CHEMICAL COMPOSITION, *IN VITRO* RUMEN FERMENTATION, AND DIGESTIBILITY OF SELECTED BROWSE SPECIES BY BUFFALOES (*Bubalus bubalis* L.) AT A SEMI-DECIDUOUS FOREST IN VENEZUELA

[COMPOSICIÓN QUÍMICA, FERMENTACIÓN RUMINAL *IN VITRO* Y DIGESTIBILIDAD DE ESPECIES LEÑOSAS SELECCIONADAS POR BÚFALOS (*Bubalus bubalis* L.) EN UN BOSQUE SEMICADUCIFOLIO EN VENEZUELA]

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SUMMARY

To determine the chemical composition and nutritive value of selected browse species by buffaloes (*Bubalus bubalis* L.) in a silvopastoral system at a semi-deciduous tropical forest (SDTF), epidermal fragments in fecal samples from 14 males of Murrah breed were evaluated. The animals grazed for 91 days in 85 ha located in Portuguesa State, Venezuela, of which 35 ha corresponded to natural grass pastures (969.3 ± 28.6 kg DM ha^{-1}), and the rest to SDTF with 463 plant ha^{-1}. Eleven botanical families and 22 species were identified, 40.9% belonged to the Fabaceae family. The specie *Guazuma ulmifolia* (Sterculiaceae) had the highest relative dominancy (60.4%) and an Importance Value Index of 162.6. 60.4% of epidermal fragments corresponded to herbaceous Poaceae, and the rest was distributed among five different species, with the highest occurrence (94.6%) for *Samanea saman* and *Sida acuta*. There were differences (P<0.05) among selected species in the proximal composition (40.9 ± 7.1% dry matter, 16.0 ± 4.1% crude protein and 2.9 ± 1.2% ether extract), neutral detergent fiber (41.6 ± 2.2%), acid detergent fiber (25.9 ± 3.8%), minerals (1.6 ± 0.59% Ca and 0.1 ± 0.02% P), condensed tannins (0.9 ± 0.7%), potential gas production (67.8 ± 37.3 ml g^{-1} DM), and *in vitro* organic matter digestibility (54.2 ± 8.0%). In general, the edible fraction of selected browse species evaluated here was similar to other tropical browse forages and confirms the strategic value of this biomass in buffalo nutrition at silvopastoral system. Furthermore, these browse species could be employed in agroforestry systems to achieve a better integration of livestock production in tropical areas.

Key words: Selectivity; digestibility; tannins; agroforestry.

RESUMEN

Para determinar la composición química y el valor nutricional de las especies seleccionadas por búfalos (*Bubalus bubalis* L.) en un sistema silvopastoril en bosque tropical semicaducifolio (BTSC) fueron evaluados fragmentos epidérmicos en muestras de heces de 14 machos de la raza Murrah. Los animales pastaron durante 91 días en 85 ha localizadas en el estado Portuguesa, Venezuela, de las cuales 35 ha correspondieron a pastos naturales (969.3 ± 28.6 kg MS ha^{-1}) y el resto a un BTSC con 463 plantas ha^{-1}. Once familias botánicas y 22 especies fueron identificadas, perteneciendo el 40.9% a la familia Fabaceae. La especie *Guazuma ulmifolia* (Sterculiaceae) tuvo la mayor dominancia relativa (60.4%) y un Índice de Valor de Importancia de 162.6. El 60.4% de los fragmentos epidérmicos correspondieron a herbáceas de la familia Poaceae, y el resto se distribuyó en cinco especies diferentes, con la mayor presencia (94.6%) para *Samanea saman* y *Sida acuta*. Hubo diferencias (P<0.05) entre especies seleccionadas en la composición proximal (40.9 ± 7.1% MS, 16.0 ± 4.1% proteína cruda y 2.9 ± 1.2% extracto etéreo), fibra detergente neutra (41.6 ± 2.2%), fibra acida detergente (25.9 ± 3.8%), minerales (1.6 ± 0.59% Ca y 0.1 ± 0.02% P), taninos condensados (0.9 ± 0.7%), producción potencial de gas (67.8 ± 37.3 ml g^{-1} MS) y digestibilidad *in vitro* de la materia orgánica (54.2 ± 8.0%). En general, la fracción comestible de las especies leñosas seleccionadas fue similar a otros forrajes tropicales leñosos, y confirma el valor estratégico de esta biomasa para búfalos en un sistema silvopastoril. Adicionalmente, estas especies leñosas pueden ser empleadas en sistemas agroforestales para obtener una mejor integración de la producción animal en áreas tropicales.

