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CHEMICAL COMPOSITION OF LEGUMINOUS TREE FOLIAGE AND EFFECT OF POLYETHYLENE GLYCOL ON GAS PRODUCTION AND *IN VITRO* DIGESTION PARAMETERS

[COMPOSICIÓN QUÍMICA DEL FOLLAJE DE ÁRBOLES LEGUMINOSOS Y EFECTO DE POLIETILENGLICOL EN LA PRODUCCIÓN DE GAS Y PARAMETROS DE DIGESTIÓN IN VITRO]

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SUMMARY

The objective was to determine the chemical composition, digestibility and *in vitro* digestion parameters in ten legume tree foliage using the *in vitro* gas-production method with and without polyethylene glycol (PEG). The foliages with higher protein content (P<0.001) (167.1 to 180.3 g/kg DM) were *A. cochliacantha*, *L. esculenta*, *E. cyclocarpum* and *A. farnesiana*; from the total phenols (P<0.001) (365.9 to 680.6 g/kg DM) *L. divaricata*, *H. brasiletto* and *C. coriaria* and condensed tannins (P<0.001) (35.4 to 88.0 g/kg DM) *E. cyclocarpum*, *A. farnesiana*, *P. dulce*, *P. acatlense* and *G. sepium*. The *in vitro* dry matter digestibility was different (P<0.001) among the foliages. The *in vitro* gas production (IVGP), *in vitro* organic matter digestibility, metabolizable energy (ME), gas yield (GY_{24h}), short chain fatty acids (SCFA) and microbial mass production (PMM), were different (P<0.0001) among the foliage as a result of the species. The use of PEG increased (P<0.0001) IVGP, ME, GY_{24h} and SCFA in *H brasiletto*, *C. coriaria*, *L. esculenta* and *A. cochliacantha*, but affect (P<0.0001) the partition factor and the PMM. The nutritional composition and fermentation parameters in vitro between foliages differ by effect of tree and use of PEG. It is concluded that chemical composition in the foliages affect the digestibility and fermentation parameters and use of PEG increased fermentation parameters in the foliages high in secondary compounds.

Key Words: tree; PEG; tannins; phenols; microbial mass production.

RESUMEN

El objetivo fue determinar la composición química, digestibilidad y parámetros de digestión *in vitro* de diez follajes de árboles leguminosos con el uso de la técnica de producción de gas *in vitro* con y sin polietilenglicol (PEG). Los follajes con mayor contenido de proteína (P <0.001) (167.1 a 180.3 g / kg MS) fueron *A. cochliacantha*, *L. esculenta*, *E. cyclocarpum* y *A. farnesiana*, en el contenido de fenoles totales (P <0.001) (365.9 a 680.6 g / kg MS) *L. divaricata*, *H. brasiletto* y *C. coriaria* y taninos condensados (P <0.001) (35.4 a 88.0 g / kg MS) *E. cyclocarpum*, *A. farnesiana*, *P. dulce*, *P. acatlense* y *G. sepium*. La digestibilidad *in vitro* de la materia seca fue diferente (P <0,001) entre los follajes. La producción de gas *in vitro* (IVGP), digestibilidad *in vitro* de la materia orgánica, la energía metabolizable (ME), el rendimiento de gas (GY_{24h}), ácidos grasos de cadena corta (AGCC) y la producción en masa microbiana (PMM), fueron diferentes (P <0,0001) entre los follajes como resultado de la especie. El uso de PEG aumentó (P <0,0001) IVGP, EM, GY_{24h} y AGCC en *H. brasiletto*, *C. coriaria*, *L. esculenta* y *A. cochliacantha*, pero afectó (P <0.0001) y el factor de partición y la PMM. La composición nutricional y parámetros de la fermentación *in vitro* entre follajes difieren por efecto de árbol y el uso de PEG. Se concluye que la composición química en los follajes afecta a los parámetros de digestibilidad y la fermentación y el uso de PEG aumento los parámetros de fermentación en los follajes con alto contenido de compuestos secundarios.

