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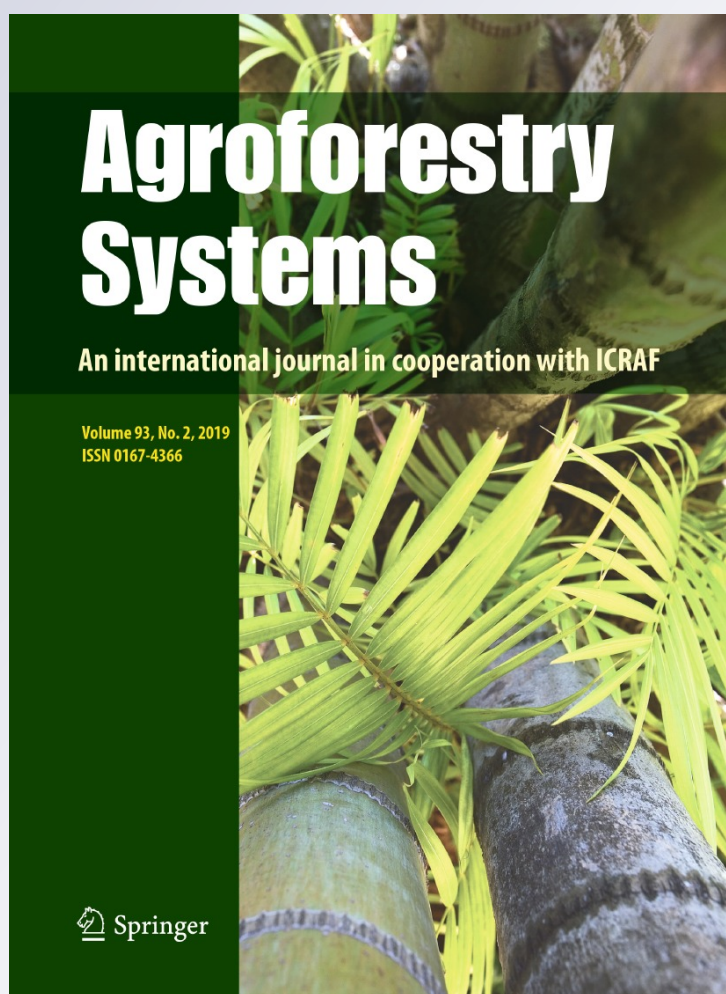
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Fruits chemical composition and potential ruminal digestion of nine tree species in dry tropic region of Mexico

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Abstract Chemical composition, in vitro gas production (GP), in vitro dry matter (DMD) and organic matter (OMD) digestibility, metabolizable energy (ME), gas yield (GY_{24h}), short chain fatty acids (SCFA) and microbial mass production (MMP) were measured in fruits of nine trees species, using in vitro gas technique with and without polyethylene glycol (PEG:MW-4000) as chelating tannins agent. The fruits with the highest protein content ($P < 0.001$) (135.5–196.0 g/kg DM) were *Leucaena esculenta*, *Pithecellobium acatense*, *Acacia farnesiana* and *Enterolobium cyclocarpum*, total phenols ($P < 0.001$) (349.8–553.1 g/kg DM) *Lysiloma divaricata*, *A. farnesiana* and *Caesalpinia coriaria* and condensed tannins ($P < 0.001$) *L. divaricata* and *E. cyclocarpum* with 95.7 and 71.7 g/kg DM, respectively. The highest DMD in fruits of *C. coriaria*, *Pithecellobium dulce*, *A. farnesiana* and *L. esculenta* ($P < 0.001$). The GP, OMD, ME, GY_{24h}, SCFA and MMP, was different ($P < 0.0001$) between fruit trees.

The PEG increased ($P < 0.0001$) the GP, ME, GY_{24h} and SCFA in the fruits of *Gliricidia sepium*, *L. esculenta* and *C. coriaria*. In conclusion, the nutritional composition and in vitro fermentation parameters differs between fruits. The increase in PEG increased the value of GP, ME, OMD, GY_{24h} and SCFA, indicating that the fruits contain phenolic compounds with biological activity that precipitate proteins.

