

Effect of polyethylene glycol on in vitro gas production of some non-leguminous forage trees in tropical region of the south of Mexico

S. Rojas Hernández, J. Olivares Pérez, M. M. M. Y. Elghandour, M. Cipriano-Salazar, B. Ávila-Morales, L. M. Camacho-Díaz, et al.

Agroforestry Systems

An International Journal incorporating
Agroforestry Forum

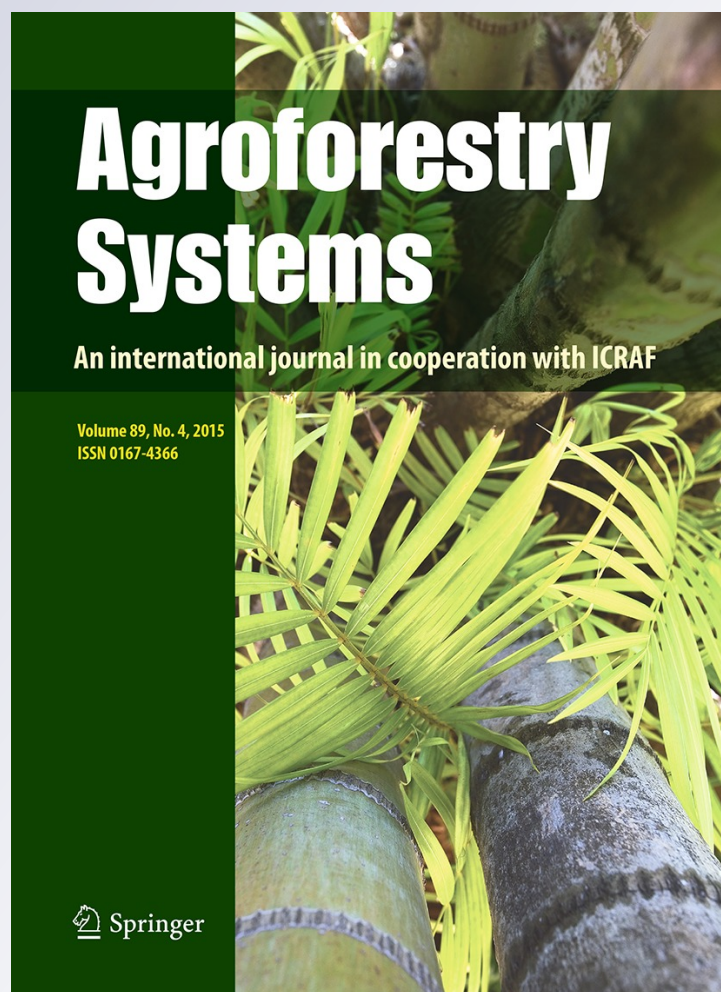
ISSN 0167-4366

Volume 89

Number 4

Agroforest Syst (2015) 89:735-742

DOI 10.1007/s10457-015-9796-8



Your article is protected by copyright and all rights are held exclusively by Springer Science +Business Media Dordrecht. This e-offprint is for personal use only and shall not be self-archived in electronic repositories. If you wish to self-archive your article, please use the accepted manuscript version for posting on your own website. You may further deposit the accepted manuscript version in any repository, provided it is only made publicly available 12 months after official publication or later and provided acknowledgement is given to the original source of publication and a link is inserted to the published article on Springer's website. The link must be accompanied by the following text: "The final publication is available at link.springer.com".

Effect of polyethylene glycol on in vitro gas production of some non-leguminous forage trees in tropical region of the south of Mexico

S. Rojas Hernández · J. Olivares Pérez · M. M. M. Y. Elghandour ·
M. Cipriano-Salazar · B. Ávila-Morales · L. M. Camacho-Díaz ·
A. Z. M. Salem · M. A Cerrillo Soto

Received: 9 June 2014 / Accepted: 16 February 2015 / Published online: 22 February 2015
© Springer Science+Business Media Dordrecht 2015

Abstract The objective of the current study was to evaluate the chemical composition and the in vitro gas production (GP) of some non-leguminous forage trees in presence or absence of polyethylene glycol (PEG). *Guazuma ulmifolia*, *Crescentia alata*, *Ficus glabrata*, *Ficus cotinifolia*, *Spondias purpurea*, *Mangifera indica*, *Licania arborea*, *Simira mexicana* were collected during the rainy season, in the Bejucos locality, State of Mexico. Metabolizable energy (ME), partitioning factor (PF_{24h}), in vitro organic matter digestibility (OMD), short chain fatty acids (SCFA) and microbial biomass production (MBP) were estimated as tools to detect the adverse effects of tannins in tree foliage. The chemical composition data were analyzed in a random design, and the in vitro digestion

parameters on a randomized design with 8 × 2 factorial arrangement. Chemical composition showed a wide variation (P < 0.05) between species. The use of PEG increased (P < 0.05) GP from the foliage of *S. purpurea*, *L. arborea*, *F. glabrata* and *G. ulmifolia*, showing activity of total phenolics and condensed tannins. Similarly, ME (5.9 MJ kg⁻¹ DM), OMD (354.5 g kg⁻¹ DM) and SCFA (2.3 mol/150 mL) increased (P < 0.05); it was higher for *S. purpurea*, because of the PEG addition effect. The PF_{24h} and MBP were different between species (P < 0.05), and decreased due to PEG addition (P < 0.05); the species with lower production was *S. purpurea*. It could be concluded that *S. purpurea* and *F. cotinifolia* represent important sources of fodder for livestock in the south region of Mexico.

