Mulberry, moringa and tithonia in animal feed, and other uses. Results in Latin America and the Caribbean

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Estación Experimental Indio Hatuey

Instituto de Ciencia Animal ICA

Mulberry, moringa and tithonia in animal feed, and other uses. Results in Latin America and the Caribbean

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Foreword

By 2050, it is forecast that there will a need to feed an additional 2 billion people, requiring 60 to 70% more animal products. In addition to the increasing human population, increases in income and urbanization are projected to drive the demand for animal products in the next two decades at almost the same rate as in the recent past, with most of the growth projected to be in developing countries. This implies a huge demand for animal feeds. Sustainability of feed production systems is already under threat due to land degradation, water scarcity, frequent and drastic climatic vagaries, along with increased competition for food, feed and fuel, and for arable land and non-renewable resources such as fossil carbon sources and minerals, including phosphorus. Moreover, currently cost of the feed in most livestock production systems is as high as 70% of the total cost of production. High quality feed resources that do not compete with human food, decrease feed cost and can be produced in tropical countries would be among key elements making livestock production more sustainable.

The foliage from *Moringa oleifera*, *Morus alba* and *Tithonia diversifolia* plants are known to be of high nutritional value for livestock. Some publications, largely on their use in ruminant production systems, are available from Asia and Africa. However, systematic studies on standardization of agronomic practices to obtain high foliage biomass from these three plants, their use as a major part of the diet for both monogastric animals (swine, poultry and rabbits) and ruminant species, and the impact of their use, have been extensively conducted in Latin American and Caribbean countries. Most of the information available is in Spanish. The high quality of the foliages, particularly the protein content, leads to reduced need for conventional and expensive feed resources such as soymeal, especially in the diets of monogastric animals. In addition, the medicinal properties of these plants improve vastly animal health, reducing the need for other medication. High animal productivity has been recorded when feeding these unconventional feeds, which in addition are easy to grow.

In our quest for novel feed resources to extend the feed resource base in developing countries, this publication aims to collate a wealth of available information on foliage production and use in different production systems in Latin American and the Caribbean countries and make it available to other areas for possible application. This document also presents industrial, food and pharmaceutical applications of different products from the three plants. It is expected that this document will promote wider use of the foliage from these plants as animal feed worldwide. Decreased use of conventional protein-rich feed resources such as oil seed cakes and meals, especially soymeal, as well as less need for expensive bought-in concentrates, are also expected. In addition, the information contained in the document will promote more sustainable production practices, such as intercropping with forage legumes.

The document is intended for use by extension workers, researchers, the feed industry, NGOs, farmers' associations, policy-makers and science managers.

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Management of *Morus alba* L. (mulberry)

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ABSTRACT

This chapter looks at *Morus alba* (mulberry) agronomic management and the principal results in Latin America and the Caribbean, including nutritional value; distribution; botany; and ecological factors influencing growth and development of mulberry. The taxonomy, botany and morphology are summarized. The characteristics of germplasm resources, vegetative reproduction capacity, propagation techniques, biomass yield and the influence of fertilizers were considered as means to increase the biomass production of mulberry. Phytosanitary aspects of mulberry are considered with respect to the incidence of pests and diseases. It is proposed that periodic monitoring be carried out regarding the presence of both noxious and beneficial organisms present in the field, which would facilitate the establishment of timely and efficient management regimes for the control of pests and diseases of the crop. The use of agro-ecological management practices that contribute to maximizing yield and gaining greater benefit from the crop are considered.

Keywords: Agronomic management, botany, pest control, *Morus alba*

INTRODUCTION

Woody plants with good potential for forage production have extended the options for livestock feed production, particularly in tropical regions (Boschini, 2003). These plants are much more efficient than grasses in the capture of light energy and in biomass production. Due to the depth reached by their roots, they act in the subsoil to recover water and nutrients, prevent soil compaction and help maintain soil physical and chemical properties, without destabilizing populations and the functioning of soil micro- and macro-organisms that decompose organic matter and transform it into available elements for plants. Among the promising species is mulberry (*Morus alba*, L.), which shows great adaptability to tropical conditions, is easily integrated into livestock production systems and offers elements of security and sustainability in feed supply and environmental maintenance (Benavides, 1999).

Mulberry also stands out because its nutritional value is one of the highest among non-legume tropical forages and is characterized by good foliage production potential. Nutritionally, it has 15–25% crude protein (CP), high energy level, good mineral composition, and an *in vitro* dry matter digestibility of 75–90% (Benavides, 1999). Yulistiani *et al.* (2015) state that, because of its CP content and degradability, this plant is considered to be a good provider of fermentable energy for ruminants consuming low-quality forages, because it favours the degradation of plant cell walls by the rumen micro-organisms.

1. DISTRIBUTION AND BOTANICAL CHARACTERISTICS

1.1 Origin

Mulberry trees have been exploited since ancient times and they began to be systematically planted for sericulture around 4500 years ago (Cifuentes and Kee Wook, 1998). The two oldest reports that include mulberry are from the Ming dynasty in China (Xiangrui and Hongsheng, 2001). Hence it is considered one of the oldest crops of the world.

Mulberry has its origin in Asia, seemingly in China or India. There is evidence that in that continent, from around 5000 years ago, sericulture began, and with it the domestication of mulberry (Ye, 2001). However, the fact that it is a cosmopolitan species has made it difficult to accurately place the origin of this plant; nevertheless, several authors point at the Himalaya as the most likely origin (Benavides, 2000; Sánchez, 2001; Datta, 2002). At the same time, Li (2001) classified the sites of origin of M. alba into five regions: (1) east of the Asian continent; (2) Malaysian archipelago; (3) southwest Asia; (4) western Africa; and (5) north, central and south America.

1.2 Distribution and adaptation

The genus *Morus* has been distributed almost worldwide, in temperate as well as tropical areas. Only the species *M. rubra* is native to the Americas, and the Australian continent is the only one with no known naturally occurring mulberry species (Sánchez, 2002)

China, India and Brazil are the major growers in terms of this crop per area unit, although Brazil has most cultivars as a result of active genetic crossing (de Almeida and Fonseca, 2002a).

In addition, with the development of sericulture projects in different regions of the world, mulberry is now present in many countries. The greatest development and expansion of the plant has occurred in Asia, with more than one million hectares being present in each of China, India and Japan; in Europe, it is used in Italy, France, Spain and other countries; in the Americas it extends from the United States of America to Argentina, including Mexico, several countries of Central America and the Caribbean, Brazil and Colombia. In northern and eastern Africa there is also important development in its utilization (Sánchez, 2002).

The wide range of distribution of mulberry demonstrates its high capacity of adaptation to diverse climate conditions. Thus, Lim *et al.* (1990) stated that mulberry grows well in temperatures between 13° and 38ºC, with an optimum range between 24° and 28ºC, and Datta (2002) reports annual rainfall levels best between 600 and 2500 mm. It requires a relative humidity of from 65 to 80%, and sunshine of 9–13 hours/day (Cifuentes and Ham-Kim, 1998), and is cultivated from sea level to 4000 masl (Ting *et al.*, cited by Benavides, 2000), although it grows better between 800 and 1500 masl (FAO, 1990).

M. alba is adapted to different soil types, preferring those with higher fertility and with good organic matter content (Cifuentes and Ham-Kim, 1998). It generally grows well on porous and deep soils in flat topography, with slopes less than 40% (Domínguez *et al.,* 2002). It prefers a pH between 6.5 and 6.8, although it is tolerant of acidity and of salinity

(Datta, 2002). It develops better on well-drained soils, with a minimum phreatic level at 1 m, of medium clayey-sandy or sandy-clayey texture, and granular and blocky structure (Cifuentes and Ham-Kim, 1998).

2. INFLUENCE OF ECOLOGICAL FACTORS ON THE GROWTH AND DEVELOPMENT OF MULBERRY

The growth and development of mulberry is highly influenced by ecological factors. The close relation that is shown among them determines the performance of this plant in different environments. Cifuentes and Kee Wook (1998) described the effect of these factors on the performance of the plant. The most important ones are considered below.

2.1 Light

This plant grows well in environments with sufficient light and is eminently heliophilous. Adequate illumination contributes to good leaf biomass production and quality. Silkworms fed with leaves that did not receive enough light are not healthy.

2.2 Temperature

On sunny days with temperatures of 30ºC, the photosynthetic rate is 2 mg DM/100 cm^2/hour; while on a cloudy day it is half that. On a rainy one it falls to 30% (Cifuentes and Kee Wook, 1998).

Temperature is a highly influential factor in normal plant growth, although this genus is very cosmopolitan; when temperatures are below 12°C, the plant reduces its growth, and in very cold countries with snowfall, a latency period occurs in the buds. If the temperatures are very high (>40°C) there is an imbalance between photosynthesis and respiration, which stops plant growth.

2.3 Water

Water is also a very important factor in this plant, essential for nutrient transport processes, regulation of cell turgor, and all physiological processes in general.

The whole mulberry plant can comprise up to 60% water; the leaves have the highest water content, between 70 and 80%. From 280 to 400 mL of water are required to synthesize 1 gram of dry matter.

The soil must contain between 70 and 80% of its water holding capacity to achieve good mulberry growth, which may vary according to the soil type. In the presence of hydraulic stress, the plants can stop growth and the buds and terminal leaves wilt or dry out.

Prolonged water excess in the soil and the environment (because of cloudy and rainy days), mainly in heavy soils, can affect nutrient absorption and thus the nutritional quality of the leaves.

2.4 Soil

The texture, structure, pH and content of macro- and micro-elements in the soil all influence normal mulberry growth. Neither clayey and heavy soils nor sandy and light ones are suitable. A sandy-clayey or clayey-sandy (medium texture) soil are suitable for normal growth.

Mulberry tolerates highly varied pH levels, between 4.5 and 9, but it grows better on neutral soils with pH from 6.5 to 7. From a chemical point of view, the C:N ratio should be less than 10, which facilitates the availability of nitrogen, phosphorus and sulphur. Good soil phosphorus content promotes growth of the root system and the absorption of nutrients. High contents of calcium (>6 meq/100 g of soil), magnesium (>2.5 meq/100 g of soil) and potassium (>0.35 meq/100 g of soil) are desirable. The cation exchange capacity should be >20 meq/100 g of soil, which will hold nutrients and release them slowly for the crop.

3. TAXONOMY AND BOTANICAL DESCRIPTION

Several authors have studied its taxonomy, but seemingly there has been agreement on order, family and genus, unlike what has occurred with species and varieties. Mulberry belongs to the division Spermatophyta, class Magnoliatae, subclass Dicotyledoneae, order Urticales, family Moraceae, genus *Morus* (Diccionario Enciclopédico Espasa, 1984).

In the taxonomic classification, *M. alba* differs from other species of the genus *Morus* because it shows pistils with distinctive long styles, protuberance within the stigma, small leaves lacking hairs or with protuberance in the young stage, veins on the lower surface and obloid purplish soroses from 1.0 to 2.5 cm. It is known by various common names: “Amoreira” (Brazil); “Mallbeerbaum” (Germany); “Mullberry” (English-speaking countries); and “Kurva” and “Tut” (Africa).

Due to the many common names, the systematization of *M. alba* has been tricky, which does not help the taxonomic arrangement and hinders homogeneity in the classification worldwide (Cappellozza, 2002).

Cappellozza (2002) describes mulberry as a woody, perennial, deciduous tree, of low to moderate size and semi-deciduous, of fast growth, monoecious or dioecious, with a deep root system. It has a roundish and branched crown, with greyish bark on a trunk that grows up to 60 cm in diameter.

4. MAIN MORPHOLOGICAL CHARACTERISTICS OF THE PLANT

4.1 Root system

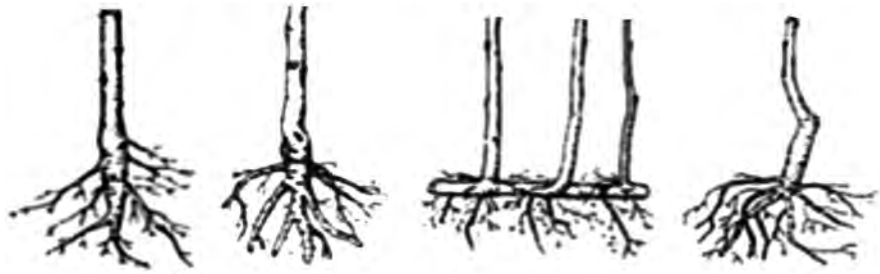
The root system of mulberry consists of a main root, lateral roots and fibrous or absorbent roots, although it varies according to the propagation method used (Figure 1).

When propagation occurs by sexual or botanical seed, the seed radicle develops into a main root, which is known as the anchoring or tap root. From the main root other lateral roots develop, which grow outward, and are known as first-order lateral roots, and so forth. The finest roots (<1 mm diameter) are called fibrous roots or absorbent hairs. The edge between the soil surface and the aerial parts of the plant is called the root collar. Generally speaking, the root system spreads itself in a circular pattern.

When propagation occurs by grafts, layers or stakes, adventitious roots are formed without a main root and with an irregular arrangement.

The mulberry root is bright yellow in colour, especially when young, while old roots are dark yellow. The root surface is covered by lenticels, which serve as openings and closures for gas exchange.

FIGURE 1
Shapes of the mulberry roots according to the form of propagation

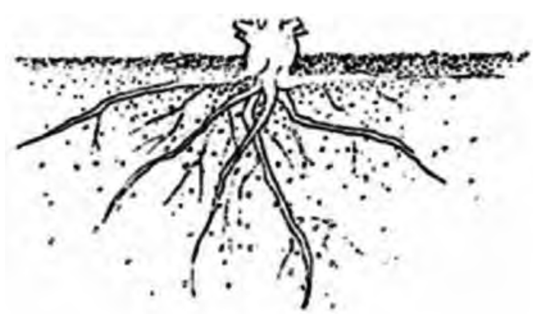


by seed by graFt by layers by stakes

Source:Cifuentes and Kee Wook, 1998.

FIGURE 2
Distribution of the mulberry roots according to the soil dept

0 cm ,,,,,,,,,,,,,,,,,,,,,
29%
20 cm ,,,,,,,,,,,,,,,,,,,,,
37%
40 cm ,,,,,,,,,,,,,,,,,,,,,
22%
60 cm ,,,,,,,,,,,,,,,,,,,,,
8%
80 cm ,,,,,,,,,,,,,,,,,,,,,
4%



Source:Cifuentes and Kee Wook, 1998.

The root system of mulberry is deep and its spreading in the soil depends on the soil texture, organic matter content, cultivation system and management.

In general, the soil area covered by the root system is 1.5 times the radius of its crown. The root that is near the soil surface and which is more or less horizontal, is termed the horizontal root and can absorb and utilize the nutrients and water of the topsoil; while the lateral roots, which grow diagonally or vertically are the vertical roots, which can effectively use the available nutrients and water in the deeper layers of the soil. The depth of the roots is related to the shape of the plant: a small plant will have relatively smaller deep roots, and vice versa (Figure 2).

4.2 Buds

The bud is the initial body of the branch, leaf and flower. Depending on the type, the characteristics and its morphological structure, buds are very important for its cultivation, selection and breeding, with buds varying in size and form according to the cultivar.

4.3 Bud types and characteristics

The buds are divided into terminal and lateral buds, according to the growth position on the branches. Those which are located at the growing points of the terminal branches are called terminal buds, while those located in the axes of the leaves are called lateral or axillary buds. If the terminal buds die, the terminal growing points stop developing and then the lateral buds will start to grow.

According to their physiological state, buds can be active or dormant. At first, the axillary buds appear green in colour and with new growth they gradually take new shape and increase in number. At this point the axillary buds show colours characteristic for the cultivar. In countries with seasonal climate they are called winter buds and usually sprout the following year, but in some lines they can sprout in the same year and they are then called active buds. The winter buds that do not sprout the following spring are called dormant buds and have a very long life.

There are also leaf buds, flower or mixed buds. The leaf buds are those that will develop into a leaf, the flower buds will produce one or more flowers, and the mixed ones can produce leaves as well as flowers

4.4 Shape and structure of the bud

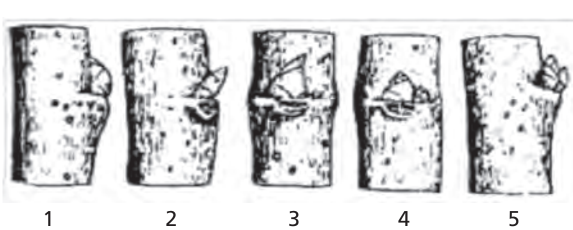
Just like the branches, the buds can show different colours: coffee, violet-coffee, yellowish-coffee, greenish-coffee, etc. Many of the buds are adhering to the stems or branches, but a few stand proud, while others show a slight tilt to one side in the tip. They can be shaped as a regular triangle, an acute triangle, an oval or other. Their shape is an important basis for the identification of cultivars (Figure 3).

Looking at a vertical section of a bud, there is a central axis, which is a miniature stem, on whose tip the growing spot [meristem] or a growth cone is observed, its cells being in a state of active division and growth. At the centre of the axis there are alternate layers of small prominences that constitute the leaf primordia, which will later develop as the true leaves (Figure 4).

4.5 Stem

As a whole, the trunk, branches and twigs of mulberry are usually called stems. Their main function is to transport water and mineral salts upwards and send downwards the products

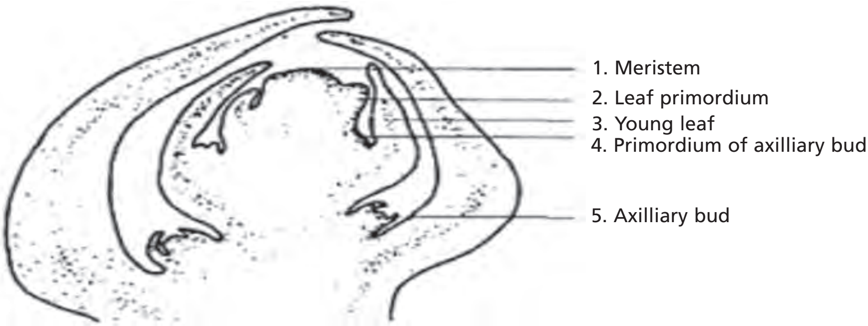
FIGURE 3
Appearance of the buds



1. Adhering to the branch
2. Tip standing proud of the branch
3. Slanted at the tip
4. With accesory bud
5. With dorsal accesory bud

Source: Cifuentes and Kee Wook, 1998.

FIGURE 4
Vertical section of a mulberry bud.



SOURCE: Cifuentes and KeeWook, 1998.

of photosynthesis and other organic materials. There is a principal trunk, with several lateral branches, which are again divided, and categorized as primary, secondary, etc.

In mulberry cultivation, the aim is to encourage stem production, in order to maximize leaf production.

4.6 Shape of the branches and twigs

Under normal conditions, the length, size and colour of the mulberry branches, as well as the length of the internodes, are stable and sufficiently basic to distinguish cultivars. In general, the branches and twigs have three growth forms: erect; open or spreading; and drooping or pendant. The erect types have the plant crown concentrated, which provides good ventilation and makes them suitable for denser plantings and mechanized cropping. Although the spreading branch types make good use of space, they cannot be used at high planting densities.

The length and size of mulberry branches depend on cultivar, age of the plant, soil conditions, climate effects, pruning methods, quantities of organic matter applied, and management.

Since branches constitute the main objective of mulberry production, the harvest of the leaves must be rational and the plants must be well cared for in order to promote vigorous growth and produce high leaf yields.

Mulberry is a plant that can survive pruning, but its recovery ability depends on the cultivar, age, fertilization method and general management of the plantation.

4.7 Leaves

The mulberry leaf consists of three parts: the petiole, stipule, and leaf lamina or blade. Its shape and structure vary between cultivars and environmental conditions.

The petiole is the organ that joins the leaf blade to the branch or twig and serves as passage for the water and nutrients. The stipule grows on both sides of the base of the petiole. It falls early from the plant and disappears as the leaf matures. It cannot be seen when the leaf is

FIGURE 5
Shapes of mulberry leaves

SOURCE: Cifuentes and Kee Wook,1998.

old. The leaf blade or lamina is the main part of the leaf. It can have one or two basic shapes, called entire or lobed leaves. The entire leaves can be classified in turn as cordate, elliptic, ovate, etc. (Figure 5).

Lobed leaves are classified according to the number of lobes into bi-, tri-, tetra-, etc. -lobed. The morphological characteristics of the tip, margins, base and veins of the leaf blade vary with cultivar. Its shape, thickness and colour change and reflect both genetic and external factors.

4.8 Flower, fruit and seed

Most mulberry flowers are monosexual [monoecious], although some of them are bisexual [dioecious]. They are small and sessile and form a raceme around an axis, and this structure is termed the catkin. The sex of the flowers depends on the cultivar, so that some are monoecious, others are dioecious and others androgynous. The staminate (male) as well as pistillate (female) flowers are in separate racemes.

The staminate flower (male) comprises four sepals and four stamens (Figure 6). The stamen is composed of one filament and an anther. When the flower is in bloom, the filament is extended outwards and the anthers split, showing a quantity of yellow pollen balls, which spread far and wide at the mercy of the wind. The mulberry plant is thus anemophilous.

The flower pistil is made up of four sepals, the ovary, the style and the stigma (Figure7). The sepals wrap the ovary closely. The ovary appears as a green ball, containing the ovule, in the centre of which is the embryo sac. The style length, which changes with cultivar, is one of the most important bases for the classification of mulberry plants.

On the tip of the style is the stigma, which splits and assumes the shape of cow horns. On it, dense and whitish projections grow, which can secrete a sugary juice that is able to stick the pollen.

When the stigma narrows down and shows a white colour, it means that the embryo sac is mature and it is fertile. When pollen grains land on the stigma, they absorb the mucus secreted by the stigma and it stimulates them to grow towards the embryo sac, thus completing insemination. After insemination, the stigma withers and the ovary and sepals gradually develop. Then they droop and become fruits, jointly developing into a sorosis.

FIGURE 6
Staminate (male) mulberry flower.

SOURCE: Cifuentes and Kee Wook, 1998.

FIGURE 7
Pistillate (female) flower and vertical section of a mulberry ovary.

SOURCE: Cifuentes and Kee Wook, 1998.

The sorosis (Figure 8) is green at first, but it gradually acquires colour, and the mature fruit becomes white, red or violet-black.

The seeds are yellowish, varying from coffee-yellow to bright yellow, and are fleshy and ovate in shape. They are made up of a coat, the embryo and the endosperm. The endosperm stores a large amount of fat, ash and proteins, which unite to satisfy the needs of the seed and its development.

The main pollinating agent of mulberry is the wind. Most species are diploid with 28 chromosomes (2n=28); nevertheless, triploid species are also extensively cultivated due to their adaptability, growth, vigour and leaf quality (Cappellozza, 2002). Machii *et al*.(2002) stated that this genus is rich in ploidy levels, because triploid varieties have been found in *M. bombycis,* and tetraploid, pentaploid and hexaploid forms in *M. cathyana*.

FIGURE 8
Mulberry sorosis and seeds

SOURCE: Cifuentes and Kee Wook, 1998.

In the 1800s and, 1900s, taxonomic investigators proposed several divisions of the genus *Morus*, mainly based on the presence or absence of style in the flower, the protuberance and hairiness of the stigma, the inflorescence, the sorosis, the base of the bilobed style, and the morphology of the leaf, mainly the shape of the base.

5. GERMPLASM RESOURCES

Most specie of the genus Morus are located in Asia, especially in China (24 species) and Japan (19). The American continent is also rich in specie of this genus. Morus is poorly represented in Africa and Europe, and it is not naturally present in Australia (Datta, 2002).

In China there are more than 1000 recognized cultivars, coming mainly from four species: *Morus alba*, *M. multicaulis*, *M. bombycis* and *M. atropurpurea*. Chinese scientists classified the genus *Morus* into 14 species and one botanical variety. This classification was made based on the characteristics of the female flower related to the length of the styles (Huo, 2002). In India there are many mulberry species, and in the Himalayan mountains *M. alba, M. indica, M. serrata* and *M. laevigata* grow wild. Cultivars of *M. multicaulis*, *M. alba*, *M. tartarica* and *M. nigra* have been introduced into India. Most cultivars in India belong to *M. indica* (Datta, 2002).

In Japan, Machii *et al.* (2002) classified the genus *Morus* into 24 species and one subspecies. Most of the cultivars belong to *M. bombycis* Koidz, *M. alba* L. and *M. latifolia* Poiret. The National Institute of Agrobiological Sciences in Japan has a germplasm bank with more than 1300 accessions, from Japan and other countries, kept in the field and in greenhouses (Machii, 2001). In Japan, Machii *et al.* (2002) studied 260 genotypes that are part of the collection of the National Institute of Sericulture and Entomology Science of Japan (Datta, 2002).

In Indonesia there are several species, among them the most important are *M. alba* (var. *tatarica* and var. m*acrophyla*), *M. nigra* and *M. multicaulis.* In Viet Nam there are more than 100 cultivars of *M. alba*, *M. nigra* and *M. laevigata* (Sánchez, 2002).

However, in spite of the increasing interest in this plant in Asian countries, outside of Asia the availability of mulberry is much more limited. Thus, for example, in Brazil, mulberry was introduced from Asia in colonial times; it is a known plant, present in gardens and other field and city sites, and is commercially associated with sericulture. Most cultivars belong to *M. alba*. In Sao Paulo state there is an active germplasm bank at the Animal

Science Research Station in Galia, belonging to the Institute of Zootechnique, with other collections at the University of Sao Paulo and at the Agronomic Institute of Parana. At the Research Station of Galia there is a germplasm bank with 88 clones and lines belonging to three collections. There is little information about the clones that are commercially used, although the companies have spread the clones cvs Miura and Korin, which, along with cv. Calabreza, occupy most of the cultivated area (de Almeida and Fonseca, 2002a).

In Italy and other European countries, such as Greece, France and Spain, although the origin and time of first introduction of mulberry is not very clear, it is evident that its development was related to sericulture (Cappellozza, 2002). According to this author, mulberry has not been much studied in Italy, although there is considerable experience in using it to feed the silkworm (*Bombyx mori*); the species most used commercially is *M. alba*, with many lines and spontaneous hybrids. Frequently the same cultivar has different local names, which obscures its true taxonomy. The largest mulberry collection is found in the sericulture section of the Animal Production Research Institute of Padova, Italy, with 51 accessions.

In Cuba there is a germplasm collection with 22 accessions (Martín *et al*., 2014), which are part of the genebank of the Estación Experimental de Pastos y Forrajes "Indio Hatuey". They were introduced mainly from Costa Rica, Brazil, China and the Republic of Korea. The first species and varieties of mulberry in Cuba were introduced between 1824 and 1848 (Tirelli, 1939), but it is not very easy to find naturalized plants on the island.

As can be appreciated, globally several studies have been conducted and large collections of species and lines of this genus are conserved, but due to the effects of diffusion and development of sericulture in different countries, many lines have been distributed under different names, and they have even been classified as different species.

A study conducted by Sharma *et al*. (cited by Machii, 2001) shows that genotypes from different geographical origins and that supposedly belong to different species, can have high genetic affinity. Thus, in Cuba it would be necessary conduct further taxonomic studies of the existing genotypes and those that might be introduced in the future, to prevent mistakes that could affect the results of the research and the utilization of this plant.

The first studies in Cuba about mulberry was provided about the planting system for mulberry using stakes, as well as the delicate care of the silkworms *Bombyx mori*, acquired for the promotion of sericulture in the island

In the botanical dictionary written by Roig (1965), three mulberry species and two varieties are described for Cuba: *Morus alba* L, *Morus multicaulis* Perr. and *Morus nigra* L. and the varieties: *Morus alba* var. *calabresa* and *Morus alba* var. *moretti*.

In the, 1900s and 2000s introductions were made from different countries, in order to use it for livestock feeding, but later used for other purposes.

In the period, 1990–2014, a large set of studies were conducted in Cuba, related to the use of mulberry as animal feed, mainly at the Research Station "Indio Hatuey".

In the 1990s there was a boom in Cuba in the use of trees for cattle feeding in agro-silvi-pastoral systems. In this sense, in 1994, during the First International Workshop on Silvipastoral Systems in Cuba, Jorge Evelio Benavides, researcher of the Tropical Agronomic Center for Research and Teaching (CATIE) in Costa Rica, presented a report on the potential of this plant as feed for animals.

TABLE 1. Accessions, dates and provenance of the mulberry introductions at Station "Indio Hatuey"

Accession	Key	Form	Date	Provenance	Quantity
Acorazonada	1822-49	Pieces	20/4/1995	Costa Rica	
Tigreada	1822-49	Pieces	20/4/1995	Costa Rica	
Criolla	1822-49	Pieces	20/4/1995	Costa Rica	
Doña Betty	1822-49	Pieces	20/4/1995	Costa Rica	
Morus alba	1833	Stakes	27/6/1995	Costa Rica	44
Morus alba	1894	Stakes	9/10/1996	Costa Rica	1000
Morus alba	1980-1984	Stakes	27/12/2000	Brazil	
YZ-64			27/12/2000	China	51
Z-13-6			27/12/2000	China	
Z-40			2005		
Z-15-7			2005	Republic of Korea	
Z-56-4			2011		
Ichinose				Republic of Korea	
Super morera				Republic of Korea	
Cheongol				Republic of Korea	
Ppong				Republic of Korea	
Universidad				Spain	
Universidad nueva				Spain	
Universidad Mejorada				Spain	
Yu-12			2011	China	
Yu-62			2011	China	
Murcia			2011	Spain	

Since then, in the framework of collaboration with the Station "Indio Hatuey" and through the contribution of Leopoldo Fernández, seeds from *Morus* cultivars were introduced in order to assess them under Cuban edaphic and climatic conditions, and see how results compared with the promising results that had been reached in the humid tropic of Costa Rica. To that end, a research programme was developed in various provinces of the country, to evaluate the nutritional quality of the mulberry leaves and acceptability by livestock, not only cattle, but also goats, pigs, rabbits, sheep and poultry.

Table 1 shows the varieties that were introduced at the "Indio Hatuey" experimental station (Martín *et al.,* 2014).

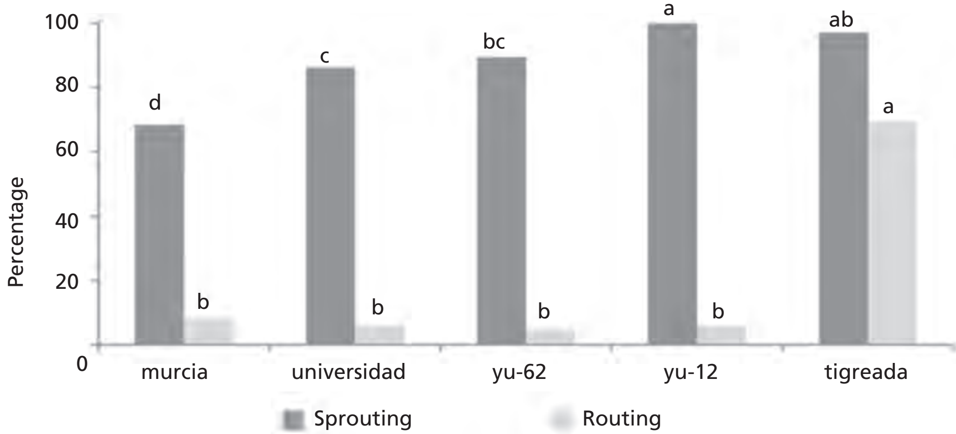
6. EVALUATION OF GERMPLASM VEGETATIVE REPRODUCTION CAPACITY

Initially the mulberry germplasm in Cuba consisted of the cultivars Indonesia, Criolla, Acorazonada and Tigreada, for feeding monogastric and ruminant animals. Along with these four cultivars, cv. Cubana, which had been introduced from Ethiopia in the, 1980s, was also naturalized (Noda *et al.*, 2004).

Later, in 2000, the Institute of Zootechnology of Brazil provided two selections (IZ-40, IZ-64) and three hybrids (IZ-15/7, IZ-13/6, IZ-56/4), and in 2005 the varieties Ichinose, Super morera, Cheongol and Ppong were introduced, from the Republic of Korea, all of them taxonomically *M. alba*.

Later, in 2011, the Station "Indio Hatuey" acquired six more cultivars: Universidad, Universidad Nueva, Universidad Mejorada, Yu-12 and Yu-62, which were introduced from

FIGURE 9
Percentage of sprouted and rooted propagules in each variety



Different letters for sprouting and rooting differ significantly at *P*< 0,05. Sprouted propagules: SE ±3.6* Rooted propagules: SE ±0.6*

China, and cv. Murcia from Spain. Their agronomic and productive performance in Cuba is under investigation. Their seeds have good germination performance, but the cultivars do not show good rooting efficiency when cuttings are used for propagation.

The vegetative reproduction capacity of the various lines were tested during 80 days, and also the effect of naphthalenacetic acid (NAA) and hydrological regime on the sprouting and rooting of the propagules of the recently introduced cultivars.

In the first study, five mulberry varieties were analysed (Tigreada, Yu-12, Yu-62, Murcia and Universidad). The branches were divided into three parts (basal, medium and apical), giving 15 treatments. The quantity and percentage of sprouted and rooted propagules, the number of branches, the number of leaves, and the weight of the root system, were evaluated.

Yu-12 had all cuttings that sprouted, similar to cv. Tigreada (used as control), which showed 98%. The others had lower values (Yu-62 – 91%; Universidad – 87%; and Murcia – 69%).

Concerning rooting, Tigreada differed significantly from the others, reaching 70% of the total planted cuttings. Yu-12, Yu-62, Universidad and Murcia showed low percentages, which varied between 4 and 8% (Figure 9).

The emergence of sprouts and the rooting of the plants are shown in Table 2. The propagules showed important differences, according to the position they occupied along the branch from which they were taken.

The highest sprouting percentages (93 and 90%) were obtained when using the basal and medium parts of the branch, with no significant differences; but they did differ significantly from the apical part, which had the lowest response (84% of total plants). Seemingly, the cuttings of the terminal parts are less effective for propagation, associated with the presence of pithy tissues with immature buds, unlike the cuttings from the middle and basal zones (Machado, 2011).

TABLE 2. Effect of the part of the branch planted on the percentage of sprouted and rooted plants.

Branch part	Sprouted plants (%)	Rooted plants (%)
Basal	93[a]	21
Middle	90[a]	17
Apical	84[b]	17
SE (±)	3.6*	2.6[n.s]

Notes: Different letters indicate significant differences $P \leq 0.05$.

TABLE 3. Effect of the variety×part of the branch interaction on the number of leaves

Cultivar	Part of the branch	Number of leaves
Tigreada	Basal	29.23[a]
	Middle	23.81[b]
	Apical	20.73[c]
Yu-12	Basal	6.90[de]
	Middle	4.46[efg]
	Apical	3.10[fg]
Yu-62	Basal	3.95[fg]
	Middle	3.48[fg]
	Apical	3.18[fg]
Murcia	Basal	8.30[d]
	Middle	5.53[ef]
	Apical	4.41[efg]
Unversidad	Basal	5.84[def]
	Middle	3.40[fg]
	Apical	2.53[g]
SE± of the interaction		5.77

Notes: Different superscripts in each row indicate significant differences ($P<0.05$).

TABLE 4. Effect of the cultivar on root weight

Variety	Root weight (g)
Tigreada	2.67[a]
Yu-12	0.42[b]
Yu-62	0.07[b]
Murcia	0.15[b]
Universidad	0.30[b]
SE(±)	0.02

Notes: Different superscripts in each row indicate significant differences ($P<0.05$).

No reports were found in the literature regarding the asexual reproduction capacity of the cultivars Universidad, Yu-12, Yu-62 and Murcia, which are efficiently reproduced by botanical seed.

The effect of the interaction of the variety and the branch part was also significant for the number of leaves sprouted in each treatment (Table 3).

Tigreada, planted from the basal part of the branch, had the most leaves (29), and differed significantly from the others ($P<0.05$).

No interaction was found for the weight of the roots, but a significant effect of the factors was found separately. Cv. Tigreada differed significantly from the others in the weight of its roots (Table 4), which could have been due to its effectiveness in reproduction by propagules. Only this variety reached a weight of its roots higher than 1 g (2.67) at 80 days after planting.

At the same time, cuttings from the basal part of branches favoured higher root mass, and differed from the cuttings from middle and apical parts (Table 5).

The results clearly matched the ones obtained by Martín (2004) and Noda *et al.* (2004), showing that cv. Tigreada has high capacity and response for efficient sprouting from planting propagules. However, Yu-12, Yu-62, Universidad and Murcia did not have this performance when reproduced by stakes.

Likewise, the part of the branch used for propagation had a significant effect, especially those that were extracted from the basal or middle parts. This indicates that mulberry has a similar performance to that of many asexually-propagated plants, attributed to greater nutritional reserves in those regions, as for many trees and shrubs (Boschini and Rodríguez, 2002).

The experiment to study the effect of the growth hormone NAA and watering regime on the sprouting and rooting of propagules of the new cvs Yu-62 and Universidad mejorada, compared with cv. Tigreada without being inoculated, showed variable results.

Rooting, quantity of sprouted and developed propagules, weight of the leaf area and weight of the roots were higher in the inoculated varieties, although in no case did they exceeded cv. Tigreada without treatment. However, the methods used seem to be encouraging for these new mulberry cultivars, which until then had propagated poorly from cuttings, which is important from an agricultural point of view, because it is an easy and fast way to preserve the characteristics of the mother plant (Boschini and Rodríguez, 2002).

TABLE 5. Effect of the branch part planted on the root weight of the new plants.

Branch part	Root system weight (g)
Basal	1.08[a]
Middle	0.50[b]
Apical	0.59[b]
SE(±)	0.02

NOTES: Different letters in each row indicate significant differences ($P<0.05$).

7. PLANTING AND BIOMASS YIELD

As noted earlier, this plant can be propagated by botanical seeds, stakes, layering and grafts. It is possible to use small micro-stakes with one or two buds, which are put in germinators and transplanted afterwards to the field, although the stakes can be planted direct to the field.

Studies have been successfully carried out on *in vitro* propagation methods, whose results have created the basis for the accelerated multiplication of varieties and ecotypes, as well as for the exchange of germplasm (Prieto *et al.*, 1999; Salas and Agramonte, 2002). Nevertheless, the main propagation method is still the vegetative one, by stakes (Benavides *et al.*, 1986).

In Cuba, following the experience developed in Costa Rica by Benavides *et al.* (1986), an inter-row distance of 1 m, with 40–50 cm between plants, are recommended for mulberry planting. From the practical experience obtained through the agronomic studies conducted by Martín (2004), it is felt that other planting densities that achieve more plants per unit area should be studied, to evaluate the effect on total and edible biomass production.

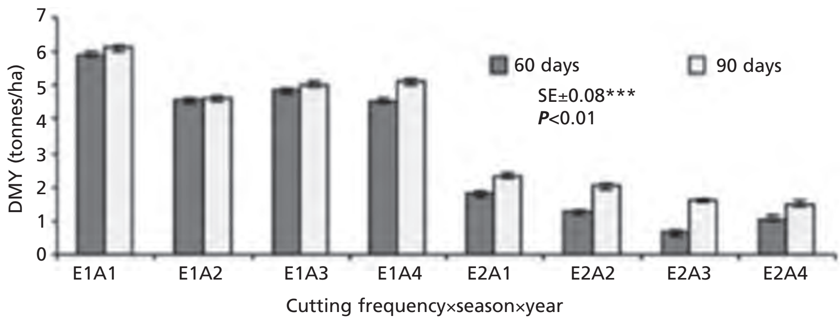
The research conducted at the Station "Indio Hatuey" on a lixiviated ferralitic red soil (Hernández *et al.,* 1999), showed that there was significant interaction ($P<0.001$) among cutting frequency, season and year of exploitation, for the leaf dry matter yield (LDMY) (Figure 10).

As can be seen in Figure 10, the LDMY was higher in the rainy season, and this yield tended to decrease with plant age, which can be explained by a progressive attrition of youth vigour of forage plants (Machado and Seguí, 1997).

In the rainy season the cutting frequencies of 60 and 90 days showed similar values of LDMY in all the years studied, but in the dry season the values were higher with the 90-day frequency. This indicates that in the rainy season the 60-day cutting frequency can be used and in the dry season, the 90-day frequency.

Also in this research, the effect of the variety×cutting frequency interaction (V×C) on leaf yield was studied. Cv. Indonesia, with the 90-day frequency, gave most yield (7.45 t DM/ha/year), while cvs. Cubana and Tigreada had LDMY from 6.94 to 7.34 t DM/ha/year. When cut at 120-day intervals yield was 7.06 t DM/ha/year. The lowest LDMY was obtained from cv. Cubana cut at 60-day intervals (5.74 t DM/ha/year), but it did not differ

FIGURE 10
Influence of the cutting frequency×season interaction on the dry matter yield of mulberry leaves



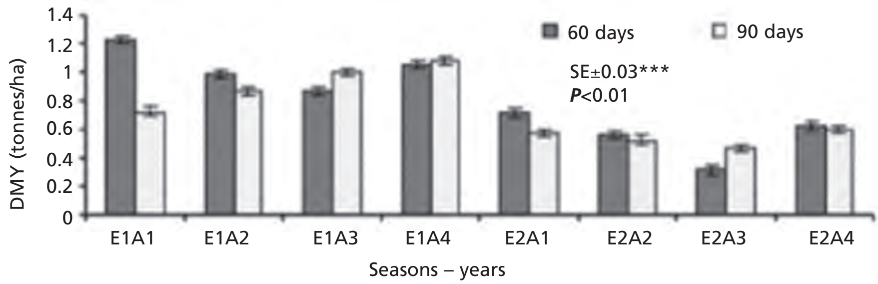
Note: E1- rainy season; E2- dry season. A1: first year; A2: second year; A3: third year; A4: fourth year.

significantly from that obtained from cvs. Indonesia and Tigreada at the same cutting frequency (6.29 and 6.08 t DM/ha/year, respectively).

The results of this study over four years indicated that to obtain the highest DM production of leaves annually, all the evaluated varieties can be used, but with different cutting frequencies. Thus use cv. Indonesia with a 90-day cutting frequency, cv. Acorazonada with any of the three frequencies, and cvs. Cubana and Tigreada with 90- and 120-day intervals between cuttings. This result indicates that there are differences in the performance of the varieties before the cutting frequencies used in the Total LDMY (TLDMY), which can be explained by the characteristics of each cultivar, among other factors. In the literature reviewed there are no studies reporting such interactions, as only the effects of the principal factors are reported separately. The fresh stem dry matter yield (FSDMY) showed significant differences ($P<0.001$) in the cutting frequency×season×year (F×S×Y) interaction. Figure 11 shows that the FSDMY decreased with the years, although it showed a slight recovery in the last year studied. As can be observed, the FSDMY was affected by the interaction of the factors cutting frequency, season and year of exploitation, and the highest yields were achieved in the rainy season. Apparently, in this season, the production of fresh stems is stimulated, because the vegetative growth processes are more favoured at that moment of the year. The unstable performance of the FSDMY in the different years was due to variable climate characteristics in the experimental period.

At the same time, the edible biomass production (leaves + fresh stems) of mulberry was determined by the interactions of variety×cutting frequency×season (V×C×S) and cutting frequency×season×year (C×S×Y), which indicated the performance of each variety for different cutting frequencies in the different seasons. Figure 12a shows that the variety Acorazonada showed the highest edible biomass production in the rainy season with the 60-day cutting frequency (6.35 t DM/ha), although it did not differ significantly from the variety Indonesia in the same season, but cut every 90 days (6.45 t DM/ha). At the same time, the Tigreada variety produced very similar values in the rainy season when cut with frequencies of 60 (6.02 t DM/ha) and 90 days (6.19 t DM/ha).

FIGURE 11
Influence of the cutting frequency×season×year on the fresh stem dry matter yield of mulberry



Note: E1- rainy season; E2- dry season. A1: first year; A2: second year; A3: third year; A4: fourth year.

The effects of the C×S×Y interaction (Figure12b) were very similar to the ones obtained in the LDMY, because leaves are the main component of the edible biomass. It was reported that in both seasons, this variable had different results between the first year and the other three; however, there were little differences among the cutting frequencies in each of the years, especially in the rainy season. In the dry season of each year the 90-day cutting frequency produced the highest quantity of edible biomass DM, results analogous to those reported by Martín *et al*. (2002).

In the reviewed literature there are no results published in which the effects of these interactions on the edible biomass production can be observed.

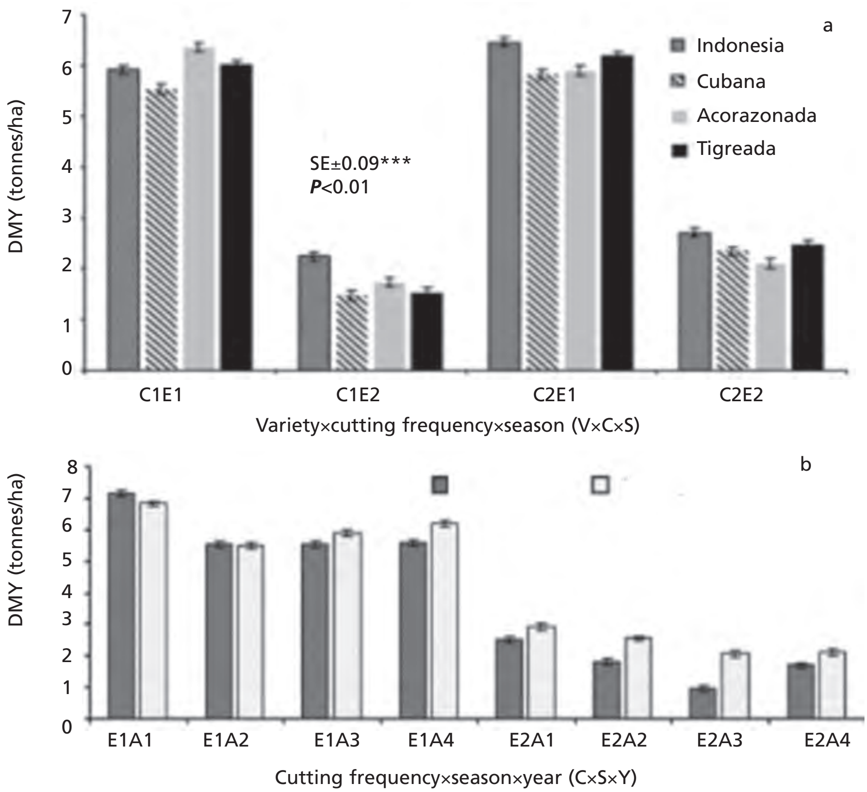
Under the experimental conditions this indicator showed a higher value in the rainy season, which proves the seasonality of the edible biomass production of this plant and indicates how advisable it is to utilize it efficiently in this season. For this purpose it could be preserved as silage (Ojeda *et al*., 2000) or as high-quality protein meals (García *et al.*, 2002), in order to be used in the seasons of feed scarcity.

The results show that the edible biomass production is determined by the interaction of factors such as variety, cutting frequency and season, which has undeniable scientific novelty, because they had never before been reported in the literature and show that, although this species has high ecological plasticity (Benavides, 2002), it will be necessary to evaluate the performance of these factors and their interactions at the different sites it is intended for, in order to determine optimal utilization.

Another aspect studied in this research was the woody stem total dry matter yield (WSTDMY) of this plant, which showed V×C×S interaction (P<0.001). Figure 13 shows that the factor cutting frequency as well as climate season had a marked effect on the performance of this indicator. The highest WSTDMY values were obtained in the rainy season with the 90-day cutting frequency and cv. Indonesia. In the rainy season the values were not significant.

The woody stem production can contribute to energy production, a very important aspect to achieve sustainability in livestock production systems. In this sense, Satiya *et al*. (1994) stated that mulberry can be incorporated in integrated production systems,

FIGURE 12
Influence of the interaction: (a) variety×cuttingfrequency×season (V×C×S); and (b) cutting frequency×season×year (C×S×Y), on the dry matter (DM) production of the edible biomass of mulberry



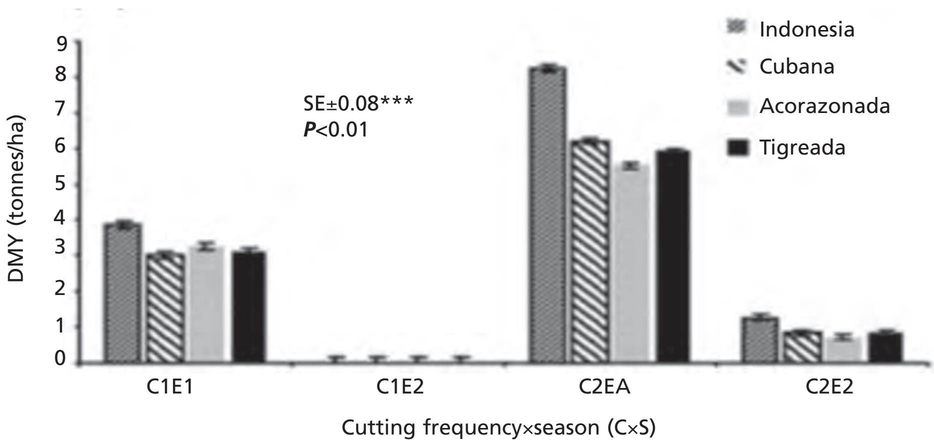
NOTE: C1; C2-cutting frecuency (60; 90 days); E1; E2-rainy and dry season; A1; A2; A3; A4-first, second, third and fourth year.

because the leaves are used as feedstuff, and the woody stems for the production of edible mushrooms. The unused residues can be taken to biodigesters for biogas production and the effluents can be used as fertilizers. The thicker woody stems are used as firewood to provide energy (T.R. Preston, pers. comm.).

The bark of the woody stems is an important feed source for some animal species. Shayo (1997) reported that the mulberry bark has 7.8% CP; 6.1% ash; 46.8% neutral detergent fibre (NDF) and 36.9% acid detergent fibre (ADF). The determinations of edible biomass, where only the fresh stem is considered by the evaluator to be the only edible fraction, are a little subjective, because in evaluations in which the whole mulberry plant was supplied as integral forage, 80% of intake was achieved, a much higher value than the edible biomass percentage found in mulberry (Benavides, 2002).

The above show the possible forage and energy value of the woody stem fraction in this species, for which future studies should be conductedto elucidate.

FIGURE 13
Influence of the V×C×S interaction on the woody stem dry matter yield of mulberr



NOTE: C1; C2-cutting frecuency (60; 90 days); E1; E2-rainy and dry season.

The results of the total biomass dry matter yield (TBDMY) of mulberry showed V×C×S (P<0.001) and V×S×Y (P<0.01) interactions. The V×C×S interaction (Figure 14a) indicated that cv. Indonesia, with the 90-day cutting frequency in the rainy season, showed the highest values. This result shows that the biomass production of mulberry in Cuba has a marked seasonality, similar to what occurs with traditional forages in the tropics, such as *Pennisetum*, *Panicum* and others (Machado and Seguí, 1997).

At the same time, the V×S×Y interaction (Figure 14b) showed the effects of the climate characteristics of each year on the performance of this indicator. As can be seen in Figure 15b, the rainy season of year two was the least productive, because the rainfall 841 mm was much less than the average for the season, which was 1035 mm over the previous 15 years, and much lower than the average of the rainy seasons of the four years in which the experiment was conducted (1217 mm).

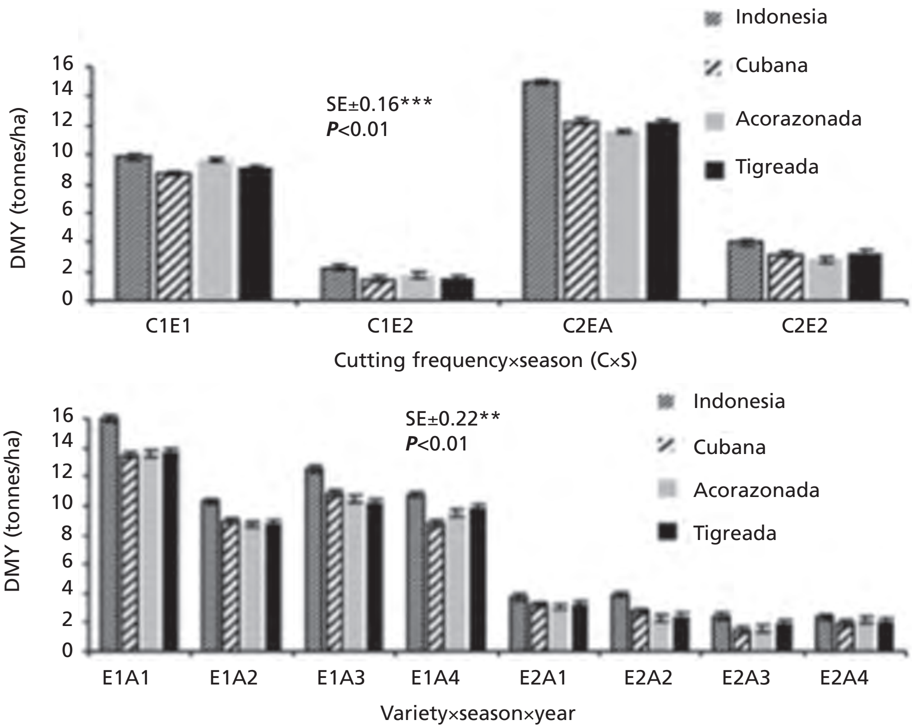
Several researchers state that the genetic and climate factors can have a marked influence on the production of the different components of the total biomass of mulberry; thus, in Asia, work in China (Chen*et al.*, 2009), India (Datta, 2002) and Japan (Machii, 2001), as well as in Italy (Cappellozza, 2002) and Brazil (de Almeida and Fonseca, 2002a) stand out, where large germplasm collections have been evaluated and genotypes adapted to different soil and climate conditions have been selected.

The results conclude that the highest annual DM production was achieved by cv. Indonesia, with the 120-cutting frequency in the rainy season. This genotype was also higher in the dry season, which shows its good tolerance of stressful drought conditions.

8. FERTILIZERS AS A MEANS TO INCREASE BIOMASS PRODUCTION OF MULBERRY

The most used source to supply nutrients to mulberry and achieve appreciable increases in biomass production has been poultry litter. Its application has had significant effects (P<0.001)

FIGURE 14
Influence of the interaction: (a) varietyxcutting frecuencyxseason, and (b) variety×season×year on the total biomass DM yield of mulberry



NOTE: C1; C2-cutting frecuency (60; 90 days); E1; E2-rainy and dry season; A1; A2; A3; A4-first, second, third and fourth year.

TABLE 6. Effect of the N doses (as poultry litter) on the dry matter yield (t/ha/year) of leaves, edible biomass and total biomass of mulberry

N doses	LDMY	kg DM/kg N	EBDMY	kg DM/kg N	TBDMY	kg DM/kg N
100	6.1[c]	61.0	7.5[c]	73.9	13.48[c]	134.80
300	6.8[b]	22.7	8.2[b]	27.4	15.58[b]	51.93
500	7.3[a]	14.6	8.8[a]	17.5	16.84[a]	33.68
SE	0.06***		0.07***		0.14***	

NOTES: Values with different superscripts in columns differ at $P<0.05$ (Duncan, 1955) ***$P<0.001$
LDMY = leaf dry matter yield; EBDMY = edible biomass dry matter yield; TBDMY = total biomass dry matter yield.

on DM production among the evaluated agronomic indicators. Results are summarized in Table 6.

The average leaf dry matter yield (LDMY) during the four years increased with increasing doses of poultry litter, and reached 7.3 t/ha/year with a dose of 500 kg N/ha. A similar performance was shown by the edible biomass DM yield (EBDMY) and the total biomass DM yield (TBDMY), with 16.84 t DM/ha/year.

Nevertheless, the efficiency of N utilization by the plant was greater with 100 kg/ha of N, being 134.80 kg DM/kg N. These results show the potential of poultry litter to increase mulberry yield. This result matches those obtained by Takahashi and Kronka (1989), who proved the effectiveness of poultry litter as fertilizer in mulberry, by accompanying it with several sources of chemical fertilizers.

In another study, Benavides *et al.* (1994) found a marked effect on the DM yield of this plant when applying increasing doses of N as goat manure. The highest dose of nitrogen fertilizer used in this assay (480 kg N/ha/year) showed a similar effect to the N applied as chemical fertilizer.

Both chemical and organic fertilization, combined or separate, has been widely studied in mulberry (Lim *et al.*, 1990; Kabir, Roy and Ray, 1991). Beneficial effects of the application of biofertilizers have also been found (Das *et al.*, 1995; Fathima *et al*., 2000).

Siswanto (1994) and Shankar and Rangaswamy (1999) found that doses of N between 400 and 450 kg/ha/year produced the greatest biomass yields in mulberry when combined with applications from 150 to 200 kg/ha/year of K.

In Cuba, where there is certain culture among farmers about the use of organic fertilizers and some biofertilizers, it is possible to use other fertilization sources, for which it would be necessary to study their performance and their combination with chemical fertilizers.

9. PESTS

For more than 5000 years mulberry has been the only feedstuff for the silkworm, due to its excellent nutritional qualities, among which is the high protein and energy content. In addition, it is used in several regions of the world for animal feeding (Benavides, 1999, 2002; Lara *et al.*, 2002; Martín *et al.*, 2007).

In Cuba, it has become a known and used plant species throughout the country by state agricultural and livestock production farms, as well as by farmers who show great acceptance, especially for feeding small species in the different livestock production sub-programmes of urban agriculture. In addition, under laboratory conditions, it has been successfully used as aqueous extract for the control of gastrointestinal nematodes of cattle (Soca *et al.*, 2002).

The phytosanitary attention to the plants of high protein content, especially those destined for cut-and-carry, constitutes an important element to be taken into consideration in their management, in order to offer higher-quality forage for the livestock of the tropics.

In this context, there are antecedents on the phytosanitary performance of this plant, conducted in China by Ting *et al.* (1988). Both studies agree in their results by acknowledging that the pathogens (primarily fungi, bacteria and mycoplasmas) associated with *Morus alba* are numerous, including: *Gibberella moricola, Pseudomonas mori, Aecidium mori, Rosellinia necatrix, Phyllactinia moricola* and *Helicobasidium mumpa.*

However, Medina *et al.* (2007), in Venezuela, report little incidence of pests and diseases found on this plant in the nursery stage, ascribing it to the possible presence of some secondary metabolites in these plants which, although they do not constitute anti-nutritional factors due to their low concentration, show protective and repellent functions, as in the case of simple phenols (oxyresveratrol), coumarins (umbelliferone and scopoletin), steroidal saponins and isoprenes (García, 2003). Other studies in Puerto Rico (Ramos and Valencia, 2011) also refer to the tolerance of *Morus alba* to pest attack.

TABLE 7. Relation of pest-insects and diseases associated with *Morus alba* in Cuba

Scientific name	Common name	Part affected	Reference
		Insect pest	
Maconelicoccus hirsutus	Hibiscus mealybug	Leaves, stems	Veitía, 2012.
Tetranychus sp.	Spider mite	Leaves	
Glyphodes sybillalis Walker.	Lepidoptera or Pyralidae	Leaves	Bruner *et al.*, 1975; Veitía, 2012.
		Diseases	
Oidium sp.	Oidium	Leaves	Veitía, 2012.
Cercosporella mori	Cercosporella spots	Leaves	Lezcano and Alonso, 2002.
Colletotrichum spp.		Leaves	Vargas *et al.*, 2002.
Cylindrosporium spp.		Leaves	

In Cuba, after the introduction of this plant in the country, several entomological and phytopathological studies were conducted in order to know the pests and pathogens that affect this plant (Table 7).

A major phytosanitary problem emerged in 2012, with the introduction in the country of the fearsome polyphagous pest *Maconellicoccus hirsutus* Green (Hemiptera: Pseudococcidae), known as hibiscus mealybug, whose life cycle is shown in Figure 15. This insect feeds on the sap of the host plant and injects toxic saliva while it feeds. This process causes malformation of leaves and fruits and produces stunted leaves and sprouts. This feeding habit of the hibiscus mealybug can cause the death of its host.

The presence of cottony, waxy and white structures, in the terminal parts of the stems and branches, are indicative of severe infestation. When the pest infests the fruits, it can cover them completely with the white waxy secretion, causing their death or withering The spider mite, also known as red spider (*Tetranychus* sp.) (Figure 16), is reported by Veitía (2012) as another potential pest in *M. alba*.

The attack of two different species of larvae, commonly known in Cuba as hairy caterpillar, *Estigmene acrea*, Drury (Lepidoptera: Arctiidae) (Figure 17) and *Maenas* sp. Poey (Lepidoptera: Arctiidae) (Figure 18), were detected in *Morus alba* by Martínez and Ramírez (2014; pers. comm.).

These same researchers also reported the presence of *Spodoptera latisfasia* Walker (Lepidoptera: Noctuidae), whose black larvae, have a tenuous central light brown line along the whole body. On both sides of the body it shows two orange-brown lines with white spots and between both lines a greyish-brownish strip, with a cephalic capsule orange-brown in colour (Figure 19A), of velvety texture.

Another abundant pest found in *M. alba* is the insect *Spodoptera latisfacia*, which consume a great quantity of the leaves of the plant (Figure 19B). Also the larvae of *Glyphodes sybillalis* Walker. (Lepidoptera: Pyralidae) (Figure 20A) cause great damage to foliage (Figure 20B).

Among the micro-organisms that attack this plant, *Oidium* sp. (Figure 21), whose whitish spots are visible through the leaf underside, and *Cercosporella mori* (Figure 22) have been found, although the latter only reached the slight phase (5% of the area affected) in 4 mulberry genotypes (cvs Acorazonada, Cubana, Indonesia and Tigreada). In the study, cv. Tigreada was the least affected by the fungus, before and after three

FIGURE 15
Life cycle of the hibiscus mealybug (*Maconellicoccus hirsutus*)

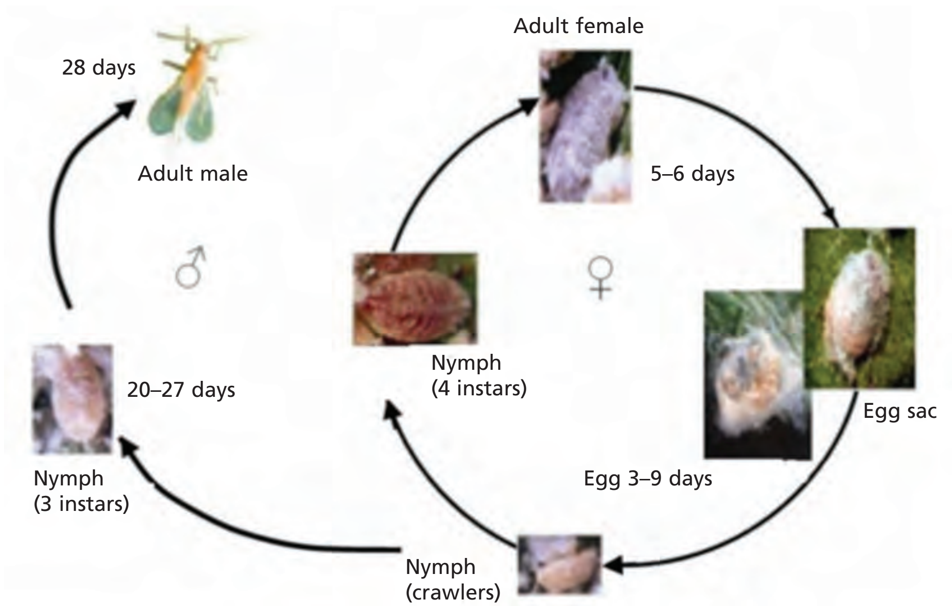
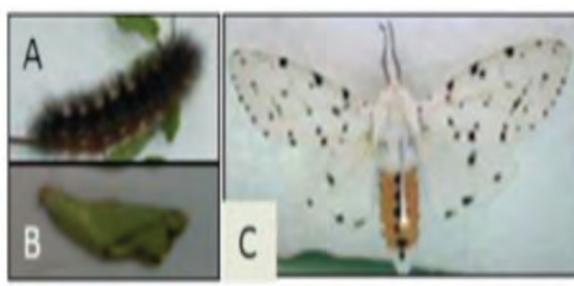


FIGURE 16
Spider mite (*Tetranychus* sp.)

FIGURE 17
***Estigmene acrea* (A) Larvae, (B) Pupa, (C) Adult**



prunings made at 60, 90 and 120 days (Lezcano and Alonso, 2002).

Vargas *et al.* (2002) detected, at the end of the establishment stage of *Morus alba* cv. Criolla, attacks of phytopathogenic fungi of the genera *Colletotrichum* and *Cylindrosporium*, which clearly affected leaf production.

9.1 Control measures

In Cuba, great emphasis is placed on the regulation of the hibiscus mealybug (*Maconellicoccus hirsutus*), as

FIGURE 18
Larva of Maenas sp.

FIGURE 19
(A) Larvae of *Spodoptera latisfacia* in mulberry leaves and (B) symptomatology of the damage (skeletonization)

it is a pest recently introduced to the country and is considered highly polyphagous and for which more than 200 hosts have been recorded (N. Valenciaga, 2014, pers. comm.). The main phytosanitary measures are:

- Maintain the systematic surveillance in the areas planted with mulberry and in nearby zones, in order to determine the real dispersal around the farm.
- Apply the bioregulator *Cryptolaemus montruozieri* (adults or larvae) at a rate of 800 to 1000 individuals/ha.
- Avoid unnecessary traffic by staff working in the affected areas and also going from more affected to less affected areas, taking extreme security measures to limit dispersal from the farm.
- Respect the movement of other plant materials authorized by the inspectors of the

FIGURE 20
***Glyphodes sybillalis:* (A) Adult; (B) Larvae feeding on mulberry leaves**

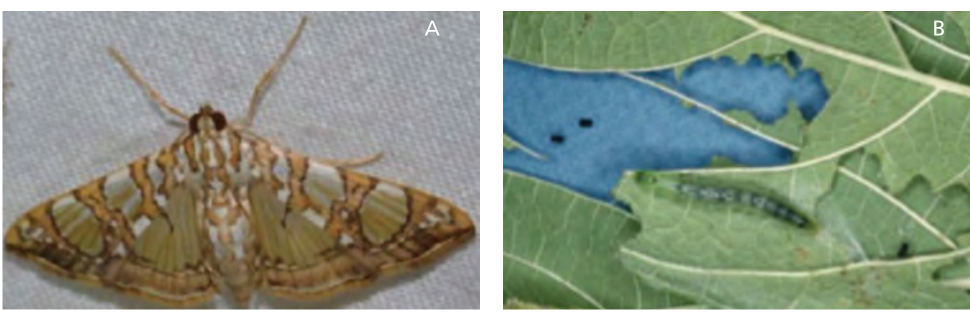


FIGURE 21
Symptoms of the attack of Oidium

FIGURE 22
Symptoms of the attack of Cercosporella

Plant Protection Territorial Station (ETPP) responsible for the region, through the Free Transit Phytosanitary Certificate.

- Perform sanitation pruning in the fields with disease indexes of 4 and 5 in order to reduce infestation levels. The results of pruning could be used for feeding cattle and other mammals of the farm. If the green mass volume is too high, it should be burned or buried at a depth of >1 m.

As other identified organisms do not constitute very frequent pests in this crop, no control alternatives are indicated for them.

Nevertheless, the periodic monitoring of the presence of noxious organisms and the beneficial fauna present in the fields is recommended, which would allow the establishment of timely and efficient management for the control of eventual pests and diseases of the crop, using agroecological management practices that contribute to maximize yields and obtain greater benefits from the crop.

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Chemical composition and nutritive value of mulberry (*Morus alba*) in animal feeding

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ABSTRACT
All mulberry species, especially *M. alba*, are useful plants that combine good chemical composition and nutritional quality, with distinctive agronomic features. As forage, *M. alba* has exceptional bromatological characteristics, with CP content >20% DM and IVDMD >80%. *M. alba* has amino acid composition similar to that of soybean meal; it is considered a significant amino acid source, of which half are essential amino acids. Total ash content can exceed 15% depending on soil fertility, although normally between 10 and 15%. Leaves contain a great quantity and diversity of macro- and micro-elements, and considerable calcium accumulation is found in cell idioblasts. *In vivo* experiments using goats showed that leaves had a digestibility >78%, and i*n vitro* analysis confirmed a disappearance percentage of between 80 and 90%. Mulberry contains a wide range of secondary metabolites that act as defence mechanisms against pests and diseases and as reserves of specific organic compounds. *M. alba*'s adaptability to tropical conditions means that this plant can ameliorate current constraints in ruminant and monogastric diets, since its foliage is equivalent in many ways to commercial concentrate feeds.

Keywords: nutritive value, chemical composition, digestibility, secondary metabolites, *Morus alba.*

INTRODUCTION
In tropical Latin America, low cattle productivity is directly related to poor availability of grasslands and low nutritive value of pastures, where seasonal performance of grass species leads to limited biomass supply in the rainy season with consequent poor response (Sánchez, 2002; García *et al.*, 2006). As pastures alone do not cover ruminant nutritional requirements for adequate milk and beef production, some trees and shrubs are a good alternative source for utilization as supplementary feed, characterized by high protein contents and good digestibility compared with the majority of pastures (Simón, 1998). There are many legume species with good forage characteristics, but other species also have recognized potential. In this respect, *Morus* species are notable as forage sources, due to their excellent biomass production, chemical composition, high ruminal degradability, adaptability to different climate and soil conditions, as well as availability. This chapter presents data on nutritive value of *Morus alba* as a potential source for animal feed.

1. CHEMICAL COMPOSITION AND NUTRITIVE VALUE

All mulberry species, especially *M. alba*, are agriculturally extremely interesting plants; its chemical composition and quality, from a nutritional point of view, combine with the distinct character of the species (García *et al.,* 2006). As forage, it has exceptional bromatological characteristics, with crude protein (CP) contents >20% dry matter (DM) and *in vitro* DM digestibility (IVDMD) >80%. It shows an amino acid content similar to that of soybean meal; it is a major amino acid source, of which half are essential amino acids (Sánchez, 2002). Total ash content can be 15% or more, depending on soil fertilization status, although the normal range is between 10 and 15% (Shayo, 1997).

Leaves are rich in quantity and diversity of macro- and micro-elements (Noda, 1998), with notable calcium accumulation observed in cell idioblasts (Sugimura *et al.,* 1999). The plant has good vitamin levels, mainly groups B and C, particularly nicotinic and pantothenic acids, riboflavin (Ho-Zoo and Won-Chu, 2001) and ascorbic acid (0.3% DM) (Singh and Makkar, 2002).

Its nutritive value has been comprehensively studied. Jegou, Waelput and Bronschwig (1994), in an *in vivo* experiment using goats, demonstrated that leaves had a digestibility >78%, and *in vitro* analysis confirmed a disappearance percentage of between 80 and 90% (Rodríguez, Arias and Quiñónez, 1994).

Similarly, in studies by González, Delgado and Cáceres (1998), ruminal degradability of leaves and tender stems, using nylon bags, was >80% at 48 h, demonstrating greater digestibility of these portions compared with other traditional forages, such as *Leucaena leucocephala* (Tolera, Seyoum and Sundstol, 1998).

Additionally, Schmidek *et al.* (2002) reported degradation values of 93.3% DM, 97.0% CP and 84.9% neutral detergent fibre (NDF). By means of the gas production technique, Bing *et al.* (2001) concluded that the maturity stage of the leaf, as well as the period of the year, influenced the amount of gas produced, while Makkar and Becker (1998) demonstrated that young leaves had twice the potential for gas production (60.2 mL/200 mg) compared with mature leaves.

Studies carried out by Boschini (2006) in Costa Rica indicate that mulberry DM yield was more than 30 t/ha/year, with exceptional bromatological quality, with foliar CP >20%, IVDMD 79%, and 89% for CP. This author completed a very interesting study at the Experimental Station "Alfredo Volio Mata" of the University of Costa Rica, Cartago province, on the nutritive value of mulberry at different regrowth stages. There were differences between the nutritional elements, with the exception of the hemicellulose content in stems, NDF and its compounds, as well as the leaf cell wall CP through the four growth stages.

In the whole plant, the concentration of lignin and protein combined with NDF showed similarity regardless of age. The stem DM contents were slightly lower than in the leaf at 70 days of growth, similar at 85 days, and higher in stems from 98 days, with values in the whole plant of 17 to 22.3%, increasing between 70 and 112 days of regrowth. Values for CP, ether extract, ash and fibre in the stem were similar at 70 and 84 days, and from that age the protein and the ether extract decreased, while the concentration of the cell wall increased. A similar effect was observed in leaves and consequently in the whole plant.

These results demonstrated that, from the four sources of energy utilizable by ruminants, stems showed the maximum non-fibrous carbohydrate concentration at 84 days of regrowth, while CP and fatty acids were higher at 70 and 84 days over the following growth stages. Initially the stem presented the lowest concentration of NDF, increasing significantly from 98 days on. In the leaves, the concentrations of non-fibrous carbohydrates were statistically similar in the four ages, while CP and fatty acids attained maximum levels at 70 days, decreasing as regrowth age advanced. The highest concentration of total digestible nutrients was observed at 84 days in the stems, while it was similar in the leaves throughout the different ages studied. The values for digestible energy and for maintenance and production, as well as net energy for maintenance, growth and production, were similar to total digestible nutrients in stems; however, in the leaves, they showed an important difference at 70 days, with a slight decrease at 84 and 98 days, and decreasing slightly again at 112 days. The whole plant had a similar pattern to that seen in leaves.

Ruminal CP degradability showed that the soluble fraction in stems is at least double that in leaves and, in both cases, peaked at 70 days and slightly decreased as regrowth age advanced. The potentially degradable in-rumen fraction showed a maximum value at 70 days for both parts of the plant, being at least triple in leaves compared with stems. The rate of degradation in stems was similar in all ages, whereas in leaves it was higher at 70 days, lower at 84 days, and decreasing slightly at 98 and 112 days. The proportion of degradable protein in rumen was >85% in stems and 60% in leaves.

González, Delgado and Cáceres (1998) reported high ruminal degradability of leaves and tender stems of this species, being >89% at 48 h; other authors have reported its high nutritive value determined *in vitro* (Rodríguez, Arias and Quiñónez, 1994; Benavides, 2002) and *in vivo* (Jegou, Waelput and Bronschwig, 1994).

1.1 Performance of the nitrogenous fraction

According to García *et al*. (2006), *M. alba* also stands out from other multi-purpose trees by the particular characteristics of its nitrogenous fraction, since although it is comparable to that shown by the majority of tropical forage legumes, it has superior protein quality (Benavides, 1999; González, Delgado and Cáceres, 1998).

From a qualitative point of view, the literature is divided regarding the main protein in the leaves. Sánchez (2002) indicates ribulose-1.5-biphosphate carboxylase (RuBisCO) as the main protein in the species, whose active centre is responsible for CO_2 fixation (Kellogg and Juliano, 1997). Yamashita and Ohsawa (1990) reported that 43% of the t_N in *M. alba* was RuBisCO.

Conversely, Singh and Makkar (2002) indicated prolamine, isolated from the alkaline alcoholic extract of the leaves, to be an important protein, containing 12.6% of t_N distributed, mainly, as insoluble N in HCl, amides and mono- and di-aminated acids. Also the primary and secondary structures of two glycoproteins denominated Moran A and Moran 20K, with molecular weights of 7.50 and 21.86 kilodalton (kDa), respectively, with anti-diabetic activity, have been purified and characterized (Kim *et al.*, 1999). The solubility of the nitrogenous fraction, although it is not high (17.3% t_N in borate-phosphate buffer and 15.7% t_N in phosphate buffer), is comparable to that of *Leucaena leucocephala* and species of the genera *Dendrocalamus*, *Artocarpus* and *Ficus*, indicating the non-protein nature of the soluble N. In contrast, legume species, such as *Acacia catechu*, *Albizia stipulate* and *Bauhinia variegate,* show greater t_N solubility (Singh and Makkar, 2002). Other authors, using the same analytical technique, report a t_N solubility <36% (Sarma, Singh and Bhat, 2000).

