastures in north-eastern Spain). The weighted averages for Specific Leaf Area (SLA) and carbon isotope ratio ( $\delta^{13}\text{C}$ ) of the most frequent species ( $n=134$ ) were calculated for each community. SLA increased with water availability and grazing intensity. Overall, a lower SLA was accompanied by a higher  $\delta^{13}\text{C}$  and C concentration. The  $\delta^{13}\text{C}$  values decreased with grazing intensity, particularly in more water limited conditions. We argue that changes in species composition in terms of life span and resource acquisition affect leaf characteristics at the community-level. Grazing promotes short-lived species, with fast resource assimilation rates, by releasing them from the competition of slow growing, late successional species which show a more conservative strategy in resource assimilation.

Keywords: carbon isotope, climatic gradient, leaf physiology, sheep, succession.

## Introduction

At present many ecosystems are experiencing unprecedented environmental changes in scale and rate. Climate and disturbance modify species composition by filtering out from the species pool, the species lacking the adaptations (functional traits) to persist under a particular set of conditions (de Bello *et al.*, 2005). Changes in the composition of plant physiological characteristics at the community-level can have very large effects on the functioning of ecosystems (Reich *et al.*, 1998; Richardson *et al.*, 2005). Leaf characteristics play a particularly important role in carbon assimilation, water fluxes and energy balance. In particular, leaf size and specific leaf area reflect general adaptations to water and nutrient availability (declining with decreasing availability: Reich *et al.*, 1998). The leaf carbon isotope ratio ( $\delta^{13}\text{C}$ ) integrates information about the regulation of carbon dioxide and water fluxes (Kahmen *et al.*, 2005). In this study we addressed whether climate and grazing affected leaf characteristics at the community-level, and the possible underlying ecophysiological mechanisms of the changes.

## Materials and methods

Five locations were selected along an altitudinal and climatic gradient in north-eastern Spain, ranging from semi-desert to subalpine conditions (de Bello *et al.*, 2005). In each location a gradient of grazing intensity was identified as a) abandoned for more than 10 years, b) visited a few times a year or c) frequently visited per year. A factorial design was used: 3 sheep grazing intensities x 5 localities x 2 aspects (north-, south-facing slopes) x 2 replicates = 60 plots. Species composition and frequency were sampled in a 10 x 10m plot, divided into 100 1m<sup>2</sup> subplots.

The most frequent species in the experiment (134) were chosen for leaf sampling (ca. 27 per location) following standard procedures (Reich *et al.*, 1998). Five representative plants were selected for each species and a portion of two twigs for each individual was cut. Leaf area and leaf dry weight and SLA (area/mass, mm<sup>2</sup> mg<sup>-1</sup>) were measured (5 plants x 2 twigs x 1 leaf). For small leafed-plants, more leaves on a twig were sampled and treated as one bulked sample. The  $\delta^{13}\text{C}$  and carbon concentration (leaf-C)

were measured for each species by bulking together all the leaves of a particular species. The weighted averages of leaf characteristics were calculated for each plot (sum of the absolute frequency of each species x its value, divided by the total species frequency). A nested ANOVA was used to test the effect of grazing, location and aspect on leaf characteristics. Locations were nested by climatic conditions, grouping them into 'dry' (the three driest locations with mean annual rainfall between <325 and 460 mm) and 'moist' (the remaining two: rainfall between <780 and 950 mm).