S. Rojas Hernández · J. Olivares Pérez ·
M. Cipriano-Salazar · B. Ávila-Morales ·
L. M. Camacho-Díaz
Unidad Académica de Medicina Veterinaria y Zootecnia,
Universidad Autónoma de Guerrero, Km. 3.5 Carretera
Cd. Altamirano-Iguala, CP 40660 Cd. Altamirano,
Guerrero, México

M. M. M. Y. Elghandour · A. Z. M. Salem (✉)
Facultad de Medicina Veterinaria y Zootecnia,
Universidad Autónoma del Estado de México, Toluca,
Estado de México, México
e-mail: asalem70@hotmail.com; asalem70@yahoo.com

M. A Cerrillo Soto
Facultad de Medicina Veterinaria y Zootecnia,
Universidad Juárez del Estado de Durango, Durango,
México

Keywords Gas production · Polyethylene glycol ·
Tannins · Tree foliage

Introduction

Ruminant production in the dry tropic areas including Mexico had faced problems of low availability of forages with poor nutritional quality of grasses and/or crop residues especially in dry season (Carranza et al. 2003; Narvaez et al. 2011). Therefore, farming system in such regions has to survive on such feed resources for most part of the year (Melaku et al. 2010; Debela

and Tolera 2013; Salem et al. 2013a). Fodder trees and shrubs may be considered as important feed resources to provide forage for grazing ruminants, particularly when traditional feed sources are scarce (Salem et al. 2013a). Simple treatments (Salem et al. 2013b) or additives (Salem et al. 2007) for such sources can improve their nutritive values as ruminant feeds. However, species containing less than 8 % CP cannot provide minimum levels of ammonium required by ruminants for optimum microbial activity in the rumen (Norton 1994; Salem et al. 2007). However, there are studies showing the presence of anti-nutritional compounds (total phenolics; TPH and condensed tannins; CT) in these plants (Salem 2005; Camacho et al. 2010a; Salem et al. 2013b; Hernández-Muciño et al. 2015), which inhibit the action of enzymes and the activity of the microorganisms in the rumen, further limiting the degradation of nutrients and reduce the production of volatile fatty acids (Salem et al. 2007; Camacho et al. 2010b). High levels of foliage CT can have detrimental effects on palatability, intake and digestibility; therefore affect animal behavior (Salem et al. 2004; Dey et al. 2008).

The in vitro gas production method, have been used to evaluate the nutritional value of feedstuffs. Increased interest in use of non-conventional feed resources has led to an increase in use of this technique, since gas production can provide useful data on digestion kinetics of both the soluble and insoluble fractions of feedstuffs (Togtokhbayar et al. 2015). The ease of measuring fermentation end products makes this method more efficient than other in vitro methods for studies on phytochemicals, plant secondary metabolites and feed additives (Makkar 2005).

Polyethylene glycol (PEG-4000 or 6000) is a non-nutritive synthetic polymer having high affinity to form complexes, especially with the CT-proteins (Salem et al. 2007; Elahi et al. 2014). Accordingly, PEG can prevent the release of protein from tannin-protein complex, and has been used to mitigate the adverse side effects of compounds on ruminal fermentation. However, an increased on in vitro gas production and degradation parameters with PEG addition was due to its signal of tannin activity on foliage (Getachew et al. 2000; Elahi et al. 2014). The use of PEG to neutralize CT has proved to be useful for protein-tannin complexes displaces due to the ability of CT to interact more strongly with PEG than they do

with protein (Elahi et al. 2014). Thus, supplementation with PEG has been used to alleviate the negative effects of tannins on livestock (Salem et al. 2006; 2007). However, their affects depend on the nature of the tannins, and the nature of the formed tannin-feedstuff complexes.

The aim of this study was to evaluate the chemical composition of five foliages, and the effect of adding PEG during incubation on in vitro gas production (GP), metabolizable energy (ME), partitioning factor (PF_{24h}), in vitro organic matter digestibility (OMD), short chain fatty acids (SCFA) and microbial biomass production (MBP) as tools to detect the adverse effect of tannins in the foliage of non-leguminous trees.

Materials and methods

The work was performed in the nutrition laboratory of the University center of Temascaltepec, Universidad Autónoma del Estado de México, México (18° 58' 43" North latitude, the parallel 19° 13' 54", West longitude of the meridian 99° 48' 50" to 100° 14' 20" meridian), in turn, the annual rainfall varies between 800 and 1600 mm, with a height of 2250 meters above sea level (Municipal Encyclopedia 2008).