1.2 Crude protein

There are numerous studies in which the CP content has been established in the edible parts of the mulberry species, with a focus on animal feed and sericulture; in intensive cultures in India, contents of up to 39% have been obtained (Singh and Makkar, 2002).

The plant part is the most obvious differentiating factor in CP concentrations (Espinoza and Benavides, 1996). Other factors – such as: genotype (Yonkang, 2002); chemical or organic fertilization (Benavides, 1994); as well as the basal soil fertilization and the type of fertilizer (Ramos *et al.*, 2002) – also influence the levels of this indicator. As for most woody plants, factors such as season (González and Cáceres, 2002), environmental conditions, cutting height (Martín *et al.,* 2002) and plantation density (Boschini, Dormond and Castro, 1998, 1999) also affect to some extent the nitrogenous content.

With all this empirical material obtained from investigations, stable performance patterns are known, based on the physiology of mulberry plants submitted to cutting frequencies (García, 2003). In this sense, regrowth age is a determining factor in CP concentration. The highest contents are observed in the most intensive defoliation frequencies, which produce leaves of lower regrowth age. From 90 days after cutting, in the majority of arboreal plants, including mulberry, similar contents of this indicator are attained (Boschini, 2002a).

The influence of chemical fertilization on the CP concentrations in this species is a matter of argument. The application of 480 kg N/ha/year as NH_4 and NO_3 produces significant increases in leaf concentrations (Benavides, 1994); while with the same nitrogen application (480 kg N/ha/year) from urea, poor effect was noticed in the CP contents in leaves and in total biomass (Rodríguez, Arias and Quiñónez, 1994). At the same time the application of organic fertilizer (0 to 480 kg N/ha/year) as manure showed no significant increases in the edible parts of the plant (Benavides, 1994).

In Cuba, Martín (2011), at the Estación Experimental de Pastos y Forrajes "Indio Hatuey", studied the nutritive value of four mulberry genoypes according to fertilization, cutting frequency and season, analysing separately the different parts of the plant. The leaf CP content values are set out in Table 1. All factors studied had a significant effect ($P<0.05$) on the CP content of mulberry leaves (CP_L) in the first year. The fertilization levels and the cutting frequencies were significantly different. The highest CP_L values were attained with fertilization of 500 kg N/ha/year (19.2%) and a cutting frequency of 60 days (19.9%). Benavides *et al.* (1994) reported similar results, finding significant differences between different organic fertilization levels (goat manure), favouring the highest dosage, and between the cutting frequencies, where best results were for cutting at earlier ages. There were differences between seasons of the year of approximately 3% between seasons. From the lines studied, cv. Indonesia had the highest values but did not differ significantly from cv. Acarazonada and cv. Tigreada, while the local Cuban line had the lowest percentages.

Espinoza and Benavides (1996) did not find significant CP leaf differences among lines in three contrasting environments in Costa Rica, including two of those assessed in this experiment (cvs Indonesia and Tigreada). There were significant differences between locations; the highest value was for cv. Coronado (24.8%) and the lowest was cv. Paquera (15.1%), this latter under climatic conditions similar to those of Cuba.

In the fourth year, only the cutting frequency factor had significant effect ($P<0.05$) on the CP content of leaves. Cutting every 60 days gave the highest CP percentages and dif-

TABLE 1. Effect of the variety, fertilization (kg N/ha/year), cutting frequency (days) and season of the year on the CP content (%) of leaves and tender stems of mulberry in the first and fourth year of the experimental period

Parameter	CP of leaves		CP of tender stems	
	First year	Fourth year	First year	Fourth year
		Cultivar		
cv. Indonesia	18.86^{a}	19.89	9.15	9.66
cv. Cubana	17.57^{b}	19.22	9.26	9.44
cv. Acarazonada	18.82^{a}	19.12	9.42	9.39
cv. Tigreada	18.32^{a}	19.84	9.18	9.58
SE ±	0.26 **	0.27^{ns}	0.18^{ns}	0.17^{ns}
		Fertilization		
100 kg N/ha/yr	17.54^{c}	19.65	9.12	9.54
300 kg N/ha/yr	18.26^{b}	19.4	9.12	9.5
500 kg N/ha/yr	19.23^{a}	19.5	9.52	9.51
SE ±	0.22***	0.23^{ns}	0.15^{ns}	0.15^{ns}
		Cutting frequency		
60-day interval	19.87^{a}	22.12^{a}	9.19^{ab}	10.01^{a}
90-day interval	16.90^{c}	17.92^{b}	9.62^{a}	9.56^{b}
120-day interval	18.27^{b}	18.31^{b}	8.95^{b}	9.94^{c}
SE ±	0.22***	0.23***	0.15**	0.15***
		Cutting frequency		
Rainy season	16.95^{b}	19.77	8.94^{b}	9.56
Poor rainy season	19.81^{a}	20.38	9.87^{a}	10
SE ±	0.18***	0.24^{ns}	0.17***	0.12^{ns}

NOTES: Means with different superscripts in each variable differ at $P<0.05$ (Duncan, 1955) * = $P<0.05$ ** = $P<0.01$ *** = $P<0.001$. ns = not significant.

fered significantly from those at 90 and 120 days. In this 4th year, the 90-day frequency had the lowest CP values but these did not differ significantly from those at 120 days.

It is noteworthy that the fourth year CP_L contents for all parameters exceeded those reached in the first year by approximately 1%; this variation coincides with the decreases found in the CP contents between these years. This implies a relationship between both indicators, reflecting similar performance from pastures. CP percentages found in leaves by Boschini (2002a, b) fertilizing with 150 kg N/ha/year (as ammonium nitrate), but under different climatic conditions, were higher than those found in this research, and with a marked decreasing tendency as plants aged.

Regarding CP percentage, in the literature there is a wide range of values, ranging from 18.6% (Shayo, 2002) to 25.6% (Almeida and Fonseca, 2002a), as response to the different conditions under which plantation have been developed. For this reason it is not sensible to presuppose values, but rather to assess the performance of the plant in the different ecosystems.

Boschini, Dormond and Castro (1998, 1999) and García (2003) have indicated that 90 days is an inflection point in the physiological processes of this species; in plants, the carbon chains are transformed for the synthesis of woody material, and the process needs a lot of endogenous compounds. For this reason, the nitrogen content in leaves could decrease. With increasing plant age, the leaf-stem relationship changes, as formation of structural compound is favoured.

The CP content of tender stems (CP_{TS}) differs for different cutting frequencies and between seasons the first year. Regarding cutting frequency, at 90 days the highest absolute value was attained, which differed significantly from that at 120 days, but not from 60 days. The poor rainy period had the highest values and differed from the rainy season. Among the lines and the fertilization levels there were no significant differences.

In the fourth year there were only significant differences between the cutting frequencies, and the highest value was at 60 days, which differed from 90 and 120 days. The percentages of CP_{TS} in both years for all factors were very similar for this indicator.

Espinoza and Benavides (1996) obtained differences in the CP_{TS} among the varieties studied and the locations, with results favouring cv. Indonesia (12.4%) and the locality of Coronado (13.9%). In this study it was confirmed that mulberry is a woody forage with low crude fibre (CF) and high CP, mainly in leaves, which constitute the most important part of the edible biomass of this plant.

Martín *et al.* (2007) determined the amino acid composition of the Cubana, Indonesia, Tigreada and Acarazonada genotypes, using the 90-day cutting frequency material; plants were fertilized with poultry manure at a rate of 300 kg N/ha/year. Results demonstrated that the amino acid pattern matched that reported for edible biomass of other Asian varieties; the significant levels of proline, glutamine, glycine and valine stood out. These are amino acids related to probable resistance mechanisms to water stress. The amino acids were found in similar proportions among the lines. The highest protein quality was found in cv. Indonesia and the highest non-protein nitrogen concentration (NPN) was in cv. Acarazonada (D. García, 2009, pers. comm.).

1.3 Fibre content

Crude fibre (CF) variations in leaves in the first and fourth years of the experimental period are set out in Table 2.

There were differences in the leaf CF percentage (CF_L) of the first year between the factors variety (V), cutting frequency (C) and season (S) not being the same between the fertilization levels. The cvs Cubana, Acarazonada and Tigreada differed from cv. Indonesia, which showed the lowest CF_L percentages. The cutting frequencies of 60 and 120 days differed from the poorer 90-day values. In the rainy season this variable was greater than in the poor rainy season. In the fourth experimental period, performance was similar, with differences between lines, cutting frequencies and seasons, with no detectable fertilizer effect. However, in all treatments the percentages were lower than those of the first year.

On comparing results obtained with those of other tree-like plants, there was lower CF than values reported in the literature. Smith (1992) indicated CF ranging between 22 and 36% in seven trees considered adequate for animal consumption.

González and Cáceres (2002) on studying 10 tree- and shrub-like plants found that mulberry was one of lowest for CF. This confirms one of the main characteristics of this species. At the same time, the CF_L values obtained in this experiment were similar to those indicated by other authors under different climatic and edaphic conditions from this study (Uribe, 2002; Singh and Makkar, 2002), which indicates that this could be mainly ruled by genetic factors.

In the CF percentage of tender stems (CF_{TS}) in the first year, there were differences in the fertilization factors (F), cutting frequency (C) and season (S) ($P<0.05$), not being the

TABLE 2. Effect of variety, fertilization (kg N/ha/year), cutting frequency (days) and season of the year on the crude fibre content (%) of leaves (CF_L) and tender stems (CF_{TS}) of mulberry in the first and fourth years of the experimental period

Parameter	CF_L		CF_{TS}	
	First year	Fourth year	First year	Fourth year
Cultivar				
cv. Indonesia	12,21^b	10,87^b	37,03	34,09
cv. Cubana	13.44^a	10.94^b	37.8	33.94
cv. Acarazonada	13.07^a	11.42^a	37.34	33.83
cv. Tigreada	13.42^a	10.53^b	37.87	33.91
SE ±	0.17 ***	0.16**	0.39^{ns}	0.15^{ns}
Fertilization				
100 kg N/ha/yr	12.99	10.87	37.31^b	33.94
300 kg N/ha/yr	13.07	10.96	36.79^b	33.89
500 kg N/ha/yr	13.05	10.99	38.43^a	33.99
SE ±	0.15^{ns}	0.14^{ns}	0.34**	0.13^{ns}
Cutting frequency				
60 days	13.15^a	9.29^c	35.77^b	32.53^c
90 days	12.69^b	11.02^b	38.18^a	34.11^b
120 days	13.26^a	12.51^a	38.57^a	35.19^a
SE ±	0.15*	0.14***	0.34***	0.13***
Season				
Rainy	14.48^a	9.66^b	41.4^a	32.70^b
Poor rainy	11.35^b	10.65^a	32.55^b	33.94^a
SE ±	0.13***	0.09***	0.35***	0.13***

NOTES: Means with different superscripts in each variable differ at $P<0.05$ (Duncan, 1955). * = $P<0.05$. ** = $P<0.01$. *** = $P<0.001$.

same among genotypes (Table 2). From the fertilization levels studied, the highest values for this parameter was found in the 500 kg N/ha/year treatment (CF_{TS} = 38.4%) which differed significantly from the 100 and 300 kg N/ha/year treatments.

The 120-day cutting frequency gave the highest values for CF_{TS}, although not very different from the 90-day cutting regime treatment, while for the season factor, the rainy period had the highest values, differing from the poor rainy. In the fourth year there were differences between the cutting frequency and the season of the year, but not between the variety and the fertilization levels.

Similarly in leaves, the CF_{TS} percentages in the fourth year showed a substantial decrease, of approximately 4%. For fertilization and cutting frequency factors there was equally a decrease of some 4% between first and fourth years. In the rainy season the decrease of this variable between the first and fourth years was almost 8%, but in the poor rainy season they were very similar.

1.4 Mineral content

Data on mineral composition of a plant destined for animal feed is essential, in view of the nutritional implications of covering the maintenance and production requirements through a ration mainly based on forage. In this sense, if it is considered that mulberry is preferably used as a supplement, then this assumes greater importance.

McDowell *et al.* (1988) indicated that in the tropics an adequate mineral balance in the diet must be guaranteed. These authors demonstrated that when there are deficient factors, then growth, gestation and milk production, as well as animal fattening, are seriously compromised, without considering increased opportunist diseases.

Few studies report variations in the mineral content of mulberry. However, results from Martín (2011) indicated that calcium content in leaves (Ca_L) differed among cultivars, cutting frequencies and seasons in both the first and fourth years. There were significant interactions among the factors in both years.

Cvs Indonesia and Tigreada did not differ in the first year (2.71 and 2.68%) or in the fourth year (2.34 and 2.20%), but did differ from cv. Acarazonada and cv. Cubana in the first year (2.52 and 2.53%) and from cv. Acarazonada in the fourth year (1.86%).

In the first year, the 90- and 120-day frequencies did not differ, but both differed from that of 60 days, where there was a Ca_L percentage increase with increased cutting frequencies. In the fourth year the 90-day frequency attained the highest value (2.44%) and was significantly different from both the 60-day (2.00%) and the 120-day (2.14%) treatments.

In both years, the poor rainy period showed the highest values (2.73 and 2.43%), and the rainy period differed significantly (2.32 and 2.02%).

Fertilization increase favoured a slight rise of Ca_L, but not significantly. In tender stems, calcium content (Ca_{TS}) was significantly different ($P<0.05$) between cultivars and seasons in the first and fourth years. In the poor rainy period, Ca_{TS} was significantly higher than in the rainy (1.85 vs 1.67 and 1.21 vs 1.38% for each year, respectively).

Phosphorus percentage in leaves (P_L) showed significant differences between the different cutting frequencies and seasons in the first year, but only between seasons in the fourth year. The frequencies of 60 (0.23%) and 90 days (0.22%) were similar, but both differed from the frequency of 120 days (0.16%) in the first year.

Phosphorus in leaves differed between seasons in the two years, but from the biological point of view it is not of great importance since differences were very small. Regarding tender stems, phosphorus (P_{TS}) was similar for all factors in the two years, with only small differences between seasons in favour of the rainy period (0.25 vs 0.14%) in the first year.

In contrast, potassium content in leaves (K_L) exhibited differences between season for the first year, between varieties for the fourth year, and in both years in the fertilization and the cutting frequencies cv. Cubana had the highest numerical value in both years (2.53 and 2.25%). Increased fertilization favoured the potassium content in leaves; these increments were significant only in the first year. Although the fertilization levels of 300 and 500 kg N/ha/year (2.53 and 2.59%, respectively) gave similar values, they differed from 100 kg N/ha/year (2.29%).

For cutting frequency effects, results were significant in the two years, but there was not the same tendency in them. In the first year, the 90-day cutting regime showed best results (2.67%), but in the fourth year it was the 60-day cutting regime that had the highest numerical value (2.24%). In tender stems, the potassium content (K_{TS}) differed between the fertilization levels and the cutting frequencies in the first year, and between seasons of the fourth year.

The highest fertilization levels (300 and 500 kg N/ha/year) did not differ (2.21 vs 2.23%), but differed clearly from the 100 kg N/ha/year (1.82%) treatment. The increased fertilization levels favoured increased of K_{TS}, as for the leaves.

The highest potassium content (2.33%) in the first year was attained with the 60-day cutting frequency, which differed from the potassium percentage attained with the 90- and 120-day regimes. Between the seasons in the fourth year, the rainy showed the highest result (1.89 vs 1.49%) for potassium.

The ash content of mulberry leaves (Ash_L) was different in the first year for all levels of the factors under study. In the fourth year there were differences between the varieties and the seasons of the year. Among the lines, cv. Tigreada was superior in numerical values in the two years (11.91 and 12.31%) but did not differ from cv. Indonesia (11.76%) in the first year or from cv. Acorazonada in the fourth year (11.66%). Cv. Cubana had the lowest values (11.38 and 10.41%) for this parameter in both years.

Fertilization increase enabled an improvement in Ash_L, with the 300 (11.71%) and 500 kg N/ha/year (11.92%) treatments similar, but both superior to 100 kg N/ha/year (11.29%) in the first year. In the fourth year the same pattern was maintained, but without significant differences between the various treatments. Cutting frequencies exerted very similar effects on leaf ash in both years. The rainy season gave the highest percentage of leaf ash (12.43 and 11.91%) in the two years, respectively.

In tender stems, all treatments influenced the ash content (Ash_{ST}) in the first year. In the fourth year, the only effects were on cvs Indonesia and Acorazonada, which exhibited the best results for this parameter in both years, and differing from the other lines, with the exception of cv. Tigreada in the first year.

Fertilization had a similar effect to that produced in the leaves in both years, but in the cutting frequency this effect was inverse, as increasing the cutting interval reduced the percentage of Ash_{TS}. The effect of the seasons was similar to that produced in leaves: the rainy period showed the highest content of this variable (7.91 and 7.49% for both years).

As can be seen, mulberry has an adequate mineral content in leaves and tender stems, especially its high calcium, potassium and ash contents. These results agree with those reported by other authors (Benavides *et al.*, 1994; Espinoza and Benavides, 1996; Singh and Makkar, 2002; Datta *et al.,* 2002; Uribe, 2002; Benavides, 2002).

Among the parameters studied, the variety, cutting frequency and season of the year were those with the greatest effects; this explains the influence of genetic factors, and of agronomic and climatic management on the performance of these indicators. Schmidek *et al.* (2002) found differences in the mineral content of diverse mulberry clones.

Almeida and Fonseca (2002b) found differences in bromatological composition with different cutting frequencies; these authors also indicated substantial seasonal differences in the mineral content in various mulberry clones.

Calcium and phosphorus contents found by Devendra (1992) and Smith (1992) in trees used as animal feed are lower than those of mulberry, which means that this plant has nutritional advantages regarding other uses with similar purposes. In the same sense, in view of the importance minerals have for milk production (Gutiérrez, 1991) mulberry forage has good potential for the feeding of milch cows.

The high mineral content in leaves and in tender stems and the high protein percentage and low crude fibre content in its leaves, make this plant into a forage source with desirable characteristics and with potential for substituting totally or partially for concentrates from cereals for the feeding of different animal species.

2. SECONDARY METABOLITES AND POTENTIAL ANTI-NUTRITIONAL FACTORS

García *et al.* (2006) stated that as for any higher plant, mulberry contains a wide range of secondary metabolites in its edible biomass: some of these compounds have arisen by co-evolution with herbivorous organisms; other are synthesized in certain physiological stages of the plant; in the regulation of the metabolic processes; as defence mechanisms against pests and diseases; and as reserves of specific organic chains (García, Ojeda and Montejo, 2003).

According to the review by Duke (2005), *M. alba* leaves contain volatile compounds such as alcohols (n-butanol and b-g-hexenol), aldehydes (methylethyl-acetaldehyde, *n*-butyl-aldehyde, isobutyl-aldehyde, valeraldehyde, dexaldehyde, μ-bhexenal), aliphatic cetones (acetone, methyl-ethyl-cetone, metyl-hexyl-cetone), butylamine, and volatile fatty acids (acetic, propionic and butyric). Also, it contains calcium malate, succinic and tartaric acids, xanthophyles, carotenoids, phythates (forming 18% of the total phosphorus), isoflavonoids (quercetin 3-glucoside), nitrogenous bases (adenine, choline and trionelline), isoprenoids (citral, linalile acetate, linalool, dihydromorine acetate, dihydrokaenferol, 2, 4′,6′-tetra-hydroxi-benzofenone, maclurine and 2% DM of stylbeno-hydroxy-resveratrol.

Through the use of phytochemical screening developed by García, Ojeda and Montejo (2003), it was indicated that from a total of 15 groups of metabolites, there was found simple phenolic compounds, flavonoids, coumarins, secondary carbohydrates, steroids, alkaloids and saponins; these appeared in all varieties and treatments investigated, though the presence of the same groups of compounds is one evidence of the marked genetic component of the secondary metabolism in the *Morus* genus (Ashok, Vincent and Nessler, 2000.).

At the same time, tannins that precipitate proteins; proantocianidines/catequines (condensed tannins); cardenolides; phytoquinones; and cyanogens – chemical groups with higher toxicity indices – are absent.

These results agree regarding the innocuousness of these compounds in ruminant feeding, with nutrition studies in which mulberry was the predominant diet. However, more

TABLE 3. Range of variability of the main secondary metabolites in *M. alba*

Plant part	Group of secondary metabolites (as %DM)			
	Total polyphenols	Flavonoids	Coumarin	Sterols
		Rainy period		
Leaves	2.25 – 2.89	1.56 – 1.72	0.45 – 0.78	1.20 – 2.10
Tender stems	1.45 – 1.95	1.60 – 1.65	0.55 – 0.64	0.94 – 1.13
		Poor rainy period		
Leaves	2.10 – 2.76	1.58 – 1.64	0.65 – 0.68	1.11 – 1.90
Tender stems	1.05 – 1.65	1.51 – 1.55	0.58 – 0.74	0.54 – 0.97

Source: García, 2003.

studies are required for elucidating with certainty the true deleterious properties of the lectins, phyto-oestrogens and saponins as major metabolites with anti-nutritional potential in monogastric animals.

In summary, the levels of the secondary metabolites with greater toxicological potential and found in the edible mass of *M. alba* are shown in Table 3.

3. FINAL REMARKS

From the, 1990s, Central America and Caribbean countries have studied the introduction and multiplication of mulberry species native to the Asian zone. Since then, numerous investigations for characterization and utilization of these plants in animal feed, complemented with studies on the agronomy, physiology and chemical composition of the species, have been carried out. These have confirmed its true forage potential, reflecting its biomass contribution and protein quality.

Its high adaptability to tropical conditions has allowed this plant to mitigate current restrictions in ruminant and monogastric diets. *Morus* foliage is comparable in many parameters with commercial concentrate values. Also, its high palatability facilitates its consumption during the dry period, either as fresh forage or conserved.

Experience gained in sericulture regarding the management of the species has been extrapolated to extend its culture in animal production systems. In this respect, the most treated agronomy aspects have been plantation density, dosages and types of fertilizer, and harvesting regimes.

Likewise, the characterization and evaluation of the main factors influencing the chemical composition have been mainly based on classical bromatological indicators that do not clarify the true nature of the fractions.

Additionally, the benefits and dangers associated with the presence of secondary metabolites, the anthelmintic activity of its extracts, as well as the advantages that derive from plant phytohormones, are aspects that could be successfully exploited in the near future.

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Utilization of mulberry in non-ruminant feeding

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ABSTRACT

Morus alba is a species becoming widespread in Latin America, Cuba and the Caribbean region, and receiving attention regarding its agronomy, chemical composition and role in the productive performance of ruminants. This chapter reviews studies using the plant in the feeding of non-ruminant animals. The type of forage was analysed: whole plant forage; foliage alone (leaves, petioles and terminal growth points); or leaves only. The effects on physiological response (apparent nutrient retention; morphology of the gastro-intestinal tract in poultry; and apparent nutrient digestibility in pigs, rabbits and guinea pigs), as well as nitrogen balance (using several cultivated genotypes in pigs) and its effect on lipid metabolism, with a marked reduction of abdominal fat in poultry, were also studied. Inclusion levels for mulberry in non-ruminant diets according to species and animal categories are suggested to assure efficient biological responses. The imbalance between information from basic research and the practical application on-farm is an obvious target for systematic investigation in future years

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Keywords: Physiological response, performance trials, non-ruminant species, *Morus alba*

INTRODUCTION

In the Latin America and Caribbean area a great number of investigations have been carried out with different sources of alternative feeds for monogastric species (poultry, pigs and rabbits). Partly this is due to the great variety of the existing forage resources and also by the need to reduce the feeding costs that constitute between 60 and 70% of the expenses invested in such process (Casamachín *et al.,* 2007). In spite of this, its inclusion level in the rations varies due to two main characteristics: the presence of fiber and of anti-nutritional factors (Savón, 2005). Although monogastric species, fowls present different anatomical and physiological characteristics, but after an adaptation process, it has been demonstrated that they can start to digest, in part, the fibre components (Martínez, 2010; Al-Kirshi *et al.,* 2013), and they show more resistance to anti-nutritional factors than do pigs and rabbits.

Within these sources, *Morus alba* stands out for being a promising plant that can be used in the feeding of monogastric animals, owing to its interesting nutritive value previously discussed in Chapter II. However, in spite of being a species widespread in the region and widely studied regarding its agronomy aspects, chemical composition and in the productive performance of ruminants (Medina *et al.,* 2009), its utilization has been limited in monogastric species.

For this reason, this chapter reviews some studies of the plant's use in the feeding of poultry, pigs, rabbits and guinea pigs. It is necessary to make clear that in the discussion the category of what foliage was studied. "Edible biomass" is considered the whole plant foliage, while the foliage itself is classified as leaves, petioles and terminal ends of the stems. The third category is leaves alone. Obviously the main difference between these three mulberry products is that the cell wall content decreases and the content of nitrogenous compounds increases as one moves from Edible biomass to primarily leaves.

1. MULBERRY IN POULTRY FEEDING

1.1 Preparation of leaf meal and inclusion levels studied

Leaf meal is commonly used for poultry feeding, and is prepared according to Casamachin, Ortiz and López (2007) and Bustamante (2008). After 60 to 70 days of regrowth, stem cutting at 50 cm above soil level is practiced. The cut material is dried for 5 to 7 days spread out and exposed to sunlight, reducing the humidity to between 20 and 25%. Next the thickest stems are removed for preparing meal. Later they are reduced to a particle size of 3 mm in a hammer mill and stored in bags until use.

Different inclusion levels of leaf meal have been studied for poultry categories. The most common were 5, 10 and 15% (Casamachin, Ortiz and López, 2007), however, 3, 6 and 9% have been employed for layers (Suda, 1999), 4, 8 and 12% (Ortiz *et al.,* 2010) in broilers, as well as 10, 20 and 30% in free range chickens (Olmo *et al*., 2012).

Casamachín *et al.* (2007) concluded that the inclusion of 5% mulberry meal in the broiler diet is an option for obtaining greater net field benefit, since production costs are reduced. At the same time, Bustamante (2008) reported that including up to 10% of mulberry in feed for this animal category does not affect weight gain and conversion in the final production stage, and Itzá *et al.* (2010) indicated that 8% can be included in the diet for chickens older than 35 days of age.

Results regarding the optimum level that must be used differ depending on the indicators measured, the region where the study is realized, the mulberry variety and the line of fowl utilized, among other factors. Medina *et al.* (2009) consider that in Latin America and the Caribbean in the last 20 years is when the greatest amount of trials with mulberry have been developed, and on analysing results, the overall conclusions are inconsistent. Therefore, further studies on this topic are needed, particularly in view of the potential benefits both economic and productive possible for small- and medium-scale producers of the region.

1.2 Apparent nutrient retention

When fibre-rich feed is used for fowls, the effect of their incorporation in the ration must be studied, especially because of the digestive challenge from large amounts of fibre (García and Martínez, 1999). Consequently, it is important to study the composition of the material that will be used, the degree of best nutrient utilization, as well as its effect on the digestive process, since they could constrain biological efficiency by reason of the effect on consumption, total digestibility and rate of passage or rate of transit (Rodríguez *et al.,* 2003).

Mulberry is a voluminous substrate, affecting nutrient consumption in birds (Itzá *et al.*, 2010). For this reason, it requires an initial adaptation period to increase the fermentative capacity of the animal. As consequence, nutrient availability and its absorption at intestinal level is increased (Maynard *et al.,* 1981; Duke, 1997; Hernández *et al.,* 2006). Nonetheless, this absorption is limited due to the anatomical and physiological characteristics of the fowl digestive tract. Together with the fore-going, the semi-liquid consistency of faeces suggests increased rate of passage of the liquid phase, which could also could favour the nutrient outflow from the digestive tract, reducing digestibility by the increased excretion (Donkoh *et al.,* 1991; Hien and Hung, 1996).

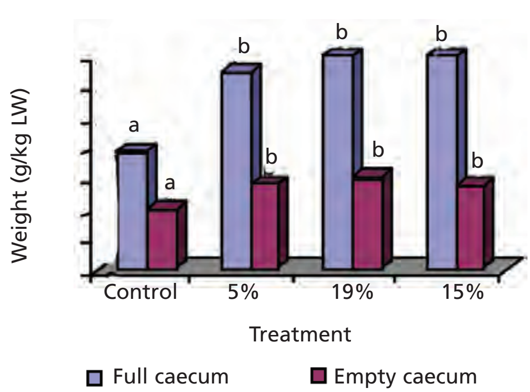
Al-Kirshi *et al.* (2013) observed high dry matter digestibility of mulberry in hens, attributing it to the fact that the NDF fraction showed higher coefficients of digestibility, which were also superior to that of broilers (29 vs. 27%, respectively). Apparent metabolizable energy values in this study were of 7.62 and 7.52 MJ/kg for hens and broilers, respectively. Herrera *et al.* (2014) working with naked neck chickens found no observe differences for the apparent retention of the nitrogenous fraction on when including coarse, tree-like content up to 9% in the ration. However, data published on energy digestibility and different nutrients and chemical compounds are scarce.

1.3 Effect on the morphology of the gastrointestinal tract

The use of fibrous materials in the feeds, as in the case of mulberry, provokes modifications in the gastrointestinal tract (GIT) morphology of monogastric animals. Itzá *et al.* (2010) reported increased intestinal tract weight on including leaf meal of the plant in the diet. Within this, gizzard weight stands out, which is related to the filling effect produced on increasing the NDF content of the diet, since this organ fulfills the function of reducing the particle size of the digesta (Rodríguez *et al.,* 2006). The gizzard, besides being a grinding organ in the fowl, acts as a filter, retaining or allowing the entry of particles into the duodenum. The coarse particles are retained until reaching the critical threshold size, probably determined by the pylorus diameter (Mateos *et al.,* 2006). For this reason, the physical properties of the mulberry meal are also related to the performance observed.

Regarding the caeca, an increase of the relative weights (full and empty) was observed in animals fed diets containing mulberry foliage meal with respect to the control group (Figure 1) (Bustamante *et al.,* 2010). Also, the length of the right and left caeca (Figure 2) differed between the control and the 10% substitution of mulberry foliage meal treatment, being higher in the latter. This could be due to the physiological adaptation of the fowl provoked by the

FIGURE 1
Relative weights of full and empty caeca of broilers consuming mulberry foliage meal.



NOTES: [a,b]Values with different letters between columns differ significantly at $P<0.05$ (Duncan, 1955).

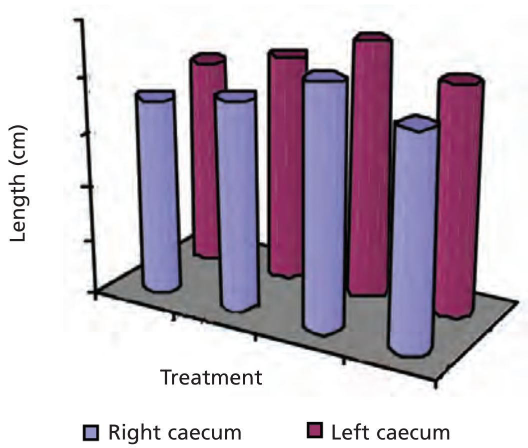
Photograph 1. Apparent retention test in heterozygote naked neck chickens
(Taken from Herrera, 2014)

increase of the fibre dwell time in these organs, which was reported by Eastwood (1992) and Carew *et al.* (2003), as to increase the digestive capacity, microbial mass and final fermentation products.

In fact, the total SCFA concentration (Table 1) also increased and consequently, pH was reduced in the treatments including mulberry, even below the levels reported by Huyghebaert (2003) in the caeca of fowls (5.8–6.8). This response agrees, according to Dunkley *et al.* (2007), with the inclusion of high levels of fibre in the diet of monogastric animals (as in the case of mulberry), since the fermentative processes in the caecum are favoured and contribute to the great microbial diversity.

At the same time, the SCFA produced in the GIT make a relative small contribution, but important to the energy metabolism in chickens (17%) (Marrero, 1998). These metabolites mainly came from the microbial attack of the different fibrous fractions of the feed and in diets such as those including increasing levels of mulberry foliage, so the increase in its production could imply energy gains for the animal.

FIGURE 2
Lengths of the right and left caeca of broilers consuming mulberry foliage meal in the ration



In NH_3 concentration, the control showed higher concentrations regarding the rest of the treatments (Table 1). This decrease perhaps is the result of the greater microbial protein synthesis. Currently it is known that, in fowl, the microflora of the lower intestinal tract is extremely capable in the utilization of this substrate (Preest *et al.,* 2003).

In general, the caecum in the digestive tract of fowls is considered the main site of fibre digestion, due to its great fermentative activity (Savón, 2002), owing to the presence of bacteria and cellulolytic fungi (Rodríguez *et al.,* 1996). Therefore, results obtained suggest that the fibre of the mulberry foliage meal leads to increased digestive activity in this organ.

TABLE 1. Total SCFA concentrations, ammonia and caecal pH values in broilers fed mulberry foliage meal in the diet

Parameter	Treatment			
	Control	5%	10%	15%
Total SCFA mmol/L	49.60	73.28	81.00	64.73
NH_3, mmol/L	2.43	1.16	1.32	1.05
pH	6.74	5.54	5.58	5.70

SOURCE: Adapted from Bustamante *et al.,* 2010.

The relative weights of the pancreas and liver of fowls are not affected with the inclusion of mulberry leaves (Dorigan *et al.,* 2011). However, these authors reported hepatic histological sections showed steatosis and multiple focal necroses with 15% and 30% of mulberry in the broiler ration. Also, changes in nucleus morphometry were detected in both pancreatic acini and hepatocytes. Generally, these lesions are linked to dietetic or toxic factors and are reversible once the originating cause is eliminated. Yadav and Nade (2008) demonstrated the presence of tannins and saponins in mulberry meal, perhaps responsible for the histological changes observed.

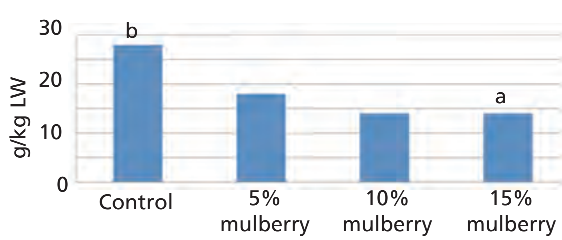
1.4 Effect of mulberry on the lipids

In studies carried out at the Institute of Animal Science, Cuba, with a male hybrid breeding broiler, Martínez *et al.* (2010) found that on including mulberry leaf meal in the diet, the blood indicators haemoglobin and haematocrit did not show differences between treatments. However, cholesterol decreased. It has been demonstrated that high levels of fibre in fowl rations reduce cholesterol and lipid absorption at intestinal level (Savón, 2005). This physiological effect is due to the soluble fraction of the fibre (pectins) and also to the lignin (Salas *et al.;* 2008, Pittaway *et al.,* 2008). Albert (2006) stated that mulberry foliage meal has high solubility and that the lignin values were relatively low (7.6%).

At the same time, this result could be related to the high levels of SCFA found in the caecum with the inclusion of this plant by Martínez *et al*. (2010), since it is stated that they have hypocholesterolaemic characteristics (propionate, acetate and butyrate) provoking inhibition of the HMG-CoA reductase enzyme (Endo *et al.,* 1999; Hara *et al.,* 1999).

Bustamante (2008) noticed that abdominal fat decreased with mulberry inclusion in the ration of chickens (Figure 3), which was to be expected due to the known reducing effect of the fibre on lipid deposition (Marrero, 1998). This effect is very beneficial, since according to Duke (2001), mulberry is used in treatments for some diseases, among them, diabetes and hypertension as well as cholesterol deposition.

FIGURE 3
Abdominal fat of broilers consuming mulberry foliage meal



NOTES: [a,b]Values with different letters within columns differ significantly at $P<0.05$ (Duncan, 1955), SE ±2,76*, $P<0.05$.

Nonetheless, studies carried out do not investigate further the metabolic aspects of the use of mulberry in fowl, which would be very useful for arriving at conclusions on the benefit it could bring to human health.

1.5 Productive performance and yield of poultry carcass

Suda (1999) fed layers (White Leghorn) diets containing different levels of mulberry foliage meal (3, 6 and 9%) and found that the productive indicators were very similar to those found with the traditional diet. However, when Tateno *et al.* (1999) included up to 15% leaf meal of the plant it was found that egg quality was reduced significantly.

In quails, Hermana *et al.* (2014) observed that with 10% of the shrub, feed consumption and egg weight were not affected, however, the amount of these decreased, which was attributed to the high levels of crude fibre in the ration. Bermúdez and Roa (2013) incorporated 10, 15 and 20% of mulberry leaf meal in the diet from the 2nd to the 23rd laying weeks and obtained better performance of the treatment with 15% regarding the laying percentage (78.92%), while with 20% there was a notable worsening (69.60%) of the variable under study.

Regarding the inclusion of mulberry leaf meal in broiler production, Herrera *et al.* (2009) utilized levels of 3, 6, 9 and 12% of mulberry in the diet (Table 2) and observed reduced weight gain in the greater treatment, as well as greater conversion. Casamachín *et al.* (2007) reported similar performance in Ross chickens in Cauca valley. Nevertheless, Bustamante (2008) in the finishing stage of a Cuban commercial hybrid noticed that live weight gain and conversion was affected with 15% substitution of the plant in the ration with an increase in feed consumption. This latter indicator also increased with ISA MPK chickens (Itzá *et al.,* 2010). Chickens are very susceptible to the quality of the feed supplied by reason of the modification of its metabolism based on breeding for rapid growth (NRC, 1994).

In free range chickens, Olmo *et al.* (2012) reported that the inclusion of 30% mulberry foliage decreased live weight from 42 to 70 days, as well as feed consumption and the conversion factor, however, the viability did not show differences regarding the control. In all cases studied for the review of the data on the effect of mulberry on poultry feeding it was confirmed that the mortality index was very low throughout the whole productive cycles, indicating that all the management treatments for the different lots throughout the trials were adequate, besides being beneficial the absence of toxic substances in high amounts in the mulberry that threaten the normal functioning of the immunological system and health of the fowl. Lastly, it is possible to emphasize that maintaining this relatively low index in poultry exploitation is vital, since it takes special significance at the time of the technical and economical settlement of the lot (Leyva *et al.,* 2012).

Regarding the yield of the edible portions there were no differences between treatments (5, 10 and 15% mulberry) in broilers compared with a traditional diet based on

TABLE 2. Productive indicators of broilers fed different levels of mulberry (*M. alba*) leaf meal

Parameter	Mulberry leaf meal (%)				
	0	3	6	9	12
Food consumption, g	5989	5805	5916	5941	5786
Weight gain, g	2748	2699	2696	2693	2655
Conversion index	2.41	2.47	2.59	2.61	2.7

Source: Adapted from Herrera *et al.,* 2009.

TABLE 3. Effect of the inclusion of mulberry meal on the relative weight (%) of the carcass of naked neck chickens at 91 days of age

Indicator (%)	Inclusion of mulberry leaf meal, %				SE(±) Significance
	0	3	6	9	
Carcass yield	77.00^{b}	74.00^{c}	75.00^{c}	79.00^{a}	0.48***
Thighs + legs	22.30^{a}	22.96^{a}	20.62^{b}	19.22^{b}	0.52***
Breast	25.21^{a}	25.96^{a}	23.31^{b}	21.73^{b}	0.59***
Carcass weight, kg	2.50^{a}	2.40^{ab}	2.30^{bc}	2.20^{c}	0.05***

Notes: ***Different letters within the same line differ significantly P <0.05 (Duncan, 1955), ***P<0.001.
Source: Adapted from Herrera *et al.,* 2014.

TABLE 4. Flavour, texture and juiciness of the breast meat and carcass pigmentation of heterozygote naked neck chickens with different inclusion levels of mulberry leaf meal

Indicator	Inclusion of mulberry leaf meal, %				SE(±) Significance
	0	3	6	9	
Flavour	1.67	1.50	1.42	1.08	0.15
Texture	1.75	1.58	1.42	1.33	0.14
Juiciness	1.83	1.58	1.50	1.92	0.14
Pigmentation	2.17^{c}	2.83^{b}	3.33^{ab}	3.67^{a}	0.16***

Notes: ^{a,b,c}Different letters within the same row differ significantly P<0.05 (Duncan, 1955), ***P< 0.001, Flavour: 1-2 normal, 2-3 others, Texture: 1-2 tender, 2-3 tough, Juiciness: 1-2 juicy, 2-3 dry, Pigmentation: 1 white, 2 creamy, 4 intense yellow.
Source: Adapted from Herrera *et al.,* 2014.

maize-soybean (Bustamante, 2008). However, Herrera *et al.* (2014) worked with naked neck chickens and the inclusion of 3, 6 and 9% mulberry leaf meal in the diet. Results of carcass yield are set out in Table 3. Carcass weight, legs +thighs and breast, was similar in the control and with 3%, however, they differed when 6 and 9% of mulberry leaf meal were included.

The sensory analysis carried out by Herrera *et al.* (2014) can be seen in Table 4. The flavour, tenderness and juiciness indicators did not show differences between treatments. Fowls consuming mulberry leaf meal presented greater pigmentation (visual observation) than the fowls of the control treatment. Machii (2000) and Moller *et al.* (2000) found the same effect in the egg yolk of grazing hens. Also, it was confirmed by Casamachín *et al.* (2007), Itzá *et al*. (2010) and Olmo *et al.* (2012), in the skin of chickens fed mulberry meal.

Alvarado (1996) mentioned that fowl consuming high levels of NDF have higher weight of the digestive tract which directly affects carcass yield. At the same time,, there are other factors that can influence the diversity of performances observed, both among the productive indicators and carcass parameters, as in the case of the genetic traits, since, seemingly, the most specialized genotypes are more sensitive to yield reduction of the edible portions in fowl.

At the same time, Díaz *et al.* (2013), in Venezuela, assessed the productive performance of fattening quails receiving 10 and 20% mulberry leaf meal as replacement of a concentrate feed with 17.5% of protein prepared for broilers. As result they found that the final weight, average daily weight gain and feed consumption were higher in quails receiving mulberry leaf meal, however, feed conversion was better in the control treatment, recommending thus new studies based on the use of mulberry leaf meal in quail feeding, but with lower substitution percentages.

2. USE OF MULBERRY IN PIG FEEDING

Arbuscular and shrub species can contribute to reducing the lack in the tropics of protein-rich feeding resources suitable for pig production (Sarría, 2003). Among other species, mulberry (*Morus alba*) stands out by being a non-legume much earlier and used for livestock production very early on (Sánchez, 2000; Preston, 2006), used mainly in ruminants.

Mulberry has more advantages than disadvantages for its use in pig feeding (Ly, 2005, 2006), although not a customary practice in areas where this alternative has been studied in pig production. Nonetheless, with the passage of time valuable information has been accumulated that is worthwhile examining.

The use of mulberry foliage for pig rearing has been mainly investigated from two points of views, that of the performance traits of the animals, or its digestive utilization in this species. Regarding the studies realized by Cuban researchers (Milera, 2011), there is not much data on animal responses (Leiva, López and Quiñones, 2002; Contino, 2007; Contino *et al.,* 2006, 2008), with more information from assessments of the mulberry nutritive value and factors influencing them (Ly and Pok Samkol, 2014).

2.1 Balance and rectal N digestibility

There is not much available information on N balance in pigs fed mulberry foliage. Nonetheless, existing data suggest considerably high values for rectal digestibility and N balance, in pigs fed variable levels of this foliage in the diet, but constituting the third part or its half. Mainly, foliage was cut with a frequency of no more than 60 days, sun dried and later ground (Table 5). In these experiments carried out in Cambodia, the mulberry foliage consisted exclusively of leaves and petioles of terminal stems of one mulberry plantation subject to periodic cutting and fertilized with effluent of biodigestors charged with pig manure (Chiev Phiny *et al.,* 2009). The fertilization level was 100 kg N/ha per year, and the cultivated variety was a broad-leaf genotype of Vietnamese origin, but identity otherwise unknown. Some characteristics of this foliage are also shown in Table 5. At the same time, pigs were of the Vietnamese genotype Mong Cai, whose effective advantage for better utilization of the fibrous fraction of the diet is not well elucidated.

These balance experiments were amplified with another where the levels of inclusion of this type of foliage, were studied as well as the influence of the pig genotype (Chiev Phiny, Preston and Ly, 2003) fed non-conventional tropical diets using locally available feeding resources. In these experiments it was confirmed that high levels of mulberry foliage supplied as leaf meal in the diet, evidently favoured the N balance of pigs.

In the same way, it was noticed that the N balance was still more favourable when a

TABLE 5. N balance in growing pigs fed mulberry(1) foliage meal included in non-conventional tropical diets

	Mulberry foliage % in the diet	
	30	50
Foliage composition		
Lignin	2.9	2.8
Total N, %	3.52	3.55
NDF-N, % of the total	30.0	35.0
Diet composition, % in dry basis		
Total N	2.85	3.24
NDF	26.30	23.59
Initial weight of pigs, kg	14.5	23.7
Utilization of dietary N		
Rectal digestibility, %	83.6	77.5
Retention, % of consumption	64.5	60.5
Retention, % of digestion	75.7	82.0
Source of data:	Ly *et al.*, 2001	Ly *et al.*, 2004

Notes: (1) Cutting frequency, not more than 60 days (leaves and petioles given in the form of sun-dried foliage meal.

TABLE 6. N balance in pigs fed mulberry(1) foliage. Effect of inclusion and animal genotype

	Mulberry foliage,% in the diet				Genotype	
	0	15	30	50	Mong CAI	Yorkshire
			Diet composition, %DM			
Crude fibre	5.1	6.6	8.1	10.1	–	–
Total N	2.75	2.68	2.61	2.53	–	–
WHC, g water/g DM	0.50	1.32	2.14	3.23	–	–
Initial weight of pigs, kg	–	–	–	–	14.2	14.2
			Dietetic N utilization			
Rectal digestibility, %	73.5	72.6	69.3	71.1	68.4	74.9
Retention, % consumption	41.0	39.8	42.9	54.1	40.9	48.0
Retention, % digestion	55.5	54.8	61.2	80.5	59.4	63.6

NOTES: (1) Cutting frequency not greater than 60 days (leaves and petioles given in form of foliage meal, sun-dried).
SOURCE of data: Chiev Phiny, Preston and Ly, 2003.

type of genetically bred individual, although exotic, was used compared with another local, Indo-Chinese. These pigs were Vietnamese, Mong Cai, presumably native to the basin of the Red River.

Additional studies also revealed in these same pigs that the numerical values of the N balance were more favourable when mulberry was supplied fresh, chopped, than as sun-dried foliage meal (Table 7). This information is very interesting, especially when thinking about pig rearing in a family agriculture system, since it offers options in the feeding strategy when the mulberry is supplied as cut-and-carry.

The experiments of N balance in growing pigs were accompanied by *in vitro* assessments on the digestibility of mulberry foliage and matched data obtained in the tests with animals, given the high degree of confidence of this type of tests, fast, economic and easy to carry out (Carvajal, 2010). Table 8 shows data relative to the *in vitro* foliage digestibility, either side of the ileal-rectal junction.

Data on the nutritive value of the mulberry foliage were confirmed by others realized *in vivo* in Venezuela (González, Tepper and Ly, 2006)) and *in vitro* (Pok Samkol *et al.,* 2011), but not by the Colombian figures published by Leterme *et al.,* (2005). The Venezuelan mulberry foliage of 90-day cutting contained 2.32% N and 34.55% NDF on a DM basis, while 60-day cuts had 2.50% N and 32.15% NDF. Leterme *et al.* (2005) found similar averages for young and old leaves, based on 30- and 120-day cuts. The data are summarized in Table 9.

TABLE 7. N balance in growing pigs fed fresh vs sun-dried mulberry(1) foliage

	Mulberry foliage, 45% in the diet	
	Fresh	Dried
Consumption, g DM/kg live weight		
Supply	40.0	40.0
Actual consumption	38.2	36.3
DM rectal digestibility	95.6	90.5
Utilization of dietetic N	83.9	82.4
Retention, % of consumption		
Rectal digestibility, %	75.0	74.7
Retention,% consumption	56.6	47.4
Retention,% digestion	75.3	71.7

NOTES: (1) Cutting frequency not greater than 60 days (leaves and petioles given in the form of foliage meal. Mong Cai × Large White pigs of 15 kg start weight. SOURCE of data: Chiev Phiny *et al.,* 2003).

TABLE 8. *In vitro* ileal (pepsin/pancreatin) and rectal digestibility of mulberry(1) foliage meal for pigs

	In vitro digestibility,%		
	Dry matter	Organic matter	N
Pre-rectal	55.5	58.1	45.0
Post-ileal	63.7	65.2	50.5

NOTES: (1) Cutting frequency no more than 60 days (leaves and petioles given in the form of sun-dried foliage meal).
SOURCE of data: Caro and Ly, 2012.

TABLE 9. Rectal N digestibility of mulberry foliage determined by difference

	Origin of the foliage		
	Colombia	Venezuela	Cambodia
	Mulberry characteristics		
Type of foliage	Leaves	Leaves	Leaves
Diet levels, %	0–35	0–20	0–50
Cutting age, days	30/120	90	60
N, % DM	3.10/2.72	2.32	2.96
NDF, %	21.8/27.8	34.55	28.5
	Rectal digestibility,%		
DM	55.0[1]	64.6	80.0[2]
N	33.0	43.6	65.0[2]
Source of data:	Leterme *et al.* (2005)	González, Tepper and Ly, 2006	Chiev Phiny, Preston and Ly, 2003

NOTES: (1) Average value for both types of leaves. (2) Calculated by regression.
SOURCE: Crampton and Harris, 1969.

2.1.1 Studies of cultivated varieties of mulberry

Ileal and rectal nutrient digestibility in pig diets containing mulberry foliage was also assessed relative to the type of cultivated variety of mulberry. The most notable difference compared with Cambodian studies was the cutting frequency, 90 or 120 days, and the type of foliage, known as edible biomass and including stems. These results showed that the nutritive value of the mulberry foliage was rather poor. In studies made with ileum-rectum-tomized pigs, Domínguez *et al*. (2007) found very low values for the ileal N digestibility, as set out in Table 10.

Regarding the N balance, as well as rectal digestibility with mulberry foliage meal from the type of edible biomass (cutting age: 120 days), see Table 11. These data showed that the use of edible biomass with an advanced cutting age would not be in any way a viable option for using mulberry foliage as a protein source for pig feeding.

Even when age of the edible biomass was reduced from 120 to 90 days (Domínguez *et al.,* 2004), this type of feeding resource would not be advisable to be used in pig feeding, owing to the low ileal DM digestibility and of the organic matter. Digestibility N was equally low, after being rectified (Domínguez, 2006). At the same time, the digestive indices pre-ileal or pre-caecal were not very different numerically when 90-day cuts were compared with 120-day for this type of mulberry foliage (Domínguez *et al.,* 2005).

From another angle, apparently neither cutting age (90- or 120-day) nor the type of mulberry variety cultivated in Cuba, tested in these experiments, seemed to influence the *in vitro* digestibility indices of the ileal type. This information is set out in Table 12.

TABLE 10. Ileal digestibility in Cuban Creole pigs fed mulberry[(1)] foliage meal

	Mulberry foliage, %	
	0	20
Diet composition, % DM		
Crude fibre	1.72	5.85
N	2.37	2.24
WHC, g water/g DM	1.43	2.41
Ileal digestibility, %		
In vivo		
Dry matter	79-9	69.0
Organic matter	78.5	68.3
N	65.9	56.9
***In vitro*[(2)]**		
Dry matter	–	36.2
Organic matter	–	35.2
N	–	35.8

NOTES: (1) Edible biomass from the heart-shaped cultivar, cut every 120 days (N, 1.74; crude fibre, 20.0%). (2) Digestibility corresponding only to the foliage.
SOURCE of data: Domínguez *et al.,* 2007.

2.2 Performance traits

First studies completed with pigs fed mulberry were possibly those of Trigueros and Villalta (1997) who used levels of mulberry leaf meal between 0 and 15%. Later, Osorto (2003) in Yucatán and Leiva *et al.* (2004) in Cuba determined performance traits in pigs fed mulberry foliage.

In Venezuela, Araque *et al.* (2005) supplied mulberry leaf meal to growing-fattening pigs at levels between zero and 24% together with trichanthera leaf meal (Table 13). Araque *et al.* (2005) found that mulberry was better than trichanthera and that a diet with 24% mulberry leaf meal did not show changes in feeding conversion compared with a soybean-based diet, but there was a decrease in daily weight gain due to reduced voluntary feed intake, attributed to the fibrous nature of arbuscular foliage.

González, Tepper and Ly (2006) evaluated the influence of mulberry foliage meal, in this case from the point of view of its inclusion in conventional diets of grains and cereals or in alternative *guarapo* (sugar cane juice) diets, and found how to influence these diets by the introduction of different levels of mulberry meal.

The performance test of González, Tepper and Ly (2006) (Table 14) show that with any sugar cane juice and mulberry meal combination supplied to pigs during the first fattening

TABLE 11. N balance in pigs fed mulberry[(1)] foliage. Effect of the cultivar

	Foliage meal, 30%		
	None	Tigreada	Acorazonada
Foliage composition, %			
NDF	–	19.5	21.8
N	2.15	2.19	2.17
NDF-N	–	43.8	42.9
Initial weight of pigs, kg	37.2	37.2	27.2
Utilization of dietetic N			
Rectal digestibility, %	82.1	70.3	64.4
Retention, % consumption	64.2	52.9	49.2
Retention, % digestion	78.2	75.0	76.2

NOTES: (1) Edible biomass cut every 120 days (N, 1.74; crude fibre, 20.0%).
SOURCE of data: Domínguez *et al.,* 2004.

TABLE 12. Ileal *in vitro* digestibility (pepsin/pancreatin) of mulberry foliage meal. Influence of the cultivated variety in Cuba

Cultivar	*In vitro* digestibility, %		
	Dry matter	Organic matter	N
Cutting age 90 days			
Tigerish	37.7	33.1	28.0
Heart-shaped	46.5	42.3	33.8
Doña Betty	48.4	44.9	30.1
Cuban	47.3	42.9	33.7
Cutting age 120 days			
Tigerish	36.1	35.2	37.2
Heart-shaped	36.2	35.2	36.8

SOURCE of data: Domínguez *et al.,* 2004, 2005.

TABLE 13. Performance traits in pigs fed sugar cane juice or cereals, with variable levels of mulberry leaf meal

Mulberry meal, %	Concentrate				Sugar cane juice			
	0	8	16	24	0	8	16	24
Initial weight, kg	41.9	42.1	39.3	38.4	38.8	41.1	39.8	39.7
Final weight. kg	60.5	62.3	56.5	53.5	58.3	61.8	55.7	54.6
Daily consumption								
DM, kg DM	1.46	1.66	1.61	1.46	1.64	1.67	1.54	1.47
Sugar cane juice, g DM	–	–	–	–	1.42	1.45	1.35	1.32
Concentrate, g	–	–	–	–	223	220	192	150
Protein, g DM	242	261	246	234	94	91	75	60
Daily gain, g	664	723	614	542	695	741	568	533
Conversion, kg/kg	2.21	2.31	2.63	2.71	2.36	2.26	2.72	2.77

SOURCE of data: González, Tepper and Ly, 2006.

TABLE 14. Feed consumption, concentrate saving and additional potential of pig meat production in diets with mulberry foliage meal (MFM)

	Conc[entrate]	Conc. + MFM	Conc. + MFM + Molasses B	Conc. + MFM + sweet potato
	Feed consumption per animal (kg)			
Concentrate	262	200	200	200
Mulberry meal	–	58	58	58
Molasses B	–	–	105	–
Sweet potato meal	–	–	–	62
Total	262	258	363	320
Fattening days	96	96	96	96
Conc. saving, kg/animal/stage	0	62	–	–
Weight increase of one pig/stage, g	63.8	55.3	53.1	56.6
Meat production from 100 pigs, kg	6380	7244	6956	7415
Production differences, g	–	864	576	1035

Source: Adapted from Herrera *et al.,* 2012.

stage (40–60 kg), protein consumption was below 50% of a conventional diet, while daily gain of the animals only fell by approximately 20% and the worsening of feed conversion was only 12.5%, if the data of the extreme diets from the experiment of González, Tepper and Ly (2006) are used.

These results might imply that probably during the second fattening stage, with animals adapted to the consumption of diets with coarse characteristics, more benefit could be derived from this new feeding resource. At the same time, the notable saving in purchased protein supplement might outweigh the cost of including up to 24% mulberry meal in the feed. Even so, 8% mulberry foliage meal in conventional diets or not could be a maximum in daily gain of pigs and a minimum in DM conversion.

Later, Herrera *et al.* (2012) studied the effect on pig production based on concentrates with the contribution of 25% of the protein through mulberry meal, and also to determine if it was necessary to include an additional energy source. In this case the energy sources were sweet potato meal and molasses B. As result it was found that weight gains between treatments were similar, but differed from the control. This response indicates that changing concentrates to alternative sources introduces modifications in nutrient availability and assimilation. The concentrate balance (Table 14) denotes that there are savings and productive benefits in the experimental treatments, which is an advantageous for production.

The substitution of 25% of the crude protein of the concentrate by mulberry gives a saving over 96 days of 62 kg of commercial concentrate. For 100 pigs, this saving is 6.2 t, permitting the fattening of 31 additional pigs with the same concentrate supply.

Results indicated that mulberry incorporation increases the productive potential but are superior if the sweet potato meal is also included, while the utilization of final molasses as energetic source does not favour animal response and may even be detrimental.

3. USE OF MULBERRY IN RABBIT-BREEDING

Rabbit-breeders in the tropics consider a primary constraint to be a lack of a constant source of feed of good nutritional quality throughout the year, at an acceptable cost that allows adequate productive and economic benefits.

In Latin America and the Caribbean there is poor information on the utilization of trees such as mulberry for rabbit nutrition, as well as its effect on the digestive physiology and health of the rabbit, aspects that obviously influence animal performance and productivity.

3.1 Nutrient digestibility

3.1.1. Growing rabbits

Studies of digestive physiology by Dihigo (2005) at the Institute of Animal Science (ICA) of Cuba showed that mulberry in the rabbit has low DM *in vitro* digestibility at stomach level, with values not surpassing 34.9%. This could be due to the presence of some anti-nutritional factors such as the saponins that interfere in protein digestibility, a hypothesis that must be confirmed.

Nonetheless, the digestibility of the fibrous fraction (NDF) of the mulberry, the same as that of the proteins was high with the use of the caecal inoculum of the rabbits. Table 15 shows the *in vitro* and *in vivo* digestibility values (determined by various methods). Moreover, high production of short chain fatty acids (SCFA) was observed, with a fermentative pattern toward propionic acid.

Nieves *et al.* (2005a) studied *in vivo* the apparent digestibility of the CP, DM, OM, energy, CF, NDF and ADF in mulberry foliage and diets with mulberry inclusion. It was determined both in a basal mixture and directly by substitution of the test ingredient for also establishing the digestible energy and digestible protein contents of the mulberry foliage. These researchers found that the content of digestible energy and crude protein decreased when included or the diet was based on mulberry. Values found for NDF (36%) and ADF (23.5%) indicate that mulberry can cover the fibre requirements of the rabbits (De Blas and Wiseman, 2003). The fibre and protein contents allow proposing this forage as ideal raw material for rabbit diets.

Similarly, Nieves *et al.* (2005b) in Venezuela assessed the *in vivo* digestibility of mulberry nutrients and *Leucaena leucocephala* (leucaena) up to 30% in the diet, obtaining values of 61.1% dry matter digestibility (DMD), 65.2% organic matter (OMD), 63.1% gross energy (GED), 72.2% crude protein (CPD) and 45.7% neutral detergent fibre (NDFD), which were higher than those obtained for leucaena.

Later, an experiment was carried out by Nieves *et al.* (2005c) for determining the nutrient digestibility in mulberry foliage compared with *Arachis pintoi* (forage peanut), *Ipomea batatas* (sweet potato foliage), *Trichanthera gigantean* (trichanthera) and leucaena, by substitution of the test ingredient of the basal diet. For that, iso-energetic and iso-proteic diets were formulated with 30% inclusion of the foliages under study. Results showed that the digestible energy and protein content was similar for all foliages except the batatas foliage. The contribution of fibre, energy and digestible protein represent an interesting potential for the use of these foliages as ingredients in rabbit diets.

TABLE 15. Nutrient digestibility (%) studies in mulberry by various in vitro and *in vivo* digestibility methods

	DM	CP	NDF	ADF	SCFA[1]	pH	Method	Author
Mulberry	73.0	78.3	59.0	–	161.5	5.3	*in vitro* caecal	Dihigo *et al.*, 2004
Mulberry	50.7	72.2	26.1	–	–	–	*in vivo*	Domínguez *et al.*, 2005

NOTES: (1) expressed in mmol/L.

TABLE 16. *In vitro* digestibility (%) and pH variations of the diets with the inclusion of mulberry forage meal

Diet	pH value			Digestibility (%)		
	Stomachic	Pancreatic	Caecal	DM	CP	NDF
Alfalfa control	2.9	2.8	2.9	50.4	61.4	68.8
50% mulberry	6.2	6.0	6.0	28.8	61.9	68.8
100% mulberry	6.9	6.5	6.4	36.6	47.2	52.8

Source: Taken from Versallo (2015, unpublished data).

TABLE 17. Values (mmol/L) of total short chain fatty acids (TSCFA) and individual SCFAs in the caecal content of diets including mulberry forage meal (50 and 100%) and alfalfa control

		Alfalfa control	50% mulberry	100% mulberry
TSCFA	mmol/L	73.84	91.10	103.60
Acetic	mmol/L	34.70	49.50	59.89
Propionic	mmol/L	18.36	14.39	1056
Butyric	mmol/L	12.42	17.83	22.46
Propionic/butyric	mmol/L	1.47	0.80	0.47

Source: Taken from Vasallo (2015, unpublished data).

Recently, Vasallo (2015, unpublished data) determined the *in vitro* digestibility of the nutrients (DM, CP and NDF) using the pepsin-pancreatin method (Vervaeke *et al.* 1989) and the caecal contents of rabbits (Pascal *et al.,* 2000) of diets where the alfalfa was substituted (50 and 100%) by the mulberry variety Yu-62. As result, it was found that diets with forage meal showed higher nutrient digestibility than the alfalfa control diet and also had an adequate acid-base balance in the pancreatic and caecal phases (Table 16).

Together, an increase in the production of SCFAs, mainly acetic and butyric was observed with a lower proportion of propionic/butyric, indicating high quality of the fibre for the rabbit (Table 17).

3.2 Productive performance

3.2.1 Growing rabbits

Several studies have been conducted for assessing the indicators of productive performance in growing rabbits receiving diets including mulberry foliage meal. Nieves (2004) evaluated the inclusion of mulberry foliage in a commercial balanced diet and found that consumption was not affected, being of the order of 50.4 and 60.0 g/animal/day, demonstrating that the rabbit accepted satisfactorily this forage as a feed ingredient.

Previously, Vargas *et al.* (2002) assessed in a backyard system the productive performance of growing rabbits fed two levels of mulberry leaf meal using sweet potato foliage as complement of the ration. Also the meat production costs were analysed based on the feeding employed. Results showed that the productive performance of the animals exceded 20 g/day of gain. Similar findings were attained by García-Soldevilla *et al.* (2001) on substituting 25 g or 50 g of feed by mulberry leaf meal. Regarding the cost per kg live weight, it was shown that this produced rabbits at a cost at that time of US$ 0.60/kg.

Performance studies by Sanguinés *et al.* (2006) demonstrated that mulberry could substitute for up to 80% in commercial feed for rabbit fattening without affecting daily weight

TABLE 18. Productive performance in replacement does receiving 20 and 30% mulberry foliage meal as substitute for commercial feed

	Control	20% mulberry	30% mulberry
Animal consumption, g	11.02	10.39	11.41
Conversión kg/kg	3.63	3.90	4.48
Initial weight, kg	1.67	1.71	1.73
Final weight, kg	3.02	2.65	2.53
Daily weight gain	19.22	13.40	11.78

Source: Marote, Sanguinés and Lara, 2005.

gain. The period for attaining commercial weight (2 kg) was extended by some 20 days, but overall production cost was reduced by more than 50%, representing a significant saving for backyard production systems.

3.2.2 Replacement rabbits

Marote, Sanguinés and Lara (2005) determined productive performance indicators in replacement does receiving 20 or 30% mulberry foliage meal in the form of nutritional mini-blocks as substitution for commercial feed (Table 18). There were differences in feed conversion but not in daily DM consumption. Daily weight gain with 20% mulberry differed from the control, but can be considered satisfactory for tropical regions, and was higher than that observed by Dinh *et al.* (1991), who obtained 11.6 g/day by utilizing mini-blocks with shrub leaves of mulberry. It is noteworthy that the cost per kilogram of meat produced was lower for 20% mulberry inclusion (US$ 11.96 versus US$ 13.92 for the control). This will be very beneficial in backyard production systems.

3.2.3 Doe breeders

López and Montejo (2011) evaluated the productive performance of crossbred does in a feeding system based on the utilization of mulberry and other local available feeds. Feeding included *ad libitum* mulberry forage, chopped sugar cane (*Saccharum officinarum*) (100 g DM/breeder), fresh sweet potato (*Ipomea batatas*) vine (90 g DM/breeder) and *criollo* feed (60 g DM/breeder).

During the evaluation period there were a total of 60 kindlings and four abortions representing 93.75% and 6.25% of the total gestations, respectively. The cause of the abortions could be motivated by the *ad libitum* consumption of the fresh mulberry forage at the beginning of the evaluation period which according to García (2004) presents high concentrations of coumarin (0.6% of the edible DM) and other anti-nutritional factors such as the flavonoids (1.3% of the edible DM), which can affect the maintenance of the does' gestation when consumed in large quantities in the diet. However, this abortion percentage is within typical values for the species, especially in young animals (López, Montejo and Lamela, 2011).

From the 420 born alive, 312 young rabbits were weaned at 45 days old, representing 74.3% survival during lactation, higher than that found by Reynaldo, Capote and Soca (2002) in a traditional system based on a commercial concentrate, but with weaning at 35 days old, where there was a 70% survival in the young rabbits. Also, it is significant that using crossbred animals and under these management and feeding conditions, seven young rabbits born alive per kindling were obtained. In the case of the performance of the weaned litters per kindling it was an average of 5.2 throughout the whole evaluation period – a similar result to that found by Reynaldo, Capote and Soca (2002) using feeding based on commercial concentrate and a traditional breeding system. Also, it was similar to that found by La O (2007) in doe breeders fed sweet potato vine, sugar cane and sunflower seeds (5.4), and slightly lower than that reported by this same author in doe breeders fed teramnus (*Teramnus labialis*) forage, sugar cane and sunflower seeds.

The average of young rabbits born alive per kindling was 7, while the average weaned was 5.2 with a weight of 874 g/animal. Daily mean gain during the lactation period was 18 g/day. Mortality percentage was 26%, most of which (20.7%) occurred in the first lactation stage (0–20 days).

Productive data previously cited confirmed the positive effects of the mulberry diet as forage, complemented with other local feeds, however, they also indicate that when this is supplied *ad libitum* in rabbit feeding it provokes not only reproductive disorders in the does, but also digestive problems in the young animals. Therefore, to avoid these undesirable effects it would be advisable to limit mulberry forage to between 25 and 40% in the diet, depending on the nature and level of fibre in the other forage sources in the ration.

Later, López, Montejo and Lamela (2011) analysed the productive performance of crossbred does during four months, but using also fresh mulberry (0.30 kg), ground sugar cane (0.25 kg), glycine (*Neonotonia wightii*), 0.40 g and criollo feed 0.06 kg. An average of 6.4 young rabbits alive were obtained per kindling, with 0.054 kg LW at birth and 5.4 young rabbits were weaned at 45 days of age with a weight of 0.694 kg. Also there was 84.4% survival during the lactation stage. Mean daily gain during lactation was 0.014 kg/animal/day. Although the number of weaned animals and their weight were lower, the high survival index (84.4%) is noteworthy. These results confirm previous studies where fresh mulberry was included in the ration for doe-breeders.

4. USE OF MULBERRY IN GUINEA PIGS

4.1 Nutrient digestibility

First studies in Cuba on the digestibility of mulberry foliage meals in guinea pigs were conducted by Albert *et al.* (2005). For that, guinea pigs of 21 ± 2 days of age of the Macabea breed with a live weight of 231 g were used. These authors determined *in vitro* with pepsin pancreatin the pre-caecal digestibility of the dry matter and protein of four feed mixtures (diets) identified by their main component *Medicago sativa* (alfalfa) T0, following the NRC (1994) recommendations, mulberry T1, trichantera T2 and *Erythrina poepeggiana* (eritrina) T3. Diets were supplied at a rate of 60 g of feed/animal/day. Alfalfa T0 was the control. The simulation of intestinal digestion for DM, CP of the foliage meals over 12 hours showed an increase of the DM and CP digestibility with time, with values reaching more than 50% for mulberry, trichanthera and erytrina that can be classified as a good digestive utilization of nutrients. Mulberry and trichantera did not differ, while erytrina showed lower values. The high digestibility of the mulberry and trichanthera could be due to the lower NDF and nitrogen content linked to the NDF of these feeds, which are relatively low in the mulberry compared with erytrina. These results were superior to those obtained by Ly *et al.* (2001) on assessing the *in vitro* digestibility of mulberry and trichantera in pigs (Figure 4).

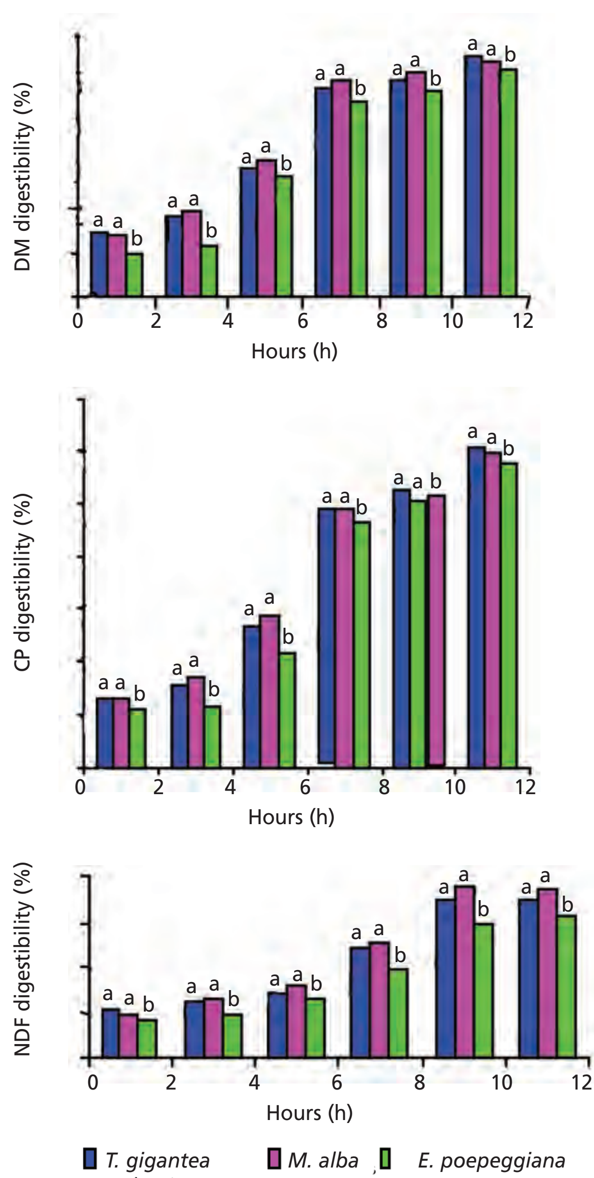
It is important to note that the fibrous fraction is only 34.6% digested in these species. This is due to the fact the main digestion of the structural carbohydrates occurs at caecum level given the characteristics of the micro-organisms living in this organ.

According to studies realized by Burgos (1978, cited by Caycedo (2000) the main micro-organisms present in the caecum of the guinea pig are of the *Bacteroides*, *Clostridium* and *Enterobacter* genera, among others that are known contributors to the degradation of the fibrous fraction of feeds.

It must be pointed out that in the case of ruminants, digestion of the fibrous fraction of arbuscular species reaches between 55 and 65% (Benavides, 2002), due to the presence of micro-organisms similar to those found in the caecum of monogastric species (Dierick *et al.*, 1985).

FIGURE 4

***In vitro* pre-caecal digestibility of DM, CP and NDF**



With short fermentation times (10–12 hours), the amount of fibre digested is limited and mainly depends on the proportion of soluble fibre (pectins, β glucanes, oligosaccharides) partially digested in the small intestine and it is the most readily available fraction for the micro-organisms (Carabaño, García and De Blas, 2001). Additionally, Albert (2006) determined nutrient digestibility by an *in vivo* method in growing guinea pigs receiving diets in which the alfalfa contained in a basal diet was substituted by mulberry foliage, trichanthera and erytrina meals.

DM digestibility values of the foliage meals were similar between treatments and the control demonstrating that its presence in the GIT did not affect negatively the digestive process. DM digestibility (90.3%) was higher than that obtained by Fernández (2002) who found values of 80.2% for the mulberry in the form of meal. Nonetheless, Flores *et al.*

TABLE 19. *In vivo* digestibility of diets with *T. gigantean*, *M. alba* and *E. poeppigiana* in growing guinea pigs compared with a *Medicago sativa* control

	Digestibility (%)			
	Medicago sativa	*Trichanthera gigantea*	*Morus alba*	*Erytrina poeppigiana*
DM	91.4	89.9	90.3	89.6
CP	80.2	73.9	71.2	70.8
OM	90.0	84.9	89.9	83.3
NDF	73.7	69.5	70.5	63.7

SOURCE: Adapted from Albert, 2006.

TABLE 20. *In vitro* fermentation indicators of the meals studied

Digestibility	*Medicago sativa*	*Trichantera gigantean*	*Morus alba*	*Erythrina poeppigiana*
SCFA mmol/L	100.5	108.5	120.45	105.15
pH	6.5	6.5	6.4	6.6

SOURCE: Taken from Albert, 2006.

(1998) achieved IVDMD of 74.5 for the mulberry. Labrada *et al.* (2002) reported 85% DM digestibility for mulberry when supplied to guinea pigs, rabbits and pigs.

Protein digestibility differed between the control (alfalfa) and mulberry with values of 80.2% and 71.2%, respectively, but not between this and the remaining foliage meals. Results obtained can be attributed to the content of this nutrient in the forages, since protein digestibility is the direct reason of this in the feed where the faecal metabolic-N represents a constant amount, regardless the nitrogen of feed origin (Mc Donald, 1995; Apráez, 2002).

Moreover, Aliaga (1979), cited by Apráez (2002), mentioned the high digestibility of the protein in guinea pigs and this is possibly due to the caecal fermentation and consequent caecotrophy, since it is a digestive strategy that extracts forage protein with high efficiency. Therefore, high fibre levels do not affect negatively CP digestibility in guinea pigs in view of the consumption of soft faeces carried out by this species.

The slight increase of the CP digestibility found in mulberry and trichanthera can be attributed to the particle size which was smaller for these species than for erytrina and formed a greater microbial attack substrate. Regarding NDF digestibility, it was observed that the mulberry showed higher values (63.8%). This could be given by its low NDF content, lower lignin percentage, lower volume, lower particle size and to the low tannin content found in the species, which agrees with the findings of Estévez, Pedraza and Guevara (2001).

From the microbial fibre fermentation of the foliages, SCFAs are obtained as final products constituting between 0.2 and 1% of the caecal content from glucose. Table 20 shows total SCFA values. SCFA production in the intestine is closely related to the type and level of fibre in the diet, therefore, in the study, performance similar to NDF degradation was found.

The caecum of the guinea pig provides adequate conditions (size, stable pH, anaerobiosis and regular nutrient entry to be site of a stable and dense microbial flora.