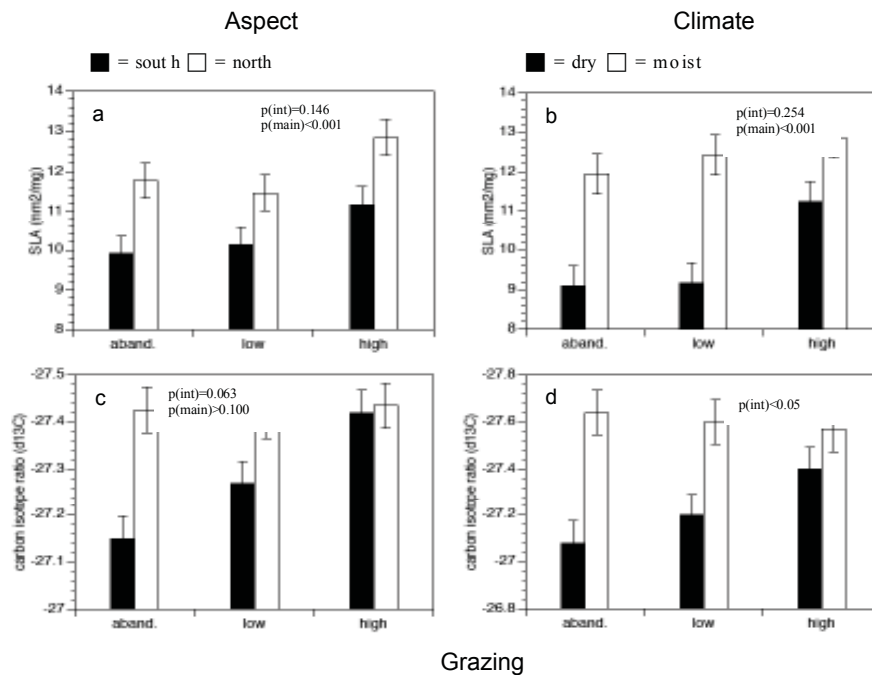


Figure 1. Weighted averages of leaf characteristics in communities under different grazing regimes (high-, low- intensity, abandonment), climate (dry vs. moist locations) and slope aspect (south- and north-facing). Graphs show specific leaf area (SLA; a-b) and carbon isotope ratio ( $\delta^{13}\text{C}$ ; d-c. Nb different scales). Bars denote +/-SE. P values show the significance of the ANOVA factors and their interactions (int=interactions; main=main factors).

## Results and discussion

The variability of leaf traits at the community-level was explained by the ANOVA models (adjusted  $R^2 \geq 0.79$  for all characteristics). For SLA, the main effects of the environmental factor considered were additive (no significant interactions;  $p > 0.05$ ). SLA increased with grazing intensity, moist conditions and north-facing slopes (Fig. 1). Other leaf characteristics were mainly affected by the combined effect of climate, aspect and grazing (Fig. 1). The leaf  $\delta^{13}\text{C}$  tended to decrease (i.e. more negative) with grazing in the water stressed conditions (dry locations and south-facing slopes; Fig 1) and generally was more negative towards moist conditions. A higher SLA was correlated to lower  $\delta^{13}\text{C}$  ( $r = -0.702$ ) and leaf-C ( $r = -0.807$ ) while a positive correlation with leaf area was not strong ( $r = 0.451$ ).

This study shows that environmental changes, such as climate and grazing have a direct impact on the importance of strategies existing at the community-level. The response observed at the community-level could be determined to a large extent by compositional shifts (de Bello *et al.*, 2005) in response to changes in resource availability (Reich *et al.*, 1998). The reduction of SLA with increasing exposure or dry conditions is consistent with the general view that this characteristic is a part of a suite of traits

associated with stress tolerance (Reich *et al.*, 1998). SLA is also negatively correlated with life span (Reich *et al.*, 1998). Short lived species, normally promoted by grazing (de Bello *et al.*, 2005), have leaves with higher SLA than long-living species (with lower SLA). This reflects the trade-off between short-life span species, with high assimilation rates, and long-life span species with a more conservative strategy in resource assimilation based on nutrient retention, long-term photosynthetic efficiency and reduced water loss (Reich *et al.*, 1998). Thus, communities under decreased grazing were dominated by long-living species (de Bello *et al.*, 2005) and tended to show lower SLA and less negative  $\delta^{13}\text{C}$  (Fig. 1). Both grazing and less water stress (as in moist locations and north-facing slopes) tended to increase SLA. This suggests that disturbance increases resource availability by exerting a negative effect on more competitive species. The impact of grazing on SLA and  $\delta^{13}\text{C}$  was generally more marked under dry conditions (Fig. 1). This confirms that grazing promotes fast growing species more markedly under water stress (de Bello *et al.*, 2005) and suggests that a release of resource availability with disturbance occurs at higher rates in more resource limited environments.

### Acknowledgements

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# A methodology for assessing economic and environmental effects of climate change

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## Abstract

An example is given for the assessment of the effects of climate change on grass yields and on some environmental indicators.

Keywords: climate change, carbon sequestration.