Collection of foliages

Foliage of the species *Guazuma ulmifolia*, *Crescentia alata*, *Ficus glabrata*, *Ficus cotinifolia*, *Spondias purpurea*, *Mangifera indica*, *Licania arborea*, *Simira mexicana* were collected during the rainy season, in the Bejucos locality, State of Mexico. Foliages from 18 trees of each species (young and mature leaves) were taken, and the foliages of six trees were mixed to make a sub-sample (1 kg), and thus obtained three subsamples. At the same time, dehydration process (in the shade) was carried out, and subsequently, was milled in a Willey type mill with a 1 mm screen, in order to process the samples.

Chemical composition of foliage

Crude protein (CP) of foliage (AOAC 1990) was analyzed, and well as neutral detergent fiber (NDF) and acid detergent fiber (ADF) (Van Soest et al. 1991), in vitro dry matter digestibility (DMD; g/kg DM–Theodorou et al. 1994), based on Menkey and

Steingass (1988) and amended by Herrero and Jessop (1996), total phenols (TPH $\text{g kg}^{-1}/\text{DM}$) (Folin Ciocalteu) and condensed tannins (CT g kg^{-1} DM) (butanol-HCl) (Waterman and Mole 1994) were determined (Table 1).

In vitro gas production technique

One gram of each foliage was digested in triplicate in bottles (160 mL), to estimate the in vitro GP and the in vitro digestibility of dry and organic matter (DMD and OMD). To assess the biological activity of tannins (Makkar 2005), samples were incubated (39 °C) with or without PEG (4000 PM, Sigma[®]), in a ratio of 2 to 1, based on the gas producing technique, proposed by Theodorou et al. (1994), and on the methodology of Menke and Steingass (1988) and amended by Herrero and Jessop (1996). Rumen fluid of three sheep was collected in a flask (39 °C), before the morning feeding with a standard diet in a 70:30 ratio of forage and concentrate, respectively. Rumen fluid was collected using a portable vacuum pump (Barnant Company, USA), and was immediately transported to the laboratory. The ruminal fluid was maintained at 39 °C, under an atmosphere of CO₂. Bottles without samples or rumen fluid were used as targets, as well as bottles containing rumen fluid only, in order to compensate the GP in the absence of substrate.

The gas volume (mL/g DM of incubated sample) was recorded every hour for the first 8 h, then every

4 h until 60 h, and subsequently at 72, 84 and 96 h of incubation using the pressure reading technique (RPT; DELTA OHM, Italy) of Theodorou et al. (1994).

Measurements

At the end of incubation (i.e. 96 h), the bottle contents was vacuum filtered using sintered glass crucibles (porosity no. 1, and 1 micron pore size, Pyrex, Stone, UK). The residue was dried at 105 °C for 4 h, where degradation potential of DM was estimated. The DMD at 96 h of incubation (g kg^{-1} DM) was calculated as the difference between the DM of initial substrate less the non-degraded DM.

ME and OMD were estimated with the equations proposed by Menke et al. (1979), which use GP at 24 h, adjusted with target:

$$\text{ME (MJ/kg DM)} = 2.20 + 0.136 \text{ GP} + 0.057 \text{ CP (\%)}$$

$$\text{OMD (\%)} = 148.8 + 8.89 \text{ GP} + 4.5 \text{ CP (\%)} + 0.0651 \text{ ash (\%)}$$

where ME = metabolizable energy, OMD = in vitro organic matter digestibility, GP24 = gas production at 24 h ($\text{g mL}/0.2$ DM), CP = crude protein percentage, XA = ash percentage.

The production of short chain fatty acids (SCFA, mmol) was calculated with the equations proposed by Getachew et al. (2000) in both the presence and

Table 1 Chemical composition (g kg^{-1} DM) of non-leguminous fodder trees in the southern Mexico State

Species	CP	OM	NDF	ADF	DMD	TPH	CT
<i>Guazuma ulmifolia</i>	167 ^a	944 ^a	392 ^{bc}	238 ^{bc}	491 ^b	38.2 ^c	41.5 ^d
<i>Crescentia alata</i>	132 ^{ab}	935 ^a	502 ^a	315 ^a	470 ^b	21 ^f	9.8 ^f
<i>Ficus glabrata</i>	173 ^a	909 ^{ab}	384 ^{bc}	232 ^{bc}	446 ^b	88 ^c	102.4 ^b
<i>Ficus cotinifolia</i>	117 ^{ab}	854 ^c	392 ^{bc}	270 ^b	391 ^b	11.1 ^g	43.5 ^d
<i>Spondias purpurea</i>	111 ^{ab}	922 ^a	236 ^e	165 ^d	468 ^b	76.7 ^d	24.4 ^e
<i>Mangifera indica</i>	45 ^c	901 ^{ab}	358 ^{cd}	253 ^{bc}	411 ^b	160.4 ^a	43.5 ^d
<i>Licania arborea</i>	81 ^{bc}	859 ^c	400 ^b	264 ^b	384 ^b	162.4 ^a	195 ^a
<i>Simira mexicana</i>	99 ^b	936 ^a	351 ^d	216 ^c	633 ^a	96.4 ^b	64.4 ^c
SEM	33.06	18.96	21.71	21.05	4.2	4.11	3.64
<i>P value</i>	0.0002	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001

CP, Crude protein, OM, organic matter, NDF, neutral detergent fiber, ADF, acid detergent fiber, DMD, in vitro digestibility of dry matter, TPH, total Phenols, CT, condensed tannins

^{abcdefg} Different superscripts following means in the same column indicate differences at $P < 0.05$

absence of PEG, using the gas volume at 24 h, in species whose protein content varies from 5.4 to 27 %, in total phenols from 1.8 to 25.3 % and in total tannins 0.2 to 21.4 %:

In the absence of PEG: $SCFA = 0.0239 GP - 0.0601$.