Palabras clave: árbol; PEG; taninos; fenoles; producción de masa microbiana.

INTRODUCTION

The low productivity of livestock in tropical regions is due to low availability and the low nutritional quality of foods that are used as basal diet (Olivares et al., 2011), consequently, the nutrition of ruminants depends of the animal ability to ferment the food, and produce nutrients such as short chain fatty acids (SCFA) and microbial biomass. The fodder trees can complement protein and dry matter in ruminant production, during critical periods of the year (Olivares-Perez *et al*., 2011), however, its foliage contains phenolic compounds, especially tannins (Patra and Sexena, 2010; Mokoboki *et al*., 2011). *In vitro* gas production (IVGP) is a technique that estimates the activity of the tannins by using polyethylene glycol (PEG) on microbial activity and digestibility of the diet in the rumen (Bueno *et al*., 2008; Mbugua *et al*., 2008). The PEG neutralizes the effects of tannins (Patra and Sexena, 2010; Olivares *et al*., 2013). When the animals are fed fodders with high in condensed tannins the PEG improves the digestibility and the final products of fermentation during digestion (Njidda and Ikhimioya, 2010; Jimenez *et al*., 2011). The objective was to evaluate the nutritional value (quality, characteristics) and in vitro fermentation parameters of tree foliage incubated without and with PEG, for alternative use in ruminant feed.

MATERIAL AND METHODS

Study area

The study was conducted in Tejupilco, State of Mexico, between the parallels 18º 45' 30" and 19º 04' 32" north latitude and between meridians 99º 59' 07" and 100° 36' 45" west longitude, altitude 1340 m with a climate A (C) wg, summer rains, average temperature of at least 15 ºC and maximum 30 °C, and mean annual rainfall of 1014 mm.

Foliage sampling

The foliage was collected from leguminous native species such as *Acacia cochliacantha, Enterolobium cyclocarpum, Pithecellobium dulce, Acacia farnesiana, Lysiloma divaricata, Pithecellobium acatlense, Gliricidia sepium, Leucaena esculenta, Haematoxylum brasiletto* and *Caesalpinia coriaria* (Olivares-Perez *et al*., 2011).

Chemical composition

Three samples were randomly collected during the rainy season (June to August) (0.5 kg DM basis, each one pooled of 18 trees, i.e. of three transects by 6 ranches). Samples were dried at 40°C for 48 h in the shade to obtain a constant weight then ground in Willey-mill of one mm screen size. Ground samples were analyzed for ash and organic matter (OM) content (AOAC 2000), CP by a Kjeldahl method (AOAC 2000; ID 954.01). Acid detergent lignin (ADL), neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined by methods of Goering and Van Soest (1970) and Total phenolic content (TP) (Folin Ciocalteu) and condensed tannins (CT) (butanol–HCl) with method described by Waterman and Mole (1994).

In vitro gas production with and without PEG

In vitro gas production (IVGP) and *in vitro* digestibility of dry and organic matter (IVDMD and IVOMD) were determined by the gas production technique modified by Herrero and Jessop (1996). Rumen fluid was collected via oral tube using a portable bomb (BARNANT COMPANY, USA) of three sheep F1 adult (Katahdin x Dorper) fed with standardized diet to concentrated (30%) and forage (70%).

Approximately one gram of tree sample was weighed and incubated at 39°C (Incubator, Binder Company, Germany), with and without polietyleneglycol (PEG-4000 MW, Sigma®) at a ratio of 2 g of PEG by 1 g of sample (i.e., three bottles for each foliage, three bottles with and without PEG, more three bottles as blank with rumen fluid only) to assess the biological activity of tannins (Waghorn, 2008). The readings of gas volume were recorded each hour during the first 8 h, then every 4 h until 60 h, later at 72, 84 and 96 h of incubation, using the reading pressure technique (RPT; DELTA OHM, Italy).