## Introduction

Climate change will affect agriculture through a large set of interacting processes which will influence both crop production and emissions to the environment, and the effects may vary substantially depending on natural and economic conditions. This presentation concerns some preliminary results using a methodology that bridges over microbiological processes in the soil, plant growth and farm practice. The methodology is implemented in a modelling structure called ECECMOD, which has been used for analyzing the effect of policies for reducing pollution from Norwegian agriculture (grassland and arable crops) under current climate conditions. The simulated yield levels, agronomic practice and nitrogen emissions in four climatically different regions were well in accordance with observations (Vatn *et al.*, *in press*). When using global scenarios for simulations of climate change, it is necessary to downscale the global simulations to regional weather forecasts (including corrections for local altitude). In a previous validation of such downscaling, we found moderate deviations in air temperature and precipitation distribution which could critically impair the correct simulation of plant growth. These errors were corrected for by a set of algorithms, securing reasonable agreement for historical weather (observed/simulated). Two weather scenarios are never identical, however, and apparently minor differences may have consequences when the data are used to drive dynamic nonlinear soil/plant models. Comparisons of simulations based on measured and downscaled control periods is thus recommendable, to evaluate the quality of the downscaled climate scenarios.

## Methods

The coupled models for grass growth (ENGNOR) and for biological soil processes (SOILN\_NO) were used to simulate the daily plant growth and nitrogen uptake, the soil microbial growth and decay of soil organic materials, as well as the nitrogen loss through leaching and denitrification. The COUP model was used to estimate the soil heat and water balances. As an example of future weather, the Hadley A2 climate scenario for the period 2071-2100 (S\_7000) was used, downscaled to the Oslo region by the Norwegian project Regional Climate Development under Global Warming, RegClim (<http://regclim.met.no/>). The modelled harvested yields, the annual N loss and changes in the active humus pool of the soil were compared with those obtained using observed weather data for the period 1961 – 1990 (O\_6190, Table 1).

In order to check the adequacy of the downscaling procedure, soil/plant model simulations based on historical weather (1961-1990) were compared with simulations based on downscaled weather for the same period (S\_6190). The soil characteristics and the initial soil organic matter were set as for a local loamy soil with a long cultivation history with cereals as the major course. The start and end of the growth period each year were based on a temperature threshold. The first harvest was estimated around heading based on a combined temperature and daylength function. In order to take advantage of the prolonged growing season in the future scenarios, the criteria for the date of the second and third harvest

were modified. No effect of increased CO<sub>2</sub> concentration in the atmosphere was included in the simulations.

Table 1. Some statistics of the selected weather scenarios. Standard deviations of annual values in brackets.

	Mean temperature, degree C		Precipitation, mm		Mean actual/potential transpiration ratio from 2 <sup>nd</sup> to 3 <sup>rd</sup> grass harvest
	Oct-Mar	Apr-Sept	Oct-Mar	Apr-Sept	
O_6190	-0.66 (1.58)	+12.2 (0.65)	339 (85)	418 (104)	0.89 (0.15)
S_7190	+3.36 (2.42)	+15.6 (1.29)	436 (115)	333 (98)	0.72 (0.21)

## Results and discussion

Comparison of the simulated plant growth and nitrate leaching for the control period shows quite similar results when using observed and downscaled weather as model input (K\_6190 versus O\_6190, Table 2), suggesting that the downscaling was adequate for our purpose. The future scenario gave about 10% higher yield, and roughly unaltered nitrogen loss (leaching + denitrification). One factor which significantly restricted plant growth with the future scenario was drought stress in late summer (Table 1).

Table 2. Dry matter yield and nitrogen loss with three weather scenarios. Standard deviation in brackets.

N application	K_6190		O_6190		S_7100		S_7100 – O_6190
	DM	N	DM	N	DM	N	DM
(g N m <sup>-2</sup> yr <sup>-1</sup> )							
9	450 (98)	1.82 (0.81)	457 (62)	1.89 (0.88)	508 (119)	1.91(0.71)	51
22	804 (140)	2.76 (1.30)	813 (99)	2.69 (1.22)	887 (189)	2.54 (1.03)	74
Unlimited	895 (155)	-	904 (125)	-	994 (202)	-	90

The temperature threshold for the end of the growth period in the autumn was chosen as observed in Norwegian ecotypes (when the average temperature during the last seven days sinks below 5° C). More southern cultivars do not stop growth at all, or they do it at much lower temperature, and thus produce higher yields in the first year but usually fail to survive during the winter. A pertinent question is thus what genotype characteristics should be used in the parameterization of the plant model in order to provide realistic prognosis, knowing that even with a warmer climate occasional episodes of frost and ice would reduce the survival of non winter hardy cultivars.