In the presence of PEG: $SCFA = 0.0207 GP + 0.0521$.

The PF_{24h} was estimated by the equation proposed by Blümmel et al. (1997), who used the truly degraded organic matter (mg), and the gas volume produced at 24 h:

$$PF_{24h} = (\text{organic matter truly degraded, mg})/\text{mL gas.}$$

Production of microbial mass (MBP) in milligrams was calculated with equations using the total gas volume at 24 h, the PF_{24h} , the stoichiometric ratio (2.2), the difference of “a” factor (non-degraded substrate of the OM) minus the factor “b” (degraded substrate of the OM), in order to obtain truly undegraded OM (France et al. 1993):

$$MBP \text{ (mg)} = ((a - b) - \text{stoichiometric factor} \times \text{total gas volume, mL})$$

Statistical Analysis

Data of foliage chemical composition were analyzed by general linear models, using a completely randomized design; the means were compared with Turkey's test ($P < 0.05$) procedures in SAS (2002); statistical model: $Y_{ij} = \mu + T_i + e_{ij}$, where Y_{ij} = response variable (CP, ash, OM, ADF, NDF, TPH, CT and DMD) treatment ($i = 1, 2, 3, \dots, 8$ trees) on repeat ($j = 1, 2, 3$); μ = general mean, T_i = effect of treatment (i), e_{ij} = random error of treatment (i) on repeat (j) $n-1$ ($\sigma^2, 0$) terms.

Data of degradability were analyzed by general linear models, using a completely randomized design in an 8×2 factorial arrangement, means were compared with Tukey's test ($P < 0.05$), procedures in SAS (2002); statistical model: $Y_{ijk} = \mu + A_i + B_j + (AB)_{ij} + e_{ijk}$, where: Y_{ijk} = response variable (GP, OMD, ME, SCFA, PF_{24h} and MBP) on repeat ($k = 1, 2, 3$), level i of ($A = 1, 2, 3, 4 \dots 0.8$ trees) and level j of ($B = 1, 2$, where: 1 = without PEG, 2 = with PEG) μ = general mean, A_i = effect of A factor to i level, B_j = effect of factor B at level j ;

$(AB)_{ij}$ = interaction $A \times B$ effect to the i, j level; e_{ijk} = random error, in terms of $n-1$ ($\sigma^2, 0$).

Results and discussion

Regarding to the chemical composition of the analyzed species, the content of CP was higher ($P < 0.0002$) for *G. ulmifolia* (167 g kg^{-1} DM) and *F. glabrata* (173 g kg^{-1} DM); however, NDF and ADF were higher ($P < 0.0001$) with *C. alata* than other species. *S. mexicana* had the highest DMD ($P < 0.0001$) with 633 g kg^{-1} DM (Table 1). The nutritional composition of assessed species was within the evaluated range for non-leguminous species in tropical regions (Pinto et al. 2010; Plata et al. 2009; Moforte et al. 2005). Species containing less than 8 % CP did not provide the minimum ammonium level for proper ruminal functioning and bacteria growth (Norton 1994). El-Hassan et al. (2000) reported that high concentrations of NDF in forages are associated with lower consumption, while a high concentration of ADF is associated with low ruminal digestibility. However, the average values of fiber fractions (Table 1) contained in the studied species was relatively low (502 g kg^{-1} DM, NDF and 315 g kg^{-1} DM, ADF).

All species contained TPH in the range of 21 to 162.4 g kg^{-1} DM. Both of *L. arborea* and *F. glabrata* species had high contents of CT (Table 1). The contents of TPH and CT in the species are similar to those reported by Moforte et al. (2005) and García et al. (2008). High contents of CT could impact the digestion processes in the rumen, affecting the growth of rumen bacteria and enzyme activity (Makkar 2005; Salem et al. 2006), even depress consumption or reduce foliage digestibility (Salem et al. 2006). Biological activity of CT depends on two factors: their concentration and structure. However, Dey et al. (2008) conclude that moderate levels 1–4 % of condensed tannins produce positive effects, when coupled with dietary protein forms complexes, preventing their degradation in the rumen.