Also, Albert (2006) found that the inclusion of mulberry foliage meal replacing 30% of the alfalfa in diets did not provoke modification of the intestinal morphology of the guinea pig. The length of the small intestine was not affected by the consumption of these meals,

TABLE 20. Performance traits of guinea pigs fed arbustive meals

Parameter	*Medicago sativa*	*Trichantera giganteae*	*Morus alba*	*Erytrina poeppigiana*
Initial weight,g	230.37	230.13	230.03	230.26
Final weight, g	1032.66	1030.5	1028.09	1020.43
Weight gain, g	802.28	800.37	798.09	790.17
Daily LWG, g	12.33	12.31	12.27	12.15
DM consumption				
Consumption g DM/ animal/day	54.2	56.75	56.00	55.5
Feed conversión g/DM/g feed	4.39	4.61	4.56	4.56

TABLE 21. Absolute weight and weight relative to the metabolic weight of guinea pig organs receiving forage meals

Organ	*Medicago sativa*	*Trichanthera gigantea*	*Morus alba*	*Erythrina poeppigiana*
Metabolic weight g/$g^{0.75}$	182.16	181.88	181.56	180.54
Intestines (absolute weight, g)	118.36	118.08	118.05	117.1
Relative to the metabolic weight, g/$g^{0.75}$	0.64	0.64	0.65	0.64
Stomach (absolute weight, g)	10.05	10.00	9.97	9.75
Relative to metabolic weight, g/$g^{0.75}$	0.05	0.05	0.05	0.05
Small intestine (absolute weight, g)	27.8	26.9	26.55	26.35
Relative to metabolic weight, g/$g^{0.75}$	0.15	0.14	0.14	0.14
Large intestine (absolute weight, g)	90.56	91.18	91.5	90.75
Relative to metabolic weight, g/$g^{0.75}$	0.49	0.50	0.50	0.50

mainly due to the fact that the time of passage through this organ is very fast and that important changes do not occur in this segment (Dihigo *et al.* 2002). Likewise, the relative weight to the metabolic weight did not differ between treatments in spite of this being an indicator of high sensitivity (Table 21).

Since feed consumption was similar for mulberry foliage meal it could be assumed that these results indicate that the digestive system of guinea pigs is better prepared for this type of diets than other monogastric species.

4.2 Performance traits

Several studies have been conducted evaluating the effect of the inclusion of mulberry foliage on the productive performance indicators in diets of growing guinea pigs. Flores *et al.* (1995) utilizing mulberry foliage and concentrate achieved weight gains of between 9.3 and 9.7 g/animal/day, respectively. Similarly, Ceballos *et al.,* 1995 (cited by Caycedo, 2000) using the same diet found weight increases of 9.5 g/animal/day.

Regarding the average daily gain, Chauca (1999) and Forte *et al.* (2000) supplied mulberry to guinea pigs and obtained gains between 10 and 15 g/animal/day. At the same time, Fernández (2002) attained weight gains of 11.98 g/animal/day utilizing concentrate and fresh mulberry. These results were slightly lower than those attained by Albert *et al.* (2005) (Table 22).

Results from this investigation are intermediate level to those obtained by other workers. These differences are the result of the sum of influencing factors, considering genetic potential (Chauca, 1999) to be one of the most important besides feeding factors, such as

TABLE 22. Performance traits of guinea pigs fed arbustive meals

Parameter	*Medicago sativa*	*Trichantera giganteae*	*Morus alba*	*Erytrina poeppigiana*
Initial weight,g	230.37	230.13	230.03	230.26
Final weight, g	1032.66	1030.5	1028.09	1020.43
Weight gain, g	802.28	800.37	798.09	790.17
Daily LWG, g	12.33	12.31	12.27	12.15
DM consumption				
Consumption g DM/ animal/day	54.2	56.75	56.00	55.5
Feed conversión g/DM/g feed	4.39	4.61	4.56	4.56

the energetic density of the feed, nutritional contribution, amount supplied, digestibility and vegetative stage in the case of the forage.

At the same time, Ruedas and Albert (2002) assessing diets containing 50% mulberry and 50% *Pennisetum purpureum* (Napier grass) attained a conversion of 6.5, which could be due to the poor nutritional quality of the Napier grass. Another aspect that must be considered is the relative production costs for guinea pigs fed meals of tree species. On analysing production costs it was observed that the lowest cost in Cuban pesos was for the treatments based on shrub species compared with the control diet, since that is expensive due to its high alfalfa content.

5. FINAL REMARKS

The wide availability of mulberry (*Morus alba*) in the Latin America and Caribbean area makes it a plant resource that merits further research to optimize its utilization for the production of monogastric animals, and especially in fowl. The use of mulberry promotes sustainability in local animal production by reducing reliance on imported inputs and promoting small-scale animal production.

According to the information given in this review, mulberry is a non-legume arbuscular species that can be used in the tropics for pig rearing. This plant can be used as permanent culture for periodical cutting, integrated into animal production systems, particularly of pigs. Mulberry provides a protein source locally available as fresh forage or as meal. It can be fed at rather high inclusion levels, taking always into account that the better digestive use of the nitrogenous compounds, high by itself, can be manipulated in such a way as they may be more nutritive while containing less fibrous material. In these integrated systems, the mulberry for pigs would be one of the important protein sources that can be incorporated in the feed formula, particularly in family agriculture. Important factors influencing herd response are cutting age and plantation fertilization management.

It is advantageous to include mulberry foliage meal for growing-fattening pigs, probably with economic advantage when it is circa 10% (dry basis) of the daily ration, in both conventional and non-conventional diets. Animal response in the fattening period when more inputs are required in the pig production system suggest that mulberry can be a plant resource capable of being usefully integrated into pig rearing, perhaps through a perennial plantation with periodic cutting and fertilized with pig effluent. This is a new concept and deserves further consideration.

Studies of productive performance in growing rabbits for backyard systems show that it is possible to replace up to 80% of commercial feed with mulberry foliage meal, corroborated by the high fermentation of the NDF of this feed. At the same time, results showed that utilization of mulberry forage and other local resources gives encouraging productive indices in crossbred does, giving seven young rabbits alive per kindling, and from these the weaning of more than five with a weight of 874 g at 45 days of age, allowing weight gains of 18 g/day during the lactation stage. However, indications are that mulberry in rabbit diets should not exceed 40% inclusion

Mulberry foliage meal, given its physico-chemical characteristics and the results obtained both in studies of digestive utilization and in biological and economic animal response, is a promising source for guinea pig diets, with results similar to those attained for alfalfa, which is used as a reference species due to its excellence for such purposes.

Finally, there is an important imbalance relating the amount of information produced through basal investigation to the practical use given to this plant under production conditions on farms. This constitutes an obvious and fundamental aspect that deserves investigation in future years.

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Mulberry (*Morus alba*) in ruminant feeding

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ABSTRACT

Mulberry *(Morus alba)* is a promising resource for animal feeding due to its good adaptability to tropical conditions and its easy integration into livestock production systems. It also offers security elements and sustainability in feeding and environmental sustainability. This chapter considers aspects of the utilization of *Morus alba* in ruminant nutrition. In calves and goats under grazing conditions it was used as a feed supplement. In dairy cows there were no differences in milk production when mulberry substituted for concentrate feed, changing from 100/0 to 25/75. Sheep responses showed that the inclusion of an energy-rich supplement in a mulberry diet gave the animals a better nutrient balance and improved ruminal activity, and, on the whole, promoting more complete ration consumption. Mulberry, owing to its high nutritional value and low secondary metabolite content, can be considered as a woody plant with good potential for producing forage for animal feed.

Keywords: feed supplement, nutrient balance, ruminant feeding*, Morus alba*

INTRODUCTION

Woody plants with great potential for forage production have led to a change in the approach of the livestock production systems. They are much more efficient resources than grasses for capturing daylight energy and producing biomass; they act on the subsoil to extract water and recover nutrients. On account of their root depth they avoid soil compacting and help maintain physical and chemical properties without destabilizing the micro- and macro-organisms that decompose organic matter and transform it into available elements for plant nutrition (Boschini, 2003). Among the most promising resources is the mulberry, with great adaptability to tropical conditions and easy to integrate into livestock production systems; also it offers security elements in feed supply, and sustainability in environmental aspects (Benavides, Lachaux and Fuentes, 1994).

Mulberry contains crude protein concentrations between 15 and 28% in the dry matter (DM); 15% crude fibre (CF); from 33 to 46% neutral-detergent fibre (NDF); 28 to 35% of acid-detergent fibre (ADF); 5% lignin; 2.42–4.71% calcium; and 0.23–0.97% phosphorus (Table 1) (Singh and Makkar, 2002). Total contents of tannins and phenols reported are very low (1.8% as tannic acid equivalent).

TABLE 1. Bromatological composition of the species (%)

Species	CF	CP	Ca	P
Panicum maximum	28.2	7.0	0.5	0.17
Morus sp. (whole)	16.6	24.6	1.8	0.42
Morus sp. (chopped)	20.3	20.5	2.5	0.79

NOTES: CF = crude fibre; CP = crude protein; Ca = calcium; P = phosphorus.
SOURCE: Milera *et al.*, 2011.

These characteristics of the mulberry suggest its possible utilization as supplement in diets for animal species, and particularly for ruminants. In that respect, research studies have demonstrated its advantageous use in feeding of the species that are the general focus of this chapter.

1. MULBERRY FOR LARGE ANIMALS – GRAZING CALVES

In Cuba, as in other countries of the region, studies have looked at the use of mulberry as a component of the diet for grazing ruminants. In this, the Pastures and Forages Station "Indio Hatuey" has led by developing a wide study on the use of this shrub. Milera *et al.* (2011) assessed the potential of *M. alba* in crossbred (Holstein × Zebu) male calves of eight months of age and an average live weight at the beginning of the trial of 108 kg.

Initially, when whole forage was offered *ad libitum*, DM consumption was 3.93 kg DM/animal/day, or circa 2.6% of live weight. However, in the second stage, when mulberry was supplied chopped, consumption was 3.12 kg DM/animal/day, ca. 1.7% of live weight (Table 2), which is a reflection of the animals' increased grazing time.

In the first stage the authors observed gains of 0.717 kg/animal/day when animals received whole mulberry (2.6% of live weight – LW), while in the second stage, when it was supplied chopped, gains were lower (0.404 kg/animal/day) (Figure 1), which is a reflection of several factors, among them the restriction of grazing to four hours daily in *Panicum maximum* areas during the first stage of the trial. This contributed to the fact that the animals dedicated little time to grass consumption and consumed greater amounts of mulberry forage. In the second stage they had a longer grazing time. Also, the forage had higher values than the pasture in terms of protein and crude fibre. At the same time, the whole mulberry supplied in the first period allowed the animals greater capacity to be selective, choosing leaves and tender stems, and thus the best gains.

It was shown that mulberry possesses high quality when used as the edible biomass in feed, and better use was seen when supplied chopped. Moreover, using mulberry, the growing cattle reached daily liveweight gain (LWG) of >400 g/animal/day, without concentrate.

Other studies with grazing young ruminants were by Soca *et al.* (2010), who estimated the potentialities of mulberry for supplementation and its effects on productivity and health of young grazing bovines. For that, 20 young calves of 70 kg initial live weight were subject to two treatments under grazing conditions: (A) grazing animals supplemented with mulberry; and (B) grazing animals supplemented with concentrate and hay (control treatment). An established plantation of *M. alba* cv. Cuban on a red ferrallitic soil was used, that at time of cutting was three years post-establishment with a density of 25 000 plants/ha. Cutting height was at 100 cm, cut every 45 days. Table 3 shows the bromatological composition of all the feeds supplied for each treatment. The high protein percentages of the mulberry foliage used as supplement

TABLE 2. Mulberry consumption throughout the experiment

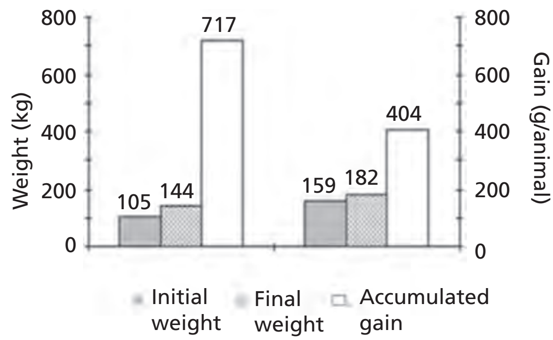
Stage	Mulberry consumption (kg DM/animal/day)	% live weight
1	3.93	2.6
2	3.12	1.7

NOTES: Taken from Milera *et al.*, 2011.

are typical for this species, and even higher than those of the commercial concentrate used commonly for such animal stock. These results are similar to those reported in other investigations, e.g. by Martín *et al.* (2007).

According to Sánchez (2002), mulberry is an important source of amino acids, of which half are essential. It also supplies substantial levels of vitamins, notably nicotinic, ascorbic and pantothenic acids, vitamin C and riboflavin.

FIGURE 1
Performance of the weight gains by stage. Initial and final weight (kg); accumulated gain (g/animal/day)



At the same time, the fibrous fraction of mulberry is low compared with other tropical forages. According to Boschini (2002), the tender stems have a similar fibrous fraction to, and in some cases lower than, usual tropical pastures, besides showing overall less lignification. Thus its nutritive value is considered good, with better digestibility than that found in the leaves of typical tropical pasture plants.

These results were reflected in liveweight and average daily gain performance (Table 4). By the end of the experiment the animals supplemented with mulberry reached higher final live weights, with significant differences compared with those fed conventionally. A similar trend was observed in the average daily LWG (>600 g/animal/day). Results in this study were lower than those reported by Milera, Sánchez and Martín (2010) using the whole plant as supplement under housed conditions.

2. MULBERRY IN MILK PRODUCTION WITH GRAZING COWS

The potential of mulberry forage in bovine milk production was determined by Milera *et al*. (2011) in a study over 140 days with recently calved crossbred Holstein × Zebu cows, without supplementation.

TABLE 3. Bromatological composition of the feeds supplied (%)

Type of feed	DM	CP	CF	Ca	P	Ash
Mulberry	26.13	20.56	14.75	2.68	0.23	8.51
Commercial concentrate	96.00	12.98	15.61	2.51	0.83	10.39
Pangola grass hay	68.40	4.25	37.03	0.58	0.19	–

NOTES: DM = dry matter; CP = cude protein; CF = crude fibre; Ca = calcium; P = phosphorus.
SOURCE: Soca *et al.*, 2010.

TABLE 4. Live weight (kg) and average daily LWG performance

Treatments	Initial weight (kg)	Final weight (kg)	Average daily LWG (g/animal/day)
Supplementation with mulberry	70.00	186.06	630
Supplementation – conventional	70.88	169.01	538
SE±	NS	1.70***	0.86*

NOTES: *P<0.05; ***P<0.001; NS = not significant.
SOURCE: Soca *et al.*, 2010.

In the first period, whole forage was supplied *ad libitum* with four hours of restricted grazing in areas of improved grasses (*Panicum maximum* and *Digitaria decumbens)*, without fertilization or irrigation, and in the second period animals grazed 8–12 hours on the above mentioned grasses, but with *Leucaena leucocephala* cv. Peru occupying 10% of the grazing area). The chopped mulberry forage was supplied at a rate of 1% of live weight.

The lowest CP value and highest CF was noted in the chopped forage, since animals selected the most tender parts, mainly the leaves (Table 5) when supplied uncut. On analysing the chemical composition of the grasses, these showed an acceptable CP content despite no fertilizer having been applied for more than 10 years.

In the first period when the edible part of the forage (leaves and tender stems) were supplied, maximum DM consumption was observed at 30 days (10.88 kg), stabilizing later (7.86 kg) until 53 days. In the second period (87 days), when the forage supplied was reduced to 1% live weight, a better use of the material offered was found, with average consumption of 5.1 kg DM per animal.

Daily milk production in the first 30 days of the trial was 12 litres when cows averaged 84 days of lactation; at the end of the trial, average production was 10.6 L/animal/day with no significant differences detected when the two stages were analysed, that is, *ad libitum* for the 53 days or restricted in the last 83 days of evaluation.

The lactation curve (Figure 2) is typical: high production maintained to the 200-day mark of lactation, reflecting the high nutritional value of the feed supplied. The requirements for medium-potential animals were covered without supplementation with concentrates. Production obtained in this experiment with crossbred animals resembles that found by Oviedo (1999), who used a Latin square design with mulberry (2.76%) and concentrates (1% LW each) and found a yield of 13.2 L/cow/day, and concluded that the energy contribution was similar to the concentrate, and that mulberry gave an economic benefit greater than with the forage.

However, in the second experimental stage, it was evident that with abundant availability of improved grasses, the *ad libitum* supply or high volumes of forage are not necessary, since animals could substitute grass consumption with that of mulberry, and the high nutritional value of the plant would be misused. When supplied chopped, the volume and the number of carrying trips decrease, consumption is faster and the less tender parts can be included that are completely used by the animals.

Hernández *et al.* (2011) reported daily yields of 14.8 L/cow feeding similar percentages of inclusion to that used by Milera *et al*. (2011), but with Holstein cows

TABLE 5. Bromatological composition of the species (%)

Species	CP	CF	Ca	P
Grasses	7.01	29.31	0.510	0.180
L. leucocephala	20.86	27.58	1.883	0.368
Whole *M. alba*	24.74	16.46	1.78	0.417
Chopped *M. alba*	20.16	20.8	2.46	0.787

Notes: CP = crude protein; CF = crude fibre; Ca = calcium; P = phosphorus.
Source: Milera *et al.*, 2011.

TABLE 6. Inclusion of *M. alba* forage in the rations of dairy cows

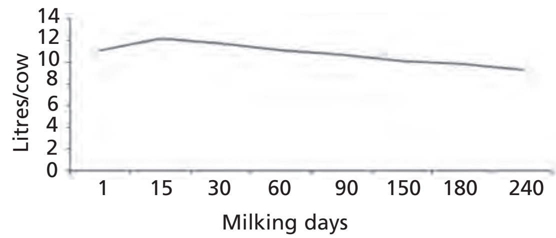
Parameter	Concentrate:forage DM relationship (kg/animal/day)		
	100:0	**60:40**	**25:75**
Concentrate	6.4	4.2	1.9
Mulberry	–	2.8	5.5
Pastures	9.3	7.8	6.2
Total	15.7	14.8	13.6

Source: Hernández *et al.,* 2011.

receiving concentrate supplementation (Table 6) and grazing in *Pennisetum clandestinum* areas. Unlike the earlier study – where the temperature averaged 23.8°C – this was carried out at lower temperatures (16.7°C) and using a short-term Latin square design.

Values found for *in vitro* dry matter digestibility (IVDMD; 80%) and digestible energy (DE; 3.5 Mcal/kg DM) were high and similar to those obtained by other authors, and justify the response in milk yield and concentrate substitution by this forage. If the feeding costs alone are considered, it can be inferred that there is greater net income and there can be a better benefit:cost relationship per animal by replacing concentrate with mulberry.

FIGURE 2
Lactation curve in animals of medium potential without concentrate supplementation



Source: Milera *et al.*, 2011.

The authors considered that *M. alba* is a forage that can be used for animals of medium potential by substituting the concentrate supplement, allowing yields of more than 8 L/cow/day when improved pastures are used.

Other trials carried out by the researchers of the Pastures and Forages Station "Indio Hatuey" included those of Lamela *et al.* (2010) who determined milk production from an association of *Pennisetum purpureum* cv. CT 115 with the forage trees *L. leucocephala* and *M. alba* under irrigated conditions.

P. purpureum CT-115 was established at four months (Table 7), although leucaena and mulberry took longer to establish, but all species established at the expected times. In the case of leucaena, the establishment was slower than that reported by Corbea and Blanco (2005), which was one year to 2 m tall. At the same time, the floral composition showed an acceptable population of improved species, both grasses and woody.

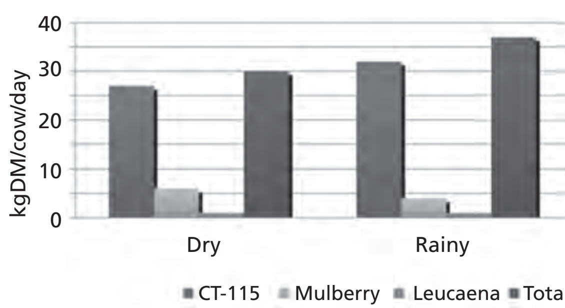
DM availability, which was >30 kg DM/cow/day (Figure 3), allowed the cows to be selective in their diet; this

TABLE 7. Feed quality (%).

Feed	DM	CP	CF	Ca	P
P. purpureum cv. CT 115	23.2	10.0	26.7	0.56	0.14
Mulberry	37.0	29.6	33.7	2.50	0.20
Leucaena	33.3	28.0	26.1	2.30	0.25
Citrus skin	16.5	7.7	12.0	18.1	1.30
Northgold	90.6	29.3	7.2	0.04	0.82

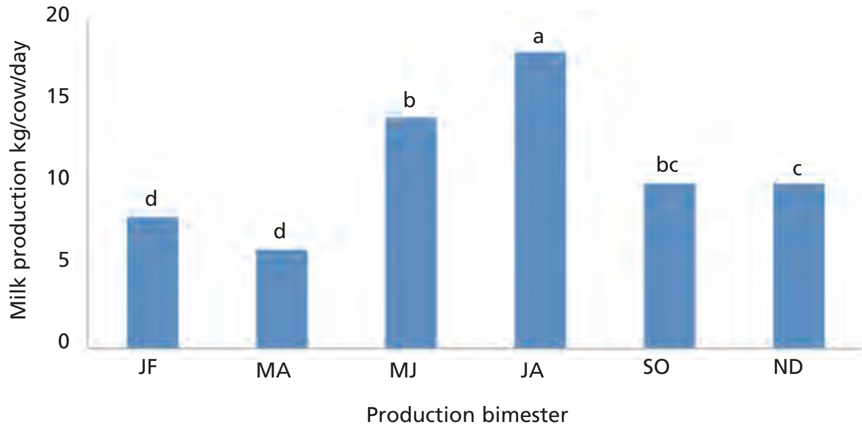
Notes: DM = dry matter; CP = crude protein; CF = crude fibre; Ca = calcium; P = phosphorus.
Source: Lamela *et al.*, 2010.

FIGURE 3
Dry matter supply (kg/cow/day) in two seasons



Source: Lamela *et al.*, 2010.

FIGURE 4
Effect of the production bimester on the association



NOTES: a,b,c,d = Values with different letters differ at $P<0.01$ (Duncan, 1955).

value matches that recommended for grazing cows on tropical pastures (Muñoz *et al.*, 2009).

At the same time, feed supply (Table 7) was of good quality due to the high CP content: cv. CT-115 reached 10% CP, and the woody species showed CP content >25%, guaranteeing therefore a diet of acceptable nutritive value for cows of medium milk potential.

The highest results in milk production were found in the rainy period, with significant differences ($P<0.01$) in particular in the July-August bimester, and the lowest in January-February and March-April (Figure 4).

Similar values were reported in a glycine + *P. purpureum* cv. CT-115 system (Pacheco, 2007) and in a silvipastoral system of leucaena with star grass or guinea grass cv. Likoni under drylot conditions, with stocking rates less than seven animals per hectare (Sánchez, 2007). In the literature it is considered that there are differences in milk production between seasons of the year (Pacheco, 2007) due to shortage in feed availability for cattle in the poor rainy period; however, when silvipastoral systems are employed with low stocking rates (1–2 cows/ha), pasture availability can be guaranteed throughout the year and these differences do not occur.

Results show that a system with woody plants in combination with grass forage under cattle farm conditions, provides medium-potential cows with acceptable levels of intake for milk production, as well as maintaining good availability of dry matter in the grassland, leading to favourable economic outcomes.

In Costa Rica, Boschini (2003) substituted the concentrate feed by fresh mulberry (*M. alba*) in the diet of 16 Jersey dairy cows. Animals were in their first or second calving, with a minimum of 120 and a maximum of 160 days of lactation. Cows were distributed in four groups. Treatments were four balanced diets using: fresh mulberry leaves; whole plant of black forage sorghum; a mixture of commercial concentrate feed and soybean cake; and the control. Experimental diets were calculated based on the substitution of the concentrate feed by the inclusion of 0, 20, 40 or 60% DM mulberry.

Total DM consumption was from 3.12 to 3.24% of live weight. There were no significant differences ($P \geq 0.05$) in total DM consumption among cows in the same treatment.

In the diet without mulberry (0%), forage was 42%, and in the diet with 60% mulberry constituted 86% of the DM. Mulberry consumption was close to 2% LW in the diet with 60%. The substitution rate was 677 g DM/animal/day of concentrate for each 1 kg DM of mulberry added to the diet, plus the substitution of 323 g/animal/day of black forage sorghum and the soybean in mixture for those diets. Protein replacement was 576 g/animal/day of the concentrate and 424 g/animal/day of black forage sorghum and soybean for each 1 kg CP mulberry added. Each 1 MJ of digestible energy of mulberry added to the diet replaced 2.62 MJ/animal/day of the concentrate energy and 1.35 MJ/animal/day of the mixture of black sorghum forage and soybean. There were differences ($P<0.05$) in the live weight of the cows and in daily milk production between the different levels of mulberry addition. However, no important differences were determined ($P>0.05$) between the initial and final weight of the animals and the variations in daily milk production were slight within the diets. Boschini (2003) recommends the addition of fresh mulberry leaves up to 60% of the total DM, providing a balanced amount of protein.

3. MULBERRY FOR SMALL RUMINANTS – GOATS

The growth rate of goats depends mainly on the quantity and quality of the feed available, besides animal health, genetic potential and sex. In most tropical regions, goats receive a poor diet in quality and insufficient in quantity; as a result, goats tend to grow very slowly, hampered in addition by diseases, infection and parasites, as well as by environmental conditions determined by the combination of humidity and high temperatures that reduce appetite and consequently feed consumption.

Mulberry, by virtue of its high nutritive value, has been used as a feed complement under grazing conditions. Thus, González *et al*. (2011) studied the effect of different levels of mulberry on consumption and growth of weaned kids. For that, the authors used 20 weaned female kids of F_1 genotypes (Criollo-Saanen, Criollo-Nubia or Criollo-Alpina breeds), averaging 10.2 kg live weight and 3 months of age. Animals were confined in an agroforestry unit for milk production in Matanzas province, Cuba. The kids were randomly distributed in four groups of five animals each; groups represented the treatments and each animal constituted a replication. These were:

A: Control. Guinea grass forage (*Panicum maximum* cv. Common) + citrus skin.

B: Guinea grass forage + mulberry DM at 0.5% of live weight.

C: Guinea grass forage + mulberry DM at 1.5% of live weight.

D: Guinea grass forage + mulberry DM at 2.5% of live weight.

The experiment had an adaptation period of 7 days and 8 weeks for data collection (60 days). Both forages came from a nearby forage bank, with a mulberry plantation of 4 ha, which was sown and established with a density of 25 000 plants/ha and cut every 100 to 120 days, with neither irrigation nor fertilization. The forage was supplied in troughs and was previously chopped to a particle size of 2 to 3 cm.

Animals were individually weighed every 7 days to calculate liveweight gain (LWG); consumption per treatment was estimated daily through the weighing of the feed supplied less that rejected. Feed conversion was established considering the feed consumption or nutrient in question relative to LWG. Total DM consumption per animal/kg LW and by metabolic weight for each treatment were also estimated.

Both total voluntary consumption of mulberry and guinea grass were significantly affected ($P<0.001$) by the level of mulberry supply; as the mulberry level increased; voluntary consumption of all nutrients increased in turn, peaking at the 1.5% inclusion level, when there was maximum ingestion of DM (544.7 g), CP (54.47 g) and metabolizable energy (ME) (4.89 MJ/animal/day).

Any additional increase in mulberry inclusion in the diet (2.5%) meant a slight decrease in the total volume consumed, since probable requirements were covered from the chemostat point of view, with the decreased grass consumption and its effect on ruminal retention of the bolus by the most fibrous proportion in its composition. At this level there was also a significant variation in the consistency of faeces, explained possibly by a greater speed of gut passage, which would also imply inefficient use of the mulberry forage.

In contrast with the results obtained by Rojas and Benavides (1994), it was observed (Table 8) that when the supply of mulberry was increased, guinea grass consumption also increased, apparently due to an improvement in the fermentative patterns of the ruminal ecology. The abovementioned authors reveal a marked selection effect on the forage consumed, above all in the case of the protein, since for each 1 kg increase in mulberry consumption, total DM consumption increased by ca 0.5 kg and a similar quantity of pasture was left.

In coincidence with the present work, Liu *et al.* (2001) demonstrated an increase in consumption of ammoniated rice straw (basal diet) by ovines when the percentage of mulberry was increase in the supply, and by González, Arece and Cáceres (2000) in an observation trial with stabled sheep.

The treatment with the most mulberry in the diet (Treatment D in Table 8) had the best feed conversion, attributed to the favourable characteristics of the mulberry protein and its relationship with energy supply.

As the mulberry forage levels were increased in the diet there was an increase in growth rate, specially in treatment D (Table 8); but when the mulberry inclusion passed 50% of supplementation in the total ration (more than 2.0% of live weight on a dry basis) its effect on the growth rate was not significant.

From this investigation it was concluded that with the inclusion of mulberry forage at 1.5% live weight, the highest DM intake (544.73 g), CP (54.47 g) and ME (4.89 MJ/animal/day) was attained. Also the increasing levels of mulberry forage in the ration (up to 1.5% of LW) produced an increase in the consumption of the accompanying grass, and

TABLE 8. Nutrient consumption according to the mulberry inclusion level in the diet

Treatment	Total	Mulberry	Guinea grass
Consumption (g DM/animal/day)			
A	392.55[a]	–	314.00[a]
B	456.04[b]	73.92[a]	382.12[b]
C	544.73[c]	97.00[b]	447.73[c]
D	380.3[d]	125.56[c]	255.00[d]
Crude protein consumption (g/animal/day)			
A	23.24[a]	–	23.24[a]
B	44.60[b]	16.27[a]	28.30[b]
C	54.47[c]	21.34[b]	33.13[c]
D	47.08[d]	27.77[c]	18.87[d]
Metabolizable energy consumption (MJ/animal/day)			
A	2.72[a]	–	2.72[a]
B	4.09[b]	0.75[a]	3.34[b]
C	4.89[c]	1.00[b]	3.92[c]
D	3.30[d]	1.25[c]	2.25[d]

NOTES: A = Control. Guinea grass forage (*Panicum maximum* cv. Common) + citrus skin; B = Guinea grass forage + mulberry DM at 0.5% of live weight; C = Guinea grass forage + mulberry DM at 1.5% of live weight; D = Guinea grass forage + mulberry DM at 2.5% of live weight. Different-lettered superscripts indicate differences at $P<0.01$ (Duncan, 1955).
SOURCE: Taken from González *et al*., 2011.

the best general indices of conversion and weight gain were achieved with the treatment with the highest inclusion level of mulberry (2.5%). However, the best nutrient utilization efficiency was considered to be the 1.5% treatment, when the grass role within the diet became optimal.

In Costa Rica, Rodríguez and Elizondo (2012), working with goats, evaluated the quality, selection, apparent digestibility and consumption of mulberry and African Bermudagrass [star grass] (*Cynodon nlemfuensis*) supplied fresh or wilted. The trial used 12 non-lactating and non-pregnant does of Saanen, Toggenburg and La Mancha breeds, with an average live weight of 37 ±5 kg. The experimental treatments were: (A) fresh grass; (B) partially wilted Bermudagrass; (C) fresh mulberry; and (D) wilted mulberry. The forage was offered to animals chopped.

The average DM consumptions (±0.09) (kg/animal/day) were: (A) 0.93; (B) 0.76; (C) 1.17; and (D) 1.12. This representing (A) 2.62%; (B) 2.09%; (C) 3.17%; and (D) 3.13% of live weight (±0.24%). CP consumption was lower with wilted Bermudagrass and higher with fresh mulberry (75.2 g vs 135.4 g/animal/day). The apparent digestibility percentage was not statistically different between treatments; it showed an average of 49.18% for DM, 59.82% for CP, 57.83% for NDF and 55.30% for ADF. This study demonstrated that the forage species influenced significantly the voluntary DM intake in goats, and that the DM content in the forages used did not noticeably affect consumption. In general, with all treatments, there were high DM consumptions, and intake by animals consuming mulberry exceeded 3% of their body weight. It could be shown that the animals were selective in their feeding due to their preference for the most digestible plant parts, i.e. those with lower proportions of cell wall.

4. MULBERRY FOR SMALL RUMINANTS – SHEEP

Mulberry has high DM digestibility and good CP, with a high speed of gut passage and a significant breakdown of all nutrients, which is why ruminal bacteria act quickly. At the same time, citrus skins – in view of their energy characteristics – are recommended as an appropriate supplement for diets having high CP levels, which aids attaining adequate balance in the protein:energy relationship of the ration. Based on these recommendations, ensiled or dried citrus skins have been utilized as supplements for growing sheep fed mulberry (*M. alba*).

For that trial (Ojeda, Arece and Cáceres, 2011) 15 Pelibuey growing male sheep, average 6 months old were used. Animals were divided into three homogeneous groups in weight (ca 21.4 kg). All were stabled in separate pens. There were feeders and collective water troughs in each pen for feeding, with complete mineral salts and *ad libitum* water. Animals were wormed two weeks prior to the start of the experiment.

Fresh forage of mulberry (*M. alba*) cut 90 days after sprouting, was taken from a plot that received organic fertilization based on poultry manure equivalent to 150 kg N/ha/year. The ration supplied consisted of fresh mulberry forage chopped and supplied *ad libitum.* The treatments were: (A) mulberry forage; (B) mulberry forage + 200 g of dried citrus skin; (C) mulberry forage + 700 g of citrus skin silage.

As evaluation parameters, supplementation should not exceed 20% of the total DM consumed, and the protein contributions of the supplements should be similar.

TABLE 9. Productive indicators of growing sheep

		Diet			SE ± Sig
		Mulberry	Mulberry+dried citrus skins	Mulberry +citrus skin silage	
Initial live weight	(kg)	21.5	21.3	21.4	
Final live weight	(kg)	26.4	26.9	27.3	
Metabolic weight	($LW^{0.75}$)	11.36	11.68	11.93	
Mulberry consumption	(g DM/kg $W^{0.75}$/day)	75.2	66.8	64.5	
Supplement consumption	(g DM/kg $W^{0.75}$/day)	0	15.9	13.8	
Total consumption	(g DM/kg $W^{0.75}$/day)	75.2	82.7	78.3	
Crude protein consumption	(g CP/kg $W^{0.75}$)	16.1	15.1	14.7	
Gain	(g/animal/day)	99.0[b]	114.5[a]	119.5[a]	10.2*
Consumption	(g total DM/live weight gain)	0.76	0.72	0.66	
Consumption	(g total CP/live weight gain)	0.16	0.13	0.12	

SOURCE: Taken from Ojeda, Arece and Cáceres, 2011.

Throughout the experimental period, DM, CP and CF were determined and, for the silages, pH and ammoniacal nitrogen N-NH_3/total nitrogen N_t (%) were also estimated. In animals, daily feed consumption was measured collectively and individual live weight was determined weekly. The trial lasted 7 weeks.

The mulberry forage supplied showed the typical bromatological characteristics of the woody species; it had outstanding high CP value (24–27%) and low CF (13–16%), giving it better nutritional value than any tropical grass (Martín *et al.,* 2007) guaranteeing a good quality basal diet. At the same time, the dried citrus skin had lower CP than in silage form (6.3% vs 7.2%). This variation is attributed to the drying process, in which losses of the volatile components containing nitrogen could occur as an effect of temperature increase.

Sheep response showed that the inclusion of an energy supplement in a mulberry diet (Table 9) allowed the animals to balance the nutrients better by incorporating a deficit element for adequate functioning of rumen activity and, as a whole, promoting a higher level of total intake of the ration. The most direct effect of this was observed with the incorporation of the citrus skin, in any of its conserved forms, as supplement, giving the highest weight gains. These gains were statistically higher than those found when supplying mulberry as sole feed, which indicates a better utilization of the nutritional potential of mulberry.

It was concluded that with the use of citrus skins either dehydrated or ensiled, response of the growing sheep is improved, although the utilization of mulberry as main feed in a diet for sheep does not contribute to the better use of the nutritive potential of this woody plant feed.

Another study using mulberry in sheep by Alpizar (2014) assessed the effect of supplementation with mulberry (*M. alba*) as concentrate replacer on the productive indicators of Pelibuey sheep under stabled fattening. Forty-eight male lambs were used and distributed randomly in four groups in a completely randomized design with 12 animals each and an initial average weight of 20.64 kg.

TABLE 10. Chemical composition and digestibility of the feeds used in the diet (%)

Feed	DM	CP	NDF	ADF	IVDMD	Ash	Ca	P
King grass	19.03	6.89	63.00	32.70	50.70	11.02	0.57	0.21
Guinea grass	21.18	10.58	65.80	36.00	52.10	9.65	0.65	0.13
Sugar cane	20.27	1.84	41.60	24.20	62.00	3.23	0.16	0.12
Mulberry	25.21	20.86	26.30	16.90	86.90	9.01	1.55	0.26
Concentrate A	81.44	21.64	14.00	5.70	92.50	5.85	1.05	0.59
Concentrate B	91.07	23.08	6.80	2.60	98.90	8.07	2.15	0.39
Mineral salts	85.67	---	---	---	---	---	14.29	6.23

Notes: DM = dry matter; CP = crude protein; NDF = neutral detergent fibre; ADF = acid detergent fibre; IVDMD = *in vitro* dry matter digestibility; Ca = calcium; P = phosphorus.
Source: Taken from Alpízar, 2014.

The four treatments substituted mulberry foliage for the usual concentrate at different supplement levels: M–1% (mulberry at 1% LW on dry basis (DB); M–0.75% (0.1 kg of concentrate/animal/day and mulberry at 0.75% of LW on DB); M–0.50% (0.2 kg of concentrate/animal/day and mulberry at 0.50% of LW in DB); and M–0% (0.3 kg of concentrate/animal/day).

Additionally, animals received a basal diet of *Pennisetum purpureum*, *Panicum maximum* cv. Likoni, sugar cane (*Saccharum officinarum*), molasses, urea and mineral salts (Table 10). Every 14 days, weight gain, feed consumption, feed conversion and some health indicators (haematocrit, infestation by gastrointestinal parasites, peripheral eosinophiles) were determined. The experiment concluded at 126 days, and at the end of the study an economic evaluation of the supplementation was made.

The mulberry showed excellent nutritional quality, with high CP content (15–20%) near to the value indicated by authors such as Sánchez (2002) and Martín *et al.* (2007), and with low fibre fractions, which were lower than those reported by Boschini and Vargas (2009). This makes this plant highly digestible, which was confirmed in the present study (Alpízar, 2014), and agrees with the part of the plant employed that did not included the woody stem. It is known that if the stem material is included, mulberry digestibility falls due to the presence of lignin (Omar, Hayo and Udén, 1999).

After 14 days all treatments showed a constant weight increase throughout the whole experimental stage, with no significant differences in the accumulated live weight at any of the weighings. Animals finished the fattening period without significant differences in the final weights, with 29.55 kg (Group M–1%); 32.16 kg (Group M–0.75%); 32.34 kg (Group M–0.5%); and 32.82 kg (Group M–0%).

There were significant differences ($P<0.05$) at each of the weighings, with treatment (M–1%) having lowest values, while group M–0.5% presented the highest consumption throughout the trial.

Responses obtained in DM consumptions in the different experimental groups could be the result of the joint ruminal biochemical reactions maximized with the synchronized protein and fermentable energy supply in the rumen, allowing better nutrient utilization (Cardozo, 2005). Mulberry and concentrates as supplements of high degradability help in the activity of the cellulytic bacteria. In this way, the balance between energy-protein, microbial growth, residual time of feeds in the rumen for their degradation, and ruminal cellulysis are improved (Suárez *et al.,* 2006).

Maximum consumption (1.047 kg DM/animal/day) was attained at 84 days by the animals of treatment M–0.50%. By the end of the experimental period, consumption (DM/animal/day) was: 0.945, 0.994, 1.004 and 1.021 kg for M–1%; M–0.75%; M–0.5% and M–0% respectively; without significant differences (P>0.05) between the last three treatments.

The final live weight of each group ranged between 29.5 and 32.8 kg and no significant differences were found between treatments, although the group receiving solely mulberry as supplement showed a lower final LW, corresponding to a lower DM consumption. Accumulated average LWGs were higher (P<0.01) in treatments M–0.75%; M–0.50%; and M–0% compared with the M–1% treatment. However, no statistically significant differences were found. It must be stressed that all treatments exhibited an average daily gain (ADG) >100 g/animal/day, considered to be very good for Pelibuey sheep.

At the same time, total DM consumption showed significant differences (P<0.01) with the highest intakes in groups M–0.75%, M–0.50% and M–0%. These values were lower than those recorded by Palma and Hurtado (1999) when evaluating different inclusion levels of *L. leucocephala* in the fattening of stabled Pelibuey sheep. They obtained DM intakes of 1.105, 1.079 and 1.058 kg DM/animal/day, when including 0, 10 and 20% of leucaena forage in the ration, respectively. These intakes were probably influenced by the quality of the complementary ration employed. However, these values were higher than those found by Reyes *et al.* (2009) using *Morus oleifera* (350 g and 500 g DM/animal/day) as a supplement to a diet based on *Panicum maximum* for crossbred (Pelibuey × Blackbelly) sheep, and finding total consumption of 730 g and 800 g DM/animal/day for each level, respectively.

An important impact of the study was that it was possible to substitute 66.6% and 33.3% of the concentrate in groups M–0.75% and M–0.50%, respectively, without significant differences in the main productive indicators of the animals, compared with animals supplemented with concentrates alone (M–0%).

From these results it is inferred that feed efficiency decreases with the gradual concentrate substitution with increasing mulberry levels. However, among the groups consuming mulberry as supplement, group M–0.75% presented the best feed conversion.

The animals showed good health indicators throughout the whole experimental period, which led to excellent productive indicators.

In México, Lara *et al.*(2007) measured the effect on the productive performance of grazing Pelibuey sheep and a mulberry (*M. alba*) protein bank. For that, 10 third-lambing ewes of 32 ±2.6 kg LW were used, considering each ewe as an experimental unit. Treatments were: control (treatment C) star grass grazing plus 250 g of concentrate feed/animal/day and restricted grazing (2 hours) in mulberry (treatment M). There was a difference (P<0.05) in ewe body condition at weaning, with values of 2.46 ±0.30 in treatment C and 2.00 ±0.24 in treatment M. Voluntary consumption (VC, as a percentage of body weight) of sheep was higher in mulberry, with 5.05 % during gestation and 2.97% in lactation regarding group C (4.28% and 2.28% respectively). Birth and at-weaning weights, as well as daily gains, were better (P<0.05) in the animals of group C, with 3.77 kg; 15.61 kg and 0.207 kg/animal/day respectively vs 2.82, 13.01 and 0.185 kg for the mulberry treatment, respectively. It should be noted that the prolificacy was lower in the control group, so the overall litter weights at lambing and weaning were higher with the inclusion of mulberry, with 5.86 and 23.41 vs 4.52 and 18.74 for treatment C. The authors concluded that with grazing of sheep in the mulberry bank restricted to 2 h daily during gestation and lactation, results can be similar to those obtained with the use of concentrates as supplementation source; hence, the utilization of mulberry is practical, since damage caused by the animals to the plants was minimal, and sprouting capacity was optimum for grazing at 70 days.

5. FINAL REMARKS

Mulberry (*Morus alba*), owing to its high nutritional value and low presence of secondary metabolites, can be considered as a woody plant of high potential for forage for animal feeding. Its calcium content allows it to be recommended for high potential cows during the initial lactation stages. However, its superiority in the Ca:P relationship could cause some infertility problems; therefore it is recommended only as part of the total diet.

Sheep experiments demonstrate that the leaves are very palatable and in bovines it shows potential degradation capable of supplying the requirements of the rumen micro-organisms. The biological availability of the nutrients contained in the foliages shows that this forage material is a promising substitute for the concentrate feeds commonly employed in dairy cattle feeding. As meal, it is a protein concentrate high in fibre and rich in energy.

With grazing dairy cows of medium potential, milk yields can exceed 8 kg/animal/day without using concentrates, and yields of 12.3 kg milk/animal/day are reported with fresh mulberry in the diet. Productive responses of ruminants fed mulberry as complement to the diet confer a high nutritional and socio-economic value to this resource under tropical conditions.

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Effect of mulberry (*Morus alba*) on the rumen ecosystem

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ABSTRACT

Studies of the effect of *Morus alba* on rumen ecosystems are very scarce, particularly in developing regions, such as Latin America. This chapter analyses investigations carried out on the effects of different mulberry genotypes on microbial populations, fermentative products and methane production under *in vitro* conditions, using rumen liquor of river buffaloes (*Bubalus bubalis*). The most promising lines for control of rumen methanogenesis were identified, as well as the mechanism involved in reducing methanogenesis in the rumen.

Keywords: Rumen ecosystem, microbial population, methanogenesis, *Morus alba*

INTRODUCTION

In Latin America there are few studies reported evaluating *Morus alba* in the rumen ecosystem. Considering that mulberry is a plant used in ruminant feeding and that in developing countries the main source of feeds for ruminants are pastures and forages, it was appropriate to carry out investigations evaluating the effect of mulberry on microbial populations and on rumen fermentation. Studies with mulberry in this region also have been focused on methane production in the rumen, since it is considered a gas with greenhouse effect, reflecting the great contribution made by ruminants to the production of environmental methane, and also for what this represents for animal production due to the energy losses that the formation of this compound causes.

1. EFFECT OF MULBERRY VARIETIES ON RUMINAL METHANOGENESIS

One of the strategies most used in the world, especially in developing countries for reducing methane production in the rumen is the use of species of trees and shrubs, since some of them present compounds that could act in the control of the rumen methanogenesis. Starting from this premise, Delgado *et al.* (2007) began the first studies in Cuba reported in the literature on mulberry evaluation for the control of methanogenesis in the rumen. In this study, on including 25% of mulberry in a basal diet of Napier grass (*Pennisetum purpureum*) cv. Cuba CT 115 the authors found that *Morus alba* reduced methane production from 26.2 mL CH_4/g fermented DM, corresponding to the control treatment of *P. purpureum,* down to 19.1 mL CH_4/g fermented DM. Later, also in Cuba, González (2010) and González *et al*. (2010, 2011), building on the earlier results, decided to extend their study with mulberry by assessing the effect of different mulberry genotypes on microbial populations, fermentation products and methane production under *in vitro* conditions, using rumen liquid of river buffaloes (*Bubalus bubalis*), and at the same time select the most promising genotype for the control of rumen methanogenesis. The mulberry lines evaluated were: Cuban, heart-shaped leaf, tiger-shaped and Indonesian with 30% inclusion in a diet based on star grass (*Cynodon nlemfuensis*).

From the previous studies it was found that the lines heart-shaped leaf, tiger shaped and Indonesian produced more methane than the control treatment of star grass during the first 24 h of fermentation in the rumen, and the Indonesian line had the greatest methane production. At the same time, the Cuban variety produced the same amount of methane as the control. Authors concluded that none of the four lines of *Morus alba* managed to decrease methane production in the rumen with 30% inclusion in the ration. However, taking into consideration that the Cuban line produced the same amount of methane as the control treatment, they recommended the evaluation of other inclusion levels of this genotype to see if it was possible to reduce methane production in the rumen.

At the same time, when these same authors assessed the effect of the inclusion of 30% of these four mulberry lines on the microbial population and rumen fermentation products, they found that as fermentation time progressed, the number of methanogens did not vary, except for the heart-shaped leaf line, where there was a decrease in the population of micro-organisms eight hours after fermentation started. When this finding was related to that of methane production they did not find the expected correspondence since they started from what was stated by various authors, namely that the methanogens are the microorganisms responsible for methane formation in the rumen (Attwood *et al*., 2008; Yáñez-Ruiz *et al*., 2008). According to this it was logical to expect that the lowest methanogen counts would belong to the Cuban genotype and not to the heart-shaped leaf line. Nonetheless, they attributed these unexpected results to the fact that counts of methanogenic micro-organisms were realized by traditional culture techniques, and that it has been demonstrated that many of these microorganisms are difficult to culture in the laboratory (Wright *et al*., 2007; Cook *et al.,* 2008). In this same sense, authors also recommended further studies employing molecular techniques for methanogen quantification.

2. EFFECT OF MULBERRY VARIETIES ON MICROBIAL POPULATION AND RUMINAL FERMENTATION

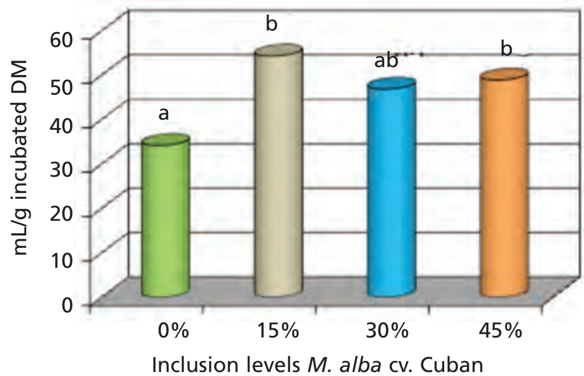
Regarding the other microbial populations of the rumen that could be quantified (total viable bacteria, cellulolytic, proteolytic and fungi) no variations were found when compared with the star grass control. However, protozoa counts were higher than for the Cuban variety.

With respect to the effect of these four mulberry varieties on the rumen fermentation indicators, it was found that even though the pH decreased, it was always maintained close to neutral. Authors state that this decrease is logical since the experiment was carried out under *in vitro* conditions and they support the criterion that in *in vitro* systems, the pH fall is due, among other factors, to the accumulation of organic acids. Ammonia concentrations in the rumen were not affected with the fermentation of any of the lines of mulberry assessed.

Taking into account all these results, authors concluded that mulberry lines heart-shaped leaf, Cuban, Tiger-shaped and Indonesian, when included at 30% in the diet do not have negative effects on the populations of micro-organisms degrading the fibre, do not affect pH or ammonia concentration in the rumen.

González (2012) considering results obtained by González *et al*. (2010 and 2011) that the Cuban genotype produced less methane than the other lines assessed and had no negative effects on the rumen ecosystem, selected this for further studies. Starting from the premise that the inclusion level of the plant in the diet is a factor influencing the fermentation and the control of methanogenesis in the rumen and that in previous studies 30% inclusion of the line Cuban has been already assessed, the author decided to bracket the inclusion level by a lower level (15%) and a higher (45%), with the objective of finding better results regarding the control of methanogenesis in the rumen.

FIGURE 1
Effect of different levels of inclusion of *M. alba* L. genotype Cuban, on methane production in rumen liquid of river buffaloes (*Bubalus bubalis*) (SE ±4.72) (*P*<0.05)



SOURCE: Taken from González, 2010.

Results were that methane production in the rumen was higher when 15% and 45% *M. alba* cv. Cuban was included with a star grass control treatment, but between the inclusion levels of this genotype there was no differences for this indicator. However the 30% inclusion showed the same performance as found by González *et al*. (2010), since it produced the same volume of methane as the control (Figure 1). At the same time, the microbial populations (total viable bacteria, cellulytic, proteolytic, methanogenic, fungi and protozoa) and the fermentation indicators in the rumen (pH and NH_3) were not affected with these inclusion levels of the line Cuban variety (Table 1).

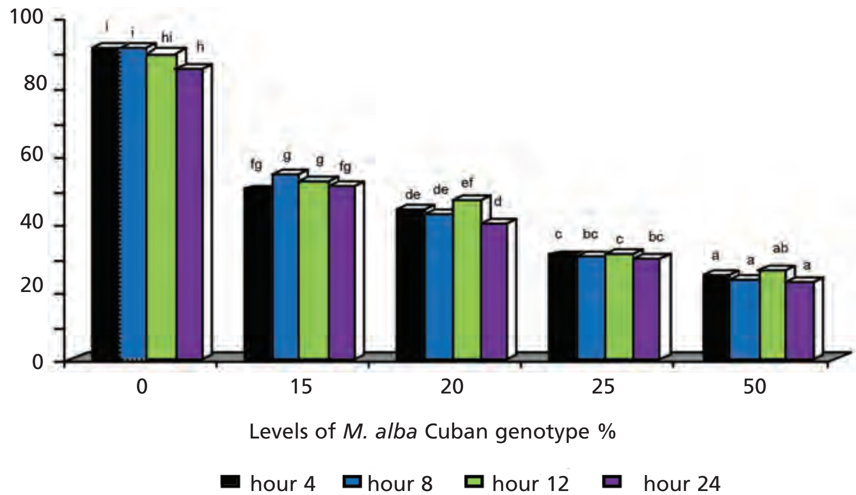
In the same way, González *et al.* (2012) in view of the results obtained by González (2010) and González *et al.* (2010) that 30% inclusion of the line Cuban showed the same methane production as the control treatment, and considering also the findings of

TABLE 1. Effect of different levels of inclusion of the mulberry (*M. alba* L. Cuban genotype) on the microbial populations and *in vitro* indicators of the fermentation in the rumen

Parameter	Treatments, *M. alba* L. Cuban genotype, %				SE
	0	15	30	45	
Total viable bacteria (10^{11} cfu/mL)	3.96 (66.33)	4.18 (79.36)	3.98 (65.43)	4.14 (74.02)	0.27
Cellulolytic bacteria (10^4 cfu/mL)	3.32 (36.34)	3.29 (28.03)	3.57 (42.38)	3.59 (40.15)	0.18
Proteolytic bacteria (10^6 cfu/mL)	3.11 (30.45)	3.22 (28.87)	3.34 (33.0)	3.48 (34.31)	0.1
Methanogenic bacteria (10^9 cfu/mL)	3.47 (54.26)	3.34 (48.33)	3.52 (47.37)	3.86 (58.30)	0.42
Cellulolytic fungi (10^3 cfu/mL)	3.20 (30.08)	3.19 (33.59)	3.10 (25.24)	2.95 (26.84)	0.20
Protozoa (10^4 /mL)	1.58 (6.25)	1.64 (6.25)	1.48 (4.98)	1.50 (5.08)	0.15
pH	6.57	6.58	6.61	6.68	0.06
NH_3 (mmol·L^{-1})	8.86	8.95	6.96	7.04	1.15

NOTES: cfu = colony forming units.
SOURCE: Taken from González, 2010.

FIGURE 2
Effect of different inclusion levels of *M. alba* L. Cuban genotype on methane production in the rumen (P<0.001)



NOTES: Different letters indicate differences at $P<0.05$, according to Duncan (1955); SE = ±2.16 for the hours at the same level of the treatments. SE = ±2.15 for the treatments at the same or different level of the hours.

SOURCE: Taken from González *et al.,* 2012.

González (2010) where 45% inclusion increased the production of this gas, they then decided to evaluate inclusion levels of *Morus alba* Cuban genotype which were between 15 and 30%, that is, they evaluated 15, 20, 25 and 30% inclusion levels, also using a basal diet of star grass.

In this study, it was found that the inclusion levels of 15, 20, 25 and 30% of the Cuban genotype reduced methane production in the rumen vis-a-vis the control treatment. Also, they observed that after four hours of fermentation all treatments including this mulberry variety started to decrease the methane production in the rumen (Figure 2). The authors noted that the reduction in methane production attained with 25% inclusion of the Cuban variety coincided with that previously published by Delgado *et al.* (2007). Also they indicated that 30% inclusion of this mulberry variety was the level that less methane produced and that the production of this gas was 3.8 times lower than the control and that, therefore, was the optimum for reducing methanogenesis. However, the authors state that even when 30% inclusion of the Cuban variety was the optimum for reducing the methanogenesis in the rumen, the levels of 15, 20 and 25% of this variety also attained the same objective.

It is noteworthy that in this research study the 30% inclusion of the Cuban genotype has led to reduction of methane production in the rumen, when papers published by González (2010) and González *et al.* (2010) always maintained similar methane production as the control treatment. Regarding this, González (2010) states that this could be due to the fact that in the experiments where 30% inclusion had a similar performance to the

TABLE 2. Effect of the different levels of *M. alba* L. Cuban genotype on fermentation indicators in the rumen

Parameter	Treatments, *M. alba* L.,%					SE ± E.E
	0	15	20	25	30	
Acetic (mmol/L)	25.30	20.95	20.55	19.12	17.82	2.06
Propionic (mmol/L)	5.34	5.06	4.56	4.28	4.53	0.43
Butyric (mmol/L)	0.67	0.73	0.56	0.52	0.55	0.08
SCFA (mmol/L)	31.44	26.90	25.68	23.75	22.24	2.59
NH_3 (mmol/L)	10.00	10.62	10.24	13.40	14.02	1.36
N-NH_3 /mg/100 mL)	14.01	14.87	14.33	18.76	19.63	1.90

Notes: [a,b,c] Different letters indicate differences (P<0.05) according to Duncan (1955). ***P<0.001
Source: Taken from González *et al.*, 2012.

control, the standard errors for the variable methane production were high as result of the loss of some samples during manipulation.

Concerning the effect of the inclusion of 15, 20, 25 and 30% of the Cuban genotype on the fermentation indicators in the rumen and the microbial populations, the authors found that the concentration of total or individual short chain fatty acids (SCFA) or NH_3 were not affected. However the lowest acetic-propionic relationship corresponded to the inclusion level of 30%. This allowed the authors to confirm what Russel (1998) and Lila *et al*. (2005) indicated regarding that methane production is related to the acetic-propionic ratio and that according to McAllister and Newbold (2008) the lowest methane production is associated with a tendency toward the lowest acetic-propionic relationship, and vice versa.

González (2010) on evaluating the Cuban variety as a strategy for controlling the methanogenesis in the rumen, considered the statement of Soliva *et al.* (2003) that due to the fact that one of the greatest merits of the ruminants is the ability for using the fibre, any strategy leading to reduction of methanogeneis in the rumen must be implemented in such a way that the digestion of the fibrous fraction is not decreased, nor affect the microbial populations intervening in this process. This reason, together with that of assuming the recommendations made by González *et al* (2010) regarding quantifying the microbial populations in the rumen by molecular techniques, with special emphasis on the methanogens, led to the populations of cellulolytic micro-organisms being quantified (*Fibrobacter succinogenes*, *Ruminococcus flavefaciens*, fungi) and rumen methanogens, using PCR in real time. The conclusion was that none of the inclusion levels of the Cuban variety assessed (15, 20, 25 and 30%) produced significant alterations in the populations of *F. succinogenes*, *R. flavefaciens* and fungi.

The inclusion of *M. alba* Cuban genotype in the ration can be used as strategy for reducing methane production in the rumen, with the comfort of knowing that it will not affect the main microbial populations degrading the fibre in the compartment. The analysis regarding the methanogens present showed that the populations did not suffer damage either. To better examine the population of methanogenic micro-organisms, denaturant gradient gel electrophoresis (DGGE) was used. This is one of the molecular methods allowing the study of the structure of microbial communities (Sigler, Miniaci and Zeyer, 2004). The author found that the different inclusion levels of *M. alba* Cuban genotype assessed did not produced marked variations in the methanogen composition present in the rumen liquid. This result matched the quantification of methanogens obtained by PCR in real time.

3. FINAL REMARKS

Results obtained suggest that the mechanism by which *M. alba* Cuban genotype affects methane production in the rumen is not by direct effect on the methanogens. All indications are that it involves the modalities of methane formation.

Also, it is suggested that when mulberry is employed in ruminant feeding, specifically buffaloes, the mulberry genotype be used must be taken into consideration, since although it is certain that all favour the digestion process of the fibrous materials and do not affect the populations of microorganisms of the rumen or the fermentation indicators in the rumen (pH, NH_3 and SCFA), the genotypes heart-shaped leaf, tiger-shaped and Indonesian increase methane production in the rumen, a negative attribute for the animal and the environment. In view of this, greater use of the Cuban genotype is proposed, at inclusion levels of 15, 20, 25 and 30%, since at the same time fibre utilization is favoured and methane production is reduced in the rumen.

It must be noted that all these evaluations of the mulberry effect on the rumen ecosystem have been made under *in vitro* conditions and only in rumen liquid of river buffaloes.

Studies *in vivo* are recommended, and also that future investigations include also other ruminant species.

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Agronomy of *Moringa oleifera* (Lam.) in agricultural systems in Latin America and the Caribbean region

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ABSTRACT

This chapter analyses agronomic parameters of *Moringa oleifera* in agricultural systems in Latina America, Cuba and the Caribbean region, with an evaluation of the performance of this plant relative to soil and climate requirements, and offers general recommendations for its use and exploitation. In addition, sowing and planting methods taking into account the use of vegetative cuttings or botanical seed are analysed. Different sowing rates are evaluated, bearing in mind productive purpose and the economic implications of the use of high sowing densities. Cutting frequency relative to volume and quality of biomass and pasture useful life are discussed. The importance of pruning and the implications for the productive life of this plant is analysed. The main pests and diseases affecting moringa are described. Finally, an evaluation of seed production and a general consideration of this plant's agronomy is recommended.

Keywords: agronomy, pests, seed production, *Moringa oleifera*

INTRODUCTION

Native to south of the Himalayas, northeast of India, Bangladesh, Afghanistan and Pakistan, *Moringa oleifera* is widespread globally, having been introduced into Central America in 1920. At that time it was used as ornamental plant and for live fences (Foidl, Mayorga and Vásquez, 1999; Carballo, 2011 pers. comm.). In Cuba, moringa was introduced as an ornamental plant at the beginning of the last century (Roig, 1988).

With the mass introduction from 2011 of this marvellous tree into Cuban agriculture, cattle production had a new source for animal feed, with excellent nutritional content and digestibility for animal and human nutrition.

The use of trees and shrubs is one of the most effective ways for improving the supply and quality of forages in cattle rearing systems (Robinson, 1985; Gutteridge and Shelton, 1994). In the search for new species, this plant stands out as promising among a group of non-leguminous trees, with potential for cut-and-carry; grazing and browsing; windbreaks; and live fence systems (Folk and Sutherland, 1996).

The use of moringa as a forage tree under the edaphoclimatic conditions of the tropics of Latin America and the Caribbean demands knowledge among producers and technicians regarding the agronomy requirements of this plant in agricultural systems of the region. This implies a need to disseminate results on the topic in a succinct way in reference texts.

In view of its great ecological plasticity, moringa is capable of adapting to the most diverse edaphoclimatic conditions. Its nutritional value and high biomass yields made it an important phytogenetic resource in the production systems that can used it as feed for diverse animal categories (Pérez *et al.,* 2010). Thus, the present study has as its objective an assessment of the main agronomy requirements for the use of *M. oleifera* in agricultural systems in Latin America, Cuba and the Caribbean region.

1. SELECTION OF THE AREA

1.1 Climate

M. oleifera stands out as a plant capable of adapting successfully to different conditions (Pérez *et al.,* 2010). There are reports on the occurrence of this plant in places where annual rainfall is between 300 and 1500 mm. It is cultivated in arid and semi-arid regions of India, Pakistan, Afghanistan, Saudi Arabia and East Africa (Ramachandran, Peter and Gopalakrishnan, 1980; Reyes, 2006; Croess and Villalobos, 2008).

Furthermore, it develops adequately at altitudes from 0 to 1800 masl (Duke, 1978; F/FRED, 1994), although better results are obtained below 600 masl (Palada and Chang, 2003). In studies of cultures completed at a height of 1200 masl, seeds germinated properly, but plant growth was very slow.

Moringa has a wide range of adaptation to different temperatures. García (2003) states that in Central America this plant is localized in zones with average temperatures between 6 and 38°C, although is capable of withstanding low temperatures, but not below 2–3°C. In addition, it is important to note that once temperatures drop below 10°C the flowering process is harmed, so in these cases dispersal must rely on vegetative cuttings. In this respect, Falasca and Bernabé (2008) stated that in its natural habitat, annual mean temperatures show large fluctuations. During the coldest months it is between –1°C and +3°C, while in the warmest months it is from 38°C to 48°C.

1.2 Soil

The soil is one of the limiting factors in plant development, with its characteristics determining potential, in most cases through pH. In this sense, Reyes (2006) indicated that moringa thrives in soils with pH between 4.5 and 8, but prefers neutral or slightly acid soils. Moreover, it requires loam to loamy-clayey soils. It does not tolerate clayey or vertisols, nor those with bad drainage. It adapts to hard or heavy soils and also to poor sandy loam soils. The field where it is planted must have good drainage, since this plant does not withstand waterlogging. In general, it can be said that it is a species of great ecological plasticity, since it is to be found under a wide range of soil, rainfall and temperature conditions.

Moringa can establish well in most soils, without added fertilizer, since its wide and deep root system allows efficient absorption of soil nutrients (Palada and Chang, 2003). However, as moringa is a tree with the capacity to generate high volumes of biomass, it must be remembered that high productivity implies great extractions of nutrients from the

TABLE 1. Nutrient extraction with different productivity in moringa

Productivity (t DM/ha/year)	Nutrient extraction (kg/ha/year)								
	Ca	P	Mg	K	Na	Cu	Zn	Mn	Fe
130	1612	338	429	1924	24.7	0.68	3.1	4.6	45.7
100	1240	260	330	1480	19.0	0.53	2.4	3.5	35.2
80	992	208	264	1184	15.2	0.42	1.9	2.8	28.1
60	744	156	198	888	11.4	0.31	1.4	2.1	21.1
40	496	104	132	592	7.6	0.21	0.9	1.4	14.0
20	248	52	66	296	3.8	0.10	0.4	0.7	7.0

Source: Taken from Foidl, Mayorga and Vásquez, 1999.

soil (Table 1) and hence requires a good fertilization programme to maintain production stable over time.

Earlier data was confirmed by the studies conducted by Reyes (2004), who obtained optimum yields with a density of 750 000 plants/ha during the first year. However, these yields were not maintained over time due to soil nutrient depletion in the absence of fertilization. At the same time, Arauz and Romero (2009) assessed different fertilization levels (0, 50, 100 and 150% of the culture requirements). These authors confirmed that fresh matter yield increased as the nutritive requirements of the plants were better satisfied.

Other authors, such as Alfaro and Martínez (2008), recommended the application of nitrogen sources for favouring the protein formation that constitutes the greatest potential of this plant.

In India, it was demonstrated that the application of 7.5 kg of manure plus 0.37 kg of ammonium sulphate per tree tripled the leaf sheath yield of this plant (Alfaro and Martínez, 2008).

On evaluating the effect of the application of poultry manure (0, 5 or 10 t/ha) on the growth and yield pattern of moringa plants, Uchenna, Uchenna and Baiyeri, 2013) observed that plants cultivated with 10 t/ha showed the greatest response for height, stem thickness and number of leaves in the first 12 weeks after sowing, and gave greater yield. In contrast, flowering was earlier in plants fertilized at 5 t/ha.

More recently, in Cuba, Lok and Suárez (2014), studying the effect of the application of different manures and biostimulants on biomass production and on some agrochemical indicators in cv. Supergenius, found that the best contributions to productivity (6.61 t DM/ha) and soil fertility (P: 136.56 ppm; Ca: 1.89%; Mg: 0.38%; and OM: 0.83%) were obtained when a combination of 25 t/ha of cattle manure and 20 kg/ha of EcoMic were applied.

2. SOWING AND PLANTING

2.1 Sowing season

Suitable sowing time selection is a key element in the establishment process, especially ensuring that there is appropriate soil moisture in the seedbed to give effective germination. According to Reyes (2004) the optimal season varies according to locality, but, in general terms, sowing must be carried out during the rainy period, when soil humidity is adequate for germination and establishment. This author also stated that for places with well defined rainy periods, sowing must be done at the beginning of the rainy season. However, in areas with irrigation, it can be sown in any season of the year provided the frequency and water volume applied to the soil is guaranteed to ensure germination and survival of plants during the establishment stage.

2.2 Establishment methods

Establishment can be made both with botanical seed and by cuttings. This will depend on factors such as seed availability. In certain localities, flowering is difficult, due to climatic conditions, and then establishment must be by cuttings. It is important to know that trees obtained from seeds produce stronger and deeper roots; thus in arid and semi-arid regions, sowing by botanical seed is preferable.

2.3 Use of botanical seed

When sown, botanical seeds germinate after about 10 days if fresh, but the germination percentage decreases as the seeds age. Moringa seeds have no latency period and can be sown as soon as they are mature, a factor to be taken into account by the producer at the time of buying the seed.

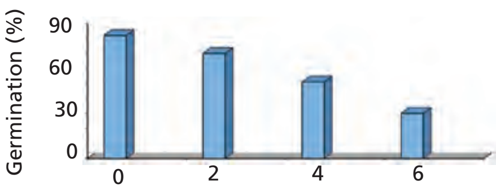
Nevertheless, seed quality loss during storage is an irreversible process, starting from harvest time. This increases in periods of high temperatures and relative humidity, when biochemical changes are induced in the seed, and fungi and insect attacks are more prevalent. At the same time, low temperatures favour greater seed longevity. In that regard, Villa-Ramos, Fernández-Olano and Diez-Núñez (2013) assessed the percentage of germination of the seeds of *M. oleifera* post-harvesting (Figure 1). A significant fall in germination percentage and vigour was found as the storage time increased, and the natural aging of these seeds was a slow and progressive process.

Seeds stored under poor conditions that favour development of insect pests, such as *Carpophilus* spp. (Coleoptera-Nitidulidae) usually cause an important reduction in germination capacity.

The declining germination of seeds as storage time increases was also found in *Albizia lebbeck* seeds (Navarro, 2003). At the same time, under commercial production conditions, Sharma and Raina (1982) and Padilla, Fraga and Suarez (2012) found fresh seeds of this plant having high germination rates (between 60 and 90%), while Villa-Ramos, Fernández-Olano and Diez-Núñez (2013) found germination percentages between 88 and 71% in seeds harvested in fields treated with organic fertilizer.

Sharma and Raina (1982) indicated that seeds of this species do not require pre-germination treatments. However, germination can be favoured by soaking the seed for 24 h in water at room temperature before sowing. Although it is true that some reports indicate that soaking is a favourable option, others say this practice is not necessary (Medina *et*

FIGURE 1
Change in seed germination of moringa during storage



Source: Ramos *et al.*, 2013.

al., 2007a). In Cuba, Padilla, Fraga and Suarez (2012) studied the effect of soaking the seeds of this plant for 0, 24 and 48 h on germination percentage and growth indicators of the plants. Pre-germination soaking for 24 h advanced germination, with 86% sprouting between days 11 and 15 after sowing, giving the young plants a good start (Photograph 1). As *Moringa oleifera* is a plant of rapid growth, a pre-germination soaking treatment for 24 h accelerates establishment ahead of weeds. However, pre-germination soaking for 48 hours is not recommended because it can affect the total germination percentage of seeds by encouraging development of rots.

In Brazil, Cardoso *et al.* (2006) studied the influence of the seed position and sowing depth on the emergence and development of plantlets. A depth of 2 cm gave better rate of emergence, and seeds placed with the apex upward produced greater emergence and aerial biomass production.

In India, the germination percentages of seed of *M. oleifera* were 60, 48 and 7.5% at 1, 2 and 3 months after harvesting, respectively (Sharma and Raina, 1982). However, Villa-Ramos, Fernández-Olano and Diez-Núñez (2013) found that seeds maintained adequate germination percentages even 4 months after harvest (51%), which could be attributed to lower humidity and temperatures in Cuba. Nevertheless, there germination decreased with storage time.

Another factor reducing seed germination over time is seed rots, which occurs with greater frequency under uncontrolled storage conditions. This was found by González, Hernández and Mendoza (1998) in *Leucaena leucocephala* cv. Cunningham seeds stored at environmental temperature, where, as the storage period was prolonged, rotten seed numbers increased. In this case, seeds were also damaged by micro-organisms, reflected in poor germination and quality of the germinated seeds.

Therefore, there is need to ensure good storage conditions, since the micro-organisms and harmful insects proliferate more when relative humidity and temperature in store are higher. Fungi are the micro-organisms that most abound in the microflora associated with the stored seeds and, in turn, constitute the most influential cause of loss and quality decline during storage. This study isolated *Aspergillus flavus*, *Penicillium* spp., *Cladosporium herbarum* and *Fusarium* spp.

2.4 Plantation establishment by cuttings and seedlings

Moringa cuttings cut at the end of the dry season show 90% survival. For obtaining these high stem germination percentages, the cuttings are left to take root, and later transplanted to the final field site, which must have a good moisture level. Once cut, it is good practice for the cuttings to be rooted by placing them vertically under shade and burying the stem 10 cm in the soil. In regions of Asia, cuttings from large braches from 1 to 1.4 m long and 4 to 5 cm in diameter are usually planted during the rainy season; also planted as posts in humid soil where they easily root and in a few months reach the size of a tree (Carballo, 2011 pers. comm.). On the trees obtained from cuttings, fruits appear 6 months after planting. In this respect, Ramachandran, Peter and Gopalakrishnan (1980) concur with the length and diameter of the cuttings mentioned above, but admitted that in arid and semi-arid regions it is better to establish by sowing with botanical seed, thus producing deeper roots.

Results obtained in Cuba suggest that for producing forage or seeds, cuttings used for planting must have a diameter >8 cm and a length of 1.2 m, whereas when they are going to be used as live posts the length must be of 1.5 to 1.8 m. For both purposes, it is recommended that cuttings must be removed during the waning moon stage at the end of the dry period, to be planted out at the end of May and beginning of June, once the rainfall has stabilized.

3. NURSERY OF MORINGA SEEDLINGS IN BAGS

At the Institute de Tecnología de las plantas of the Universidad Central "Marta Abreu" of Las Villas, Cuba, a methodology for the *in vitro* propagation of moringa was developed through the cutting of scions from a small bank of donor plants in the field. In this respect, Jiménez and Agramonte (2012) confirmed that plants obtained by scions and those from *in vitro* culture showed better growth than those obtained by spikes in the initial growth stage in the field. This result opens the possibility of a sustainability alternative for the intensive multiplication of this multi-purpose plant when introduction is proposed of new cultivars of different origins for exploitation at commercial scale.

Medina *et al.* (2007b) reported that, in Venezuela, transplantation of moringa seedlings to the field can be practiced from the seventh week in the nursery. However, it is more general to transplant when the seedlings reach a height of 30 to 40 cm, with excision of the terminal bud before planting. When it is not possible to plant direct into soil, then use plastic bags filled with 3 parts earth and 1 part sand, planting two or three seeds in each bag at 0.5 cm depth. Germination will occur within two weeks. Extra plants must be removed, leaving one in each bag. Plantlets can be transplanted after four to six months when they reach 60–90 cm height.

4. CHARACTERIZATION OF EIGHT ACCESSIONS OF MORINGA UNDER NURSERY CONDITIONS

In Cuba there is very little information on *M. oleifera* performance when sown using seeds or seedlings. Therefore, at the Estación Experimental de Pastos y Forrajes "Indio Hatuey", Toral *et al.* (2013) carried out trials to characterize the performance of eight lines of *M. oleifera* under nursery conditions. The lines (Table 2) were eight accessions of moringa introduced from abroad or collected in-country.

Figure 2 shows results from the germination tests of the *M. oleifera* seeds under controlled conditions. Lines Plain and Holguín had best results, with 84% and 80%, respectively, while Guatemala (49%) and Paraguay (60%) had the poorest germination.

Germination rates reported in the literature (Jahn, Musnad and Burgstaller, 1986; Nautiyal and Venhataraman, 1987) fluctuated between 60% and 90% for fresh seeds, and most of the accessions in the Cuban trial were in this range. Also it is noted that the length of seed storage influences its germination rate. The low germination rate

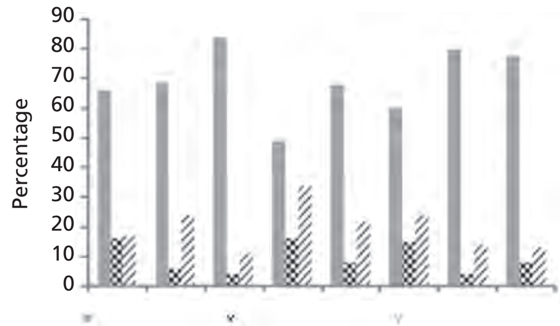
TABLE 2. Accessions assessed

	Plant material	Source
1	PKM-1	Introduced from India
2	Supergenius	Introduced from India
3	Plain	Introduced from India
4	Guatemala	Introduced from Guatemala
5	Criolla-Granma	Collected in Cuba
6	Paraguay	Introduced from Paraguay
7	Holguín-Mayarí	Collected in Cuba
8	Matanzas-Ciudad	Collected in Cuba

registered in accessions Guatemala and Paraguay in the study could be related to the time from harvest, since the seeds used were sown three months after ripening. At the same time, seeds received no pre-germination treatment, and it has been shown elsewhere that this can help to break seed dormancy and thus improve the germination rates (González and Navarro, 2001). There were significant numbers of rotten seeds, between 11 and 34%. This could be due to poor seed storage conditions that allowed a lot of infestation by pathogens. In this respect, Navarro (2009) found that viability loss during storage could be caused by insect or fungal attacks that destroy the physical integrity of the seed, or by loss of viability with age.