A major question is whether an increased biomass production, and thus a higher litter input into the soil, will compensate for the accelerated rate of decomposition under a warmer climate. With continuous fallow the annual decays of the active humus was estimated to be around 3 – 4 g C m<sup>-2</sup> yr<sup>-1</sup> above the decay rate under present weather conditions. We also estimated the increase in humus for the transition from nearly permanent cereal monoculture to continuous grass leys. In the present example the future weather scenario will reduce the accumulation of the humus by continuous leys by 22 – 26 % (Table 3). The difference between present and future weather is almost independent of fertilization.

To compensate for the accelerated humus decay the warmer scenario, the annual input of fresh plant material would have to be 30-40 g C m<sup>-2</sup> yr<sup>-1</sup>, equivalent to 60 – 90 g DM dead roots. An appropriate question is whether the grass model adequately simulates root growth under a changed climate.

Table 3. Estimated annual changes in soil carbon during 30 years of leys or black fallow following a long lasting cereal monoculture. The present fertilization practice is around 22 g N m<sup>-2</sup>yr<sup>-1</sup>. In the last row, C in the active humus pool at approximately equilibrium (>150 years with leys) when nitrogen supply is unlimited.

Treatment and N application (g N m <sup>-2</sup> yr <sup>-1</sup> )	O_6190	S_7100 Annual C sequestration (g m <sup>-2</sup> yr <sup>-1</sup> )	S_7100 - O_6190
Fallow, 0 N	-16.4	-20.0	-3.57
Leys, 9 N	13.9	10.3	-3.68
Leys, 22 N	16.8	13.0	-3.84
Leys, no N limitation	17.6	17.7	-3.90
Total active humus around equilibrium			
Leys >150 years	6340	5190	-1150

Measurements of root growth of grasses under field conditions are rare and often inadequate. In general, under a warmer weather the root fraction of the total plant biomass is expected to decrease. Allocation to roots is relatively at a maximum when plants are younger, and decline with age. In aged plants, probably no more than 20 % of new photosynthesis products are allocated to roots. In this case there is also little increment of tillers below cutting height. Thus, an additional root production of 60 - 90 g could occur when the increment of the harvestable yield is ca.  $[(60 \text{ to } 90) \cdot 0.8] / 0.20 = (240 \text{ to } 360) \text{ g DM m}^{-2}$ , which is much higher than indicated by using plant parameters for Norwegian varieties.

### Conclusions

The results suggest that yield increase would be moderate (around 10%), that the net mineralization rate of soil organic matter will increase by more than 20 %, and that soil organic matter will decline in response to the predicted climate change. As pointed out by IPCC, however, prognosis regarding the effects of climate changes should be based on a number of different scenarios. The present simulation included only one, and the result is thus very uncertain. In addition, we suggest that a selected choice of alternative parameterization of the biological models should be used to illustrate the effect of different genotypes, and also to provide a wider perspective for the evaluation of the model results.

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# Role of biogeographical, ecological and management factors on plant diversity in grasslands

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## Abstract

Semi-natural grasslands are recognized as one of the most species-rich habitats in Europe. They are also within the most vulnerable to climate change. In order to assess the relative role of the expected driving factors in the distribution of plant species diversity, we carried out a survey on subalpine grasslands in the Eastern Pyrenees taking into account biogeographical, ecological and management factors. Using Geographical Information Systems and related methodologies, we defined patches of grasslands above 1800 m in all the studied mountain ranges. The patches were classified according to size, shape and connectivity. We assessed the number of species in 10 x 10 m plots distributed at random on patches with different characteristics and under different climatic conditions. Several ecological situations were considered, in which slope, aspect, altitude, macro and microtopography changed. Different management regimes were taken into account: cattle grazing, sheep grazing, mixed grazing. Plant species richness was successfully modelled based on the considered variables. Species richness decreased with altitude, and cattle-grazed areas showed higher number of species per plot than other grazing managements.