Keywords Degradability · Gas production · PEG · Trees species

Introduction

The productivity of livestock in tropical regions is related to the low availability and nutritional quality of foods that are commonly used as basal diet (Salem et al. 2006). The nutritional status of ruminants depends on their ability to ferment and obtain nutrients from their diet, such as short chain fatty acids (SCFA) and the microbial mass (Salem et al. 2006). The leguminous trees produce fruit (up to 200 kg per tree per year) (Ngwa et al. 2002; Olivares-Perez et al. 2011). In addition, the fruits contain useful amounts of protein and can serve as a nitrogen supplement in ruminant feeding during the dry season of year, when feed is scarce (Mlambo et al. 2008). However many

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trees contain high levels of tannins, which may limit the use of its foliage or fruit to feed animals (Olivares et al. 2013; Salem et al. 2013; Salem 2012), this requires investigation of the chemical composition, in vitro fermentation parameters and digestibility of dry matter. The in vitro gas production (GP) is a technique that has been used to estimate the activity of tannins by use of polyethylene glycol (PEG) on microbial activity, and diet digestibility in the rumen (Elahi et al. 2014; Salem et al. 2007; Makkar 2005). The PEG reduces or neutralizes the effects of tannins, by forming tannin–PEG complexes (Salem et al. 2007, 2013; Waghorn 2008). Thus the PEG avoid that the free tannins bind to dietary nutrients, in addition to dissociate the tannins–nutrient complex preformed (Lorenz et al. 2014; Salem et al. 2013). It has been shown that when animals are fed forages high in condensed tannins, adding PEG improves the digestibility of the substrate and the final products of the fermentation during digestion of feed (Jiménez et al. 2011). The objective of the study was to evaluate the chemical composition and effect of the species and the addition of PEG on the in vitro parameters (i.e., GP, degradability, SCFA) in nine fruit trees used as feed in ruminants diet in the tropical area of Mexico.

Materials and methods

Study area

The region of Tierra Caliente, Altamirano province of Guerrero State, located at 18°20'30" north latitude and 100°39'18" west longitude on the left bank of the Rio Cutzamala and 340 m a.s.l. The predominant climate type is Aw0 by classification Koppen is the driest warm sub-humid region, with summer rains. The average temperature was of 28 °C and annual rainfall of 1010.7 mm.

Fruits studied

Fruits of leguminous trees native to the study area were used: *Acacia cochliacantha*, *Enterolobium cyclocarpum*, *Pithecellobium dulce*, *Acacia farnesiana*, *Lysiloma divaricata*, *Pithecellobium acatense*, *Gliricidia sepium*, *Leucaena esculenta* and *Caesalpinia coriaria* (Olivares-Perez et al. 2011).

The samples collected (during the months of December–February of 2014) were mature fruits of legume trees. Three individual samples of 0.5 kg (each one pooled from 18 trees, i.e. collected randomly from three transects from 6 ranches) were randomly collected. The samples were dried at 40 °C for 48 h in the shade to obtain a constant weight, and then ground in a Willey-mill (Thomas Wiley® Mini-Mill, USA) of one mm screen size. Ground samples were analysed for dry matter (DM) by drying at 105 °C for 24 h in a forced air oven (Humboldt, model H-30140, USA). Ash content was measured after igniting samples in a muffle furnace (Benchtop muffle furnace model 4800, California, EE.UU.) at 550 °C for 12 h (AOAC 2000). The organic matter (OM) was calculated after by the difference between DM cremated and the residual ash (AOAC 2000). The CP was determined by the Kjeldahl method (AOAC 2000; ID954.01). Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined by the method of Van Soest (1994). Total phenolic content (TP) and condensed tannins (CT) were estimated according to the method TP-Folin–Ciocalteu and CT-butanol–HCl, respectively, described by Waterman and Mole (1994).

