In vitro fermentation, digestibility and methane production of tropical perennial grass species

Belete Shenkute Gemeda[A,B] *and Abubeker Hassen*[A,B]

[A]Department of Animal and Wildlife Sciences, University of Pretoria, Pretoria 0002, South Africa. [B]Corresponding authors.

Emails: beletegemeda@gmail.com; Abubeker.hassen@up.ac.za

Abstract. This study characterised 16 tropical perennial grass species in terms of *in vitro* methane output and related their digestibility and rumen fermentation with methane output. The grass samples were collected, dried in a forced oven, and ground and analysed for nutrient composition. *In vitro* gas production and organic matter digestibility (IVOMD) were determined using rumen fluid collected, strained and anaerobically prepared. A semi-automated system was used to measure gas production through *in vitro* incubation at 39°C. *Anthephora argentea* and *Stipagrostis ciliate* produced the highest concentration of methane in terms of g kg^{-1} digestible dry matter (DDM) and g kg^{-1} digestible organic matter (IVOMD). *Cenchrus ciliaris*, *Setaria verticillata* and *Panicum coloratum* produced the lowest ($P < 0.05$) methane when expressed in terms of g kg^{-1} DDM and g kg^{-1} IVOMD. Ash, ether extract, non-fibrous carbohydrate, neutral and acid detergent insoluble nitrogen, and crude protein were negatively correlated with methane production. Methane production positively correlated with neutral and acid detergent fibre, cellulose and hemicellulose. It is important to focus on screening and selecting perennial grass with higher nitrogen content and low methane production to mitigate methane production under tropical conditions.

Additional keywords: digestibility, fermentation, methane, perennial grass, tropical.

Introduction

Enteric methane (CH_4) production in ruminants decreases their energy utilisation efficiency and contributes to the global greenhouse-gas effect. Methane is produced under anaerobic conditions by rumen microorganisms, called methanogenic archaea, that gain energy by reducing CO_2 with H_2 to form CH_4 (Leng 2008). Ruminal microbes convert major portions of carbohydrate and protein in feed to volatile fatty acids, microbial protein, methane and CO_2. Enteric methane production depends primarily on quantity and quality of the diet (Van Soest 1994; Beauchemin *et al*. 2009), the nature of fermented carbohydrates (Santoso *et al*. 2003), the concentrations of neutral (NDF) and acid (ADF) detergent fibres (Hindrichsen *et al*. 2003), the ratio of acetate to propionate in fermented feeds (McAllister *et al*. 1996), and type and maturity stages of forage consumed by the animal (Arthington and Brown 2005).

Mitigation of methane production from ruminants by altering the diet is an effective way to decrease enteric methane production (Singh *et al*. 2012). An increased proportion of concentrate in ruminant rations is generally associated with a reduction in methane emission per unit of feed intake and per unit of animal product (Johnson and Johnson 1995; Lovett *et al*. 2003). However, in many tropical and subtropical livestock production systems, ruminants receive small quantities of concentrates, if at all, because of unavailability and cost. Therefore, under such systems, it is important to focus on tropical grassland forages and to design effective feed-based mitigation strategies. Previous research has suggested that increased forage quality will reduce methane emissions per unit of weight gain (Mc Geough *et al*. 2010) or per unit of animal product (Moss 2000), due to improvement in animal productivity.

Characterisation of tropical grasses and relating those attributes to potential methane production is important for selection and improvement through breeding. Methane production is mainly related to the extent of organic matter digestion and the profile of volatile fatty acids produced and fermented (McDonald *et al*. 2011). Thus, methane output from a wide range of grass species can be studied using *in vitro* gas-production techniques; this method is inexpensive and widely used (Tavendale *et al*. 2005). For tropical grass species in Africa, little information is documented on their potential methane outputs and the correlation with their nutrient constituents. Therefore, this study was undertaken with the aim of providing information on grass species commonly found in the Kalahari Desert of Southern Africa. The specific objectives were: (*i*) to characterise the grass species in terms of chemical composition and related attributes; (*ii*) to compare *in vitro* gas production and methane output of various tropical grass species; and (*iii*) to relate methane production of grass species to their chemical composition, digestibility and *in vitro* gas production attributes.

Materials and methods

Study area description

The study was carried out in North West Province of South Africa, in the Thorny Kalahari Dune Bushveld, which covers 2000 ha with an altitude of ~900–1100 m a.s.l. It is a fenced area with rotation-grazed paddocks. The carrying capacity is 13.2 tropical livestock units (TLU) and the land is used for livestock farming (96%), including beef cattle, sheep and goats. Soils are red, excessively drained sandy soils with high base status; dunes are present and there are elevated concentrations of copper, which is bound in the soil in the form of the secondary copper hydroxyl mineral atacamite ($Cu_2(OH)_3Cl$) (Le Roux 2013). The area has highly erratic rainfall that ranges from 150 to 350 mm per year; however, it barely exceeded 150 mm during the study period or the previous 2 years. The wettest months are usually January–April and the temperature extremes range from winter lows reaching –10.3°C to summer highs of up to 45.4°C (Rooyen 2001).

The study area has sparsely scattered trees, mainly camel thorn (*Acacia erioloba*), false umbrella thorn (*Acacia luederitzii*) and shepherd's tree (*Boscia lehmanniana*). The dominant herbaceous plants are perennial rather than annual grasses. Some of the dominant perennial grasses include *Eragrostis* spp., *Schmidtia* spp. and *Stipagrostis* spp.

Grass sampling and chemical composition analysis

The study area was categorised as lightly grazed and heavily grazed according to grazing histories and condition of the rangeland. In each grazing site, eight transects, each 7.5 m long, were set up in the canopied and uncanopied sub-habitat. Data on floristic composition, total plant cover and individual species cover were collected using the quadrat point method (Daget and Poissonet 1971). To obtain dry matter (DM) of aboveground parts of vegetation, we used the formula of Le Houe'rou (1987).