Keywords: biodiversity, subalpine, land-use, climate, connectivity.

## Introduction

During the last decade, particularly after the Earth Summit in 1992, the loss of biodiversity has been pointed out as one of the main environmental problems worldwide. Semi-natural grasslands are recognized as one of the most species-rich plant habitats in Europe (WallisDeVries *et al.*, 2002) but, at the same time, they are among the most threatened ones. In a global scale, it is estimated that grasslands areas are declining due to the development of agriculture. The quality of the surviving grassland is also declining due to human-induced modifications like agriculture, fragmentation, overgrazing and invasive plants and animals. In addition, mountain grasslands have been identified as potentially threatened areas by ongoing climate change (Guisan *et al.*, 2000). Consequently, it is increasingly important to understand the factors that regulate diversity composition at different scales in order to assess potential future impacts. Connectivity has been long recognized as a fundamental factor determining species distributions (MacArthur *et al.*, 1967). There is widespread agreement on the significance of abiotic factors in community assembly (Sebastià, 2004). In addition, grazing is known to have a strong effect on vegetation, influencing ecosystem processes and biodiversity composition (Bullock *et al.*, 2001). We conducted an extensive survey in order to determine the role of the main driving forces influencing diversity distribution: biogeographical, abiotic and biotic factors (including management).

## Materials and methods

We defined patches of grassland above 1800 m in nine mountain ranges in the Eastern Pyrenees, using GIS Arc-Info (ESRI, 1991). Grassland areas were defined from the existing maps based on CORINE (EU, 1991). We carried out an extensive survey in 63 grassland patches above 1800 m. The actual patch area (rather than the projected one) was computed using Arc-Info. An isolation index was calculated using Fragstats (McGarigal *et al.*, 2002). Climate was obtained from the *Atlas Climàtic de Catalunya* (Ninyerola *et al.*, 2000). Samples were distributed considering: (a) altitude (1800-2000m / > 2000m) (b) slope (0-20° / 20°-30° / >30°), (c) aspect (north / south), (d) management (sheep, cattle, mixed). In each

of the sampled grasslands we set a 10 x 10 m plot. Species presence was determined by a floristic inventory in each of these plots.

## Results and discussion

Plant species richness was modelled with some accuracy based on the following variables: abiotic (mean annual temperature and macrotopography), management (type of grazing animal) and biogeographical (patch area and proximity index).

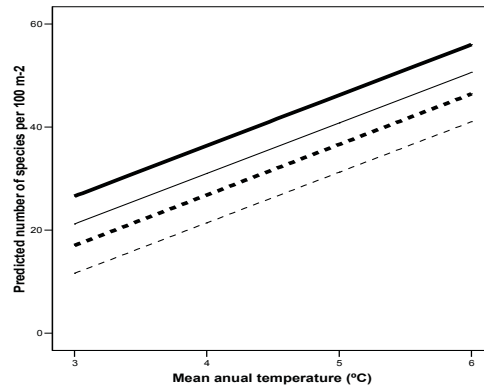


Figure 1. Relationship between the predicted number of species from the model combining biogeographic, abiotic and management factors and mean annual temperature. Continuous lines indicate cattle-grazing and discontinuous, other grazing management. Thin lines indicate prediction for 300 ha size patches and thick lines for 3000 ha size patches.

Modelling indicated that species richness was highly dependent on abiotic factors, probably in relation to resource availability. We found a strong positive relationship between mean annual temperature and species richness ( $P < 0.001$ ). Topographically protected areas (hollows and other convex landforms) were associated to a higher number of species per plot than exposed ones ( $P = 0.07$ ). These results suggest that rigorous environmental conditions act as an environmental filter and strongly select the most resistant species both at the regional large scale at which climate would act, and at the smaller local scale defined by topography. In addition to abiotic factors, grazing management affected species diversity. Cattle-grazed areas had higher species richness than sheep-grazed areas ( $P < 0.001$ ). This is probably explained by the fact that cattle are non-selective grazing animals, while sheep are much more selective and contribute to the negative selection of the most palatable species and the extension of aggressive non-palatable plants which then become highly dominant species (Sebastià, 2004).