In vitro gas production of fruits with and without PEG

In vitro gas production (GP) and in vitro digestibility of dry and organic matter (DMD and OMD) were determined by the gas production technique, proposed by Theodorou et al. (1994). Rumen fluid was collected via an oral tube connected to portable vacuum pump (Barnant Company, USA), in the morning (07:00 h) from three Creole male adult goats, adapted for 30 days to the diet with 30% of concentrated (16% of CP and 2.5 Mcal ME) and 70% of foliage (oat hay with 7% of CP and 1.8 Mcal ME).

One gram of fruit tree sample was weighed, without and with of polyethylene glycol (PEG-4000MW, Sigma) (2 g) (i.e., six bottles for each fruit of the nine fruits of the leguminous trees to which were added three bottles with PEG and three bottles without PEG, and three more bottles as blank with rumen fluid only), to assess the biological activity of tannins in 160 mL serum bottles (Waghorn 2008). With the use of an automatic dispenser (Jencons, Hemel Hemstead, England), 90 mL of serum reduced buffer containing

micro- and macro-elements, were added to the bottles. A reducing agent of resazurin were prepared in flasks at 39 °C under a CO₂ atmosphere to turn into a light pink colour; 10 mL was subsequently added of rumen fluid (previously filtered through four layers of gauze) in each bottle, during the procedure the solution was maintained in an anaerobic environment by the addition of CO₂ and the bottles were incubated at 39 °C (Incubator, Binder Company, Germany). The gas volume was recorded each hour during the first 8 h, then every 4 h until 60 h, and later at 72, 84 and 96 h of incubation, using the reading pressure technique (pressure transducer, RPT; DELTA OHM, Italy) of Theodorou et al. (1994).

Estimation of degraded substrate

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

The ME and OMD were estimated with the equations proposed by Menke et al. (1979), which uses gas production at 24 h of 0.2 g sample, adjusted with white:

$$ME \text{ (MJ/kg DM)} = 2.20 + 0.136 \text{ GP}_{24} + 0.0057 \text{ CP} \tag{1}$$

$$OMD \text{ (\%)} = 14.88 + 0.889 \text{ GP} + 0.45\text{CP} + 0.0651 \text{ XA} \tag{2}$$

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

The production of short chain fatty acids (SCFA, mmol), were calculated with the equations proposed by Getachew et al. (2004), in the presence and absence of PEG, using the gas volume at 24 h:

$$\text{In absence of PEG : } SCFA = 0.0239 \cdot \text{Gas} - 0.0601. \tag{3}$$

$$\text{In presence of PEG : } SCFA = 0.0207 \cdot \text{Gas} + 0.0521. \tag{4}$$

The effective gas volume produced (GY_{24h}) was estimated with the equations proposed by Blümmel et al. (1997), using the degraded organic matter (mg) which was obtained of treating the residue with a neutral detergent solution to corrected microbial biomass and the gas volume at 24 h:

$$GY \text{ 24h} = \text{mL gas} / \text{degraded organic matter, mg}. \tag{5}$$

The microbial mass production (MMP) was calculated with equations, using the total gas volume at 24 h, the stoichiometric factor (2.2), the difference of the factor “a” (undegraded substrate of the OM) minus the factor “b” (degraded substrate of the OM) to obtain the undegraded factor of the OM (Blümmel et al. 1997):

$$MMP \text{ (mg)} = ((a - b) - \text{stoichiometric factor} \times \text{total gas volume, mL}). \tag{6}$$

The kinetics of in vitro fermentation on fruits (treatment) without and with PEG was assessed using the model of France et al. (2000):

$$A = b \times \left(1 - e^{-c(t_{Lag})}\right) \tag{7}$$

where A is the gas volume production at time t, b is the asymptotic gas production milliliter per gram DM, c is the speed the gas produced (h) of fraction b of slowly fermentable food, and t_{Lag} is the starting time of the fermentation of NDF (SAS 2002).

Experimental design and statistical analysis

The data variables were analysed by general linear models and the means were compared using the Tukey test (P < 0.05), procedures in SAS (SAS 2002).