From each transect, four randomly selected sub-sites were taken. The herbaceous vegetation was cut at ground level using a 50 cm by 50 cm quadrat and immediately identified and categorised into perennial grasses, annuals, legumes and sedges. Perennial grasses were separated into species and put in paper bags for further study. Sampling was done when most pasture plants were fully grown and were flowering (important for identification). The perennial grass species common to both study sites were used for determination of nutritive value, and *in vitro* fermentation and methane production.

The grasses were kept under a shaded area until transported to the Nutrition Laboratory of University of Pretoria, where they were oven-dried (55°C for 48 h). For all *in vitro* studies and chemical composition analyses, the samples were ground to pass through a 1-mm sieve in a Willey mill (Arthur H. Thomas, Philadelphia, PA, USA). The samples were analysed for organic matter (OM) by ashing in a muffle furnace at 550°C (AOAC 2002). Crude protein (CP) was measured according to the combustion method (AOAC 2002) for nitrogen (N) on a LECO FP-428 Nitrogen and Protein analyzer (LECO Corporation, St. Joseph, MI, USA), and ether extract (fat) was done according to AOAC (2002) procedures. The NDF and ADF contents were determined using an ANKOM200/220 Fibre Analyzer (ANKOM Technology, Fairport, NY, USA) based on the methods described by Van Soest *et al*. (1991). Sodium sulfite and heat-stable amylase were used in the analysis of NDF. Lignin (ADL) was determined by solubilisation of cellulose with sulfuric acid in the ADF residue (Van Soest *et al*. 1991). The N contents of NDF and ADF (i.e. neutral (NDIN) and acid (ADIN) detergent insoluble N) were determined by the CP method referenced above and expressed exclusive of residual ash. The non-fibre carbohydrate (NFC) content of feeds was calculated by subtraction of CP, NDF, fat and ash from total DM (Sniffen *et al.* 1992). Hemicellulose was estimated from the difference between NDF and ADF, while cellulose was estimated as the difference between ADF and ADL.

In vitro *gas production measurement*

Collection of rumen fluid from donor sheep

Rumen fluid was collected before the morning feeding from two ruminally cannulated Merino wethers fed on *ad libitum* lucerne (*Medicago sativa*) hay. Approximately 500 mL rumen fluid was collected from each donor animal, mixed, strained through four layers of cheesecloth, and transferred to a pre-heated thermos flask. In the laboratory, the flask contents were emptied into an industrial blender and simultaneously purged with CO_2 to maintain anaerobic conditions (Grant and Mertens 1992). After blending, the rumen fluid was transferred in a large glass beaker that was kept inside a 39°C water bath purged with CO_2 and continuously stirred as recommended by Goering and Van Soest (1970). Thereafter, 15 mL rumen fluid was added to 25 mL buffer solution in the respective incubation bottles.

Buffer media preparation, sample incubation and gas measurement

The buffer solution, macro-mineral solution and micro-mineral solution were prepared in large quantities and utilised as required following the procedure described by Goering and Van Soest (1970). The micro-mineral solution was prepared with a slight modification whereby $MgSO_4.7H_2O$ was replaced with $MgCl_2.6H_2O$ to reduce the amount of SO_4 in the media, as suggested by Mould *et al*. (2005). It was stored in a dark glass bottle to maintain the quality of the solution. In the morning, before the start of the experiment, appropriate amounts of distilled water, rumen buffer solution, and macro- and micro-mineral solutions were mixed with tryptose and prepared 0.1% (wt/vol.) resazurin. Appropriate amounts of L-cysteine hydrochloride were weighed and added directly to the rest of the solution once all of the chemicals were dissolved. As soon as L-cysteine hydrochloride was added, the buffer solution was placed in a 39°C water bath and bubbled with CO_2. The serum bottles were then sealed with a rubber stopper and left at 39°C until the buffer solution was clear, which indicated that the solution was sufficiently reduced.

A semi-automated gas production system was used to measure gas production through *in vitro* incubation at 39°C, according to Theodorou *et al*. (1994). The system consists of a digital data tracker (Tracker 220 series indicators; Omega Engineering Inc., Laval, QC, Canada) connected to a pressure transducer (PX4200-015GI; Omega Engineering Inc.) with a needle on the tip. Approximately 400 mg of each grass-feed sample was weighed into 120-mL serum bottles, then 40 mL

rumen fluid + medium was added under a stream of CO_2 to each of the serum bottles closed with rubber stoppers and crimp seal caps. A needle was inserted through the rubber stopper of each serum bottle for ~5 s to release small amounts of gas that might have built up since the start of incubation. All serum bottles were placed in the incubator and the rotary shaker was turned on at 120 rpm. Gas pressure was taken at 2, 4, 8, 12, 24, 32, 48, 54 and 72 h. To quantify the gas production derived from the culture medium and the rumen inoculums, two blanks were included in every analysis. Two replicates were used in each run and four different runs were executed for every grass sample included in the study. The pressure and gas volume were recorded at different times and were added to the values of the previous readings. Thus, the cumulative pressure and gas volume of the fermentation were obtained. Fermentation was terminated after 72 h by removing serum bottles from the incubator and placing them on ice. Supernatants were immediately pipetted and stored at 208C until analysed for
− ammonia-N (McDonald *et al*. 1960) and volatile fatty acids
. (Ottenstein and Bartley 1971)

In vitro *digestible organic matter determination*

The *in vitro* digestible organic matter (IVOMD) content was determined according to the method of Tilley and Terry (1963), as modified by Engels and Van der Merwe (1967). The method involved two digestion phases. During the first digestion phase, feed samples (200 mg) were incubated in triplicate under anaerobic conditions with rumen liquor for 48 h at 39°C, with the inclusion of blanks and standards in every batch of incubation. This was followed by an acid pepsin digestion phase for 48 h at 39°C, under anaerobic conditions. Following the incubation for 96 h, the residual plant materials were collected and oven-dried at 105°C for 12 h. Ash contents were determined by combustion at 550°C for 2 h (Engels and Van der Merwe 1967).