Estimation of truly degraded substrate

At the end of incubation (i.e., 96 h), the contents of each serum bottle were filtered using sintered glass crucibles (coarse porosity No. 1, pore size µm porosity, Pyrex, Stone, UK) under vacuum. Fermentation residues were dried at 105°C overnight to estimate the potential DM disappearance.

The ME and IVOMD were estimated using the equations proposed by Menke *et al*. (1979):

$$ME\ (MJ/kg\ DM) = 2.20+0.136GP_{24}+0.0057CP$$

$$IVOMD\ (\%) = 14.88+0.889GP_{24}+0.45CP+0.0651XA$$

Where:
ME = Metabolizable energy, IVOMD = *In vitro* organic matter digestibility, GP_{24} = gas production at 24 h (mL/0.2 g DM), CP = crude protein percentage and XA = ash percentage.

The short chain fatty acids (SCFA, mmol) production was calculated with the equations proposed by Getachew *et al*. (2000):

Absent PEG: AGCC = 0.0239.Gas–0.0601

Present PEG: AGCC = 0.0207.Gas+0.0521

The partition factor (PF) and the effective gas volume produced (GY) was estimated by the equations proposed by Blümmel *et al*. (1997):

PF = (Truly degraded organic matter, mg) / mL gas

GY (volume) = mL gas / truly degraded organic matter, mg

The production of microbial mass (PMM) in milligrams, was calculated with the equations proposed by France *et al*. (1993):

PMM (mg) = ((a-b)-Stoichiometric factor (2.2) * Total gas volume, mL 24 h)

Where:
Difference of the factor "a" (substrate undegraded OM) minus the factor "b" (substrate degraded OM) to obtain the truly undegraded organic matter.

Experimental design and statistical analysis

Data were analyzed by GLM (SAS, 2000). Mean comparisons were performed using Tukey Test (P<0.05).

The variables of chemical composition of foliage were analyzed by general linear models, using a completely randomized design, statistical model:

$$Y_{ij} = \mu + T_i + \xi_{ij}$$

Where:
Y_{ij} = response variable (CP, Ash, OM, ADF, NDF, ADL, CT, TP and IVDMD) of the treatment (i = 1, 2, 3, 4……10 trees) in repetition (j = 1, 2, 3 samples by tree)
μ = general mean
T_i = treatment effect (i)
ξ_{ij} = random error treatment (i) repetition (j), terms of n-1(σ^2,0).

The data variables of degradability of the substrate with and without PEG, were analyzed using a completely randomized design in factorial arrangement of 10 x 2, statistical model:

$$Y_{ijk} = \mu + A_i + B_j + (AB)_{ij} + \xi_{ijk}$$

Where:
Y_{ijk} = response variable (IVGP, IVOMD, ME, SCFA, PF, GY, PMM) in repetition (k = 1, 2, 3 samples by tree), level i of (A = 1, 2, 3, 4….10 trees) and level j of (B = 1, 2 where: 1 = without PEG, 2 = with PEG)
μ = general mean
A_i = effect of factor A at level i
B_j = effect of factor B at level j
$(AB)_{ij}$ = interaction effect A*B at level i, j
ξ_{ijk} = random error, in terms of n-1(σ^2, 0).

With correlation analysis of the relationship between the nutritional composition of the foliage with the production of short chain fatty acids, gas yield, in vitro digestibility of organic matter and dry matter was determined (SAS, 2000).

RESULTS

Chemical composition and *in vitro* dry matter digestibility

The foliages with higher CP content were *A. cochliacantha*, *L. esculenta*, *E. cyclocarpum* and *A. farnesiana*, compared to *G. sepium* and *H. brasiletto* (Table 1). The OM content was greater (P<0.01) in *C. coriaria* and low in *G. sepium* and *P. dulce*, the ash was higher (P<0.01) in *P. dulce* and *G. sepium*, indicating that contain more minerals (Table 1).