FIGURE 2

Germination tests (%). Lines were: (1) PKM-1; (2) Supergenius; (3) Plain; (4) Guatemala; (5) Criollo-Granma; (6) Paraguay; (7) Holguín-Mayarí; & (8) Matanzas-City



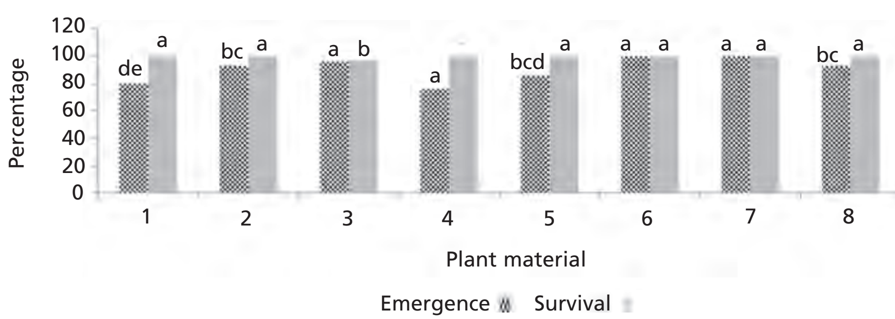
Source: Toral *et al.,* 2013.

Figure 3 shows the emergence and survival of moringa under nursery conditions. The Paraguay and Holguín accessions showed excellent emergence percentages, at 100%. Plain also stood out, with 97%. These three differed significantly from the other genotypes. Results from these lines of different origins are similar to those reported by Medina *et al.* (2007b), who evaluated the performance of *M. oleifera* and *Leucaena leucocephala* from the nursery stage until 30 days after sowing. Results showed emergence of 95% and 100%, respectively.

The lowest emergences were for Guatemala (76%) and PKM-1 (80%). This poor performance could be due to either seed storage conditions or to the 3–4 month delay

FIGURE 3

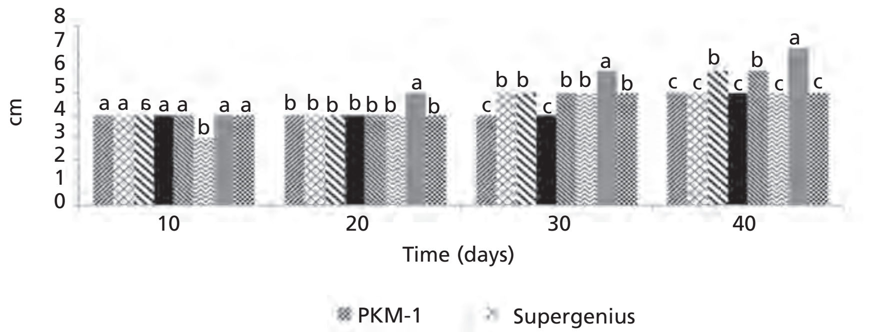
Emergence and survival (%) in the nursery



Legend: (1) PKM-1; (2) Supergenius; (3) Plain; (4) Guatemala; (5) Criollo-Granma; (6) Paraguay; (7) Holguín-Mayarí; (8) Matanzas-City. a,b,c,d,e, = Values with different superscripts differed at $P < 0.05$ (Duncan, 1955).

Source: Toral *et al.,* 2013.

FIGURE 4
Number of plantlet leaves



NOTES: a,b,c = Values with different superscripts differ at $P<0.05$ (Duncan, 1955).

between harvest and sowing. This agrees with the statements of Croess and Villalobos, (2008) concerning the loss of vitality when stored for more than two months. Seed ageing during storage is one of the main causes of reduction in seed quality and this results in loss of vigour and failure to thrive.

Plantlet emergence occurred from six days after seed sowing. The emergence index was lower than the results found by Bezerra, Momenté and Medeiros Filho (2004) on assessing the effect of substrate on the germination and development of this species in the nursery stage. These authors observed that the first emergences occurred between three and four days after sowing.

Regarding plantlet survival, there were no significant differences among the varieties of different origin with 100% survival, except Plain that with 97% was excellent. These survival percentages are similar to those reported by Medina *et al.* (2007b), who obtained 100% survival under similar conditions.

Figure 4 shows that genotype Holguín had a progressive increase in leaf number throughout the experiment, differing ($P<0.05$) from the others, which showed no increase in leaf number in the first 20 days; after that, Plain and Criolla-Granma were better than the others, showing statistically significant differences ($P<0.05$) at 30 and 40 days after sowing, although not exceeding 6 leaves per plant.

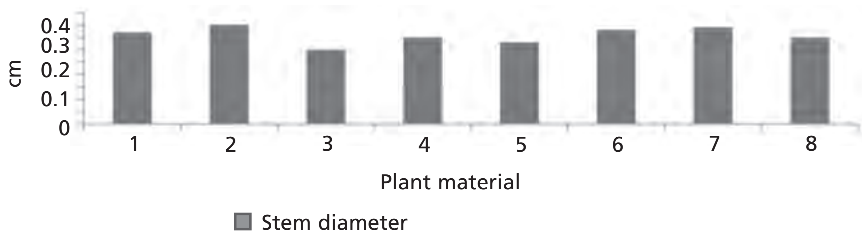
According to Moroto *et al.* (2000), *M. oleifera,* despite its non-legume status, compared favourably with legumes, as it presents a similar arbuscular branch architecture in its initial developmental stages. Such branches are typically scattered, of sturdier constitution and with numerous leaves.

Figure 5 shows plant diameter 40 days after sowing. In this respect, similar values were attained in genotypes, ranging between 0.33 and 0.40 cm.

Higher values were found by Pérez (2011) when assessing moringa under nursery conditions, but using different substrates. There, with a combination of red soil + earthworm-rich humus, plantlets had a diameter of 0.58 cm 42 days after sowing.

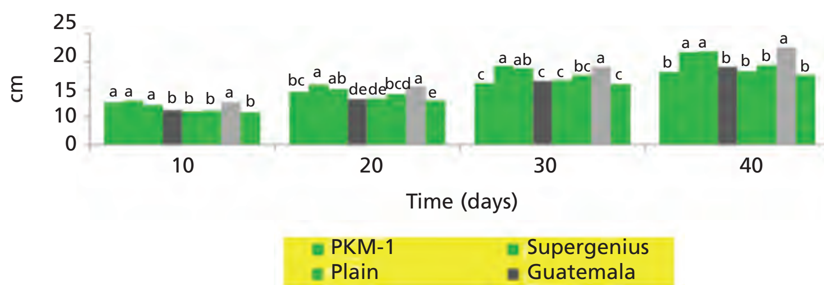
In assessments under nursery conditions, Medina *et al*. (2007b; 2011) studied the performance of moringa and other tree species on alkaline soil substrates, and noted

FIGURE 5
Stem diameter (cm) of the plantlets



LEGEND: (1) PKM-1; (2) Supergenius; (3) Plain; (4) Guatemala; (5) Criollo-Granma; (6) Paraguay; (7) Holguín Mayarí; (8) Matanzas-City.

FIGURE 6
Height of the plantlets (cm) in the different samplings



NOTES: ^{a,b,c,d,e} = Values with different superscripts differ at $P<0.05$ (Duncan, 1955).

that moringa surpassed the other species, exhibiting more rapid and progressive stem enlargement.

Concerning height (Figure 6), progressive growth was observed throughout the whole trial. Values attained showed significant differences at each measuring, with the best height values being for Holguín, Plain and Supergenius, at the end of the stage, and they did not differ. At 40 days after sowing their heights were 17.5 cm, 16.8 cm and 16.7 cm, respectively. The worst results for height were Matanzas-City, Criolla-Granma, PKM-1, Guatemala and Paraguay, with 12.45; 13.15; 13.1; 14.00; and 14.25 cm, respectively.

Accessions showed best development from 20 days, which was attributed to development of the plant root systems, thus guaranteeing water and nutrient absorption for subsequent development.

This performance pattern agrees with the results of Medina *et al.* (2007b), who stated that moringa, from the beginning of its growth, accelerates in growth terms, as it develops a very deep root system that makes greater use of soil nutriments and the available water.

Seemingly, the fast development of moringa is a peculiarity of the species. In this respect, Toral (2005), on evaluating the field establishment of 67 forage woody species,

TABLE 3. Growth dynamics (cm/day)

Days	Plant material							
	1	2	3	4	5	6	7	8
10	0.76	0.79	0.71	0.62	0.60	0.61	0.76	0.58
20	0.48	0.54	0.50	0.41	0.42	0.46	0.53	0.40
30	0.37	0.47	0.46	0.38	0.39	0.42	0.47	0.38
40	0.34	0.41	0.42	0.35	0.33	0.36	0.43	0.31

noticed that *M. oleifera* surpassed the rest as regards speed of establishment, including *L. leucocephala,* since by seven months it had reached the pre-fixed height of 2 m considered the threshold for starting exploitation.

Table 3 shows the growth dynamics of the various genotypes. The accessions Holguín, Supergenius and Plain exhibited the highest increase with 0.43; 0.41 and 0.42 cm/day, respectively, 40 days after sowing. Similar growth rate values in the nursery were found by Pérez *et al.* (2010) when they used unscarified moringa seeds. However, in a second trial, when the seeds were scarified, they found a greater growth rate, up to 0.57 cm/day, justifying what was previously reported on the process of seed scarification, namely that it creates much more favourable conditions for plantlet emergence. The plant material with the lowest growth rate was Matanzas-City, at 0.31 cm/day.

5. IRRIGATION

Moringa needs at least 700 mm of water annually, although there are reports from places on the Pacific coast of Nicaragua where it grows very well with 300 mm/yr. Good performance in places with annual rainfall of 2000 mm has been observed. Plantlets are susceptible to drought. Once established, the young trees and later stages are tolerant, and capable of surviving droughts, although when these are prolonged, plants can lose their leaves. A similar response to defoliation is produced by excess humidity, even in soils with good internal and superficial drainage (Reyes, 2006). In Cuba, when sowing is carried out during the poor rainy period, a common practice is the application of 200 m^3/ha of water every 2–3 days during the first month after sowing, and 300 m^3/ha every 9 days during the dry period. This application frequency can vary depending on the type of soil, being fewer on heavy soils with good water retention, and greater on light soils.

Wastewater can be used if it is below pH 8.5, which makes moringa a species suitable for using treated waters. In Cuba, González (2012) applied treated wastewater from pig facilities and obtained excellent results in biomass yields and plant survival in small areas of moringa sown on plots of land at a density of 100 seeds per m^2.

6. SOWING DENSITY

In forage production of moringa, as for all species, some aspects must be considered that promote its productive development. In this sense, sowing density is a determinant factor. In plantations where the sowing density is too high, there is a risk of intense inter-plant competition for the ecological niche, leading to poor yields. In this case, plants have reduced stem diameter and thinner shoots, reducing yield. In that respect, Foidl, Mayorga and Vásquez (1999) recommend a sowing density of one million plants/ha to obtain yields of 34 t/ha/cut. These authors also stated that density losses are minimal once the plants are pruned.

Nonetheless, Castillo *et al.* (2013) found that moringa leaf yield was greater (4.24 t/ha) at a density of 40 000 plants/ha, compared with higher densities.

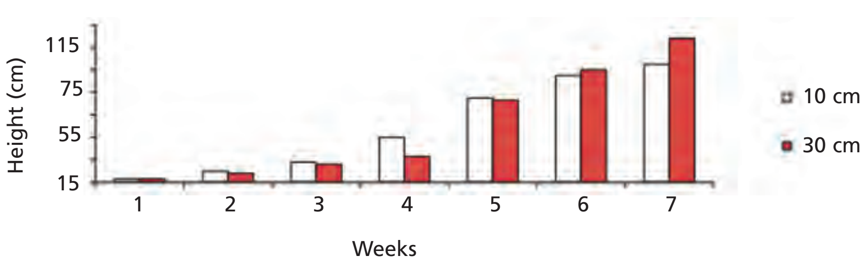
At the same time, the study of Meza *et al.* (2013) showed that in the initial phase (<100 cm tall), plants displayed less development with a density of 333 333 plants/ha, but the opposite with the lowest density (111 111 plants/ha). The highest final yield was attained with the highest sowing density.

In Cuba, the sowing of large areas of moringa indicate that the use of high sowing densities (0.5 to 1.0 million plants/ha) are not advisable due to difficulties for weed and fungal disease control. Under these circumstances, plants do not develop the vigorous stems or the deep rooting that favour abundant and strong secondary branches. In Nicaragua, Foidl, Mayorga and Vásquez (2011) has indicated that a high plant density leads to stiff competition among plants for light, and that has caused losses of up to 20% and 30% of the population per cut, which has directly reduced overall biomass yield per unit area. Additionally, the stem and shoot diameters are reduced, negatively affecting production, although large amounts of fresh matter have been obtained at the expense of the high density, but mainly in the first cuttings after sowing.

Plant growth differed between sowing densities, and the greatest initial growth was obtained from plants sown at 10 cm spacing (333 333 plants/ha). This performance matched that reported by Goss (2012, cited by Meza *et al.,* 2013), where it was noticed that a higher sowing density gave taller stems, but when plant attained heights >100 cm there was the most growth ($P<0.05$) in plants sown at 30 cm (111 111 plants/ha), as shown in Figure 7.

According to Padilla *et al.* (2014) the use of high sowing densities provokes severe depopulation as the exploitation time of the forage area is increased. They found that the population decreased over time from 45–46 plants/m^2 in the first cutting to only 11–12 plants/m^2 in the last cut (70% reduction). The decrease of the population in time was due to severe attacks of *Atta insularis* and fungi, mainly *Fusarium* spp. Performance was also affected by competition from weeds in the field, and mechanical damage produced by the cutting regime, facilitating opportunist pathogen invasion.

FIGURE 7
Plant height (cm) as a factor of sowing density



Source: Adapted from Meza *et al.,* 2013.

TABLE 4. Plant population and seed rate for different purposes in moringa

Population (plants/ha)	Sowing frame	Seed dosage (kg/ha)
	For forage use	
1 000 000	10 cm × 10 cm	328
250 000		82
160 000	50 cm × 12.5 cm	54
80 000	50 cm × 25 cm	27
	For seed production	
1 333	3.0 m × 2.5 m	
625	4.0 m × 4.0 m	
555	6.0 m × 3.0 m	

Notes: Seed assumed to be 85% germination and 3600 seeds/kg. Source: Adapted from Reyes, 2006.

In this sense, Reyes (2004) and Pérez *et al.* (2010) indicated that when high sowing densities are employed, competition among plants for nutrients, water, light and vital space increases, provoking plantlet losses that can reach 20% to 30% per cut and influence overall biomass production. This result of the plant density decrease of *M. oleifera* over time was found in Cuba when mass sowings of this plant were made using sowing densities of 500 000 and 1.0 million plants per ha.

The best practical results for yield and persistency in Cuba by producers have been obtained with sowings of approximately 10 plants/m^2 in furrows 70 cm apart, which has favoured weed control using animal-drawn equipment or specialized machinery. According to Palada and Chang (2003), a good population for forage production is achieved by sowing 50 cm between plants and 1.0 m between furrows. In Nicaragua, Reyes (2006) recommends sowing in furrows 40 cm apart and with 10 to 20 plants per linear metre, equivalent to 250 000 to 500 000 plants/ha. According to this author, the sowing density to be used must be closely linked to the productive purpose (Table 4).

Another aspect to borne in mind with the use of high sowing dosages is the cost, since a dosage of 328 kg of seed, at a price of 41.00 pesos/kg, costs some 13 500 pesos/ha. The practical experience in Cuba at a commercial scale has confirmed that the best results are attained with sowing frames ranging between 0.7 and 1.0 m between rows, guaranteeing sowings of approximately 10 seeds/m^2. This notably reduces the establishment cost and facilitates the use of machinery and agricultural implements, as well as animal drawn vehicles, for culture labour and weed control.

Evidently the scientific basis for sowing densities for moringa still needs further research to establish recommendations for various uses, and to adjust to the typical machinery and implements of the primary producer, suitable for machine cultivation for weed control, while optimizing sowing rates to maximize establishment and reduce cost per ton of forage produced. Not less important would be to find the biological responses of the plants over time regarding persistency and biomass production per plant and per ha, when high, medium or low sowing densities are used in forage areas for animal feed (Photograph 1).

7. HEIGHT AND CUTTING FREQUENCY

Cutting height together with the cutting frequency will influence the useful lifespan of forage areas, as well affect the quality and quantity of biomass produced. In this regard, most studies cut at heights of 10, 20 and 30 cm, confirming that the 10 cm cutting height affects biomass production and population over time. There are studies indicating that the first cut should be at 5 or 6 months after sowing; that cutting must be performed with a well sharpened machete every 45 days in the rainy season and every 60 days in the dry season; and at a height of 20 cm from the soil. Moringa has good sprouting capacity and production of fresh forage when sown at high densities, although the cutting response is

Photograph 1. A vigorous stump in a low sowing density plot.

negative when the stem diameter is small (5–10 mm) indicating that its shooting capacity is limited (Reyes, 2006). At the same time, Manh, Dung and Ngoi (2005) in Viet Nam proposed that plants can be harvested 7 times per year, with fresh biomass yields of 43 to 52 t/ha, while in Nicaragua, Reyes (2006) found maximum annual yields of dry matter of 24 to 10.4 t/ha during the first and second years, respectively, when cutting was every 75 days.

Padilla *et al.* (2014) used cutting frequencies of 45 and 60 days on the Cuban cv. Criolla sown at 100 seeds/m² in a red ferrallitic soil, and monitored the population over time. There were significant differences in the number of plants/m² at the beginning and end of the experiment for the cutting frequencies of 45 and 60 days (Table 5), and the population decreased in time from 45–46 plants/m² at the first cutting, to only 11–12 plants/m² in the last (a 70% reduction). This population decrease in *M. oleifera* over time reflected the knowledge gained in Cuba when massive sowings of this plant were made using sowing densities of 500 000 and 1 million plants/ha. These high populations, together with low cutting heights, make manual and mechanized weed control difficult, provoking the death of plants due to pests and diseases. Sowings with high densities of 1 million plants/ha implies high seed and labour costs. (See Photographs 2 to 6).

According to Padilla *et al.* (2014), when biomass production between the first and last cuttings was compared, it was found that the lowest yield was when harvested every 45 days (Table 5). However, for both cutting frequencies there was yield reduction when after two years the last cutting was compared with the first.

On comparing biomass production for two years in the rainy and poor rainy periods according to the cutting frequency (Table 6) in this same experiment, it was evident that the best yields of forage in the rainy season and in total were attained with the 60-day cutting

TABLE 5. Influence of exploitation time and cutting frequency on the population and yield of *M. oleifera*

Parameter	Time	Cutting frequency		SE±
		45 days	60 days	
Yield, t DM/ha	Initial	2.46[bc]	2.99[c]	0.17*
	Final	0.43[a]	1.93[b]	
Population plants/m²	Initial	6.81[b] (46.38)	3.42[a] (11.70)	0.16***
	Final	6.71[b] (45.02)	3.24[a] (10.50)	

NOTES: ( ) Original means; data transformed according to √x.
* = $P<0.05$; *** = $P<0.001$.
SOURCE: Padilla *et al.*, 2014.

Photograph 2. Seedbeds – density 1 million plants/ha.

Photograph 3. Rustic dibbing equipment for marking holes for placing the seed.

Photograph 4. Using the holes for placing the seeds.

Photograph 5. Men sowing.

frequency. The low yields found in this experiment (7 t DM/ha) are linked to heavy attacks of *Atta insularis,* with greater frequency in the dry period, when only one cutting was feasible due to the severe damage by this pest. Yield in the dry period was similar for the two cutting frequencies used. In the second year of the cutting frequency did not affect yield in the rainy period or in total. Nonetheless, in the dry period, yield was higher when cutting was every 60 days.

Photograph 6. Established field.

Reyes (2006) indicated that there is no connection among studies developed regarding the response of biomass production of moringa under differing cutting frequencies. Reyes (2006) indicates that the local studies are needed to adjust for edaphoclimatic conditions and different moringa genotypes

The best performance of *M. oleifera* from the Criolla de Cuba genotype in terms of persistency and biomass production when harvested every 60 days, matches other studies that found a better response at higher cutting frequencies. Thus, González (2012), in the western region of Cuba on a red ferrallitic soil and studying the growth curve of the Supergenius genotype, found that the best performance for quantity and quality of biomass was from 90-day-interval cuts.

Santiesteban *et al*. (2012) carried out other studies with the Criolla genotype in the eastern region of Cuba in an alluvial soil of Cauto Valley. The indicators analysed were effect of cutting height (10, 20, 30 or 40 cm) and cutting frequency (45 or 60 days) on biomass production for animal consumption. Padilla *et al.* (2012b) on studing cutting frequencies of 10, 20 and 30 cm, concluded that cutting height affects plant yield, but the higher cutting height favoured plant density improvement, leaf/plants and secondary twigs/plant, mainly in the third and fourth cuts. This could be an indicator for moringa cutting height until further studies are made combining height and cutting frequencies in order to optimize forage production. It is concluded that the best cutting height of the plant should be between 20 and 30 cm. It is recommended to extend this research for a longer period. They found a tendency towards increasing biomass as height and cutting frequency increased, with greatest values from a cutting height of 40 cm and a cutting frequency of 60 days. Also, in Mexico, Castillo *et al.* (2013), when assessing the effect of pruning frequency (60 vs 90 days) on total aerial biomass yield, found that overall yield was better with 90-day cuts, giving 10.83 t/ha. In addition, the chemical composition was similar in the leaf and edible stem of the plants for the cutting frequencies studied. Even though it is certain that low or medium cutting frequencies and heights will give more biomass, it must not be forgotten that these values come from studies using high sowing densities with the crop managed for production purposes, but at a small scale. There will therefore be uncertainty regarding outcomes over time regarding productivity and lifespan of the forage areas. This uncertainty will become more evident when monitored at a larger scale, with the need to make rational use of mechanization for increasing productivity and reducing production costs, primarily for weed control.

TABLE 6. Performance of biomass production during two years according to the season and the cutting frequency

Years	Period	Cutting frequency		SE±
		45 days	60 days	
First	Rainy	5.41	7.05	0.37*
	Dry	0.28	0.47	0.08
	Total	5.59	7.51	0.30**
Second	Rainy	2.46	2.12	0.30
	Dry	0.83	1.98	0.21**
	Total	3.29	4.01	0.48

NOTES: **P*<0.05; ***P*<0.01. SOURCE: from Padilla *et al*., 2014.

Another important management aspect is that when low population densities are used, more vigorous plants are obtained and with more branches (Photograph 6). To exploit this, it will be essential to cut at approximately 1.20 m from the soil, and to get more sprouts per stump, giving a vigorous tree with good potential for biomass or seed production.

8. PRUNING

Pruning constitutes an important part of the management to give greater yield. When trees are cultivated for forage or for fruit, they need to be pruned to restrict top development and stimulate new lateral branch growth (Ramachandran, Peter and Gopalakrishnan, 1980). On this topic, Nautiyal and Venhataraman (1987) reported that pruning moringa gave 4 to 8 vigorous sprouts per stump. In general, moringa is a tree that is capable of producing new sprouts quickly; (Foidl, Mayorga and Vásquez, 2011).

In plantations destined for seed production, pruning is used to control maximum plant height; decrease negative effects of meteorological events; eliminate damaged branches; eradicate low branches obstructing access to the area; and facilitate fruit harvest. Pruning thus stimulates appearance of young stems, favouring the flowering and fructification that contributes to better seed yields per plant and per ha.

The importance of and need for pruning can be summarized as managing to attain a healthy, adequate and balanced plant structure, in such a way that will promote efficient productivity (Farrés, 2001).

According to Iglesias (2014), the main objectives of pruning can be summarized as:

- good distribution of branches;
- adequate air circulation;
- better conditions to withstand the fruit weight;
- better daylight penetration inside the top;
- better plant health;
- lower pest and disease incidence;
- greater flowering and fructification;
- rejuvenation of old plants;
- height control;
- harvesting planning; and
- control of fruit size.

Various types of pruning can be used:

- Training or conduction pruning has the objective of shaping a plant structure that facilitates its management and promotes greater production.
- Cleaning up or maintenance pruning consists of removing sick or unproductive branches from sprouts, or reducing the number of branches to maintain the structure needed, and to rid the plant of all structure hindering its efficient functioning.
- Production pruning consists of the eradication of all branches that have already produced and of others that are unproductive. On occasion, removing fruits to guarantee the quantity and quality of the next harvest.
- Rehabilitation and rejuvenation pruning is made when a plantation has lost its total or partial productive capacity and needs to recover.

Falasca and Bernabé (2008) stated that *M. oleifera* has a tendency to quickly produce a tall tree with few branches. In one or two years, most leaf production could be out of reach for picking. Hence it must be pruned to maintain an accessible plant height.

Moringa tolerates pruning very well, producing vigorous new shoots after each cut, with four to eight sprouts per stump. Trees cultivated for fruit and for forage are frequently pruned to restrict top development and to promote new branch growth. After pruning, each cut must be treated to avoid possible fungal infection.

9. SEED PRODUCTION

In view of the importance of seeds for propagation, it is interesting to know the seed production behaviour of moringa. Parrota (2003) indicated that fruit production during the first two years of life of the tree tends to be poor. However, from the third year, a single tree can produce from 600 to 1600 kg of fruit per year. CAC (2005) reported that seed content

Photograph 7. Ornamental moringa tree with good seed production.

per fruit ranges between 12 and 25, so each tree can produce from 15 000 to 25 000 seeds per year. Elsewhere, Mohammed *et al.* (2003) obtained yields of 3000 kg seed/ha, a yield comparable with soybean.

The fruit is a dehiscent triangular capsule, 20–40 cm long, although there are other genotypes from India that exceed 100 cm. Seeds are blackish, rounded and with a tissue by way of “wings”. There are variations in seed weight according to genotype, from 3000 to 9000 seed/kg. Between 3600 and 4000 seeds/kg, with an average of 3800 seeds/kg in the Criolla of Cuba genotype (Colectivo de Autores, 2014) (Table 7).

As mentioned earlier, Becker and Nair (2004) did not recommend a sowing frame of 1.0 × 1.0 m (= 10 000 plants/ha) for seed production, since as time elapses, trees must be eliminated to provide the vital area for each plant and to avoid competition among them. Likewise, Bosch (2009) stated that, for seed production, between 700 and 1100 trees must be sown per hectare, with a sowing frame of 3×3 m or 3×4 m, where the individual productivity of each tree will be better since it has access to more nutrients and more light, with less competition among them and higher growth and production.

In Cuba the highest seed production obtained was 350 to 450 kg/ha/year. Old trees with a good top in live fences or as garden ornamentals are capable of producing a high number of capsules (Photograph 7).

10. PESTS

Pest and disease incidence in *M. oleifera* varies. Foidl, Mayorga and Vásquez (1999) claimed that ants, such as the Large-headed ants (*Atta* spp.), and the *Mocis latipes* moth are the worst pests on the plants immediately after germination, normally responsible for a single attack and not returning, although these authors consider that control measurements must be implemented to reduce the damage they cause. In studies by Reyes (2004) under nursery conditions, the pests most affecting the plants in the first development stages are *Atta* sp. and *Mocis latipes*. This author also refers attacks of *Coccus* sp. and to a lesser degree by *Aceria sheldoni*.

Since the 1980s, studies carried out by Duke (1983) reported various pest organisms. Among them he mentioned the fruit fly (*Gitona* spp.) infesting the fruits and causing rotting.

Also different species of weevils (*Myllocerus discolor* var. *variegates*, *M. undecimpustulatus*, *M. tenuiclavis*, *M. viridanus* and *Pitochus ovulum*) that attack the leaves of young plants and recently planted stumps. He also notes attacks by parasitic plants, such as *Dendrophthoe flacata,* and phytopathogenic microorganisms: fungi attacking the plant include *Cercospora moringicola*, *Sphaceloma morindae*, *Puccinia moringae*, *Oidium* spp. and *Polyporus gilvus*.

In India, attacks from various caterpillars are reported, causing defoliation. The bud worms, *Noordia moringae*, *Noordia blitealis* Wlk, *Diaspidotus* spp. and *Ceroplastode cajani* are reported to be capable of causing serious damage. Also mentioned are the aphid *Aphis craccibora*, the stem drillers, *Indarbela tetraonis* (Moore) and *Diaxenopsis apomecynoides,* and the fruit fly *Gitona* spp. It is suggested that in any part of the world when *M. oleifera* is introduced, the local pests are less numerous (Anon., 2002).

Other studies in southern India emphasize that *Moringa oleifera* is very susceptible to wind damage, and note that this plant under humid conditions can be affected by the root-rot fungus *Diplodia* sp., and under seedbed conditions must be protected against termite attack (Ramachandran, Peter and Gopalakrishnan, 1980). In addition, a rotting of the fruit caused by *Cochliobolus hawaiiensi* has been is reported (Kshirsagar and D'Souza, 1989). At the same time, Ullasa and Rawal (1984) state that this plant hosts *Leveillula taurica,* which is a mildew that causes serious damage in papaya (*Carica pap*aya L.) fruit nurseries in the South Asian region.

Of major pests attacking moringa, the following have been mentioned: *Spodoptera* spp., *Phantomorus femoratus* and *Atta* spp., the last-named being of greater economic importance although in low population densities (García, 2003).

There are also researchers that assert that moringa is resistant or immune to pests and diseases (Medina *et al.,* 2007a) including phytoparasitic nematodes (Cuadra *et al.*, 2012). This could be attributable to some statements made to the effect that moringa leaves and seeds contain substances having bactericidal and fungicidal activity (Anon., 2010). In consequence there is apparently proof that moringa leaves incorporated into the soil prevent the attack of the plantlet fungi *Pythium* spp. (Anon., 2001).

Table 7 lists the pests and diseases that have been reported from Cuba and associated with some type of damage to *M. oleifera*.

In Cuba, *Atta insularis* Guerin (Hymenoptera:Formicidae) (Figure 8-A) is currently one of the organisms threatening the survival of the *M. oleifera* plantlets. The insects defoliate the leaves and young stems (Figure 8-B), to transport them to their burrows to provide a substrate for producing the fungus on which they feed.

Aphids (*Aphis* spp.) (Hemiptera:Aphididae) (Figure 9) were observed occasionally on young plants of *M. oleifera* that were produced under organoponic conditions (Valenciaga *et al*., 2012).

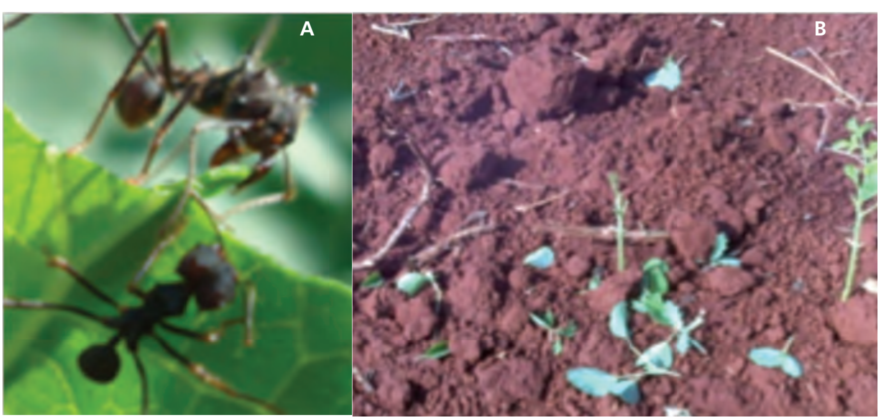
Valenciaga *et al.* (2012) also found attacks of a small scolytidae in moringa sowings. It pierced the plant, consuming the internal tissues. This attack allowed the secondary entry of other insects and phytopathogenic micro-organisms that caused rot and plant death.

Similarly, Alonso *et al.* (2012) assessed damage by insects and mites, as well as the infestation index of pathogenic micro-organisms in moringa sowings under organoponic conditions in the Nicaragua and Supergenius genotypes, and they found in both plant materials the presence of the insect *Ascia monuste* L. (Lepidoptera:Pieridae) (Figure 11).

TABLE 7. Pests and diseases associated with *M. oleifera* under Cuban conditions

Scientific name	Part of the plant attacked	Reference
		Insect pests
Atta insularis (Guér.)	Leaves	Campos, 2012
Aphis sp.	Stems, sprouts	Valenciaga *et al.,* 2012
Coleoptera: Scolytidae	Stems	Valenciaga *et al.,* 2012
Ascia monuste L.	Leaves	Alonso *et al*., 2012
(Unidentified)	Plantlets	Alonso *et al*., 2012; Campos, 2012.
Apate monachus (F.)	Stems	Martínez, Sánchez and Avilés, 2012
Stigmene acraea (Drury)	Leaves	Valenciaga *et al*., 2012; Martínez and Ramírez, 2014
Gitona sp.	Fruits	De la Maza, 2014; Valenciaga, unpublished data
		Diseases
Fusarium sp.	Stems	Alonso *et al*., 2012; Castellanos and Meléndez, 2012, Valenciaga *et al*., 2012
Colletotrichum spp.	Stems	Valenciaga *et al*., 2012
Colletotrichum spp.	Stems	Castellanos and Meléndez, 2012
Colletotrichum demathium	Stems	Alonso *et al.,* 2012
Curvularia spp.	Leaves	Castellanos and Meléndez, 2012
Helminthosporium spp.	Leaves	Castellanos and Meléndez, 2012
Alternaria spp.	Leaves	Castellanos and Meléndez, 2012
Cercospora spp.	Leaves	Castellanos and Meléndez, 2012
Cladosporium spp.	Leaves	Castellanos and Meléndez, 2012
Phoma spp.	Leaves	Castellanos and Meléndez, 2012

FIGURE 8
A. *Atta insularis*; B. *Atta insularis* defoliation symptoms



Apate monachus (Coleoptera:Bostrichidae), known as Free negro (Figure 12) was detected by Martínez *et al.* (2012) attacking the stems of *M. oleifera*. The females lay its eggs in the dry branches and the pest develops all its cycle inside the tree, the adult is fed from the live tissue of the xylem and provokes deterioration and progressive death of the host. The complete lifecycle is estimated to take from 87 to 190 days.

FIGURE 9
A. Aphids on *M. oleifera* sprouts; B. Nymphs and adults of *Aphis*

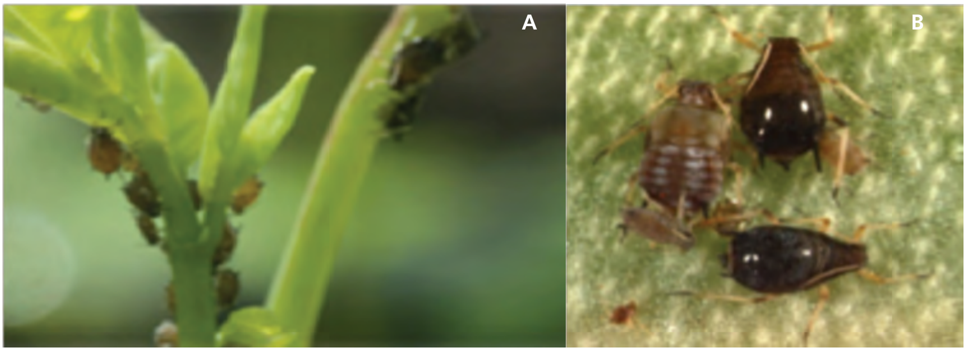


FIGURE 10
Adult scolytidae (Coleoptera:Scolytidae)

Recommended control measures include the removal of all dry branches, both within the plantation area and in adjacent areas. This helps to reduce the possibilities of invasion by adults that attack the live wood. Another control measurement is the burning of dead wood to eliminate the larvae of this insect that are developing inside the dry branches.

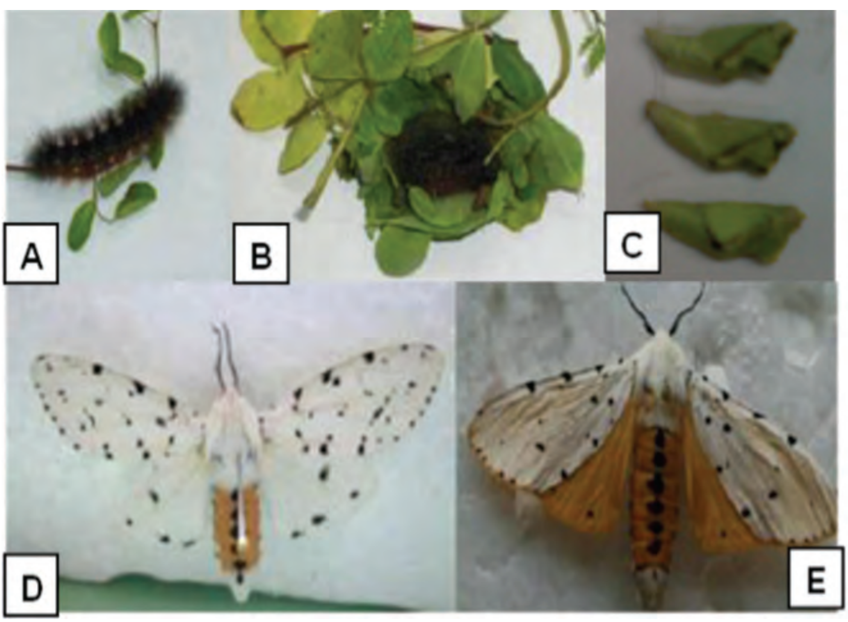
Studies by Valenciaga *et al.* (2012) in *M. oleifera* revealed the presence of and damage caused by hairy or shaggy larvae, colloquially known from its appearance as Hairy bear cubs. Later, Martínez and Ramírez (2014) described it as *Stigmene acraea* (Drury) (Lepidoptera:Arctiidae) known as marsh or salt caterpillar (Figure 13).

The adult is a moth of night habit and presents sexual dimorphism, as can be seen in Figures 13-D and 13-E. The adult female has two pairs of white wings, at the borders

FIGURE 11
***Ascia monuste* (left) Larva, (right) Adult moth**

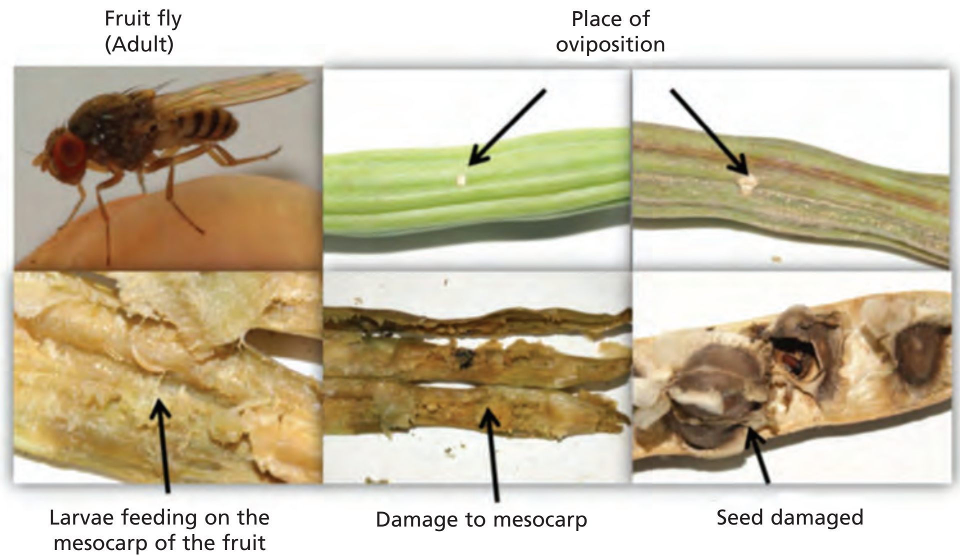
FIGURE 12
A. Free negro (*Apate monachus*). B. Insects feeding on *M. oleifera* stems

FIGURE 13
***Stigmene acraea* (Drury). (A) Larva or caterpillar. (B) Pre-pupa phase. (C) Pupae. (D) Adult female. (E) Adult male**



of the front wing irregular points of black colour are observed in lines, while in the rest of the wing the irregular points are disperse. The abdomen is brown, with central black spots on the dorsal zone at each segment. Ventrally, the abdomen is white with the same pattern of dark spots. Males show different wing pattern coloration. The first pair of wings match with those of the female, but the second pair is of brown colour, with similar black spots.

FIGURE 14
Fruit fly (*Gitona* sp.) and damage caused to *M. oleifera* fruits



In Cuba, moringa fruits affected by the fruit fly, *Gitona* spp. (Diptera:Drosophilidae), have been found (de la Maza, 2014) (Figure 14), and the fly is considered the main pest of moringa fruit in almost all regions of the world. The adult female oviposits in the fruit. The eggs hatch, releasing larvae whose morphology is typical of the order: vermiform without head and legs. These develop, feeding on the mesocarp of the fruit, causing rot and seed damage. The opening made by the fly facilitates secondary entrance of other harmful agents, including larvae and adults of coleopterae, lepidopterous larvae, acarus and phytopathogenic micro-organisms that accelerate the fruit damage (Valenciaga, unpublished data).

FIGURE 15
Symptomatology of *Colletotrichum demanthium* in *M. oleifera*

FIGURE 16
Symptomatology of *Fusarium* sp. in *M. oleifera*

To counteract the harmful organisms in moringa, establishment of a complex living barrier is recommended for fields adjacent to the plantation, and to gradually substitute the live barriers of this plant by others in order to keep increasing plant biodiversity and therefore favour the permanent presence of bioregulators. In addition, systematic removal of dead material, soil management and systematic insecticide application plans at fructification in moringa are advisable to protect the sheaths from attack by the fruit fly.

Alonso *et al.*, 2012 found two fungal pathogens (*Colletotrichum demathium* and *Fusarium* sp.) 22 days after sowing moringa genotype Supergenius (Figures 15 and 16), with infestation indices higher than 50%. The plantation was devastated.

The symptoms and signs of *Colletotrichum demanthium* are spots, more or less rounded, whose centre is light brownish with dark brown or brown border, surrounded by a greyish mycelium. The disease becomes visible in the older leaves (face or reverse) in the centre or border of the leaflet. Alonso *et al.* (2012) reported that as the disease advances it causes drying and yellowing that lead to withering, chlorosis and total drying of the foliage.

The symptoms and signs provoked by *Fusarium* sp. consist of the appearance of sunken spots of whitish browny-grey colour in the stem, with stem slimming at the insertion of the first branches, with a consequent decrease in the number of secondary roots and their absorbent hairs (Alonso *et al.,* 2012).

The presence of a range of fungal genera (including *Colletotrichum*, *Alternaria*, *Curvularia*, *Fusarium*, *Cladosporium*, *Phoma*, *Helminthosporium* and *Cercospora*) were determined under organoponic sowing conditions (Castellanos and Meléndez, 2012).

Among the control measures recommended for minimizing fungal damage in moringa are previous applications of the antagonist *Trichoderma harzianum* strain A-34 (Alonso *et al.*, 2012). Since *Trichoderma* is a fungus that acts on the pathogenic fungi, García and Padilla (2012) advise its application to the soil at a rate of 40 L/ha, or 10 kg diluted in water, at the last harrowing. Regarding seed, it is suggest that they be soaked in water for 5 minutes and air-dried under shade, or left to scarify by the water immersion method for 24 h at room temperature. This protects against the pathogenic fungi and should reduce any loss of seed viability from this cause.

11. FINAL REMARKS

Moringa is acknowledged as a plant with high ecological plasticity and one that adapts well to varied climate and soil conditions. However, edaphoclimatic factors have a strong influence on the yield and lifespan of it as a forage crop. It does not prosper in drought or in waterlogged soils, as excessive rainfall in fertile and well-drained soils provokes leaf fall. Together with this, it is prone to attack by pests and diseases that on occasions appear opportunistically, with devastating effects on plant health.

Recently, harvested seeds have been shown to have good germination, approximately 90%, but this decreases over time, being more apparent when stored at environmental temperatures. The use of such seed for sowing was successful and gave plants with deep root systems, guaranteeing more vigorous trees with tolerance of unfavourable climatic factors. Moringa can be reproduced by vegetative means, but the cutting is preferably at the end of the dry period when they are more than 8 cm diameter, and must be planted when rainfall is stabilized at the beginning of the rainy period, to favour the appearance of shoots and abundant and vigorous secondary branches.

The sowing and plantation methods for moringa vary from one region to another, reflecting availability of machinery and suitable implements for husbandry by the primary producer and for sowing and culture, as well as adequate labour. The experience acquired in recent years in Cuba with the introduction and exploitation of this plant for massive cattle raising areas, indicate a need for continuing study in the search for practical methods for weed control that also reduces possible attacks by the pests and diseases that are the main causes of the limited persistency of forage areas of moringa.

Sowing densities used will depend on purpose, whether for forage or for seed. Most data available on forage production is for the use of high sowing densities, and the information is repetitive. It will therefore be necessary to identify what we still do not know on forage production performance when using low to medium sowing densities for forage purposes. Here further investigations and trials are needed using methods and sowing patterns adjusted to the machinery and implements typical of the region and that facilitate mechanical weed control.

Practical experience has demonstrated that when sowing plots are used with the equivalent of 500 000 and 1 million plants/ha, survival of plantlets is very much affected by weed invasion. The need to hand-weed limits the maintenance of forage areas, besides increasing establishment costs. Moreover, the labour requirement influences the useful and productive life of the culture and susceptibility to pest attacks, mainly *A. insularis.* The fungus *Fusarium* sp. has also caused damage of economic importance.

Regarding height and cutting frequency, it is generally proposed that the first cutting should be at 5 or 6 months after sowing, then cutting with a sharp machete every 45 days in the rainy season and every 60 days in the dry season, at height of 20 cm from the soil. Certainly, low-to-medium frequencies and cutting heights will favour biomass quality, but it must not be forgotten that these parameters came from studies where high sowing densities are used and the culture is managed for small-scale productive purposes. Thus, there is lack of security that output will be similar when subject to different cutting patterns and frequencies. This uncertainty will become more evident when working at a larger scale and implies use of mechanization for increasing productivity and decreasing production costs, mainly in weed control. The previous assessments have led us to think that it will be necessary to have trials with more frequencies and cutting heights to enable this plant to express its genetic potential and have a longer population persistency without compromising the nutritive value of the biomass produced.

Pruning in areas destined for seed production needs to control maximum plant height, decrease the negative effects of weather extremes, eliminate damaged branches, remove low branches obstructing access to the area, and facilitate fruit harvest. Pruning stimulates shooting, flowering and fruiting, contributing to higher seed yields per plant and per hectare.

Trees cultivated for forage react well to pruning for promoting growth, and they sprout vigorously producing new shoots per stump, giving improved quantity and quality of biomass.

For seed production, moringa has a high production potential. However, despite the acknowledged potential, there are few scientific investigations in Cuba and other tropical regions of Latin America and the Caribbean that can provide precise recommendations on sowing distances, nutritional requirements and harvesting, phytosanitary protection of the plants and seeds, as well as technologies for harvest and post-harvest handling of the seeds. This implies a need for further scientific research in this field.

Considering all aspects of *M. oleifera* regarding the incidence and damage due to pests and diseases, and their quick appearance and damage symptomatology seen in Cuba in the different phenological phases, coupled with the expanding growing area of this plant in the country, it is recommended that there be frequent monitoring of the culture, with a search for agroecological control alternatives for protecting plant populations, and thus minimizing damage, which will enhance the potential benefits offered by the plant.

AUTHORS ACKNOWLEDGEMENTS

The information presented in this chapter does not represent a technical standard practice for moringa agronomy, nor was that its purpose. Rather, we will feel satisfied if to some extent this material serves as a guide for technical work by producers or teachers, while new research studies could elucidate the many unknowns that still exist.

Photographs supplied by authors.

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Nutritional value of *Moringa oleifera* (moringa) for animal feeding

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ABSTRACT

Moringa oleifera (moringa) is a very useful plant in animal feeding, reflecting its good nutritional characteristics due to its protein and vitamin contents, making it useful as a supplement for dairy and beef cattle, as well as for poultry, fish and swine feeding. Besides its high nutritional quality, it produces a good quantity of green biomass in the field, especially during the dry period, making this plant a good potential source of feed for cattle. This chapter discusses the importance of determining the nutritional value of forages, and refers to studies on the nutritional value of moringa that have been carried out in Latin America and elsewhere. It demonstrates the nutritional value and chemical composition of moringa forage (ash, soluble carbohydrates, fibre, nitrogen, mineral and vitamin content), digestibility, and the resultant nutritive value of moringa forage. There are some anti-nutritional factors. The results show that, from its chemical composition, *M. oleifera* may be considered an excellent option for supplementing cattle and small ruminants in low-input production systems, and its values are comparable with other forages widely used in cattle rearing systems in the Latin American tropical region. Its low content of anti-nutritional factors and the high biological availability of its nitrogen compounds give it a high nutritional value for non-ruminant species that are more susceptible to secondary metabolites and demand proteins of high biological value. Interest in its use as a supplement for ruminants and non-ruminants rations has recently increased, since this plant can provide a large quantity of protein and other elements, particularly macro- and micro-elements, with high digestibility, which can improve the nutritional value of feed.

Keywords: nutritive value, chemical composition, digestibility, secondary metabolites, *Moringa oleifera*

INTRODUCTION

Arboreal species are noted for their good nutritional value, due to their high protein content, readily fermentable carbohydrates, the degradable fibre that is better than in tropical grasses, and their positive effects on nitrogen (N) metabolism in the rumen. These elements make it possible to increase the productivity of animals fed mainly on pastures (Rubanza *et al.,* 2007).

Although *Leucaena leucocephala* is the most widely introduced arboreal species for livestock production in Cuba and in many tropical or subtropical countries (Shelton, 1996), there has been a growing interest in recent years in the introduction of other multipurpose arboreal species for livestock, where the following are of particular importance: *Moringa oleifera, Morus alba, Gliricidia sepium* and *Trichanthera gigantea*.

Moringa oleifera (moringa) is a tree belonging to the Moringaceae family; it is originally from the southern foothills of the Himalaya Mountains and is now grown in practically all tropical, subtropical and semi-arid regions of the world. It can grow under drought conditions, but its intensive production using irrigation and fertilization can increase yields to up to more than 100 t/ha. It is known by several common names, such as: marango, moringa, reseda, radish tree, ramrod tree, angela, asparagus tree, pearl tree, ben tree, tree of life, drumstick tree, horseradish tree, ben oil tree or benzoil tree and tree of miracles (Martin *et al.* 2013). In Latin America and Central America, the moringa or marango was introduced and naturalized in 1920 as an ornamental tree, and it was used as a green fence and as a wind shielding tree curtain.

This is a very important tree in animal feeding because of its good nutritional characteristics, given by its protein and vitamin content, serving as supplements for dairy and beef cattle, as well as for poultry, fish and swine feeding (Garavito, 2008).

Besides its high nutritional quality, it produces a good amount of green biomass in the field, especially during the dry period, making this plant a good potential source of feed for cattle (Reyes-Sánchez, Sporndly and Ledin, 2006b; Nouman *et al.,* 2014). Its potential is, however, still unclear for animal feeding, and only a few, scattered studies have been made of its nutritional value in Latin America. The purpose of this chapter is to point out the importance of determining the nutritional value of forages, and to refer to studies on the nutritional value of moringa, with a focus on studies that have been mainly carried out in Latin American countries.

1. NUTRITIONAL VALUE AND CHEMICAL COMPOSITION OF FORAGES

In Latin America, the Biomass Department in Nicaragua considers moringa to have high nutrient content. Also, a large number of trials were made using moringa or marango leaves as forage for cattle (beef and dairy), and as feed for swine and poultry.

With moringa leaves constituting 40–50% of the feed, milk production in dairy cattle and weight gain in beef cattle increased by 30%. Birth weight, with an average of 22 kg for local Jersey cattle, increased by 3–5 kg.

An ecological feed concentrate for beef cattle can be produced in the form of a meal or pelleted. It can contain 15% protein, 5% fat, 11% humidity, 18% fibre, 15% ash, with a price that would be equivalent to 55% of equivalent commercial products, produced mainly with pasture grasses, legumes and high-protein-content arboreal plants that are dried, ground, pre-cooked or extruded, and pelleted.

In the tropics, the inclusion of moringa foliage in the basal diet for ruminants, which is generally based on gramineous pastures with moderate or low nutritional value, reduces the need for concentrate supplementation (Reyes-Sánchez, Sporndly and Ledin, 2006b). The meal from the foliage of this arboreal plant is a potential source of protein to supplement low quality forages, and may be used to replace commercial concentrate constituents for dairy cattle (Mendieta-Araica *et al.,* 2011).

The recommendation of moringa as animal feed is based on analyses of its chemical composition, which includes CP, fibre, ashes, etc. (Nouman *et al.,* 2014), alongside other studies *in vitro* to estimate its degradation kinetics and the presence of anti-nutritional factors and their effects (García *et al.,* 2008; Rodríguez *et al.,* 2014a)

In relation to the studies carried out at the Nutrient Analysis Laboratory of Bogota, Columbia, its leaves and stems 30 days after planting contain up to 30% protein, 6% fat and 15% fibre, besides the vitamins and minerals that surpass many other products used for human consumption. Its protein content is similar to poultry egg, twice that of milk, with triple the potassium of bananas, 3.6 times the calcium found in milk, seven times the vitamin C of oranges, and 3.6 times the vitamin A of carrots. At the same time, biomass production is high at 45 days, normally reaching up to 80 t/ha per cut and giving 8 cuts per year, although in practice only up to 30 t are obtained on good land with sufficient amounts of an organic fertilizer.

At this age, bromatological analyses reported 29.34% protein, 5.86% fat and 15% fibre in plants grown at their definitive site in Melgar, Tolima, while plants grown in nurseries (in bags) at 40 days in Acacías, Meta, showed only 18.94% protein and 3.84% fat, since the plastic bag prevented the main root from accessing the soil nutrients.

1.1 Ash content in moringa

In studies carried out in Latin America, moringa shows ash contents of between 12.18% and 44.3% (Oliveira *et al.,* 1999; García *et al.,* 2006; Reyes-Sánchez, Sporndly and Ledin, 2006b), and their mean value is higher than the range of 8.8–13.4% reported by other authors (Makkar and Becker 1996, 1997; Al-Masri, 2003; Reyes-Sánchez, Sporndly and Ledin, 2006b) and higher than the values reported for the most common forage species of Central America and the Caribbean (González and Cáceres, 2002). However, Bressani (2007) in Guatemala and Rodríguez *et al.* (2014a) using the Supergenius genotype in Cuba reported lower values (8.8 and 8.2%, respectively).

Many factors can influence ash concentration in plants. These factors include the mineral content of the soil and its availability for the plant, the type of soil and its pH, as well as the state of growth of the plant (Lukhele and Van Ryssen, 2003). In Nicaragua, the cutting frequency of a moringa cultivar was shown to affect the ash content, while this indicator was not affected by the planting density per unit area (Reyes-Sánchez, Sporndly and Ledin, 2006b).

1.2 Soluble carbohydrates content

Besides proteins, the highest proportion of soluble components of forage is carbohydrates; basically sugars that normally accumulate when photosynthesis produces more than that needed for plant growth. Plant species vary in type and concentration of soluble carbohydrates that are stored in leaves and stems. Temperature, plant maturity, fertilizers and nutrient availability have significant effects on the soluble carbohydrate content of plants (Norton and Poppi, 1995).

In Latin America, the only carbohydrate determinations reported are from Nicaragua and Guatemala, where it was found that moringa leaves contained from 10.0% to 24.1% soluble carbohydrates (Foidl, Mayorga and Vásquez, 1999; García *et al.,* 2006; Alfaro and

Martínez, 2008). At the same time, fresh moringa leaves contain metabolizable energy (ME) in a range of 9.5 to 12.3 MJ/kg DM (Becker, 1995; Foidl, Mayorga and Vásquez, 1999).

1.3 Fibre content

Plant tissues are formed by different types of cells, each having specific functions. The anatomical structure affects the speed of digestion of plant tissues. The primary cell walls are formed mainly by micro-fibrils of cellulose interwoven with hemicellulose (xiloses, arabinoses) (Norton and Poppi, 1995).

Although certain species, such as *M. oleifera,* have the C_3 photosynthetic pathway, making them less efficient in capturing CO_2, their digestibility is greater than that of grass pastures with the C_4 metabolic pathway. Hence, their leaves have less vascular tissue and sclerenchyma, as well as less lignin content, making the material more accessible to the action of rumen micro-organisms and microbial enzymes, with a higher degradation rate, less ruminal retention time and greater voluntary intake (Herrera, 2006).

In moringa plants harvested in Nicaragua, Reyes-Sánchez, Sporndly and Ledin (2006b) reported that the NDF and ADF contents of the plant material formed by leaves, petioles and stems of up to 5 mm in diameter, were in the range of 151–564 g/kg DM, and of 92–515 g/kg DM as reported by other researcher for NDF and ADF, respectively (Makkar and Becker, 1996, 1997; Foidl, Mayorga and Vásquez, 1999; Oliveira *et al.,* 1999; Aregheore, 2002; Al-Masri, 2003). At the same time, the moringa meal used by Mendieta-Araica *et al.* (2011), with plant material from Nicaragua, showed an NDF content of 161 g/kg DM. This value is similar to the NDF content of 159 g/kg DM previously reported by Richter, Siddhuraju and Becker (2003), where only leaves were used; while this is lower than the NDF content of 306 g/kg DM found by Murro, Muhikambele and Sarwatt (2003), who included branches in the moringa meal.

The variations observed in fibre content are due to various factors, particularly agro-climatic conditions, soil type and fertilization, age of the trees, leaf maturity, cutting frequency and parts of the plant harvested (leaves, petioles, branches, stems). Moreover, it is known that moringa plant density per unit area does not affect NDF and ADF content (Al-Masri, 2003; Reyes-Sánchez, Sporndly and Ledin, 2006b).

Lignin content differs in moringa foliage meal depending on the age of the plant material used and the different proportions of leaves, green branches and stems included (Aregheore, 2002). Young moringa stems are generally of high quality, which may decrease more rapidly than in leaves as their epidermis and fibrous cells are transformed into secondary cell walls and the lignin content increases with time.

1.4 Nitrogen content

Its high protein content is one of the most widely mentioned advantages of moringa foliage (Thurber and Fahey, 2009). This shrub is an excellent option for providing protein supplement for ruminants in productive tropical systems, because the CP content of the moringa foliage (47%) and of the leaf meal (64%) are greater than the typical tropical grass pastures and forages that are normally consumed by cattle (Minson, 1990; Soliva *et al.*, 2005).

Reports from the literature show that fresh moringa leaves contain between 193 and 264 g CP/kg DM, while branches and stems contain 72 and 62 g/kg, respectively (Makkar and Becker, 1996, 1997; Foidl, Mayorga and Vásquez, 1999; Aregheore, 2002). In Latin America, CP contents ranging from 179 to 335g CP/kg DM have been reported for this species (Oliveira *et al.,* 1999; Reyes-Sánchez, Sporndly and Ledin, 2006b; Bressani, 2007; Mendieta-Araica *et al.,* 2009). In Nicaragua, Becker (1995) reported 230 g CP/kg DM in fresh moringa leaves, and Mendieta-Araica *et al.* (2011) found that CP content of fresh moringa was ca 241 g/kg DM, and the silage contained 226 g/kg DM, which is higher than the value previously reported (144 g/kg DM) by Mendieta-Araica *et al.* (2009), probably because thick branches and stems were not included in the most recent study. At the same time, in Cuba, Rodríguez *et al.* (2014a) reported CP contents of 222 g CP/kg DM for foliage meal of moringa plants (Supergenius genotype) established ten months earlier.

The CP content varies according to the fraction of the plant, with the leaves having the highest value (Garavito, 2008). At the same time, the CP of the moringa foliage meal may vary considerably as a function of the amount of small stems and branches included in the foliage. Fujihara *et al.* (2005) analysed different fractions of the moringa (leaves, seed cakes, twigs and stems) and found that the leaves and the seed cakes had CP contents of approximately 250–300 g/kg DM, while the leaves with twigs had CP at 195 g/kg DM. The CP content of twigs alone was lower, but this fraction can be used for animals with lower nutrient requirements, such as dry cows that readily consume this fraction (Nouman *et al.,* 2014).

Table 1 shows the changes in the chemical composition of moringa, on changing the age or the part of the plant that is harvested. In all cases, except for the stems, the CP content exceeded 20%.

At the same time, the moringa leaves are an excellent source of amino acids, including lysine (Makkar and Becker, 1996, 1997; Foidl, Makkar and Becker, 2001; Ferreira *et al.,* 2008; Newton *et al.,* 2010; Mendieta-Araica *et al.,* 2011). Moringa is also rich in two amino acids that are generally deficient in other feeds, such as methionine and cysteine, which have contents of 14.14 and 8.36 mg/g DM, respectively (Makkar and Becker, 1996).

The dry leaves and fresh pods of this plant are also a good source of amino acids. High levels of arginines, valine and leucine are found in dry moringa leaves and fresh pods, while no serine, glutamate, aspartate, proline, glycine and alanine were found in these parts (Freiberger *et al.,* 1998; Fuglie, 2000). Moringa seeds also have good amounts of all amino acids except for valine, lysine and treonine (Oliveira *et al.,* 1999).

TABLE 1. Chemical composition of *M. oleifera*

Parameters		Study 1		Study 2		
		Leaves and stems		Plants at 54 days		
		Young	Well developed	Leaves	Stems	Leaves and stems
DM	(%)	66.86	34.90	89.60	88.87	89.66
CP	(%)	21.59	26.74	24.99	11.22	21.00
EE	(%)	3.73	3.80	4.62	2.05	4.05
Ash	(%)	9.83	10.63	10.42	11.38	10.18
DE	(Mcal/kg DM)	2.99	2.93	2.81	1.99	2.43
ME	(Mcal/kg DM)	2.45	2.39	2.30	1.63	1.99

NOTES: EE = ether extract; DE = digestible energy.
SOURCES: Study 1 is based on Pérez *et al.,* 2010; Study 2 is from Garavito, 2008.

The studies carried out in the region include those showing that the N content of leaves and young stems does not decrease with plant maturity, and it is not affected by the sun drying method used for dehydration (Mendieta-Araica *et al.,* 2011). Furthermore, it was demonstrated that the nutrient content of moringa is less sensitive to cutting intervals than grasses such as *Pennisetum* sp., and moringa has better digestibility and CP content for longer periods (Reyes-Sánchez, Ledin and Ledin, 2006a).

1.5 Mineral and vitamin content

Tropical forages generally contain less macro-minerals (calcium, phosphorus, magnesium, potassium, sodium, sulphur, etc.) than the species from temperate regions (McDowell and Valle, 2000). In a study made with more than one thousand samples of forages harvested in Latin America, it was observed that they were low in calcium, phosphorus and magnesium (McDowell *et al.,* 1977). At the same time, Devendra (1977) reported low calcium contents in 103 samples of forages harvested in the Caribbean region (less than 3.0 g Ca/kg DM).

However, the moringa leaves are rich in nutrients such as calcium, iron and potassium, which are essential for weight gain and milk production (Newton *et al.,* 2010; Mendieta-Araica *et al.,* 2011). Furthermore, the leaves have high available calcium (18.8 mg Ca/kg DM) and insoluble oxalate contents, which are not harmful to animals (Noonan and Savage, 1999; Radek and Savage, 2008; Nouman *et al.,* 2014). Moreover, when assessing the chemical composition of moringa and another five arboreal species in the state of Trujillo, Venezuela, it was found that there were no important variations between the arboreal species in relation to the levels of P (0.12–0.21%, vs moringa 0.20%), Ca (2.71–3.33%, vs moringa 3.10%) and Mg (1.71–2.26%, vs moringa 1.94%); and the maximum concentrations of K and Na were observed in *M. oleifera* (2.65 and 0.24%, respectively) (García *et al*., 2006).

Vitamins are organic macromolecules with complex molecular structures that are essential, in very small amounts, for ruminant health, growth and reproduction. Since adult ruminants can directly produce or receive many of the vitamins they need, as ruminal fermentation products, these animals generally depend only on the supply of vitamins A and E in their rations. Although fresh forages are potential sources of vitamins A, E, D, niacine and thiamine (McDowell, 1989), the levels found are variable and depend on the interaction of different factors such as climatic conditions, species and plant variety, state of maturity of the plant and preservation and storage method of the plant material (Ballet, Robert and Williams, 2000).

Moringa is known to be an important source of vitamins. Its foliage contains significant amounts of vitamins A, B and C, carotene, ascorbic acid and iron (Makkar and Becker, 1996; Alfaro and Martínez, 2008; Ferreira *et al.,* 2008). Compared with other feeds, the nutrient content of moringa for each 100 grams of edible part shows a higher content of vitamin A than found in carrots, more vitamin C than in oranges, more calcium than in cow milk and more potassium than in bananas (Pérez *et al.,* 2010).

2. DIGESTIBILITY OF MORINGA FORAGE

Digestibility is an indicator of the nutritive value of the components of a ration (Beever and Mould, 2000). Forage digestibility is a simple measure of the availability of the nutrients, usually expressed in terms of disappearance of dry material or organic material, but it does not provide any information on the composition of the degraded or absorbed nutrients (Norton and Poppi, 1995).

Moringa is a plant with very good digestibility. At least under *in vitro* conditions, the range of degradability of its DM was 64.8 to 79.0% (Makkar and Becker, 1996, 1997; Foidl, Mayorga and Vásquez, 1999; Aregheore, 2002; Al-Masri, 2003; Manh *et al.,* 2003). At the same time, in Cuba, Pérez *et al.* (2010) reported a digestibility of 70.5% of the dry matter and 65.5% of apparent digestibility of the protein. Furthermore, in studies made in Nicaragua, the fresh moringa leaves had a DM digestibility *in vitro* of 79.7% (Becker, 1995).

Mendieta-Araica *et al.* (2011) observed that the digestibility of the DM and of the OM averaged 74 and 77%, respectively. Additionally, the values obtained were in the DM digestibility range that has been reported by other authors (Murro, Muhikambele and Sarwatt, 2003; Nouala *et al.,* 2006) and no differences were found in the digestibility of these indicators for moringa and soybean meals.

Also, Mendieta-Araica *et al.* (2011) found no differences between the digestibility of the CP of moringa and soybean meals. This shows that the CP content of moringa is as digestible as that of soybean, which is one of the conventional protein sources most widely used in cattle feeding. Furthermore, the digestibility of the moringa silage in this study was higher than that of other silages obtained from other arboreal species (Mendieta-Araica *et al.,* 2011).

In the case of the study carried out by Rodríguez *et al.* (2014a), the estimated digestibility at 96 h of incubation was lower than the values reported previously. However, the degradability of the DM was similar between moringa and *L. leucocephala* and the degradability of the NDF and the OM was higher than that of this legume.

In vitro and *in situ* techniques have also been used to measure or predict digestibility. Although these techniques do not provide information on voluntary intake, they have become very popular because of their simplicity, low costs, repeatability and the amount of information they generate. One of these is the *in vitro* technique of gas production (Menke *et al.,* 1979; Theodorou *et al.,* 1994). Also, these procedures have been enhanced with methodologies that estimate protein degradability and the amount of nutrients that bypass ruminal degradation (Norton and Poppi, 1995).

In Cuba, Rodríguez *et al.* (2014a) used this technique to study fermentation kinetics and the degradability of the nitrogen fraction of the Supergenius moringa genotype, compared with other protein species (*Morus alba, L. leucocephala, T. gigantea*). Moringa fermentation produced more gas than *L. leucocephala* and *T. gigantea*, but less volume than *M. alba*. The gas production potential was similar. However, the values of maximum speed of gas production were similar for moringa and *M. alba* (8.08 and 8.36 mL/g MOinc/h, respectively). The similarity of gas production between both species was attributed to their high readily fermentable carbohydrate content (García *et al.,* 2008). Thus, their fermentation profile is comparable to concentrates and indicates a better nutritive value than traditional forages (Jayanegara *et al.,* 2010).

Regarding degradability of the nitrogen fraction, estimated after 24 h by the method of Raab *et al.* (1983) that combines the amount of gas produced and the N-NH_3 content, Rodríguez *et al.* (2014a) observed that the degradability for the moringa N (43%) was lower than that observed for *M. alba* and *L. leucocephala* (53 and 80%, respectively). This lower degradability of the moringa protein in the rumen is one of the reasons why it is considered that its CP is of better quality for ruminants than the CP of the widely used legume leaves used as supplements in the productive systems of the tropical Latin American and Caribbean region, such as *G. sepium* and *L. leucocephala*. This is due to its higher over-pass protein levels (47% vs 30 and 41%, respectively according to Becker, 1995). At the same time, García *et al.* (2008) reported that moringa showed a higher post-ruminal CP digestibility than *M. alba*.

However, these studies are not conclusive, since other *in vitro* studies showed that the proportion of potentially degradable protein in the lower parts of the digestive tract was less in moringa than in *L. leucocephala*, which is the arboreal species that is most widely used as a protein supplement in cattle feeding in the tropical regions (Fujihara *et al.,* 2005). These differences may be due to the characteristics of the plant materials evaluated.

3. OTHER RESULTS ON THE NUTRITIVE VALUE OF MORINGA

In Cuba, Montejo *et al.* (2014, unpublished data), carried out a study where their hypothesis was that the mixture of the moringa "piscidium" meal with soybean meal would improve feed quality and produce a better nutritional utilization of the plant. They took into account that the moringa tree could produce more than 1 000 "piscidia" per year, with a productivity of over 7 kg of "piscidia" per plant. At the same time, its industrial use, when harvesting the seeds for oil extraction, could create contamination, making it a technology that would not be environmentally friendly, but it could instead be used to potentiate animal production (Foidl, Makkar and Becker, 2001; Fuglie, 2000), To demonstrate this, an *in vitro* ruminal fermentation test was carried out where nine treatments of moringa "piscidium":soybean were evaluated (100:0; 90:10; 80:20; 70:30; 60:40; 50:50; 40:60; 20:80 and 0:100). The chemical composition of moringa "piscidium" and soybean (Table 2), gas production and true digestibility were determined as part of this study.

In relation to the gas production of the treatments, these researchers (Montejo *et al.,* 2012) found that on increasing soybean percentage there was an increase in gas production.

Most studies have considered the amount of gas produced to be equivalent to substrate degradation. However, it should be noted that a portion of the substrate degrades and joins anabolic pathways for the synthesis of microbial biomass (Makkar, 2000). The magnitude of the microbial synthesis cannot be estimated solely from *in vitro* gas production because this is only a reflection of catabolic processes, such as the formation of volatile fatty acids (VFAs). Although there is an inverse relationship between the production of gas or VFAs and microbial biomass (Blümmel, Makkar and Becker, 1997), this ratio is not constant because there is great variability in the production of microbial biomass per unit of ATP

TABLE 2. Bromatological composition (g/kg DM)

Feedstuff	DM	Ash	CP	Lipids	NDF	ADF
Moringa "piscidium"	912.24	41.39	30.32	16.46	747.14	635.57
Soybean	935.81	65.98	908.30	10.69	—	—

Source: Montejo *et al.*, 2012.

generated during fermentation (YATP) (Pirt, 1975; Blümmel, Makkar and Becker, 1997). Current nutritional concepts seek to select foods based on high microbial synthesis during fermentation. Therefore, selection should be based on gas production *in vitro*, else it would be selecting precisely against peak performance in microbial biomass.

Furthermore, Montejo *et al.* (2012) note that including soybean, even when gas production is increased, is not an indication of utilization of the feedstuff, since the degradation of the incubated ration has two outcomes: (1) that using the micro-organisms for their nutrition and to form cellular structures, which are ultimately used by the animal to increase post-ruminal protein; and (2) the nutrients that do not become structural parts of the biomolecules are turned into gas, which is not really giving the nutrient evaluated a good use (Foidl, Makkar and Becker, 2001). This was the reason why the calculation or estimation of true digestibility of the moringa pods were considered necessary; there was thus an increase in true digestibility that was proportionate to the inclusion of soybean, up to 100%, which is more than 98% digested.

For a better assessment of how the animals are able to use the ration, calculations were made of the microbial protein that would be obtained with each treatment in the study. It was estimated that the microbial protein values decrease with increasing soybean in the ration.

4. ANTI-NUTRITIONAL FACTORS

Anti-nutritional factors are those substances produced through the secondary metabolism of plants that can, through different mechanisms, exert negative effects on the nutrition of the animals consuming them. The anti-nutritional factors found in forages include tannins, saponins, alkaloids, non-protein amino acids, cynogenic glycosides and terpenes. These are generally undesirable compounds for animals because of their bitter taste, poor palatability or because they are indigestible (Nouman *et al.,* 2014). However, in low concentrations, some of these compounds may be beneficial to the animals, thus improving the nutritive value of the feed and having positive effects on health (Reed *et al.,* 2000).

Anti-nutritional factors of moringa are minimal (Pérez *et al.,* 2010). Moringa leaves contain insignificant amounts of tannins, a saponin content that is similar to that of soybean meal and they have no trypsin or amylase inhibitors or other anti-nutritional factors such as cyanogenic glycosides (Makkar and Becker, 1996, 1997). At the same time, in moringa the tannins and phytates are 12.0 and 21.0 g/kg DM, respectively, while the leaves of *L. leucocephala* may contain tannins and phytates that are equivalent to 29.4 and 1.4 mg/100 g DM, respectively (Udom and Idiong, 2011).

The results of the phytochemical study of *Moringa oleifera* made in different geographical areas of Cuba and in India are shown in Table 3.

Low levels of secondary compounds were found in the samples evaluated in these studies. While in Cuban cultures no saponins were found, in samples from India their content was low. Sharma and Paliwal (2012) isolated saponins from the pods of this plant with a chemo-preventive effect.

García *et al.* (2008), on analysing the levels of secondary metabolites of several arboreal species from Venezuela, observed that *M. oleifera* was within the group with the highest concentrations of total phenols. Nonetheless, the levels of the compounds found for these

TABLE 3. Phytochemical analyses of *Moringa oleifera* made in Cuba (provinces of Camagüey and Mayabeque) and in India

Secondary metabolites	Mayabeque	Camaguey	India
Tannins	++	+	+
Condensed tannins	+	++	—
Saponins	0	0	+
Alkaloids	++	0	—
Flavonoids	+	—	+
Terpenes	++	—	+
Glycosides	-	—	+
Steroids	+	—	+
Source	Scull *et al.,* 2012	Pedraza *et al.*, 2013	Nepolean, Anitha and Renitta, 2009

NOTES: ++ moderate; + poor; — absent, not analysed.

species are, in general, equivalent to those reported in tropical forages (0.2 to 4.0%) and they did not cause digestive problems at the levels fed (Makkar, 2003). García *et al.* (2008) also found similar results for tannin contents, which were in the range that would favour beneficial processes for ruminants, not only by the formation of bypass protein and the later decoupling of the tannin-protein complex (because of the drastic change in pH at the entrance to the abomasum), but also because the presence of phenols of different structural complexities in the ration could favour productivity and health; this is because they inactivate formation of free radicals, which affect the extent of the production period of the animals (Makkar, 2003; García and Medina, 2006).

The quantification of the biological effect (BE) of the tannins through the use of the gas production technique and employing polyethylene glycol as an inactivating agent for tannins, is a simple, thrifty and practical method to estimate the effect of these secondary compounds in ruminal fermentation of plants containing them, without having to characterize the chemical nature of the tannins or to determine their concentration (Rodríguez *et al.*, 2013). In a study in Cuba, Rodríguez *et al.* (2014b) observed that in up to 24 h of incubation there were no differences in the BE of the tannins of moringa (Supergenius genotype) and *L. leucocephala* in *in vitro* gas production. However, at 96 h of incubation, the highest BE was found for tannins from *L. leucocephala* compared with moringa. At the same time, the tannins of *L. leucocephala* had a higher influence on the potential (15 vs 7%) and the maximum speed of gas production (24 vs 13%). It must be considered that the biological effects of these secondary metabolites found in both species, are considered to be moderate, and are in relation to the low concentration and activity reported for these compounds in those species (Makkar *et al.,* 1996; García *et al.*, 2008; Rodríguez *et al.,* 2013). At the same time, Rodríguez *et al.* (2014b) also observed that the moringa tannins did not affect the degradability of the OM and the NDF, nor the concentration of NH_3 (BE in the range of 1 to 2%).