Methane production measurements

Methane production was measured from the duplicate bottles incubated with each grass sample at 2, 12, 24 and 48 h. The methane concentration was determined by gas chromatography (8610C Gas Chromatograph (GC) BTU Gas Analyzer GC System; SRI Instruments GmbH, Bad Honnef, Germany). The GC was equipped with a solenoid column packed with silica gel and a flame ionisation detector. Gas production from each bottle was recorded and gas samples were immediately taken using a Hamilton syringe. The sampled gas was injected manually (pull and push method of sample injection) into the GC, which was already calibrated with standard methane and CO_2. Two blanks were included for correction of methane produced from the inoculum in each run, and two runs were executed for each samples. The measured methane concentration was related to the respective total gas measurement in order to estimate its concentration (Tavendale *et al*. 2005), and subsequently converted to energy and mass values using 39.54 kJ L^{-1} CH_4 and 0.716 mg mL^{-1} CH_4 factors, respectively (Santoso *et al*. 2007).

Calculations and statistical analyses

Non-fibrous carbohydrate was calculated as:

$$\mathrm{NFC} = 100 - (\mathrm{CP} + \mathrm{fat} + \mathrm{ash} + (\mathrm{NDF} - \mathrm{NDIN}))$$

Metabolisable energy (ME, MJ kg^{-1} DM) was estimated according to Menke and Steingass (1988) as:

$$\mathrm{ME\,(MJ\,kg^{-1}\,DM)} = 2.20 + 0.136\,\mathrm{IVGP24\,(mL\,per\,0.5\,g\,DM)} + 0.057\,\mathrm{CP\,(\%\,DM)}$$

where IVGP24 is *in vitro* gas production over 24 h. Methane production was calculated as:

$$\mathrm{CH_4\ produced\,(g\,g^{-1}\ digested\ DM)} = ((\mathrm{gas\ production\ 24\,h} \times ([\mathrm{CH_4\ 24\,h}]) - \mathrm{gas\ produced\ blank\ 24\,h} \times [\mathrm{CH_4\ blank\ 24\,h}]))/\mathrm{g\ digested\ DM}$$

according to Chaves *et al.* (2006).

The rate and extent of gas production was determined for each grass species by fitting gas production data to the non-linear equation (Ørskov and Mcdonald 1979):

$$y = b\,(1 - \mathrm{e}^{-ct})$$

where y is gas production at time t; b is the slowly fermentable fraction (g kg^{-1} DM); and c is the rate (% h^{-1}) of fermentation of fraction b.

The experimental design used in the study was a completely randomised design. The data were statistically analysed using the GLM option of SAS (2002) and differences among the means were determined using Tukey's test. The *in vitro* incubation times were used to fit non-linear regression models using the NLIN procedure (SAS 2002).

Result

Plant cover, density and dry matter yield

At the lightly grazed site, there was a significant difference ($P < 0.05$) between the canopied and uncanopied areas for total plant cover and DM yield. However, there was no significant ($P > 0.05$) difference between the two areas at the heavily grazed site in terms of the two parameters. Total plant cover and the DM yield values tended to be higher under tree canopy (Table 1).

The mean perennial species cover (% of DM) at the study sites is shown in Table 2 (only species presenting a cover >0.5% are indicated). At the lightly grazed site, the perennial grass species cover showed significant differences ($P < 0.05$) for some *Aristida vestita*, *Schmidtia pappophoroides*, *Stipagrostis ciliate and Stipagrostis obtuse.* The highest cover of these species was recorded under the tree canopy and it varied from 0.99% to 10.3% in the canopied sub-habitat. The most abundant species at both grazing sites were *Schmidtia pappophoroides*, *Stipagrostis ciliate*, *Stipagrostis obtuse and Stipagrostis uniplumis*.

Chemical composition

Table 3 summarises the chemical compositions of grass species used in the study. Significant ($P < 0.05$) variation in terms of chemical composition was recorded for different grass species. The highest ash concentration was recorded for *Setaria verticillata* (147 g kg^{-1} DM) and the lowest value for *Panicum coloratum* (20 g kg^{-1} DM). CP content in the grass species ranged between 20 and 126 g kg^{-1} DM. The highest CP content was

Table 1. Mean total plant cover, dry matter and density of perennial species occurring under shrubs and tree canopies and in open areas in (lightly grazed site) and outside (heavily grazed site) the study area

*$P < 0.05$; n.s., not significant

	Lightly grazed site			Heavily grazed site		
	Canopied	Uncanopied	Sub-habitat effect	Canopied	Uncanopied	Sub-habitat effect
Total plant cover (%)	66.8	51.0	*	14.0	13.2	n.s.
DM yield (kg ha^{-1})	1200.0	1045.0	*	713.0	700.0	n.s.
Perennial grasses (plants m^{-2})	721.0	658.0	*	480.0	479.0	n.s.
Annual grasses, legumes and sedges (plants m^{-2})	479.0	387.0	*	233.0	221.0	n.s.

Table 2. Mean perennial grass species cover (% of dry matter) occurring under tree canopies (Can.) and in open areas (Uncan.) in (lightly grazed site) and outside (heavily grazed site) the study area

*$P < 0.05$; n.s., not significant

Scientific name	Common names	Lightly grazed			Heavily grazed		
		Can.	Uncan.	Sub-habitat effect	Can.	Uncan.	Sub-habitat effect
Anthephora argentea	Silver wool grass	3.62	3.53	n.s.	2.01	1.98	n.s.
Aristida vestita	Herder silk grass	5.96	1.18	*	2.01	1.87	n.s.
Brachiaria ciliaris	Black foot brachiara	0.99	1.02	n.s.	0.05	0.01	n.s.
Centropodia glauca	Gha grass	4.33	4.29	n.s.	1.45	1.12	n.s.
Cynodon dactylon	Couch grass	1.36	0.09	n.s.	0.08	0.0	n.s.
Cenchrus ciliaris	Foxtail buffalo grass	0.58	0.78	n.s.	0.05	0.00	n.s.
Digitaria eriantha	Common finger-grass	0.68	0.67	n.s.	0.01	0.0	n.s.
Eragrostis trichophora	Hairy love grass	1.36	1.12	n.s.	0.6	0.11	n.s.
Panicum coloratum	Small buffalo grass	1.00	0.97	n.s.	0.98	0.58	n.s.
Pogonarthria squarrosa	Herringbone grass	1.26	0.60	n.s.	0.65	0.45	n.s.
Setaria verticillata	Bur bristle grass	2.68	2.48	n.s.	0.25	0.01	n.s.
Stipagrostis uniplumis	Silky bush man grass	10.3	9.95	n.s.	3.2	3.09	n.s.
Schmidtia pappophoroides	Kalahari sand quick	7.67	2.02	*	2.87	2.88	n.s.
Stipagrostis ciliate	Long bushman grass	5.83	1.59	*	2.37	2.20	n.s.
Stipagrostis obtuse	Small bushman grass	7.91	2.58	*	2.32	2.02	n.s.
Tricholaena monachne	Blue-seed grass	2.98	2.05	n.s.	0.12	0.10	n.s.