The variables of chemical composition of fruits were analysed by completely randomized design, statistical model:

$$Y_{ij} = \mu + T_j + \xi_{ij} \tag{8}$$

where Y_{ij} is response variable (CP, ash, OM, ADF, NDF, CT, TP and DMD), μ is general mean, T_i is treatment effect (j = 1, 2, 3...9 fruits) and ξ_{ij} is the error in terms of n - 1 (σ²,0).

The data variables of the degradability of the substrate and fermentation kinetic with and without

PEG, were analysed using a completely randomized design in factorial arrangement of 9 × 2, statistical model:

$$Y_{ijk} = \mu + A_i + B_j + (AB)_{ij} + \xi_{ijk} \quad (9)$$

where Y_{ijk} = fermentation characteristic (GP, OMD, ME, SCFA, GY₂₄, MMP, b , c and t_{Lag}), μ is general mean; A_i is the substrate effect ($i = 1, 2, 3 \dots 9$ fruits), B_j is the PEG treatment effect ($j =$ with PEG and without PEG); AB_{ij} is the first-order interaction and ξ_{ijk} is the error in terms of $n - 1$ (σ^2 , 0) (SAS 2002).

Results

Chemical composition of the fruits and in vitro dry matter digestibility (DMD)

According to the contents of CP, the fruits are classified into three groups ($P < 0.0001$), the high protein content (135.5–196.0 g/kg DM) which represent the fruits of *L. esculenta*, *P. acatlense*, *A. farnesiana* and *E. cyclocarpum*, the average protein content (101.7–110.8 g kg/MS) which represent the fruits of *A. cochliacantha*, *P. dulce* and *L. divaricata*, and finally the low protein content (48.4–93.0 g/kg DM) that integrating to the fruits of *G. sepium* and *C. coriaria* (Table 1).

The OM content was higher ($P < 0.0001$) in the fruits of *L. divaricata*, *G. sepium* and *C. coriaria*. The

ash content was higher ($P < 0.01$), in the fruits of *P. dulce*, *L. esculenta* and *A. cochliacantha* indicating that they contain more minerals. The content of NDF and ADF was higher ($P < 0.001$) in the fruits of *A. cochliacantha*, *G. sepium*, *L. divaricata* and *P. acatlense* respectively and DMD was greater ($P < 0.001$) in the fruits of *C. coriaria*, *P. dulce*, *A. farnesiana* and *L. esculenta*, respectively (Table 1).

Fruits high in TP ($P < 0.001$) were *C. coriaria*, *L. divaricata* and *A. farnesiana*, the fruits of the other trees contained low levels of phenols. In all fruits, the CT content was low; however, the fruits of *L. divaricata* and *E. cyclocarpum* had more CT compared to the fruit of the other trees (Table 1).

Effect of PEG in gas volume produced

The fruits had significant effect on the gas volume production at 24, 48 and 96 h of incubation ($P < 0.0001$) (Table 2) were observed. The fruits with higher gas volume production during digestion at 24, 48 and 96 h were *A. farnesiana*, *P. dulce* and *E. cyclocarpum* respectively. Fruits with minor gas production in the three incubation times were of trees *P. acatlense*, *G. sepium*, *L. esculenta*, *L. divaricata* and *A. cochliacantha* respectively.

Incubation with PEG increased ($P < 0.0001$) the gas volume production during digestion in the three incubation times of the fruits *G. sepium* and *C. coriaria* only. The fruit × PEG interaction was

Table 1 Nutritional composition (g/kg DM) of the fruits of leguminous trees of Mexico