Species richness per plot increased with increasing patch area ( $P < 0.001$ ), whereas surprisingly, species richness per plot increased with the isolation index ( $P < 0.001$ ). This might be explained by the particular isolation index used. The distance between two studied patches calculated by Fragstats using the proximity index takes into account the distance from cell centre to cell centre, instead of edge-to-edge. This distance is obviously bigger as patch area increases. Consequently, large grassland patches seemed more isolated than they really were, which means that the index used was not the most suitable for our study.

## Conclusions

Abiotic and management factors, as well as grassland patch size and connectivity, explained much of the variability in species richness distribution. However, the effect of connectivity would probably be

better analysed through more suitable indices than those available in standard programmes and more research is necessary for our study area.

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## Modelling of grassland yields in consideration of drought

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### Abstract

Grassland with its different characteristics covers an area of 1.61 million hectares, which represents more than 50 % of the Austrian agriculturally used area. Grassland is managed by 115,000 grassland and cattle farmers, mostly running small structured enterprises. Over the past years, the global climate change has affected parts of Austria by the occurrence of intensive drought periods, which caused heavy drought damages on agricultural areas. Grassland yield, which is influenced by many factors, can be estimated by different models, mapping certain natural processes in a simplified way. The prerequisite for a state-wide analysis of grassland yield is the integration of these models into a Geographic Information System (GIS). The soil water balance is the basis for the simulation of growing processes. The precipitation is compared with the potential evapo-transpiration and the soil water balance model is developed according to the FAO method, considering the field capacity. The growth and yield model is based on the data of the soil water balance and results in a multiple regression in order to calculate the quantity of grassland yield. The results of this work will be the fundamentals of an insurance model for drought damages on grassland and will therefore help to protect the existence of grassland and cattle farmers in drought endangered regions.

Keywords: drought, GIS, grassland, water balance, yield.

### Introduction

Grassland yield, which is influenced by many factors, can be estimated by different models, mapping certain natural processes in a simplified way. The prerequisite for a state-wide analysis of grassland yield is the integration of these models into a Geographic Information System (GIS), since the model parameters have a well-defined spatial reference.

Grasslands of different types cover an area of 1.61 million hectares, which is more than 50% of the Austrian agricultural land. They are managed by 115,000 grassland and cattle farmers, mostly running small to medium size enterprises that depend on the stability of the fodder production. (BMLFUW, 2004).

Over the past years, the global climate change has affected parts of Austria by the occurrence of intensive drought periods, which caused heavy drought damages on agricultural areas, especially in the Northeast, East and Southeast of Austria (Figure 1). In the year 2003, the drought damages on grassland amounted to about 300 million Euros. An appropriate insurance against drought damages on grassland is not yet available, because qualified, scientifically based models do not exist so far.

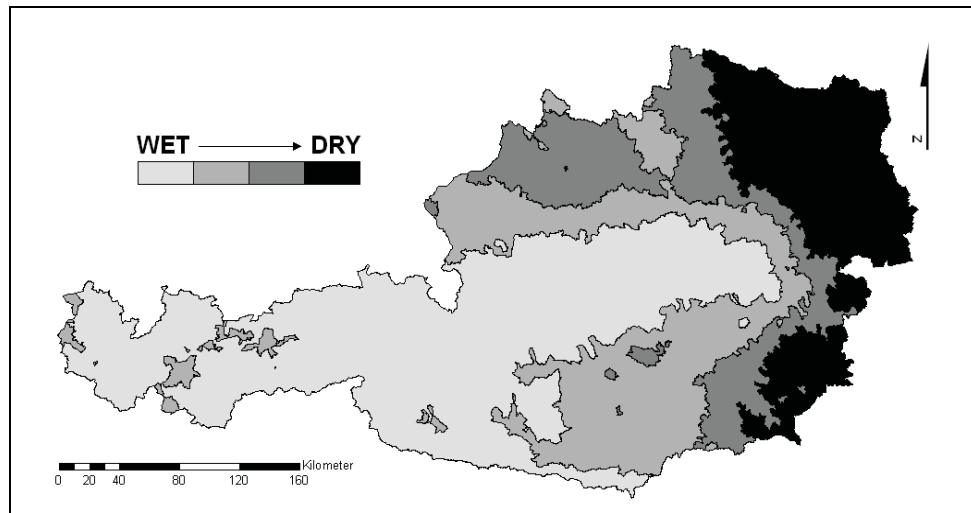


Figure 1. Spatial distribution of droughty endangered areas in Austria.

