Morphological characteristics, nutritive quality, and methane production of tropical grasses in Brazil

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Abstract – The objective of this work was to evaluate three tropical forage species for their in vitro methane (CH_4) production and organic matter degradability, in order to determine the relationships between forage grass nutritive quality and CH_4 production. Guinea grass (*Megathyrsus maximus*), palisade grass (*Urochloa brizantha*), and signal grass (*Urochloa decumbens*) were evaluated. Palisade grass showed the highest organic matter, neutral detergent fiber, acid detergent fiber, lignin, and lower-crude protein content. Signal grass had the highest values for hemicellulose and neutral detergent fiber-nitrogen, and the lowest-cellulose content. Guinea grass and signal grass showed a higher-total gas production than palisade grass. Besides, Guinea grass showed an increased CH_4 production, and palisade grass showed lower value for truly degraded organic matter, and reduced partitioning factor, in comparison to signal grass. An increased CH_4 production was observed in cases of lower hemicellulose and paratitioning factor. The nutritive value and CH_4 production of forages may be employed as parameters, aiming at the sustainability of ruminant production.

Index terms: *Brachiaria*, *Megathyrsus maximus*, *Urochloa*, livestock, methane production.

Características morfológicas, qualidade nutritiva e produção de metano de gramíneas tropicais no Brasil

Resumo – O objetivo deste trabalho foi avaliar três espécies de gramíneas tropicais quanto à produção de metano (CH_4) in vitro e à degradabilidade da matéria orgânica, para determinar as relações entre a qualidade nutritiva das gramíneas e a produção de CH_4. O capim-colonião (*Megathyrsus maximus*), o capim-braquiarão (*Urochloa brizantha*) e o capim-decumbens (*Urochloa decumbens*) foram avaliados. O capim-braquiarão apresentou os maiores teores de matéria orgânica, fibra em detergente neutro, fibra em detergente ácido e lignina, e o menor teor de proteína bruta. O capim-decumbens apresentou os maiores teores de hemicelulose e de nitrogênio em fibra de detergente neutro, além do menor teor de celulose. O capim-colonião e o capim-decumbens apresentaram maior produção total de gases do que o braquiarão. Além disso, o capim-colonião apresentou maior produção de CH_4, e o capim-braquiarão, menor degradabilidade verdadeira da matéria orgânica e reduzido fator de partição, em comparação ao capim-decumbens. Maior produção de CH_4 foi verificada em casos em que os valores de hemicelulose e fator de partição foram menores. A qualidade nutricional e a produção de CH_4 das forragens avaliadas podem ser utilizadas como parâmetros a serem empregados, que visem a sustentabilidade da produção de ruminantes.

Termos para indexação: *Brachiaria*, *Megathyrsus maximus*, *Urochloa*, pecuária, produção de metano.

Introduction

One of the advantages of grassland-based livestock production is the ruminant ability to transform fibrous feed of relatively low-nutritional value into products for human consumption. Meanwhile, in this process, enteric methane (CH_4) is produced under anaerobic conditions in the gastrointestinal tract of these animals, via methanogenic Archaea which use carbon-dioxide (CO_2) and hydrogen (H_2) to produce CH_4 during the microbial degradation of feed (McAllister & Newbold, 2008). This process is responsible for a significant loss of dietary energy (5–10%) that could be redirected potentially towards the production of milk and meat (Eckard et al., 2010; Madsen et al., 2010).

Brazil has the largest commercial herd of ruminants in the world, and a great part of these animals is kept on tropical pastures (Bueno et al., 2015) which comprises approximately 170 million hectares of grasslands (Bustamante et al., 2012). The genus *Urochloa* (Syn. *Brachiaria*) is the most dominant, representing 50% of pastures of the country because of its remarkable adaptability and productivity in tropical climate conditions (Cabral et al., 2014; Demarchi et al., 2016). However, despite being more challenging and demanding for soil fertility, the use of *Megathyrsus maximus* is increasing as an option to forage grass due to its high-leaf production and nutritional value, factors that may allow the achievement of a superior animal performance (Fernandes et al., 2014).