Palabras clave: Selectividad; digestibilidad; taninos; agroforestería.

INTRODUCTION

Buffalo (*Bubalus bubalis* L.) herds are usually managed as a triple purpose systems (milk, meat and draft-power), taking advantage of their rusticity, high fertility rates and ability to adapt to different climatic conditions (Borghese and Mazzi, 2005). Additionally, this specie show higher efficiency in the use of fibrous resources, superior dry matter intake and digestibility due to an adjusted selectivity of the forage quantity and quality, as well as to a higher nitrogen recycling and retention time of the feed (Ichinohe *et al*., 2004; Corrêa *et al*., 2008).

In tropical America, buffalo herds are distributed over large extensions of savannas with presence of sparse forest formations, under a rainy regime with marked seasonality which determines a wide annual variability in the quantity, quality and structure of forage biomass produced. During the dry season, to increase the offer and nutritional value of forage, animals are allowed to have access to the forest when annual availability of grasses and other herbaceous plants are reduced. These seasonal silvopastoral systems present higher biological, economic, social and ecological sustainability than other traditional systems (Corrêa *et al*., 2008).

Due to their protein crude content (16.5 to 23.2%), neutral detergent fiber (39.5 to 49.8%), gross energy (15.0 to 20.3 kJ g^{-1} DM), degradability of dry matter (41.3 to 73.2%), biomass of trees and shrubs present in tropical forests can contribute to improve the quality of the animal diet (García and Medina, 2006). Even though there are no studies that evaluate the selectivity by buffaloes in silvopastoral conditions in tropical dry forests, evaluations with sheep (García and Medina, 2006) and cows (Baldizán *et al*., 2006) point out that it is related with the participation of secondary metabolites in the foliage, where stand out polyphenols, alkaloids, saponins, terpenes, and phytic phosphorus. In humid forest, it has been found that, in spite of a marked preference for grasses, up to 32.7% of the buffalo diet can be formed by shrubby dicots (Bekhuis *et al*., 2008).

To improve buffalo efficiency in the seasonal grazing of these tropical forests, it is required to generate information about its feeding habits, as well as the chemical and nutritional characterization of woody species with forage importance. This information would allow building programs for sustainable management that would add value to the forests and promote an active role of the farmers on the biodiversity conservation of these ecosystems. The study therefore, aimed to investigate the chemical composition and nutritional value of woody plants selected by buffaloes grazing a tropical semi-deciduous forest in Venezuela.

MATERIALS AND METHODS

Study site

The study was carried out during the dry season (January to May 2010), at the San Nicolás Experimental Farm (8º 49´ 58´´ N and 69º 48´ 00´´ W) of the Universidad Central de Venezuela, located in Portuguesa State, Venezuela. During the experimental period, the cumulative rainfall was 246.8 mm, mean temperature 27.0 ± 0.72ºC, potential evapotranspiration 892.5 mm, and relative humidity of 63%. The soils were classified as Fluventic Haplustepts and Vertic Endoaquepts (Abarca, 2005), with clay fine mixed, non acid and isohypertermic. The total experimental area (85 ha) was delimited by a perimetrical fence and consisted of a semi-deciduous tropical forest with low level of human intervention (50 ha), composed by herbaceous and graminaceous vegetation (35 ha) dominated by *Cynodon nlemfuensis*, *Sporobolus indicus*, *Axonopus sp*., *Mimosa pudica*, and *Hyptis suaveolens*. Paddocks had a mean yield of 969.3 ± 28.6 kg DM ha^{-1} and a leaf:stem ratio of 0.53 ± 0.15, evaluated according to Greig-Smith (1983).

Botanical composition and browse species sampling

Botanical composition of the woody species in the forest was determined in 12 transects of 100 to 200 m length and 2 to 4 m wide, by the quadrant point technique (Muller-Dumbois and Ellemberg, 1974) with 10 m distance between points. Considering a height equal or lower than 2 m as the susceptible browsing strata by buffaloes, monthly samples (900 g) of green foliage (leaves, leaflets, reproductive structures, and woody material with diameter less than 6 mm) were collected for chemical analysis. Ten woody plants per species selected by the animals were chosen randomly, observing that all plants had similar age and phenological stage. Leaves and flowers of the browsed species were pressed, labeled, and dried for latter identification at the Botanical Herbarium Victor Manuel Badillo (MY) of the Universidad Central de Venezuela.