Fruits	DM	CP	Ash	OM	ADF	NDF	DMD	TP	CT
<i>L. esculenta</i>	73.1	196.0	51.8	948.1	237.4	343.6	361.0	51.2	45.0
<i>A. cochliacantha</i>	99.4	110.8	58.4	941.5	383.8	550.1	154.0	46.1	50.3
<i>G. sepium</i>	72.7	93.0	38.2	961.8	347.8	510.5	174.0	91.0	48.6
<i>E. cyclocarpum</i>	72.0	135.5	42.0	957.9	138.5	213.4	272.0	28.2	71.7
<i>A. farnesiana</i>	97.8	153.1	46.8	953.1	162.1	230.3	335.0	397.3	24.0
<i>P. dulce</i>	70.9	101.7	65.3	934.6	147.2	209.1	356.0	29.9	17.9
<i>L. divaricata</i>	99.1	107.6	25.4	974.5	349.5	507.2	125.0	349.8	95.7
<i>C. coriaria</i>	98.7	48.4	33.2	966.7	61.5	102.6	446.0	553.1	26.9
<i>P. acatlense</i>	98.5	170.2	42.6	957.3	278.3	400.0	252.0	49.0	48.0
SEM	14.00	3.02	4.77	4.77	8.41	4.90	65.71	31.10	2.60
P value [†]	–	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001

DM dry matter (%), CP crude protein, OM organic matter, NDF neutral detergent fibre, ADF acid detergent fibre, DMD in vitro dry matter digestibility, TP total phenols, CT condensed tannins, SEM standard error of the mean

[†]Tukey test

Table 2 Cumulative gas production (mL/g DM) without (–) and with (+) polyethylene glycol (PEG) in fruits of leguminous trees of Mexico

Fruits	PEG	In vitro gas production (mL/g DM)		
		GP _{24h}	GP _{48h}	GP _{96h}
<i>A. farnesiana</i>	–	71.7	133.9	220.0
	+	107.9	182.5	277.4
<i>P. dulce</i>	–	115.8	171.4	212.0
	+	136.8	201.5	238.7
<i>P. acatlense</i>	–	34.6	57.8	79.0
	+	38.1	65.7	93.8
<i>G. sepium</i>	–	21.3	49.3	82.2
	+	55.7	104.0	177.2
<i>A. cochliacantha</i>	–	33.1	52.4	79.3
	+	46.2	69.7	108.5
<i>E. cyclocarpum</i>	–	96.1	151.0	185.3
	+	109.4	172.0	204.8
<i>L. divaricata</i>	–	19.0	49.3	94.8
	+	28.7	66.1	119.6
<i>L. esculenta</i>	–	24.1	48.1	86.3
	+	56.6	86.3	136.0
<i>C. coriaria</i>	–	62.8	89.0	126.2
	+	156.5	198.4	260.4
SEM		14.52	17.90	21.33
<i>P</i> value [†]				
Fruit		0.001	0.001	0.001
PEG		0.001	0.001	0.001
Fruit × PEG		0.001	0.001	0.001

GP gas production (24, 48 and 96 incubation hours), SEM standard error of the mean

[†]Tukey test

significant ($P < 0.001$) on the gas volume production in the fruits of the species during digestion with and without the addition of PEG in the three incubation times (Table 2).

In vitro fermentation parameters of fruits with and without PEG

Effect of the fruits on the OMD and ME of fruits ($P < 0.0001$) was observed (Table 3). The fruits with higher OMD and ME content were of *A. farnesiana*, *P. dulce* and *E. cyclocarpum* respectively. The addition of PEG increased OMD in the fruit of *C. coriaria* ($P < 0.0001$), interaction of the fruit × PEG ($P = 0.027$) was also observed.

The effect of the fruits was observed in the gas yield to 24 h (GY_{24h}) and short chain fatty acids (SCFA) ($P < 0.0001$) (Table 3). The GY_{24h} and synthesis of SCFA was higher in fruits of *A. farnesiana*, *P. dulce*, *E. cyclocarpum* and *C. coriaria* respectively. The effect of fruits was also observed in the MMP ($P < 0.0001$); the MMP was higher in fruits of *G. sepium*, *P. acatlense*, *A. cochliacantha*, *L. divaricata* and *L. esculenta*, respectively.

The addition of PEG increased ($P < 0.0001$) the fermentation parameters GY_{24h} and SCFA in fruits of *G. sepium*, *L. esculenta* and *C. coriaria*. The addition of PEG decreased ($P < 0.001$) the MMP in the fruits of *A. farnesiana*, *P. acatlense*, *L. esculenta* and *C. coriaria* during digestion. Also an interactive effect of fruit × PEG ($P < 0.05$) on the parameter of fermentation (GY_{24h}, SCFA and MMP) was observed (Table 3).