Inclusion of PEG in fermentation of non-leguminous fodder trees (Table 2) results in a significant increase ($P < 0.0001$) of GP in *S. purpurea*, *G. ulmifolia*, *F. glabrata* and *L. arborea* species. In addition, there is a positive effect on the species \times PEG interaction at the 24, 48 and 96 h (Moforte

Table 2 In vitro gas production of non-legume forage species, without (–) PEG or with (+) PEG

Species		Incubation time		
		24 h	48 h	96 h
<i>G. ulmifolia</i>	–	44.1 ^{ef}	88.1 ^{def}	117.0 ^{efg}
	+	64.5 ^{bcd}	130.1 ^c	157.8 ^{cde}
<i>C. alata</i>	–	66.1 ^{bcd}	170.9 ^{ab}	210.6 ^{ab}
	+	65.7 ^{bcd}	171.1 ^{ab}	211.0 ^{ab}
<i>F. glabrata</i>	–	46.1 ^{def}	81.1 ^{ef}	97.9 ^g
	+	63.8 ^{bcd}	117.3 ^{cde}	149.1 ^{cdef}
<i>F. cotinifolia</i>	–	80.9 ^b	150.9 ^{bc}	189.8 ^{abc}
	+	78.8 ^b	154.5 ^{abc}	199.8 ^{ab}
<i>S. purpurea</i>	–	71.1 ^{bc}	139.3 ^{bc}	173.6 ^{bcd}
	+	109 ^a	190.6 ^a	223.0 ^a
<i>M. indica</i>	–	51.8 ^{cdef}	89.6 ^{def}	122.4 ^{efg}
	+	63.6 ^{bcd}	119.3 ^{cde}	145.3 ^{def}
<i>L. arborea</i>	–	36.0 ^f	64.9 ^f	82.7 ^g
	+	54.1 ^{cdef}	120.8 ^{cd}	150.0 ^{cdef}
<i>S. mexicana</i>	–	36.6 ^f	73.8 ^f	115.2 ^{fg}
	+	34.5 ^f	75.3 ^f	123.1 ^{efg}
SEM		6.8	12.6	13.7
<i>P</i> value				
Species		<0.0001	<0.0001	<0.0001
PEG		<0.0001	<0.0001	<0.0001
Species × PEG		0.0001	0.0008	0.0010

^{abcdefg} Different superscripts following means in the same column indicate differences at $P < 0.05$

et al. 2005; Arhab et al. 2009). The addition of PEG overcomes the tannins effects over nutrients availability, as indicative of the accumulated GP, when forming the PEG-tannins complex (Arhab et al. 2009; Elahi et al. 2014). Foliage with high content of CT not always respond similarly when incubated with PEG; it is possible that this difference is due to the chemical composition and structure of tannins of each species (Salem et al. 2007; Elahi et al. 2014). Arhab et al. (2009) studied the effect of of tannins present of *Aristida plumosa*, *Danthonia forskahlii*, *Astragalus gombiformis*, *Genista saharae*, two date palm fractions (leaves and racemes), and vetch-oat hay taken as control on in vitro GP and reported overall increase in GP (20.2 %), with the exception of *Danthonia* and *Aristida*. Getachew et al. (2002) conclude that the foliage containing TPH and CT between 40 and 20 g kg⁻¹ DM does not induce the expected increase in GP by the addition of PEG. This differs from the

results of this study for *G. ulmifolia*, which shows an increase (Table 1) of GP, as a result of PEG.

In turn, the ME was different ($P < 0.0001$) between species, by PEG addition (Table 3). *S. purpurea* has the highest ME content (5.9 MJ kg⁻¹ DM). There is a positive correlation between ME calculated and in vitro GP (Table 2).

Elahi et al. (2014) incubated *Prosopis cineraria* with PEG, and showed increased GP due to PEG addition. In the present study, the *C. alata* and *F. cotinifolia* species showed no PEG effect on GP and ME, which could be influenced by the lack of complex formation between the PEG and tannins.

The use of PEG increased ($P < 0.0001$) the OMD (Table 3) in *G. ulmifolia* (275 g kg⁻¹ DM) and *S. purpurea* (354 g kg⁻¹ DM). This coincides with studies in tree foliage (Salem et al. 2007; Arhab et al. 2009), when increased OMD in fodder shrubs species obtained due to the effect of PEG incubation; unlike Moforte et al. (2005), who by incubating PEG with 15 shrub species and found no differences in OMD. However, the difference in the degradability of some species could be due to the degree of lignification of NDF (Elahi et al. 2014). The CT interfere with adhesion of rumen bacteria to feed particles, affecting the bacterial population and ruminal fermentation, and reducing OMD (Elahi et al. 2014). When the foliage is incubated with PEG, PEG-tannins complex was formed, which facilitated the substrate digestion by bacteria, improving OMD.

The PF_{24h} decreased ($P < 0.0001$) by addition of PEG (Table 3) to *L. arborea*, *F. glabrata*, *G. ulmifolia* and *S. purpurea*, which were the species showing a higher GP by effect of PEG. Arhab et al. (2009) stated that addition of PEG can promote GP with decreasing PF_{24h} of *Aristida plumosa* and palm leaves forages without affecting PF_{24h} in *Genista saharae*, *Danthonia forskahlii* and vetch-oat hay. The effect varied due to the limited ability of PEG to completely inhibit the negative effects of tannins (Frutos et al. 2004), which depends on stereochemistry and chemical structure of tannins (Elahi et al. 2014). The increased of in vitro GP could be the result of a low partitioning of nutrients for protein synthesis, which reduces the value of PF_{24h}. In turn, this is due to the tannins formation of complex with proteins, which are insoluble in neutral detergent and contribute to non-degradable fraction (Makkar et al. 1998). Plants with a high PF_{24h} value are,