However, around 70% of the Brazilian pasture areas show some degree of degradation (Dias-Filho, 2014; Cherubin et al., 2016), which affects their nutritive value and contributes negatively to the efficiency of the system, impairing animal performance, and increasing the CH_4 emission per unit of generated products. In addition to its impacts on the system productivity, CH_4 emission by ruminants is also important for the environmental impact of livestock production, since this activity contributes 33% of the anthropogenic emissions of this greenhouse gas, which has a global warming potential 28 times as higher as that of CO_2 (Eckard et al., 2010; Hünerberg et al., 2015).

Sustainable pasture-based ruminant production may be defined as a system that ensures the adequate generation of animal products and profitability of farming with minimal impact on the environment, making it possible to future generations the use of the land, besides counting with the best use of locally available resources and animal welfare as important aspects of the system (Wathes et al., 2013). By this definition, the amount and quality of forage fed to animals are considered crucial factors, especially regarding the mitigation of enteric CH_4 emissions. Since the ruminant livestock sector is a major contributor to climate change (O'Mara, 2011), it is essential to investigate and determine the most important factors which affect both directly and indirectly the greenhouse gas emissions in animal production.

The objective of this work was to evaluate three tropical forage for in vitro CH_4 production and the organic matter degradability, in order to determine the

relationships between forage grass nutritive quality and CH_4 production.

Materials and Methods

The experiment was carried out in accordance with the Brazilian laws of ethics in animal experimentation, and it was approved by the Ethics Committee on Use of Animals of the Escola Superior de Agricultura Luiz de Queiroz (CEUA-Esalq/USP).

Samples of three forage species: the cultivar Áries of Guinea grass (*Megathyrsus maximus* (Jacq.) B.K.Simon & Jacobs), palisade grass (*Urochloa brizantha*, Syn. *Brachiaria brizantha*), and signal grass (*Urochloa decumbens*, Syn. *Brachiaria decumbens*) were collected from plots located at the experimental station Água Limpa, of Universidade de Brasília, Brasília, DF, Brazil, at 1,014 m altitude. According to the Köppen-Geiger's classification, the climate is an Aw type, with hot rainy summers, and cold dry winters (Nimer, 1989). The predominant soil in the area is classified as an Oxisol, which is acidic showing high-aluminum and low-calcium and magnesium contents (Libano & Felfili, 2006).

In December 2007, Guinea grass (15 kg seed ha⁻¹), palisade grass, and signal grass were sown (40 kg seed ha⁻¹) in three different paddocks (0.375 ha each), combined with P and K fertilization (20 kg ha⁻¹ P_2O_5, and 60 kg ha⁻¹ K_2O), followed by N fertilization (70 kg ha⁻¹ N) thirty days later. Each paddock (0.375 ha) contained only a single grass species, and was divided by electric fences into six parcels of 0.0625 ha. Seventy days after sowing, thirty-six Santa Inês lambs aged four months [21.8±4.14 kg body weight (BW)] were used for grazing in each paddock (12 animals per paddock) for seven days, totaling 35 days of experimental period. From December 2007 to May 2008, the environmental conditions were 21.2ºC of mean temperature (27.5ºC maximum, and 14.9ºC minimum), 83% mean relative air humidity, 163.9 W m⁻² mean solar radiation and 1,241.8 mm of accumulated rainfall. Four samples of 0.25 m² were collected in random spots in each parcel, leaving plant residues with 20 cm height (grazing portion of the forage) prior (pre-grazing) to the entrance of animals in each experimental parcels, which was carried out respecting a 7-day interval.

The collected material was weighed and, then, dried at 60ºC, for 72 hours in air-forced oven. After

that, samples were weighed for determining grass morphological characteristics (kg ha^{-1}) such as available biomass, leaf, stem, and senescent material. Grass nutritive value was assessed by chemical analysis and in vitro gas-production assay, at the laboratory of animal nutrition of the Centro de Energia Nuclear na Agricultura (Lana-Cena/USP).

Dry matter (DM), organic matter (OM), and crude protein (CP) concentrations were determined according to Horwitz et al. (2011). Neutral detergent fiber (NDF) was analyzed according to Mertens (2002), and acid detergent fiber (ADF) and lignin (ADL) were determined sequentially, following the methodology of Van Soest et al. (1991). Hemicellulose (HEMI) and cellulose (CEL) were calculated by the differences between NDF, ADF, and ADL. Also, nitrogen bound to NDF (N-NDF) was determined according to Sniffen et al. (1992).