Fermentation kinetic of fruits

The total volume of gas (*b*) was affected significantly by the type of fruit and the addition of PEG (Table 4), was also evident the interactive effect of PEG × fruit in this parameter. The *b* was higher ($P < 0.001$) in the fruits of *A. farnesiana*, *P. dulce* and *E. cyclocarpum* with 233.4, 215.9 and 179.6 mL/g DM, respectively. The effect of PEG on the *b* was significant ($P < 0.001$) only in the fruits of *G. sepium* (without PEG: 106.5 mL/g DM vs. With PEG: 200.2 mL/g DM) and *C. coriaria* (without PEG: 113.7 mL/g vs. DM with PEG: 252.7 mL/g DM).

The degradation rate (*c*) and the time of colonization for fibre degradation (*t*_{Lag}) were affected ($P < 0.001$) only for the fruits (Table 4). The *c* was higher in the fruits of *P. dulce* (0.07%/h), *E. cyclocarpum* (0.06%/h) and *C. coriaria* (0.06%/h) and *t*_{Lag} was higher in the fruit of *L. divaricata* (10.4 h).

Discussion

Chemical composition of the fruits and in vitro dry matter digestibility (DMD)

The medium and high protein content in the fruits of *A. cochliacantha*, *P. dulce*, *L. divaricata*, *L. esculenta*, *P. acatlense*, *A. farnesiana* and *E. cyclocarpum*,

Table 3 In vitro fermentation parameters without (–) and with (+) polyethylene glycol (PEG) in the fruits of leguminous trees of Mexico

Fruits	PEG	OMD	ME	GY _{24h}	SCFA	MMP
<i>A. farnesiana</i>	–	347.6	5.02	206.0	2.23	494.7
	+	412.0	6.00	261.9	2.25	350.6
<i>P. dulce</i>	–	422.8	6.23	299.5	2.97	297.4
	+	439.9	6.50	310.8	2.85	259.1
<i>P. acatlense</i>	–	290.4	4.11	118.2	0.76	633.3
	+	296.6	4.20	128.4	0.81	619.5
<i>G. sepium</i>	–	231.5	3.31	90.4	0.45	721.5
	+	292.4	4.24	190.3	1.17	585.0
<i>A. cochliacantha</i>	–	261.4	3.73	125.0	0.73	665.7
	+	284.7	4.09	161.8	0.97	613.6
<i>E. cyclocarpum</i>	–	383.4	5.59	250.7	2.23	405.0
	+	407.0	5.95	268.8	2.28	352.1
<i>L. divaricata</i>	–	234.1	3.33	79.2	0.39	723.8
	+	251.3	3.59	114.2	0.61	685.5
<i>L. esculenta</i>	–	284.1	3.97	84.6	0.51	662.9
	+	342.0	4.86	165.6	1.19	533.4
<i>C. coriaria</i>	–	283.9	4.18	221.2	1.44	577.9
	+	416.8	6.21	319.4	2.86	429.5
SEM		30.41	0.463	23.48	0.360	30.88
<i>P</i> value [†]						
Fruit		0.001	0.001	0.001	0.001	0.001
PEG		0.001	0.001	0.001	0.001	0.001
Fruit × PEG		0.050	0.010	0.010	0.050	0.001

OMD 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), SCFA short-chain fatty acids (mmol/g DM), MMP microbial mass production (mg/g DM), SEM standard error of the mean

[†]Tukey test

respectively, covering the minimum required (80 g/kg DM) to ensure the smooth functioning of the rumen microflora (Van Soest 1994). This ensures the sustenance of nitrogen in the diet of ruminants in the tropics during the dry season, when the trees produce their fruit and fodder used for feeding the animals is scarce and of poor quality (Mlambo et al. 2008; Olivares-Perez et al. 2011; Salem et al. 2006, 2017).