Table 3 Contents of ME, OMD, PF, SCFA and MBP in non-leguminous forage species without (–) PEG or with (+) PEG, at 24 h incubation

Species		ME	OMD	PF _{24h}	SCFA	MBP
<i>G. ulmifolia</i>	–	4.4 ^{bcde}	238 ^{efgh}	5.4 ^{ab}	1.0 ^{fg}	664.5 ^{ab}
	+	4.9 ^{bcd}	275 ^{bcde}	4.3 ^{bcd}	1.4 ^{cdef}	583.3 ^{bcde}
<i>C. alata</i>	–	4.7 ^{bcd}	276 ^{bcde}	4.2 ^{bcd}	1.5 ^{bcde}	578.2 ^{cde}
	+	4.7 ^{bcd}	276 ^{bcde}	4.2 ^{bcd}	1.4 ^{cdef}	579.7 ^{cde}
<i>F. glabrata</i>	–	4.4 ^{bcde}	244 ^{efgh}	5.4 ^{ab}	1.0 ^{efg}	654.4 ^{abc}
	+	4.9 ^{bcd}	276 ^{bcde}	4.3 ^{bcd}	1.3 ^{cdef}	583.9 ^{bcde}
<i>F. cotinifolia</i>	–	5.1 ^b	308 ^b	3.8 ^{cd}	1.9 ^{ab}	514.1 ^e
	+	5.1 ^b	304 ^{bc}	3.9 ^{cd}	1.7 ^{bc}	522.5 ^e
<i>S. purpurea</i>	–	4.9 ^{bcd}	286 ^{bcd}	4.1 ^{cd}	1.6 ^{bcd}	557.3 ^{de}
	+	5.9 ^a	354 ^a	3.3 ^d	2.3 ^a	404.8 ^f
<i>M. indica</i>	–	3.9 ^e	250 ^{defgh}	4.8 ^{bc}	1.2 ^{defg}	636.2 ^{abcd}
	+	4.2 ^{cde}	271 ^{cdef}	4.3 ^{bcd}	1.3 ^{cdef}	589.4 ^{bcde}
<i>L. arborea</i>	–	3.7 ^e	226 ^{gh}	6.3 ^a	0.8 ^g	694.9 ^a
	+	4.2 ^{de}	258 ^{defg}	4.8 ^{bc}	1.1 ^{efg}	622.9 ^{abcd}
<i>S. mexicana</i>	–	3.9 ^e	224 ^h	6.4 ^a	0.8 ^g	695.9 ^a
	+	3.8 ^e	220 ^h	6.4 ^a	0.7 ^g	704.3 ^a
SEM		0.260	1.2	0.4396	0.1554	27.3
<i>P value</i>						
Species		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
PEG		<0.0001	<0.0001	<0.0001	0.0005	<0.0001
Species × PEG		0.0094	0.0001	0.0171	0.0004	0.0001

(–) without PEG, (+) with PEG
 ME, Metabolizable energy (MJ/kg DM), OMD, in vitro digestibility of organic matter (mg/g DM), PF_{24h}, the partitioning factor at 24 h of incubation (mg OMD, ml gas), SCFA, short chain fatty acids (mol/150 mL), MBP, production of microbial biomass (mg/g DM)
 abcdefgh Different superscripts following means in the same column indicate differences at P < 0.05

generally, highly digestible, a value which is highly correlated with the dry matter intake in ruminants.

Production of SCFA in the foliage samples incubated, with and without PEG, was modified ($P < 0.0001$) by the effect ($P < 0.0005$) of the species and addition of PEG (Table 3). With the addition of PEG, *S. purpurea* (2.3 mol/150 mL) showed the higher production of SCFA. Similar results were reported by Getachew et al. (2000) showing an increased SCFA production ($P < 0.05$) due to PEG addition. The addition of PEG increased isoacid concentration, also in the absence of PEG; there was greater absorption isoacids, because tannins protected protein from deamination. The addition of PEG only increases the production of butyrate (Getachew et al. 2000). However, in another study of Mohammadabadi et al. (2010) conclude that there is a positive correlation between SCFA and GP. The increase of in vitro GP and SCFA production due to the addition of PEG in the present study confirm the effect of tannins on OMD.

The MBP was different between species ($P < 0.0001$) by the effect of PEG. In order of importance, species with more MBP were *L. arborea*, *S. Mexican* and *G. ulmifolia*. With the addition of

PEG, MBP decreased in all species tested, being *S. purpurea* the one with the lowest MBP. Different results reported by Al-Masri (2007), who find no differences in MBP in shrub species by effect of PEG. Getachew et al. (2000) report that the addition of PEG to the foliage with tannins increased ruminal NH₃-N concentration, but reduced the synthesis of microbial protein. This is probably due to poor synchronization between liberated nitrogen and the fermentation of carbohydrates. Synchronizing in the rate of degradation of N and carbohydrates in the rumen is important for efficient use of NH₃-N in the rumen for the synthesis of microbial protein. Apparently, it is necessary to add energy sources to trap nitrogen resulted from fermentation, when using PEG as a binding agent in leguminous and non-leguminous trees with high tannin content.