Table 3. Mean of chemical composition (g kg^{-1} DM) of tropical grass species used the study

OM, Organic matter; CP, crude protein; EE, ether extract; NDF and ADF, neutral and acid detergent fibre; ADL, acid detergent lignin; ADIN and NDIN, acid and neutral detergent insoluble nitrogen. Within a column, means followed by the same letter are not significantly different at $P = 0.05$

Scientific name	Ash	OM	CP	EE	NDF	ADF	ADL	ADIN	NDIN	Cellulose
Anthephora argentea	44.8h	907.7bcd	52.4bc	17.4cde	806.1bcd	540.6b	93.2d	1.59o	21.6e	447.4b
Aristida vestita	41.1i	915.7abc	38.2bc	12.6cde	787.7def	490.2de	60.1ij	1.87l	17.9g	430.1bc
Brachiaria ciliaris	47.3g	904.5cde	85.2b	12.4def	776.0f	455.3f	90.5de	2.54h	18.3fg	364.8g
Cenchrus ciliaris	76.9c	873.9gh	41.0bc	19.5ab	682.3i	418.7g	58.5ij	3.28d	15.4h	360.2gh
Centropodia glauca	76.3c	882.8fgh	32.7bc	12.6cde	749.5g	457.2f	117.8a	2.84e	20.9ef	339.4ij
Cynodon dactylon	105.2b	830.0i	20.4c	23.7a	638.9i	356.3h	51.6k	5.36e	63.7a	298.3k
Digitaria eriantha	63.0e	864.4h	20.4c	10.9de	800.1cde	514.6c	102.7c	10.9a	10.9l	411.9cd
Eragrostis trichophora	33.1k	919.7abc	34.3bc	9.47e	794.9def	477.7e	67.9h	2.34i	21.0e	409.8d
Panicum coloratum	18.6n	893.3def	34.8bc	11.3cde	783.2ef	488.6de	111.3b	2.25j	2.21k	377.3fg
Pogonarthria squarrosa	29.5l	922.0abc	123.7a	18.4abc	795.4def	500.0cd	87.5ef	2.57g	21.6e	412.5cd
Setaria verticillata	146.5a	806.3j	45.8bc	13.2cde	563.8k	331.8i	70.6h	6.98b	61.8b	261.2l
Stipagrostis uniplumis	30.9m	927.0a	74.4abc	21.0b	823.0ab	486.0de	83.6g	2.23g	26.6d	402.4de
Schmidtia pappophoroides	65.7d	887.3efg	37.3bc	10.5de	704.8h	407.1g	62.6i	2.74f	47.2c	344.5hi
Stipagrostis obtuse	26.3n	926.4ab	34.5bc	12.1cde	827.2a	571.6a	70.1h	1.76m	17.2gh	501.5a
Stipagrostis ciliate	37.9j	909.5abcd	126.2a	9.47e	778.0f	514.8c	109.1b	2.16k	12.6i	414.4cd
Tricholaena monachne	51.9f	878.8fgh	39.1bc	12.9cde	756.0g	443.5f	57.6j	1.63n	6.98j	385.9ef
s.e.m.	0.35	3.63	10.4	1.27	3.72	3.71	0.81	0.004	0.45	3.73
P-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

recorded for *Pogonarthria squarrosa* (124 g kg^{-1} DM) and *Stipagrostis ciliate* (126 g kg^{-1} DM) and the lowest amount for *Cynodon dactylon* and *Digitaria eriantha* (20 g kg^{-1} DM). *Cynodon dactylon* had the highest value of NDIN (64 g kg^{-1} DM-NDF) and *Panicum coloratum* the lowest value (2.21 g kg^{-1} DM). The highest value of ADIN was recorded in *Digitaria eriantha* (11 g kg^{-1} DM-ADN) and the lowest value in *Anthephora argentea* (1.59 g kg^{-1} DM).

The NDF ranged from 564 to 827 g kg^{-1} DM, and ADF ranged from 332 to 572 g kg^{-1} DM. *Stipagrostis obtusa* had the highest values of NDF (827 g kg^{-1} DM) and ADF (572 g kg^{-1} DM), and *Setaria verticillata* the lowest NDF (564 g kg^{-1} DM) and ADF (332 g kg^{-1} DM). *Centropodia glauca* had the highest ADL (118 g kg^{-1} DM) content, whereas the lowest value was recorded for *Cynodon dactylon* (52 g kg^{-1} DM). *Stipagrostis obtusa* had the highest cellulose (502 g kg^{-1} DM) and *Setaria verticillata* the lowest (261 g kg^{-1} DM).

In vitro *gas production, organic matter digestibility and volatile fatty acid (VFA) production*

The cumulative gas production pattern from the *in vitro* fermentation of the grass species is given in Fig. 1. The total volume and pattern of gas production varied among species; however, the observed differences were not consistent for the different incubation times except for *Schmidtia pappophoroides*, which consistently produced the highest volume of gas over all incubation times. The lowest gas production was measured for *Panicum coloratum* at 2 and 48 h, and *Pogonarthria squarrosa* during 8–72 h. Gas production was, in general, lower in these two grass species compared with the others grasses studied.