The CH_4 production, the truly degraded organic matter (TDOM), and the in vitro gas production assays were conducted according to the methodology described by Theodorou et al. (1994) and Mauricio et al. (1999), adapted to a semiautomatic system by Bueno et al. (2005).

Eight adults from 6.3 to 1.7-year-old rumen-cannulated Santa Inês male sheep (60±2.5 kg of BW) were used as inocula donors. The animals fed tropical grass pasture, a concentrate mixture (0.7 kg 100 kg^{-1} BW; 70% ground corn, and 30% soybean meal), and had ad libitum access to mineral supplement and water. Before morning feeding, ruminal liquid and solid fractions were collected from each animal, and kept at 39ºC under anaerobic conditions until inoculation was carried out. Four inocula were prepared (two animals per each inoculum) adopting a 50:50 solid:liquid ratio (on a volume basis) (Bueno et al., 2005).

Half gram of each dried ground grass sample was weighed in filter bags (Ankom F57), and incubated in 160 mL bottles with 50 mL of incubation medium (Menke's buffered medium), and 25 mL of rumen inocula, to complete 75 mL of final volume and 85 mL of head space (Longo et al., 2006; Abdalla et al., 2012). The bottles were immediately sealed with 20 mm butyl septum stoppers (Bellco Glass Inc, Vineland, NJ, USA), then manually mixed and incubated at 39ºC in a forced-air oven (Marconi MA35, Piracicaba, SP, Brazil) for 24 hours. Two bottles (experimental units) were incubated per grass sample with each one of the four inocula. The same scheme was used for the internal standard samples ['Tifton' hay (*Cynodon dactylon*)] and blank (bottles without substrate, containing rumen inoculum + medium), to correct the gas production (GP) from the inoculum. Gas pressure was measured using a pressure transducer and data logger (Pressure Press Data 800) at 4:00, 8:00, 12:00 and 24:00 h, in order to determine the GP, using the equation $V = 7.365 \times p$, in which: V is the gas volume (mL); and p is the measured pressure (psi) (Araujo et al., 2011).

For the measurement of CH_4 using a gas chromatograph system (Shimadzu GC 2014, Chiyoda-ku, Tokyo, Japan), during each one of the pressure measurement events, 2.5 mL gas samples were collected from the bottles, using 5 mL syringes, and stored in 10 mL vacuum tubes, as described by Soltan et al. (2012). Gas production was expressed as (mL DM[-1]), and CH_4 was expressed as a percentage of GP (%), as well as per unit of truly degraded organic matter (mL g^{-1} TDOM).

The TDOM was determined by neutral detergent solution (NDS) treatment according to Van Soest et al. (1991), but with some modifications. At the end of the 24-hour incubation, all the Ankom F57 (Ankom Technology, Macedon, NY, USA) bags (with the nondegraded samples) were removed from the bottles, and immediately immersed in cold water (-4ºC), so that the microbial fermentation process was interrupted. All the bags were treated with NDS for one hour at 90°C, washed with hot water and, then, acetone. The DM and ash of the residual were determined. The difference between the incubated OM sample and the nondegraded residual OM was the TDOM. The partitioning factor (PF) was calculated by the ratio between TDOM (mg) and gas volume (mL) at 24-hour incubation (Blümmel et al., 1997).

The statistical analysis was performed using the software SAS v.9.4 (SAS Institute Inc., Cary NC, USA). The obtained data were subjected to analysis of variance, using the Proc GLM procedure, at 5% probability. Correlation (Proc Corr) and factor analyses (Proc Factor) were performed to verify the relationships between the plant chemical composition and the in vitro gas production variables. Stepwise (Proc Stepdisc) and canonical (Candisc) discriminant analyses were carried

out, to verify which characteristics were important in discriminating between the grass species.

Results and Discussion

In addition to the results of the present study, besides considering grasses grown in the same soil type, temperature, photoperiod, and rainfall conditions, it is important to take into account that using the same grazing management for different grass species may affect their qualitative parameters, such as morphological (Table 1) and chemical characteristics (Table 2). The correlations between grass characteristics and gas production variables are present in Table 3. To minimize possible impairment of these characteristics, it is important to consider the concept of the critical leaf area index (LAI), to determine the moment of grazing or cutting for each grass species, a condition in which the grass canopy intercepts 95% of the photosynthetically radiation and is near its maximum growth rate, without shading itself (Rhodes, 1971; Parsons et al., 1983). After this moment, the senescence and stem growth rate increase, decreasing the grass nutritive value and leaf production in many grass species (Silva et al., 2015).