Animal management

Fourteen male adult buffaloes of Murrah breed (446.9 ± 19.3 kg LW) were used in continuous grazing with free open access to the forest. Water availability and a commercial blend of minerals and salt (20% Ca, 10% P, 9% Na, 0.6% S, 0.5% Mg, 0.5% Zn, 0.25% Mg, 0.25% Cu, 0.08% F, 80 ppm I, 20 ppm Co, and 20 ppm Se) were offered to the animals on an *ad libitum* basis. To allow selectivity with usual stocking rates for these savannas, additional animals were used to obtain a grazing pressure of 6 kg DM for 100 kg LW.

Fifteen days before evaluation, all animals were dewormed with levamisol chlorhydrate (1 ml for 50 kg LW) and dosed with 1 ml for 100 kg LW of a multivitamins complex (500 000 UI ml^{-1} vit. A, 75 000 UI ml^{-1} vit. D3, and 50 UI ml^{-1} vit. E).

Fecal collection and preparation

Fecal samples were individually taken every 15 d (8:00 to 10:00 h) directly from the rectum. Once collected, samples were placed in plastic bags and stored at -18°C. Later, at the laboratory, material was dehydrated until constant weight in a forced convection oven at 65°C, grounded with a hammer mill to pass a 1 mm screen and then, 2 g were rehydrated with ethylic alcohol of 50% v/v. Samples so treated were screened (180 µ) and plant pigments removed with sodium hypochlorite (Holechek, 1982). After that, 20 ml of glycerin (10%) and four drops of tolouidine blue (1%) were added to prepare 3 semi-permanent plates (24 x 24 mm) for each sample. Evaluation of epidermic fragments was performed with a binocular microscope at 400X, using transversal views of 2 mm wide, 60 mm length and 3 mm between transversals. As reference, at the beginning of the experimental phase, digital photographic patterns of the woody species were development using as identification criteria, the presence, frequency, shape, size and distribution of trichomes, stomata, crystals, and typical epidermal cells.

Botanical and selectivity indexes

In the forest, plant density, dominance and frequency were estimated according to Muller-Dumbois and Ellemberg (1974). The Importance Value Index (IVI), a non-dimensional parameter that estimates the relative ecological success of a species on a plant community, was determined by the following equation (Barbour *et al*., 1999):

$$IVI = \Sigma\ [\text{Density (\%)} + \text{Frequency (\%)} + \text{Dominance (\%)}]$$

Selectivity of woody species was evaluated through the Ivlev Selectivity Index (ISI), where values varies between +1 (maximum preference) and -1 (total rejection), while records close to zero indicates that the species are selected in direct relationship with its density in the grazing area (Lechowicz, 1982). The formula is:

$$ISI = \left[\frac{\text{Plant 1 epidermic fragments in feces (\%)} - \text{IVI Plant 1 (\%)}}{\text{Plant 1 epidermic fragments in feces (\%)} + \text{IVI Plant 1 (\%)}} \right]$$

Chemical analyses

For the determination of phenolic compounds and *in vitro* studies, foliage samples from selected browse species were dehydrated at room temperature until constant weight in a dry and dark environment. For the other analysis, samples were dehydrated in a forced convection oven at 65°C. All samples were later ground with a hammer mill to pass a 1 mm screen and stored in amber containers. The dry matter (DM), nitrogen, fat, ash, calcium (Ca), and phosphorous (P) contents were determined using the methods of the AOAC (1999). The organic matter (OM) content was calculated as 100-ash content. Crude protein (CP) was calculated by multiplying nitrogen content with a factor of 6.25. The neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined by the method of Van Soest *et al*. (1991), adding sodium sulfite to remove the tannins-protein complex (Terril *et al*., 1994). Extracted total phenols (TP, tannin acid equivalent) were determined by a modified Folin-Ciocalteu assay (Makkar, 2001) and the extracted condensed tannins (CT, leucocyanidin equivalent) were determined using the method of Porter *et al*. (1986).