Although moringa leaves contain saponins that make it taste bitter to cattle consuming it, these compounds are not always harmful to animals (Makkar and Becker, 1996, 1997). The extracts of moringa leaves contain saponins in the range of 4.7 to 5.0 g/kg DM, for which reason they may be consumed by the animals without any adverse effects (Liener, 1994; Makkar and Becker, 1997; Price, 2000; Foidl, Makkar and Becker, 2001; García *et al.,* 2008).

Moringa leaves do not contain lectin, trypsin or amylase inhibitors (Becker, 1995; Gidamis *et al.,* 2003; Makkar and Becker, 1997; Ferreira *et al.,* 2008), but they do have glucosinolates of modified sugars (mainly glucomoringin in their leaves and glucotropaeolin in their roots) (Fahey, Zalcmann and Talalay, 2001; Bennett *et al.,* 2003; Newton *et al.,* 2010). Their concentrations in the plants vary widely depending on the soil, climate, growth stage and species or cultivar. Cartea *et al.* (2007) and Charron *et al.* (2005) reported concentrations of glucosinolates in warm and long days. Furthermore, certain glucosinolate derivatives, such as tiocarbamates, isotiocyanates and carbamates have been reported for moringa leaves (Leuck and Kunz, 1998), but their concentrations are very low compared with other phytochemicals, or they are not detected at all in other tissues of the plant (Newton *et al.,* 2010).

5. FINAL REMARKS

Based on its chemical composition, *M. oleifera* can be considered an excellent option for supplementing cattle and small ruminants in low input production systems in the tropics. Its values agree with those forages widely used and distributed for cattle rearing systems in the American tropical region (García *et al.,* 2006, 2008). Its low content of anti-nutritional factors and the high biological availability of its nitrogen compounds give it a high nutritional value for non-ruminant species, which are more susceptible to secondary metabolites and demand proteins of high biological value.

The interest in its use as a supplement for animal rations (ruminants and non-ruminants) has recently increased, since this plant can provide a large amount of protein and other elements, particularly macro- and micro-elements that can improve the nutritional value of the ration. Besides its high nutrient content, this plant shows high digestibility, thus having the factors most frequently used to select a forage species as a ration supplement or complement.

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Moringa oleifera (moringa) in the feeding of non-ruminants

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ABSTRACT
*Moringa oleifera s*tands out among non-legume trees and shrubs as a promising option for animal feed due to its high availability and excellent nutritive value. This chapter reviews results obtained in Cuba and in some Latin American countries with the use of moringa in poultry, swine and rabbit diets as partial substitute for soybean meal, maize or alfalfa. Physiological indicators are discussed, and the positive impact on monogastric animal growth, body composition and economy, in support of sustainable production. The variability of response shown by poultry, swine and rabbits in different experiments evaluating the use of leaf meals and leaf+stem meals is associated with variability in nutritive value, affect primarily by cutting age and the leaf:stem relationship of the moringa meal used. The optimum inclusion levels of moringa stem and leaf meals and moringa leaf meals must be specified for the different poultry species (geese, ostriches and quails) and in pigs, where scientific reports are scarce, as well as in rabbits, including breeding does and replacements.

Keywords: physiology indicators, productive performance, inclusion levels, non- ruminant species, *Moringa oleifera.*

INTRODUCTION
The use of non-legume trees and shrubs is a promising option for animal feeding due to its high availability and nutritive value (Lara *et al.,* 2012). Among these, the tree species moringa (*Moringa oleifera*) stands out because it is an excellent source of protein (22–36%) and minerals (Olugbemi *et al.,* 2010a). Various studies demonstrate that leaves are rich in vitamins and present low content of anti-nutritional factors (Mutayoba *et al.,* 2011). At the same time, moringa is an outstanding plant well suited to different edaphoclimatic conditions, with great ecological plasticity. It therefore constitutes a feeding alternative, especially in tropical countries.

In recent years, important studies have been conducted on the utilization of moringa foliage for the feeding of monogastric species. These investigations have been mainly on the African continent, with poultry (Elkhaifa *et al.,* 2007; Kakenga *et al.,* 2007; Olugemi *et al.,* 2010b; Banjo, 2012; Oludoyi and Toye, 2012), rabbits (Odeyinka *et al.,* 2008; Davis, 2010; Nuhu, 2010; Dougnon *et al.,* 2012; Ewuola *et al.,* 2012; Abu, Ahemen and Ikpechukwu, 2013; Ahemen, Abu and Iorgilim, 2013; Owen *et al.,* 2013; Ufele *et al.,* 2013; El-Badawi *et al.,* 2014) and in Asian countries, such as India (Rajeshwari *et al.,* 2008) and Amata and Okorodudu (2013) in rabbits.

In the Latin America and Caribbean region, countries such as Cuba, Mexico, El Salvador, Nicaragua and Colombia, have investigated the effect of both moringa foliage and meal as forage have been carried out, but few reports have been published in the scientific literature.

This chapter presents a review of the results obtained in Cuba and in some Latin American countries with the use of moringa in poultry, swine and rabbit diets as partial substitute for soybean meal, maize or alfalfa, looking at physiological indicators and impact on monogastric animal growth, body composition and economics of sustainable production.

1. MORINGA IN POULTRY FEEDING

1.1 Morphometry of the gastrointestinal tract

Recent studies show that fowls require the inclusion of fibre in the diet for stimulating the development of the upper part of the gastrointestinal tract (GIT). However, it is known that when the recommended levels are surpassed, there deleterious effects on the digestibility of nutrients, productive performance and animal health (Haoyu, 2013).

In previous experiments carried out in birds, it was demonstrated that the amount and composition of the fibre can influence on the development of the digestive system (Jamroz *et al.,* 2001). In that regard, Bustamante *et al.* (2013) determined the effect of different graded levels of moringa forage meal (0, 5, 10 and 15%) on the development of the GIT of broilers.

Tables 1 and 2 show the relative weights of the different sections of the full and empty GIT, respectively, of broilers consuming moringa forage meal in the ration. The full weight of the complete GIT increased with the inclusion of 10 and 15% of moringa forage meal (Table 1). Relative weights of the proventriculus, gizzard and small intestine decreased between the control and 15% moringa. The reduction in the weight of these organs could be explained by the low soluble fibre proportion present in this diet (15% moringa) in comparison with the rest, which could modify the rate of passage of the digestive content.

It was observed that the relative weights of the gizzard, caeca as well as the empty GIT in broilers fed diets containing 15% moringa forage meal were lower than the control (Table 2).

The length of the colon-rectum decreased with 15% moringa forage meal, while the other lengths of the intestinal segments did not differ between treatments (Table 3).

It is known that the inclusion of these fibrous sources in diets for monogastric species provokes modifications in the gastrointestinal macro-architecture through its physical properties. The magnitude of this effect depends on the physical form and chemical nature (source and origin, type of fibre, processing to which it was submitted) as well as the adaptation and typical characteristics of the animal. The main physicochemical characteristics of the dietetic fibre influencing the digestive content are the solubility and water absorption capacity.

TABLE 1. Relative weight to LW (%) of the different full sections of the GIT of chickens consuming moringa forage meal

Indicator (g/kg)	Control	Moringa forage meal (%)		
		5	10	15
GIT	172.02	189.24	213.95	209.47
Proventriculus	6.15	4.97	5.04	4.28
Gizzard	36.31	33.08	32.54	29.26
Small intestine	54.89	49.44	46.03	35.58
Caeca	5.63	4.17	5.88	4.71
Colon-rectum	2.64	2.39	2.85	2.10

Source: Adapted from Bustamante *et al.*, 2013.

1.2 Nutrient digestibility

The use of forage plants has an impact on the digestive physiology of monogastric species, since it provokes modifications in utilization of nutrients and in biological efficiency. Nieves *et al.* (2011) stated that it is of vital importance to determine the nutrient content and the digestive utilization of non-conventional diets.

Bustamante (2014) assessed the apparent faecal retention of the fibrous fraction in broilers fed moringa forage meal in the diet (see Table 4). Apparent faecal retention of the fibrous fraction showed differences for all indicators, except for the acid detergent fibre (ADF).

It was observed that chickens used with certain efficiency the fibrous fractions of the feed. According to Bertechini (2006), fowls have low capacity for digesting fibrous materials and it is estimated that adult fowls are capable of digesting up to 25% of the fibre present in the diet.

Values of NDF, cellulose and hemicellulose decreased in the treatments including moringa forage meal regarding the control ($P<0.05$). This performance could be due to the chemical composition and physical nature of the fibre present in the fibrous source. Rentería-Flores .*et al.* (2008) indicated that insoluble fibre consumption limits microbial fibre utilization, increases faecal mass and accelerates the rate of intestinal passage, and thus contributes to poorer digestive utilization of the fibrous fraction by the animal.

Nevertheless, improvement of the utilization efficiency of feeds of high fibrous content is possible by increasing the digestion of its soluble and insoluble non-amylaceous polysacchararides through biotechnological processing generating biochemical and structural modifications for increasing the nutritive value of the final product. That is why fermentative processes are employed for increasing the bioavailability and digestibility of the nutrients in fibre-rich feeds (Hardini, 2010). For obtaining fermented products, efficient micro-organisms are employed that confer probiotic properties (Savidou, 2009).

TABLE 2. Relative weight to LW (%) of the different empty sections of the gastro-intestinal tract (GIT) of chickens consuming moringa forage meal

Indicator (g/kg)	Control	Moringa forage meal (%)		
		5	10	15
GIT	77.28	70.49	72.20	64.84
Proventriculus	5.35	4.77	4.86	4.31
Gizzard	24.70	21.23	22.69	18.37
Small intestine	34.64	32.72	32.96	32.63
Caeca	2.79	2.57	2.73	2.39
Colon-rectum	1.88	1.76	2.06	1.62

Source: Adapted from Bustamante *et al.,* 2013.

TABLE 3. Absolute length of different sections of the GIT of broilers consuming moringa foliage meal

Indicator (g/kg)	Control	Moringa forage meal (%)		
		5	10	15
Small intestine	161.87	164.75	168.92	163.12
Right caecum	17.69	17.31	18.47	17.06
Left caecum	18.69	18.44	19.31	17.81
Colon-rectum	7.10	7.12	7.69	5.94

Source: Adapted from Bustamante *et al.,* 2013.

TABLE 4. Apparent faecal retention of the fibrous fraction in broilers receiving moringa forage meal in the diet

Retention (%)	Control	Moringa forage meal (%)			SE ± Sign.
		5	10	15	
DM	68.51^{a}	65.80^{ab}	62.15^{b}	60.09^{b}	1.25*
NDF	47.20^{a}	44.53^{b}	43.61^{b}	42.89^{b}	0.53*
ADF	41.76	40.25	39.38	36.52	1.85
Cellulose	47.05^{a}	37.48^{b}	38.35^{b}	38.12^{b}	1.02*
Hemicellulose	54.81^{a}	48.40 ^{ab}	40.44^{b}	42.36^{b}	2.39*

Notes: ^{a,b}Values with different letters in the same row differ significantly at $P<0.05$ (Duncan 1955) *P 0.05.
Source: Bustamante, 2014.

TABLE 5. Effect of the dry fermented product (DFP) Vitafert on the apparent faecal retention of the fibrous fraction in broilers receiving 10% moringa forage meal (MFM) in the diet

Retention (%)	Treatments				SE± Sign.
	Control	MFM 10%	DFP 2%	MFM 10% + DFP	
DM	77.71[a]	62.38[c]	76.12[a]	75.25[ab]	0.05*
NDF	47.71 [a]	40.61 [b]	46.23[a]	45.85[a]	1.52*
ADF	44.90	39.38	43.53	42.29	2.03
Cellulose	48.52[a]	39.82[b]	45.55[a]	43.16[a]	1.26*
Hemicellulose	55.91[a]	41.64[b]	49.36[a]	47.89[a]	1.35*

Notes: DM = dry matter; NDF = neutral detergent fibre; ADF = acid detergent fibre; DFP = dry fermented product; MFM = moringa forage meal. [a,b]Values with different letters in the same row differ significantly at $P<0.05$ (Duncan, 1955) *$P<0.05$.
Source: Bustamante, 2014.

In this sense, Bustamante (2014) studied the effect of the dry fermented product Vitafert on the apparent faecal retention of the fibrous fraction in broilers consuming 10% moringa forage meal (Table 5).

The dry fermented product increased the apparent faecal digestibility of NDF, cellulose and hemicellulose in the moringa forage meal for broilers. These results agree with Utama *et al.* (2013) who stated that the efficient microorganisms play an important role in the modification of nutritive compounds, due to their capacity to use the fibre as energy source for their growth and development.

1.3 Productive performance

1.3.1 Broiler chickens

Recent papers from African authors (Olughemi *et al.,* 2010a; Ebenebe, Umegechi and Aniebo, 2012; Gadziravi *et al.,* 2012) recommend the inclusion of from 5 to 10% *M. oleifera* leaf meal in broiler diets as optimum levels that do not affect the productive performance of chickens while reducing feeding cost. This statement is backed by results from Madrazo *et al.* (2012) in Cuba.

Two Cuban institutions with poultry farms (Instituto de Investigacines Porcinas (IIP) and Institute de Ciencia Animal (ICA)) carried out in unison a trial evaluating the inclusion of 0, 20 or 40% moringa forage (tender stems + leaves) meal in the diets for heavy breed chickens to study what occurred when chickens received these extreme diets with much fibre and poor metabolizable energy. Performance of the birds is shown in Table 6.

Financially, the use of 20% moringa forage meal in those diets for chickens of medium growth potential reduced the cost of the feed necessary for producing one tonne of live weight from 1505 to 1116 dollars (CENPALAB, 2012a). The level of 40% moringa meal did not reduce the feeding costs when compared with the control, but it did decrease growth and worsened feed conversion.

1.3.2 Laying hens

Recent literature recommends using between 5 and 10% of *M. oleifera* leaf meal or forage (leaves + stems) meal in the diets for laying hens, giving a productive performance equal to that obtained with the maize-soybean control diet (Kakengi *et al.,* 2007; Olugbemi *et al.,* 2010b; Pérez, 2013; Tapia, 2013; Valdivié *et al.,* 2013a).

TABLE 6. Productive performance of chickens with high levels of *M. oleifera* (stems + leaves) meal in the diets. Results from Cuban poultry farms

	Moringa meal in the diet (%)		
	0	20	40
ICA (Guayabal poultry farm)			
Average feed consumption, g/bird	3943	3773	3609
Average initial weight, g/bird	34.40	34.50	36.00
Average final weight, g/bird	1595	1569	1383
Average daily gain, g/bird	37.16	36.54	32.07
Feed conversion, g/g	2.47	2.41	2.61
IIP			
Average feed consumption, g/bird	3523	3726	3698
Average initial weight, g/bird	35.05	35.56	35.22
Average final weight, g/bird	1575.22	1514.29	1256.55
Average daily gain, g/bird	36.67	35.21	29.08
Feed conversion, g/g	2.24	2.46	2.94

In young laying hens of 20 to 25 weeks of age, Valdivié *et al.* (2012) showed the possibility of including up to 20% moringa forage meal in the diets, without affecting the productive performance of the fowls and reducing to USD 118 334 the cost of feed for one million young laying hens during those 6 weeks of life, as set out in Table 7.

This trial was carried out at three Cuban CENPALAB (Centro Nacional para la Producción de Animales de Laboratorio) poultry farms: Combinado Avícola Nacional (CAN) and the Unidad de Agricultura Militar (UAM). The latter two belong to the province of Pinar del Río, All worked with young laying hens (20 to 25 weeks of age), with positive results, confirming what was indicated by Valdivié *et al.* (2012).

In a study cited by Valdivié *et al.* (2013) and conducted in 2012 by CENPALAB at five Cuban farms, with layers between 20 and 56 weeks of age, it was found that with 20% moringa forage in the diets, layers had similar egg production to that of the maize-soybean control diet, but reduced production cost per egg from 5.8 to 5.6 cents and a kilogram of eggs from 1.22 to 1.00 dollar, i.e. 220 dollars less for each tonne of eggs produced.

During the laying peak of the layers (28 to 46 weeks of age), levels of 30 and 40% of *M. oleifera* meal in the diets reduced egg production significantly (see Table 8). With levels of 30% of moringa forage meal, egg production during the laying peak did not exceed 74% of the laying, and with levels of 40% of moringa forage meal it reached on average only 60% of the laying (Valdivié *et al.,* 2013a, b).

M. oleifera at levels of 30 to 40% in the diets for laying hens did not provoke deaths, favoured yolk pigmentation and limited the growth or fat deposition of layers during the

TABLE 7. Performance and cost of the feed consumed during six weeks by young layers

Indicator	Moringa meal (%)		SE ±
	0	20	
Initial live weight, g/bird	1264	1306	11
Live weight at 25 weeks, g/bird	1620	1657	36
Average weight of one egg, g/egg	47	50	1
Yolk pigmentation (Roche scale)	5	7	0.3*
Laying in week 25, %	58	66	3*
Cost of the feed per million young layers. USD	1 459 804	1 341 470	-

Notes: *$P<0.05$

TABLE 8. Performance of laying hens L-33 during the laying peak with levels of 30 and 40% moringa meal in the diets

Indicator	Moringa meal (%)		
	0	30	40
Viability, %	98	96	100
Final live weight, g/bird	1761	1683	1611
Laying, %	87.96	74.44	60.15
Total feed consumption, g/bird/day	109	118	120
Consumption of traditional feed, g/bird/day	109	83	72
Moringa consumption, g/bird/day	0	35	48
Crude protein consumption, g/bird/day	17.35	17.29	16.54
ME consumption, MJ/bird/day	13.16	12.49	12.45
Crude fibre consumption, g/bird/day	3.15	6.40	7.68
Grams of feed/egg	125	158	201
Grams of traditional feed/egg	125	111	121
Grams of moringa meal/egg	0	47	80
Broken eggs, %	0.017	0.021	0.049
Shell-less eggs	0.026	0.093	0.012
Average egg weight, g/egg	58.47	59.02	58.10
USD/t of feed	429.35	346.80	331.45

laying peak. Growth limitation was associated with an overestimation of the contribution of the metabolizable energy made by the moringa forage meal, which must be less than 15.08 MJ/kg ME.

1.3.3 Mule ducks (male Cairina moschata*×female White Peking)*

The possibility of fattening mule ducks efficiently was confirmed with diets containing 20, 30 and 40% moringa forage meal during the starter, growth and finishing stage, respectively, with higher carcass + viscera yields and reduced cost in US dollars per ton of carcass + edible viscera (Table 9).

It was noteworthy that ducks in the feeding system with moringa forage meal in the diet did not have abdominal fat when a breast dissection was carried out, and also for the legs for meat, skin and bones. It was found that the treatment with 20-30-40% moringa forage meal produced more meat and less skin than the control treatment, which was associated with less sub-cutaneous fat deposition (Table 9). This confirms that the ME value of the moringa forage meal must be below 7.95 MJ/kg.

These results with mule ducks obtained at ICA in 2013 confirm those attained in Cuba in 2012 by the CENPALAB and the Goose Plan, which are summarized in Table 10.

There was no information on the use of moringa meal in the feeding of geese and ostriches, but if they were palatable for them, its levels in goose feeding must be as high as those found in these studies with mule ducks, and in the case of ostriches could be used as total replacer of alfalfa in industrial feeds. All this must be confirmed in further investigations.

2. MORINGA IN PIG FEEDING

The available information on the use of moringa for pig feeding is rather limited and in practice is restricted to studies of its nutrient composition. Nonetheless, moringa is one of the forages fit for swine due to the high amount of protein that these animals require,

TABLE 9. Inclusion of *M. oleifera* forage meal in diets for mule ducks from 1 to 60 days of age

Indicator	Moringa forage meal in the diet (%)		SE±
	0	20-30-40	
Viability, %	100	99	-
Initial live weight, g	39	40	-
Final live weight, g/bird	2691	2595	25*
Feed consumption, g/duck	11.79	13.22	-
Feed conversion	4.38	5.10	0.05*
Yield (%)			
Carcass + edible viscera	73.21	75.93	0.52*
Carcass without neck	60.36	63.48	0.59*
Abdominal fat	0.58	0	0.38*
Breast	16.39	15.71	0.43*
Carapace	13.50	14.49	0.65
Legs	14.71	15.60	0.40*
Yield of the breast			
Skin	20.42	12.91	0.97*
Fat	56.17	61.55	1.72*
Bones	23.41	25.84	1.27
Yield of legs			
Skin	23.22	13.20	3.07*
Meat	57.82	67.20	2.88*
Bones	17.95	19.63	0.85
Feed cost to produce one ton of carcass + edible viscera			
Dollars/tonne	2365.39	2182.31	-
%	100	92.26	-

NOTES: *$P<0.05$.
SOURCE: Mesa *et al.,* 2013 (unpublished data).

TABLE 10. Performance of fattening mule ducks on including moderate or high concentrations of moringa forage (tender stems + leaves) meal in the diets from 1 to 63 days of age

Parameter	Control without moringa	Moringa forage meal in the diet (%)	
		Starter (20%) Growth (30%) Finishing (40%)	Starter (30%) Growth (45%) Finishing (60%)
Viability, %	83.0	91.5	86.5
Initial live weight, g/bird	37	37	38
Final live weight, g/bird	2693	2848	2616
Feed conversion, g/bird	5.04	5.02	5.69
USD in feed/ton of live weight	1987	1638	1638

SOURCE: CENPALAB, 2012b.

apart from the plant having virtually zero presence of anti-nutritional factors (Foild, Mayorga and Vásquez, 1999; García *et al.,* 1999).

Some research reports are noted below that assessed digestive indicators and productive performance of pigs receiving moringa foliage meal in the diet as replacer for commercial concentrate.

2.1 Apparent nutrient digestibility

Leiva and López (2012) conducted one of the first investigations in Cuba to study the effect of moringa on the digestive indicators of growing pigs. For that, moringa forage (leaves + tender stems) meal was used and the nutrient digestibility was assessed with the substitution of 20, 40 and 60% of the commercial concentrate.

Results showed that dry matter (DM), organic matter (OM), crude protein (CP) and NDF digestibility remain stable with the inclusion of 20% moringa forage meal in the diets of growing pigs, as shown in Table 11, and decreases with 40% moringa meal in the ration and even more with 60% moringa meal, indicating that from a digestive point of view the level of 20% of moringa forage meal is excellent for growing pigs and that higher levels worsen to some extent the efficiency in the utilization of feeds.

Later, García and Macías (2014) at the Instituto de Investigaciones Porcinas of Cuba, conducted a digestive evaluation of the *M. oleifera* foliage meal in pre-fattening castrated male pigs. Animals received a control treatment of Dominican commercial feed and two experimental treatments where 10 and 20% of the diet was substituted by moringa foliage meal. It should be noted that the commercial feed contained 22% protein while the moringa leaf meal had 18%.

Results from the apparent faecal digestibility (Table 12) indicated that the diet including 20% moringa meal showed a notable decrease in DM, OM and N digestibility compared with the 10% diet and the control, while ash digestibility was similar between treatments. If digestibility values obtained in this experiment are compared with those reported by Hernández *et al.* (2011) in studies realized with different commercial mixtures of Dominican, Mexican and Cuban origin for pigs of this category, the DM and OM digestibility showed lower values in this study in comparison with the sample of home origin. Nonetheless, N digestibility presented values much higher than those previously reported, considering that all diet had a similar bromatological composition and adequate for this nutritional category (NRC, 1998).

Riopérez and Rodríguez (2003) have indicated that starter feeds for piglet feeding must contain between 18 and 20% protein, since one of the main objectives of the diets during this stage is to enhance piglet growth without overloading its digestive capacity.

The digestibility values of the diet where 20% moringa was included were lower than those of the other two diets, but considering the nutritional category of the pigs under study, in which fibre inclusion is very limited when these values are compared with other foliage diets, it can be observed that are similar to the results reported by Domíngez (2006)

TABLE 11. Apparent digestibility of diets with 0, 20, 40 and 60% moringa meal for growing pigs

Apparent digestibility, %	Moringa forage meal, %			
	0	20	40	60
DM	84.35	83.13	75.94	70.14
OM	86.42	84.93	76.81	71.94
Ash	88.40	84.16	73.23	67.22
N	81.21	80.31	75.23	64.56

SOURCE: Taken from Leiva and López, 2012.

on substituting 30% of the diet by mulberry in growing-fattening pigs.

The nitrogen balance (Table 13) in diets used in the experiment showed similar nitrogen consumption. From the dietetic point of view, the inclusion of moringa meal did not influence positively the nitrogen balance. An increase of nitrogen outflow in faeces was observed as the inclusion percentage increased, provoking decreased retention as consumption percentage. However, estimated nitrogen retention as percentage of digestion maintained similar values.

Results of this experiment indicated that in the pre-fattening category (from 33 to 75 days of age) *M. oleifera* leaf meal can substitute 10% the commercial concentrate without affecting the digestive utilization indicators. However, when the growing pig category is analysed it is possible to include up to 20% of moringa forage meal.

TABLE 12. Faecal digestibility of pigs receiving moringa foliage meal

Apparent digestibility, %	Moringa leaf meal, %		
	0	10	20
DM	81.6	80.5	78.5
OM	83.1	81.7	79.7
Ash	67.9	69.2	66.7
N ×6.25	86.3	83.9	82.5

SOURCE: Adapted from García and Macías, 2014.

TABLE 13. Faecal digestibility of pigs receiving moringa foliage meal

Nitrogen balance, g/day	Moringa leaf meal, %		
	0	10	20
Consumption	22.6	22.4	21.6
Outflow			
In faeces	3.08	3.59	3.79
In urine	3.41	3.29	3.83
Total			
N digested, g/day	19.5	18.8	18.2
Retained, g/day	16.1	15.5	14.3
Retained, % of consumption	71.1	69.5	65.8
Retained, % digested	82.5	82.8	79.9

SOURCE: Adapted from García and Macías, 2014.

2.3 Performance traits

Pérez, Torres and Mendieta (2001) at the University of Nicaragua studied three levels of moringa foliage meal in substitution for three levels of commercial feed in piglets from 45 days of age until the fattening stage. These authors concluded that it was possible to substitute the commercial feed by 20% moringa foliage meal, although average gain and conversions decreased compared with the control diet but values were acceptable from the biological viewpoint.

At the El Guayabal farm of the Cuban Institute of Animal Science, Quintana, Martínez and Valdivié (2012) fattened pigs with a commercial feed of medium quality and live weight gains attained were 10% lower with the use of 40% moringa forage (stems + leaves) meal as substitute for the medium quality feed (Table 14). These live weight gains are appropriate for small Cuban pig producers (468 g/pig/day) in view of its economic viability. In this experiment, as found for ducks, it was striking the poor fat content of the meat and carcass.

At Plácido farm, in Cárdenas, Cuba, González (2012, unpublished) substituted 15% of an industrial protein-vitamin-mineral concentrate named Nuprovin by 15% of moringa forage meal with excellent results when molasses B was used as metabolizable energy (ME) source for pigs during the first fattening stage. Table 15 summarizes live weight and weight gain attained in this trial.

The difference noticed between the Cuban and the Nicaraguan studies regarding the inclusion level of *M. oleifera* forage meal in the diet of fattening pigs can be due to the various reasons discussed earlier, in Chapter II, regarding nutritive value, among them the edaphoclimatic conditions, cutting age, leaf:stem proportion (in forage meal), etc. These

TABLE 14. Pig fattening during 150 days with 40% moringa forage (stems + leaves) meal in the diet

Parameter	Feed	60% feed + 40% moringa meal
Number of pigs	10	10
Viability	100	100
Initial live weight, kg	19.35	19.45
Final live weight, kg	97.30	89.60
ADG, g/pig/day	520	468
Carcass yield	81.20	80.0
Leanness	medium	very lean

Notes: ADG = average daily live-weight gain.
Source: Quintana, Martínez and Valdivié (2012, unpublished; taken from El Guayabal farm records).

TABLE 15. Performance of fattening pigs with diets of molasses B + Nuprovin and molasses B + Nuprovin + 15% moringa forage meal

Indicators	Molasses B + Nuprovin	Molasses B + 15% moringa forage meal
Viability, %	100	100
Initial live weight, kg	26.8	26.1
Final live weight, kg	35.0	35.1
ADG, g/pig/day	405	450

Notes: ADG = average daily live-weight gain.
Source: González (2012, unpublished; taken from Plácido Farm, Cárdenas, records).

TABLE 16. Apparent faecal nutrient digestibility in rabbits fed different levels of moringa forage meal

Parameter	Moringa forage meal, %		
	0	15	30
DM	74.63	77.45	78.14
CP	73.01	82.75	85.32
NDF	45.89	48.83	50.27
ADF	55.14	67.40	68.68
Hemicellulose	60.66	58.44	62.25
Cellulose	30.65	39.84	41.36

Notes: DM = dry matter; CP = crude protein; NDF = neutral detergent fibre; ADF = acid detergent fibre.
Source: Taken from Caro, 2014.

aspects must be considered when the inclusion levels of this plant are determined in pig diets and, in general, in all species.

3. MORINGA IN RABBIT FEEDING

3.1 Apparent nutrient digestibility

In the evaluation of the nutritive value of the feeds, besides its chemical composition, the effect of the digestive, absorption and animal metabolism processes must be considered. Digestibility trials allow examining the proportion of absorbable nutrients present in a ration. Consumption and digestibility and are two of the main parameters defining the quality of a feed (Rodríguez *et al.,* 2007).

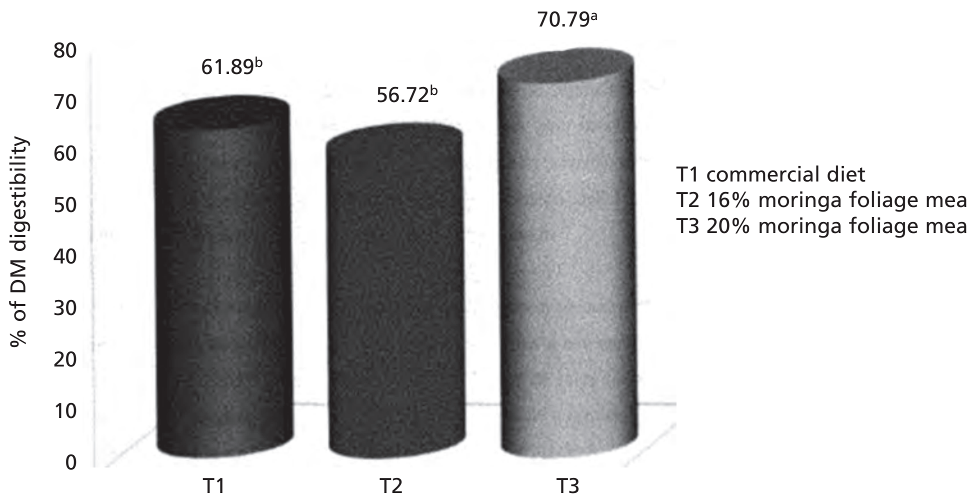
The utilization of forage plants has an impact on the digestive physiology of the rabbit, since it provokes modifications in use of nutrients and in biological efficiency. For this reason, determination of the nutrient content and the digestive utilization of the diets are necessary when these non-conventional resources are incorporated (Nieves *et al.,* 2011).

In this sense, Caro (2014) employed moringa foliage meal as protein and fibre sources for substituting soybean meal and wheat bran in rabbit diets. This author determined the effect of different inclusion levels (0, 15 and 30%) on apparent faecal nutrient digestibility in growing rabbits. With the inclusion of moringa forage meal (see Table 16), it was noted that apparent faecal digestibility of the protein, NDF, ADF and cellulose increased.

Values of apparent faecal DM digestibility are higher than those reported by González and Herrera (2012) (see Figure 1), who substituted totally (T2) and partially (T3) the soybean meal by moringa foliage meal.

These authors found that the best digestibility was obtained in animals consuming 20% moringa foliage meal (70.79%). In general, the values of apparent DM digestibility reported in the present paper are higher than those obtained by other authors assessing other non-conventional sources for rabbit fattening (Cordero *et al.,* 2010, using *Manihot esculenta;* and Nieves *et al.,* 2011, working with *Tithonia diversifolia*).

FIGURE 1
Percentage of seafood sales in supermarkets and food chains in Beijing, 1998



Notes: [a,b]Different letters between columns indicate significant differences of $P<0.05$.

Source: Taken from González and Herrera, 2012.

The increase in the apparent faecal digestibility of the fractions relative to the fibre in the diets containing moringa (see Table 16), could be determined by a higher retention time of the digesta in the caecum that would produce an increase of the fermentative activity (García *et al.,* 1999), According to Gidenne *et al.* (2000), on increasing the fibre in the diet for rabbits its digestibility is improved due to an increase both in quantity and quality of the caecal microbiological activity, since in this type of immature forage the esterified xylose residues are not present with acetyl groups. These esters prevent fibre degradation in more mature forages through a restriction of the specificity of microbial enzymes by polysaccharides.

Results shown suggest that the high nutrient digestibility of the moringa forage meal could supply rabbits with the necessary energy for maintenance.

3.2 Productive performance

3.2.1 Growing-fattening rabbits

Studies by González and Herrera (2012) reported that animals consuming diets with 20% moringa foliage meal showed the best results for all the indicators studied (except for consumption), in comparison with those consuming 16% (Table 17). It is interesting to note out that groups fed moringa had a similar performance to those of the control group. These results suggest that the inclusion of foliage meal did not affect feed acceptance in fattening rabbits.

At the same time, Caro (2014) observed that the inclusion of forage meal of the moringa Supergenius genotype in the diet favoured increased final live weight and weight gain compared with the control diet. It is important to stress that this non-conventional

TABLE 17. Productivity indicators in fattening rabbits fed diets containing moringa foliage meal

Indicator	Moringa foliage meal, %		
	0	16	20
Final liveweight, g	2296	2007	2438
Consumption, g/day	110.50	111.60	113.10
ADG, g/day	19.17	16.05	20.67
Conversion, g/g DM	5.72	6.92	5.42

NOTES: ADG = average daily live-weight gain.
SOURCE: Adapted from González and Herrera, 2012.

TABLE 18. Performance traits in rabbits consuming diets with increasing levels of moringa forage meal during the growing-fattening stage

Parameter	Moringa forage meal, %		
	0	15	30
Initial live weight, g	885	885	887
Final live weight, g	1957	1999	2003
Consumption, g/day	102	95	92
ADG, g/day	23.83	24.75	24.80
Conversion, g/g DM	4.30	3.86	3.75

NOTES: ADG = average daily live-weight gain; DM = dry matter.
SOURCE: Adapted from Caro, 2014.

protein source promoted a favourable productive performance in the rabbits, with 100% viability (see Table 18).

There was a decrease in consumption with increasing inclusion levels of moringa forage meal. Lara *et al.* (2012) stated that feed consumption is influenced by the level and type of fibre of the diet, which affects ingesta accumulation in the caecum through its effect on intestinal motility. Performance could be due to poor contribution from the source under study, and to the content of insoluble fibre of the diet. Gidenne (1992) indicated that the insoluble fibre stimulates consumption since it increases the rate of passage, which favours the caecotrophagia. Another factor that could have influence is the coarseness of the moringa forage meal, that inhibits greater consumption due to physical factors, that is, to the capacity of the digestive tract (García, 2006).

Animals consuming the diets with moringa forage meal showed a tendency to decrease the minimum value of feed conversion.

In another study, Diz (2013) substituted the commercial concentrate with higher levels of moringa forage meal (20, 30 and 40%) and concluded that with the utilization of 40% in the diet, favourable productive results are obtained during the growing-fattening stage (see Table 19). Nonetheless, values reported with 30% were controversial in comparison with those described by Caro (2014) (see Table 18).

All previously mentioned studies have in common that the diets were supplied as meal, which is not the most advisable since it tends to affect the values of feed conversion. In rabbits, for increasing feed utilization, it is recommended to use granulated diets or in the form of blocks.

Investigations at CENPALAB (2012) and replicated in two productive units (Plan 160 and Los Jardines) looking at the possibility of including up to 50% moringa forage meal supplied as granulated diets for rabbits, showed it did not affect the productive performance of the animals. Also, the cost of one tonne of rabbit meat in Cuba was reduced significantly. It is interesting to note that with the inclusion of 75% pelleted moringa forage meal, a live weight gain of 22.38 g/rabbit/day was attained (see Table 20). At the same time, Rodríguez (2010) used multi-nutritional blocks containing as protein source moringa forage meal at different inclusion levels (0, 25, 50 and 75%) and observed that weight gain during the fattening stage was similar to that of rabbits consuming the commercial concentrate and the 50% inclusion. However, it must be stressed that in these studies very high levels of moringa meal were used in the diets, without taking into account the effect that this could provoke on the digestive physiology of the animal.

In the tropics, moringa has also been included in different schemes and in non-conventional feeding systems as the only protein, fibre and/or mineral source(s) or in combination with other alternative sources. In this respect, García (2013) evaluated consumption and live weight gain in rabbits fed three diets based on: (1) mulberry (*Morus alba*) foliage; (2) *Bauhinia variegata* foliage; and (3) moringa (*M. oleifera*) foliage, compared with commercial feed (see Table 21).

The diet containing moringa gave more weight gain and the lowest feed conversion index compared with the other diets based on protein foliages. Consumption of the moringa diet was considered acceptable, which was reflected in the other indicators assessed (weight gain, feed conversion and growth rate).

TABLE 19. Productive parameters of growing rabbits consuming diets with increasing levels of moringa forage meal

Parameter	Commercial concentrate	Moringa forage meal, %		
		20	30	40
Initial live weight, g	1000	1000	1000	1000
Final live weight, g	2250	2220	2170	2220
ADG, g/day	20.74	20.37	19.63	20.30
Feed conversion	7.24	7.37	7.67	7.40

NOTES: ADG = average daily live-weight gain.
SOURCE: Adapted from Diz, 2013.

TABLE 20. Productive performance of rabbits during 42 fattening days with pelleted moringa meal in the diets

Indicators	Moringa forage meal, %			
	0	25	50	75
Viability, %	97.9	97.9	87.5	93.7
Initial live weight, g	702	682	720	740
Final live weight, g	1874	1871	1831	1675
ADG, g/rabbit/day	28.59	29.70	27.10	22.38
Feed conversion, g/g DM	3.37	3.32	3.88	5.81
Feed cost/tonne meat ($)	2140.2	1710.4	1538.8	1526

NOTES: ADG = average daily live-weight gain.
SOURCES: Taken from CENPALAB (2012a, b).

At the same time, Smarth *et al.* (2013) assessed the productive performance of rabbits (see Table 22) on four feeds, namely: *T. labialis* + sugar cane stalks + sunflower seeds; *M.*

TABLE 21. Productive performance of Cuban Brown rabbits fed four alternative feeds during the fattening stage

Parameter	Treatment			
	Control	Moringa	Mulberry	*B. variegate*
Final live weight, g	2175	2119	2190	2121
ADG, g/day	40.54	32.71	29.29	26.54
Feed conversion	2.94	3.12	3.81	3.14[a]

NOTES: ADG = average daily live-weight gain.
SOURCE: Adapted from García (2013).

TABLE 22. Productive performance of Cuban Brown rabbits fed four feeds systems during the fattening stage

Parameter	Feeding systems			
	Teramnus labialis + sugar cane + sunflower	*Moringa oleifera* + sugar cane + sunflower	*Morus alba* + sugar cane + sunflower	*Rosa sinensis* + sugar cane + sunflower
Initial live weight, g	595.00	595.00	595.16	595.33
Final live weight, g	2602.16	2696.33	2418.33	2162.00
ADG, g/day	22.30	23.51	20.25	17.43
Conversion, g/g DM	6.80	6.28	6.73	7.02

NOTES: ADG = average daily live-weight gain; DM = dry matter.
SOURCE: Adapted from Smarth *et al.*, 2013.

TABLE 23. Reproductive indicators in White New Zealand breeding does fed four alternative feeds during the fattening stage

Parameter	Moringa foliage meal, %			
	0	25	50	75
Number of young rabbits weaned	2.75	2.00	3.75	2.50
Number of young rabbits born alive	4.75	4.75	5.75	3.25
Number of stillborn rabbits	2.50	1.50	0.75	1.75
Average weight of young rabbits born alive, g	37.50	30.75	34.50	37.2
Average weight of stillborn rabbits, g	30.37	12.42	19.62	10.75
Average weight of young rabbits at weaning, g	166.70	173.80	212.78	312.10
Average weight of does at the beginning of the experiment, g	2310	2200	2140	2260
Average weight of does at the end of the experiment, g	2660	2360	2620	2550

SOURCE: Adapted from Cornejo & Paredes, 2011.

oleifera + sugar cane stalks + sunflower seeds; *M. alba* + sugar cane stalks + sunflower seeds; and *H. rosa-sinensis* + sugar cane stalks + sunflower seeds. Forages were fresh supplied at a rate of 600 g/day, with 150 g/day sugar cane and 25 g/day sunflower seeds.

The best productive response was obtained with the animals consuming moringa-sugar cane-sunflower, with 100% viability. This also gave the best carcass yield and yield of edible parts.

In general, results achieved are encouraging for rabbit rearing in tropical climates.

3.2.2 Breeding does

Cornejo and Paredes (2011) evaluated the effect of multi-nutritional blocks containing moringa foliage meal as protein source at different inclusion levels (0, 25, 50 and 75%) on breeding does (see Table 23).

Results found show similar performances since diets containing moringa foliage meal did not compromise foetus development and, consequently, did not affect birth or mortality of young rabbits or influence litter or doe weights.

The number of young rabbits weaned showed differences between treatments, increasing this indicator in breeding does consuming the blocks containing 50% moringa foliage meal. However, when compared with a group of does fed a granulated commercial concentrate weanings were fewer. The authors indicate that part of the effect could be due to the low milk yield of the does as a result of the stress provoked by the change of feed and the mode of presentation. All this must be confirmed in subsequent investigations.

4. FINAL REMARKS

There is the possibility of including 10% moringa forage meal in broiler rations without affecting the morphometric indicators of the GIT.

The positive effect of the dry fermented product Vitafert on the apparent faecal retention of NDF, cellulose and hemicelluloses was evident in broilers consuming moringa forage meal.

With moringa leaf meal it was demonstrated that inclusion levels do not worsen bird growth, which is greater than growth based on other stem + leaf meals.

The variability of response shown by poultry, swine and rabbits in different experiments evaluating the use of leaf meals and leaf + stem meals is associated with the variability in the nutritive value, due essentially to cutting age and the leaf:stem relationship of the moringa meal used.

The study showed that moringa meal can be used as total and/or partial substitute for the protein in diets for fattening rabbits and breeding does without negative effects on consumption, live weight gain, digestibility or reproductive indicators.

The possibility of including high levels of *M. oleifera* forage meal in the diets for poultry, swine and rabbits was demonstrated by the adequate growth rate, egg production, viability, health, egg quality, higher leanness in meats and good flavour of the final products (meat and eggs). Replacing part of imported feeds (soybean meal, maize and alfalfa meal) could reduce feeding costs per tonne of live weight, tonne of carcass and per million eggs produced.

The optimum inclusion levels of moringa stem and leaf meals and moringa leaf meals must be specified for the different poultry categories (geese, ostriches and quails) and in pigs, where scientific publications are scarce, as well as in rabbits, including breeding does and replacements.

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Moringa oleífera (Lam.) in ruminant feeding systems in Latin America and the Caribbean region

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ABSTRACT

The characteristics of *M. oleifera* are excellent for animal nutrition. This chapter discusses responses to *Moringa oleífera* (Lam.) in ruminant feeding systems in Latin America, Cuba and the Caribbean region. The results indicate that the highest live weight gain and greater feed consumption of animals supplemented with moringa are due to the cell contents, with high levels of energy-rich constituents that are known to stimulate microbial protein synthesis. Productive performance increased. Results using *M. oleifera* as a complement to poor quality fibrous diet in sheep, goats and bovines demonstrated the feasibility of increasing beef and milk production with stable maintenance of health indicators. The increased ruminal fermentation due to the supply of protein with high biological value possibly explains the positive responses obtained.

Keywords: productive performance**,** fibrous diets, milk production, *Moringa oleifera.*

INTRODUCTION

The Western part of the contemporary world has increased the use of chemical products for greater agricultural production, but with some disastrous side-effects, both for human health and for agriculture and livestock production. At the same time, other regions advance rapidly, and there is rediscovery of traditional customs and habits, as well as re-evaluating the immense richness of the flora and its potential for providing solutions to many problems in the so-called Developing Countries. One of the most prominent plants in recent years in that sense is *Moringa oleifera,* which originates from the Himalayas to the north of India, but that nowadays grows in abundance in the tropics worldwide.

Moringa can be propagated sexually by means of seed, or clonally by asexual cuttings, even in poor soils; it withstands long drought periods and grows well under arid and semi-arid conditions. It is a hardy species, requiring little agronomic attention, and grows rapidly, up to four metres in a year, with a nutritive value suitable for various animal species.

In general, ruminant rearing is often carried out on the least productive sites, with less fertile soils, in the great majority of tropical countries. There is a constant search for plants and new feeds that can be used for animals under such conditions. In this context, *M. oleifera* seem able to meet such needs. This chapter reviews briefly reports found on the use of moringa for ruminant feeding in Latin America and the Caribbean.

According to Martín *et al.* (2013) the nutritional characteristics of *M. oleifera* are excellent. Consequently, it is used as forage on a large scale in various African countries and in Nicaragua. It has high productivity of fresh matter compared with other pastures, such as alfalfa, and high values are attained with a sowing density of one million plants per hectare. Its leaves and the pressed cake of its seeds can be used in rations for animal feeding (Pérez *et al.,* 2010). Leaves can be used either direct or after extraction with ethanol. In an investigation at the Institute of Animal Production in the Tropics and Subtropics (in Hohenheim, Germany), it was demonstrated that the amino acid composition of moringa leaves is comparable with that of soybean, and it was confirmed that the digestible protein index (DPI) of its leaves in the intestines is higher than that of various conventional protein supplements, such as coconut cake and cotton, groundnut, sesame and sunflower seedcakes.

The high levels of crude protein (CP) and DPI make moringa leaves a good protein supplement for high-production cattle. At the same time, leaves extracted with ethanol are even better ingredients for feeds, since besides its high protein content, it has almost no tannins, lectins, trypsine inhibitors or flatulence factors, and its saponin and phytate levels are low. In Nicaragua, good results have been obtained with the use of mixtures of *M. oleifera* leaves with molasses and sugar cane straw (Radovich, 2011). Also, the use of leaves of this plant has been tested in pisciculture and vermiculture (Cova *et al.,* 2007).

During 2008–2009, investigations with moringa were conducted in Sinaloa (Autonomous University of Sinaloa), Mexico. The adaptability of the culture to the climatic conditions of central Sinaloa was demonstrated. The sowing density used was one million plants per hectare, in a poor soil, yielding 180 t/ha of fresh forage under irrigated conditions, and 80 t/ha of fresh forage in the rainy season. Results suggest that with more efficient irrigation management, sowing density, cutting frequency and fertilization, these yields could be increased substantially.

Data from laboratory analyses indicate that the apparent dry matter digestibility (DMD) of moringa in sheep was 70.5%, almost equal to that of alfalfa (75%). The difference is due to the fact that moringa has a higher indigestible fibre proportion. At the same time, studies of apparent protein digestibility in moringa indicated that it is 65.5%, versus 63% in alfalfa, owing probably to higher percentage of soluble protein in moringa. Currently, the second stage of the project is underway, looking to improve moringa yield by optimizing culture and fertilization conditions, as well as varying irrigation intensity and sowing density.

Garavito (2008) indicated that in view of the shortage of good quality grasses for the feeding and nutrition of bovines, pigs and equines, either in extensive or intensive husbandry on soils preferably below 1000 masl, a new, economic and excellent option used widely worldwide, but little used in Colombia, is the culture of *M. oleifera*. This shrub has been traditionally used in Asian and African countries as human and animal feed, as well as for water purification, with special application in treating malnourished children and preventing blindness, among other uses, It is a source of zeatin, a cytokinin plant growth hormone, used as accelerator and multiplier in the production of traditional cultures through its leaf extract. This, together with the stems, presents excellent potential for bioethanol production.

1. MORINGA AS FORAGE FOR BOVINE CATTLE

Studies conducted in Honduras with steers fed star grass hay and a supplement of moringa leaves showed greater liveweight gain (380 g/day) and feed consumption, compared with those without supplementation. These studies indicate that the high liveweight gain and higher consumption in the supplemented animals with moringa are due to the constituents of its cell content, that have high levels of energy releasing substances known to increase microbial protein synthesis. Becker (1995) noticed that the fermentation rates of rations with different hay:moringa proportions produced variations in the fermentation, compared with when both feeds were supplied separately. This indicates evidence of a possible associative effect in the digestion and assimilation of both feeds when supplied together.

Alternatively, plants that are considered to increase the protein level in the ration have a positive effect on consumption, as it stimulates an increased level of efficiency in metabolizable energy utilization, produced by greater microbial activity.

Trials in different parts of the world with bovines, pigs, sheep, goats and poultry, have shown important yield increases in both weight gain and milk production. These results have been, as is logical, much more spectacular with animals from a deficient diet than in those with a balanced diet.

In studies in Latin America and the Caribbean on this plant, it was found that the forage obtained contains between 16 and 20% protein in dry matter (DM), although it can be even higher (values similar to those of alfalfa, and higher than the 12% of forage sorghum).

In Nicaragua, the Biomasa organization has shown that supplying moringa leaves at 40–50% of the total ration, improved milk yield in cows, and weight gain in calves increased by 30%. Also neonate animals weighed between 13% and 22% more than the average (Table 1).

Nevertheless, authors indicate that these observations must be considered with caution and repeated over time.

Also, they assessed the cutting of moringa re-growths at intervals between 35 and 45 days, depending on the management conditions. Growth between cuts can reach 120–150 cm. The cut material – stems, branches and leaves – are chopped and supplied to the animals. Up to 27 kg of fresh material/animal/day has been supplied to the animals.

At the beginning of moringa feeding an adaptation period is recommended, mixing it with other feed supplied to the cattle. Moringa can be used as protein complement or as complete diet.

M. oleifera as fresh forage for dairy cattle has been studied in various trials. Results have shown no decrease in milk production in grazing animals supplemented with concentrates and later transferred to grazing and moringa supplementation. Cost with moringa under these conditions were 10% lower than with concentrates.

In addition, in Nicaragua, Rodríguez (2011) studied dairy cows supplemented with fresh or ensiled *M. oleifera* versus a control diet based on *P. purpureum* cv. CT-115 + commercial concentrate, as basic

TABLE 1. Data from trials in Central America. Daily consumption 15 kg per adult ruminant

Parameter	Animals with moringa	Animals without moringa
Milk production	10 litres/day	7 litres/day
Daily gain	1200 g/day	900 g/day
Birth weight	23–26 kg	20–22 kg
Multiple calvings	3 per 20 calvings	1 per 50 calvings

Source: Reyes, 2004.

diet for dairy cows. This author assessed the effect of the experimental diets on consumption and digestibility, milk yield and composition, as well as the organoleptic characteristics of the milk and cheese. DM consumption of the moringa treatments was higher than the control. Fresh moringa had the highest consumption of organic matter (OM), crude protein (CP), neutral-detergent fibre (NDF) and acid-detergent fibre (ADF) compared with the control diet. The highest digestibility was shown in the control rather than in the moringa treatments (fresh or ensiled), except for CP digestibility. Milk production of the ensiled moringa treatment was slightly lower (9%) relative to the other two treatments.

Milk composition was similar between treatments. However, milk from the fresh moringa treatment showed a grass flavour and aroma, differing from the other two treatments, despite having normal colour and appearance. There were no organoleptic differences between the milk from the control treatments and the treatment with ensiled moringa. Similar results were found in the cheese prepared from the milk of the three treatments. The financial analysis favoured the moringa treatments, therefore, it was concluded that moringa silage can be used for feeding dairy cattle in large quantities for producing the same amount and quality of milk as conventional diets.

Studies in Cuba at the enterprise “Niña Bonita”, with medium to high potential Holstein cattle and substituting Norgold commercial concentrate in the diet showed that the 33% moringa inclusion level gave best performance, with no changes in milk production or composition (García-López and Reyes, 2012, unpublished).

At the Instituto de Ciencia Animal in Mayabeque, Cuba, García-López and González (pers. comm., 2014) carried out evaluations using calves. Results indicated that it can be a feeding alternative even when dependant on very poor quality feeds (bagasse pith from sugar cane mills) for the bulk of the diet. Table 2 shows the percentile composition of the diets used.

Daily gain (329 g/day vs 239 g/day) and haematochemical components determined indicated better performance in animals consuming moringa with bagasse pith, perhaps influenced by higher percentages of vitamins in moringa relative to maize and soybean,

TABLE 2. Characteristics and chemical composition of the starter integral diet

Feed	Control (%)	Treatment (%)
Sugar cane bagasse	30	16
Maize	46	44
Moringa	0	32
Soybean	22	6
M-V premix	1	1
Monocalcium phosphate	0.25	0.25
Calcium	0.5	0.5
Common salt	0.25	0.25

Macro nutrient analysis	DM %	CP %	CF %	ME (MJ/kg)	Ca %	P %
Control	85.5	16.5	10.42	11.67	0.5	0.4
Treatment	85.8	16.3	10.74	11.11	0.41	0.3

NOTES: DM = dry matter; CP = crude protein; CF = crude fibre; ME = metabolizable energy; Ca = calcium; P = phosphorus.
SOURCE: Reyes, 2004.

since the remaining components (CP and ME) remained stable and similar. Nonetheless, in both treatments, gains were low with respect to that expected for this growth stage (450–500 g) relative to the quality of the fibre used (bagasse pith residue from sugar cane juice mills). Nevertheless, results indicated good utilization of bagasse pith, which basically is an element of great environmental pollution.

2. UTILIZATION OF MORINGA IN THE FEEDING OF GOATS AND SHEEP

2.1 Goats

In recent years a group of researchers from the Instituto de Ciencia Animal (ICA) of Cuba have been developing alternative protocols for utilization for basal diets of integral mixtures with a fibrous base, including woody forages, together with improved lines of Napier grass (*Pennisetum purpureum*), for different home production systems in the Latin America region, with positive results in the productive performance of cattle, sheep and goats.

Even though there is poor information regarding *M. oleifera* consumption in goat feeding, feeding protocols have been developed (Gutiérrez *et al.*, 2014) with confined Alpine breed billy goats fed mainly a mixture based on increasing levels (20, 40 and 80% of DM) of *M. oleifera* cv. Supergenius, substituting *Pennisetum purpureum* clone OM-22. Plants of approximately 50 or 27 days growth and with chemical compositions of 21.28% DM, 19% CP, 10.18 MJ/kg DM, 66.34% NDF, 64.93% DM digestibility (at 50-day growth), and 26.40% CP, 7.90 MJ/kg DM, 81.84% NDF and 52.49% DMD (at 27-day growth) were cut and carried in the morning and later chopped to 3–5 cm particle size. In general, results showed increased voluntary consumption corresponding to the proportion of moringa in the mixture, being highest for 80% inclusion of moringa in the mixture, with absolute and relative values of: 1.37 kg/day, 3.58% LW, 88.99 g/kg $LW^{0.75}$ and for moringa of 1.10 kg/day, 2.87% LW, 71.34 kg $LW^{0.75}$.

In studies conducted with goats during the development stage, supplemented with *M. oleifera* leaves at inclusion levels of 9, 27 and 36% DM, consumptions were: 251, 335 and 311 g/day, respectively. Similarly, on supplementing nanny-goats with levels of 20% or 50% of moringa foliage, DM consumptions were 50.9 and 51 g/$kg^{0.75}$ with average daily gains of 86 and 78 g/day, in contrast to non-supplemented animals that only attained 55 g/day.

It should be stressed that moringa forage can be supplied alone, in contrast to other forage plants such as leucaena; goats show prefer this forage to be offered suspended in the air rather than in a trough, after a minimum adaptation period (2 days). In spite of this, the supply method does not affect the ME and OM digestibility.

2.2 Sheep

Reyes-Sanchez, Ledin and Ledin (2006) carried out studies on Santa Rosa farm of the Universidad Nacional Agraria in the Department of Managua, Nicaragua, to assess the productive performance of sheep fed a basal diet of guinea grass (*Panicum maximum* Jacq.) and supplemented with different levels of *M. oleifera*. For that, 18 crossbred (Pelibuey × Black belly) lambs were used, with average initial weights of 20 ±2 kg. Animals were wormed, treated with vitamins and distributed in three treatments: *Panicum maximum* ad libitum; *P. maximum* ad libitum + 0.35 kg DM *M. oleifera*; or *P. maximum* ad libitum + 0.50 kg DM *M. oleifera*. The variables studied were: total DM consumption (TDMC), average daily gain (ADG) and feed conversion (FC). Results showed that *M. oleifera* forage as protein supplement for sheep consuming a basal diet of *P. maximum* increases weight gain and improves total DM consumption and feed conversion. Animals attained ADG of 118 g/day with FC of 6.78 kg feed per kg ADG, in contrast with those consuming 0.730 kg animal/day, which only gained 91 g/day with worse conversion (8.02 kg feed per kg ADG).

At the same time, on presenting results from the project on intensive culture of *M. oleifera*, Pérez *et al.* (2010) indicated that it represents an alternative for forage production with high protein content for sheep feeding in the central zone of Sinaloa, due to its adaptability and low production cost. In addition, it has 70.5% apparent DMD and 65.5% apparent protein digestibility.

Martín *et al.* (2010) pointed out that grease-removed moringa cake, due to its high protein content, is a raw material of interest for animal feeding. In recent research, six non-traditional oleaginous plants growing in Cuba were compared, and *M. oleifera* had one of the highest protein contents (68.6% DM) in pressed cake. The evaluation of that cake as additive in the diet for sheep has been the subject of recent studies. In an investigation with 24 lambs fed hay *ad libitum* and restricted amounts of soybean meal and *M. oleifera* cake over 45 days, it was demonstrated that the addition of the cake resulted in better ruminal fermentation and live-weight gain (LWG) directly proportional to the level supplied. Additionally, this cake had a higher CP content and lower NDF than soybean meal, and did not affect hay intake, digestibility or the nitrogen balance. At the same time, it was demonstrated that the proteins present in the cakes have an antibiotic effect and those in which grease was totally removed do not contain the majority of the secondary metabolites of the plants, such as: tannins, saponins and trypsin, and amylase inhibitors (Makkar and Becker, 1996).

3. MIXED SILAGES (MORINGA + *PENNISETUM PURPUREUM*) FOR RUMINANTS

Many tree and shrub species by their nature have forage potential and in their natural distribution under tropical conditions are used as multipurpose agricultural components. There are advantages from the utilization of such a resource in the tropical region, and an obvious option for development now and in the future. This biomass can be utilized for grazing and for cut-and-carry in many cattle production systems and in ruminant species, as well being the practical focus of applied research centres.

Reflecting their nature as forage potential and their natural distribution, many tree and shrub species, under tropical conditions, are multipurpose (Toral, 2005; Escalante, 2006). They have advantages in cattle feeding and there is no doubt that their use is a viable option currently and with future potential in tropical regions. This applies to biomass used both for grazing, and for cut-and-carry as used for many livestock production practices and ruminant species. As a technique, the use of multipurpose forages are topics for many protocols and specific actions of research centres.

The use of multipurpose-forage-based models provides potential for achieving profitability and sustainability in systems, in harmony with the environment. These elements justify the application of different technologies at national level and different tropical regions, as a means to diminish reliance on imported supplemental feed sources to cover animal requirements (Ojeda *et al*., 2006).

Earlier reports by researchers (Suárez *et al*., 2011) state that there are deciduous trees that lose their leaves because of blooming or due to the season, which is convenient for using this produced biomass and avoiding, by means of cutting, the expiration of leaves. This gives a preserved and regrowth biomass during dry seasons.

It is an unresolved issue that part of the productive potential of these agroforestry systems are lost, even though appropriate technologies are known that could remedy this situation (Ojeda *et al*., 2006). In this sense, the use of mixed ensiling of this tree forage in mixtures with grasses would contribute to increasing the quality of this fibrous material.

Studies by Gutiérrez *et al*. (2015) demonstrated that 60% substitution of pennisetum for moringa in pre-dried silages shows better quality, with values of DM (27.76%), CP (19.47%), OM (80.39%) and ash (20%), and a significant reduction of NDF (55.98%) (Table 3). This does not match the findings of Mendieta-Araica *et al*. (2009) when they used the whole plant of moringa, with an average of 14% CP and 58.3% of NDF.

Regarding dry matter, as a control indicator of quality and intensity of fermentation process (Vallejo, 1995), the values obtained in studies of Gutiérrez *et al.* (2015) are superior by 25% to those reported by Boschini and Elizondo (2003) and Huerta and Polo (2007), who point this indicator as a necessary factor for the conservation of ensiled forages, responsible for the decrease of losses due to effluents, for the predominance of lactic acid bacteria and achieving proper pH. Ojeda *et al*. (2006) note that values around 37% DM are more effective indicators determining fermentation, such as fermentation intensity, butyric acid production, and buffering capacity, the last-named being determined by the nitrogen content, which, when released by bacteria, increase the initial buffering capacity.

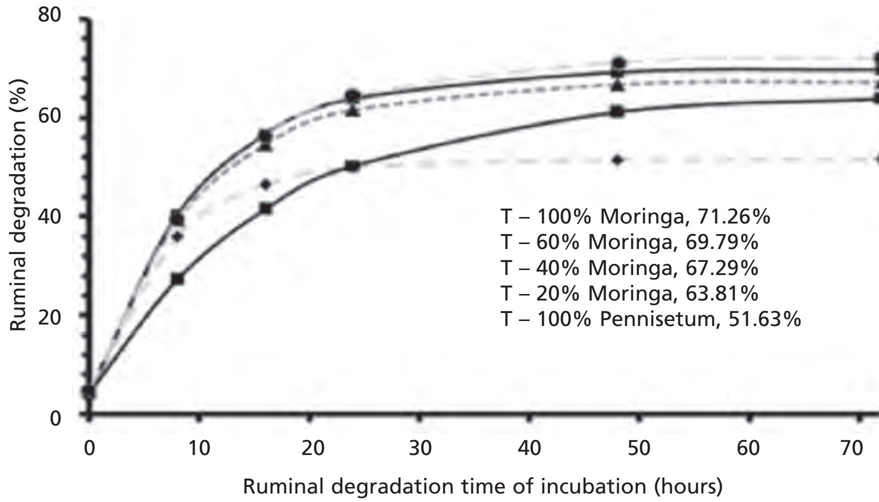
Although, according to Gutiérrez *et al*. (2015), in this type of mixed silage (*Pennisetum purpureum* + *Moringa oleifera*), regression analysis considered a 54% substitution of pennisetum by moringa in the ensiled mixture to be an optimum level, ensuring good fermentation, which corresponds to a contribution of CP = 20.34% (R2 = 94.13, ± SE = 0.01, $P<0.001$) and NDF = 53%. Regarding protein concentration, it tended to increase as the moringa increased in the mix, with a range of 17 to 20% of CP, according to the content (20, 40 or 60% moringa) in the mixture. This values are superior to those found in mixed silages of leucaena and gliricidia and of king grass, with about 12% of CP (Santana, 2000) and studies by Pinto *et al*. (2010), with different substitution levels (20, 40, 60 and 80%) of *Pennisetum purpureum* by *Leucaena leucocephala* (9.4 vs. 16% of CP) and those found by Gutiérrez *et al.* (2014) in mixed silages of *Tithonia diversifolia* + *Pennisetum*

TABLE 3. Chemical composition (% DM) of from micro-silos, based on different ratios of *Pennisetum purpureum* to *Moringa oleifera*

Pennisetum: moringa ratio	DM	CP	NDF	Ash	OM
100% pennisetum	31.98	13.66	68.15	21.33	78.66
80:20	27.70	17.29	60.46	21.22	78.80
60:40	27.28	18.52	55.41	21.74	78.19
40:60	27.76	19.47	55.98	19.82	80.39
100% moringa	26.60	20.71	59.99	19.66	80.33

Notes: DM = dry matter; CP = crude protein; NDF = neutral detergent fibre; OM = organic matter.
Source: Gutierrez *et al.,* 2015.

FIGURE 1
Curve of *in situ* ruminal degradation of DM



Source: Based on model proposed by Orskov and McDonald, 1979.

purpureum cv. Cuba CT-169, with tithonia inclusion levels of 20, 40, 60 and 80% and protein values of 8, 11, 14 and 17% CP, respectively.

Regarding kinetics of ruminal degradation of dry matter, Gutiérrez *et al.* (2015) found the best response with a mixture of 40% *P. purpureum* + 60% *M. oleifera*. These authors observed that, after the first 24 h of incubation, there was a tendency to stabilize and, after 72 h, the maximum value of degradability (69.79%), potential degradability (69.79%) and dry matter effective degradation (78.34%) was obtained (Figure 1). This response is similar to that stated by Gutiérrez *et al.* (2013), with 65.10% DM degradability after one plant was ensiled, which confirms the fast degradation of its cell wall and its use by ruminal micro-organisms during digestion as a nitrogen and energy source. It was also confirmed that anti-nutritional factors within moringa do not affect its digestibility (Reyes, 2004).

4. MORINGA – SOME DRAWBACKS AND PROPOSALS

Some persons think that moringa taints milk. This is easily solved by milking the animals more than three hours after last consumption.

The greater weight of calves at birth can be problematic in some dairy cattle breeds. This problem can be mitigated by either inducing premature calving (10 days seems sufficient), or limiting moringa consumption by pregnant cows presenting this problem.

Some precautions are advisable with moringa supply: since it is a high protein feed, it must be balanced with feed and energy sources rich in fibre, which are generally easily available. Table 4 shows concentrations of fibre and protein in the diet recommended for calves and fattening cattle.

TABLE 4. Recommended consumption in Central America of fibre and protein: maximum advisable in the diet

	Protein (%)	Fibre (%)
Lactating calves	18	26–30
Fattening cattle	12–14	36

5. FINAL CONSIDERATIONS

Moringa, by its characteristics, can be utilized in Latin America and Caribbean communities not only for improving animal feeding but also for developing sustainable systems. Its use in ruminant feeding in this area is low compared with the wider application in human feeding and health. Nonetheless, results obtained with its use as a complement to poor quality fibrous diets in sheep, goats and cattle have demonstrated the feasibility of increasing beef and milk production while maintaining stable health. The increments in ruminal fermentation due to the supply of a protein of high biological value match the positive responses obtained up to present. The similarity of the productive results from substituting commercial concentrates of high cost by moringa confers on this shrub a positive value from both economic and social viewpoints. The control of the inconveniences that its use could cause, together with the precautions that must be taken for its correct application in the different feeding systems, are essential premises for attaining better exploitation of moringa, sometimes termed “the tree of the miracles” or “the green manna of the Tropics”.

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Agronomy of *Tithonia diversifolia* in Latin America and the Caribbean region

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ABSTRACT

This chapter analyses the agronomy of *Tithonia diversifolia* in Latin America, Cuba and the Caribbean region. Distribution, botanical characteristics, germplasm, growth and development of 29 plant lines collected in various locations of Cuba and suitable for grazing by animals are discussed. In addition, germination capacity of the botanical seed of these lines was studied. Other aspects considered in the productive performance of tithonia are the effect of the plant on soil fertility; planting method; biomass production; cutting (frequency and height); and plant spacing. Observations confirm its excellent resistance to diseases and pests, and its attraction and feeding source for insects – functions necessary in order to produce with minimum environmental impact when using *T. diversifolia* in a silvipastoral system.

Keywords: agronomy, germplasm, plant materials, productive performance, *Tithonia diversifolia*

INTRODUCTION

The serious damage to natural resources and the present economic and social crises in many countries have re-awakened interest in establishing accelerated but sustainable agricultural development. This will only be achieved insofar as the production strategies are consistent with the rational use of the ecosystem (Iglesias *et al.,* 2011). In this context, examining agricultural activity in agroforestry systems is a valid, necessary and relevant approach in research investigations and training for agricultural development in the tropics. Various studies have demonstrated that, in cattle production in tropical America, a change from pasture monocultures to mixed vegetation, combining grasses, legumes and woody plants in sown areas, increases photosynthesis, improves nutrient recycling, restores biota and soil fertility, and increases biodiversity (Murgueitio *et al.*, 2011). Murgueitio (2005) indicates that cut-and-carry systems, as well as mixed forage banks, are ideal for the conservation of the fragile slope soils and humid ecosystems very much used in peasant production and dairy units. In view of the diversity of forage trees and shrubs, there is the urgent need for studying and recommending promising species for specific agroecological environments and agricultural production systems, both for biomass productivity and for nutritive value.

Mahecha and Rosales (2005) described *Tithonia diversifolia* as a species with good biomass production capacity and fast recovery after cutting, according to sowing density, soils and vegetative stage.

On evaluating the possibility of developing a suitable strategy, work with *T. diversifolia* started. Botanically it is a herbaceous plant of the Compositae family (Asteraceae), native to Central America (Nash, 1976; Murgueitio, 2005). This species is part of the Cuban flora. It has great root volume; a special ability to extract soil nutrients, even though they appear in short supply; and is widely adapted and distributed in the tropical zone. It is found from sea level up to 2400 masl in places with rainfall between 800 and 5000 mm/year. The plant is tolerant of acidity and low soil fertility. It has rapid growth, its biomass production varies between 30 and 70 t/ha/year of fresh forage (Mahecha and Rosales, 2005; Zapata and Silva, 2010). It has good foliage nutritional value (Ibrahim, Villanueva and Mora, 2005) and can accumulate as much protein in the leaves (up to 33%) as some legumes. It has high phosphorus content and also high dry matter digestibility (DMD) and presence of oils in leaves and flowers. It has 39.8% total sugars and can accumulate high C concentration in its aerial biomass (>77 t/ha/year). *T. diversifolia* has been recognized as a useful plant as improver of the general fertility of soils, mainly when managed as green manure (Crespo, Ruíz and Álvarez, 2011) either by incorporating it into the soil or managed as accompanying culture (Ríos, 2002). This is a plant that prevents erosion (Murgueitio and Ibrahim, 2004). It is used in living fences as flora for apiculture, in medicine and in bovine silvipastoral systems. It is cut as forage for feeding pigs, sheep, rabbits, bovines and buffaloes. It shows potential for feeding both ruminant and monogastric animals (Mahecha and Rosales, 2005; Wambui, Abdulrazak and Noordin, 2006) and its forage has good nutritive value (De Souza Junior, 2007).

Interesting results were found at the Institute of Animal Science (ICA) of Cuba by a multidisciplinary group (Ruiz *et al.*, 2010) developing a national research project using genotypes of *T. diversifolia* collected in Cuba, and proposing technical protocols for biomass production, cutting performance and grazing, as well as its use for improving the biological and physiological performance of animals used for genetic improvement.

1. DISTRIBUTION AND BOTANICAL CHARACTERISTICS

Tithonia diversifolia is native to Central America and has spread from there to South America and the Caribbean. It is a species of great ecological plasticity, being widely distributed in the tropical area. It is naturalized in Cuba. *T. diversifolia* belongs to the order Rubiales; Family Compositae or Asteraceae; Tribe Helianthea.

Flower Leaf Plant

Photograph 1. Images of typical Tithonia diversifolia
Source: Supplied by authors.

The *Tithonia* genus includes approximately 10 species, and *T. diversifolia* is a perennial plant. In Cuba two species have been identified: *Tithonia rotundifolia* and *T. diversifolia*. In Venezuela and Colombia it is known as *botón de oro*. In Cuba it is recognized as island marguerite, marguerite, Mexican marguerite and margaritone throughout the country, and specially in Victoria de la Tunas, Macagua and Remedios (Roig, 1928, 1974).

2. GERMPLASM EVALUATION

Luévanos *et al.* (2010) in Mexico crossed the cultivated HA 89 sunflower *Helianthus annuus* and wild species with ornamental *Tithonia rotundifolia*. Results indicated that all hybrid plants obtained were sterile, did not produce pollen and were incapable of forming seed. These authors conclude that chromosomal manipulation or tissue culture techniques are required for the development of fertile hybrids with ornamental potential.

Ruiz (2010) and Ruiz *et al.* (2010) studied the growth of 29 lines of *T. diversifolia* collected in the central-west part of Cuba. This was the first reported study of this type realized in Cuba or the Caribbean area. The researchers indicated that the leaf component accounted for 60.21% and 74.83% of the variability (see Tables 1 and 2). In the study it was established that the variables of greatest significance were total leaves (green, yellow, dry and fallen) per plant and stems per plant, all with a positive relationship. This result showed the importance of considering the growth performance of leaves when evaluating these materials.

At the same time, the second component was named structure, and accounted for 20.95% and, 19.50% of the variability (see Tables 1 and 2), where this biological component is essential for the selective comparison of the materials under study. For both seasons, plant height and height of the first leaf stood out, and for the dry season the thickness of the stem alone, but there were parameter differences between lines in the rainy and dry seasons, with indications of variability in the collections.

In general, the germplasm evaluated indicates that there is probably material available with potential for biomass production for both the dry and rainy periods. An important element that must be considered for future work is that these lines,

TABLE 1. Matrix of typical plants corresponding to average rainfall at two year evaluation

Parameter	Component	
	Leaves	Structure
Stem height. cm	0.04	0.98
No. stems/seedling	0.94	-0.31
No. green leaves/seedling	0.98	-0.11
No. yellow leaves/seedling	0.90	-0.07
No. dry leaves/seedling	0.95	-0.19
Thickness first branch. mm	0.41	-0.33
No. Flowers/seedling	0.28	-0.47
Height first green leaf. cm	-0.05	0.97
No. fallen leaves/seedling	0.98	0
No. total leaves/seedling	0.99	-0.10
Individual Value	6.02	2.09
% of variance accounted for	60.21	20.95
% Accumulated	60.21	81.16

TABLE 2. Matrix of parameters corresponding to the dry season average of two years of evaluation

Indicators	Components	
	Leaves	Structure
Height. cm	0.39	0.89
No. stems/seedling	0.99	0.03
No. green leaves/seedling	0.95	0.26
No. yellow leaves/seedling	0.94	0.28
No. dry leaves/seedling	0.91	0.22
Stem thickness. mm	0.04	0.93
Height of 1st green leaf. cm	0.34	0.91
No. fallen leaves/seedling	0.86	0.46
No. total leaves/seedling	0.94	0.35
Individual value	6.73	1.75
% of variance accounted for	74.83	19.50
% Accumulated	74.83	94.34

according to their group, must be worked according to the particular characteristics of their development. Four groups were formed for each season.

On analysing individually the performance of the measurements for the dry season for the 29 lines of *T. diversifolia* collected in the central-west part of Cuba (Group 1: 1, 2, 3, 5, 6, 7, 8, 9, 10, 11, 12, 19, 21, 22, 23, 24, 25, 26 and 28; Group 2: 4, 14 and 17; Group 3: 13, 15, 16, 18, 20 and 27; and Group 4: 29) although plant material 29, included in group 4, showed the highest values for all measurements taken, it was not always positive for the indicators number of fallen leaves (558) and height of the first green leaf (52 cm). It was observed that lines in Groups 1 and 2, although they had a quite similar performance, also showed differences, namely that the plants from group 1 had fewer stems/plant (32), and those from group 2 were shorter in height (71 cm) and with fewer fallen leaves (232). Also Group 2 had lower stem thickness (5.7 mm) and lower height to the first green leaf (31 cm) of all the material evaluated.