The NDF content was higher (P<0.001) in foliages of *E. cyclocarpum* and *P. acatlense* compared to *A. cochliacantha*, *L. esculenta*, *P. dulce*, *L. divaricata*, *C. coriaria*, *G. sepium* and *H. brasiletto*. The FDA content was higher (P<0.001) in the foliage of *P. acatlense* compared to *L. esculenta, A. cochliacantha*, *L. divaricata* and *C. coriaria* (Table 1).

The ADL content was higher (P<0.001) in the foliage of *E. cyclocarpum*, compared to *A. cochliacantha*, *L. esculenta*, *P. dulce*, *L. divaricata*, *P. acatlense*, *C. coriaria*, *G. sepium* and *H. brasiletto* (Table 1).

The TP content was higher (P<0.001) in *L. divaricata*, *H. brasiletto* and *C. coriaria,* compared to *E. cyclocarpum*, *A. farnesiana*, *P. dulce*, *P. acatlense* and *G. sepium* (Table 1). The CT content was higher

(P<0.001) in *H. brasiletto*, *A. cochliacantha*, *L. esculenta*, *P. dulce*, *L. divaricata* and *P. acatlense*, compared to *E. cyclocarpum*, *A. farnesiana*, *C. coriaria* and *G. sepium* (Table 1).

The IVDMD was higher in foliages of *P. acatlense*, *A. cochliacantha*, *P. dulce* and *L. divaricata*, compared to *L. esculenta*, *A. farnesiana*, *C. coriaria* and *H. brasiletto* (Table 1).

Gas production with and without PEG

The effect of species (P<0.0001) the foliages with higher volume of gas produced during digestion at 24 and 48 h were *G. sepium*, *A. farnesiana* and *P. dulce* and to 96 h incubation were *G. sepium*, *A. farnesiana*, *P. dulce*, *C. coriaria* and *L. divaricata* (Table 2).

The effect of PEG (P<0.0001), the foliages with higher volume of gas produced during digestion in the three times of incubation were of *H. brasiletto*, *C. coriaria*, *L. esculenta*, *L. divaricata* and *A. cochliacantha*, the addition of PEG to these foliages increased the gas volume produced up to 27.7 and 73.2% (Table 2). The interaction tree*PEG was significant (P<0.0001) on volume of gas produced by the foliage during digestion in the three incubation times (Table 2).

Fermentation parameters with and without PEG

An effect of the species on the IVOMD (334.3 to 368.3 g/kg DM) and ME content (4.2 to 5.2 MJ/kg DM) (P<0.0001) (Table 3), was observed in foliages that had higher (P<0.0001) *in vitro* gas production to 24 h (63.4 to 88.4 mL/g DM) (Table 2), as *P. dulce*, *G. sepium* and *A. farnesiana*, compared to *H. brasiletto*, *C. coriaria*, *L. esculenta*, *L. divaricata* and *A. cochliacantha* (Table 2 and 3).

The PEG (P<0.0001) increased the IVOMD and ME content between 22.3 and 32.9% in foliage of *H. brasiletto*, *C. coriaria*, *L. esculenta* and *A. cochliacantha*; they also had an interactive effect of tree * PEG on *in vitro* digestibility observed in the organic matter and content ME (P<0.0001) (Table 3). The GY_{24h} and SCFA were higher (P<0.0001) in *G. sepium* and *A. farnesiana*, compared to the observed in *P. acatlense*, *H. brasiletto*, *C. coriaria*, *L. esculenta*, *L. divaricata*, *E. cyclocarpun* and *A. cochliacantha* (Table 3). Also the PF and PMM differed between species (P<0.0001) (Table 3).

The addition PEG increased (P<0.0001) GY_{24h} and SCFA up to 83.6% and 93.7% respectively in *H. brasiletto*, *C. coriaria*, *L. esculenta*, *L. divaricata* and *A. cochliacantha* (Table 3). The PEG affected (P<0.0001) the PF, PMM in foliages of *H. brasiletto*, *C. coriaria*, *L. esculenta*, *L. divaricata* and *A. cochliacantha* during digestion (Table 3). In addition it was observed an interactive effect of the tree * PEG (P<0.0001), on the fermentation parameters ($PGE_{24\ h}$, FP_{24h}, SCFA and PMM) (Table 3).