***In vitro* gas production and digestibility**

Browsed foliage samples were tested for *in vitro* ruminal fermentation with a gas pressure transducer technique as described by Mauricio *et al*. (1999). Foliage DM (1 g), with or without extra 1 g polyethylene glycol with relative molecular mass 8000 (PEG 8000, Merck®), were weighed into serum bottles with 168 ml capacity. Bottles were filled with 90 ml of a mixture of mineral and buffer solution and 4 ml of reducing agent (Menke and Steingass, 1988). After adding 10 ml of rumen fluid, eight replications of each treatment were incubated in a pre-heated water bath at 39°C. Additionally, four blanks with buffer solution, reducing agent, and rumen fluid were incubated in each experiment. The mixed rumen content (50:50 solid and liquid phase) was collected at 07:00 h from two rumen cannulated cows (429.5 ± 19.6 kg LW). Cows grazed on a medium-quality pasture dominated by *Cynodon nlemfuensis*, and supplemented daily with 2 kg of concentrate (2.7% N, 7.6% ash, and 37.2% NDF). The serum bottles were sealed by a rubber stopper and gas pressure was set to zero using a transducer and a LED digital readout voltmeter (Red Lion®, Model DP5-1/8 DIN). Gas pressure was recorded at 3, 6, 9, 12, 24, 36, 48, 60, 72, and 96 h after the inoculation. The gas data were fitted to an exponential model according to France *et al*. (1993).

Once readings of gas pressure were done, the bottles were placed in an inverse water bath (4°C). The contents were filtered into glass cresols with porous

porcelain plates (pore # 1) previously weighted, to be later dehydrated at 105°C until constant weight. Apparent digestibility (48 and 96 h) was calculated by weight difference between the substrate for the organic matter (OMd) and NDF (NDFd) fractions.

Statistical analysis

The relative frequency of epidermal fragments in feces was analyzed by non parametric statistics, using the Kruskal-Wallis's test, and the chemical composition and nutritive value were examined by a completely randomized design, considering each species as a treatment. ANOVA was conducted on data thorough a linear model. Those variables that showed significant effect by treatments were subjected to Tukey's test. Correlation analyses were used to establish associations between chemical, *in vitro* gas production, and digestibility parameters. The information was processed using the statistical software SAS (2001), with a significance level of $p<0.05$.

RESULTS

The floristic inventory showed the presence of 11 botanical families that grouped 22 identified woody species, 40.9% belonged to the Fabaceae, followed in importance by the Malvaceae, Moraceae and Polygonaceae, which together represented 27.3% of woody species, while 31.8% of the species was distributed in the other six families (Table 1). *Guazuma ulmifolia* (Sterculiaceae), *Pithecellobium lanceolatum* (Fabaceae), *Samanea saman* (Fabaceae) and *Guadua angustifolia* (Poaceae) represented 80.2% of the total species (463 woody plants/ha). *G. ulmifolia* showed high dominance in the community due to its high density and plant development and also the highest IVI, which together with *S. saman* and *P. lanceolatum*, accumulated 71.3% for this index.

A high proportion of the epidermic fragments in the feces corresponded to graminaceous forage, and the other was distributed among five woody species with the highest participation for *S. saman* and *Sida acuta* (Table 2). A small proportion (3.7%) of the fragments did not show morphoanatomic characteristics that could allow its botanical identification. This limitation is due to the integrity degree of the fragments when consumed by the animals, the sensitivity to degradation during its passage through the gastrointestinal tract, and to refringency to colorants used (Bekhuis *et al*., 2008).

The chemical composition of foliage of selected browse species is shown in Table 3. *I. laurina* and *S. saman* had the highest CP content, and *I. laurina* had the highest NDF. Ca content ranged from 0.84 to 2.21%, while P content did not show significant differences among species. TP and CT varied widely, with the highest values in both cases for *C. caracasana*.

There were a low level of hemicelluloses (15.7 ± 5.4%), and the high proportion of ADF in the cell wall suggests high contents of lignin and cellulose.

The *in vitro* gas parameters, OMd, and NDFd values varied significantly ($P<0.05$) among browsed species (Table 4). The rate of DM degradation was similar among species and the time needed for microorganism to *in vitro* colonize the vegetal tissue (To) was highest for *S. acuta* and *R. spinosa*, and similar among the other species. T½ ranged from 23.1 to 11.6 h, with the lowest value for *R. spinosa* (Table 4). The accumulative gas production varied widely with PEG 8000 addition to the culture medium (Fig. 1), and ranged from 2.1 to 78.1% at 48 h, and 2.0 to 117.1% at 96 h.