In the rainy season, the groups were Group 1: 1, 2, 3, 5, 6, 7, 8, 9, 10, 11, 12, 22, 23, 24 and 26; Group 2: 4, 13, 19, 21, 25 and 27; Group 3: 14, 17 and 29; and Group 4: 15, 16, 18, 20 and 28. Group 3 showed the highest development values, but with some non-desirable characters, including stem thickness (7.3 mm), number of flowers/plant (152) and number of fallen leaves/plant (414). In general, Groups 1 and 2 showed similar performance for the measurements taken, and group with the plants with intermediate development. Group 1 has the tallest plants (251 cm) of all the material assessed, as well as also the highest values for height to first leaf (169 cm). Group 2 possesses more fallen (291) and dry (107) leaves per plant, and greater stem thickness (7.2 mm).

Another element showing the importance of this study is the limited information available on the evaluation of tithonia material in other countries. From a consultation made by González, Román and Santacruz (2006) at the Herbarium of the University of Guadalajara (IBUG), Mexico, it was noted that there were specimens of this species collected in the State of Jalisco at different elevations. These authors also found no reports of evaluation experiments with this species having been carried out in Mexico, and they report first information on the performance of two lines collected in this zone regarding sprouting of cuttings under nursery conditions.

No decisions have been agreed regarding whether the evaluated materials are clones or ecotypes, since that will require knowledge of their genotypic characters and also to relate them to their native environment, as climate and soil, and as well as aspect, affect yield.

The data analysed allow us to report for the first time some characteristics of the development of *T. diversifolia* materials collected in different Cuban regions. Equally, it is indicative of the probable existence of a range of different genotypes. The variability found could be used strategically in future programmes of varietal improvement. Moreover, this shrub plant demonstrates good potential for use in silvipastoral systems.

3. PERFORMANCE OF SOME MORPHOLOGICAL COMPONENTS OF OUTSTANDING PLANT MATERIALS

Finding information in the literature on investigations related to evaluation of tithonia germplasm is difficult.

On this topic, Ruiz *et al.* (2012a,b,c,d) have published papers where the individual performance of groups of outstanding material were evaluated, and determined the models

FIGURE 1
Dynamics of the seedling height of five lines

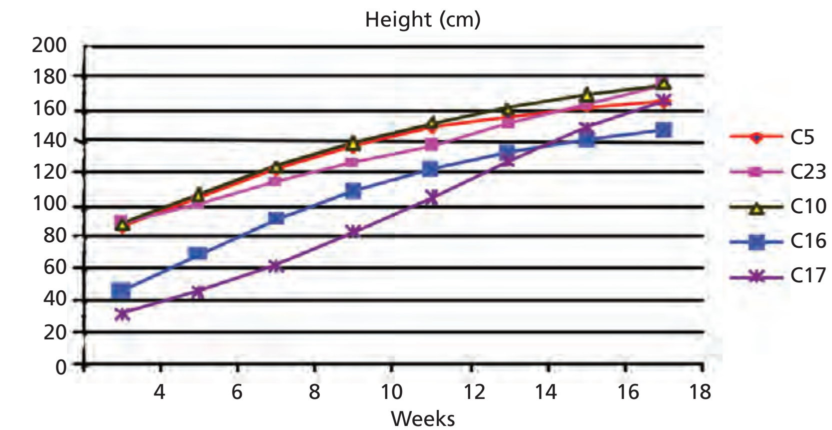
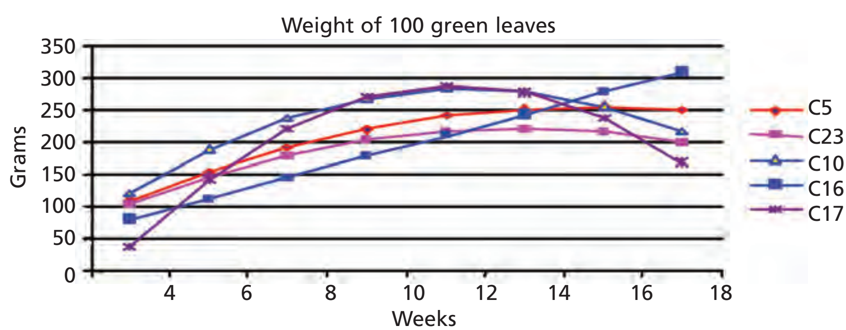


FIGURE 2
Dynamics of the weight of 100 green leaves for the five lines



of best adjustment for each measurement under study. More recently, these same authors (Ruiz *et al.*, 2013b) studied the performance of prominent *T. diversifolia* materials collected in Cuba regarding some morphological components throughout the year, and found that all variables expressed as DM did not show adequate goodness of fit in any of the models used. At the same time, they indicated that Richards' model was not adequate for describing the performance of any of the variable studied. On assessing the rainy season materials, lines 5, 10 and 23 exhibited similar dynamics, beginning with seedling heights above 80 cm in the 4th week, and starting to stabilize growth from the 14th week (Figure 1). Lines 16 and 17 presented slow growth in the first weeks, with 17 the slower growing.

The greatest weight of 100 green leaves was shown by Line 10 (Figure 2), while line 16 had the lowest weight of them all from the 4th week. Line 10 had high total fresh forage weight until the 14th, week when it started to decrease rapidly, although it was more than the rest until the 16th week (Figure 3).

FIGURE 3

Dynamics of the variable total weight of fresh forage in 1 m for lines 23, 10 and 16

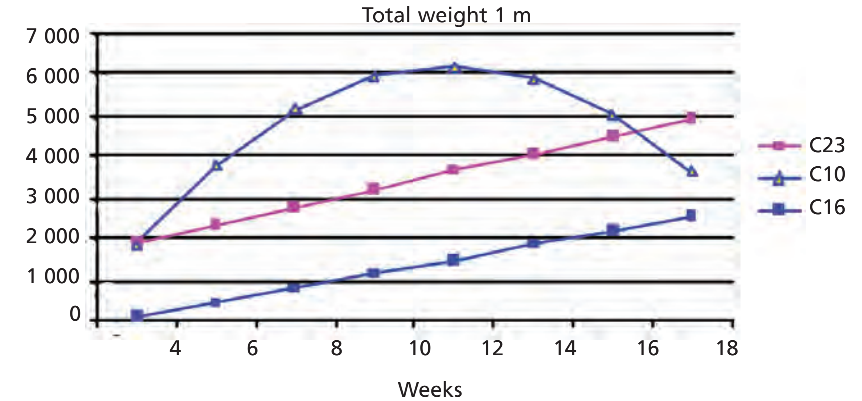
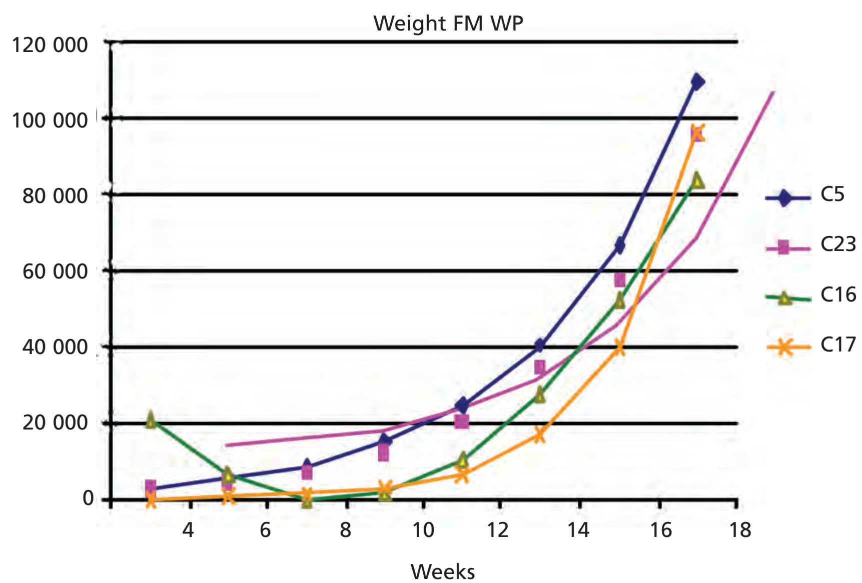


FIGURE 4

Dynamics of the variable weight of the fresh matter (FM) of the whole plant (WP) for all the lines



These authors (Ruiz *et al.*, 2013b) suggested that during the dry season, when performance stability was not found for any of the variables, one might think that these collections could not express their maximum potential because of environmental constraints. Lines 5 and 23 exhibited similar dynamics: a slow start, with seedling heights above 30 cm from the 4th to the 10th weeks, increasing faster until the 18th week, when they reached >125 cm and 143 cm, respectively. Lines 10, 16 and 23 had little variation in height over time and had lower values. Lines 5 and 23 had the highest values from the 8th to 18th weeks, while lines 16 and 17 had lower weights of 100 green leaves. The weight of the fresh matter (FM) of the whole plant was most in lines 5 and 23, but with smaller difference than with Lines 16 and 17 (Figure 4). It can be concluded that the growth potential of line 10 was high, while line 23 was intermediate and 16 was low. Line 5 can be considered of intermediate and line 17 low potential. In the dry season the materials evaluated presented a different performance. All plants had slow growth in the first weeks after cutting for all parameters studied. The lines presenting high growth indicators are 5 and 23, while 10 is intermediate and 16 and 17 low. The information found allowed us to develop future studies related to biomass production either for cutting or for grazing.

4. GERMINATION

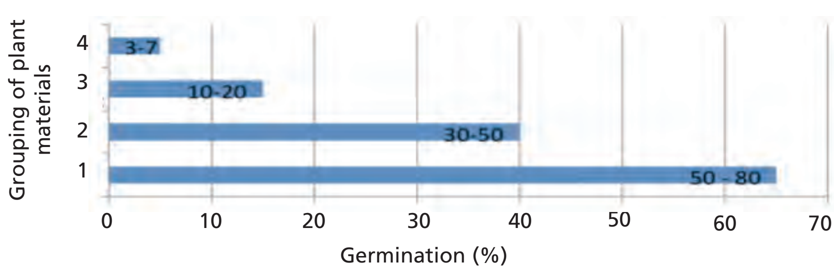
The study of this plant mainly focused on its capacity to produce biomass and its quality. There are no studies analysing the different plant parts of this species. Only the investigations reported by Ruiz *et al.* (2009a; 2010) in Cuba are applicable. Papers reporting germination of tithonia are few, and mostly look at variables for improving germination.

Agboola, Idowu and Kadiri (2006), studying the germination of fresh seed of *T. diversifolia,* noted values of 30%, and that to obtain higher percentages it was necessary to submit the seed to treatment, and that humid heat (80–100ºC) and light increased germination up to 70%.

The germination capacity of the true seed of the 29 lines under evaluation ranged from 3 to 73% (Figure 5) and it was shown that 12 of them had values higher than 28%. In a general way, 72% of the lines being assessment had germinations >50% in the first 7 first days, and 28% in the first two weeks.

In an experiment by Ruiz *et al.* (2010a) screening 29 lines of tithonia, 9 were selected (3, 5, 10, 13, 16, 17, 23, 24 and 25) as being representative of the four groups, defined by their growth and development characteristics.

FIGURE 5
Grouping of *T. diversifolia* lines according to germination capacity 28 days after sowing



Group 1: 4, 13, 17, 19 and 25; Group 2: 2, 14, 15, 16, 20, 21, 22, 24 and 26; Group 3: 1, 3, 5, 6, 8, 9, 11, 23, 28 and 29; and Group 4: 7, 10, 12, 27 and 30.

FIGURE 6
Germination capacity of lines of *T. diversifolia* at 28 days after sowing

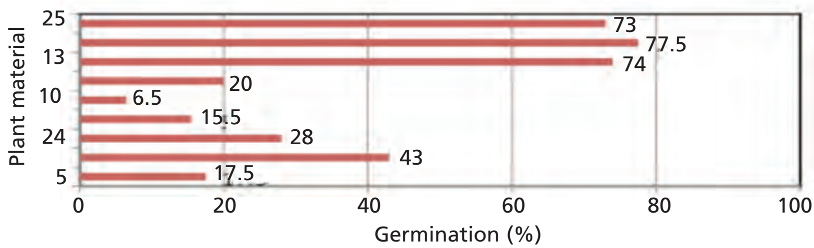
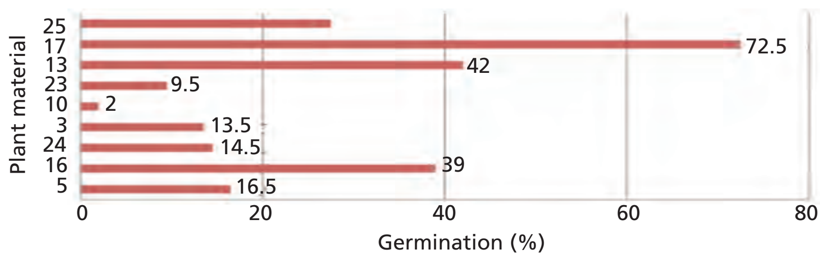


FIGURE 7
Germination capacity of outstanding lines of *T. diversifolia* at 7 days after sowing



In Figure 6 it can be seen that only line 10 showed low germination, with the highest observed in lines 13, 17 and 25. In addition to this, on analysing the value attained in the first week (Figure 7), lines 3, 5, 16 and 17 had final germination >90%, and lines 13, 23 and 24 were >50%.

For the lines with total germination below 20%, further studies of seed production parameters are required, as these lines are outstanding for biomass production both for cutting and for grazing (Ruiz *et al.*, 2014).

The germination capacity indicated for each plant line studied indicates the possibility of sowing this plant by true seed and the importance of studying further the factors influencing seed production, so as to increase in quantity and quality this resource.

5. SOIL FERTILITY

First experiments with this plant in Cuba showed that it develops adequately under the varied soil and climatic conditions of the country (Ruiz *et al.*, 2010). The performance of numerous ecotypes was studied, looking at productive balances, nutrient composition, animal acceptability and other aspects (Ruiz *et al.*, 2014).

5.1 Nutrient content

In a study in nine localities of Kenya on 257 ha sown with tithonia, the production potential was estimated to be 530 t FM/ha, equivalent to 84.8 t DM/ha/year (Research Report, 2000), whereas Wanjau, Mukalama and Thijssen (2010) found values <55 t DM/ha/year.

TABLE 3. Mineral content in *T. diversifolia* (%)

Parameter	Growth stage				
	Early growth	Pre-flowering	Median flowering	Complete flowering	Post-flowering
Calcium	2.30	2.14	2.47	2.4	1.96
Phosphorus	0.38	0.35	0.36	0.36	0.32
Magnesium	0.05	0.05	0.07	0.06	0.06

Concerning mineral content, Navarro and Rodriguez (1990) report that calcium and phosphorus contents decreased as the plant matured, while magnesium increased slightly (Table 3).

At the same time, Wanjau, Mukalama and Thijssen (2010) found phosphorus concentrations in leaves of 27 to 28 g/kg, whereas Research Report (2000) indicated calcium contents of 35 g/kg, and 4.1 g/kg of magnesium.

Phosphorus content in tithonia is considered higher than in other shrub species. Rodríguez (1997) reported values of 0.20, 0.28 and 0.33% in *Erythrina fusca*, *Erythrina edulis* and *Erythrina poepiggiana,* respectively, whereas Gómez (1997) encountered values of 0.17–0.22% in *Gliricida sepium* leaves and 0.37% in *Trichantera gigantea* leaves.

According to Jama *et al.* (2000), the foliar biomass of tithonia is high in nutrients, averaging approximately 3.5% N, 0.37% P and 4.1% K on a DM basis.

5.2 Effect on soil fertility

Although this plant is recommended for various uses in animal feeding (Mahecha *et al.,* 2007; Wambui, Abdulrazak and Noordin, 2006; Mahecha and Rosales, 2005) many authors regard it highly as green manure for soil fertility improvement (de Oliveira *et al.,* 2007; Jama *et al.,* 2000). In this respect, Ríos (1998), Ramírez *et al.* (2005) and Research Report (2000) highlighted that the high biomass production, its high N, P and K contents and its fast decomposition capacity in the soil makes it very effective as green manure.

In a two-year experiment carried out by Ikerra, Semu and Mrema (2006) the effect of tithonia as green manure (at 2.5, 5.0 or 7.5 t/ha) was evaluated combined with phosphoric rock or with triple superphosphate (at 40 kg P/ha) on the total phosphorus content and assimilable phosphorus in the soil and its effect on maize grain yield in a chromic acrisol soil in Tanzania. Tithonia significantly increased pH, interchangeable Ca and inorganic P, and reduced the interchangeable Al and phosphorus absorption. The application of phosphoric rock alone had a liming effect and increased the soluble phosphorus, while the combination of tithonia with the rock had better effect. At the same time, the application of superphosphate alone produced acidification, not found when it was applied together with tithonia. The application of green manure of tithonia for two consecutive years increased by 70% the yield of maize grain compared with the control treatment. The agronomic effectiveness of the phosphoric rock was increased from 46% in the first year to more than 142% in the second year, indicating that the slow initial dissolving action of the rock improved thanks to the joint application with tithonia. It was demonstrated that tithonia can improve phosphorus availability in that soil, through the modification of its properties related to the P transformation and its availability.

Identification and evaluation of this plant as green manure in bean culture in loam soils of Costa Rica was realized by de Oliveira *et al.* (2007). Results showed that this plant had high phosphorus, calcium and potassium contents in its leaves (>2500 ppm) and gave a grain yield increase of 127 kg/ha compared with the control.

Borrero (1998), working with a tropical slope agro-ecosystem in Colombia, found weight loss and N of the leaves of *Canavalia brasilensis* (CAN), *T. diversifolia* (TIT), *Cratylia argentea* (CRA), *Indigofera constricta* (IND), *Mucuna deeringianum* (MDEE) and three varieties of *Mucuna pruriens*: var. IITA (MPIT), var. TLALT (MPTL) and var. Brunin (MPBR), using nylon bags placed randomly in a field recently sown to maize. The dosage of each green manure was equivalent to 3.3 t/ha. Additionally MPIT and IND (MPIT and INDt) stems and a mixture of foliage and stems of these species (MPIT× and IND×) were appraised to identify the effect of the stems on N decomposition and release.

After 20 weeks, weight and N losses showed a negative exponential pattern. The decomposition of the materials followed the order: TIT > IND > MPTL > MPBR > CAN = MPIT = MDEE > MPIT× > INDx > CRA = MPITt > INDt, whereas N release was in the order: IND > IND× > MDEE > MPBR > TIT = CAN > MPTL > MPIT > INDt > MPIT× > CRA > MPITt. Weight loss correlated significantly with the initial content of N, K, Ca, Mg, lignin (L), acid detergent fibre (ADF), neutral detergent fibre (NDF), *in vitro* digestibility and the relationships C/N, L/N and (L + polyphenol)/N, while N loss did it with the initial content of N, L, ADF, NDF, *in vitro* digestibility and the relationships L/N and (L+ polyphenol)/N. The inclusion of stems with leaves modified the loss patterns of weight and N, suggesting possible interactions between materials in the mixture. The authors recommend that these green manures can be used satisfactorily as substitutes for nitrogenous chemical fertilization in view of the amount of N that they can contribute to the soil.

In another study, Jama *et al.* (2000) concluded, after reviewing results obtained in Africa and in Asia on the application of green manure of *T. diversifolia* in various cultures, that it has been recognized as an effective source of nutrients for increasing yields in rice, maize and vegetables. Also, the green biomass of tithonia has a high nutrient content, averaging 3.5% N, 0.37% P and 4.1% K in DM and that biomass is rapidly decomposed after its application to the soil. In some experiments they found that the application of green manure produced increases in rice yield comparable to those obtained with NPK fertilizers. In addition, the incorporation of 5 t/ha of DM of tithonia reduced phosphorus absorption and increased microbial biomass in the soil. However, these authors explain that eventually some external application of nutrients will be required for sustaining tithonia production when its biomass is continually cut and transported to agricultural fields.

The investigation of Crespo, Ruíz and Álvarez (2011) in Cuba recommended dosages of 12 t/ha of green manure (2.5 t/ha dry basis) of tithonia for accelerating the establishment and biomass production of *Pennisetum purpureum* cv. Cuba CT-169. With that application, approximately 43 kg/ha N, 10 kg/ha P, 30 kg/ha K, 36 kg/ha Ca and 20 kg/ha Mg were incorporated. In the first cut after pasture establishment there was no effect of the treatments on plant height; however, the number of seedlings per unit area, the number of tillers/plant and the DM yield of CT-169 were significantly increased with the application of 12 t/ha but without difference with 24 t/ha (Table 4). At the second cutting (residual effect) performed three months after the first, no difference between treatments was found

TABLE 4. Effect of the green manure of tithonia on the yield indicators of *P. purpureum* clone CT-169 in the first cutting (November 2008)

Green manure (t/ha)	Height (cm)	Seedlings per m^2	Tillers per plant	DM yield (t/ha)
0	240.2	2.0[a]	9.45[a]	12.46[a]
12	243.5	2.4[b]	10.65[b]	18.62[bc]
24	247.2	2.5[b]	10.40[b]	16.20[b]
SE±	0.3	0.1**	0.30**	1.10***

NOTES: [a,b,c] = Means with different superscript per column differ at $P<0.05$ (Duncan, 1955) **$P<0.01$ ***$P<0.001$
SOURCE: Crespo, Ruíz and Álvarez, 2011.

TABLE 5. Effect of green manuring with tithonia on the yield indicators of *P. purpureum* clone CT-169 at the second cutting (residual effect)

Green manure (t/ha)	Height (cm)	Seedlings/m^2	Tillers per plant	DM yield (t/ha)
0	231.0	1.15a	16.1a	9.20a
12	215.8	1.30b	23.5b	10.70b
24	215.6	1.44c	29.7c	12.50c
SE±	0.4	0.03*	1.5***	1.20**

NOTES: a,b,c = Means with different superscript per column differ at $P<0.05$ (Duncan, 1955). *$P<0.05$ **$P<0.01$ ***$P<0.001$.
SOURCE: Crespo, Ruíz and Álvarez, 2011.

TABLE 6. Effect of green manure on the level of some chemical and physical soil indicators

Green manure dosage (t/ha)	Parameter						
	Density g/cm^3	OM %	Total-N %	P ppm	K %	Ca %	Mg %
0	1.01	3.5	0.20	9.2	45.8	0.9	0.12
12	0.79	3.7	0.24	8.9	47.6	1.1	0.14
24	0.68	4.2	0.30	9.4	50.5	1.0	0.14
SE±	0.06	0.1	0.09	1.2	3.2	0.3	0.04

NOTES: OM = organic matter; N = nitrogen; P = phosphorus; K = potassium; Ca = calcium; Mg = magnesium.
SOURCE: Crespo, Ruíz and Álvarez, 2011.

for plant height, but the parameters seedlings/m^2, tillers/plant and DM yield increased significantly with increasing green manure application (Table 5).

In addition, the application of this green manure decreased the apparent density and increased OM and total-N contents of the soil, with no difference among the dosages applied (Table 6).

The decrease in the soil density and the increase in its OM and total N contents seem to have also benefited forage performance.

The beneficial effect of this green manure on the improvement of soil properties was pointed out by Kumar *et al*. (2003) and Muraoka *et al.* (2002) in rice fields. These authors attributed its positive effect primarily to the percentage of stable water aggregates and the increased OM content.

5.3 Planting

It is essential to know the best way to plant tithonia for different edaphoclimatic conditions in order to obtain satisfactory growth during the first stages, and in this way avoid weed competition that could eliminate the plants.

Work by Ruiz *et al.* (2009b) indicates that good plant development of tithonia can be obtained when stems are planted at the bottom of a furrow. Table 8 shows no differences in plant height, while for the other two parameters there was significant difference. The worst performance was found when the stem was planted by burying it at one end.

Concerning the part of stem to be used for planting (Tables 7 and 8), this showed no significant difference for the parameters measured. Ríos (1999) and Ramírez *et al.* (2005) suggested that the apical part of the stem of this plant must not be used owing to its poor propagation capacity, probably due to the poor development of the conductive tissue of the buds at this level. This was also apparent in our study.

It must be highlighted that the way of planting tithonia stems flat in the bottom of the furrow gives the possibility of obtaining more stems per linear metre, which is of great importance for attaining higher biomass production that is stable over time. Also, the characteristics of the individual plants of tithonia in the treatment where the stems were buried by one of its points show weaker plants with fewer leaves, lower weight and lower weight of the whole plant.

To all the abovementioned can be added that in observations of the root system of stems planted in each of the treatments, there is greater volume of roots and more per linear metre in the treatment where the stems were planted flat in the bottom of the furrow.

TABLE 7. Effect of the planting method and the stem section to be planted on biomass production of a tithonia plant

Treatment	Per plant		
	No. of green leaves	Weight 100 green leaves	Weight, g DM
	Planting method		
Flat in the bottom of the furrow	4.8 (19.35)	758	125
Buried at one end	4.1 (16.35)	676	99
SE ±	0.1**	25**	7**
Stem section			
Median	4.4 (19.7)	718	128
Basal	4.3 (16.6)	742	103
SE ±	0.1	9	9

NOTES: Original values transformed according to $\sqrt{x + 0.375}$. ***P*<0.01.
SOURCE: Ruiz *et al*., 2009.

TABLE 8. Effect of the planting method and the stem section to be planted on the biomass production of tithonia per linear metre

Planting method	Yield		
	No. of tillers/m	Height, m	Yield, kg DM/m
Flat in the bottom of the furrow	7 (45)	3.2	6.81
Buried at one end	6 (37)	3.2	3.37
SE. ±	0.2**	0.8	0.91***
	Stem section		
Median	7 (46.0)	3.30	5.68
Basal	7 (45.0)	3.20	4.47
SE. ±	0.5	0.05	0.45

NOTES: Original values transformed according to $\sqrt{x}$. **P<0.01, ***P<0.001.
SOURCE: Ruiz *et al*., 2009.

Probably this is due to greater contact between the stem surface and the soil. Also, note that the soil used in this experiment was fast draining, which does not favour the treatment of planting the stem by one end, where the contact surface with the soil is much smaller.

In a review by Escobedo *et al.* (2008) they indicated the capacity of this plant for multiplication, and noted the importance of good selection of the stem section for planting.

The type of cutting used for establishment influences biomass production (leaves and green stems) of the plants generated. With these woody cuttings, greater yields are obtained (Ospina, 2002) reflecting the more complete development of the conductive tissue.

In a trial of vegetative propagation in Cauca Valley, Colombia, by Ríos (2002) and Jama *et al.* (2000), cuttings from the first one-third or more woody part of the stem, from the second one-third or intermediate zone, and last one-third or the most tender part of the stem, were used. Results showed that the worst performance was found for the more tender part, with the lowest percentage of establishment and number of roots. Similar results were found by Tun (2004) and Ramírez *et al.* (2005). Meanwhile, Ortiz *et al.* (2014) in Mexico reported that the best sowing method for *T. diversifolia* is as a cutting from a proximal stem part and planted at a slant, covering at least one bud.

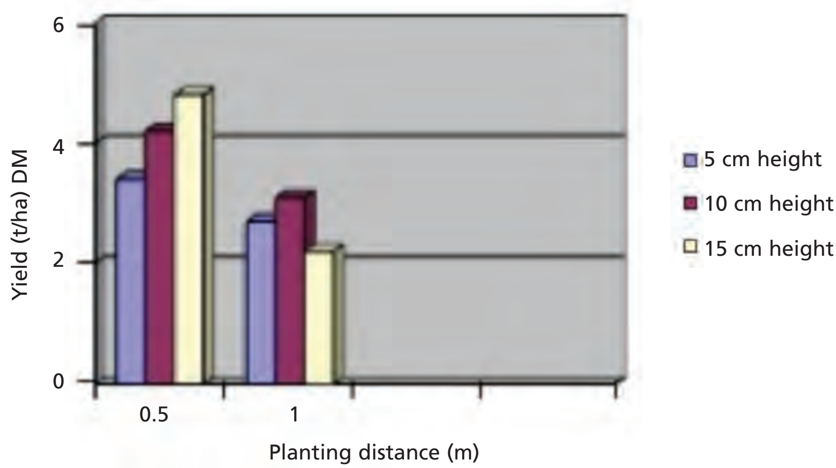
From the above, it can be concluded that tithonia planting is best carried out by laying the stem in the bottom of the furrow and using the basal portion, giving plants with better development, more population and greater biomass production.

6. BIOMASS PRODUCTION

Foliage production of tithonia as a plant for cutting is well known in Latin American countries (Ríos and Salazar, 1995; Ríos, 1998; Wanjau *et al.*, 1998; FAO, 1999). Up to the present there are no Cuban reports on this aspect, although results from those investigations reported match reported studies from other Latin America regions (Ruiz *et al.,* 2012c).

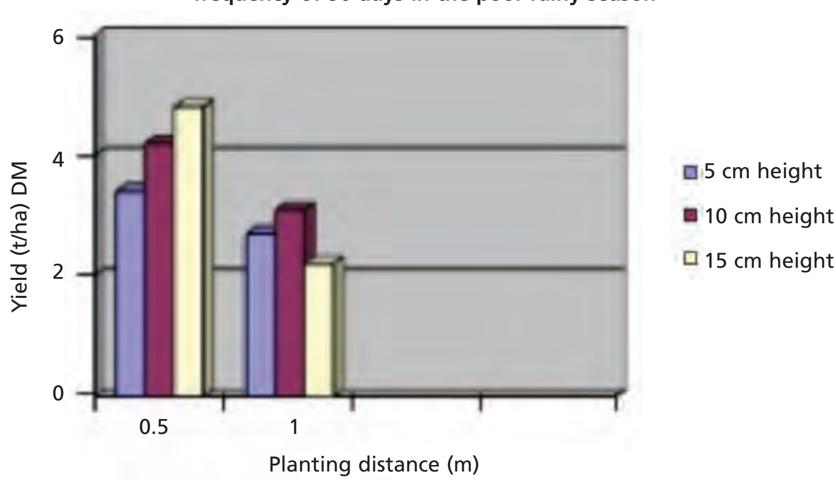
On analysing aspects related to the planting distance, cutting frequency and height, among others, it was possible to confirm that in both experiments there was interaction for the factors under study. Partey (2011) reported similar results, indicating that height and cutting frequency and their interactions affected significantly the dry matter production of *T. diversifolia*. The best results were attained when cutting was every two months at 50 cm height. The highest yield was produced during the rainy season ($P<0.001$) with the lowest planting distance, and there was no effect related to cutting height (Figure 8). This performance could be associated with the fact that this had the highest population per m^2. At the same time, during the poor rainy period (Figure 9) the lower planting distance also exhibited better performance with respect to yield, and there was the effect of the cutting height with lower yield at 5 cm ($P<0.01$). This could be influenced by the season, since in plants cut at this height fewer reserves remain in the stems for the next sprout (Figure 9). These results match those of Ramírez *et al.* (2005) in studies in the north-central area of Yucatan, Mexico, with this same plant. These authors studied the sowing distance (0.5 × 1.0 m = 20 000 plants/ha; 0.75 × 1.0 m = 13 333 plants/ha; and 1.0 × 1.0 m = 10 000 plants/ha) and cutting height (25 and 50 cm). The highest biomass production was found with the highest density (5 450 kg DM/ha) and increased 27% when plants were cut at 50 cm. In this sense, Ríos (2002) reported potential forage production of 31 t/ha at

FIGURE 8
Effect of planting distance and cutting height on tithonia yield with a 60-day cutting frequency in the rainy season



Source: Ruiz *et al*., 2012c.

FIGURE 9
Effect of planting distance and cutting height on tithonia yield with a cutting frequency of 90 days in the poor rainy season

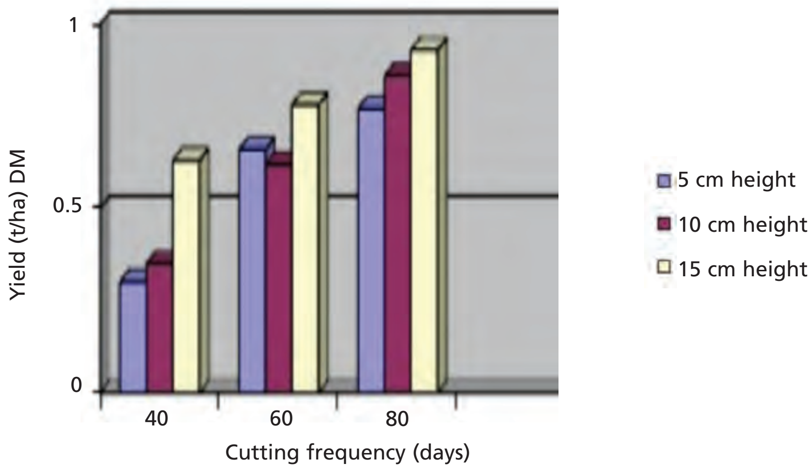


Source: Ruiz *et al*., 2012c.

a planting density of 0.75 m × 0.75 m, and 21.2 t/ha at 1 m × 0.75 m, without significant differences. However, he added that it is possible to btain higher yield per unit area with a density of 0.5 m × 0.75 m, although some phytosanitary risks were inherent in such a dense planting.

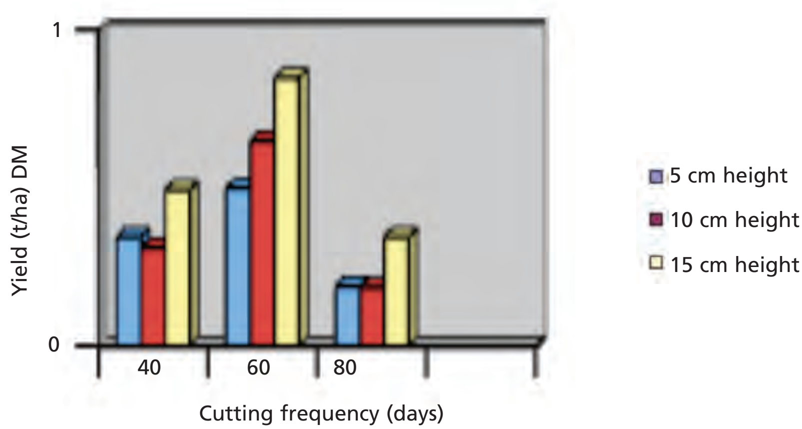
Regarding recovery after cutting, Ríos (1998) reported that with plant densities of 0.75 m × 0.75 m, plants grew 6.2 cm when cut every 21 days, 19 cm when cut every

FIGURE 10
Effect of cutting frequency and height on tithonia yield in the poor rainy season



Source: Ruiz *et al*., 2012c.

FIGURE 11
Effect of cutting frequency and height on tithonia yield in the rainy season



Source: Ruiz *et al*., 2012c.

35 days, 44 cm when cut every 49 days, and 180 cm when cut every 110 days. Therefore, the most appropriate time for harvesting the forage for feeding purposes, without damaging the culture is in its pre-flowering stage, was cutting every 49–50 days), with a potential biomass production of 31.5 t/ha.

In an earlier study, Ruiz *et al.* (2012c) looked closer at the combination of frequency and cutting height when analysing the effect of cutting height. The best performance ($P<0.001$) was found from a cutting height between 10 and 15 cm, and an 80-day frequency during the poor rainy season (Figure 10). In this season it was clear that biomass production decreased with the 40-day frequency and heights of 5 and 10 cm, whereas the cutting height of 15 cm gave the best yields ($P<0.001$), together with the 60-day frequency for the rainy season (Figure 11). It is clear that the 80-day frequency is much too extended and produces the lowest yield, because of leaf loss as plants age.

Results obtained in these studies confirm those reported by Soto, Rodríguez and Russo (2009), who studied the yield of various shrub plants (*Erythrina poeppigiana*, *Gliricidia sepium*, *T. diversifolia* and *Morus alba*) and indicated that aerial biomass production was affected by pruning age. According these authors, the aerial and digestible biomass decreased as pruning age was reduced, with higher yields obtained at 26 weeks. In this context, Zavala *et al.* (2007) concluded that the cutting frequency (6, 10, 14, 18, 22 or 26 weeks) should be every 18 weeks to achieve a more palatable plant with greater utilization by the animals. In this respect, Polo (2010) suggests that tithonia should be cut at between 8 and 12 weeks of sprout, a period in which a forage of good nutrient content and good biomass productivity is obtained both in the poor rainy and in the rainy season.

On studying the bromatological characteristics, Lezcano *et al.* (2012) in Cuba concluded that *T. diversifolia* foliage showed variations in its nutritive quality, in the two phases of its physiological cycle evaluated (30 and 60 days) in the rainy period (RP) and poor rainy period (PRP). Nonetheless, in both phases the forage exhibited significant values of CP (29.79% and 28.69%) and CF (5.29 and 5.27%) in the edible fractions, in both RP and PRP. The leaf plus tender stem fraction had the best values for the indicators measured for their use in animal feeding.

Other authors used flowering degree as a physiological indicator of the plant for harvest timing. De Souza (2007) studying different cutting times for (pre-flowering, flowering and post-flowering) in *T. diversifolia* showed that the lowest yield is produced in the pre-flowering stage, and the greatest post-flowering. In a general sense, there is a tendency to more production with tighter spacing (0.50 × 0.75 m; 0.75 × 0.75 m; and 1.0 × 0.75 m). This was also reported by de Oliveira *et al.* (2007). In reports of CIPAV (2009) harvesting is recommended before flowering, approximately every 50 days with cutting at 10 cm above the soil. Parada (2006) suggests performing the first tithonia cut when plants attain the stage of floral buds (75%), coinciding with the appearance of the first flowers. Studies by González, Ruiz and Díaz (2010) pointed out that *T. diversifolia* yield was not affected when sown either in standing or flat manner, but planting must be done with the median part of the stem. The highest yield is attained at distances of 0.50 m between furrows for both seasons. The plantation must be cut at heights between 10 and 15 cm above soil, with cutting frequencies of 60 days in the rainy season and 80 days in the poor rainy season.

7. GRAZING

T. diversifolia has been traditionally used as cattle feed, but rarely for direct grazing (Rúa, 2011). Interesting practices applied by Colombian producers are known, but few published technical papers were found on this topic.

The literature indicates that this plant is mainly used for cut-and-carry forage (Mahecha and Rosales, 2005; Murgueitio, 2005; Zapata and Silva, 2010). Also, it is clear that despite some mentions of the use of *T. diversifolia*, especially from farmers, in animal feeding, few investigations have been carried out worldwide on this topic (CIPAV, 2009). Nonetheless, in some Central American and Caribbean countries, tithonia has started to be valued as a shrub species with high potential for integration into grazing systems, combined with grasses and legumes in silvipastoral arrangements, since under these circumstances economic benefits are more attainable, considering the labour cost implicit in cut-and-carry.

In this respect, studies at CORPOICA, Colombia, confirmed that *T. diversifolia* was one of the shrub plants that cattle usually selected when grazing during the summer season, when forage availability falls to really critical levels (Sánchez., Bueno and Pérez, 2002).

At the same time, work by García *et al.* (2008), studying cattle preference for the foliage of twelve species with potential for agrosilvipastoral systems in Venezuela, indicate that the most consumed species by bovines under stabling conditions were: *Pithecellobium pedicellare*, *Leucaena leucocephala*, *Morus alba*, *Guazuma ulmifolia*, *Chlorophora tinctoria* and *Cordia alba*. Foliage from *Gliricidia sepium*, *T. diversifolia*, *Moringa oleifera*, *Azadirachta indica* and *Samanea saman* were moderately accepted. while *Trichantera gigantea* consumption was very low. Preference for the forages was not related to chemical composition, quantified levels of secondary metabolites nor forage degradability.

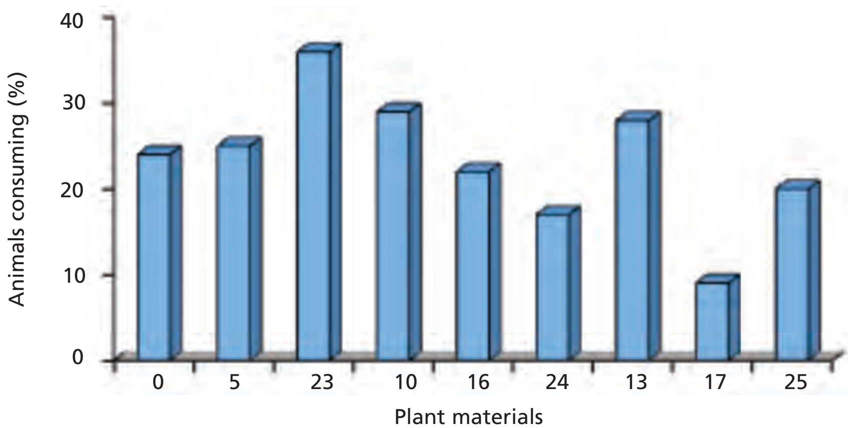
Studies carried out at the Estación Experimental Rafaela of INTA, Argentina (cited by Soto, Rodríguez and Russo, 2009) indicate that *T. diversifolia* showed a decrease in dry matter digestibility as forage matured, but there was a recovery at 26 weeks that could be attributed to new sprouts from the more mature stems. This factor could clearly influence grazing systems where it is impossible to attain total utilization of the available material at each grazing. In addition, grazing stimulates plant sprouting and favours a greater mixture of older leaves with new sprouts.

Ruiz *et al.* (2009a; 2013a) reported on various tithonia lines collected in Cuba that can be grazed by bovines, and specifically evaluated them in dairy cattle. These authors, on assessing under grazing the available material of tithonia from the germplasm bank of the Instituto de Ciencia Animal (29 lines) found some (lines 15, 20 and 28) that were not appetizing for the animals, while lines 3, 7, 8, 9, 10, 11 and 12 were the most grazed, being 100% browsed, alongside lines 1, 2, 5 and 6 with 80% foliage removal. Also they consider that the rest of the lines reaching levels between 10 and 40% must also be considered for future investigations.

These authors, Ruiz *et al.* (2013a), stated that animal behaviour when grazing the lines that reached removal percentages between 50 and 100% showed (Figure 12) that the number of animals grazing tithonia differed. In lines 24 and 17 the percentage of animals consuming tithonia was <20%, while among the rest of the lines, values were greater, with lines 23, 10 and 13 outstanding with values close to 30%. This should be compared with results found in leucaena by Castillo and Ruiz (2005), who reported grazing values ranging between 17 and 20%. The above results indicate the potential for grazing of all lines except line 17.

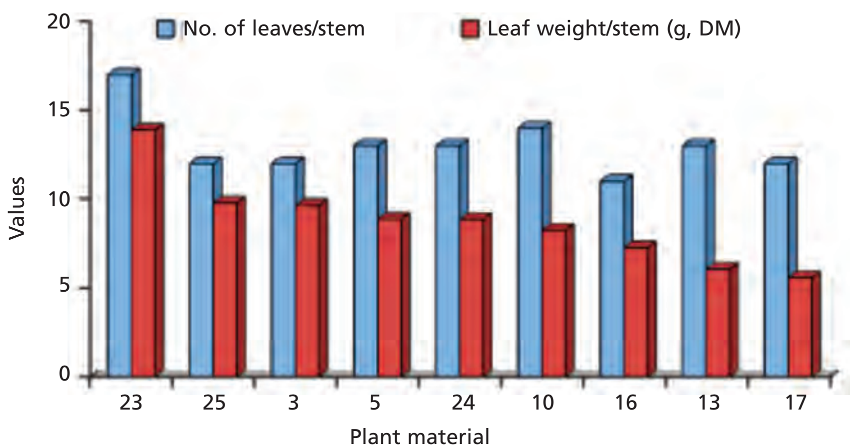
In other studies, Ruiz *et al.* (2009a; 2013a) assessed outstanding lines of *T. diversifolia* under non-irrigated conditions with simulated grazing, and plant response to grazing was measured through the indicators number of leaves per stem and leaf weight per stem. It was concluded that line 17 must not be used for grazing. However, line 3 could be used for grazing throughout the year. In the rainy season, lines 10 and 23 could be employed,

FIGURE 12
Animal performance grazing different lines of *Tithonia*



Source: Ruiz *et al.,* 2013a.

FIGURE 13
Characteristics of *T. diversifolia* lines grazed during the rainy period



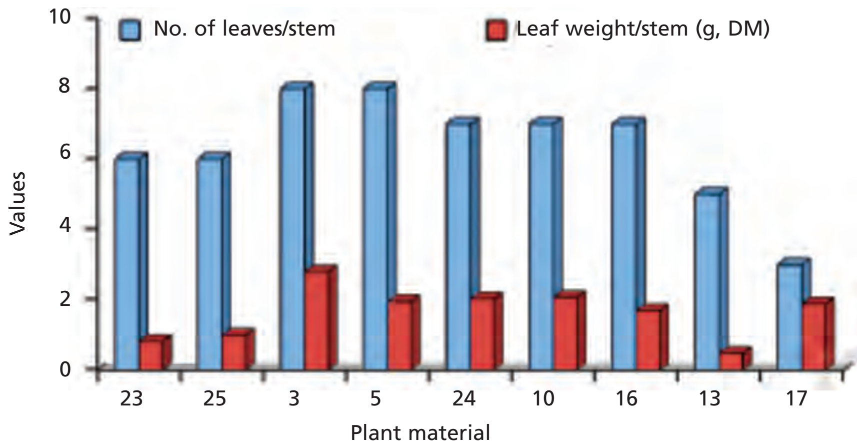
Sources: Ruiz *et al.*, 2009, 2013a.

and line 24 in the poor rainy season. Lines 5, 10, 13, 23, 24 and 25 showed intermediate performance (Figures 13 and 14).

7.1 Distance between furrows

Spatial distribution of trees in grazing agro-ecosystems has a direct relationship with the behaviour of the animals, and markedly influences their productivity. Mobility, heat stress and the good use of the available biomass are elements that can be affected when the density of the tree component is not considered, as it is a decisive element in the establishment of silvipastoral systems.

FIGURE 14
Characteristics of *T. diversifolia* lines grazed in the poor rainy period



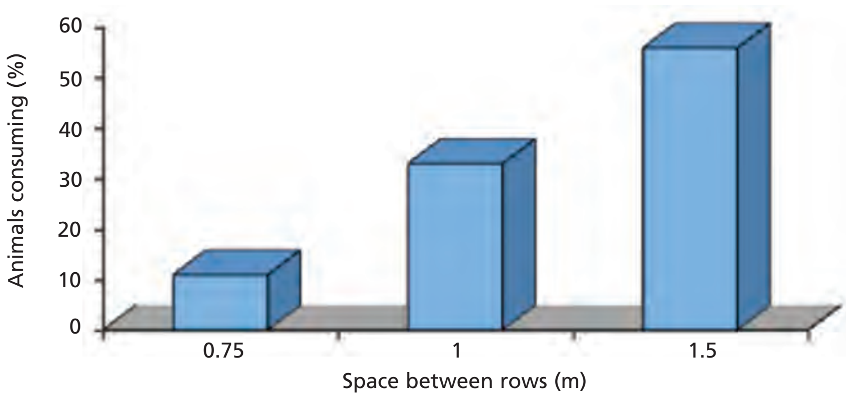
Sources: Ruiz *et al*., 2009, 2013a.

In Central America, work by various research centres show that the inter-plant distance influences the yield. Trials In Yucatan, Mexico, found greatest tithonia production (5450 kg DM/ha) with 0.5 × 1.0 m spacing, and stated that as the planting distance increased yields decreased. Estimated green forage production in Colombia is from 30 to 70 t/ha, depending on the plant density, soils and vegetative stage (Navarro and Rodríguez, 1990; Ríos and Salazar, 1995).

The effect of spacing can be assessed in tithonia plants not only by yields from grazing, but also by animal performance, since too dense plantations can cause difficult animal behaviour when grazing, leading to high plant losses as a result of trampling and even reduced use due to the restricted mobility of the animals within the area. Similar results were found by Ruiz *et al.* (1995; 2003) on studying *L. leucocephala* as a multipurpose tree for animal production in the tropics. Studies by Ruiz *et al.* (2013a) with tithonia confirmed results, and pointed out that as spacing between furrows was increased (from 0.75 to 1.50 m) the number of animals eating this plant increased (Figure 15). This is of importance at the time of establishing an exploitation protocol for this species, and opens a new possibility for the use of this shrub plant in silvipastoral systems.

On studying the sowing distance between furrows (2, 3, 4 or 5 m), Alonso *et al.* (2012) reported that during grazing, leaf consumption for all the spacings studied were high, since the rejection percentage of the plant component did not exceed 13%. In all rotations, the number of stems damaged was low with no significant difference among evaluated treatments. Also, it indicates differences for the animals' rejection, since in the third rotation with the lowest values (0.66, 0.57 and 0.50) for the distances of 3, 4 and 5 m, respectively (Table 9), the percentage utilization was similar for all the treatments, with values up to 41.25%. In general, the authors consider that this result indicates adequate exploitation of this plant in grazing systems.

FIGURE 15
Animal behaviour facing different inter-plant spacing in tithonia



Source: Ruiz *et al.*, 2013a.

Alonso *et al.* (2012) evaluated productive performance over time for each plant spacing (Figure 16), and observed a marked seasonal variation in tithonia availability. In the rainy period (grazing rotations 1 and 2) the availabilities found were higher than in the poor rainy period (grazing rotation 3).

In this same study it is indicated that the desirable characteristics of this plant for grazing were evident from the indicators number of stems harmed and number of stems per cutting (Figure 17). Both can reflect the growth capacity and response of the plant before the pressure exerted by the animals during and after grazing. It was evident that the average number of stems damaged in the three grazings was low, with similar values in all spacings studied, which varied between 1.03 and 1.37. With the greatest exploitation time under grazing, tithonia displayed an increase in the number of stems per cutting – an element that can reflect its persistency capacity.

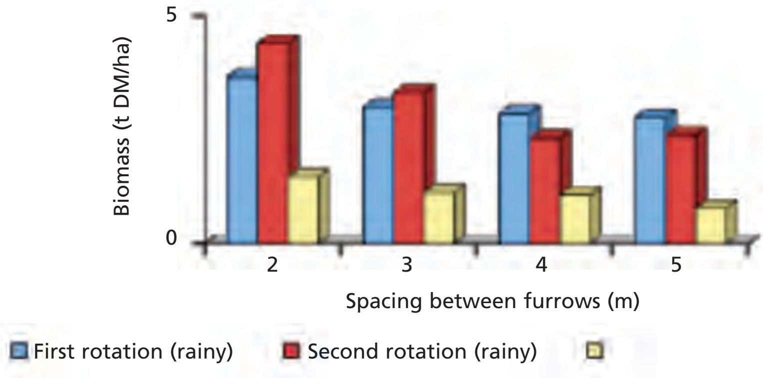
On studying the behaviour of the animals grazing in each of the sowing distances, it was seen in all cases that there was adequate acceptance of tithonia for grazing, which was more obvious in the second grazing (Figure 18). This can be related to the adaptation period that the animals always require when facing a new feed. This criterion was confirmed on observing that the percentages of animals grazing tithonia was always lower during the first day of observation in each grazing cycle, with average values of 44.4%.

TABLE 9. Productive performance of *T. diversifolia* at different inter-plant spacings during the third grazing cycle

Inter-plant spacing	Height (cm)	Stems per cutting	kg/plant		DM (%)	t DM/ha		Percentage (%)		No. of stems damaged
			FW	DW		Available	Rejected	Used	Rejected	
2 m	64.1	17.73	0.59	0.12	19.75	1.47	1.06	27.40	6.84	0.70
3 m	66.2	16.60	0.67	0.13	20.29	1.12	0.66	40.81	8.54	0.48
4 m	88.6	17.90	0.89	0.17	19.51	1.07	0.57	41.25	4.27	0.53
5 m	88.0	16.38	0.80	0.15	19.32	0.78	0.50	31.72	3.46	0.65

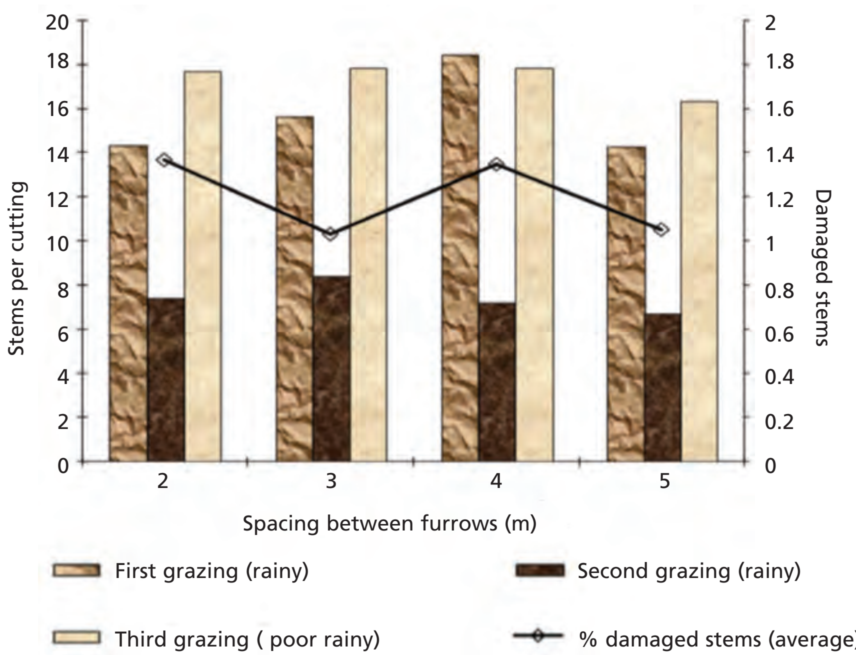
Notes: DM = dry matter; FW = fresh weight; DW = dry weight.
Source: Alonso *et al.*, 2012.

FIGURE 16
Biomass availability with different spacing of *T. diversifolia* and the grazing rotation



SOURCE: Alonso *et al.*, 2012.

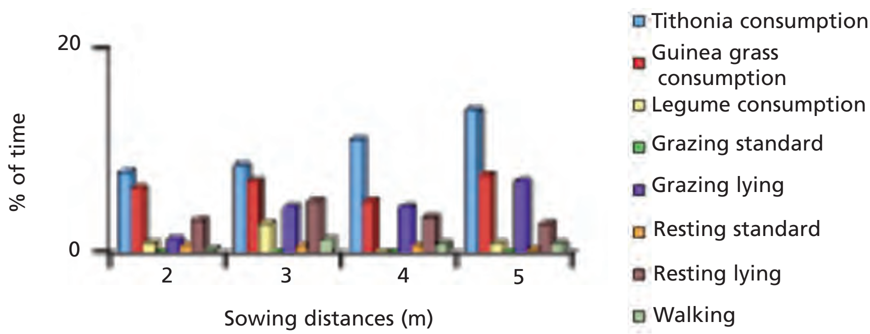
FIGURE 17
Performance (number of stems per cutting and number of damaged stems) in *T. diversifolia* grazed at different plant spacings



SOURCE: Alonso *et al.*, 2012.

The time dedicated to tithonia grazing was less with greater plant density, since the movement of the animals when grazing was constrained in the higher density plantation, and affected consumption activity, even for the associated grasses. The widest spacing

FIGURE 18
Behaviour of the animals during the second grazing in *T. diversifolia* different inter-plant spacing. Mean of the three grazing times



Source: Alonso *et al.*, 2012.

favoured the time dedicated to shrub consumption within the system, showing a linear increase during the second grazing period (Alonso *et al.,* 2012).

Results found in these studies indicate better productive performance from *T. diversifolia* under grazing with furrow spacing of three and four metres. However, these results are preliminary, since spatial arrangements other than line sowing could increase and improve the productive performance of this plant under grazing.

7.2 Time from establishment to starting grazing

Four intervals before starting grazing were assessed, defined by plant heights of 50, 100, 150 and 200 cm, and the conclusion was that grazing should be started when plant height is between 100 and 150 cm following the establishment cut (Alonso *et al.,* 2013). Animal access to the available forage decreases as plant height increases, and this makes management of the plantation difficult due to reduced grazing.

7.3 Grazing frequency

Four grazing frequencies were evaluated according to the season of the year. In both seasons, four different rest periods were studied. In the rainy period the rest days were 30, 60, 90 and 120 days, while in the poor rainy period these increased to 60, 90, 120 and 150-day rests (Alonso *et al.,* 2015).

During the rainy period it was found that the productive capacity of the plantation reached an accumulated DM availability of 2.28 t/ha. With 90 days of rest, only two grazing periods were possible, the DM availability (2.64 t/ha) was similar to that found with the 60-day cycle. Differences in plant height between these grazing frequencies (169 vs 204 cm) could indicate better use of the available material in a grazing frequency with 60 days of rest, since all the available material would be below 200 cm.

The plantation in the poor rainy period had the lowest availability of plant biomass (1.36 t DM/ha), which differed from the other intervals studied. The greatest biomass availability (2.93 t DM/ha) in this period was observed with 90 days of rest, when plant height reached only 184 cm. Plant height found in this treatment did not differ from the 150-day rest period, but this latter case allowed only a single grazing.

Another element directly related to the height attained by the plant under grazing is the relatively better use that the animals made of the available material. Figures 19 and 20 show the percentage exploitation for the different grazing frequencies according to the poor rainy season. The best percentage exploitations were 53.1% for the 60-day rest cycle in the rainy period, and 14.6% for the 90-day cycle in the poor rainy period (Alonso *et al.*, 2015).

Lastly, the information available in the international literature databases such as Scielo, EBSCO and Science Direct, as well as in journals with high impact indices, show that investigations with *T. diversifolia* have been first and foremost directed to agronomy, bromatological and nutritional studies for its use in cut-and-carry systems, or as green manure, with very little reported on its grazing possibilities.

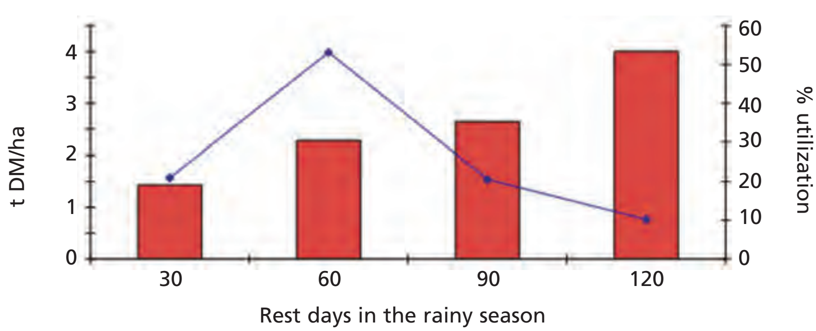
In general, the few studies indicate that *T. diversifolia* must be planted for grazing at a distance of 3 or 4 m between furrows. Grazing should start when the plant has a height of between 100 and 150 cm after the establishment cut. Animal access to the available material is constrained when grazing starts at higher heights, making management difficult with inefficient utilization of the plants under grazing. A system of 45–60 days rest in the rainy period and 70–90 days in the poor rainy period probably provides the best productive performance from the plant under continuous rotational grazing.

8. PESTS

Tithonia diversifolia is a species that is considered a medicinal shrub since it has been employed successfully for worming kids, malaria prevention and curing fevers and stomach disorders (Roig, 1974; Martínez, 2011). In addition, biocidal qualities have been detected in it, and researchers refer to repellant properties of the extract and foliage of this shrub in the fight against termites (*Nasutitermes* spp.) (Adoyo, Mukulama Enyola, 1998), cutting ants (*Acromyrmex* spp. and *Atta* spp.) (Giraldo *et al.,* 2006), whiteflies (Aguiar *et al.,* 2003; Bagnarello *et al.*, 2009) and seed weevils (*Callosobruchus maculatus*) (Adedire and Akinneye, 2005; Kolawole and Okonji, 2011). Recently, aqueous extracts of *T. diversifolia* produced using traditional methods in Cuba were

FIGURE 19

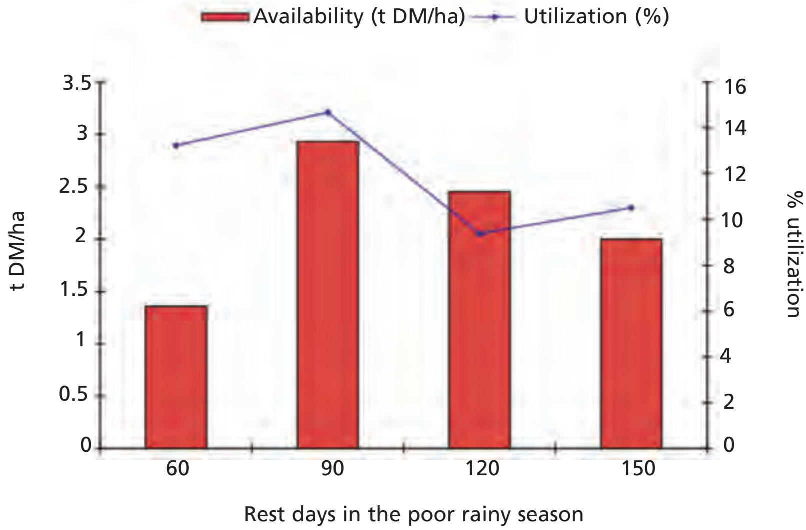
Biomass availability (t DM/ha) and percentage of utilization of *T. diversifolia* in grazing systems with different rest periods according to the rainy season



SOURCE: Alonso *et al.*, 2015.

FIGURE 20

Biomass availability (t DM/ha) and percentage of utilization of *T. diversifolia* in grazing systems with different rest periods according to the poor rainy season



Source: Alonso *et al.*, 2015.

employed with encouraging results for controlling a type of destructive ant (*Atta insularis*) (Guérin) (Lara, 2014; unpublished data) that is an endemic species whose spread and attack has proved it to be a pest difficult to control.

Regarding the incidence and damage on *T. diversifolia* caused by pests and diseases, studies report that it is a plant tolerant of pest and diseases, since only on occasion has it been seen to be attacked by some leaf eaters in larval or grub stages. Investigations under nursery conditions confirm it as having few pests and diseases, indicating the excellent resistance of this plant to such organisms (Medina *et al.,* 2009). These results are possibly related to the presence in the foliage of some secondary metabolites, such as terpenoids, lactonics and coumarins with repellent action reported in some studies (Ríos, 1999). The plant has attributes that function as attractants and feed source for insects, including pollinators, honey producers and biological controllers. These functions are vital for production without agrochemicals, since it allows the system to reach a balance between insect populations and other arthropods for production with a minimum of negative environmental impact.

Table 10 lists arthropods associated with *T. diversifolia* plantations used for grazing in a silvipastoral system with native pastures and observed over a year (Valenciaga, N., 2014, unpublished).

Regarding tithonia damage in the study by Alonso *et al.* (2015), the incidence has been slight since only occasional isolated plants, >20%, have been seen to have been attacked, confirming the plant's phagodeterrent activity.

TABLE 10. Arthropod fauna associated with a silvipastoral system of *T. diversifolia* used for grazing

Class	Order	Family	Scientific name
Insecta	Hemiptera-Homoptera	Cicadellidae	*Empoasca* sp. *Hortensia similis* (Walk.) *Draeculacephala cubana* (M y B.) *Thamnotettix* sp.
		Membracidae	*Stictocephala rotundata* (Stal.)
	Hemiptera-Hemiptera	Miridae Lygaeidae Reduviidae	*Polymerus cuneatus* (Dist.) *Pachybrachius* sp. *Zelus longipes* L.(1)
	Coleoptera	Chrysomelidae	*Diabrotica* sp. *Epitrix* sp. *Systena basalis* (Duval) *Colaspis brunnea* (L.) *Oedionychus pictus* (Fab.) *Cryptocephalus* spp. *Anisostena cianoptera* (Suffr.)
		Coccinellidae Lycidae	*Cycloneda sanguinea*(1) *Dieomus bruneri* (Chpn.) *Thonalmus suavis* (J.D)
		Cerambycidae	*Oxymerus aculeatus* lebasi
	Diptera	Otitidae	*Euxesta stigmatia* (Loew.)
		Dolichopodidae	*Condylostylus* sp.
		Chamaemyiidae	*Leucopis* sp.
		Chalcididae	*Spilochalsis femorata* (Fab.)
		Syrphidae	1 morpho-species not identified[1]
		—	6 morpho-species not identified
	Lepidoptera	Noctuidae —	*Spodoptera* spp. *Hedylepta indicata* (F.) 1 morpho-species non identified
	Orthoptera	Tettigonidae	*Conocephalus* sp.
	Dermaptera	Forficulidae	*Doru taeniatum* (Dohrn.)
	Hymenoptera	Formicidae	*Wasmannia auropunctata* (L.)(1) *Paratrechina longicornis* (F.) (1)
		Ichneumonidae	*Coccygominus rufoniger*(1)
		Apididae	*Apis mellifera* (L.)
Arachnida	Araneae		5 morpho-species not identified
Gasteropoda			1 morpho-species not identified

Notes: (1) Known bioregulator.
Source: N. Valenciaga, 2014, pers. comm.

9. FINAL REMARKS AND RECOMMENDATIONS

9.1 Germplasm evaluation

Reported for the first time is an integral study on the growth and development characteristics of 29 plant lines of *T. diversifolia* collected in different locations in Cuba. Variability found could be strategically used in future programmes of varietal improvement. Information is given on the capacity of this plant to multiply by true seed under Cuban conditions.

The possibility is indicated of having a selection among genotypes of tithonia collected in Cuba that can be grazed by bovines. This suggests the new possibility of providing other shrub plants for utilization in silvipastoral systems.

9.2 Agronomy

9.2.1 Green manure

A beneficial residual effect is produced from this manure in forage yield. A suitable dosage is 12 t/ha fresh basis, which increases significantly the yield of the successor forage and decreases soil apparent density and improves its OM, total N, potassium and magnesium contents.

9.2.2 Planting

Tithonia planting must be carried out by laying the stem in the bottom of the furrow, and using cuttings from the basal or median part of the shoot, which achieves better development, greater population and higher biomass production.

9.2.3 Forage production

Greater yield is obtained at spacings of 0.5 m between furrows for both seasons, and the plantation must be cut at between 10 and 15 cm height with cutting frequencies of 60 days in the rainy and 80 days in the poor rainy seasons.

9.2.4 Pests

This plant, despite its reported pest resistance, should be further monitored for associated arthropods, according to the different production objectives, in both seasons of the year (rainy and poor rainy), and optimize the use of the plant for pest control in Cuba.

9.3 Recommendations

Results of the integrated study on the potential of alternative sources abundant in the tropics, such as *T. diversifolia,* should be provided to producers, teachers, researchers and students pre- and post-diploma.

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Nutritive value of *Tithonia diversifolia* for animal feeding

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ABSTRACT
This chapter demonstrates the nutritive value of *Tithonia diversifolia* for animal feeding. Nutrient contents are very high compared with other species. The ether extract content and its mineral concentration (Ca and P) vary according to vegetative stage. CP can range between 14.8 and 28.8%, with the lowest protein values found in the advanced flowering stages, while the highest values are obtained in the advanced growing and pre-flowering stages. The characteristics of the nitrogenous fraction are favourable in *T. diversifolia* meal, with 71.0% of total N content being amino acids ($N\text{-}NH_2$) and 17.3% nitrogen associated with the insoluble fraction of the dietetic fibre. Nutrient degradation is within the range of values for tropical plants. It discusses role of some secondary compounds, such as tannins; regarding some macronutrients, the nitrogen concentration and other macromolecules linked to the lignocellulosic complex in the plant material True digestibility ranges from 72 to 85% for DM and 60 to 70% for OM. The results show that *Tithonia diversifolia* is a forage alternative suitable to be widely employed in sustainable systems of animal production.

Keywords: nutritive value, nutrient degradation, secondary metabolites, *Tithonia diversifolia*

INTRODUCTION
In view of the typical characteristics of tropical pastures, with low levels of digestible protein and high fibre, the foliage of shrub legumes and arbuscular plants has been used in many cases as a nutritional element in ruminant supplementation in the tropics, mainly during the periods of forage shortage. Many of these species have nutritional values higher than those of pasture forages and can produce large amounts of edible biomass, and are more sustainable in the longer term than pasture forages under zero fertilization conditions (Mahecha *et al.,* 2008). Nonetheless, there is evidence that non-legume plant species, such as *Tithonia diversifolia,* accumulate both nitrogen in its leaves similar to legumes, has high phosphorus levels, a large root volume, a special ability for recuperating scarce soil nutrients, a wide range of adaptation, tolerates acid conditions and low soil fertility, is very robust, and can withstand pruning at soil level and burning. In addition, it has rapid growth and low input and management demands for its culture (Calle and Murgueitio, 2008).

This shrub grows as a weed in border paths, is fast-growing even under unfavourable conditions, and multiplies easily by cuttings. It can produce up to 275 t/ha/yr of fresh material (some 55 t/ha DM/yr). Its cut stems are supplied as cut-and-carry feed for cattle without pastures (Olabode, 2007).

Other properties of *T. diversifolia* are that it improves nutrient recycling, prevents erosion, reduces the effects of animal soil trampling and offers high biomass productivity without agrochemical inputs, as well as being ideal for cut-and-carry systems and for the conservation of fragile soils. It is used in farm and dairy production (Murgueitio *et al.,* 2013). The objective of the present chapter is to assess the nutritive value of *T. diversifolia* due to its high potential value for animal feeding.

1. NUTRIENT CONTENT AND NUTRITIVE VALUE

In Colombia, Navarro and Rodríguez (1990) assessed the nutritive content of *T. diversifolia* (leaves, petioles, flowers and stems ≤15 mm diameter), at five developmental stages. These authors found that the dry matter (DM) varied from 13.5 to 23.23% and the crude protein (CP) ranged between 14.8 and 28.8%. The lowest protein values were found in the advanced flowering stage (89 days), while the highest values were found in the advanced growing stage (30 days) and pre-flowering stage (50 days). The ether extract content also varied depending on the vegetative stage, from 1.4 to 2.43% (Table 1). These protein contents are within the range reported by Devendra (1995) for leaves of 12 tree species (14 to 36.6%) and by Benavides (1994) in a database covering 24 trees and 22 shrub species (10.9 to 42.4%).

Regarding the ether extract content (indicator of the fat content), this is low compared with that reported for various forage species (2.1–6.5%) by FAO (1993), and the 1.4–6% listed by Norton (1994). The ether extract from the bromatological analysis made by Rena Pérez (unpublished) on *T. diversifolia* meal was characterized by being very coloured (greenish shades). Therefore, in this fraction, it was considered that some pigments, perhaps carotenes or others, could be present.

The high content of crude protein found in *T. diversifolia* by Navarro and Rodríguez (1990) has been confirmed by Wanjau *et al.* (1998), 28.75%; Solarte (1994), 18.9%; Vargas (1994), 21–25%; and Rosales (1996), 24.2% (Table 2). The starch content found in *T. diversifolia* exceeds that found in species in wide use in bovine feeding, such as *Leucaena leucocephala* (15.6%), *Gliricidia sepium* (10.9%), *Erythrina poeppigiana* (10.5%), but less than found in *Trichanthera gigantea*. For structural carbohydrates, Rosales (1996) found values of 35.3% for neutral-detergent fibre (NDF) and 30.4% for acid-detergent fibre (ADF), being in the

TABLE 1. Nutrient contents of *T. diversifolia*

Developmental stage	% fresh basis	% dry basis	
	DM	N ×6.25	EE
Advanced growth	14.1	28.5	1.93
Pre-flowering	17.2	27.5	2.27
Medium flowering	17.2	22.0	2.39
Complete flowering	17.7	20.2	2.26
Elapsed flowering	23.2	14.8	2.43

NOTES: DM = dry matter; EE = ether extract.
SOURCE: Navarro and Rodríguez, 1990.

TABLE 2. Protein and carbohydrate content in *T. diversifolia* foliage

Crude protein (% in DM)	24.2
Soluble protein as% of total protein	40.2
Total water-soluble carbohydrates	7.6
Sugars as % of soluble carbohydrates	39.8
Reducing sugars as % of soluble carbohydrates	35
Neutral-detergent fibre (NDF),% in DM	35.3
Acid-detergent fibre (ADF), % in DM	30.4

SOURCE: Rosales, 1996.

low range found by this author for 11 shrub and 9 tree species (28.2–72.5% and 21.8–62.8%, respectively).

The characteristics of the nitrogenous fraction were also favourable in the *T. diversifolia* meal (Table 3). Pérez (unpublished data) found a total nitrogen content of 3.35% (20.93 CP) of which 71.0% was N of amino acids (N-NH_2) and 17.3% was N associated with the insoluble fraction of the dietetic fibre. There was a total amino acid content (TAAC) of 36.9%. These results indicate that a lot of the N present in the *T. diversifolia* meal is of easy availability. It was also found that the combustion values (gross energy) were acceptable in the products used in animal feeding.

TABLE 3. Bromatological composition of *T. diversifolia* meal

Total nitrogen (TN), % of DM	3.35
Nitrogen, N-NH_2, % of DM	2.38
N-NH_2, % of TN	71.0
Total amino acids, % of TN	36.9
Nitrogen associated with insoluble dietetic fibre (NIDF) %	0.58
NIDF, % of TN	17.3
ADF, % of DM	19.4
NDF, % of DM	24.0
Gross energy, MJ/kg DM	16.5

SOURCE: R. Pérez, unpublished data.

1.1 Mineral content

Just like the protein content, Navarro and Rodríguez (1990) found that Ca and P in *T. diversifolia* decreased as the plant matured (Table 4). Ca varied from 2.25 to 1.65% and P from 0.39 to 0.32%, while Mg increased from 0.046 to 0.069%. Wajau *et al.* (1998) reported P concentrations of 0.27 to 0.28% in the leaves. P content in *T. diversifolia* is considered high compared with other species commonly used in agroforestry.

Age has a marked effect on the nutritional value of *T. diversifolia*. This is demonstrated by studies carried out in Cuba at different regrowth ages during two periods of the year at ages of 60, 120 and 180 days. DM, neutral-detergent fibre (NDF), acid-detergent fibre (ADF), hemicelluloses, total phenols, total condensed tannins, condensed tannins linked to the fibre, and free condensed tannins increased their content (29.47%; 50.51%; 32.12%; 32.12%; 18.39%; 6.47 g/kg; 13.11 g/kg; 10.12 g/kg and 2.99 g/kg, respectively) by age 180 days, while the CP, cellulose, cellular content, *in vitro* digestibility and the cell wall digestibility decreased, peaking at 60 days (28.95%; 21.08%; 56.34%; 78.59% and 76.61%) (Verdecia *et al.,* 2011).

Other investigations by Lezcano *et al.* (2012) characterized bromatologically some essential nutritional components of *T. diversifolia* at two stages of the physiological cycle (30 and 60 days) in the rainy (RP) and poor rainy (PRP) periods. These authors analysed the edible fractions (leaves, tender stems and leaves + tender stems) at two stages of the physiological cycle, measuring DM, CP, crude fibre (CF), calcium (Ca), magnesium (Mg) and ash. Tithonia foliage showed variations in its nutritive quality. At 30 days, the best CP values (29.79% and 28.69%), Mg (0.094% and 0.210%) and ash (16.32% and 20.59%) for the

TABLE 4. Mineral content in *T. diversifolia*

Mineral	Growth stage				
	Advanced growth	Pre-flowering	Medium flowering	Complete flowering	Elapsed flowering
Calcium	2.3	2.14	2.47	2.4	1.96
Phosphorus	0.38	0.35	0.36	0.36	0.32
Magnesium	0.05	0.05	0.07	0.06	0.06

SOURCE: Navarro and Rodríguez, 1990.

TABLE 5. Chemical composition (%) of *T. diversifolia* ecotypes

Ecotype	DM	ASH	OM	NDF	CP
TITONIA 3	88.76	21.97	78.02	38.38	18.26
TITONIA 5	89.12	20.11	79.88	34.09	19.21
TITONIA 6	88.41	17.72	82.27	37.57	23.61
TITONIA 10	88.87	20.15	79.84	36.24	19.72
TITONIA 13	88.85	16.88	83.11	32.62	25.91
TITONIA 17	88.12	16.04	83.95	37.41	26.40
TITONIA 23	89.21	19.04	80.95	38.31	24.62
TITONIA 24	88.65	19.15	80.84	41.83	20.81
TITONIA 25	88.77	17.51	82.48	38.54	20.79
Standard deviation (±)	0.33	1.86	1.86	2.68	3.03

NOTES: DM = dry matter; OM = organic matter; NDF = neutral-detergent fibre; CP = crude protein.
SOURCE: Adapted from La O *et al.*, 2008.

RP and PRP, respectively, were found with similar properties for the edible fractions for the two physiological stages in the two periods evaluated.