Overall, the specific grazing management for each grass can increase the use efficiency of forage by resulting in better canopy structure and nutritive value (Pereira et al., 2015; Anjos et al., 2016), and should be taken into account when discussing the suitability of each of these grass species for the sustainable livestock production in Brazil.

As to the chemical composition, Guinea grass and signal grass showed better nutritive value due to their high-protein and low-fiber contents (Table 2), which leads to improvement of productivity of ruminant livestock in tropical regions. The superior quality characterized by the higher-protein and low-cellulose contents for signal grass was associated with a decrease of their CH_4 production (Table 2).

Rumen microbiota requires a minimum crude protein of 70-80 (g kg^{-1} DM) to optimize the breakdown of cell wall components, otherwise, the use of diets with lower-protein levels than these, may result in a reduced feed intake (Van Soest, 1994). Therefore, in a ruminant production system based on grass species, with crude protein content lower than 70 mg kg^{-1} DM (as Palisade grass, Table 2), nitrogen supplementation is an alternative that can be used, in order to improve feed intake and digestion by the animals (Sampaio et al., 2010).

The amount of feed intake affects enteric fermentation and CH_4 production, even when these are also moderated by feed digestibility and animal characteristics (Hegarty et al., 2007; Liu et al., 2017). In general, increasing feed intake reduces CH_4 per unit of consumed feed, which is usually attributed to a shorter time of digesta retention in the rumen, and,

Table 1. Morphological characteristics of the three experimental grass species[1].

Grass species	Days						Mean	SEM
	0	7	14	21	28	35		
Available biomass (kg DM ha^{-1})								
Guinea grass	5,691A	4,200A	3,697B	5,384A	3,212B	4,012A	4,366B	379.16
Palisade grass	8,511Aa	4,606Ab	7,732Aab	8,166Aab	7,151Aab	6,345Aab	7,085A	367.12
Signal grass	6,185A	6,195A	6,438AB	6,371A	5,941AB	4,724A	5,976A	367.12
Leaf (kg ha^{-1})								
Guinea grass	361A	787B	891B	977B	482B	192B	615B	80.73
Palisade grass	740Ab	1,159Aab	1,711Aa	1,823Aa	1,730Aa	1,314Aab	1,413A	78.17
Signal grass	698Ab	1,070Aab	1,602Aa	1,643Aa	1,313Aab	843ABab	1,195A	78.17
Stem (kg ha^{-1})								
Guinea grass	853Ab	3,173Aab	2,628Bab	3,898Aa	2,463Bab	3,926Aa	2,824B	297.34
Palisade grass	2,530Ab	3,377Aab	5,876Aa	6,099Aa	5,049Aab	4,801ab	4,622A	287.90
Signal grass	1,897A	4,729A	4,588AB	4,365AB	4,285AB	3,572A	3,906AB	287.90
Senescence (kg ha^{-1})								
Guinea grass	349A	487A	151A	490A	268A	293A	340A	43.04
Palisade grass	233A	95B	111A	246A	398A	161A	207A	41.67
Signal grass	352A	402AB	239A	374A	248A	301A	319A	41.67

[1]Means followed by equal letters, uppercase within a column and lowercase within a row, do not differ by Student's test, at 5% probability. SEM, standard error of the mean.

consequently, to less extensive fermentation (Pinares-Patiño et al., 2003; Yan et al., 2010; Hammond et al., 2013). However, it is important to consider that variations of feed intake not always affect the whole tract digestibility (Molano & Clark, 2008).

Furthermore, our results showed that grasses with higher biomass amount (Table 1) tend to increase the cell wall fraction, and to decrease the soluble contents of the plants, thus reducing the feed quality (Table 2). Improving forage quality, both through feeding with lower-fiber and higher-soluble carbohydrates can reduce CH_4 production (Beauchemin et al., 2008; Hristov et al., 2013). Rodríguez et al. (2007) reported

that a proper diet synchronization of available nitrogen and energy for microbial utilization should lead to a decreased CH_4 production. Enhancing forage quality (for instance, low-fiber and high-soluble carbohydrates) also tends to increase voluntary intake and to decrease retention time in the rumen, promoting energetically more efficient post-ruminal digestion, and reducing the proportion of dietary energy converted to CH_4 (Eckard et al., 2010). Overall, the inverse relation between feed intake level and CH_4 yield offers an opportunity to lower emissions while increasing animal productivity (Liu et al., 2017), contributing to the sustainability of livestock production.