The correlations between chemical composition and *in vitro* parameters of the browsed species foliage are shown in Table 5. CT was positively correlated to ADF and negatively correlated CP. TP content was positively correlated to CT and T½, and negatively correlated to a, b, To, and OMd. Additionally, OMd was negatively correlated to T½, and positively correlated to a, b, and To. The a parameter was strongly and positively correlated to To, OMd, and NDFd, and negatively to T½.

DISCUSSION

Compared with other tropical forests with actual or potential nutritional value for silvopastoril systems (Valencia *et al*., 2004; Zent and Zent, 2004; Mohandass and Davidar, 2009), this semi-deciduous community showed an important floristic diversity. The presence of the Fabaceae is outstanding, which besides its nutritional importance for the ruminant, possesses an aggregate value to the edaphic component because it adds nitrogen and participate in Ca and P recycling (Zent and Zent, 2004). *G. ulmifolia*, *S. saman*, and *P. lanceolatum* showed a high forage potential value because IVI relates to voluntary intake (Mandaluniza *et al*., 2011) and to species spatial distribution which in turns, it is one of the most influential factors for grazing buffaloes to access a particular plant (Melletti *et al*., 2007).

Table 1. Botanical composition and Importance Value Index (IVI) of browsed species at a semideciduous tropical forest in Venezuela.

Families	Species	Index			
		Density (%)	Frequency (%)	Dominance (%)	IVI
Arecaceae	*Acrocomia aculeata*	0.4	0.5	0.5	1.4
Boraginaceae	*Rochefortia spinosa*	3.0	0.9	1.6	5.5
Chrysobalanaceae	*Parinari campestris*	0.3	1.9	1.1	3.3
Fabaceae	*Enterolobium cyclocarpus*	0.4	3.9	0.3	4.6
	Erithrina fusca	1.7	1.9	2.4	6.0
	Inga laurina	0.5	1.0	0.2	1.7
	Machaerium humboldtianum	0.7	1.1	0.5	2.3
	Pithecellobium lanceolatum	6.4	11.2	7.2	24.8
	Pterocarpus sp.	1.8	1.9	1.3	5.0
	Samanea saman	6.2	6.8	13.5	26.5
	Senna pallida	1.3	2.9	0.6	4.8
	Senna sp.	0.6	1.8	0.3	2.7
Poaceae	*Guadua angustifolia*	6.0	5.8	1.9	13.7
Malvaceae	*Hibiscus sp.*	1.3	0.2	0.2	1.7
	Sida acuta	1.3	0.9	0.3	2.5
Moraceae	*Morus sp.*	0.4	0.5	0.1	1.0
	Cecropia peltata	1.1	2.9	1.3	5.3
Rubiaceae	*Genipa americana*	0.4	0.3	0.1	0.8
Piperaceae	*Piper sp.*	0.2	0.5	0.1	0.8
Polygonaceae	*Coccoloba caracasana*	0.5	5.8	0.3	6.6
	Triplaris caracasana	0.2	4.8	0.2	5.2
Sterculiaceae	*Guazuma ulmifolia*	61.6	40.6	60.4	162.6
Non identified[2]		3.7	1.9	5.6	11.2

NI, botanically non identified browsed species.

Even though, buffaloes showed an alimentary habit as essentially ungulate grazers, feces analysis showed an important proportion of epidermic fragments of woody species. In similar studies carried out with African buffaloes (*Syncerus caffer nanus*) grazing on a tropical humid forest with open access to savannas, Macandza *et al*. (2004) found that 32.7% of fragments in feces corresponded to woody species, being leaves the fraction with the highest values (26.5%). In these African animals, it has been documented that their grazing habits are strongly related to the quality of the available plant biomass (Melletti *et al*., 2007).

Table 2. Epidermal fragments in the feces and Ivlev Selectivity Index (ISI) by buffaloes in a silvopastoral system.

Families	Species	Epidermal fragments (%)	ISI
Boraginaceae	*Rochefortia spinosa*	2.01c	0.15c
Fabaceae	*Inga laurina*	0.04c	0.49b
	Samanea saman	18.1b	0.53b
Malvaceae	*Sida acuta*	17.1b	0.78a
Polygonaceae	*Coccoloba caracasana*	0.1c	-0.38d
Poaceae		60.4a	
NI		2.3c	
SEM		17.2*	0.32*
p value		0.03	0.02

NI, non identified species; Poaceae only refers to gramineous plants; [abc]Means followed by different letters within a column are significantly different

Table 3. Chemical composition (% DM) of browse species foliage selected by buffaloes.