Conclusions

Foliage trees can be used as animal feed resources in dry tropic areas over the world, where low availability of conventional animal feeds. Chemical composition

and in vitro GP and digestibility of the tested foliage trees showed different nutritive values (*S. mexicana* > *G. ulmifolia* > *C. alata* > *S. purpurea* > *F. glabrata* > *M. indica* > *F. cotinifolia* > *L. arborea*). Improved GP with PEG addition before fermentation (*S. purpurea* > *C. alata* > *F. cotinifolia* > *G. ulmifolia* > *L. arborea* = *F. glabrata* = *M. indica* > *S. mexicana*) were obtained. *S. purpurea* and *F. cotinifolia* had the highest OMD, ME, and SCFA compared to the other species. Therefore, both of *S. purpurea* and *F. cotinifolia* can be used as animal feed resources for livestock in the south region of Mexico.

References

- Al-Masri MR (2007) An in vitro evaluation of some drought-tolerant native plants range in terms of ruminal microbial nitrogen, microbial biomass fermentation and their characteristics utilizing a gas-production technique. *Trop Grassl* 41:292–300
- AOAC (1990) Official methods of analysis, 15th edn. Association of Official Analysis Chemists, Washington, DC, p 2044
- Arhab R, Macheboeuf D, Aggoun M, Bousseboua H, Viala D, Besle JM (2009) Effect of polyethylene glycol on in vitro gas production and digestibility of tannin container containing feedstuffs from north African arid zone. *Trop Subtrop Agroecosyst* 10:475–486
- Blümmel M, Makkar HPS, Becker K (1997) In vitro gas production: a technique revisited. *J Anim Physiol Anim Nutr* 77:24–34
- Camacho LM, Rojo R, Salem AZM, Mendoza GD, Lopez D, Tinoco JL, Albarran B, Montañez VOD (2010a) In vitro ruminal fermentation kinetics and energy utilization of three Mexican tree fodder species during the rainy and dry period. *Anim Feed Sci Technol* 160:110–120
- Camacho LM, Rojo R, Salem AZM, Provenza FD, Mendoza GD, Aviles F, Montañez VOD (2010b) Effect of season on chemical composition and in situ degradability in cows and goats in adapted and unadapted of three Mexican browse species. *Anim Feed Sci Technol* 155:206–212
- Carranza MMA, Sanchez VLR, Pineda LMR, Cuevas GR (2003) Quality and forage potential of species of tropical deciduous forest of the Sierra de Manantlán, Mexico. *Agrociencia* 37(2):203–210
- Debela E, Tolera A (2013) Nutritive value of botanical fractions of *Moringa oleifera* and *Moringa stenopetala* grown in the mid-Rift Valley of southern Ethiopia. *Agrofor Syst* 87:1147–1155
- Dey A, Dutta N, Sharma K, Pattanaik AK (2008) Effect of dietary inclusion of Ficus leaves as a protectant infectoria of proteins on the performance of lambs. *Small Rumin Res* 75:105–114
- Elahi MY, Nia MM, Salem AZM, Mansouri H, Olivares-Pérez J, Cerrillo-Soto MA, Kholif AE (2014) Effect of polyethylene glycol on in vitro gas production kinetics of *Prosopis cineraria* leaves at different growth stages. *Ital J Anim Sci* 13:363–368
- El-Hassan SM, Lahlou A, Newbold CJ, Wallace J (2000) Chemical composition and degradation characteristics of foliage of some African multipurpose trees. *Anim Feed Sci Technol* 86:27–37
- Encyclopedia of the Municipalities of the State of Mexico (2008) Tejupilco Township. 114 pp
- France J, Dhanoa MS, Theodorou MK, Lister SJ, Davies DR, Isac D (1993) A model to interpret gas accumulation profiles with in vitro associated ruminal degradation of feeds. *J Theor Biol* 163:99–111
- Frutos P, Hervás G, Giráldez FJ, Mantecón AR (2004) An in vitro study on the ability of polyethylene glycol to inhibit the effect of quebracho tannins and tannic acid on rumen fermentation in sheep, goats, cows, and deers. *Aust J Agric Res* 55:1125–1132
- García DE, Medina MG, Clavero T, Humbría J, Baldizan A, Dominguez C (2008) Preference fodder trees by goats in the lower area of the Venezuelan Andes. *Revista Científica U. Z. XVIII* 005:549–555
- Getachew G, Makkar HPS, Becker K (2000) Effect of polyethylene glycol on in vitro degradability of nitrogen and microbial protein synthesis from tannin - rich browse and herbaceous legumes. *Br J Nutr* 84:73–83
- Getachew G, Makkar HPS, Becker K (2002) Tropical browses: contents of phenolic compounds, in vitro gas production and stoichiometric relationship between short chain fatty acid and in vitro gas production. *J Agric Sci (Camb)* 139:341–352
- Hernández-Muciño D, Sosa-Montes E, Ceccon E (2015) *Leucaena macrophylla*: an ecosystem services provider? *Agrofor Syst*. doi:10.1007/s10457-014-9751-0
- Herrero M, Jessop NS (1996) Relationship between in vitro gas production and neutral detergent fiber disappearance in three tropical grasses. *J Anim Sci* 62:682–692
- Makkar HPS (2005) In vitro gas methods for evaluation of feeds containing phytochemicals. *Anim Feed Sci Technol* 123–124:291–302
- Makkar HPS, Blümmel M, Becker K (1998) Application of an in vitro gas method to understand the effects of the natural plant products on availability and partitioning of nutrients. In: Deaville ER, Owen E, Adesogan AT, Rymer C, Huntington JA, Lawrence TJL (eds) *In Vitro Techniques for Measuring Nutrient Supply to Ruminants*. British Society of Animal Production, Edinburgh, pp 147–150 Occasional Publication no. 22
- Melaku S, Nigatu L, Aregawi T (2010) Chemical composition, in vitro dry matter digestibility and in sacco degradability of selected browse species used as animal feeds under semi-arid conditions in Northern Ethiopia. *Agrofor Syst* 80:173–184
- Menke KH, Steingass H (1988) Estimation of the energetic feed value from chemical analysis obtained and in vitro gas production using rumen fluid. *Anim Dev Res* 28:7–55
- Menke KH, Raab L, Salewski A, Steingass H, Fritz D, Schneider W (1979) The estimation of the digestibility and metabolizable energy content of ruminant feedingstuffs from the gas production when they are incubated with rumen liquor in vitro. *J Agric Sci Camb* 93:217–222