Table 1. Nutritional composition of foliage from leguminous tropical trees from Southern Mexico (g/kg DM).

Tree foliage	CP	Ash	OM	NDF	ADF	ADL	IVDMD	TP	CT
A. cochliacantha	180.3^{a}	44.3^{bc}	955.6^{ab}	241.9^{d}	205.0^{bcde}	93.7^{bc}	474.0^{ab}	130.7^{d}	248.2^{c}
L. esculenta	180.1^{a}	54.4^{abc}	945.5^{abc}	251.2^{cd}	170.7^{de}	83.9^{bc}	207.0^{e}	220.2^{c}	397.5^{b}
E. cyclocarpum	168.5^{a}	67.2^{ab}	932.7^{bc}	357.8^{a}	246.7^{abc}	153.0^{a}	377.0^{bcd}	58.1^{e}	43.7^{f}
A. farnesiana	167.1^{a}	51.^{abc}	948.9^{abc}	325.3^{ab}	272.8^{ab}	134.6^{ab}	258.0^{de}	52.0^{e}	29.4^{f}
P. dulce	149.6^{ab}	83.8^{a}	916.1^{c}	316.2^{bcd}	242.1^{abcd}	86.4^{bc}	427.0^{abc}	88.0^{de}	134.3^{de}
L. divaricata	146.1^{ab}	50.8^{abc}	949.1^{abc}	232.1^{d}	180.1^{cde}	72.4^{c}	402.0^{abc}	680.6^{a}	179.5^{d}
P. acatlense	138.0^{ab}	58.9^{abc}	941.0^{abc}	359.1^{a}	291.4^{a}	80.9^{c}	521.0^{a}	82.7^{de}	111.2^{e}
C. coriaria	137.5^{ab}	30.8^{c}	969.1^{a}	242.1^{d}	139.3^{e}	73.7^{c}	246.0^{de}	360.9^{b}	46.2^{f}
G. sepium	103.9^{b}	70.8^{ab}	929.1^{bc}	265.7^{bcd}	218.3^{abcd}	93.3^{bc}	322.0^{cde}	35.4^{e}	26.8^{f}
H. brasiletto	95.1^{b}	54.7^{abc}	945.2^{abc}	283.9^{bcd}	242.7^{abcd}	92.2^{bc}	260.0^{de}	385.8^{b}	614.5^{a}
SEM	19.9	12.54	12.54	9.82	30.5	21.19	47.1	37.3	16.4
[1]P value	<0.001	<0.01	<0.01	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

CP: crude protein, OM: organic matter, NDF: neutral detergent fiber; ADF: acid detergent fiber, ADL: acid detergent lignin, TP: total phenols, CT: condensed tannins, IVDMD: *in vitro* dry matter digestibility (%); SEM: standard error of the means.

[1]Different letters in columns indicate mean differences (Tukey: P<0.05).

Table 2. Accumulated gas volume (mL/g DM) with and without polyethylene glycol (PEG) of ten tropical legume tree foliage.