The grass species showed high variability in terms of gas production parameters and constants (Table 4). A higher *b*-value (gas production from the slowly fermentable OM) was recorded for *Schmidtia pappophoroides* (69.7 mL per 0.4 g DM) and the lowest *b*-value for *Panicum coloratum* (26.3 mL per 0.4 g DM). The potential gas production value was highest for *Schmidtia pappophoroides* (35.3 mL per 0.4 g DM), and lowest for *Pogonarthria squarrosa* (10.7 mL per 0.4 g DM). The rate of gas production was highest (0.057 mL h^{-1}) for *Eragrostis trichophora* and lowest (0.029 mL h^{-1}) for *Pogonarthria squarrosa*.

The total and individual short-chain VFAs and ammonia-N concentration are presented in (Table 5). The total and individual short-chain VFAs varied among perennial grasses. *Anthephora argentea* produced large amounts of total VFAs and individual fatty acids, whereas *Panicum coloratum* contained the lowest amounts, except isobutyric acid.

Methane production and its association with IVOMD, VFA and chemical composition

Methane production (mL g DM^{-1}) and its percentage concentration (v/v) in the total gas differed ($P < 0.05$) among the studied grass species and incubation periods (Table 6). Methane production and its concentration (%) from tested perennial grasses differed significantly ($P < 0.05$) at different periods of incubation. The volume of methane production was higher ($P < 0.05$) from *Stipagrostis ciliate*, *Anthephora argentea* and *Aristida vestita* during the early incubation period (2 h), whereas *Stipagrostis ciliate* and *Aristida vestita* produced significantly ($P < 0.05$) higher volumes of methane during 12–48 h of

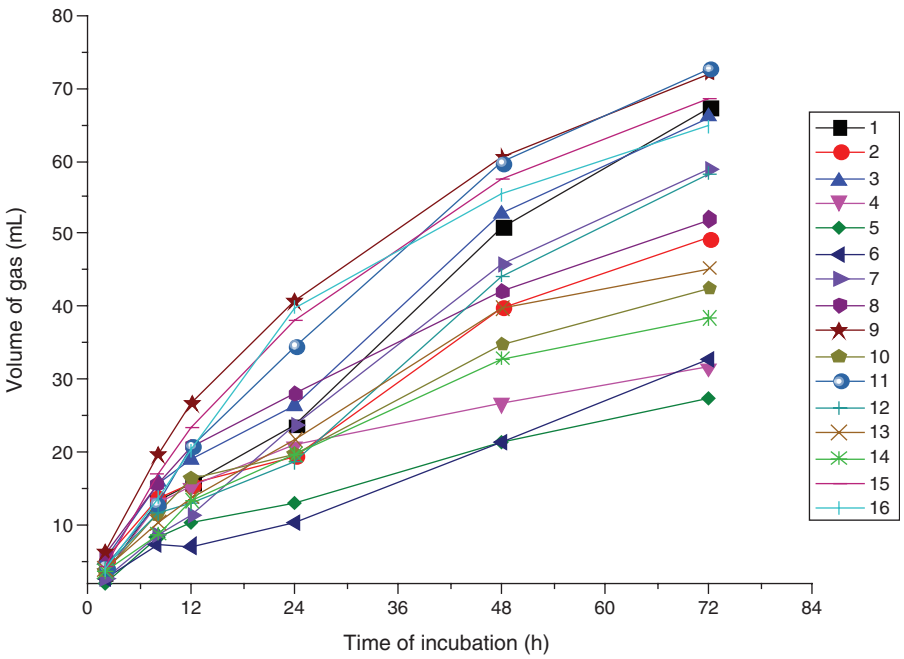

Fig. 1. Gas production (mL per 400 mg DM) pattern of tropical perennial grasses used in the study. 1, *Anthephora argentea*; 2, *Brachiaria ciliaris*; 3, *Cenchrus ciliaris*; 4, *Eragrostis trichophora*; 5, *Panicum coloratum*; 6, *Pogonarthria squarrosa*; 7, *Setaria verticillata*; 8, *Stipagrostis uniplumis*; 9, *Schmidtia pappophoroides*; 10, *Centropodia glauca*; 11, *Stipagrostis obtuse*; 12, *Aristida vestita*; 13, *Tricholaena monachne*; 14, *Stipagrostis ciliate*; 15, *Cynodon dactylon*; 16, *Digitaria eriantha*.

incubation. The lowest volume of methane was recorded for *Stipagrostis uniplumis* during the early incubation period (2 h), whereas *Cenchrus ciliaris* and *Panicum coloratum* produced the lowest volume during 12–48 h of incubation.

Methane production expressed in mass (methane production, g kg^{-1} DM and g kg^{-1} IVOMD) and eructated energy (%ME) also varied significantly ($P < 0.05$) among the studied grasses (Table 7). *Cynodon dactylon* and *Tricholaena monachne* contained significantly higher ME (12.3 MJ kg^{-1} DM) and IVOMD (61%), whereas *Schmidtia pappophoroides* (6.21 MJ ME kg^{-1} DM) and *Stipagrostis ciliata* (40.7%) contain the lowest ME and IVOMD, respectively. The studied perennial grasses showed significant variation in their ME, IVOMD and ammonia-N contents. *Anthephora argentea* and *Stipagrostis ciliate* produced the highest concentration of methane, both in terms of g kg^{-1} DM and g kg^{-1} DOM. *Cenchrus ciliaris*, *Setaria verticillata*, and *Panicum coloratum* produced the lowest ($P < 0.005$) methane when expressed in terms of both g kg^{-1} DM and g kg^{-1} IVOMD. Methane production expressed in terms of eructated energy was higher ($P < 0.05$) from *Anthephora argentea* and *Stipagrostis ciliate*, and this ranged between 0.335% and 1.12% across the grass species.