The CF content in the tender stems at 60 days was the most representative in both periods (5.29 and 5.27%, respectively). Overall, the leaf + tender stem fraction attained the best values of the indicators measured for its use in animal feeding.

In Brazil, studies by Ronan *et al.* (2010) showed the effects of plant spacing and the growth phase on the nutritive value of *T. diversifolia,* and confirmed that there were effects and interaction between these factors and the nutritive quality of the plant. CP contents differed from those indicated by Navarro and Rodríguez (1990), who found lower levels after flowering.

In Cuba, La O *et al.* (2008) characterized nine *T. diversifolia* ecotypes (Table 5) collected in the central and western regions. These researchers found that the protein and NDF values were those maintaining greater standard deviation regarding the rest of the indicators studied, with protein and NDF values ranging from 18.26 to 26.40% and 14.79 to 25.74%, respectively. These results are related, in part, to what was said by some authors (Stewart and Dunsdon, 1998; La O *et al.,* 2003a; Mahecha and Rosales, 2006; Verdecia *et al.,* 2011; Ruiz *et al.*, 2014) of trials carried out in the tropics, in general, on the influence of the environment, cutting frequencies and phenological stage on the variation of the quality indicators. Nonetheless, in the ecotypes studied it was evident that all the studies of *T. diversifolia* agreed on its high content of the nutritive constituents of interest for animal feeding studies.

TABLE 6. Nutrient content (%) of *T. diversifolia* vs *P. purpureum* cv. Cuba CT-115

%	*Tithonia diversifolia* (SD)	CT-115 (SD)
OM	91.5 (± 1.2)	93.42(± 1.3)
CP	25.63 ((± 0.7)	11.16 ((± 0.3)
NDF	33..36 (± 0.9)	68.23 (± 1.7)
Ash	8.50 (± 0.2)	6.58 (± 0.4)

NOTES: OM = organic matter; NDF = neutral-detergent fibre; CP = crude protein; SD = standard deviation.
SOURCE: Adapted from La O *et al.,* 2009.

In general, *T. diversifolia* ecotypes studied by these authors had adequate nutritional value of the foliage for ruminant feeding (Table 5) and the protein contents obtained are within the range of values reported by Devendra (1995) for leaves of 12 species of tropical trees (14–36.6%), by La O *et al.* (2003b) for 12 genotypes of *L. leucocephala* (14–30.6%), and Verdecia *et al.* (2011) for *T. diversifolia* with different cutting ages under the conditions of Granma province, Cuba, while the structural carbohydrates are similar to those reported by Rosales (1996), who found a content of 35.5% for NDF in this same shrub species.

In addition, a marked difference in the nutritive value has been found between this species and grasses of interest for ruminant feeding under current tropical conditions (Table 6), and the important contribution that tithonia can offer to complement ruminant feed in the tropics.

TABLE 7. Dry matter degradability in the rumen *(in sacco)*

Hours	Degradability, % DM
0	33.0
12	50.8
24	83.3
48	90.2
72	92.8

SOURCE: Rosales, 1996.

In general terms, *T. diversifolia* is characterized by high contents of total nitrogen, to a larger extent in the form of amino acids that decreases progressively as plants age, as well as having good mineral contents, especially phosphorus (Navarro and Rodríguez, 1990). However, in spite of the quality of this plant regarding its chemical composition, studies are still needed regarding the productive response of the animals consuming it directly or as a plant derivative (forage or silage).

2. *IN VITRO* FERMENTATIVE POTENTIAL AND *IN SITU* RUMINAL DEGRADABILITY OF *T. DIVERSIFOLIA*

Rosales (1996) assessed the *in sacco* degradability of 20 arbuscular shrubs, among them *T. diversifolia.* This author found that 33% of the dry matter of the foliage of this species was completely water soluble, half degraded by 24 h and 90% was degraded at 48 h (Table 7). *T. diversifolia* was one of the three species showing high degradability in the assessment. *T. diversifolia* foliage degradability values found at 48 h were higher than those reported by FAO (1993) in *L. leucocephala* (79%), *Gliricidia sepium* (82.1%) and *Enterolobium cyclocarpum* (87.6%).

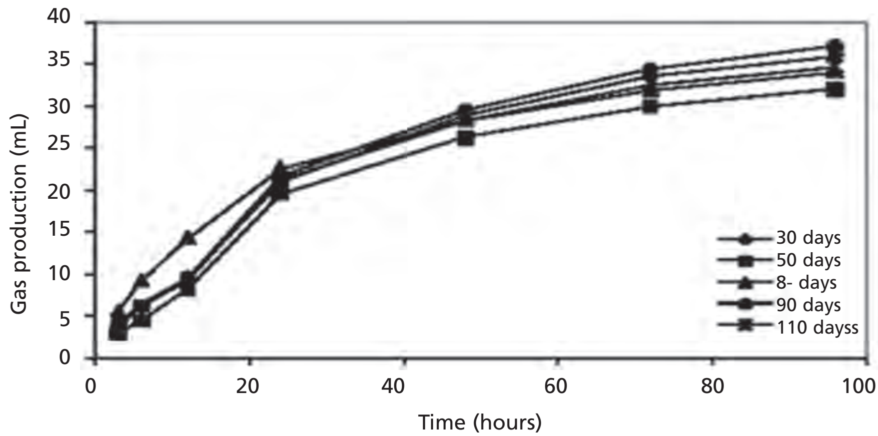
Vargas (1994), in an *in sacco* degradability trial of *T. diversifolia* foliage, found DM degradability of 72% at 24 h, and protein degradability of 79%. These results indicate that more than 50% of the foliage DM is degraded at 24 h. This matches the results of Rosales (1996).

The above data show that *T. diversifolia* leaves have good degradability of both DM and protein, since with only a short dwell period in the rumen, degradation was extensive, characteristics that should be used in animal nutrition.

3. FERMENTABILITY

The potential fermentability of *T. diversifolia* has also been evaluated and results match with those of the high degradability obtained in the rumen. Results show a rapid fermentation. Rosales (1996) found an accumulated gas production in *T. diversifolia* of 195 ml after an *in vitro* incubation period of 166 h. Values obtained for *T. diversifolia* were within the high range in this evaluation among 20 species analysed (81–230 ml). Similarly, they were within the high range obtained by Wood, Johnson and Powell (1993) from 19 species of Bolivian plants (38–223 ml). Also, Rosales (1996) found a fermentability rate of the highly fermentable compounds of 3.66 ml/h, and 0.76 ml/h for the slowly fermentable compounds. The fermentability rate of the highly fermentable compounds were considered within the range obtained in the assessment (1.82–4.12 ml/h). The fermentation rate of the slowly fermentable compounds was within the medium range obtained (0.20–1.74 ml/h).

FIGURE 1
***In vitro* fermentative potential of *T. diversifolia* with different cutting ages**



Source: La O *et al.*, 2008.

Results in this evaluation indicate that *T. diversifolia* has high DM fermentability and therefore fast nutrient availability from the fermentation.

More recently, studies developed at the Instituto de Ciencia Animal in Cuba (Ruiz *et al.*, 2008) with different *T. diversifolia* ecotypes demonstrated the great variability in the phenological characteristics of growth and productivity. Moreover, marked differences have also been identified in the fermentative potential of the organic matter of different ecotypes of this plant (La O *et al.,* 2008) and it has been reported that it reduces methanogen populations, with a beneficial effect on the ruminal microbial ecology, by modifying the populations of protozoa, bacteria and cellulolytic fungi (Galindo *et al.,* 2012)

Figure 1 shows the accumulated gas production by *in vitro* OM fermentation of samples with different cutting frequencies of *T. diversifolia* (for 30, 50, 70, 90 and 110 days of regrowth) during the *in vitro* incubation period of up to 96 h of incubation in the experiment of by La O *et al.* (2008) at the Instituto de Ciencia Animal, Cuba. The kinetic performance was characterized by an increase in gas production with the exposure time of the samples to micro-organism attack, with higher values in gas production at 90 h. This is attributed to the concentration of easily fermentable carbohydrates and nutrients which are present before the regrowth, and to the fact that rumen micro-organisms and enzymes first attack the easily available carbohydrates, and then later, with the fibre colonization and its fermentation, increased gas production occurs.

The influence of *T. diversifolia* on grass fermentation potential by virtue of its nutrient contribution is one of the main elements in obtaining optimum exploitation of the benefit goodness that this plant offers. Figure 2 shows the accumulated gas production by the OM fermentation during the incubation period of different levels (0, 15, 30 and 100%) of *T. diversifolia* as complementing *Pennisetum purpureum* cv. Cuba CT-115 (La O *et al.,* 2008). The performance in terms of accumulated gas production was characterized by an increase of production with exposure time of the combinations to micro-organism attack,

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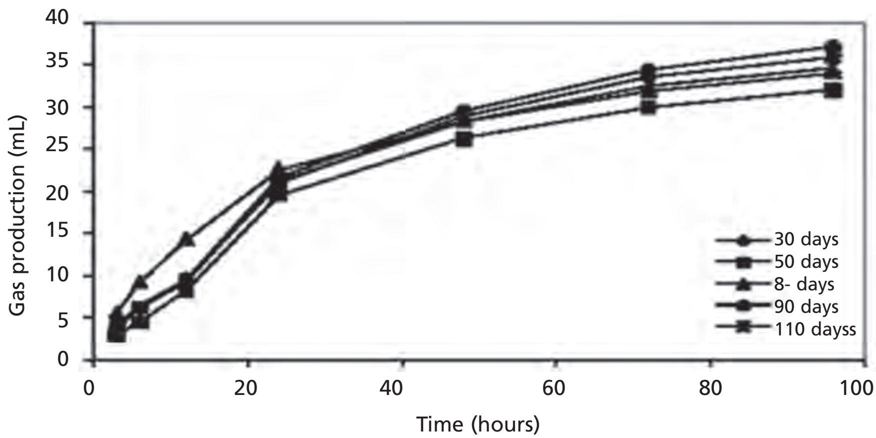

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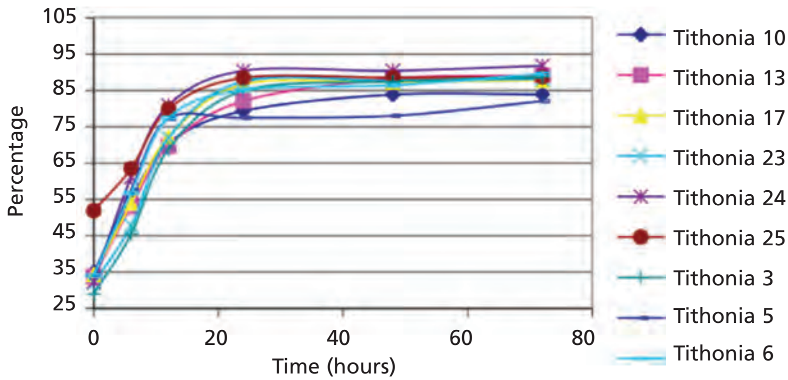
FIGURE 3
Dynamics of *in situ* ruminal degradation of DM of different tithonia ecotypes



In this respect, the great contribution to the rumen regarding degradable nutrients have been identified in different *T. diversifolia* ecotypes regardless of its variable nutrient content. Therefore, the use of this plant as grass complement in the tropics constitutes a viable option.

The dynamics of ruminal nutrient degradation (Figure 3) as well as the effective DM degradability values in different ecotypes of *T. diversifolia* for unequal constants of ruminal turnover rate (*k*) have not displayed similar performance in all plant materials (La O *et al.,* 2008) with values from 26.29 to 86.55% of effective DM ruminal degradation. These nutrient degradation contents are within the range of values for tropical plants reported by Mupangwa, Nogongoni and Harmidikuwanda (2003); La O *et al.* (2003a, b; 2008; 2012) with different seasonal legumes as well as trees and shrubs including some creeping plants and *T. diversifolia* with different cutting ages (La O *et al.*, 2008) and much lower than those found by Rosales (1996) on studying DM degradability of this plant with increasing times of ruminal fermentation up to 72 h.

The low degradation values obtained by La O *et al*. (2008) in one ecotype (No. 5, Figure 3) were attributed to possible effects of some secondary compounds such as the tannins with regard some macronutrients, and to the nitrogen concentration and macromolecules linked to the lignocellulosic complex in the plant material, aspects raised by La O *et al.* (2003a) in leucaena, and Savón and Scull (2002) and Nieves *et al.* (2011) in *T. diversifolia.* Hence, the identification of the factors that could limit the utilization of this ecotype in the rumen is a challenge. Furthermore, in that respect, Stewart and Dunsdon (1998) showed that 45% of the variation in *in vitro* digestibility of tropical legumes was represented by the variation in the tannin content. Nonetheless, these same authors indicated that it is not clear what beneficial or harmful effect tannin could have in tropical legume genera, and recommend considering this aspect with extreme caution until finding evidence on the specific nutritional effects of each one of the tropical tree and shrub species.

4. *IN VITRO* OM, DM, ADF AND NDF DIGESTIBILITY OF *T. DIVERSIFOLIA* ECOTYPES

The estimates of *in vitro* apparent digestibility of DM (IVADMD) and of OM (IVAOMD) in *T. diversifolia* studies at Autonomous University of Juárez City, Chihuahua, Mexico (La O *et al.,* 2008) assign lower values to the true digestibilities of both constituents (IVTOMD and IVTOMD) (Table 9), with values ranging from 72.25 to 79.77% for IVADMD and 57.71 to 66.20% IVAOMD, compared with 81.08 to 85.66% IVTDMD and 65.27 to 70.22% IVTOMD for the ecotypes studied ($P<0.01$ and $P<0.001$). These variations are logical, bearing in mind the nature and particular chemical characteristics of the ecotypes evaluated, as well as the diverse sources of variation that can influence digestibility, among which are the intrinsic characteristics of each ecotype and the relationships established between the macromolecules in its interaction with the rumen environment. These values are considered to be much higher than those reported by Verdecia *et al.* (2011) on conducting digestibility studies in *T. diversifolia* from poorly drained soils in Granma province, Cuba. In that respect, D'Mello (1992) and La O *et al.* (2003a) emphasized the importance of knowing the variability in the chemical composition, and the utilization degree, of some tropical forage resources in which variabilities of up to 45% in some phytogenetic resources are reported. This aspect could be formed in *T. diversifolia* according to the great amount of ecosystems where this plant appears. At the same time, La O *et al.* (2008) point out the importance of realizing *in vitro* assessments with fast methodologies and of great repeatability, within which are some that do not provoke animal invasion and that allow recognition of the feed as a favourable option for tropical use.

The fibrous fraction contents, its composition and utilization by ruminants are an interesting element, due to its significance for ruminants. In that respect, La O *et al.* (2008) on studying the apparent and true digestibilities of the cell wall and ADF (Table 10) noted similar performance to that of DM and OM with analogous values regarding the tendency of the digestibilities, both as apparent and as significant differences ($P<0.01$, $P<0.001$)

TABLE 9. *In vitro* apparent and true digestibility of DM and OM of different ecotypes of *T. diversifolia*

Ecotype	IVAOMD	IVTDMD	IVAOMD	IVTOMD
TITONIA 3	73.96[a]	83.65b	57.71[a]	65.27[a]
TITONIA 5	76.99[b]	83.94[bc]	61.50[b]	67.05[ac]
TITONIA 6	75.52[ab]	81.47[a]	62.14[b]	67.03[ac]
TITONIA 10	73.25[a]	83.39[b]	58.48[a]	66.58[ab]
TITONIA 13	79.77[c]	84.48[c]	66.30[c]	70.22[d]
TITONIA 17	76.33[b]	83.28[b]	64.08[bc]	69.92[d]
TITONIA 23	78.35[bc]	85.66[d]	63.43[b]	69.34[d]
TITONIA 24	72.25[a]	83.38[b]	58.41[a]	67.41[bc]
TITONIA 25	74.58[ab]	81.08[b]	61.52[b]	66.87[ac]
SE	0.63**	0.22**	0.68**	0.54**

NOTES: IVAOMD = *in vitro* apparent digestibility of DM; IVTDMD = *in vitro* true digestibility of DM; IVAOMD = *in vitro* apparent digestibility of OM; IVTOMD = *in vitro* true digestibility of OM; [abcd]Means in the same column differ at $P<0.01$**.

SOURCE: Taken from La O *et al.,* 2008.

TABLE 10. *In vitro* apparent and true digestibility of NDF and ADF of different ecotypes of *T. diversifolia*

Ecotype	IVANDFD	IVTNDFD	IVAADFD	IVTADFD
TITONIA 3	28.39[bc]	32.11[c]	11.55[a]	13.06[a]
TITONIA 5	26.25[a]	28.62[ab]	12.20[a]	13.31[a]
TITONIA 6	28.38[bc]	30.61[bc]	14.97[b]	16.15[c]
TITONIA 10	26.54[a]	30.22[b]	11.34[a]	12.91[a]
TITONIA 13	26.03[a]	27.56[a]	14.22[b]	15.06[bc]
TITONIA 17	28.56[bc]	31.16[b]	13.68[b]	14.93[bc]
TITONIA 23	30.02[c]	32.82[c]	11.60[a]	12.68[a]
TITONIA 24	30.22[c]	34.88[d]	11.91[a]	13.75[a]
TITONIA 25	28.74[bc]	31.25[bc]	14.84[b]	16.13[c]
SE	0.67**	0.69**	0.40 ***	0.40 ***

Notes: IVANDFD = *In vitro* apparent digestibility of NDF; IVTNDFD = *In vitro* true digestibility of NDF; IVAADFD = *In vitro* apparent digestibility of ADF; IVTADFD *In vitro* true digestibility of ADF;
[abcd]Means in the same column differ at **P<0.01, ***P<0.001.
Source: Taken from La O et al., 2008.

present in plant materials. However, future studies should elucidate from the productive point of view the correspondence and validation of these results. Therefore, research studies with beef or dairy production animals are a challenge.

Results obtained by La O *et al.* (2008) in IVAD of DM, OM, NDF, ADF, as well as in IVTD of DM, OM, NDF, ADF, highlighted the differences between the *T. diversifolia* ecotypes studied, identifying as a result those with favourable digestibilities and possibly usable as elements for incorporation of this plant into ruminant diets.

Results from the analysis of the chemical composition, gas production, *in situ* ruminal DM degradability and apparent and true nutrient digestibility in different ecotypes of *T. diversifolia* suggest a high nutritional value for this species. Nonetheless, physiological studies are necessary in animal trials where cutting frequency and inclusion level of the ecotype are related to the degree of utilization of these nutrients by the animal, as well as the effect of some secondary metabolites on the physiological and productive responses to this plant.

5. SECONDARY METABOLIC COMPOUNDS

The Americas are the continents where tithonia has been most studied and used in animal feeding systems (Gallego, Mahecha and Angulo, 2014), not only owing to its high edible biomass production (Nieves *et al.*, 2011; Ruiz *et al.*, 2014), but also in view of its nutritive quality (Mahecha and Rosales, 2006). However, there is insufficient knowledge of the compounds or secondary metabolites of this plant, mainly due to the great diversity and complexity of the compounds in its tissues (García *et al.*, 2008).

In an analysis of secondary metabolites in *T. diversifolia* by Rosales (1992) there were no phenols or tannins, while Vargas (1994) found a low level of phenols and absence of saponins.

Ríos (1998) reported that a coumarin, possibly cholinine, was found, but its level was not quantified. There were no manifestations of intoxication in bovines and rabbits to which forage of this species was supplied for various periods.

In later studies, Togma, Kobayashi and Usui (2001) indicated that secondary metabolites of tithonia are variably produced according to the genus, family and even the species. The phytochemical composition of this plant can change according to the nutritional conditions of the soil and other environmental characteristics, such as insolation, water availability, depredation and diseases (Waterman and Mole, 1994).

Results from the phytochemical studies reported in the scientific literature reveal great variability in the presence of secondary compounds in tithonia. Table 11 shows results from an analysis of secondary metabolites in Cuba (Scull, Savón and Ramos, 2008) and in Mexico (Hinojosa *et al.,* 2013).

TABLE 11. Secondary compounds present in the tithonia forage meal

Secondary compounds	*T. diversifolia* (Scull, Savón and Ramos, 2008)	*T. tubiformis* (Hinojosa *et al.*, 2013)
Tanins	moderate	slow
Alkaloids	slow	slow
Flavonoids	absent	slow
Saponins	absent	absent
Triterpenes	absent	slow
Anthocyanidins	slow	
Reducing sugars	moderate	
Coumarins	absent	slow
Quinones	absent	slow
α amino group	abundant	
Resins	absent	
Cardiotonic G.	absent	

The general chemical composition of the tithonia samples in both studies showed a diverse combination of secondary metabolites. Results obtained demonstrated that in both cases the plants contained tannins and alkaloids, although in low concentrations, and saponins were not found. In this investigation the presence of flavoids, terpens or coumarins was not verified

Similar results were attained by Alarcón and Navarro (2012) on assessing the quality of tithonia foliage and the presence of saponins was not found. Nonetheless, Gutiérrez *et al.* (2010) when studying the phytochemical composition of *T. diversifolia* cultivated in Mexico found saponin concentrations of 0.76 g/100 g. These results are similar to those obtained by Otusanya and Ilori (2012) when the composition of bioactive substances was studied in organic extracts of this plant. García and Medina (2006), determining saponins in forage plants, considered saponin contents ≥3.5% to be high. The concentration of this metabolite in studies where it was found is similar to those found in the foliage of other plants commonly consumed by cattle (Makkar *et al.,* 2007).

Saponins are a group of molecules of varying structure, such as steroids and triterpenes; hence the diverse biological activity. Their tenso-active properties make the cell membranes alter. Their haemolytic activity and capacity to reduce serum cholesterol are some of the more important characteristics (Oleszek, 1990). Wu *et al*. (2007), Gómez *et al.* (2011) and Arabski *et al. (*2012) demonstrated the anti-inflammatory and anti-viral effects, among others, of these compounds. Reporting results from phytochemical investigations, Odeyemi *et al*. (2014) attributed the antimicrobial properties of tithonia extracts to the presence of secondary compounds, such as the polyphenols, flavonoids, alkaloids and triterpenes.

Results reported from Kenya by Ezeonwumelu *et al.* (2012) indicated the presence of saponins, alkaloids, flavonoids, tannins and triterpenes in *T. diversifolia.* Uduak and Emana (2013) investigated the presence of secondary metabolites in plants cultivated in Nigeria and found *inter alia* saponins, tannins and flavonoids. However, Miranda *et al.* (2014) in studies in Brazil with this same forage species found polyphenols, tannins and flavonoids, but no saponins.

TABLE 12. Total polyphenols (%) in different tithonia ecotypes

	Leaves	Stem	Whole plant
Low	2.67	3.17	3.14
Medium	6.10	2.46	3.33
High	5.23	4.03	-

Source: Scull, Savón and Ramos, 2008.

Otusanya and Ilori (2012) verified the presence of terpenes in leaf extracts of this plant. The sesquiterpene lactones, which are a recognized class of terpenoids, are indicated as characteristic secondary metabolites of the species in the Asteraceae family (Bohlmann and Zdero, 1990). These compounds are colourless and with a bitter flavour, leading in many cases to poor plant acceptability by animals. Toledo *et al.* (2014) refer to a wide spectrum of biological activities that are attributed to these substances, and for the first time indicate strong effects of the tyrotundin compounds, 3-0 methyl ether, tagiatinin F and guaianolide, against *Leishmania* parasites in its intra and extracellular life.

Scull, Savón and Ramos (2008) using phytochemical screening essays for evaluating *T. diversifolia* forage with different cutting ages (30, 50, 70, 90 and 110 days) demonstrated that from 70 days the presence of secondary metabolites in the plant increased. Similar results were obtained by Verdecia *et al.* (2011), who reported that the concentration of total condensed tannins and free condensed tannins increased with plant age, and who relating this to the increase in biomass maturity. Nonetheless, Lezcano *et al.* (2012) did not find differences in the presence of secondary compounds relative to the season or phenological stage.

Scull, Savón and Ramos (2008) evaluated the presence of total polyphenols in *T. diversifolia* with high, low or medium growth, collected in the central-western region of Cuba, and reported values between 2.46–6.10% (Table 12). However, results found by García *et al.* (2008) and Verdecia *et al.* (2012) were lower (1.46 and 0.65%) than those shown in Table 12. These differences could be related to age of the plants used in each study.

Studies by Santacoloma and Enríquez (2010) reported differences in the concentration of phenolic compounds in samples collected in two different localities of Colombia. Similar results were obtained by Martínez *et al.* (2011), who evaluated total polyphenols and tannins in *T. tubiformis* cultivated in two municipalities of Toluca Valley in the state of Mexico.

In an analysis of secondary metabolites in *T. diversifolia* made by Mahecha and Rosales (2005), the plant was reported as a source of carotenoids that could be used to improve egg quality.

Mwanauta, Mtei and Ndakidemi (2014) indicated that the non-volatile fraction of *T. diversifolia* is a source rich in flavonoids and sesquiterpene lactones, while in the essential oil, hydrocarbide monoterpenes such as beta-ocimen, alpha-pinene and limonene predominate. Usually plants synthesize a great diversity of secondary metabolites, and according to the environmental conditions one or other functional group will predominate.

In recent years the number of publications and investigations related to the mechanisms involving bioactive molecules in biological activity attributed to the plants has increased. Toledo *et al.* (2014) in an *in vitro* trial reported a group of sesquiterpene lactones isolated from tithonia leaves with strong antimicrobial activity, while Bouberte *et al.* (2006) found a new isocoumarin, tithoniamarin, with anti-fungal and herbicide effects. Ezeonwumelu *et al.* (2012) reported anti-diarrhoeic activity with leaf extracts of *T. diversifolia* in rats. Miranda *et al.* (2014) demonstrated a marked antioxidant potential in flower extracts from tithonia, and Mwanauta, Mtei and Ndakidemi (2014) reported antioxidant activity in leaf extract of the plant.

Undoubtedly, the diversity and low content of secondary metabolites in tithonia make this plant a feed option that can be used for mitigating feed shortage in countries with low inputs and resources. It is recommended that investigations regarding the bioactive compounds present in this species be implemented, reflecting its great variability in response to edaphoclimatic conditions. In this way, more knowledge of the possible effects of the inclusion of the plant in different animal diets can be attained.

6. FINAL REMARKS

T. diversifolia foliage shows variations in its nutritive quality depending on its vegetative stage. In stages of advanced growth (30 days) and pre-flowering (50 days) there were higher protein values. These results, together with those of Ríos (1998) on the recovery capacity of the plants in successive cuttings (19 cm/35 days and 44 cm/49 days using plant densities of 0.75 × 0.75 m), could imply that the most appropriate time for forage harvesting for feeding without harming the crop, is in its pre-flowering stage (cutting every 49–50 days) when potentially biomass production of 31.5 ton/ha is possible.

Data on the nutritive and feeding value of the *T. diversifolia* forage, although it is still scarce, indicate its potential use as supplement in animal feeding. In general terms, *T. diversifolia* foliage is characterized by a high content of total nitrogen, of which a high proportion is amino acids, with a high phosphorus content, fast degradability and fermentation at ruminal level, a low N proportion linked to the insoluble dietetic fibre, and low content of fibre and secondary metabolism compounds. In addition, the presence of pigmenting substances is presumed. These results, when analysed in comparison with other forage species in extensive use in animal feeding, such as *L. leucocephala*, *G. sepium* and *Erythrina* spp., as well as acceptability results and productive responses obtained in sheep, broilers and layers, show the potential for its use in both monogastrics and ruminants. If one includes with these evaluations the various observations on its use in animal feeding, coupled with previous knowledge on effective management and response of this culture, this species becomes a forage alternative widely acceptable in sustainable systems of animal production.

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Use of *Tithonia diversifolia* in non-ruminants

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ABSTRACT

Tithonia diversifolia is a new feeding option that can be used to bridge feed shortages, particularly in tropical countries that experience periodic or regular insufficient supplies and resources. Very little research has been reported in tithonia in non-ruminant species. This chapter offers information on investigations carried out in Cuba and Latin America with the use of tithonia for feeding non-ruminants, notably in poultry, swine and rabbit feeding. For each species it reports the effect of this plant on several digestive physiology aspects, such as morphology of the gastrointestinal tract of replacement chicks for layers; growing swine; nutrient digestibility in growing swine and rabbits; performance trials in broilers; layers; weaned and growing pigs, breeding does; and growing rabbits. It helps establish inclusion levels for tithonia in conventional diets, as well as the improvement or similarity of productive indicators for monogastric species.

Keywords: digestive physiology, performance trials, non ruminants, *Tithonia diversifolia*

INTRODUCTION

The production of biofuels by the wealthy countries of the northern hemisphere using cereals and soybeans, places the rest of the world and particularly many tropical countries in a difficult situation, since most of those countries are not efficient in producing maize and soybean for animal feeding. However, it is known that because of the biodiversity of the tropics and its climatic conditions, trees and bushes produce high yields of biomass per unit area, making research into their use of great importance for animal feeding (Ly, 2005; Savón *et al.,* 2005a, b; Nieves *et al.,* 2011).

Tithonia (*Tithonia diversifolia*) because of its agronomic characteristics, its adaptation to a range of economic conditions, its nutritive value and bromatological composition, demands little tillage and produces high yields of biomass (70–90 t/ha). It can be consumed by many animal species. It is undoubtedly a new feeding option that can be used to compensate for shortage of feed, especially in tropical countries having limited supplies and resources (Pérez *et al.,* 2009).

1. USE OF *TITHONIA DIVERSIFOLIA* IN POULTRY FEEDING

The research on physiological and nutritional effects of the use of *T. diversifolia* in poultry feeding in Latin America is scarce. Almost all papers refer to consumption and productive performance of broilers, the development of the birds, and egg quality of laying hens. Some of the results obtained are considered below.

1.1 Morphology of the gastrointestinal tract

1.1.1 Replacement chicks for layers

In Cuba, Almeida and Savón (2011) evaluated the effect of substituting the feed of replacement layers up to 12 weeks of age with different levels of tithonia (*Tithonia diversifolia)* forage meal, looking at productive and morphometric indicators. Experimental rations were based on a control meal (maize-soybean-wheat) and three rations with substitution of 5, 10 and 15% of the control by tithonia forage meal. Results show an increase of the relative live weights of the caeca, both filled and empty, without modifying the other organs of the gastro-intestinal tract (GIT). (Tables 1 and 2).

In the case of the filled organs (Table 1), the similarity between the control and experimental treatments indicate that the feed consumed was not retained for a long time in these organs. These results agree with those of Duke (1997) and Hetland, Svihus and Choct (2005), who state that the dimension of the physiological effects of the fibre in poultry on certain organs depends on the physical form and the chemical nature of the feed. At the same time, they expressed that on increasing the amount of the tithonia forage meal there was an increase in the relative weight of the GIT, which was defined by the increase of the caeca. This increase is the result of greater entrance of fibrous particles into those organs for their microbial digestion (Marrero, 1999**).**

On eliminating the digesta from the organs (Table 2) there was also an increase of the relative weights of the caeca of birds receiving the experimental treatments (10 and 15%) of tithonia forage meal. This is the response to a physiological adjustment produced by the microbial mass and the final fermentation products (Eastwood, 1994). These results confirm those of Savón (2005), who indicated that the increase in weight of the organs corresponds to their specific metabolic functions.

At the same time, the substitution of the control feed by the levels of tithonia foliage meal studied (10 and 15%) showed an increase in consumption compared with the control, and similar live weights of the birds receiving the different treatments. The increase in consumption indicates that for the chicks to cover the shortage of energy produced by including this fibrous source, they had to increase consumption to maintain the levels of metabolizable energy required for growth and maintenance. In relation to final weight, this is considered a very important result since one of the main aspects of the future layers is that they must have appropriate live weights to later enable good body development.

TABLE 1. Weight relative to LW (%) of the different filled sections of the GIT of replacement chicks of 12 weeks of age consuming tithonia forage meal

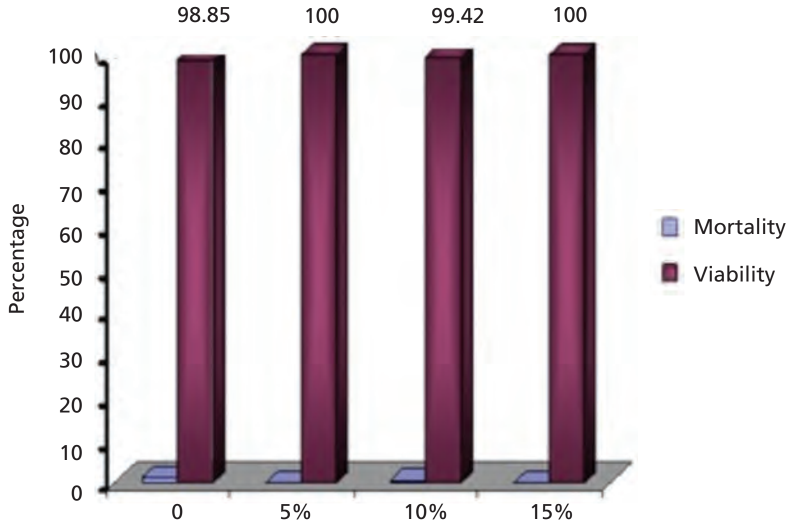
Organ	Treatment			
	0	5	10	15
GIT	63.1	74.5	77.6	79.0
Proventriculus	4.4	5.6	4.4	4.9
Gizzard	32.0	41.4	35.4	38.9
Small intestine	32.0	36.1	36.4	34.8
Caeca	6.8	6.6	7.7	9.1
Colon+rectum	5.1	4.9	4.2	4.3

TABLE 2. Weight relative to LW (%) of the different empty sections of the GIT of replacement chicks of 12 weeks of age consuming tithonia forage meal

Organ	Treatment			
	0	5	10	15
GIT	45.8	46.1	48.4	47.5
Proventriculus	4.4	5.7	4.4	5.2
Gizzard	25.9	28.3	26.2	28.9
Small intestine	28.7	29.4	27.9	28.9
Caeca	4.3	5.2	6.2	7.4
Colon+rectum	2.9	2.5	2.8	3.8

Source: Taken from Almeida and Savón, 2011.

FIGURE 1
Mortality and viability of layer replacement chicks of 12 weeks of age consuming 0, 5, 10 or 15% of forage meal containing tithonia



These authors also assessed other health indicators, with blood tests of replacement chicks receiving different levels of tithonia forage meal. They found that viability remained over 98% (Figure 1), while health indicators were found to be in the physiological ranges of 7–13% and 23–55 for haemoglobin and haematocrit, respectively.

These results suggest that it is possible to substitute up to 15% of the commercial feed by tithonia forage meal without affecting the relative weights of the organs of the GIT (except for the caeca), final live weight and the haemoglobin and haematocrit of layer replacement chicks up to 12 weeks of age.

1.2 Productive performance of tithonia in poultry

1.2.1 Broilers

In Colombia, Vargas (1994) carried out a biological test with 13 forage species, including *Tithonia diversifolia*, in seven-day-old chicks, where 20% of the commercial concentrate was replaced by dry ground forage of each species, for seven days. Weight gain and consumption of chicks fed tithonia was in the range of 75–99% of the control, which the author considered to be very high in relation to the other species evaluated. There was a trend toward higher weight gains of chicks with higher protein content, lower saponin and phenol content and a higher ration digestibility. Feed conversion was in the range of 125–150% compared with the control. *T. diversifolia* was finally classified as one of the foliages having the highest potential for non-ruminant feeding.

Although few studies have been carried out in poultry in Latin America, in Indonesia, Susana and Tangendjaja (1988), evaluated the effect in poultry of the foliar protein of tithonia obtained as a concentrate, isolating it from other components, mainly fibre, in terms of weight gain and feed consumption. The treatments were: the control without tithonia concentrated protein; a ration with 10% concentrated foliar protein; and a ration with 20% concentrated foliar protein. The feed was offered as meal. Weight gains at four weeks did not differ between treatments and feed intake was not affected by the tithonia foliar protein concentrate. These results indicate that the tithonia concentrated foliar protein can be used in poultry rations at a level of up to 20% without adverse effects.

1.2.2 Layers

Odunsi, Farinu and Akinola (1996) evaluated the influence of *Tithonia diversifolia* leaf meal in layer rations on the development of the birds and egg quality. Six groups of 72 layers of the commercial line Nera Black four months after the start of laying were fed commercial concentrates and a ration containing 0, 5, 10, 15 and 20% *T. diversifolia* leaf meal. Egg production was stable in all rations. Voluntary intake varied from 96.3 g/animal/d for a ration containing 20% of tithonia to 107 g/animal/d for the commercial concentrate. Feed conversion in terms of kg of feed consumed per dozen eggs was better for the ration containing 15% tithonia meal, while the commercial concentrate had the highest cost of feed consumed per dozen eggs. All rations produced positive net weight gains. The egg yolk index, shell thickness and egg weight were not significantly affected by the ration. Egg yolk colour score was higher for all rations containing tithonia compared with the commercial concentrate. There was no mortality during the 12 week evaluation. These results show great potential for the use of tithonia in layers, where the recommended contribution is 15% as a percentage of the ration.

At the same time, Mahecha and Rosales (2005) confirmed in Colombia that tithonia is a source of carotenoids that serve as a pigment for hen eggs.

2. USE OF TITHONIA IN SWINE

There have been very few studies globally with the use of tithonia in swine. A summary of the main papers shows that there is still much to do so that this crop can be used efficiently in swine feeding.

2.1 Nutrient digestibility and morphometrics of the GIT

2.1.1 Swine growth

Studies examining digestive processes in swine fed tithonia in Cuba were carried out by Pinto (2009). Three experiments determined chemical characteristics, physical properties and nutritive value of swine rations with different proportions of tithonia forage, green matter, as well as its effect on nutritional and blood indicators. The physical and chemical characterization of rations was carried out, and a correlation matrix developed. DM digestibility and NDF were determined by *in vitro* digestibility with the use of caecal and faecal inocula in the pigs. Finally, an *in vivo* study was carried out with 12 pigs of the commercial cross Yorkshire×Landrace×Duroc of 32 kg live weight, to determine the blood indicators: haemoglobin, haematocrit and differential blood count at the start and after 25 and 50 days of supplying the feed.

Rations with the 10 and 20% substitution with tithonia had a lower NDF content than the control ration (31.4, 34.0 and 38.1% respectively). An interaction was observed

TABLE 3. Interaction between the rations with different percentages of substitution of tithonia forage meal and the different inocula in relation to NDFD (%).

	Feed			SE± Sign.
	Control	10%	20%	
Caecal NDFD	40.09 [b]	40.11 [b]	43.74 [bc]	2.83**
Faecal NDFD	28.73 [a]	49.62 [c]	47.86 [bc]	

NOTES: [abc] Means with different superscripts in each row differ significantly at $P<0.05$ (Duncan, 1955); **($P<0.01$)

between the rations and the inocula for NDF digestibility. NDFD was higher in rations with 10% and 20% substitution of tithonia, both with the use of the caecal and faecal inocula, compared with the results for NDFD in the control ration (Table 3). This indicates that the caecal inoculum may be used to estimate the NDFD of the rations, because the conditions within the caecum is ideal for microbial growth and thus there is a greater nutrient fermentation; this segment has the highest calibre and volume of the large intestine (LI) of swine (Álvarez, 2002; Morales, 2002).

At the same time, the high NDFD coefficients found in these treatments could be due to the presence of tithonia and its physicochemical characteristics, as the plant has high levels of carbohydrates and water soluble proteins (Navarro and Rodríguez, 1990). In relation to DMD, similar values were found among treatments despite using different rations and inocula.

As a result of the study of *in vitro* digestibility of the NDF and DM of tithonia forage meal a linear regression was obtained with values of DMD ($R^2 = 0.94$) and NDFD ($R^2 = 0.65$) that were very high for both inocula used.

There was evident interaction between rations and sampling time in the behaviour of the eosinophils, showing a higher value (1.7 [C×10^9/L]) in animals consuming the control ration at first sampling, differing from the rest of the interactions. In this respect, this result indicates that the eosinophils are above their physiological levels [0.11–1.1 ±0.45 C×10^9/L] (Sotolongo, Rubio and Castilli, 2009; Gélvez, 2009) and therefore the animals at that time had higher parasitic rates, since the eosinophils are able to damage directly or indirectly the parasite and to reduce the damage released by their presence when modulating the hypersensitivity reactions. It has been found that these cells in the presence of parasitic antigens have a low medullar generation time and emerge from the medulla in 18 hours, showing higher serum levels in the cases, named eosinophilia (Borrás *et al*., 2002).

The substitution of 10% and 20% of tithonia in rations, showed no negative effect on the eosinophil performance in any age that animals were evaluated, the levels of this indicator were gradually decreasing as the animals were fed with these rations. This could be due to the *Tithonia diversifolia* presence, since this plant destroys the cattle intestinal parasites and is useful for treating parasitic infections in children (Wanjau, Makulama and Rhysen, 1998; Ríos, 2002)

From another point of view, Savón, *et al*. (2008), used 32 kg liveweight pigs to study the effect of replacing the control feed with 10 and 20% of tithonia on GIT morphometry. These authors found no obvious differences between the treatments in the absolute, relative and metabolic weight of the digestive organs, accessory organs nor of the spleen. That was the reason it was suggested that it was feasible the use up to 20% of *Tithonia diversifolia* leaf meal replacing the grains and cereals diet.

It can be concluded that the use of 10 and 20% replacing the tithonia forage meal diet did not cause alterations in nutritional value, nor in food digestibility, morphometric indicators of the digestive organs and GIT of growing pigs, and that the haematological indicators were within the normal physiological range.

2.2 Productive performance of pigs

2.2.1 Weaned pigs

In pigs of 33 to 61 days old, Mora *et al.* (2007a) in the Instituto de Ciencia Animal in Cuba, included 0, 5 and 10% of tithonia forage meal taken with with 60 days between cuts. There were no differences in animals weight gain for the 5% inclusion level, but there was for 10%. Similarly for food conversion. Thus, it could suggest for animals in the pre-fattening period up to 14 kg to not include more than 5% of tithonia forage meal.

In an experiment of Fasayi and Ibitayo (2011), on the African continent with weaned pigs, soybean meal was progressively substituted by levels of 0, 10, 20 and 30% of tithonia leaf meal. There was a significant reduction of food intake in the range of 390 g/day in the control diet against 264 in the diet with 30% of tithonia. The same tendency existed for liveweight gain and feed conversion. These authors found nitrogen retention of 2.87 g/nitrogen/pig for the animals fed 10% tithonia, 2.26 g/nitrogen/pig for the control without tithonia, and negative retentions of -2.60 and -1.70 g/nitrogen/pig for the treatments with 20 and 30% tithonia, respectively. Due to the similarity in the control performance and that of 10% of tithonia leaf meal, the authors considered that it was feasible its use up to this level in weaned pigs.

2.2.2 Growing pigs

In further studies, Herrera *et al.* (2013) evaluated the effect of inclusion of sorghum, tithonia and mulberry in the diet of growing and fattening pigs. These authors did not find distinctions between liveweight gain for control treatments and that of soybean and sorghum, but there was between these and the other treatments, regarding to feed, sorghum and tithonia (570 g) and feed, sorghum and mulberry (524 g). The feed conversion (kg DM/kg of LW) did not differ between treatments. The changes in weight gain could be attributed partly to the energy efficiency of voluminous food being lower than that of concentrate (Ly and Macías, 1995).

In another paper, Mora *et al.* (2007b) reported using 24 pigs of ca 31.7 kg liveweight and 85 days old to observe the effect of substituting 0, 10 or 20% of feed with tithonia forage meal. These animals were distributed in pens with eight animals per treatment. There was a decrease of the progressive performance with the tithonia forage meal inclusion in rations. The daily liveweight gain considerably differed in favour of the control treatment, and a similar effect was found with the feed conversion, which matched that reported in Mora *et al.* (2007a) when fed levels of tithonia forage meal greater than 5% in weaned piglets.

There are several factors that might be influencing these results. Some authors state that tithonia is not readily consumed by pigs (Sarria *et al.,* 1992), so by reducing food intake due to the presence of alkaloids (Lezcano, 2013) that give a bitter taste, liveweight gain becomes less and it is possibly that these animals cannot reach the same weight as other animals at the end of the fattening period. Other factors that could influence the results might be a superior fibre concentration, which causes the diet to be more voluminous (Savón, 2005).

In order to assess food intake, Oliva *et al.* (2014, unpublished) conducted a preliminary study, and selected a group of 12 animals of ca 26 kg liveweight, which were kept in individual cages to measure intake pattern, adaptation to rations and performance over 14 days of feeding. Three rations were used: a control with maize and soybean meal, and other two with 20% of tithonia or moringa, respectively. It was observed that, in the early days, the animals in groups with foliage meals included in the feed did not like to consume the rations, especially that of tithonia, in contrast to the control, which had a good intake. Ten days after the beginning of the test, intake was monitored, which involved determining the time when the animals remained consuming the feedstuff for a period of 90 minutes (Table 4). Intake was controlled every minute and, later, intakes were grouped into stages of 30 minutes at the end of this period of observation.

TABLE 4. Intake pattern (10th day of food intake)

Parameter	Treatment		
	Control	20% Tithonia	20% Moringa
30 minutes	26.75	29.75	28.7
60 minutes	9.0	23.2	16.2
90 minutes	13.25	11.96	16.25
Total tithonia	49.0	64.91	72.0
Total % tithonia	54.4	72.1	67.9
Food intake g per 90 min.	1320	400	770

SOURCE: unpublished data from Oliva, 2014 study.

TABLE 5. Animal performance during adaptation stage

Parameter	Treatment		
	Control	20% Tithonia	20% Moringa
Initial weight, kg	27	26	26.3
Final weight, kg	38	30.5	34
Daily gain, g	785	321	544
Animal intake, kg/day	2.08	1.16	1.55
Food conversion	2.7	3.61	1.55

SOURCE: Taken from Oliva (2014, unpublished data).

Animals consuming the treatment of feedstuff without foliage kept eating for 49 minutes, which was equivalent to 54.4% of the time when food was provided. The time in front of a trough used by animals fed moringa and tithonia was higher compared with the control. The time pigs were in front of the trough did not correspond to their intake. This way, concentrate intakes were the highest, followed by those of feedstuff.

The zootechnical performance obtained at 14 days after providing the previously described rations (Table 5) clearly showed that tithonia rations are not attractive to pigs and it requires a long period of adaptation. Animals consuming tithonia only ate 48.0% of the control intake and those that consumed moringa represented 74.5%. There was a pig that never adapted to tithonia intake. Therefore, the mean daily gain was very limited due to feed intake, apart from the fact that the worst food conversion was that of the diet with tithonia, which was much lower than that determined by moringa. This demonstrated that there are factors affecting the digestive use of this feed.

Nevertheless, Pedroso (2008) reported gains of more than 600 g/day in pigs weighing 20 kg which were fed with a ration containing sorghum and feedstuff complemented with 30% of pre-dried and ground tithonia, without detecting health problems or other deficiencies. This author states that tithonia forage (especially when it is fresh) is rejected at the beginning, but, later, the animals adapt and normally consume it. These authors also recommend supplying forage in form of feedstuff pre-dried and ground with grains, because it has been demonstrated, in comprehensive studies, that sesquiterpene lactones have a marked influence on monogastric acceptability, because these compounds give a distinct bitter flavour (Personiou *et al.,* 1987; Villalba and Provenza, 2005).

Obviously, there is a difference regarding the ability of pigs to use tithonia rations, and the studies so far indicate that further studies with this feed are needed in pigs, to use the good bromatological composition and agronomic characteristics of the crop. In this sense, it is recommended that attention be focused on anti-nutritional and physiological factors, as well as performance of tithonia in pig feeding.

3. USE OF TITHONIA FOR FEEDING RABBITS

Tithonia, due to its nutritional potential and forage contribution, is a feed traditionally used by producers for feeding rabbits, because it has a very good acceptability when it is freshly supplied in the form of meal or included in the rations for fattening rabbits. In Latin America, some studies have looked at the physiological and nutritional effects of tithonia in rabbits.

3.1 Apparent digestibility of nutrients

The use of forage for feeding rabbits affects the digestive process, nutrient use and biological efficiency due to changes in passage rate (García *et al*., 1999). Therefore, it is important to determine nutrient content and digestive use of rations when these unconventional resources are included.

In Venezuela, Nieves *et al.* (2011) determined the dry matter digestibility (DMD), organic matter digestibility (OMD), crude protein digestibility (CPD), energy digestibility (ED), neutral detergent fibre digestibility (NDFD) and hemicellulose digestibility (HEMD) in rations that included increasing levels (0, 9 and 18%) of tithonia forage for fattening New Zealand×California rabbits (individually housed), through the indirect method of insoluble acid ash, in order to estimate the digestibility of these fractions in tithonia forage. The content of protein and digestible energy was also analysed. Tithonia forage presented high protein (18.5), NDF (32.9) and CF (17%) contents, so it was proposed as an ingredient in rabbit rations, which had appropriate values of these fractions, according to the nutritional requirements for fattening rabbits indicated by Blas and Wiseman (2003).

It was found that the DMD, OMD, CPD and HEMD were similar for all treatments (Table 6). The average values obtained for DM, OM and CP were slightly lower than those reported for meal rations including similar levels of *Trichanthera gigantea* forage (Nieves *et al.,* 2001) or *Leucaena leucocephala* (Nieves *et al.,* 2002). Similarly, Nieves *et al.* (2008) reported slightly superior values for DMD, OMD and CPD with granulated rations

TABLE 6. Apparent digestibility of DM, CP, gross energy, CF, NDF and OM in rations with the inclusion of tithonia forage for fattening rabbits

Digestibility (%)	Treatments (levels of tithonia forage meal) (%)		
DM	51.12	53.45	51.25
CP	68.57	60.11	64.08
Gross energy	54.89	43.09	53.62
CF	27.91	33.23	45.12
NDF	26.84	31.57	67.44
Hemicellulose	44.20	45.37	47.24
OM	51.99	54.87	52.60

NOTES: CP = crude protein; NDF = Neutral Detergent Fibre; OM = organic matter
SOURCE: Adapted from Nieves *et al.* 2011.

that included 30% of *Morus alba* (mulberry), *Leucaena leucocephala* (leucaena), *Trichantera gigantea* (naranjillo) and *Arachis pintoi* (pinto peanut). This comparison suggests that rations with inclusion of tithonia forage have acceptable nutrient digestibility.

Furthermore, digestibility of fractions related to fibre (NDF and CF) was superior in the rations that included a higher proportion of the studied foliage (Table 6). This result suggests that arnica forage favours higher fibre digestibility, which could be explained by a higher content of fermentable fibre in the caecum from this ingredient. The values found in this case are higher than those observed in earlier studies.

At the same time, the values of faecal digestibility of nutrients from tithonia forage meal, estimated by difference, were DM (55.80), OM (55.19%), hemicellulose (39.18%), digestible energy (8.74 MJ/kg) and digestible protein (109.6 g/kg). These values are within the range found by Raharja *et al.* (1986) and García *et al.* (1999) for fibrous ingredients used in rations for rabbits. These references, based on obtaining nutritional value of fibrous ingredients widely used for formulating rations for rabbits, indicate the promising nutritional value of tithonia forage for rabbits.

3.2 Tihonia effect on productive performance of rabbits

In Colombia and other tropical countries the use of tithonia foliage for rabbit does and fattening animals is a common practice (Rosales 1992, 1996; Ríos and Salazar, 1995; Ríos, 1998). Foliage is mixed with concentrate and pasture in the feeding adaptation phase and later it is utilized as alternative protein source. Also Rodríguez (1990) pointed out that quail and rabbits have good consumption of the forage without its being chopped, up to a stem diameter of 1.0–1.5 cm , specially when it is offered tender (around 50 days cutting interval), with good nutritional value.

Nieves *et al.* (2011) analysed the productive performance of fattening rabbits receiving 0, 9 and 18% of tithonia forage replacing a control diet of maize-rice polishing-wheat bran and soybean. DM intake (115.30, 118.57 and 113.77 g/day) was similar among rations and supported daily weight gains in rabbits (18.17, 18.15 and 20.93 g, for 0, 9 and 18% of tithonia forage inclusion, respectively). These values agree with those observed for these variables when unconventional rations were used for rabbits under tropical conditions.

Later, Nieves *et al.* (2012) reported that a restriction of 40% of the feed balanced with tithonia forage, provided *ad libitum*, reduced the daily weight gain due to poor DM and nutrient intake. However, moderate restriction in the supply of concentrated feed (20%) and the *ad libitum* offer of tithonia forage, produced growth similar to that observed with commercial feed provided *ad libitum*, with a similar DM intake.

4. FINAL REMARKS

4.1 Poultry feeding

In layer replacement chicks it is possible to replace up to 15% of commercial feed by tithonia forage meal without affecting the relative weights of the GIT (except for the caeca), final weight and health indicators. These were confirmed in layers, with tithonia leaf meal giving:

- better feed conversion/kg of feed consumed per dozen eggs;
- egg yolk index and shell thickness similar to those of the concentrate ration;

- egg yolk more highly coloured than with concentrate alone; and
- no mortality during 12 weeks.

4.2 Swine feeding

The use of 10 and 20% of tithonia forage meal is recommended in growing-finishing pigs, as it does not cause alterations in nutrient digestibility, morphometric indicators of digestive organs and GIT accessories. This was confirmed in performance trials. An adaptation period is necessary to introduce consumption of the forage due to its bitter taste.

4.3 Rabbit feeding

Tithonia forage presents a high content of digestible nutrients for growing rabbits and constitutes an alternative food source for feeding this species under tropical conditions. The content of digestible protein and energy in tithonia foliage highlights the potential for use for feeding rabbits.

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Feeding of cattle, sheep and goats with *Tithonia diversifolia* in Latin America and the Caribbean

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ABSTRACT

This chapter shows principal results for utilization of *Tithonia diversifolia* in cattle, sheep and goats in Latin America and the Caribbean. In goats, sheep and calves it has been used in complete diets supplying a total nutrient feed in a single ration. Among the benefits of this approach for calves in tropical countries are: more controlled consumption of feed; improved utilization of total nutrients in the ration; development of the papillae and ruminal muscles; and increased productive performance. Tithonia is recommended as a strategic option for reducing need for supplementation of dairy cows, and thus increasing efficiency of dairy systems. Results obtained indicate the nutritional potential of *T. diversifolia* as feed for various animal types and ruminant categories. There is a need for future studies on this and other plant species for animal feeding.

Keywords: integrated diets, total nutrient utilization, ruminants, *Tithonia diversifolia*

INTRODUCTION

Agriculture in the tropics seeks improved modalities for human food and animal feed, given that the human populations are growing fast. In this context, shrubs and trees present new feeding alternatives and their use is generating new knowledge on management and exploitation systems, leading to more sustainable animal rearing. Many attempts to use these new options have been based on silvipastoral principles or other approaches. Some have been successful and others remain at the stage of seeking producer approval, as the recognition or rejection by producers will ultimately define the success of the system.

Within the enormous floristic potential of the tropics, in recent years progress has been attained in studies of one species, *Tithonia diversifolia* (Hemsl.) Gray, due to its high protein value and resistance to prolonged drought periods. *T. diversifolia* is one of the non-legume plants considered as promising for use as feed for various animal species (Mahecha, 2002), and in ruminants in particular.

Its utilization in animal feeding has been increasing in recent years. Its use is reported in cows (Mahecha and Rosales, 2005), sheep (Vargas, 1994; Premaratne *et al.,* 1998), buffaloes (Premaratne, 1990), and goats (Wambui, Abdulrazak and Noordin, 2006). Nevertheless, reports on its use in animal feeding are scarce, and further studies are needed. Therefore, this chapter focuses on results from the use of *T. diversifolia* in ruminant feeding.

1. MAIN RESULTS WITH *TITHONIA DIVERSIFOLIA* IN RUMINANTS

Tithonia diversifolia has a wide range of adaptation and it is a species with good biomass production capacity, fast growth, and requiring only low input and management demands for its culture. Its use has been demonstrated as a nutritional strategy in ruminant supplementation in the tropics, mainly in periods of forage shortage. This species has higher nutritional value than grasses, and can produce large amounts of edible biomass that are more stable in time than those of pastures under conditions of zero fertilizer application (Hernández *et al.,* 1998).

According to Rosales (1996), this plant is characterized by containing 24.2% crude protein (CP); 40.2% soluble protein; 7.6% water soluble carbohydrates; 9.8% of total sugars; 35% reducing sugars; 35.3% of neutral-detergent fibre (NDF); and 30.4% acid-detergent fibre (ADF), with a degradability between 50% at 12 h and 83% at 24 h of ruminal incubation. These properties allow wide use in ruminant feeding. Studies with sheep showed better results than those with *Leucaena leucocephala*, *Gliricidia sepium* and *Erythrina* spp. regarding animal acceptability and productive response.

In Colombia, it is used in cow feed (Ríos, 1998) and for sheep (Vargas, 1994). Excellent consumption by browsing Holstein cows has been observed at 2400 masl (E. Murgueitio, 2010, pers. comm.). Farmers of Dagua and El Dovio supplied chopped *T. diversifolia* mixed with other forages such as *Trichanthera gigantean*, *Erythrina edulis*, *Morus alba* and sugar cane tops to cows.

2. FEEDING OF GOATS AND SHEEP

The main use of this feed for goats and sheep has been in complete rations. Using this technique under tropical conditions has increased both economic and productive efficiency of flocks. Use of complete rations led to more controlled feed consumption, improved use of total nutrients in the ration, development of the papillae and ruminal muscles, and increased productive performance.

Information on *T. diversifolia* as part of a feeding alternative for small ruminants is scarce. Nonetheless, analysis of meal showed an *in vitro* DM digestibility of 63.3% and N digestibility of 65.9%. This confirms its suitability as a component in complete rations for feeding lactating kids.

A trial evaluated ways of supplying *T. diversifolia,* and the resultant feeding response in goats, with or without sugar cane juice supplementation. Treatments were:

- Adaptation phase, with four crossbred male animals (*Capra hircus*) in the post-weaning phase. Treatments consisted of: tithonia browsing with sugar cane juice; tithonia leaves in the feeder with sugar cane juice; tithonia browsing; or only tithonia leaves in feeders. This phase of the trial involved a 10-day feeding, based on a factorial Latin square (4×4) model, for a total of 40 days.
- Treatments were then finalized based on the preliminary results of the Adaptation Phase, with elimination of supply as solely plant leaf, which had led to considerable weight loss. In this way supply became chopped tithonia with sugar cane juice.

There was no significant difference in the average tithonia consumption when supplied fresh: chopped (2363 g/day) versus browsing (2391 g/day). At the same time, average consumption on a dry basis showed a difference ($P<0.05$) when sugar cane juice was included (44.75 g DM/kg LW for no juice and 55.35 g DM/kg LW for the with-juice treatment), as the supplement improved digestibility and consumption significantly. From this, the use of tithonia for browsing was recommended in the diet of small ruminants when accompanied by sugar cane juice for greater dry matter use and weight gain improvement.

A production system in Venezuela used uncut tithonia fresh forage for sheep and goat consumption as part of a diet with sugar cane tops and elephant grass (*Pennisetum* spp.). In the afternoon, forages such as *T. gigantea*, *G. sepium* and *Cassia moschata* are supplied to the animals. In other regions of the world, such as Sri Lanka tithonia has been used in nutritional trials with buffalo and sheep (Premaratne *et al.,* 1998) and in Indonesia for goats (Premaratne, 1990).

Feeding trials in Cuba used dairy goats (both sexes) of Alpine, Nubian and Saanen breeds during the rearing stage, with an average live weight of 5.97 ±0.87 kg and 53 ±10.0 days of age. Animals consumed a ration of one litre of goat milk and an integral mixture (tithonia meal, maize meal, soybean meal, urea, premix and common salt) supplied at a rate of 0.409 kg (fresh basis) as basal diet. In this case, the forage plants tithonia and *P. purpureum,* of 60 and 75 days of age, respectively, were the fibre portion of the mixture.

Tithonia forage was used to replace 50% of the protein contribution of the control mixture based on conventional protein sources (soybean meal). The mixture provided was 87.83% DM; 18.56% CP; 10.05 MJ/kg DM 16.14% CF; 33.89% NDF; and 10.58% ash. Results were DM consumption of 0.360 kg/animal/day (6% LW; 95.30 g/kg $LW^{0.75}$), 62.12 g of average daily gain and 7.53 feed conversion, showing that tithonia can be used in kids during their first life stage as a partial replacer of concentrate feeds, enhancing voluntary DM intake, improving ration efficiency and productive indicators.

3. UTILIZATION BY HAIR SHEEP

In Buga (Cauca Valley), Ríos (1998) reported results from an assessment by Vargas (1994) of tithonia acceptance by hair sheep to which two diets were supplied for five days, with 50% and 100% of the basal diet from tithonia. Plants were in the flowering stage at harvesting time. Both diets received multi-nutritional blocks (10% urea) *ad libitum* and *G. sepium* forage (3% live weight, fresh basis). The diet with 50% was completed with cut sugar cane tops, and tithonia consumption was 0.868 kg/day on a fresh basis, corresponding to 0.369 kg/day in a dry basis. In the 100% diet, animals consumed 1.668 kg/day on a fresh basis, equivalent to 0.712 kg/day dry basis. These results show the possibility of using this forage species as protein supplement, or as the only forage, in the feeding of hair sheep.

Premaratne *et al.* (1998) evaluated the effects of the type and forage supplementation level on voluntary consumption, digestion, microbial protein synthesis and growth of sheep fed a basal diet of rice straw and yucca. The assessment was carried out for 30 days in Dorset × South Down growing sheep. The average initial live weight was 20.6 kg and the final 23.7 kg. The forage used included *L. leucocephala*, *G. sepium* and tithonia at a daily supplementation level of 13 $g/kg^{0.75}$. There was a daily organic matter consumption of 40.4, 55.5, 55.0 and 54.9 $g/kg^{0.75}$, for the control (rice straw and yucca *ad libitum*), *L. leucocephala*, *G. sepium* and *T. diversifolia*, respectively. Voluntary intake of the forage supplement had a positive effect on voluntary intake of the basal diet. All diets including forage supplementation showed a higher OM digestion than the control, and results were:

control, 488 g/kg; *T. diversifolia*, 557 g/kg; *L. leucocephala*, 516 g/kg; and *G. sepium*, 526 g/kg. The corresponding daily live weight gains were 1.7, 5.2, 5.4 and 4.7 g/kg. The efficiency of microbial protein synthesis, estimated by urinary excretion of purine derivates, was low for the control (3.8 g microbial N per kg digestible OM matter consumed) and showed significant differences from the remaining treatments: 11.3, 9.0 and 9.4 g microbial N per kg digestible OM consumed for *L. leucocephala*, *G. sepium* and *T. diversifolia*, respectively. These results indicate that of the three forage species, *T. diversifolia* had the highest daily DM intake, OM digestibility and live weight gain.

In spite of these indicators of agronomic potential, adaptation plasticity and results available on the productive performance of animals, *T. diversifolia* requires further investigation of its potential for animal feeding, including measuring its effect on protein supplementation.

4. FEEDING OF YOUNG CALVES

Although in the available published literature there were no papers showing the utilization of *T. diversifolia* in pre-ruminant calves, at the Instituto de Ciencia Animal de Cuba a very interesting investigation looked at its use in this animal category. In this case, the nutritive quality of *T. diversifolia* in integral diets was related to the performance of growing calves.

With these criteria, an experiment studied *in vitro* gas production with integral diets, where the fibrous source was forage hay and included five levels of *T. diversifolia* forage meal (0, 5, 10, 15 and 20%) and 15% of this plant combined with 5% ground hay. Six integral diets (ID) were evaluated on the basis of 30% of fibrous source in the diet and different *T. diversifolia* meal levels, fed from 8 to 10 weeks of age, with ground grass hay, using as inocula the rumen of calves of 45, 75 and 120 days of age.

The highest gas productions (mL/g of incubated DM) with inocula of calves of 75 and 120 days of age were attained on diets 1 and 2, corresponding to 0 and 5% *T. diversifolia* (P<0.001), respectively, as shown in Figure 1. Different authors estimate that this effect corresponds to the highest nutrient availability for ruminal fermentation. If the composition of the integral diets with different *T. diversifolia* levels is analysed, a different soluble carbohydrate contribution (celluloses and pentosanes) could probably be involved. If this analysis is transferred, evaluating that with calf age DM consumption increases, it implies the need for future studies with higher levels of meal in the integral diet. Moreover, these findings show for the first time the use of inocula of growing animals for this type of study with integral diets, and confirm the importance of further investigations of the fermentative potential and its relationship with nutrient availability to achieve this effect according to Heinrichs and Zanton (2007) in studies with inocula from growing cattle.

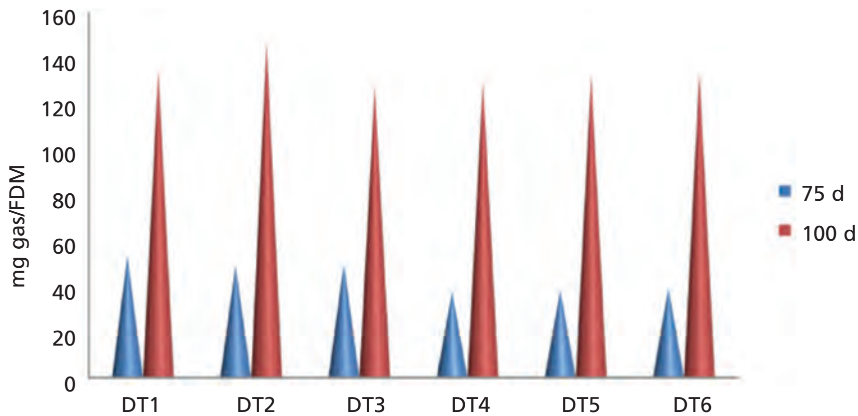
T. diversifolia by its chemical characteristics can contribute to diet composition for calves, contributing to early ruminal development and be an important nutrient source. In this respect, the assessment of the ingestive performance of calves fed different levels of *T. diversifolia* hay meal in the integral diet can be considered a factor that determines its physiological development and as consequence decides its nutritional performance.

On this topic, integral diets (ID) were studied using ground hay of *Cynodon nlemfuensis* plus meal of *T. diversifolia* Line 10, namely:

T1 – ID with 20% ground hay (control);

T2 – ID with 5% ground hay and 5% tithonia meal;

FIGURE 1
Effect of age of the inoculum donor calf on *in vitro* gas production in integral diets with *T. diversifolia* inclusion over until 72 hours



Notes: FDM = fresh dry matter.
Source: Chongo et al., 2015 (unpublished)..

T3 – ID with 10% ground hay and 10% tithonia meal; and

T4 – ID with 15% ground hay and 15% tithonia meal.

The study of feeding performance of calves at 45, 75 and 120 days of age consuming these diets indicated a greater daytime feed consumption time, increasing with the age of the animals (Martínez, 2009). In the three ages studied, it was noted that the time dedicated to consumption was greater in diets with a higher proportion of tithonia hay meal (T3 and T4) and the control (T1) at 45 and 75 days of age, while at 120 days of age the difference was only between T4 and the control. This was attributed to the fibre level in the rations, and could have influenced outcomes as the calves took longer to consume sufficient to cover their requirements. Plaza, Ruiz and Elías (1984), using different levels of ground hay in the integral diet of calves, reported that DM consumption increased up to 40% inclusion in the diet without affecting this indicator.

Concerning standing rumination, there were no differences with the inclusion of tithonia in the diet, while when animals were lying down there was different performance with the highest grass and tithonia level. In this case, animals dedicate more time to rumination ($P<0.001$), which is attributed to the manner of supplying the feed in only one session in the morning, according to what was reported by Alvarez *et al.* (2004), but with time ranges comparable to other investigations conducted by Ybalmea *et al.* (2007).

An interesting factor found with the diets where grasses or *T. diversifolia* cv. 10 were the only fibre source of the integral diet, as the time devoted to water drinking was higher ($P<0.01$) than that of the animals where the fibrous sources were combined. During the browsing act, calves with access to the integral diets with higher levels of tithonia hay (T4) and hay (T1) dedicated more time to this activity at 45 and 75 days of age, which agrees with gas production at these same ages. These results can be linked to the proportion of fibrous feeds, since the less digestible fibre needs more rumination time for reducing the particle size, and at the same time more time of action of the microorganisms for its colonization and for its efficient degradation in the rumen (González, 1995; González and Enriquez, 1997).

5. PRODUCTIVE PERFORMANCE OF CALVES

The productive performance study of growing calves related to the feed consumed by the animals indicated that the amount of fibrous feed in the ration did not affect DM, CP and ME consumption, although nutrient availability could influence its utilization as the fibrous fraction increases.

In this sense, Hintz (1995) and Quigley and Mills (2006) stated that high fibre levels in the diet tend to limit energy consumption due to the decrease of digestibility associated with the increases of fibre concentration. In our case, this could explain why calves increased consumption in the second stage as ruminal capacity increased.

Table 1 shows nutrient consumption in which there were no differences in protein and energy consumption throughout the experiment. Nonetheless, the values for CP and ME at 30–60 days seem to favour treatment T2. During the 61–120-day period, CP and ME consumption tended to be higher in T1 and T4. CP consumption throughout the experimental stage favoured T4, followed by T1 and T2, while ME consumption implies T1 was best.

There were no differences for average DM consumption at 30–60 days. However, it seems that this was favoured with diet T2. DM consumption between 61 and 120 days of age favoured treatments T1 (control) and T4 with significant differences ($P<0.05$) from T2 and T3. Total DM consumption did not differ but a tendency favouring T1 and T4 is observed. It is important to note that DM consumption related to LW reflected that treatments increased ingestion relative to CF concentration of the ration.

Klein *et al.* (1987) observed in calves consuming a pre-weaning diet that there was a greater DM consumption of the starter with 20% fibre in the post-weaning period. Nonetheless, when the starter diets were not supplied, animals consumed more DM with 10% hay. This implies that ruminal development from early stages contributes to a better utilization of fibrous diets when the animal is functionally ruminant.

At the same time, in these studies, the inclusion of lower fibre levels does not seem to influence animal performance. This agrees with Coverdale *et al.* (2004), who found no significant differences in DM consumption in calves fed fibre levels of 7.5 and 15% as alfalfa

TABLE 1. Average nutrient consumption of the feed by experimental stage

Parameter	Treatments (inclusion levels of tithonia)				SE ±
	T1 (0%)	T2 (5%)	T3 (10%)	T4 (15%)	
DM 30–60 kg/animal/day	1.17	1.27	1.07	1.20	0.11
DM 30–120 kg/animal/day	2.84	2.65	2.55	2.88	0.09
CP 30–60 g/animal/day	0.24	0.28	0.22	0.25	0.02
CP 30–120 g/animal/day	0.46	0.46	0.41	0.47	0.019
ME 30–60 MJ/day	38.91	43.01	35.61	39.29	0.92
ME 30–120 MJ/day	30.79	29.66	27.07	29.92	0.29
CF 30–120 g/animal/day	0.15	0.11	0.11	0.15	0.02
CF 30–120 g/animal/day	0.36a	0.24b	0.26b	0.37a	0.02***
DM intake/100 kg LW	3.78	3.35	3.43	3.58	0.11

NOTES: Different letters indicate significant differences ($P<0.05$) (Duncan, 1955); ***$P<0.001$

hay. However, Strozinski and Chandler (1971) found that this indicator increased up to 67.5% on including alfalfa hay, since the quality of the fibrous feed is a determinant factor in the calf. Later studies with higher inclusion levels could clarify this statement for tithonia.

Seemingly, the amount of fibrous feed in the ration did not affect DM, CP and ME consumption in the experiment, although nutrient availability could influence its utilization and it is higher as the fibrous fraction increases, which accounts for greater consumption by the calves in the second stage, together with the increased rumen capacity.

It is worthy of note that average daily gain (ADG) in live weight (LW) for the first stage (30–60 days of age) surpassed the 500 g/animal/day, with a tendency to favour T1, T2 and T4. The accumulated ADG of LW over the whole stage exceeded 700 g/animal/day, where T1 and T1 performed better. The other treatments also had good gains for the stage, demonstrating the possibility of including tithonia meal in the diets.

Daily gain for the control with grass hay did not show differences when tithonia was included in different combinations (Table 2), which is of interest since it is attractive under the present tropical cattle conditions if the high prices of the energy and protein ingredients required for calf feeds are considered.

These results demonstrated the possibility of including tithonia meal in integral calf diets and make possible new approaches and strategies in the studies for increasing the levels with age in this animal category.

Table 3 shows the productive performance of the calves. Feed conversion of DM when tithonia meal was incorporated showed results similar to that of the control treatment during the whole 30 to 120 days of age of calves, which were T1 3.45; T2 3.48; T3 3.61; and T4 3.68 kg/kg LW.

Concerning health indicators, it was confirmed that calves receiving the integral diet with different levels of *T. diversifolia* cv. 10 did not show problems during the trial. Only three diarrhoea cases occurred, but were not related to the treatment. There were no deaths or other clinical manifestations indicating health problems in the calves.

In general, the use of *T. diversifolia* in integral diets for calves showed satisfactory acceptance, with 60–80% feed ingestion between 08:00 and 20:00, regardless of the level of the diet. The *In vitro* Gas Production (IVGP) indicated satisfactory behaviour patterns for the 30–60 day old calves and evident changes in ruminal development with age. Moreover,

TABLE 2. Average daily gain (ADG) of calves fed different levels of tithonia

Parameter	Treatments (inclusion levels of tithonia)				SE±
	T1 (0%)	T2 (5%)	T3 (10%)	T4 (15%)	
ADG, g/animal/day (30–60 days)	687.18	631.98	552.28	620.03	84.38
Total ADG, g/animal/day	826.10	768.28	739.75	783.43	54.60
$MW^{0.75}$ 120 days	34.58	34.88	33.68	35.55	1.15

TABLE 3. Average live weight gain performance of calves

Parameter	Treatment (inclusion levels of tithonia)				SE±
	T1 (05)	T2 (5%)	T3 (10%)	T4 (155)	
Initial LW kg (30 days)	37.38	43.88	41.75	43.00	1.75
Weaning live weight (60 days)	58.50	63.25	58.75	62.13	3.28
Final live weight kg (120 days)	112.75	114.00	109.25	117.00	4.85

the combinations of hay and tithonia gave gains >700 g/animal/day from the integral diet, with average LW >109 kg at 120 days. The use of the plant meal in the integral diet gave normal health indicators in the calves. Results obtained indicate the nutritional potential of *T. diversifolia* for calf feeding and the need for further studies on this and other use options for calves.

6. UTILIZATION IN DAIRY COWS

According to Gallego, Mahecha and Angulo (2014), improving dairy protein content and hence milk quality, it is important to exploring new strategies for feeding systems that would increase rumen fermentation efficiency and in this way ensure adequate levels of ruminal ammonium and help improve bacterial activity and protein digestion (Hervás *et al.,* 2000). Both affect positively amino acid availability for the ruminant, giving a better protein level in the milk.

In this respect, Mahecha *et al.* (2007) assessed the production and quality of the milk of F_1 Holstein × Zebu cows supplemented with tithonia forage as partial replacer for concentrate feed. The authors did not find significant differences in milk production in the rainy season (12.5 L/cow/day) with 100% concentrate supplementation versus 12.4 L/cow/day with 35% substitution of the concentrate by fresh tithonia. In the dry season, yields were 11.71 L/cow/day with 100% concentrates, versus 12.16 L/cow/day with tithonia substitution. At the same time, there were slight differences in milk quality, favouring cows consuming the highest proportion of *T. diversifolia*.

The protein level rose to 3.82% (compared with 3.51% in cows with 100% concentrates) and butterfat reached 3.9% (compared with 3.48%). This could mean an increase of $79 per litre of milk based on the quality bonus of the product given by Colanta Enterprise in Colombia. Mahecha *et al.* (2007) also estimated that for every 100 milking cows, the producer would receive an additional annual net increase of $5 million in income from the combination of additional profit due to better milk quality, and reduced cost of concentrates.

Hence, we recommend tithonia as a strategic option for reducing supplementation of dairy cows and increasing the efficiency of milk production systems.