Tree foliage	PEG	*In vitro* gas volume (mL/g DM)		
		$PG_{24\,h}$	$PG_{48\,h}$	$PG_{96\,h}$
P. dulce	without	63.4^{efg}	109.0^{def}	140.8^{bcde}
	with	73.0^{cdef}	118.4^{cd}	151.47^{bcd}
P. acatlense	without	45.4^{hi}	66.7^{ghi}	76.5^{fgh}
	with	54.9^{gh}	78.8^{fgh}	99.4^{efg}
H. brasiletto	without	27.1^{jk}	51.0^{hij}	75.9^{fgh}
	with	72.0^{def}	127.9^{bcd}	169.5^{abc}
G. sepium	without	88.4^{abcd}	144.7^{abc}	171.1^{ab}
	with	94.8^{a}	156.0^{ab}	181.9^{ab}
C. coriaria	without	42.9^{hij}	82.0^{efg}	127.0^{cde}
	with	89.6^{abc}	167.0^{a}	198.3^{a}
L. esculenta	without	8.5^{l}	32.7^{j}	48.7^{h}
	with	76.8^{abcd}	129.2^{bcd}	161.6^{abcd}
L. divaricate	without	37.4^{ij}	66.2^{ghi}	124.6^{de}
	with	57.6^{fgh}	110.2^{de}	171.5^{ab}
E. cyclocarpum	without	41.8^{hij}	67.0^{ghi}	78.0^{fgh}
	with	54.3^{gh}	83.8^{efg}	101.7^{ef}
A. farnesiana	without	79.5^{abcde}	138.3^{abcd}	167.0^{abcd}
	with	91.5^{ab}	151.8^{ab}	173.4^{ab}
A. cochliacantha	without	20.2^{kl}	42.2^{ij}	57.0^{gh}
	with	75.5^{bcde}	135.8^{bcd}	163.4^{abcd}
SEM		5.4	9.9	13.7
[1]P value				
Tree		<0.0001	<0.0001	<0.0001
PEG		<0.0001	<0.0001	<0.0001
Tree*PEG		<0.0001	<0.0001	<0.0001

GP: gas production (24, 48 and 96 h incubation); SEM: Standard error of the means
[1]Different letters in columns indicate mean differences (Tukey: P<0.05)

Correlation analysis

The correlation analysis showed that the detergent fibers affected (r= -0.02; P <0.05) IVOMD and acid detergent lignin affect (r=-0.3; P <0.05) the production of SCFA and IVOMD, respectively (Table 4). The total phenols and condensed tannins affected the production of SCFA(r=-0.82; P<0.001) and (r=-0.41; P<0.05), IVOMD (r=-0.85; P<0.001) and (r=-0.63; P<0.01), and IVDMD (r=-0.89; P<0.01), respectively (Table 4). Crude protein favored the production of SCFA (r=0.86; P <0.001), the IVOMD (r=0.78; P <0.001) and IVDMD (r=0.43; P <0.05) (Table 4).

DISCUSSION

Chemical composition

The high protein content in the foliages of *A. cochliacantha*, *L. esculenta*, *E. cyclocarpum* and *A. farnesiana*, ensures the sustenance of nitrogen in the diet of ruminants in the tropics (Table 1), although proteins may be binding to the fiber or phenols and condensed tannins, this could diminish the nitrogen availability to the animal. The observed levels of nutrients are comparable to those reported in leguminous tree leaves by Njidda and Nasiru (2010); Seresinhe *et al*., (2012).

The TP and CT content registered in foliages of *H. brasiletto*, *C. coriaria*, *A. cochliacantha*, *L. esculenta*, *P. dulce*, *L. divaricata* and *P. acatlense* (Table 1) is compared to the reported by Tiemann *et al*. (2008); Njidda and Ikhimioya (2010) in forages tree. Gonzalez *et al*. (2006) registered the *H. brasiletto*, *C. coriaria* and *L. divaricata* as species with high content of TP and CT, similar to what was observed in this study. The foliages with a low content of TP and CT were *E. cyclocarpum*, *A. farnesiana* and *G. sepium*, in these foliages the secondary compounds may have beneficial effects during substrate degradation in ruminants (Patra and Sexena 2010; Seresinhe *et al*., 2012).

Table 3. *In vitro* fermentation parameters with and without addition of polyethylene glycol (PEG) in the foliage of ten tropical tree legumes.