Species	DM	CP	Fat	NDF	ADF	Ca	P	TP	CT
R. spinosa	53.2a	15.1b	4.1a	40.6b	23.3b	2.21a	0.13	2.0c	0.33b
I. laurina	40.0b	19.3a	1.7b	45.2a	20.8b	1.03c	0.15	3.7b	0.44b
S. saman	35.9b	20.7a	3.7ab	39.4b	26.9a	0.84c	0.14	4.2b	0.73b
S. acuta	36.3b	14.5b	3.6ab	42.2b	28.3a	1.9b	0.13	3.2b	0.65b
C. caracasana	39.2b	10.4c	1.6b	40.7b	30.1a	1.78b	0.09	6.5a	2.46a
SEM	7.9	2.2	0.6	2.1	3.2	0.3	0.04	1.1	0.8
p value	0.04	0.02	0.001	<0.01	0.04	0.03	0.41	0.01	0.02

DM, dry matter; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; Ca, calcium; P, phosphorous; TP, total phenols; CT, condensed tannins; SEM, standard error of the mean; [abc]Means followed by different letters within a column are significantly different.

The CP content was superior to that of natural and introduced tropical pastures (Minson, 1990), and above of the minimum required for ruminants (7 to 8% DM) for optimum intake and rumen performance (Van Soest, 1994). All evaluated forage showed less than 45% of NDF, so they can be considered as a fibrous resource of good quality (Melaku *et al*., 2010). The Ca content emphasizes the relative superiority of this foliage of woody species in comparison to tropical grasses with values adjusted to the estimated requirements of buffaloes with medium levels of production. However, P values indicate the need for supplementation programs for buffaloes grazing in similar forest formations (Habib *et al*., 2004).

The CT levels were in the range referred as beneficial to ruminant nutrition because of its capacity to reduce the risk of timpanism, to control parasitic infections, and to promote the nitrogen flux to the lower tract (Makkar, 2001), but inferior to expected with regards to the TP content and literature values for non cultivated woody foliage plants (Makkar, 2001; Baldizán *et al*., 2006; García and Medina, 2006; Melaku *et al*., 2010). This could be due to the traditional seasonal grazing management of these forests that do not promote an adequate plant-grazer interaction, which would make difficult a genetic fixation of phytochemical strategies of defense, such as synthesis and vacuolar storage of CT (Launchbaugh *et al*., 2001). In general, bromatological composition, fiber content, tannins, and evaluated minerals showed values similar to those of the edible fraction of other woody species with forage value (Carranza-Montaño *et al*., 2003; Guimaraes-Beelen *et al*., 2006; Melaku *et al*., 2010), and even though there were significant differences ($p<0.05$) among species, all of them can be considered nutrient sources for buffaloes grazing under these conditions.

The *in vitro* gas production parameters for some woody plants mentioned in the literature (*Acacia sp, Erythrina poeppegiana, E. fusta, E. olei, Ficus sp, Flemingia congesta, Gliricidia sepium, Inga marginata, I. ingoides, Leucaena leucocephala, Moringa oleifera and Piptadenia macrocarpa*) show highly variable ranges, for a (22 to 149 ml g^{-1} DM), b (0.02 to 0.06 ml h^{-1}), To (0.8 to 4,5 h), and T½ (11 to 28 h) that reflect the effect of age and physiological development of the plant, secondary metabolites content and environmental conditions (Wood *et al*., 1993). Compared to the *in vitro* value for a (118.7 to 229.1 ml g^{-1} DM), b (0.01 to 0.04 ml h^{-1}), To (3.1 to 12.4 h) and T½ (19.1 to 48.2 h) of fresh or dried tropical grasses (Bueno *et al*., 2005; González-Ronquillo *et al*., 2009), with the exception of *S. acuta* (a= 120.9 ml g^{-1} DM), the foliage of the evaluated woody species presented a lower *in vitro* potential gas production, which could be attributed to an inhibitory effect of abundant lignocelulolitic complexes in the cell wall of semi-deciduous plants (Van Soest, 1994), and the presence of a wide range of secondary metabolites, besides polyphenols, in woody plants of tropical forest, such as saponines, terpenes, cyanogens, steroids, trypsin inhibitors and alkaloids (Baldizán *et al*., 2006; García and Medina, 2006).