- Moforte BGE, Sandoval CCA, Ramirez AL, Capetillo LMC (2005) Defaunating capacity of tropical fodder tree: effects of polyethylene glycol and its relationship to in vitro gas production. *Anim Feed Sci Technol* 123–124:313–327
- Mohammadabadi T, Chaji M, Tabatabaei S (2010) The effect of tannic acid on in vitro gas production and rumen fermentation of sunflower meal. *J Anim Vet Adv* 9(2):277–280
- Narvaez N, Brosh A, Mellado M, Pittroff W (2011) Potential contribution of *Quercus durata* and *Adenostoma fasciculatum* supplemented with *Medicago sativa* on intake and digestibility in sheep and goats. *Agrofor Sys* 83:279–286
- Norton BW (1994) The nutritive value of tree legumes. In: Gutteridge C, Shelton H (eds) *Forage tree legumes in tropical agriculture*. CAB International, Wallingford, pp 177–192
- Pinto RR, Hernandez D, Gómez H, Cobos MA, Quiroga R, Pezo D (2010) Livestock fodder trees three regions of Chiapas, Mexico: uses and nutritional characteristics. *Univ Sci* 26(1):19–31
- Plata FX, Ebergeny S, Resendiz JL, Villareal O, Barcena R, Viccon JA, Mendoza GD (2009) Palatability and chemical composition of food consumed by captive white-tailed Yucatan (*Odocoileus virginianus yucatanensis*) deer. *Arch Med Vet* 41:123–129
- Salem AZM (2005) Impact of season of harvest on in vitro gas production and dry matter degradability of *Acacia saligna* leaves with inoculum from three ruminant species. *Anim Feed Sci Technol* 123–124:67–79
- Salem AZM, González JS, López S, Ranilla MJ (2004) Feeding behaviour patterns and water intake in sheep and goats fed alfalfa hay treated with quebracho. *Cah OPTIONS Méditerran* 59:215–219
- Salem ZM, Salem MZM, El-Adawy MM, Robinson PH (2006) Nutritive evaluations of some browse tree foliage during the dry season: secondary compounds, feed intake and in vivo digestibility in sheep and goats. *Anim Feed Sci Technol* 127(3–4):251–267
- Salem AZM, Robinson PH, El-Adawy MM, Hassan AA (2007) In vitro fermentation and microbial protein synthesis of some browse tree leaves with or without addition of polyethylene glycol. *Anim Feed Sci Technol* 138:318–330
- Salem AZM, López S, Ranilla MJ, González JS (2013a) Short to medium-term effects of consumption of quebracho tannins on total and parotid saliva production and composition in sheep and goats. *J Anim Sci* 91:1341–1349
- Salem AZM, Zhou CS, Tan ZL, Mellado M, Cipriano-Salazar M, Elghandour MMY, Odongo NE (2013b) In vitro digestibility, chemical composition, and kinetics of gas production of foliage from four fodder trees ensiled with or without molasses and urea. *J Integr Agric* 12(7):1234–1242
- Theodorou MK, Williams BA, Dhanoa MS, McAllan AB, France J (1994) A simple gas production method using a pressure transducer to determine the fermentation kinetics of ruminant feeds. *Anim Feed Sci Technol* 48:185–197
- Togtokhbayar N, Cerrillo SMA, Jigjidpurev S, Shinekhuu J, Urantulkhuur D, Nergui D, Elghandour MMY, Odongo NE, Kholif AE (2015) Effect of exogenous xylanase on rumen in vitro gas production and degradability of wheat straw. *Anim Sci J*. doi:10.1111/asj.12364
- Van Soest PJ, Robertson JB, Lewis BA (1991) Methods for dietary fiber, neutral detergent fiber, and non-starch polysaccharides in relation to the animal nutrition. *J Dairy Sci* 74:3583–3592
- Waterman P, Mole S (1994) *Analysis of phenolic plant metabolites*. Scientific Publication Blackwell, Oxford