Table 2. Chemical composition (g kg^{-1} DM), gas production (GP), methane production (CH_4), truly degraded organic matter (TDOM), and partitioning factor (PF) of the experimental grass species[1].

Parameter[2]	Guinea grass	Palisade grass	Signal grass	p-value	SEM
Organic matter (OM)	941.0B	957.0A	941.0B	<0.0001	1.68
Crude protein (CP)	102.0A	41.0B	108.0A	<0.0001	54.17
Neutral detergent fiber (NDF)	708.0B	759.0A	717.0B	0.0110	9.75
Acid detergent fiber (ADF)	466.0B	524.0A	417.0C	0.0005	11.87
Acid detergent lignin (ADL)	58.0B	82.0A	52.0B	<0.0001	2.77
Hemicellulose (HEMI)	242.0B	234.0B	299.0A	<0.0001	4.48
Cellulose (CEL)	407.0A	442.0A	365.0B	0.0028	11.01
N-NDF	45.0B	33.0B	65.0A	0.0039	4.87
TDOM (g kg^{-1} OM)	437.2A	378.0B	463.2A	0.0108	15.62
Total gas production (GP, mL g^{-1} DM)	116.1A	101.2B	111.6A	0.0245	31.92
CH_4 (%)	4.8A	3.1B	3.5B	0.0126	0.334
CH_4 (mL g^{-1} TDOM)	12.9A	8.3B	8.6B	0.0068	0.845
Partitioning factor (PF)	3.9AB	3.8B	4.3A	0.0527	0.125

[1]Means followed by equal letters within a row, do not differ by Student's test, at 5% probability. [2]N-NDF, nitrogen in NDF; values expressed on dry matter basis (g kg^{-1} DM); CH_4, methane production as percentage of GP; CH_4 (mL g^{-1} TDOM), methane production per gram of truly degraded organic matter; TDOM (g kg^{-1} OM), truly degraded organic matter. SEM, standard error of the means.

Table 3. Pearson correlations coefficients between grass characteristics and gas production.

Parameter[1]	OM	CP	NDF	ADF	ADL	HEMI	CEL	N-NDF	GP	CH_4 (%)	CH_4 (mL g^{-1} TDOM)	TDOM
Crude protein (CP)	-0.86*											
NDF	0.87*	-0.64*										
ADF	0.84*	-0.73*	0.82*									
ADL	0.88*	-0.89*	0.74*	0.87*								
Hemicellulose (HEMI)	-0.55	0.58*	-0.39	-0.85*	-0.71*							
Cellulose (CEL)	0.77*	-0.63*	0.79*	0.98*	0.77*	-0.85*						
N-NDF	-0.70*	0.69*	-0.58*	-0.82*	-0.72*	0.79*	-0.81*					
GP	-0.81*	0.65*	-0.81*	-0.65*	-0.72*	0.29	-0.58*	0.67*				
Methane (CH_4, %)	-0.44	0.44	-0.58*	-0.26	-0.51	-0.11	-0.15	0.14	0.60*			
CH_4 (mL g^{-1} TDOM)	-0.35	0.34	-0.47	-0.11	-0.40	-0.26	0.00	0.04	0.58*	0.97*		
TDOM	-0.80*	0.74*	-0.78*	-0.85*	-0.83*	0.65*	-0.81*	0.73*	0.73*	0.40	0.22	
Partitioning factor (PF)	-0.40	0.47	-0.34	-0.60*	-0.52	0.65*	-0.60*	0.40	0.09	-0.02	-0.26	0.74*

[1]NDF, neutral detergent fiber; ADF, acid detergent fiber; ADL, acid detergent lignin; N-NDF,– nitrogen in NDF; GP, total gas production; TDOM, truly degraded organic matter. *Significant at 5% probability.