Table 4. Gas production parameters of tropical perennial grasses used in the study

Units for *b* (slowly fermentable fraction) and PD (potential) are mL per 0.4 g DM; units for *c* (rate of fermentation of fraction *b*) are mL h^{-1}. With columns, means followed by the same letter are not significantly different at $P = 0.05$

Scientific name	Gas production parameters		
	b	*c*	PD
Anthephora argentea	49.29f	0.044e	23.04f
Aristida vestita	44.13i	0.046d	21.15h
Brachiaria ciliaris	45.64g	0.036j	19.01j
Cenchrus ciliaris	57.79e	0.038i	24.95e
Centropodia glauca	39.59l	0.040g	17.57l
Cynodon dactylon	60.059c	0.047c	29.0b
Digitaria eriantha	58.08d	0.046d	27.7d
Eragrostis trichophora	30.31n	0.057a	16.12m
Panicum coloratum	26.25p	0.038i	11.25o
Pogonarthria squarrosa	28.98o	0.029k	10.71p
Setaria verticillata	44.36i	0.043f	20.48i
Stipagrostis uniplumis	45.12h	0.051b	22.76g
Schmidtia pappophoroides	69.71a	0.051b	35.30a
Stipagrostis obtuse	64.34b	0.039h	28.11c
Stipagrostis ciliate	36.75m	0.039h	16.01n
Tricholaena monachne	42.6k	0.039h	18.56k
s.e.m.	0.003	0.001	0.003
P-value	<0.0001	<0.0001	<0.0001

Methane production was negatively correlated with CP (–0.357*), ash (–0.602*), ether extract (–0.299*), NFC (–0.635*), NDIN (–0.308*) and ADIN (–0.398*) of the grass species (Table 8). A significant positive correlation was noted between methane production and NDF (0.652*), ADF (0.703*), ADL (0.371*), cellulose (0.658*) and hemicellulose (0.643*). There was also a positive correlation between methane production and acetate (0.307*), isobutyrate (0.423*) and butyrate (0.323*).

Linear regression of methane production based on the studied parameters indicated that the equations developed based on NFC, NDF and ADF values can be regarded as better predictors of methane production (g kg DM^{-1}) (Table 9).

Discussion

Plant cover and dry matter yield

The higher plant cover and DM yield for the canopied sub-habitat can be attributed to the high soil fertility under trees, which might result from accumulation of soil that was swept away from

Table 5. Total and individual volatile fatty acid (mM) production, acetate to propionate ratio (A : P), and ammonia-N (mg per 100 mL), in supernatant after 72 h incubation of 400 mg DM of the studied grasses

For NH_3-N, means followed by the same letter are not significantly different at $P = 0.05$

Scientific name	NH_3-N	Acetic	Propionic	Isobutyric	Butyric	Valeric	Total VFA	A : P
Anthephora argentea	7.97m	91.76	30.29	2.68	12.21	2.92	139.9	3.03
Aristida vestita	8.71k	52.35	22.99	1.81	6.79	2.04	85.97	2.42
Brachiaria ciliaris	15.3d	47.97	19.83	1.73	6.80	2.02	78.36	2.60
Cenchrus ciliaris	8.70l	56.34	21.68	1.63	6.17	2.37	88.19	3.26
Cynodon dactylon	10.6h	59.58	18.51	1.97	7.97	2.51	90.54	2.90
Centropodia glauca	15.4c	46.47	16.85	1.70	5.93	2.10	73.05	2.20
Digitaria eriantha	13.6e	48.55	18.81	1.47	5.67	1.74	76.24	2.86
Eragrostis trichophora	11.4f	48.92	15.00	1.55	6.14	2.01	73.62	2.65
Panicum coloratum	19.5a	36.86	12.73	1.52	4.97	1.69	57.77	3.55
Pogonarthria squarrosa	5.31p	51.55	23.41	1.78	6.76	1.99	85.49	2.76
Setaria verticillata	9.18j	54.38	19.04	1.70	5.70	2.20	83.03	2.69
Stipagrostis uniplumis	18.3b	43.90	16.56	1.71	5.95	2.00	70.13	2.28
Schmidtia pappophoroides	6.66n	71.83	20.25	2.21	9.95	2.91	107.2	2.57
Stipagrostis obtuse	6.63o	48.22	17.94	1.55	5.13	1.70	74.54	2.79
Stipagrostis ciliata	9.39j	41.50	14.86	1.82	5.57	2.17	65.92	3.22
Tricholaena monachne	11.0g	52.18	20.31	1.60	6.97	1.90	82.96	2.58
s.e.m.	0.0001							
P-value	<0.0001							

Table 6. Percentage and volumes (mL g^{-1} DM) of methane production from the studied grasses
Within columns, means followed by the same letter are not significantly different at $P = 0.05$

Species	2 h		12 h		24 h		48 h	
	%	mL	%	mL	%	mL	%	mL
Anthephora argentea	2.52cde	0.81ab	7.15b	5.51a	9.02a	12.7a	8.59e	22.7b
Aristida vestita	2.86ab	0.79ab	4.59i	3.15i	5.62h	4.95k	6.02g	8.45h
Brachiaria ciliaris	2.48cde	0.63bcd	5.88g	3.58fg	6.26g	5.65j	8.52e	12.1g
Cenchrus ciliaris	2.26efg	0.18efg	6.54d	2.83j	7.95cde	4.38l	8.16e	7.68i
Centropodia glauca	2.34def	0.37def	6.51d	3.50gh	7.63de	6.14i	9.46d	14.4e
Cynodon dactylon	2.146fg	0.16fg	5.33h	3.22i	4.60i	4.50l	5.16h	7.10i
Digitaria eriantha	1.03g	0.05g	6.36de	3.66f	7.47e	6.53h	9.85d	11.77g
Eragrostis trichophora	2.578dc	0.56bcd	6.44d	4.31d	7.55e	9.85c	10.67c	21.9bc
Panicum coloratum	2.33def	0.21efg	6.03fg	3.17i	8.91a	7.28g	8.09e	14.3e
Pogonarthria squarrosa	2.51cde	0.67bc	7.96a	4.91b	8.34bc	9.11d	11.77a	22.0b
Setaria verticillata	2.39cdef	0.19efg	5.35h	2.89j	5.46h	4.02m	6.41g	6.37j
Stipagrostis uniplumis	2.01g	0.01g	6.20de	4.18e	6.90f	7.61f	7.37f	13.3f
Stipagrostis obtusa	2.44cdef	0.47cde	6.53d	4.41c	8.10cd	9.68c	8.33e	19.1d
Schmidtia pappophoroides	2.53cde	0.46cde	7.20b	4.10e	8.71ab	8.08e	11.06a	19.7d
Stipagrostis ciliata	3.10a	1.01a	6.84c	4.86b	9.18a	11.70b	11.33ab	23.1a
Tricholaena monachne	2.69bc	0.68bc	5.89g	3.42h	6.81f	6.05i	9.69d	13.7ef
s.e.m.	0.093	0.009	0.078	0.032	0.161	0.103	0.190	0.250
P-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Table 7. *In vitro* organic matter digestibility (IVOMD), metabolisable energy (ME), methane (CH_4) production, and per cent of energy lost as methane from the studied grasses after 24 h of incubation
Within columns, means followed by the same letter are not significantly different at $P = 0.05$