The difference in variation of the cumulated gas production with the addition of PEG 8000 evidenced the presence of CT with different reactivity. In this regard, in spite of *C. caracasana* showed a higher CT content than *S. acuta* ($p=0.02$), in both species PEG generated a similar increment in accumulated gas production (110.8 ± 0.14%). This indicate a higher biological activity of CT of *S. acuta*, which can be linked to variations in molecular weight, tertiary structure, distribution of prodelfidine, procianidine and propelargonidine in the molecule, isoelectric point, and compatibility of the union sites of the secondary compound (Hagerman and Butler, 1980; Guimaraes-Beelen *et al*., 2006). These characteristics are highly variable among species, and even for the

same species according to its phenological stage (Rodriguez *et al*., 2010).

This demonstrated that tannin content is not by itself an indicator of tannins activity and further studies are required to evaluate the potential impact on the productive performance of herbivorous that fed with the foliage of woody plants.

The negative correlation between TP and the *in vitro* gas production parameters and OMd confirm the detrimental effect of these compounds on the rumen microbial activity as previously indicated. However, no correlation was observed between the *in vitro* fermentation parameters and the CT levels, so this could be associated to other non-evaluated polyphenolic compounds, such as flavonoids with proven reactivity towards the PEG 8000 and with negative impact on rumen fermentation (Bodas *et al*., 2009). Gas potential production was highly correlated with the other *in vitro* fermentation parameters and OMd, which indicates its potential as a nutritional indicator for tropical woody foliage evaluation.

CONCLUSIONS

The proximal composition, fiber content, and evaluated minerals from the edible fraction of selected browse species (*Rochefortia spinosa*, *Inga laurina*, *Samanea saman*, *Sida acuta* and *Coccoloba caracasana*) by buffaloes in a silvopastoral system at a semi-deciduous tropical forest showed higher values than tropical grass pastures, so these species could be considered a nutrient source for buffaloes grazing under these conditions. The condensed tannins of this biomass showed different reactivity, and addition of PEG 8000 could be an alternative to reduce the negative ruminal effects of this secondary metabolites of these forage resources.

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Table 4. *In vitro* gas production and digestibility parameters of browse species foliage selected by buffaloes.

Species	Parameters				Degradability (%)	
	a	b	To	T½	OMd	NDFd
R. spinosa	91.8b	0.06	3.2a	11.6b	64.8a	35.1b
I. laurina	36.3c	0.03	1.1b	23.1a	48.4b	42.4a
S. saman	53.4b	0.04	1.0b	17.3a	50.0b	31.7c
S. acuta	120.9a	0.03	4.4a	18.1a	60.8a	38.7b
C. caracasana	36.5c	0.03	1.0b	23.1a	47.1b	36.6b
SEM	22.1	0.02	1.2	4.9	7.3	4.1
p value	0.03	0.12	0.02	0.03	0.02	<0.01

a, potential gas production (ml g^{-1} DM); b, rate of DM degradation (ml h^{-1}); To, lag time (h); T½, time taken for half the material in the pool described by the exponential component to be degraded (h); OMd, organic matter degradability; NDFd, neutral detergent fiber degradability; SEM, standard error of the mean; ^{abc}Means followed by different letters within a column are significantly different.