Despite the similar gas production showed by Guinea grass and signal grass (Table 2), it is important to consider that CH_4 (mL g^{-1} TDOM) is the most adequate parameter to evaluate the CH_4 mitigation potential of a given substrate, when performing in vitro assays. In this context, Guinea grass did not show to be the most suitable option due to its increased cell wall content – which limits its consumption and nutritional value (Van Soest, 1994) – and CH_4 (mL g^{-1} TDOM) production, in comparison with signal grass (Table 2). Furthermore, the increased protein level observed in those grasses contributed to ruminal DM degradability, which consequently led to a higher-gas production (Sallam et al., 2010). In such cases, the increased gas production, as a consequence of the increased degradability, may contribute towards the sustainability of the system as well, since higher-animal production indexes may be observed in those situations, leading to a higher generation of animal products.

The lower-protein content, as well as the high-cell wall and lignin found in palisade grass impaired the degradation of its organic matter possibly due to microbial inefficiency (Table 2). This fact led to a lower-

gas production and CH_4 (mL g^{-1} TDOM) than those of Guinea grass (Table 2). Therefore, it is important to emphasize that the crude protein content of palisade grass was lower than 70 g kg^{-1} DM (Table 2), which is not enough to sustain an optimal microbial activity to achieve an efficient ruminal fermentation (Van Soest, 1994).

Forages with higher-crude protein and nitrogen content were capable of promoting high-degradation rates by ruminal microbiota for the reason that nitrogen stimulates the microbial growth and ruminal efficiency (Table 2). Methane is a by-product of the breakdown of carbohydrate molecules in the rumen, and characteristics of those (structural or nonstructural ones) are determining factors regarding CH_4 production (Hristov et al., 2013). Therefore, despite the similar crude protein levels showed by Guinea grass and signal grass, the increased cellulose content observed for the *Megathyrsus* species has led to an increased CH_4 (Table 2), which explains the positive correlation observed for this plant with both CH_4, as a percentage of total gas production, and the degraded organic matter (Figure 1).
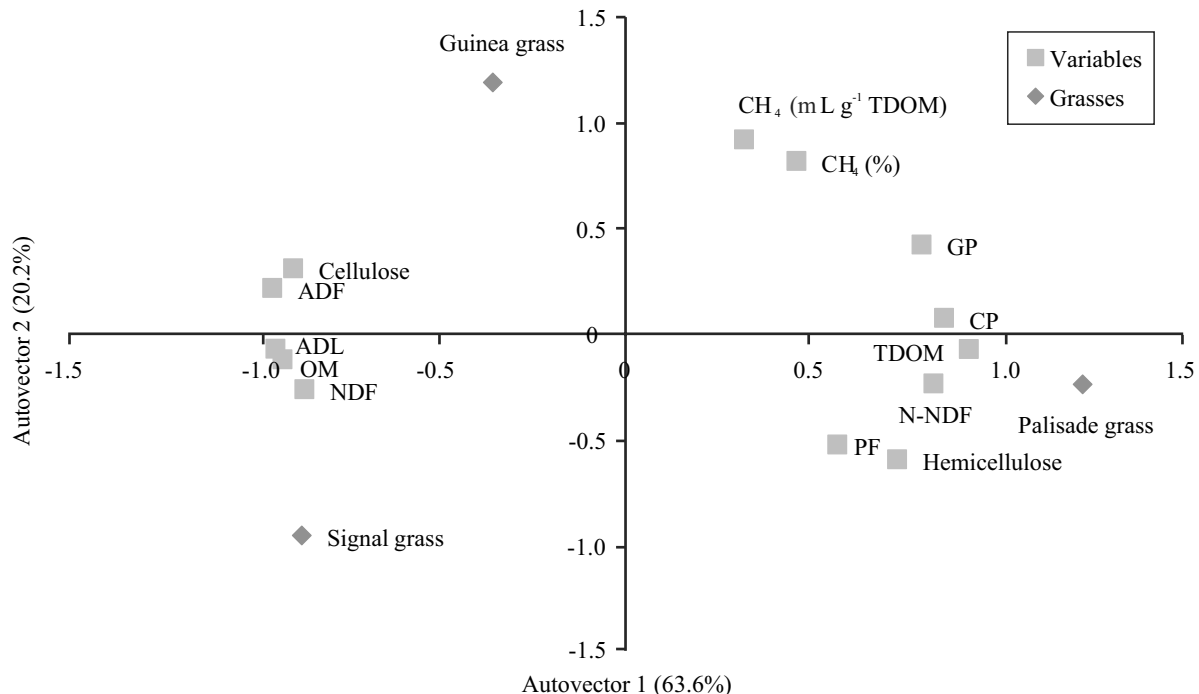