Scientific name	IVOMD (g kg^{-1} DM)	ME (MJ kg^{-1} DM)	CH_4 (g kg^{-1} DM)	CH_4 (g kg^{-1} IVOMD)	Lost energy as CH_4 (% ME)
Anthephora argentea	572.9e	11.7b	12.7a	15.6a	2.24a
Aristida vestita	548.8g	9.56g	4.95k	7.73e	1.65fg
Brachiaria ciliaris	484.6j	10.4f	5.65j	9.12d	1.92def
Cenchrus ciliaris	427.1l	9.40h	4.38l	6.33f	1.72gh
Centropodia glauca	511.8h	7.59k	6.14i	9.11d	1.82def
Cynodon dactylon	610.1a	12.3a	4.50l	7.39e	1.20h
Digitaria eriantha	420.7m	8.58i	6.53h	10.30c	1.98bcdef
Eragrostis trichophora	557.9f	11.4c	9.85c	12.7b	1.16h
Panicum coloratum	447.01k	8.16j	7.28g	10.56c	2.18abc
Pogonarthria squarrosa	582.3c	11.6b	9.11d	12.12b	2.12abcd
Schmidtia pappophoroides	407.90o	6.12m	4.02m	10.60c	1.86cdef
Setaria verticillata	577.7d	11.2d	7.61f	6.55f	0.67i
Stipagrostis ciliata	406.9n	8.15j	9.68c	14.9a	2.21ab
Stipagrostis uniplumis	446.9k	6.70l	8.08e	10.32c	1.91def
Stipagrostis obtusa	500.3i	10.7e	11.70b	12.01b	1.72ef
Tricholaena monachne	604.7b	12.2a	6.05i	9.88c	1.31gh
s.e.m.	0.004	0.06	0.103	0.144	0.032
P-value	<0.0001	<0.0001	0.001	0.001	0.001

uncanopied areas in this arid region. Moreover, in such arid rangeland, trees provide better protection of herbaceous groundcover. In addition, the greater plant cover under canopied areas has a positive effect on fertility and water balance of soil, further creating a better microclimate for palatable perennial species with a high water-use efficiency, OM decomposition and nutrient dynamics (Abule *et al*. 2005; Snyman 2005). This better performance in plant cover and DM yield from arid grassland has been reported by other researchers in tropical and subtropical conditions (Abule *et al.* 2005; Snyman 2005; Abdallah *et al*. 2008).

Chemical composition and gas production

The nutrient compositions of studied species were comparable with the values reported for South African feeds and forages by Bredon *et al*. (1987) in a similar season. However, the CP values of most species were low in terms of animal nutritional needs. The cell-wall content (NDF, ADF and ADL) values were high for the studied grasses. The low CP and the high cell-wall contents observed in this study are due to seasonal fluctuation of nutrients, which is partly caused by seasonal dynamics of the plant nutrients. Compared with the wet season, when N is translocated to actively photosynthesising tissues, resulting in a lower carbohydrate : N ratio, in the dry season nutrients

Table 8. Pearson correlation between *in vitro* methane (CH_4) production and chemical constituents of studied grasses
NDIN, ADIN: Neutral and acid detergent insoluble nitrogen; IVOMD, *in vitro* organic matter digestibility. *$P < 0.05$

Major feed components	CH_4	Fibre components	CH_4	Nitrogen component	CH_4	Fermentation characteristics	CH_4	Volatile fatty acid	CH_4
Dry matter	0.206	Neutral detergent fibre	0.652*	Crude protein	–0.357*	Gas production 24 h	–0.13	Total VFA	0.251
Ash	–0.602*	Acid detergent fibre	0.703*	NDIN	–0.308*	Gas production 48 h	–0.20	Acetate (A)	0.307*
Organic matter	0.218	Acid detergent lignin	0.371*	ADIN	–0.398*	Metabolisable energy	–0.15	Propionate (P)	0.13
Ether extract	–0.299*	Non-fibre carbohydrate	–0.635*			IVOMD	0.03	Isobutyrate	0.413*
		Cellulose	0.658*					Butyrate	0.323*
								Valeric	0.16
								A : P	0.223

Table 9. Linear regression equation to predict CH_4 (g/KG DM) from chemical constitutes ME, and IVOMD of the grasses studies
EE, Ether extract; NDF and ADF, neutral and acid detergent fibre; NDIN and ADIN, neutral and acid detergent insoluble nitrogen; ADL, acid detergent lignin; CP, crude protein; NFC, non-fibre carbohydrate