Tree foliage	PEG	IVOMD	ME	GY_{24h}	PF_{24h}	SCFA	PMM
P. dulce	Without	334.3^{abcdf}	4.7^{cdef}	189.3^{defgh}	5.3^{bc}	1.4^{efgh}	526.2^{fgh}
	With	351.4^{abcdf}	5.0^{bcdef}	207.6^{cdef}	4.8^{bc}	1.5^{defg}	487.9^{ghij}
P. acatlense	Without	295.5^{fgh}	4.2^{ghij}	153.8^{hijk}	6.5^{bc}	1.0^{ij}	604.3^{de}
	With	312.4^{efg}	4.4^{fghi}	175.6^{fghij}	5.6^{bc}	1.1^{ghij}	566.8^{def}
H. brasiletto	Without	243.4^{j}	3.4^{kl}	111.6^{lm}	9.0^{bc}	0.5^{kl}	696.7^{ab}
	With	323.2^{defg}	4.7^{defgh}	223.0^{abcd}	4.4^{bc}	1.5^{defgh}	518.1^{fghi}
G. sepium	Without	357.4^{abcd}	5.2^{abcde}	347.3^{ab}	4.0^{c}	2.05^{a}	447.9^{ijk}
	With	368.7^{ab}	5.3^{ab}	256.9^{a}	3.8^{c}	2.0^{ab}	422.6^{jk}
C. coriaria	Without	289.0^{ghi}	4.1^{hij}	147.6^{ijkl}	6.8^{bc}	0.9^{ij}	616.3^{cde}
	With	371.9^{ab}	5.4^{ab}	240.8^{abc}	4.1^{c}	1.9^{abcd}	430.9^{jk}
L. esculenta	Without	248.4^{ij}	3.4^{l}	33.9^{n}	32.7^{a}	0.1^{m}	732.8^{a}
	With	370.0^{ab}	5.3^{abc}	206.8^{cdef}	4.8^{bc}	1.6^{bcde}	460.7^{hijk}
L. divaricata	Without	284.3^{ghij}	4.0^{ijk}	131.4^{kl}	7.6^{bc}	0.8^{jk}	633.4^{bcd}
	With	320.3^{defg}	4.6^{fghi}	179.8^{efghi}	5.5^{bc}	1.2^{fghi}	552.9^{efg}
E. cyclocarpum	Without	303.3^{fgh}	4.6^{efgh}	137.6^{jkl}	7.2^{bc}	0.9^{ijk}	604.6^{de}
	With	325.6^{cdefg}	4.2^{ghij}	166.8^{ghijk}	5.9^{bc}	1.1^{hij}	554.8^{efg}
A. farnesiana	Without	368.8^{ab}	5.3^{abc}	215.7^{bcde}	4.6^{bc}	1.8^{abcd}	556.0^{hijk}
	With	390.1^{a}	5.6^{a}	234.9^{abc}	4.2^{c}	1.9^{abc}	408.5^{k}
A. cochliacantha	Without	268.8^{hij}	3.7^{jkl}	74.7^{m}	14.5^{b}	0.4^{lm}	686.6^{abc}
	With	367.1^{abc}	5.2^{abcd}	205.7^{cdefg}	4.8^{bc}	1.6^{cdef}	466.6^{hijk}
SEM		13.5	0.1	12.7	3.3	0.1	23.8
[1]P value							
Tree		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
PEG		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Tree*PEG		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

IVOMD: *in vitro* organic matter digestibility (g/kg DM); ME: metabolizable energy (MJ/kg DM); GY_{24h}: gas yield to 24 h (mL gas/g degraded substrate); PF_{24h}: partition factor to 24 h incubation (mg degraded substrate/mL gas); SCFA: short-chain fatty acids (mmol/g DM) PMM: production of microbial mass (mg/g DM), SEM: standard error mean

[1]Different letters in columns indicate mean differences (Tukey: P<0.05).

Table 4. Correlation between nutritional compositions with *in vitro* fermentation parameters.

	SCFA	GY	IVOMD	IVDMD
Neutral detergent fiber	-0.13^{ns}	0.25^{ns}	-0.2*	0.03^{ns}
Acid detergent fiber	-0.12^{ns}	0.23^{ns}	-0.14*	0.07^{ns}
Acid detergent lining	-0.3*	0.21^{ns}	-0.31*	0.19^{ns}
Crude protein	0.86***	-0.16^{ns}	0.78***	0.43*
Total phenols	-0.82***	0.06^{ns}	-0.85***	-0.89**
Condensed tannins	-0.41*	0.09^{ns}	-0.63**	0.28^{ns}

IVOMD: *in vitro* organic matter digestibility; GY_{24h}: gas yield to 24 h; SCFA: short-chain fatty acids; IVDMD: in vitro dry matter digestibility.