Figure 1. Two main factors analysis showing the relation between plant chemical composition and in vitro gas production variables. OM, organic matter; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; ADL, acid detergent lignin; GP, gas production; N-NDF, nitrogen bound to NDF; TDOM, truly degraded organic matter; and PF, partitioning factor.
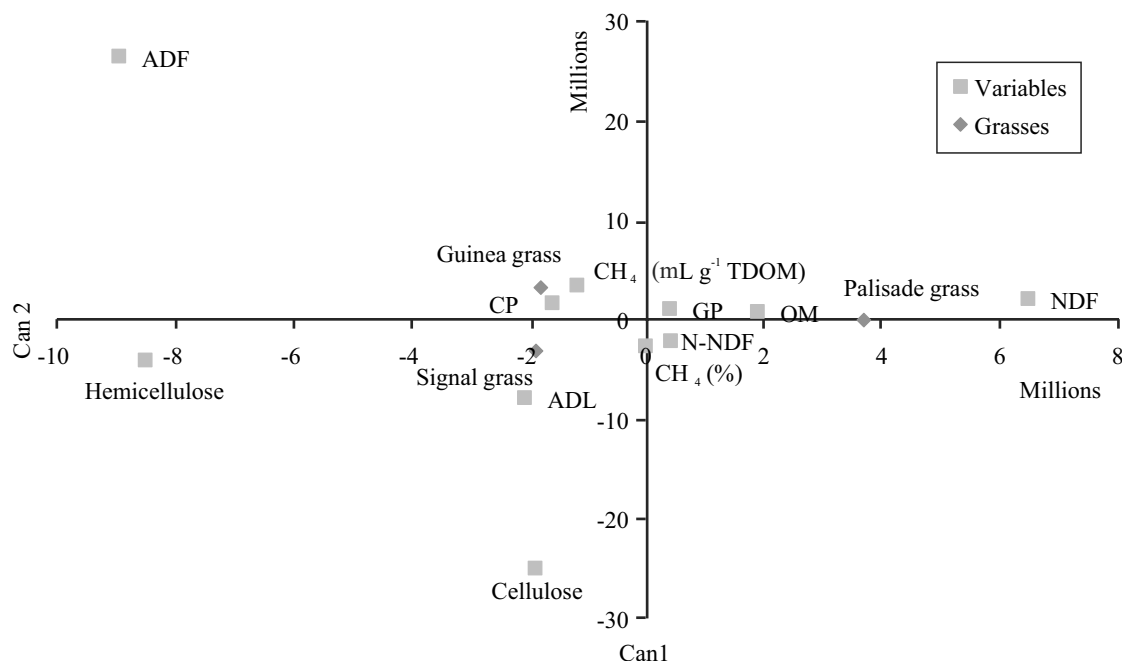
Figure 2. Discriminant canonical (Can) analysis for chemical and gas production variables of in vitro gas production of tropical grasses grazed by sheep. OM, organic matter; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; ADL, acid detergent lignin; GP, gas production; N-NDF, nitrogen bound to NDF; and TDOM, truly degraded organic matter.

The most remarkable discriminatory variable in our study was CH_4 (mL g^{-1} TDOM), which was increased in Guinea grass (Figure 2). Generaly, the *Megathyrsus* grass species shows a better nutritional quality (fiber and protein content) than the *Urochloa* (Syn. *Brachiaria*) grasses. Despite that, in the present study, Guinea grass showed a higher-ADF and cellulose content than signal grass (Table 2) which had a higher hemicelulose content (potentially more degradable fiber fraction), possibly due to forage management and soil fertility conditions employed here. However, an in vivo study is necessary to precisely characterize these grass species, considering the animal performance and its methanogenic potential, which are key factors concerning the sustainability of pasture-based ruminant production.

Conclusions

1. Chemical characteristics and nutritional quality show to be major aspects concerning CH_4 production.

2. Despite the similar organic matter degradatibility of Guinea grass with the other grass species, its poorest fiber quality led to an increased CH_4 production.

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