Equation	R^2	P
$CH_4 = 8.05 - 0.048Ash$	0.35	<0.001
$CH_4 = 7.95 - 0.1822EE$	0.07	0.039
$CH_4 = 8.07 - 00171EE - 0.0475Ash$	0.33	<0.001
$CH_4 = -22.7 + 0.035NDF + 0.0653NDIN$	0.49	<0.001
$CH_4 = -7.95 + 0.008NDF + 0.0286ADF + 0.08ADL$	0.46	<0.001
$CH_4 = -6.19 + 0.00264ADF - 0.213ADIN$	0.51	<0.001
$CH_4 = -12.1 + 0.0232NDF$	0.41	<0.001
$CH_4 = -8.08 + 0.0289ADF$	0.48	<0.001
$CH_4 = 1.72 + 0.0453ADL$	0.11	0.009
$CH_4 = 3.99 - 0.0259CP$	0.10	0.013
$CH_4 = 5.98 - 0.01CP - 0.281ADIN - 0.0304NDIN$	0.22	0.003
$CH_4 = 7.30 - 0.356ADIN - 0.0304NDIN$	0.16	0.007
$CH_4 = 8.72 - 0.0233NFC$	0.39	<0.001

accumulate as lignin, cutin, and other phenolic plant defensive substances in the cell wall (Van Soest 1994), and this influences the nutritive quality of forage. This generally results in low fermentation and OMD and consequently results in higher methane emissions. Supplementation with concentrate is important to improve digestibility and enhance propionate production. Small-scale farmers of tropical and subtropical Africa can supplement ruminants with foliage from trees and shrubs, because they remain fairly constant during the early dry period and contain a reasonable amount of CP ($\geq$14%), needed by ruminants for a medium level of production (Subba 1999).

The differences in the volume of gas produced among grass species and duration of times is mainly due to differences in the fermentable OM and fibre contents. This in turn affects the rate and extent of substrate fermentation to short-chain fatty acids, carbon dioxide and methane (Blümmel and Becker 1997). The amount of short-chain fatty acids produced is related to OMD and energy content of feed. However, methane production is an energetic loss, as the portion of animal feed converted to methane and lost through eructation (Getachew *et al*. 2005).

Production of methane and other fermentation end products

In ruminants, methane is produced by rumen microbes through anaerobic fermentation of cell contents and cell-wall contents of feed. The variation in methane production among grass species in the present study may be attributed to their significant differences in chemical constituents such as CP, ash, ether extract, ADF, NDF, ADL, NDIN, ADIN and NFC concentration. Similarly, the difference in methane production across incubation times was attributed to the difference in the rate of fermentation of these feeds. Higher methane production for *Anthephora argentea* and *Aristida vestita* grasses for most of the incubation times was due to their high cell-wall content (ADF, NDF, ADL, cellulose and hemicellulose), NFC content, IVOMD, ME and fermentation potential. In addition, the efficiency expressed as the ratio of methane to total gas produced was also comparatively higher for these grasses. On the other hand, *Cenchrus ciliaris* and *Panicum coloratum* produced the lowest amount of methane during 12, 24 and 48 h, and this might be due to the low level of cell-wall contents (ADF, NDF, ADL, cellulose and hemicellulose) and the acetate : propionate ratio. There is little information on methane production for tropical perennial grasses under tropical conditions, with which to compare our data. We assume that the higher values in our report than for temperate conditions (Getachew *et al*. 2005) might be attributed to relatively higher levels of fibre and lignin (Van Soest 1994) recorded for dry season harvested grasses (Table 1) and associated low values of digestibility, and due to the lower digestibility (13%) of most tropical grasses compared with temperate grasses (Minson 1990).

Similar to our report, there are many findings showing that methane production could be influenced by the contents and nature of cell wall digested (NDF, ADF, ADL, cellulose, and hemicelluloses) (Santoso *et al*. 2003, 2007; Singh *et al*. 2012). This is because the OM digestion, fermentation and production of short-chain VFAs of forages are mainly dependent on their structural factors and the relative proportion of cell types present in their tissues, and the existence of factors restricting microbial access to walls (Van Soest 1994). On the other hand, increasing protein in the diet is expected to decrease methane emission because of direct negative association of protein with methane (Table 7) or the replacement in the diet of methanogenic carbohydrate with protein (Pelchen and Peters 1998).

Association between methane, IVOMD, VFA and chemical composition

In the present study, a significant association was revealed between methane production and various studied parameters. Similarly, many researchers have explained the relationship between the quality of feed expressed in terms of chemical constituents and digestibility, and methane production (Santoso

et al. 2003, 2007; S i n g h *et al*. 2012). This is mainly because fermentability of feed to its end products is primarily determined by digestibility, which mainly depends on its composition. For example, VFA concentration and its relative proportions, which mainly influence methane production, are influenced by the nature and fermentation of carbohydrate (Johnson and Johnson 1995).

The positive correlation between methane production and cell-wall contents (NDF, ADF, cellulose and lignin) observed in this study is in agreement with previous reports (Moss 2000; Singh *et al*. 2012). The negative correlation between CP and methane production is in agreement with the finding of Moss (2000). Moreover, negative correlations of methane production with energy and ether extract are in agreement with previous finding by Yan *et al*. (2009) and Ellis *et al*. (2008), respectively.

Our prediction equations for enteric methane production with ADF and NDF had R^2 values 0.48 ($P < 0.01$) and 0.41($P < 0.01$), whereas equations using protein fractions and carbohydrate fractions had R^2 values 0.51 ($P < 0.03$) and 0.49 ($P < 0.01$), respectively. This shows that carbohydrate and its fractions give a better estimate of *in vitro* methane production from dry-season tropical perennial grasses. This finding is in agreement with previous finding of Johnson and Johnson (1995), who identified that carbohydrate fed to livestock has a major effect on methane production, most likely because of the effect on rumen pH and its microbial population. Similar to this finding, Santoso *et al*. (2007) and Singh *et al*. (2012) indicated that carbohydrate fractions (NDF and ADF) are better methane predictors than feed components.

Conclusion

The results of the present study showed that methane production varied between grass species, which shows room for screening species for lower methane production. The negative correlation between methane production and CP indicates that screening and selecting perennial grass forages for higher CP content will help to mitigate methane production in tropical grassland. The fact that most palatable perennial grasses produced low to medium methane might also indicate that good-quality grasses are good for methane mitigation. Moreover, in formulating a ration for ruminant animals, the use of grass with lower methane production might have potential to mitigate methane emission from agriculture.

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