*P<0.05; **P<0.01; P<0.001; ns: not significant.

The lower digestibility in *H. brasiletto* is associated to higher content of TP, CT and ADF and low CP content; in *C. coriaria* to higher content TP and low content CP; in *L. esculenta* to higher content of CT; in *A. farnesiana* to higher content of ADF, NDF and ADL (Table 1). In table 4 shows that the content of TP, CT and ADF has negatively correlated with the digestibility of the foliages.

The foliages with higher IVDMD as *A. cochliacantha* were due to low content TP, TC and FDN, average levels of ADF and ADL, and higher content of CP; in *P. dulce* was due to average levels of CP and low content of TP and CT; in *L. divaricata* it was associated to the average content of CP and low content of NDF, ADF, ADL and CT; in *P. acatlense* was associated to low content TP and CT (Table 1). In table 4 shows that CP content has positive correlation with the digestibility of the foliages. Several reports have indicated a negative relationship between high ADF, NDF, TP and CT contents and the digestibility of the substrate (Njidda and Ikhimioya, 2010; Bhatta *et al*., 2012).

Fermentation parameters with and without PEG

The higher IVGP, IVOMD and ME in the foliage of *P. dulce*, *G. sepium* and *A. farnesiana* (Tables 2 and 3) was due to low content of TP, CT, ADF and ADF in the foliage of these trees. Reports indicated that high level of TP affect the IVGP, IVOMD and ME available (Olivares *et al*., 2013).

The addition of PEG increased IVGP, IVOMD and ME in the foliages of *H. brasiletto*, *C. coriaria*, *L. esculenta*, *L. divaricata* and *A. cochliacantha*, which contained high values of TP and CT, demonstrated the biological activity of these compounds to precipitate nutrients (Tables 2 and 3). Reports indicate that the use of PEG reduce the astringency (70%) of tannins and total phenols that interfere in nutrients precipitation, which favors the action of enzymes and bacteria for the degradation of the substrate and increased the availability of nutrients (Mbugua *et al*., 2008; Patra and Sexena, 2010; Olivares *et al*., 2013).

The higher GY24h and SCFA production in foliages of *G. sepium* and *A. farnesiana* (Tables 2 and 3) is due to factors related to their nutritional composition (content of TP, CT, ADF, NDF and ADL) (Table 1). In table 4 shows that ADL, ADF, NDF, TP and CT content in the foliages has negatively correlated with the SCFA production. It is reported that higher content of TP, CT, ADF, NDF and ADL, limits the gas production (Mahipala *et al*., 2009), and GY_{24h} and SCFA production in tree foliages. Makkar (2005), reports that the measurement of *in vitro* gas production reflects the SCFA production.

The addition of PEG to foliages with high content of TP and CT increased GY24h and SCFA production, but affected the PF and PMM (Table 3). Makkar (2005) report that the addition of PEG to foliage's with higher content CT and TP, favours availability of digestible nutrients and SCFA by the increase in IVGP.

The negative effect of PEG on the PF and PMM in foliages (Table 3) is similar to the reported by Arhab *et al*. (2009) and Makkar (2005) and it is attributed to an increase in substrate degradation with low increase in gas production, i.e. the production of SCFA and microbial mass is not constant.

CONCLUSIONS

The foliage studied can be considered as a source of N to supplement low quality diets fed to ruminants. However, the high-TP, CT and cell wall constituents affect the digestibility, which suggests a selection prior to use in animal feed. The PEG interfered with the biological activity of CT and TP to precipitate nutrients in the foliage of *H. brasiletto*, *C. coriaria*, *L. esculenta*, *L. divaricata* and *A. cochliacantha* and increased IVGP, IVOMD, ME, SCFA and GY_{24h}.

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