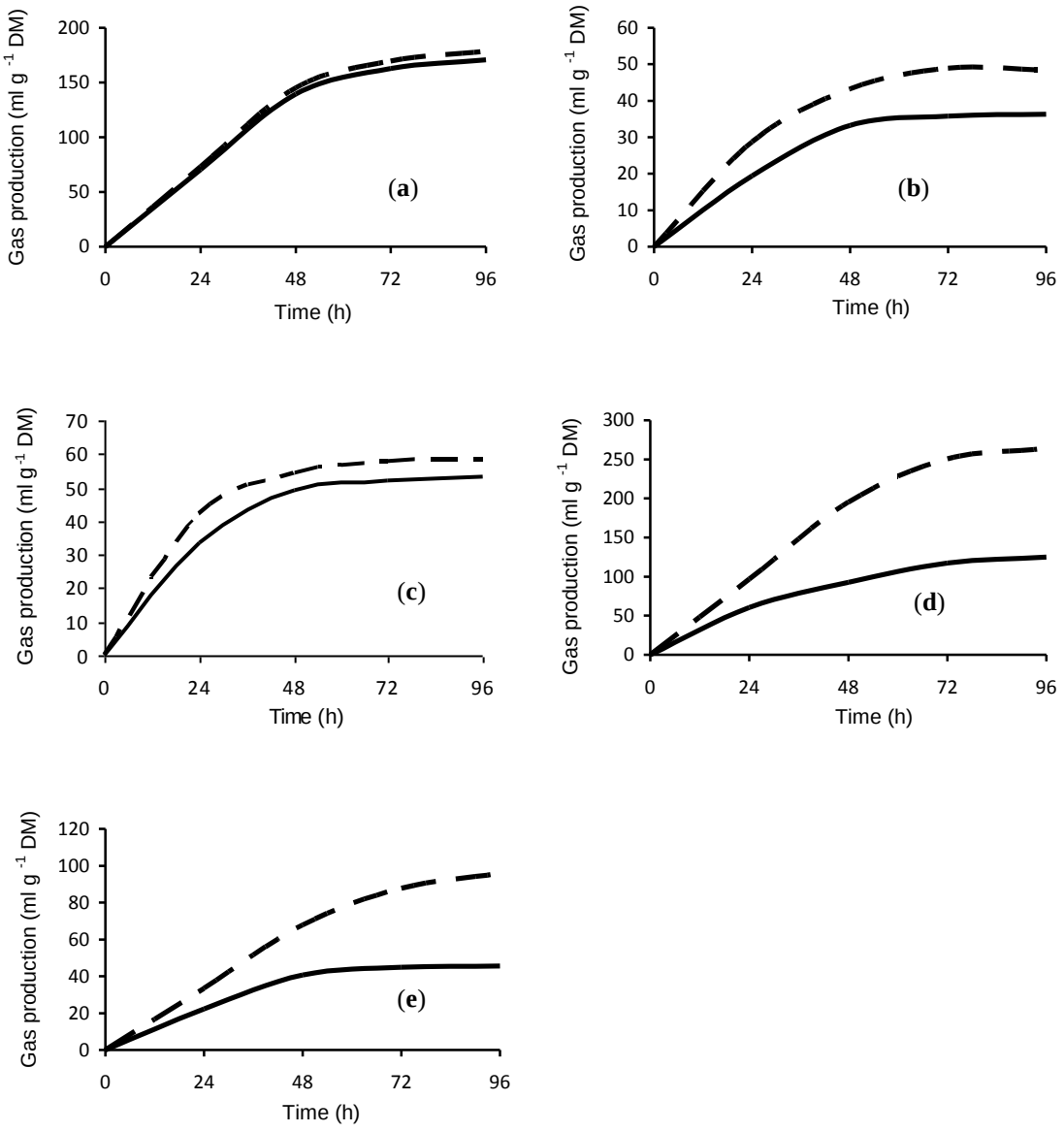

Figure 1. Accumulative *in vitro* gas production (—) and its variation with addition of polyethylene glycol 8000 (– –) in *R. spinosa* (a), *I. laurina* (b), *S. saman* (c), *S. acuta* (d), and *C. caracasana* (e) foliage

Table 5. Correlation coefficients among chemical composition, *in vitro* gas production, and digestibility parameters of browsed species foliage selected by buffaloes.

	CP	NDF	ADF	TP	CT	a	b	To	T½	OMd	NDFd
CP	1										
NDF	0.18	1									
ADF	-0.59	-0.60	1								
TP	-0.41	-0.15	0.62	1							
CT	-0.71*	-0.29	0.73*	0.92**	1						
a	-0.16	-0.14	0.14	-0.66*	-0.45	1					
b	0.09	-0.45	-0.34	-0.63*	-0.43	0.30	1				
To	-0.26	0.01	0.09	-0.64*	-0.41	0.98**	0.25	1			
T½	-0.12	0.51	0.16	0.76*	0.55	-0.64*	-0.90**	-0.56	1		
OMd	-0.14	-0.17	-0.13	-0.81*	-0.55	0.89**	0.66*	0.89*	-0.85**	1	
NDFd	-0.09	0.96**	-0.42	-0.05	-0.11	0.89**	-0.51	0.11	0.55	-0.12	1

CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; TP, total phenols; CT, condensed tannins; a, potential gas production; b, rate of DM degradation; To, lag time; T½, time taken for half the material in the pool described by the exponential component to be degraded; OMd, organic matter degradability; NDFd, neutral detergent fiber degradability; *P<0.05; **P<0.01.

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