The Austrian Federal Ministry of Agriculture and Forestry, Environment and Water Management (BMLFUW) reacted on that situation and charged the Federal Research and Education Centre for Agriculture Raumberg-Gumpenstein (HBLFA) with the development of a model aiming at the determination of drought damages on grassland. The Institute of Meteorology at the University of Natural Resources and Applied Life Sciences in Vienna (BOKU) developed a growth and yield model and provides the necessary model equations, whose coefficients are being harmonized with the results of grassland field trials (Trnka *et al.*, 2005). The results of this work will be the fundamentals of an insurance model for drought damages on grassland.

#### Materials and methods

In the year 2002, an integrated project has been started with the installation of exact grassland field trials with randomised blocks and triplicate replication on 27 different sites throughout Austria. On every field trial site there are three different types of fertilization and standardised utilisation. The data from these grassland trials, particularly yield and forage quality, are the main basis for growth and yield modelling. 80% of the yield data were used randomised to calibrate the model and the rest for validation.

The soil water balance is the basis for the simulation of growing processes. The precipitation is compared with the potential evapo-transpiration and the soil water balance model is developed according to Penman-Montheith (Allen *et al.*, 1998), considering the field capacity. First of all the potential evapo-transpiration for grass surfaces was determined on a daily basis for the entire country. The most important parameters of this model are the global radiation, respectively the daily radiation balance, the saturation deficit based on temperature and relative humidity as well as the wind. For this project, the weather measurements from 274 official stations of the Central Institute for Meteorology and Geodynamics (ZAMG) were available for the year 2003, providing the basis of all meteorological analyses.

The calculation result of the potential evapo-transpiration was included into the soil water balance model. Together with the field capacity the soil water ratio and the effective evapo-transpiration of three different soil layers from 0 to 40 cm could be determined in this way. The change of soil water balance compared with that of the day before was included as well as the possible drainage of the soil layers located above. The precipitation considering the interception was integrated into the balance calculation. In order to identify drought, it is necessary to examine a longer period and not only a cutoff date. Hence



the accumulation of results of the soil water balance calculation for a certain period is an important precondition to simulate growth.

Another important parameter for the soil water balance is the plant factor, which indicates the development status of plants. The plant factor is used for the calculation of interception and transpiration and describes a linear increase of biomass production from the beginning of the vegetation period to the first utilisation and in the same way from the next to the following utilisation. The basic requirement for this calculation is the exact determination of the vegetation period with its temperature-sensitive and elevation-dependent begin and end as well as the elevation-dependent duration of growth stages for the several utilisations (Schaumberger, 2005).

The growth and yield model is based on the data of the soil water balance and results in a multiple regression in order to calculate the quantity of grassland yield (Trnka *et al.*, 2005). For that purpose it is necessary to calculate a growth factor, which is generated from long-term and short-term water stress factors and from the resultant water availability factor. The model also considers the cultivation intensity concerning fertilisation. Using the Integrated Administration and Control System (IACS) data, the stocking rate in livestock units (LU) per hectare was calculated and used for the estimation of the N-fertilisation as another parameter of the yield equation.

## Results and discussion

The results refer to data of the year 2003. The yield was on a very low level in the eastern part of Austria and in many areas with total yield loss of the following growths. In areas of enough rainfall the yield was above-average due to the higher temperature of this year. Of course, the results of a GIS implementation can not be visualised satisfactory without maps. These results with coloured and detailed maps for every step of the model are published in Schaumberger (2005). The model results were verified on sites of the exact grassland field trials and show good correlation with a coefficient of determination about 0.7. Qualified interpolations of weather data as well as the very simplified modelling of utilisation frequency and N-fertilisation are the big challenges and also the weak points of the model. All calculations are based on the grid data model and were performed as local grid operations in ESRI ArcGIS programs. Most of the intermediate and particularly the final results had to be generated in a resolution of 50 meters. These state-wide operations with a high resolution scale on a daily basis resulted in a geodata set of more than 1.5 TByte with intensive and long computing time.

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