Total forage consumptions of *T. diversifolia* supplied to the animals in both dry and rainy seasons showed a positive acceptance by the animals, an aspect also found by Vargas (1994) on supplementing goats with 50% and 100% of the basal diet from cut forage of *T. diversifolia*. This author found consumptions of 0.868 kg/day and 1.67 kg/day on a fresh basis, respectively. Likewise, Premaratne *et al*. (1998) on comparing the use of *T. diversifolia* with *L. leucocephala* and *G. sepium* in sheep feeding, found that tithonia performed better in terms of consumption (54.9, 55.5 and 55.0 g/kg$^{0.75}$, respectively) and in increasing consumption of the basal diet. In the same way, Wambui, Abdulrazak and Noordin (2006), supplementing goats with *T. diversifolia*, *Calliandra calothyrsus* and *Sesbania sesban* foliage, attained the highest forage consumptions with tithonia (154, 146 and 145 g/day respectively).

TABLE 4. Feeding performance of cows grazing tithonia

Activity	Time in hours	%
Grazing	4.2	38
Ruminating	2.3	21
Resting	3.1	28
Walking without consuming	1.4	13

Source: Garcia-Lopez and Rodríguez (2014; unpublished).

As a complement to these research findings based on tithonia forage consumption in ruminants in cut-and-carry systems, Rodríguez, Osechas and Briceño (2000) and Mahecha and Rosales (2005) reported its browsing utilization by dairy cows and yearlings in production systems of Colombia and Venezuela, respectively, although consumption levels are not mentioned. Previous results contrast with reports from the Kenya Agricultural Research Institute (KARI, 1999), when comparing *C. calothyrsus*, *T. diversifolia, Lantana camara* and *Ficus* spp. leaf consumption by bovines, found the lowest consumptions with *T. diversifolia*.

In other investigations, the value of *T. diversifolia* was demonstrated not only in its consumption, but also in the great stability attained in production and milk quality between climatic seasons, indicating the usefulness of this plant for productive sustainability in these systems throughout the year.

Results obtained indicate that the substitution of 35% of the concentrate in the diet of the animals by *T. diversifolia* forage, under the conditions of the experiment, do not alter production or milk quality; to the contrary, there is a tendency to improve levels, although without statistically significant differences. Nonetheless, considering the short adaptation periods in a changeover design, results could be affected. Therefore, it is important to consider new studies for verifying these results and extend the use of *T. diversifolia* in animal feeding.

In Cuba, García- López and Rodríguez (2014; unpublished) working with grazing cows of medium potential, found the following: grass (daytime) + tithonia (night) + Norgold supplement: 9.5 L/cow/day; grass mixtures (daytime and night) + Norgold supplement: 7.4 L/cow/day.

The simple analysis of variance indicates higher production in tithonia grazing at night, probably due to a better synchronization of the soluble nitrogen of tithonia with the grass digestion process.

This performance was for 11 hours of tithonia grazing during the dry season, from 17:00 to 04:00 (5 cows) daily

In the same way, results can be supported by those obtained in other ruminant species. Premaratne *et al.* (1998) on comparing weight gain in sheep supplemented with *T. diversifolia*, *L. leucocephala* and *G. sepium* forage found the best live weight gains with *T. diversifolia*.

Razz and Clavero (1997) have attributed the positive results in milk production when woody legume or shrub foliage are used to the high protein and nutritive values. In that respect, Mahecha and Rosales (2005) highlighted the potential of *T. diversifolia* as forage plant since, without being a legume, it has a forage of high nutritive value with high protein and mineral contents, high dry matter digestibility, presence of oils in both leaves and flowers, and total sugars of 39.8%. Likewise, Premaratne *et al.* (1998) attributed the best results in live weight gain in sheep supplemented with *T. diversifolia* compared with other shrub woody fodder to a greater growth rate and efficiency in microbial biomass production, and an extra contribution of protein for the ruminant due to the fact that *T. diversifolia* supplies both rumen degradable protein and non-degradable protein that can be available for digestion in the small intestine.

Although *T. diversifolia* has been traditionally used as feed for cattle as a cut-and-carry forage in some Central America and Caribbean countries, this plant is being assessed as woody species with high potential for integration into grazing systems, associated with grasses and legumes in silvipastoral arrangements.

Also, studies in Colombia (Sánchez, Bueno and Pérez, 2002) demonstrated that *T. diversifolia* is one of the shrub plants that cattle commonly select under grazing during the summer season when forage availability decreases to really critical levels. In Venezuela, investigations by García *et al.* (2009), studying the forage potential of twelve species for silvipastoral systems, indicated that *T. diversifolia* was moderately accepted for its consumption while *T. gigantean* was poorly accepted.

In Cuba, Ruiz *et al.* (2013) reported on different genotypes of tithonia being grazed by dairy cows. These authors evaluated 29 lines of tithonia under grazing using material from the germplasm bank of the Institute of Animal Science. They found that 7 lines were the most acceptable for the animals, with 100% browsing. Another four lines had 80% foliage removal. The remaining lines were 10–40% browsed. These findings should be considered for future investigation.

Ruiz *et al.* (2013) also indicated that animal performance when grazing with the lines that were between 50% and 100% browsed showed that the numbers of animals grazing tithonia were different. For two lines, the percentage of animal consuming tithonia did not exceed 20%, while the rest had higher values, close to 30%. Results found in leucaena by Castillo and Ruiz (2005) report grazing values ranging between 17 and 20%, indicating the utilization for grazing possibilities of the tithonia material studied.

7. MIXED SILAGE PREPARATION FOR RUMINANTS

Gutiérrez (2012) studied mixed silages (*T. diversifolia* and *Pennisetum purpureum* inoculated with the biological product VITAFERT). In this context, the preparation of mixed silages based on woody plants was demonstrated as a conservation technique. This will allow simultaneous use of grass yields and the fermentative potential, aspects determining silage quality. Another element is the reduction in moisture content, which increases the DM and nutrient concentrations in the silage. The microbial inoculants stimulate fermentation efficiency and, at the same time, ensure the predominance of lactic bacteria and the preservation of the material.

In recent years there have been few studies reported in the Latin America region where forage woody silages have been produced mixed with tropical grasses, although the effects on fermentation, chemical composition and rumen degradability are known. Consequently, it is essential to assess the most appropriate inclusion levels for woody plants in the mixture. This will allow use of these mixed silages as animal feed in the most critical season of feed availability.

In experiments in Cuba with the utilization of microbiological products (yeasts, lactobacilli) as inocula, responses have been consistent as improvers of fermentation efficiency and DM recovery. Inclusion levels of 4.5 and 6.0% of the inocula in the ensiled mixture prepared with *T. diversifolia* and *P. purpureum* cv. Cuba CT-169, showed improvements in the chemical composition in terms of increased CP, ash concentration (% C) and reduction of the neutral-detergent fibre (% NDF). Protein values corresponded with the woody proportion in the silage mixture. Also, with the protein, the fermentable carbohydrate availability and the conservation increase if we consider that the grass used (*P. purpureum*) presented low protein levels (5.7%). The protein values exceeded the 11%

CP found in maize silages mixed with *L. leucocephala*, *Acacia boliviana,* and with different proportions of *Morus alba* ensiled with *P. purpureum* cv. Taiwan mixed with *Guazuma ulmifolia*, *Lisyloma latisiliquum*, *Piscidia piscipula* and *Albizia lebbeck,* as well as higher than the 11.51% CP obtained in mixtures of 75% mulberry + 25% sorghum.

Another element that must be considered in silages with tithonia is that the DM content showed a quadratic increase ($Y = 48.62 - 0.63X + 0.006X^2$, $R^2 = 0.63$, SE ± 2.04, $P<0.0001$) with the woody inclusion, which was 55 and 31.47% of the optimum inclusion values in the mixture and the dry matter contribution, respectively. Even though the inclusion of microbial inocula tended to improve DM degradation, it was the combination 20% tithonia+80% *Pennisetum,* with the addition of 4.5% and 6.0% inocula, where the highest response was attained, with values between 37 and 45%. These increases could be associated with increased cell wall solubility as result of the increased enzymatic activity by the inoculated micro-organisms, and the functional effect on ensiled material, ash and nitrogen availability, as well as the role that the short chain fatty acid present in the woody plant played, and the biological products as growth stimulators of the micro-organisms and their activity.

The effect of the micro-organism cultures are very variable although, in general, the nitrogen content expressed as crude protein increased with the inclusion of tithonia in the mixture with *Pennisetum*, as evident from the values attained in the mixture 80% tithonia+20% *Pennisetum*, which is logical due to the high nitrogen content and the expected fermentation at ruminal level, in view of the nature of the tithonia plant.

In studies conducted by Gutièrrez *et al*. (2010) with a mixture 20:80 tithonia:pennisetum plus 4.5% microbial inocula, they found a median dwell time of material in the rumen of 14.75 h with a total time of 30 h, a degradation rate of 13%, with 48% of the mixture degraded, a situation that would favour animal consumption and nutrition. In this mixture, the degradation increase constant (*c*) exerted a marked effect on the disappearance of the ruminal DM and was less affected by the variations in the passage rates than the other mixtures used. This performance could be attributable to the lower content of insoluble fractions, evident from the lower retention time in the rumen. This seems to indicate that in this combination greater changes were produced in the rumen microbial activity.

On analysing the kinetics during the initial microbial growth, there was a clear relationship with the stimulating activity of the inocula, associated with the existence in the ruminal fluid of more active live cells both initially and throughout the degradation process, together with the contribution made by the biological product as peptides and amino acids present in the true protein and carbonated chains utilized as energy sources.

Similarly, results obtained showed the possibility of applying pre-drying techniques with the *T. diversifolia* + *P. purpureum* cv. Cuba CT-169 forages for ensiling. The inclusion in the mixture of 4.5% or 6.0% VITAFERT increases CP content, ash concentration and reduces NDF. At the same time, higher degradation and ruminal degradability rates are attained.

8. FINAL REMARKS

Considering that *T. diversifolia* is a plant species with a wide range of adaptation and good capacity for biomass production, rapid growth, and low input and management demands for its culture, it can contribute to a nutritional strategy for ruminant supplementation in the tropics, especially in seasonal periods of forage shortage. This species has nutritional values higher than those of grass pastures and can produce large amounts of edible biomass that are more balanced in time with those of pastures without the need for fertilizers. In studies carried out with sheep, goats and cattle, this plant has demonstrated its benefits regarding consumption and palatability, which has had a positive influence on production parameters for the various ruminant species. Its use as partial substitute for concentrate feed in Holstein × Zebu cows did not affect production, or milk composition when substituting up to 35% in goats and sheep, and it was shown to improve performance and ruminal indicators. In pre-ruminant calves, *T. diversifolia* in integral diets showed satisfactory acceptance in the diet regardless of the inclusion level. The IVGP showed satisfactory performance patterns for this category which was evident from ruminal development with age. Also, the combinations of hay and tithonia meal gave gains >700 g/animal/day with average LW >109 kg at 120 days. The use of meal in the integral diet maintained normal health indicators in calves during the study. Results indicate the nutritional potentials of *T. diversifolia* for feeding of the different animal types and ruminant categories, as well as the need of future studies on this and other alternatives for animal feeding.

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Tithonia diversifolia (Hemsl.) A. Gray and its effect on the rumen population and microbial ecology

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ABSTRACT

Previous chapters studied the use of *Morus alba* (mulberry), *Moringa oleifera* (moringa) *and Tithonia diversifolia* (tithonia) in animal feeding. Here these plants are also considered as important sources of chemical compounds with pharmacological and therapeutical uses. Among the more remarkable are diabetes and cancer prevention, antioxidant, neuro-protective and antiinflamatory effects of mulberry and moringa; antihelmintic activity of mulberry and tithonia; and immunogenic effects of tithonia. Some industrial applications of these plants are discussed, such as mulberry use in sericulture or treatment of drinking.and wastewater; and biodiesel production from moringa. At the same time note was made of tithonia use as insecticide to control ants, and in order to improve degraded soils especially for phosphorus absorption.

Keywords: therapeutic properties, industrial use, *Morus alba, Tithonia diversifolia, Moringa oleifera*

INTRODUCTION

At present, the use of *Tithonia diversifolia* (tithonia) for silvipastoral purposes or as cut forage has been increasing, and according to Mahecha *et al.* (2007) can be included in ruminant diets without affecting milk production. Tithonia is widely considered as a feeding alternative in diets for high tropics dairy units. However, the few investigations carried out with this plant recommend evaluating its inclusion in animal diets with caution, so as to gain better knowledge of its possible impacts from productive, environmental and economic points of view.

This forage species has the capacity to supply important amounts of protein (Verdecia *et al.,* 2011), and its mineral content is high. Animals consume the whole plant, but prefer the leaves and flowers (Osuga *et al*., 2012).

Regarding secondary metabolites, Galindo *et al.* (2011) reported that the presence of total phenols, tannins, saponins, among other compounds, is variable. Likewise, Hess *et al.* (2006) indicate that tannins are associated with reduction in methane production, agreeing with Galindo *et al*. (2011), who found that the inclusion of 10% or 20% of *T. diversifolia* provoked reductions in the ruminal methanogen population. Also, Hess *et al.* (2006) reported that condensed tannins form protein links that will facilitate their flow to the duodenum, and there be available for the animal due to the pH change and enzymatic action.

All these arguments led to designing a strategy for controlling methane production in the rumen by exploiting plant diversity.

1 EFFECT OF TITHONIA ON RUMINAL METHANOGENESIS

Recent studies by Galindo *et al.* (2014) have shown that the utilization of the foliage of different lines of *T. diversifolia* reduces the population of methanogenic bacteria relative to a diet based on star grass (*Cynodon nlemfuensis*) (Figure 1).

The diet – mainly the fibre content – is recognized as influencing the population density of these microbial groups in the rumen. In that way, in the feeding systems based on grasses there are 10^9 to 10^{10} colony forming units (cfu) per gram of ruminal liquid for these microbial groups (Joblin, 2004), that can be modified through different means, including the use of compounds such as tannins, saponins and alkaloids, together with commercial products.

In another group of studies, Galindo *et al.* (2012) established that supplementation with 20% of *T. diversifolia* on a dry basis in the diet for ruminants produced a marked reduction in the protozoa population in the rumen, although this effect was not evident when 10% *T. diversifolia* was used. However, in the same study, there were no effects due to the inclusion of the plant on the population of ruminal cellulolytic fungi (Table 1).

At the same time, the addition of 10% or 20% of *T. diversifolia* in star grass rations produced reductions in the ruminal methanogen population, which is of great importance since these microorganisms are responsible for methane production in the rumen (Figure 2).

T. diversifolia manifested its fermentative potential previously reported by Rosales (1996) when this plant was incubated through the *in vitro* gas production technique, producing 195.4 mL after an incubation period of 166 hours, as well as a fermentation rate of 3.66 mL/h for highly fermentable compounds and 0.76 mL/h for slowly fermentable compounds. These processes depend mainly on different factors, such as the proportion and consumption level, the nutrient content, its digestibility, as well as the utilization efficiency by the animal (La O *et al.,* 2009).

FIGURE 1
Effect of different plant materials [Accessions from the ICA Germplasm collection] of *T. diversifolia* on the population of ruminal methanogens (×10^9 cfu/mL; SE± 0.22)**

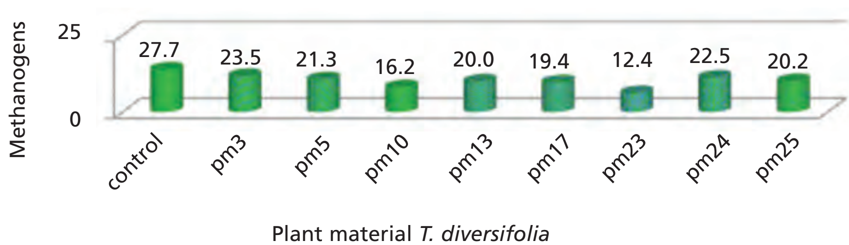


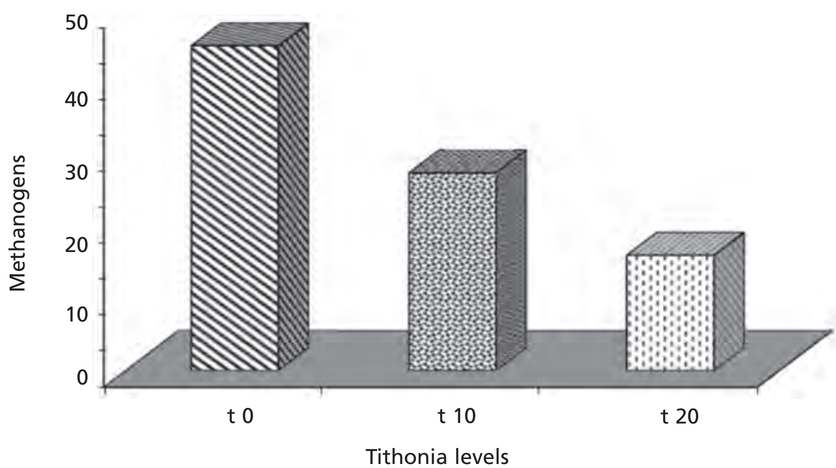
TABLE 1. Effect of *T. diversifolia* on some sectors of the ruminal microbial population under *in vitro* conditions

Parameter	% *T. diversifolia*			SE± Sig
	0	10	20	
Protozoa, 10^5 cell/mL	1.32[a] (3.75)	1.17[a] (3.25)	0.69[b] (1.5)	0.02*
Cellulolytic fungi 10^5 tfu/mL	3.26 (26.1)	3.36 (28.9)	3.23 (25.2)	0.15
Total viable bacteria 10^{11} cfu/mL	4.18[a] (65.35)	4.17[a] (65.00)	3.90[b] (49.60)	0.20*
Cellulolytic bacteria10^6 cfu/mL	3.21a (24.9)	4.00b (55.8)	3.37a (29.2)	0.12*

NOTES: cfu = colony forming units; tfu = thallus forming units. Data transformed according Ln X; original means in parentheses. a,b – means with different letters within the same row differ at $P<0.05$) (Duncan, 1955) *$P<0.05$

FIGURE 2

Effect of *T. diversifolia* on methagen population (×10^9 cfu/mL; SE± 0.31*)



The stimulating effect of the cellulolytic bacteria populations found when using 10% foliage of *T. diversifolia* could be related to the high protein content of this plant (23.91%). In that regard, studies by Galindo *et al.* (2009) showed that when feeding shrub foliage, leguminous or not, but capable of contributing protein sources, ruminal microorganisms, specifically the cellulolytics, have at their disposal compounds such as ammonia, amino acids, peptides and branched short chain fatty acids.

Galindo (2004) and Galindo *et al.* (2005, 2006) also demonstrated that condensed tannins and total polyphenols, among other metabolites, are capable of reducing the protozoa as well as the saponins. Abreu *et al.* (2003), Galindo *et al.* (2009) and Rodríguez, Fondevila and Castillo (2009) determined that the presence of these metabolites can exert similar effects, contributing to "wash" these microbial populations out of the rumen.

On evaluating 13 plant species utilizing the *in vitro* gas production technique, Galindo *et al*. (2013) found low methane production in the rumen with *T. diversifolia* when used for

TABLE 2. Effect of the foliage of different protein plants on methane production in the rumen (μL/g DM)

Plant	CH_4
Samanea saman	4.3[a]
Albizia lebbeck	5.73[a]
Azadirachta indica	8.59[a]
Tithonia diversifolia mv 23	9.2[a]
Cordia alba	11.76[a]
Leucaena leucocephala	16.38[a]
Pithecelobium dulce	20.03[a]
Moringa oleifera	25.33[a]
Gliricidia sepium	29.02[ab]
Guazuma ulmifolia	37.98[ab]
Tithonia diversifolia line 10	47.15[ab]
Enterolobium cyclocarpum	64.71[b]
Cynodon nlemfuensis	65.15[b]
SE± sig.	1.20***

NOTES: [a,b] Means with different letters differ. ***$P<0.001$.

supplementing star grass (*Cynodon nlemfuensis*) (Table 2).

2. EFFECT OF TITHONIA AND OTHER TREES ON *IN VITRO* GAS PRODUCTION

In other research, Galindo *et al.* (2012) assessed the effect of *T. diversifolia* plant material accession no. 23 on the methanogen population and ruminal ecology, compared with *S. saman* and *A. lebbeck*.

2.1 Rumen micro-organism population

Table 3 shows the effect of different plants on the population of ruminal total viable bacteria. There was no effect from the use of *S. saman*, *A. lebbeck* and *T. diversifolia* line 23 compared with the star grass (*C. nlemfuensis*) control.

The fact that the population of total viable bacteria is maintained within the range of 10^{11} cfu/mL has ecological importance within the rumen, agreeing with reports from Hungate (1966), Hoover and Miller (1991) and González (2010) regarding the total number of bacteria in the rumen of animals fed fibrous diets. For those reasons, the possibility can be proposed of using foliage of the abovementioned plants without producing depressive effects on the total population of ruminal bacteria. This helps guarantee an adequate balance between the different ruminal populations.

Table 3 also shows that the populations of cellulolytic bacteria vary considerably depending on the plant assessed. As shown, the highest population of cellulolytic bacteria was found when supplementation was with *A. lebbeck,* followed by tithonia. It should be noted that the use of *S. samen* reduced ($P<0.001$) the representation of this microbial group, leading to assessing other levels of inclusion of that plant in the diet of ruminant animals, mainly, because in feeding systems based on fibrous feeds, the main response that must be expected is increased population of micro-organismal fibre digesters, which are responsible for cellulose degradation in the rumen.

TABLE 3. Effect of the inclusion of foliage of three trees on the population of some sectors of the ruminal microbial population

Microbial group	C. n	S. s	A. l	T. d	SE± Sig
Total viable bacteria $\times 10^{11}$ cfu/mL	3.89 (36.00)	3.69 (40.00)	3.74 (42.00)	3.56 (35.00)	0.23
Cellulolytic bacteria $\times 10^{5}$ cfu/mL	2.72[b] (15.20)	2.28[c] (9.80)	3.14[a] (23.10)	2.85[ab] (17.40)	0.31 $P<0.001$
Methanogens $\times 10^{9}$ cfu/mL	4.26[a] (70.00)	3.66[b] (34.00)	3.04[c] (19.00)	2.99[c] (18.50)	0.35 $P<0.001$
Protozoa $\times 10^{5}$ cell/mL	2.18[a] (8.90)	1.92[b] (7.20)	1.79[c] (6.00)	1.87[c] (6.50)	0.13 $P<0.05$

Notes: a,b,c = Means with different letters within the same line differ at $P<0.05$ (Duncan, 1955). Data transformed according to Ln., original means in parentheses. S. s = *Samanea saman*; A. l = *Albizia lebbeck*; T. d = *Tithonia diversifolia*; C. n = *Cynodon nlemfuensis.*

Of great importance is the effect of plant foliage on the population of members of the Archaea group, or rumen methanogens. All the plants reduce the population of methanogenic bacteria relative to star grass. However, *A. lebbeck* and *T. diversifolia* line 23 are plants that reduce these specific microbial groups to a greater extent. The populations of these micro-organisms were 70; 34; 19; and 18 cfu/mL for star grass, *S. samen*, *A. lebbeck* and tithonia, respectively.

One of the most significant results of the present study was the effect of the tree foliage on the protozoa population in the rumen. In that regard, Table 3 shows that *S. samen*, *A. lebbeck* and tithonia reduce the protozoa population, and *A. lebbeck* and tithonia stand out as the most promising for the purpose.

Foliage inclusion from trees or shrubs in ruminant feeding has been demonstrated to exert a de-faunating effect by reducing ruminal protozoa populations. In that respect, Galindo, Marrero and Aldama (2000) indicated that ruminal protozoa and fungi are closely related; therefore, it is logical to think that the effect of the secondary metabolites on fungi population and cellulolytic bacteria is indirect due to the fact that protozoa absorbed enormous amounts of these microbial groups during the day. De-faunation as a strategy for manipulating rumen microbial fermentation has been used with poor quality fibrous diets.

Investigations by Hegarty (1999) demonstrated that methanogens live on the inside or adhere to the surface of the rumen ciliate protozoa, and are responsible for more than 37% of the methane emissions. When protozoan populations are minimized, rumen methane emissions are reduced by approximately 13%, this effect varying with the diet. That is why the use of the plants evaluated as having a depressive effect on the protozoan population is a promising way to decrease the methanogens (McAllister *et al.,* 1996; Tokura *et al*., 1997; Kobayashi, 2010).

The same study (Hegarty, 1999) demonstrated that *T. diversifolia* line 23 reduces the population of ruminal cellulolytic fungi in terms of time from the start of fermentation (Figure 3).

FIGURE 3
Effect of foliage inclusion of three woody plants on the population of ruminal cellulolytic fungi (×10^4 cfu/mL; SE ±0.45; *P*<0.05)

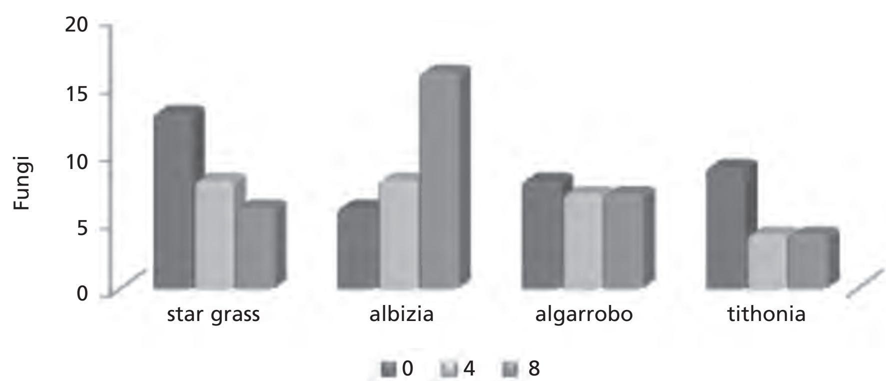


TABLE 4. Effect of the inclusion of foliage of three woody plants on pH and ammonia concentration in the rumen

Treatment	pH	NH_3, mmol/L
Cynodon nlemfuensis	6.4	15.76
Samanea saman	6.3	14.68
Albizia lebbeck	6.3	15.99
T. diversifolia line 23	6.3	15.71
SE ±Sig	0.01	1.23

These results lead us to study intrinsic factors in the plants being studied that could exert depressive effects on the population of cellulolytic fungi.

Total rumen viable and methanogenic bacteria showed modifications over time. The number of bacteria increased from 31.08 $\times 10^{11}$ cfu/mL before the start of fermentation, to 47.96 $\times 10^{11}$ cfu/mL 8 hours after fermentation started. At the same time, the methanogens over the same period were capable of doubling their initial population.

It is important to note that even though techniques capable of reducing an unwanted microbial population are used, increases of the same in time can be expected, unless the toxic effect on the populations is such that these effects are not produced.

One relevant aspect found was that *S. Saman, A. lebbeck* and *T. diversifolia* plants did not modify the pH or ammonia concentration in the rumen relative to the star grass treatment (Table 4). This response does not seem to have a close relationship with the protein contents of these plants, which had values of 18.15%, 17.18%, and 23.95%, respectively, while the protein content of star grass was 11.90%. As is well known, ruminal fermentation of proteins is very variable and as result certain amounts of ammonia (NH_3) are produced. Among the factors modifying ruminal fermentation of the proteins are its solubility and degradability.

From previous studies at the Animal Science Institute it is known that foliage from trees and shrubs (leguminous or not) show tannin concentrations which on linking to the proteins in the rumen can form complexes and reduce degradation (La O, 2001). In this way, the feeding protein will be much closer to bypass protein. Evidently, if protein degradability in the rumen is reduced, ammonia concentration is lower. Nonetheless, further studies on this effect are in progress.

3. FINAL REMARKS

T. diversolia is a shrub that stands out because of its high CP content, besides presenting secondary metabolites in sufficient amounts that make it feasible to use it for modifying rumen microbial ecology. Its use as supplement for these purposes has been demonstrated, and suggests levels of approximately 20% of the daily DM ration.

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Other uses of mulberry (*Morus alba*), moringa (*Moringa oleifera*) and tithonia (*Tithonia diversifolia)*

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ABSTRACT

Morus alba, Moringa oleifera and *Tithonia diversifolia* forages have good nutritional value and can be incorporated in the diets of both ruminant and monogastric animals, which has been extensively discussed in the previous chapters. This chapter discusses non-feed uses, for example medicinal and industrial uses including that for sericulture of these plants. Production of biodiesel from moringa oil and its other industrial uses are also presented.

INTRODUCTION

Trees and shrubs, such as mulberry, moringa and tithonia in various tropical regions, have had uses besides as animal feed, as previous chapters have stated. Thus, in the Central America region, almost all the parts of these plants have been used by humans. Leaves, flowers, fruits and roots are important due to their nutritional value and can also be used in human feeding.

These plants are important sources of chemical compounds with high pharmacological and therapeutic activity for humans and animals, besides their industrial applications. Mulberry has an essential role in sericulture. Moringa leaves are rich in vitamins and various amino acids, which are recommended for treating problems of malnutrition in children (Fuglie, 2001). Extracts from the leaves of moringa, mulberry and tithonia have a high anthelmintic and antioxidant activity (Díaz and García, 2011; Lezcano, 2013; Martín *et al.,* 2013). The uses of their seeds in food, medicine, water treatment and as fertilizers are relevant. The bark of moringa is useful for the adsorption of heavy metals, as well as for making ropes and carpets. The oils obtained from these plants are used in the perfumery and cosmetics industry as lubricant, in human feeding, and some oils, e.g. moringa, are used in the production of biodiesel.

Regarding the previous information, the objective of this chapter is to review these uses of mulberry, moringa and tithonia, apart from their use as animal feed.

1. OTHER USES OF *MORUS ALBA* (MULBERRY)

1.1 Use of mulberry in sericulture

Sericulture is the combination of rearing an insect, *Bombyx mori* (used for more than 4500 years and also known as silkworm, but it is not a worm, it is a caterpillar), with the sowing of a perennial plant, called mulberry (*Morus alba*, among other species). This activity needs neither big investments nor physical efforts, but it requires precise control of factors like temperature, humidity and cleaning of the room for rearing the worms and the food to offer, which should mainly be fresh mulberry leaves of the best quality (Díaz and Lezcano, 2011).

According to Díaz and Lezcano (2011), mulberry provides starch to the worm during its life, which they transform into a thread that can reach 500 m long in each cocoon. The silk worm produces the cocoon three or four weeks after hatching. A week later, this cocoon is ready for its harvesting and selling. In tropical areas, unlike other regions of the world, it is possible to achieve five or more silkworm generations annually.

In Cuba, sericulture was successfully introduced in 2001, at the Estación Experimental de Pastos y Forrajes "Indio Hatuey", as an agricultural alternative to provide a complementary use to the cultivation of mulberry. It is important to note that sericultural development cannot function sustainably in any country if research activities are not in place, because quality depends largely on the adaptation of technology to local socio-economic and environmental conditions.

Current consumers of silk look for quality and aesthetics of the final product. Likewise, it is possible to integrate the use of a certain amount of alternatives within sericulture, searching for a different use of silk production like pupae and mulberry for human and animal feeding, cosmetic products from oils and creams extracted from pupae and larvae, medicine and pharmacology products using mulberry and larvae, and also the use of the silkworm as a biotechnological tool.

1.2 Medicinal uses of mulberry

Most of the reports on the use of mulberry for treating many conditions come from the Asian area (Yun and Lee, 1995a, b; Kim *et al*., 1998). Only a few reports come from Latin America.

The most outstanding characteristics of *M. alba* in the medical field are for treating arterial hypertension, diabetes (Lemus *et al*., 1999) and high levels of cholesterol (Ho-Zoo and Won-Chu, 2001), although it has also demonstrated efficacy against cancer, leukemia, as a neuro-protector and immunological effect, and on skin. The following sections consider these.

1.2.1 Diabetes prevention

Its characteristics as an anti-diabetic plant are the most studied ones, and this research is directed to clarify the biochemical mechanisms involved and the compounds causing this action (Kim *et al*., 1999).

Recently, Oku *et al*. (2006) demonstrated the inhibiting effect of mulberry leaf extract on rat and human intestinal disaccharidase. These authors concluded that human digestion is inhibited when a certain level of mulberry leaves is orally ingested with sucrose and polysaccharides.

Among other experiments, after supplying mulberry leaves with a sucrose solution to rats that have not eaten, glucose levels in blood decreased significantly, depending on the mulberry leaf concentration involved. These results suggest that mulberry leaves could be used as ingredients for healthy meals and preventing diabetes.

At the same time, there are also reports of a hypoglycaemic effect of extract of *M. alba* root bark. Studies conducted on rats have demonstrated that this extract can be used for fighting the diabetes caused by lipid peroxidation (Singab *et al*., 2005). In addition, Taniguchi *et al*. (1998) and Sun-Yeou, Jian-Jun and Hee-Kyoung (2000) reported that phagomine substance, which is a pseudo-sugar obtained from mulberry leaves, favours the secretion of insulin induced by glucose, through the acceleration of some steps after the formation of glyceraldehyde 3-phosphate on the glycolytic route.

Morus alba leaf extract, dissolved in dichloromethane-methanol, showed hypoglycaemic activity due to a triterpene and two galactolipids. One of the galactolipids produced 16% of activity (Chul-Young *et al.,* 2000).

Finally, isolated hemicelluloses and peptins of *M. alba* presented stronger hypoglycaemic activity than those isolated from *Morus nigra* and *Morus rubra* (Sanavova and Rakhimov, 1997), due to the differences demonstrated among the activities of *Morus* spp.

1.2.2 Antioxidant effect

Nowadays, there is a marked interest, at global level, for the identification of antioxidant compounds in plants that represent potential drugs for use in preventive medicine and for human and animal feeding.

Oxidative stress is caused by an imbalance between the production of reactive oxygen species (ROS) and the capacity of the organism to maintain the ROS at non-toxic levels.

The antioxidant system of plants includes a wide group of compounds of a protein nature, including antioxidant enzymes, as well as non-protein compounds like vitamin E, ascorbic acid, alpha-tocopherol, glutathione, and carotenoid pigments (González *et al*. 2000). Catalase and peroxidases are among the most important enzymes due to their ability to transform the pro-oxidant compound hydrogen peroxide into water and molecular oxygen.

In Cuba, Díaz *et al.* (2010) evaluated the specific activity of catalase and guaiacol peroxidase antioxidant enzymes in *M. alba* genotypes Indonesia, Acorazonada, Criolla, Tigreada and Cuban, using fresh extracts obtained from the root, stems and leaves. The highest values of catalase and peroxidase-specific activity were generally found in leaves, followed by stems and roots. Lines Tigreada and Criolla had the highest specific activity for both enzymes. Results demonstrated the antioxidant protective role of extracts, mainly from leaves, according to important functions of these enzymatic systems in the removal of oxygen-reactive species.

Every part of mulberry, but mainly the leaves, contains a varied amount of natural antioxidants that act with synergism, which means that the overall antioxidant effect obtained is superior to the sum of separate effects.

Doxorubicin is an important and effective anti-cancer chemotherapy drug, but it may provoke cardio-toxicity, depending on the dose used, because it increases the superoxide anion in tissues, causing cellular oxidative stress. Thai scientists performed a phyto-chemical sieving of medicinal plants to analyse its cardio-protective effect against the toxicity of doxorubicin. *M. alba* showed higher antioxidant levels than the rest of plants studied. Therefore, these researchers recommended the inclusion of antioxidants from natural sources in the diet due to its cardio-protective property in patients that needed doxorubicin (Wattanapitayakul *et al.,* 2005)

Mulberry has also been used in research related to atherosclerosis. Studies were conducted in mice, which had a deficiency of the receptor for low density lipoprotein (LDL), and susceptibility to oxidative modification. Enkmaa *et al*. (2005) concluded that the intake of *M. alba* leaves in the diet diminished the intensity of the atherosclerotic lesion of the group, mainly because of the amount of flavonol glycosides in these leaves. The development of atherosclerosis could be diminished thanks to the antioxidants that prevent LDL.

In addition, epidemiological studies indicate that those diets with antioxidant substances extracted from plants are inversely associated with mortality due to coronary heart disease (Knekt *et al.,* 1996). It has also been confirmed that antimicrobial activity with extracts of leaves from *Morus alba* varieties and hybrids occurs in the face of bacterial pathogens (Díaz *et al*., 2011).

1.2.3 Fatty liver

In an experiment using rats separated at random into different groups, Kwon, Kim and Choung (2005) examined the effect of a Chinese traditional preparation for treating fatty liver disease. The preparation contained *Astragalus membranaceus, M. alba, Crataegus pinnatifida, Alisma orientale, Salvia miltiorrhiza* and *Pueraria lobata.* These authors concluded that those animals treated with the preparation showed a considerable decrease in fat accumulation in the liver caused by alcoholism, and recommended the use of this preparation in humans.

In addition, El-Beshbishy *et al*. (2006) demonstrated the hypolipidaemic and antioxidant effect of the extract of *M. alba* root bark, after supplying it to rats with increased levels of cholesterol and obtaining a reduction in levels.

1.2.4 Therapy against cancer

Anthocyanins are present as natural colouring in many fruits and vegetables, and they have been widely used due to their antioxidant properties. In addition, recent studies have shown an anti-proliferative ability against cancer. Based on that fact, Chen *et al*. (2006) obtained cyanidin 3-glucoside and cyanidin 3-rutinoside (two anthocyanins) from an extract of *M. alba* and observed their *in vitro* inhibiting effect, depending on the doses, on the migration and invasion of carcinogenic cells from a human lung suffering from an advanced metastasis. This result has a great potentiality for the development of an effective therapy against cancer.

1.2.5 Neuroprotective effect

A large number of neurological illnesses, like Alzheimer and Parkinson, has been attributed to the decrease of gamma butyric amino acid (GABA) in the brain. Recently, research demonstrated that leaves show considerable concentrations of this acid.

Therefore, a process was developed to increase the accumulation of GABA in leaves of mulberry as a result of several anaerobic treatments, and to provide a pharmacological basis for the neuro-protective action of GABA accumulation in mulberry leaves (ML) against *in vitro* and *in vivo* cerebral ischaemia. After analysing the results, it was suggested that the anaerobic treatment of mulberry leaves leads the GABA of ML to increase neuro-protection against cerebral ischaemia and it is effective in both *in vivo* and *in vitro* processes (Kang *et al.,* 2006).

Mulberry fruit also develops an important neuro-protective function, because the cyanidin 3-glucopironidase (C3G) was obtained from the fruit extract and its protective effect has been demonstrated on damaged neuronal cells. Research has demonstrated that the extract decreases cerebral ischaemic damage caused by decreased oxygen-glucose. Also verified, through an *in vivo* test, was the neuro-protective effect of the fruit, using a model with a mouse with a cerebral lesion through arterial occlusion.

1.2.6 Other important medicinal effects

There is evidence that mulberry has a beneficial effect on the immunological system because it is one of the five species of Chinese medicinal plants that are used for treating patients infected with HIV/AIDS. After several weeks of study, Thai scientists determined that the response, in most of the patients, had been positive due to a marked decrease in the number of cells infected with the virus. Nevertheless, other complementary studies are needed before using this treatment in patients that carry HIV.

At the same time, Sun–Yeou *et al.* (2000) demonstrated that flavonoids (quercetin-3-D-glucopyranosides and quercetin-3, 7-di-O-D-glucopyranosides) within mulberry showed a very marked inhibiting effect on the growth of a cell line of human leukaemia and an important collection of free radicals.

Likewise, Díaz and García (2011) reported that mulberry leaves are useful for treating skin discoloration after inhibiting tyrosinase activity, and, therefore, melanin biosynthesis. In addition, these authors also found a beneficial effect of mulberry leaf extract on the central nervous system as an antidepressant.

Finally, Díaz *et al.* (2011), in an essay of toxicity, observed that fresh extract of mulberry may inhibit gastric lesions caused by ethanol. Another result revealed the presence of anti-inflammatory activity of leaf and root extracts, as well as cicatrizing activity.

1.3 Use of mulberry as anthelmintic plant

In tropical areas, gastrointestinal parasitism is one of the main problems affecting animal production, so the study of this characteristic of mulberry has a vital importance in the Latin American region.

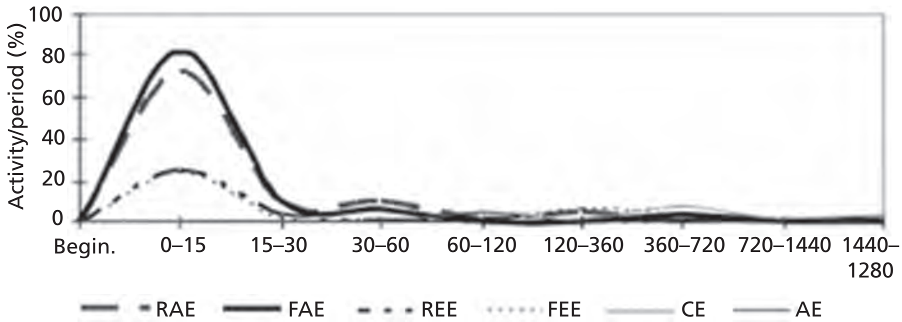
At the same time, one of the advantages of these forage shrubs and trees is that, as shown in earlier chapters, many species contain secondary metabolites with an anthelmintic action against some genera of gastrointestinal parasites, so these characteristics can be exploited in practice to counteract this problem.

In this sense, research on secondary metabolism within *Morus alba* states that this plant shows varied medicinal properties, specially anthelmintic, which are used on the Asian continent to control different diseases (Duke, 2001). Out of all the genotypes studied in Cuba, the best results have been obtained with cv. Cuban, which contains the highest concentration of total polyphenols and shows high biomass production during the dry season (García, 2003).

This way, Soca, Simón and Roque (2011) evaluated the anthelmintic potentialities of secondary metabolites of this species, considering the function of this plant in animal feeding.

During the first stage of research, studies were conducted with aqueous solutions obtained from edible biomass of *Morus alba* cv. Cuban, at different dilutions (20, 40,

FIGURE 1
Anti-larval activity of *M. alba* extracts during the evaluation periods



Source: Taken from Soca, Simón and Roque, 2011.

60 and 80% of matrix solution). During the second stage, different extractions were performed to the initial solution (matrix), in order to select, among all metabolites within this species, those with the highest anthelmintic activity. The primary solution was divided into raw ethanolic extract (REE), fractioned ethanolic extract (FEE), raw aqueous extract (RAE), fractioned aqueous extract (FAE), alkaloid extract (AE), and chloroformic extract (CE). In a general sense, mortality percentages increased dramatically up to 30 minutes, followed by less abrupt increases. *M. alba* cv. Cuban shows anthelmintic characteristics in L3 larvae of gastrointestinal nematodes under lab conditions.

As a result, these authors concluded that, out of the six extracts obtained from leaves, petioles and young stems of mulberry, RAE and FAE have the highest anti-larvae action, with a mortality superior to 80% at 60 minutes. The highest biological activity of active extracts is expressed during the first 15 minutes and it is caused by the presence of major polyphenols like quercetin, rutine, umbelliferone and resveratrol (Figure 1).

2. OTHER USES OF *MORINGA OLEIFERA* (MORINGA)

2.1 Moringa for human feeding

According to Martin *et al.* (2013), practically every part of the plant is edible. Fruits, leaves, flowers, roots and oil are highly appreciated due to their nutritional value and are used for producing different dishes in India, Indonesia, Philippines, Malaysia, the Caribbean and several African countries (Foidl *et al.,* 2001). Cooked tender leaves are used for preparing salads, soups and sausages. They can also be eaten raw, like other vegetables. Cooked flowers have a similar flavour to some mushrooms. Tender pods are very appreciated in India. They are prepared like green beans and their flavour is similar to that of asparagus. After maturation, pods become woodier and lose their qualities as food. Nevertheless, seeds can be separated from the mature pod and used as food. Mature seeds can be prepared similar to peas, or they can be fried, toasted (like *Arachis*), in infusions and sausages (Agrodesierto, 2006). In Malaysia, green pods are used as ingredients in local curry recipes. The roots are used for sausages that, due to their favor, remind one of spicy radish. Therefore, in some places, moringa is known as radish tree.

The leaves of this species present a good content of vitamins, pro-vitamins and minerals (Liñan, 2010). In addition, it has been demonstrated that it contains all the essential amino acids, including some, like arginine and histidine, that are generally found in animal protein, and are really important for the development of children. For this reason, during the last decade, FAO promoted a programme for the use of moringa, directed to child populations with high levels of malnutrition and to pregnant and lactating mothers (Fuglie, 2001). However, it should be noted that some online sources report inflated numbers when comparing this plant with other fruits and vegetables in terms of nutrient content.

Moringa oil is full of oleic acid and tocopherols. Except for its low content of linoleic acid, this oil has a chemical composition and physical properties similar to olive oil. It is used as a salad dressing in Haiti and other Caribbean islands (Foidl *et al*., 2001), without any report of negative effects, allergies or toxicity.

Liñan (2010) reported that fruits or green pods may be cooked, tender pods are edible and they are used in soups or prepared as asparagus. Roots are spicy like rustic radish and are used as spices. Mature seeds are toasted and consumed like walnuts because of their sweet, slightly bitter and attractive flavour. Seeds are oleaginous and the leaves are eaten like vegetables or salads. Flowers cooked with eggs become an exquisite dish, and the use of oil with a similar quality to that of olive oil is used for dressing salads. In Guatemala, seeds eaten like roasted walnuts.

The coagulation activity of milk, through the watery extract of seeds, has shown that it can be used for preparing cheese. It can also be used for clarifying molasses and sugar cane juice. In Colombia, the use of moringa is developed in the Agro area, with extensive sowings in the Andean and Orinoco regions and with some in the Atlantic region.

In Cartagena Bolívar, UMATA (Unidades Municipales de Asistencia Técnica Agropecuaria) have adopted another approach to the use of moringa, and it involves educative workshops, which teach the most needed population and those from poor areas to seize the nutritional advantages of each part of this tree.

Since 2009, UMATA has been developing, within the *Patios Productivos* project, some campaigns on sowing these trees in different regions of the Department and poor urban areas, for small-scale farmers to benefit from the nutritional advantages and agricultural and economic opportunities from exploitation for the extraction of cooking oil and biodiesel. Up to this date, UMATA has delivered more than 200 000 samples. Moringa has been revealed as a first-order resource, with low production cost, to prevent malnutrition and many pathologies like child blindness related to lack of vitamins and essential elements otherwise missing from the diet. This plant has a promising future in the dietary industry and as a protein food for sportsmen.

Previous studies analysiing the nutritional and dietary values of leaves, pods and seeds indicate values of macro- and micro-nutrients that characterize it as a source of proteins, fat, calcium, potassium, iron, carotenes, vitamin C, and some others, and, therefore, as an energy source.

Moringa leaf has more than 25% protein, which is the same amount as a hen egg, double that of the milk, four times the amount of vitamin A in carrots, four times the amount of calcium in milk, seven times more vitamin C than in oranges, three times more potassium than in bananas, significant amounts of iron, phosphorous and other elements.

This is an exceptionally good source of vitamins A, B and C, as well as minerals (particularly iron) and amino acids like cysteine and methionine, which contain sulphur.

2.2 Medicinal uses of M. oleifera

In many tropical countries, it is very difficult to differentiate among the feeding and medicinal uses of *M. oleifera*, because of its nutritional qualities and its medical attributes, which have been long-recognized in traditional culture. It is said that leaves, fruits, roots and seeds are useful to fight anaemia, anxiety, asthma, paralysis attack, bronchitis, flu, cholera, chest congestion, conjunctivitis, sperm deficiency, milk deficit in lactating mothers, diabetes, diarrhoea, erectile dysfunction, joint pain, headaches, sore throat, scurvy, sprain, pimples, lack of sexual desire in women, fever, gonorrhea, glandular swelling, arterial hypertension, hysteria, blood impurities, skin infections, wounds, malaria, otitis, intestinal parasites, poisonous bites, bladder and prostate problems, psoriasis, respiratory problems, cough, tuberculosis, abdominal tumors, ulcer, and some others (Fuglie, 2001). Its therapeutic uses are applied in several countries from Latin America and the Caribbean, including Guatemala, Nicaragua, Puerto Rico, Cuba and Venezuela, among others.

Despite the traditional knowledge regarding the use of moringa for many remedies and medical treatments in different countries, not everything appears in the scientific literature. The study of the chemistry and pharmacology related to its medical attributes is recent and under development. Although many of its therapeutic benefits have been tested using strict *in vitro* and *in vivo* research in modern laboratories, others have yet to be supported by clinical tests. Even though the internet nominally provides information on its healing properties, this information is based too often on empirical findings without no reference to specialized literature. In addition, a large amount of this information comes from companies that produce or distribute nutritional supplements, and preparations using this plant, so it is only a publicity value.

2.2.1 Antimicrobial activity

The use of *M. oleifera* to control different infections provoked by micro-organisms is well known, and scientific results that confirm its microbial activity have been obtained in recent years. *In vitro* studies have confirmed the effect of different parts of the plant on pathogenic micro-organisms. Inhibition of growth of *Pseudomonas aeruginosa* and *Staphylococcus aureus* by watery extracts from leaves was demonstrated by Guatemalan scientists (Cáceres *et al*., 1991). At the same time, Chuang *et al*. (2007) demonstrated the anti-fungal activity of essential oils of leaves and alcoholic extracts of seeds and leaves to fight dermato-phytes like *Trichophyton rubrum* and *Trichophyton mentagrophytes*. In addition, 44 components of essential oils from leaves were identified, which could be used for future development of medicines to treat typical skin diseases of tropical areas.

Bacteriological studies demonstrated the antimicrobial activity of moringa seed extracts, in which Gram+ve and Gram-ve bacteria flocculate the same as water colloids. Their bacteriostatic action consists of the disruption of cellular membranes due to the inhibition of essential enzymes (Suárez *et al.,* 2003). The main ingredient responsible for this activity is 4-(4′-O-acetyl-α-L-rhamnopyranosyloxy) benzyl isothiocyanate, which has a bactericidal action against several pathogenic species, including isolates of *Staphylococcus*, *Streptococcus* and *Legionella* resistant to antibiotics. The power of isothiocyanates as antibiotics was also demonstrated in a study with *Helicobacter pylori*, which is the cause of duodenal and gastric ulcers (Fahey, 2005).

2.2.2 Cancer prevention

Antitumor activity of remedies prepared with leaves, flowers and roots of *M. oleifera* is recognized in popular medicine (Murakami *et al*., 1998). Many of the anti-carcinogenic effects have been scientifically confirmed in recent years. Recently, researches revealed that hydro-alcoholic extracts of moringa fruit, due to their positive effect on hepatic cytocrhome, can be used for combating chemical carcinogenesis. Researchers arrived at this conclusion after a detailed study on genesis of skin papilloma induced by 7,12-dimethylbenzanthracene in albino rats (Bharali *et al.,* 2003).

The effects of extracts of this plant in cancer prevention are a result of the presence of phytochemical substances that regulate enzymatic activity, favouring detoxification and guaranteeing anti-tumoral activity. For instance, the inhibiting action of 4-(4'-O-acetyl-α-L-ramnopiranosiloxi) benzyl isothiocyanate and niazimicin was confirmed on forbolic ethers responsible for the early activation of antigens on lympho-blastoid cells (Guevara, Vargas and Sakurai, 1999).

2.2.3 Antioxidant activity

The various parts of *M. oleifera* contain more than 40 compounds with antioxidant activity. Among the compounds with this potential, whether by the action of collecting free radicals or by the ability to form chelating agents of metallic ions, identified in seeds of moringa, there are phenolic compounds like kaempferol and galic and ellagic acids (Singh *et al*., 2009).

In vitro studies demonstrated that extracts of moringa leaves, fruits and seeds, due to their antioxidant characteristics, protect live cells from oxidative damage associated with aging, cancer and degenerative diseases (Singh *et al.*, 2009). In addition, these studies also indicated that these extracts inhibit lipid peroxidation and bacterial *quorum sensing,* and *M. oleifera* was proposed as an ideal candidate for pharmaceutical, nutraceutical and functional feed industries. Another study revealed that the fraction extracted with ethyl acetate, which is rich in phenolic and flavonoid acids, presents the highest antioxidant power among the fractions extracted with different solvents (Verma *et al.,* 2009).

The antioxidant activity of moringa leaves varies depending on the agroclimatic and seasonal conditions (Iqbal and Bhanger, 2006). Samples from cold regions in Pakistán presented higher antioxidant activity than temperate regions of that country, while those collected in December showed higher activity than those taken in June.

Extracts from *M. oleifera* seeds can be used in antioxidant therapy to decrease geno-toxicity of arsenic and other heavy metals, in which the mechanisms of carcinogenic action are related to oxygen-reactive species. The antidote action of the seeds of this plant was demonstrated in experiments with lab rats, previously exposed to arsenic (Gupta *et al.,* 2007). It was confirmed that the powder of these seeds reduces the concentration of arsenic and protects against haematological changes and oxidative stress induced by this metal, where several phytochemicals with antioxidant and chelatant power have a significant function. Natural coagulants in moringa seed, its high content of amino acids like methionine and cysteine, and of antioxidants like vitamins C, E and ββ-carotene are responsible for reducing the oxidative stress induced by arsenic (Flora and Pachauri, 2011).

2.2.4 Anti-inflammatory activity

Due to its high content of phenols, vitamins, omega 3 fatty acids, amino acids, glutathione, sterols and isocyanates, extracts from roots and seeds of *M. oleifera* contribute, directly or indirectly, to protection against inflammatory diseases. The protective effect of seed extracts against different inflammatory pathological conditions has been demonstrated, including the relief of bronchial inflammations like asthma (Mehta and Agrawal, 2008).

In vivo experiments demonstrated that methanol and aqueous extracts of moringa roots reduce notably the oedema induced by carrageenan. This same inflammatory activity was observed in water- (Cáceres *et al*., 1991) and ethanol- (Guevara, Vargas and Uy, 1996) -soluble fractions of seeds. In the case of the watery extract of roots, reduction degree is similar to that achieved with indomethacin, a very strong anti-inflammatory drug .

From *M. oleifera*, 36 compounds, with anti-inflamatory activity, have been isolated, such as alkaloids, glucosinolates and isocyanates (Mahajan and Mehta, 2008). Alkaloids have a similar activity to ephedrine and can be useful in asthma therapy, while moringina shows bronchiole relaxation activity. Seed extracts suppress several inflammatory regulators involved in chronic arthritis (Mahajan and Mehta, 2008).

2.2.5 Antihypertensive and hypoglycaemic activity

In Indian traditional medicine, *M. oleifera* is used for treating diabetes and arterial hypertension. Popular records in Africa also report several cases of a miraculous cure of diabetes and hypertension using remedies prepared with this plant. This early scientific research on this subject has already obtained convincing evidence of many of these cases, although the confirmation of others requires more study. In recent years, research has been conducted in several different countries in order to evaluate the hypoglycaemic, anti-diabetic and hypotensive potential of moringa using bio-clinical, pharmacological and biochemical assays.

Moringa leaves show hypoglycaemic and hypotensive activity, among other biological activities (Iqbal and Bhanger, 2006). There is evidences of its potential for relieving endocrine system dysfunctions, like thyroid disorder and insulin secretion.

Several phyto-chemical substances contained in leaves and fruits of *M. oleifera* have revealed its potential for controlling diabetes and arterial hypertension. Francis *et al*. (2004) isolated and purified eight biologically active compounds from the fruits of this plant. From them, a thiocarbamate, two carbamates and a phenyl glucoside stimulated insulin secretion in pancreatic cells of rats (Francis *et al.,* 2004). At the same time, a study performed by Pakistani scientists demonstrated that the responsibility for antihypertensive activity of moringa lies in its glucoside carbamate and isothiocyanate, as well as on ββ-sitosterol and methyl p-hydroxybenzoate.

The high content of vitamins in moringa is essential for treating diabetes. D vitamin is important for the correct functioning of pancreas and insulin secretion. The presence of ββ-carotene reduces the risk of blindness due to diabetes. B-12 vitamin is very useful for treating diabetic neuropathy and vitamin C prevents the accumulation of sorbitol and glycosylation of proteins, two very important factors in the development of diabetic complications like cataracts (Liñan, 2010).

2.3 Industrial uses of moringa

2.3.1 Purification of drinking water

The use of moringa seeds for purifying water is an economically attractive option for developing countries, taking into account the high cost of many chemical coagulants (Arenales, 1991).

The required doses of seed extracts is similar to that of alum, which is usually used, but if the treatment is performed with purified proteins and not with total extract, the coagulant effect is higher. Taking also into consideration that moringa is biodegradable and non-toxic, it does not affect pH or conductivity of water, and the mud produced by coagulation is innocuous and has low volume, it can be considered as a viable substitute for alum. Although toxicity of seeds is not confirmed, Santos *et al*. (2009) suggest a thermal treatment for purified water for denaturing lectin protein, which is a known anti-nutritional factor.

2.3.2 Treatment of waste waters

There is experience with the application of *M. oleifera* for treating waste waters from different sources. The evaluation, at lab scale, of a watery extract of moringa seeds for treating waste waters resulted in a decrease of chemical oxygen demand (COD) superior to 50%. The combination of 100 mg/L of extract with 10 mg/L of alum increased the removal of COD to 64%. The simplicity of the procedure and low cost of extract support use of this treatment for its application at larger scales (Bhuptawat, Folkard and Chaudhari, 2007).

The treatment of abattoir effluents with ground *M. oleifera* seeds gave a reduction of absorbency of 25% for waste waters from the septic tank, with lower quantities of suspended organic matter, and 82% reduction in waste water in a lake, with more suspended solids (Morales, Méndez and Tamayo, 2009). The extraction of proteins from seeds with saline solutions and their further use in distillery vinasses gave a removal of 53–64% of the initial colour (Krishna, 2009). The use of moringa defatted cake in palm tree oil extraction plants eliminated 95% of suspended solids and decreased the COD by over half. Combined with a commercial coagulant, removal of solids exceeded 99% (Bhatia, Othman and Ahmad, 2007).

M. oleifera may also be useful for controlling vectors in stagnant water. Recently, it was demonstrated that seed extracts prevent the adaptation of *Aedes aegypti* larvae to control means used in the anti-vectorial fight. Larval activity is attributed to water-soluble lectin contained in extracts, which causes a growth delay of larvae and their mortality (Coelho *et al*., 2009).

2.3.3 Softening of hard water

Hardness of water, due to a high content of salts, affects capacity of soap as a cleaning agent and provokes scaling in pipes and equipment, which is inconvenient at domestic and industrial levels. The process of removal of water metallic ions is known as softening and may be performed by means of different methods, including chemical precipitation, ionic exchange, nano-filtration and others (Magaña, 2012).

M. oleifera has the ability of eliminating ions of calcium, magnesium and other divalent cations. This property was accidentally discovered in a clarification study that revealed a reduction of 60–70% in water hardness after coagulation and two hours of sedimentation. Muyibi and Evison (1995), in a study with four different sources of hard water, demonstrated that the hardness removal mechanism works by means of adsorption of soluble ions and their later precipitation, and that removal efficiency is directly proportional to the doses of moringa and not to water pH.

2.3.4 Adsorption of heavy metals

During the last decade, the use of biosorbents has gained acceptance for heavy metal adsorption, whose toxicity towards the human organism is widely recognized. Biosorbents have increased in popularity because other technologies currently used for removing toxic metals are neither effective nor economic (Reddy *et al*., 2011). Biosorbents can be prepared from agricultural and agro-industrial residues, which present a high adsorptive capacity due to the presence of polar functional groups, forming coordination complexes with metallic ions in dissolution. In addition to low cost, biosorbents show other advantages, like their high efficiency, low mud generation, high level of regeneration and the possibility of metal recovery.

Recently, it was discovered that seeds and bark of moringa can be used for heavy metal adsorption for metals such as cadmium, lead and nickel (Reddy *et al*., 2011). Regarding the high costs of the usual techniques used for toxic metal removal, Martín *et al*. (2013) stated that the discovery of the adsorptive capacity of this species is very important for developing countries. Additionally, *M. oleifera* bark allows good recovery of adsorbed metals, reaching desorption of 98% of adsorbed nickel in aqueous solutions (Reddy *et al*., 2011).

Moringa bark powder can be used effectively as a biosorbent for several adsorption/desorption cycles, as was confirmed in a kinetic study of adsorption and a strict physical and chemical characterization, using electronic microscopy, infrared spectroscopy, X ray diffusion and element analysis. The effectiveness of the material for removing lead was also demonstrated, even in the presence of other metallic cations like Na^+, K^+, Ca^{2+} and Mg^{2+} (Reddy *et al.,* 2011).

2.4 Non-edible uses of moringa oil

2.4.1 Traditional uses

The oil forms between 22 and 40% of the total weight of *M. oleifera* seeds and contains around 70% oleic acid (Martin *et al.,* 2010). Besides its edible uses, the oil, commonly known as "Ben oil", has various inedible uses, many of which date back to early civilizations, and relate to the specific physical and chemical properties.

This oil has the property of absorbing and retaining floral fragrances, making it very suitable for cosmetics and the perfume industry. In Ancient Egypt, moringa oil was used for preparing perfumes, beauty creams, sacred ointments, skin protection against infections, insect repellent, and skin and hair moisturizer and conditioner (Deon, 2006).

2.4.2 Biodiesel production

M. oleifera is an excellent option for sustainable biodiesel production in countries with arid lands because of its fast growth, drought tolerance and high oil yield.

M. oleifera has gained importance because it is one of the plant species with high oil content (35%) that yields a high quality biodiesel. Typical yield is ca 2500 kg/ha, giving ca 1500 L/ha, with a biodiesel conversion factor of 0.96, which produces ca 1400 L/ha of biodiesel.

In Panama, a group of 15 scientists from the University of Agricultural Sciences at the University of Chiriqui is working on a super-project to produce biofuels, with moringa being an alternative plant for the project, because the leaves of this tree are well known for biogas production. Additionally, moringa is considered as an excellent choice for reforestation, which can contribute to soil conservation by preventing erosion, becoming an alternative for marginal soils and areas at risk of desertification.

Between 700 and 1100 trees per hectare of *Moringa oleifera* can be sown, which start fruit production after 12 months. Once the fruit is mature and shows a dark brown colour, seed production for new planting starts, or it is toasted for consumption, like groundnut; ground for purifying water and molasses; or for extracting edible oil either for culinary purposes or for biodiesel production.

Recent research has demonstrated the potential of moringa oil for biodiesel production (Silva *et al*., 2010). Properties of products from trans-esterification of this oil, such as density, viscosity, lubricity, oxidative stability, ethane index and turbidity point, meet international standards for its use as a combustible. Recently, the optimization of alkaline trans-esterification conditions of *M. oleifera* oil for biodiesel production was reported (Rashid *et al*., 2011).

2.5 Use of seed husks and dry pods of moringa

With industrialization of *M. oleifera* for oil extraction, the residual quantity of press cake will be large, due to large amounts of solid residues created by seed husks and dry pods.

The BIOMAS-CUBA Project, led by the Estación de Pastos y Forrajes "Indio Hatuey", promotes rational use of by-products like press cakes, husks and glycerol, generated during oil extraction and the trans-esterification process when producing biodiesel. The integrated use of all the fractions generated in biodiesel production is a necessity for process sustainability.

A possible application of husks is for the production of activated charcoal. In research on carbonization, followed by its steam activation, charcoal with a highly developed micro-porous structure and a high specific area were obtained. Also evaluated was a single-stage steam pyrolysis process, which gives the charcoal a higher adsorptive capacity than when produced by the conventional method of carbonization-activation in two stages (Warhurst *et al.,* 1997). In both cases, the charcoal shows performance similar to that of commercial activated charcoal, and the production would be import replacement. Another possible use of husks is for producing resins for anionic exchange purposes (Orlando *et al*., 2003).

At the same time, Moringa polysaccharide content is relatively high and glucanes represent 28% in husks (Martín *et al*., 2010) and 32% in pods (Martín, C. and Puls, M. 2014 unpublished). The high content of glucanes, similar to other ligno-cellulosic bio-resources (Martín *et al*., 2006), encourages interest in these materials as possible substrates for ethanol production and other products of glucose bio-conversion. This requires hydrolyzing polysaccharides in order to obtain fermentable sugars. Preliminary studies demonstrated that glucane within pods (Hernández *et al*., 2010) are more easily hydrolyzed than those within husks (Martín *et al*., 2008), which can be attributed to the high lignin content in husks. Taking into account that the pod represents 64% of the fruit weight, it is clear that there is considerable potential for ethanol production.

3. OTHER USES OF *TITHONIA DIVERSIFOLIA* (TITHONIA)

There are non-legume species with nutritional characteristics that are very valuable due to their feeding qualities (Murgueitio *et al*., 2009; Ruíz *et al*., 2009), especially those containing secondary metabolites (García *et al*., 2005) and taking part of diets for animals in feeding systems as for *T. diversifolia.* Earlier, in Chapters 10, 11 and 12, it was demonstrated that it is a plant with simple requirements and can be consumed by ruminants and monogastric species, as well as other animal categories.

Its nutritional value and secondary metabolite content have contributed to other uses of tithonia, although there are few published reports based on scientific results, similar to the situation for moringa and mulberry, but it is also important.

3.1 Medicinal uses and effects of tithonia

Ríos (1998) reported that the infusion of its leaves, in Alta Verapaz, Guatemala, contains a bitter oil and it is sometimes used as a remedy for malaria and for treating eczema and skin wounds of domestic animals. In Cuba, it was macerated with alcohol as if it were arnica (Roig 1974). Meanwhile, Mahecha and Rosales (2005) noted that, in Venezuela, tithonia was used for animal health in order to decrease abortions and cannibalism among rabbits, and also to stimulate the cleaning and expulsion of the placenta. In addition, Ríos (1998) reported that cooked leaves are used for treating paralysis and as medicine for liver problems.

At the same time, Toledo *et al*. (2014), in an *in vitro* study, reported a group of isolated sesquiterpene lactones from tithonia leaves with potent antimicrobial activity, while Bouberte *et al.* (2006) found a new isocoumarin, tithoniamarin, with antifungal and anti-herbicide effects. Ezeonwumelu *et al.* (2012) reported anti-diarrhoeal activity of extracts from *Tithonia diversifolia* leaves in rats. Miranda *et al.* (2014) showed a marked antioxidant potential in tithonia flower extracts, and Mwanauta, Mtei and Ndakidemi (2014) reported antioxidant activity in extract of plant leaves.

3.2 Immunogenic effect of tithonia

In Cuba, Ortega *et al.* (2011) evaluated, in tissues of various organs of the GIT (small intestine, large intestine and spleen) of growing pigs, intake effect of a control diet with maize/soybean and two treatments that substituted 10 or 20% of this diet for two levels of tithonia forage (10 and 20%), respectively. Samples of the corresponding organs were taken for histological analysis.

As a result, these authors found that components of tithonia forage meal showed a slight immunogenic power after locally stimulating lymphocytic formations in those associated with mucus of Peyer plaques from intestine and white pulp from spleen (Malpighian corpuscle) in experimental treatments (10 and 20%) of tithonia forage meal. The highest inclusion level (20%) showed an immuno-supressive effect on spleen and a reduction of cells from the reticulo-endothelial system. Substitution percentage in the diet was demonstrated on the intestinal mucus through epithelial hyperplasia (20% of substitution) and crypts together with calceiform cells or villi shortening, which supposes the physiological need to respond to digestion of secondary metabolites present in the diet. (Photographs 1, 2, 3 and 4)

3.3. Effect of tithonia on the control of intestinal parasites

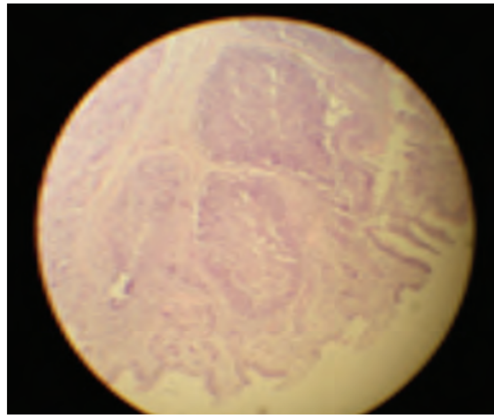
Previously, this chapter reported that gastro-intestinal parasitism is a disease of cattle, mainly in tropical countries, due to climatic factors prevailing in these regions, such as humidity.

Soca, Simón and Roque (2011) demonstrated the potential of trees and shrubs in the control of gastrointestinal nematodes in young cattle under Cuban conditions. Later, Lezcano (2013) analysed the effect of *T. diversifolia* on controlling intestinal nematodes when calves were supplemented in two seasons, during dry period (DP) and rainy period (RP) (Figures 2 and 3).

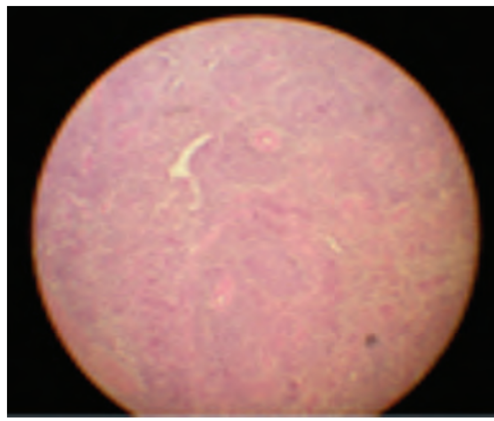
Figure 2 shows that the group of calves, supplemented with *T. diversifolia*, did not exceed values of 150 eggs per gram, considered to be the threshold for effects on animal production (Hansen and Perry, 1994). However, the parasites from the unsupplemented group reached up to 500 eggs per gram during the dry period.

Figure 3 shows that calves (Group a) supplemented with *T. diversifolia* presented significantly lower values in the faecal counting of eggs in comparison with the group b calves not supplemented with this forage, which reached 3500 eggs per gram.

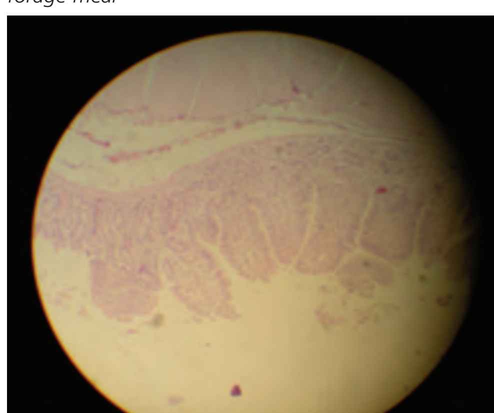
These values confirm those stated by Coop and Kiriasakis (2001), who note that protein diets influence host/parasite relationships directly, increase the resistance of hosts and show more adequate immunological responses, improve patho-physiological effects, and clinical signs are less severe (Torres, 2005).



Photograph 1. Large intestine. Chain of Peyer plaques (10% of tithonia forage meal)



Photograph 2. Spleen. Splenic tissue with two reactive Malpighian corpuscles, 10% of tithonia forage meal



Photograph 3. Small intestine. Long villi and separated from the intestinal mucus. Tithonia forage meal at 10%

Photograph 4. Small intestine. Shortening of intestinal mucus villi, 20% of tithonia forage meal

Tithonia diversifolia, from a bromatological point of view, was characterized by presenting significant values of CP and CF in the edible fraction of leaf plus young stem, during both studied periods, and, from a phyto-chemical point of view, there was a considerable and remarkable amount of alkaloids during the rainy and dry periods, respectively.

In vivo efficiency of *Tithonia diversifolia* in calves was evident in a reduction in faecal counts of eggs (150 eggs per gram and 450 eggs per gram) during dry and rainy periods, respectively.

Finally, Savón *et al.* (2010) corroborated the favourable effect of tithonia on reducing intestinal parasites, as confirmed in growing pigs. These researchers provided 10 and 20% tithonia forage meal as a partial replacement of maize/soybean in control feedstuff of pigs

FIGURE 2

Results of faecal counting of eggs from gastro-intestinal nematodes in the rainy period. Group a received tithonia; group b did not

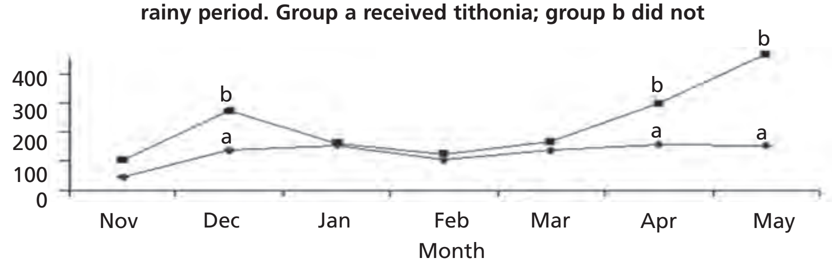
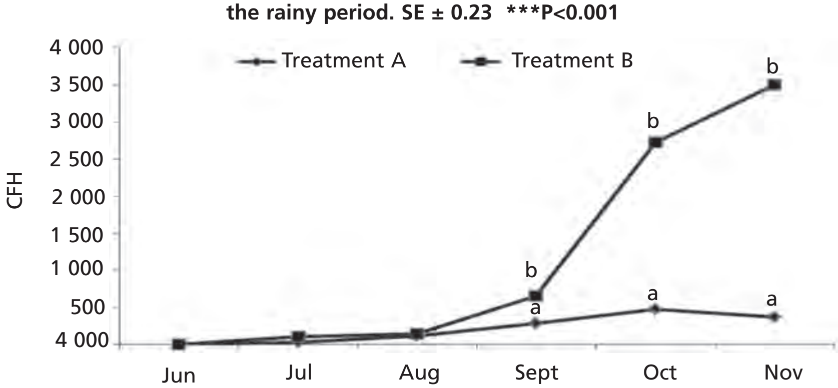


FIGURE 3

Results of faecal counting of eggs from gastro-intestinal nematodes during the rainy period. SE ± 0.23 ***P<0.001



that were not dewormed. By analysing the faeces, these researchers observed that animals receiving 20% of tithonia showed no presence of *Ascaris lumbricoides* while the feedstuff control treatment presented a high incidence of this endo-parasite.

3.4 Other reported uses

Mahecha and Rosales (2005) indicate that tithonia can be used as insecticide to control ants (bibijaguas in Cuba), which improves degraded soils (especially for phosphorus absorption). Giraldo *et al.* (2006) also reported that extracts and plants have insecticidal properties, which turns this shrub into a protector of other plants and crops that are useful for humans as food and timber.

This plant has great potential thanks to its tree characteristics and its chemical properties. In Colombia, it is used as a live fence, flora for apiculture, and as an ornamental plant. Similarly, there is information in the literature on its use in other countries for attracting beneficial insects in crops, livestock straw bed and green manure for crops such as rice. This last use is one of the most widespread in some countries, like the Philippines.

In Costa Rica, tithonia has been used at experimental level to increase the production of beans in improved fallow lands, and in Kenya it has been used for maize (Wanjau *et al*., 1998).

4. FINAL REMARKS

Mulberry, moringa and tithonia possess an undeniable nutritional value that provides great potential for feeding all animal species. Secondary metabolites, contained in all parts of these plants (leaves, roots and stems), but mainly in leaves, favour other uses in feeding, and in human and animal health. Some of them, like moringa and mulberry, can also have other uses and industrial applications.

It is important to note that, for moringa and mulberry, the action mechanisms of some of the metabolites responsible for the therapeutic and pharmacological effects, and other medicinal uses, have been studied mainly in Asia. However, in Latin America, there are few reports. Nevertheless, various uses of tithonia have been less studied and are known only by popular empiricism, since there are very few reports Their action on the control of gastro-intestinal nematodes has been more extensively researched.

It is necessary to promote research in Latin America to develop other therapeutic and medicinal uses in human and animal health, as well as industrial applications that enable a better understanding of the properties of these plants.

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All photographs supplied by authors.

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This publication presents state-of-the-art information on forage production from *Morus alba*, *Moringa oleifera* and *Tithonia diversifolia* plants, their nutritive value and use in both monogastric and ruminant production systems in Latin American and Caribbean countries. It also discusses industrial, food and pharmaceutical applications of different products from the three plants. The information has been collated to promote wider use of the foliage from these plants worldwide. The document is intended for use by extension workers, researchers, the feed industry, NGOs, farmers' associations, policy-makers and science managers.