The observed levels of nutrients (OM, CP, NDF and ADF) are comparable to those reported in the fruit of other species like *Acacia nilotica*, *Acacia erubescens*, *Acacia sieberiana*, *Acacia erioloba*, *Piliostigma thonningii* and *Dichrostachys cinerea*, respectively (Mlambo et al. 2008; Rojas Hernández et al. 2015); in fruits of *Spindus saponitaria*, *Enterolobium cyclocarpum* and *Pithecellobium saman* (Hess et al. 2003) and species of the genus *Acacia* (Rubanza et al. 2005).

The contents of CT observed in the fruit of the nine species evaluated is comparable to that reported by Hess et al. (Hess et al. 2003) in fruits of *Spindus saponitaria*, *Pithecellobium saman* and *Enterolobium*

cyclocarpum. However, the content of TP observed in the fruits of *C. coriaria*, *L. divaricata* and *A. farnesiana* are higher than those reported in fruits of different acacias by Rubanza et al. (2005). Moreover considering the low content of TP and CT in the fruits of *L. esculenta*, *A. cochliacantha*, *G. sepium*, *E. cyclocarpum*, *P. dulce* and *P. acatlense*, could be used for animal feed. This suggestion is based on the knowledge that the secondary compounds in low concentrations in feeds (< 6.0%) can exert beneficial effects during substrate degradation in the rumen (Salem et al. 2017).

The higher DMD in the fruits of *C. coriaria*, *P. dulce*, *A. farnesiana* and *L. esculenta* was associated with low NDF and ADF; on the contrary, the low DMD of the fruits of *A. cochliacantha*, *G. sepium*, *L. divaricata* and *P. acatlense* is due to its high content of NDF and ADF. The content of CT in the fruits of the trees did not affect DMD, perhaps due to its low concentrations (Elahi et al. 2014; Salem et al. 2007; Salem 2012; Tiemann et al. 2008).

Table 4 Fermentation kinetic without (–) and with (+) polyethylene glycol (PEG) of the fruits of leguminous trees of Mexico

Fruits	PEG	<i>b</i> (mL/g DM)	<i>c</i> (%/h)	<i>t</i> _{Lag} (h)
<i>A. farnesiana</i>	–	233.3	0.03	3.01
	+	285.2	0.04	0.93
<i>P. dulce</i>	–	215.9	0.07	2.23
	+	230.5	0.07	2.09
<i>P. acatlense</i>	–	78.21	0.04	0.08
	+	95.20	0.04	0.04
<i>G. sepium</i>	–	106.5	0.03	3.99
	+	200.2	0.03	3.15
<i>A. cochliacantha</i>	–	76.4	0.04	0.89
	+	106.6	0.04	0.00
<i>E. cyclocarpum</i>	–	179.7	0.06	1.03
	+	196.7	0.06	2.59
<i>L. divaricata</i>	–	118.8	0.03	10.38
	+	146.8	0.03	5.84
<i>L. esculenta</i>	–	90.8	0.04	5.57
	+	133.9	0.04	0.14
<i>C. coriaria</i>	–	113.7	0.06	0.00
	+	252.7	0.06	0.93
SEM		25.60	0.001	2.821
<i>P</i> value [†]				
Fruit		0.001	0.001	0.001
PEG		0.001	NS	NS
Fruit × PEG		0.001	NS	NS

b total volume of gas, *c* degradation rate % by hours, *t*_{Lag} colonization time in hours for fibre degradation

[†]Tukey test

Effect of PEG in gas volume produced

The GP being higher in fruits of *A. farnesiana*, *E. cyclocarpum* and *P. dulce* ($P < 0.0001$), is due to the lower content of NDF and ADF in the fruit of these species and not the content of TP and CT. Several reports have linked high contents of negative ADF and NDF with GP and digestibility of the substrate (Elahi et al. 2014; Getachew et al. 2004; Salem 2012). Mbugua et al. (2008) report that the cumulative gas production is linearly related to the NDF degradability; indicating that NDF observed in the fruits is slightly digestible. Tiemann et al. (2008) reported that low doses of 25 mg/g DM did not affect the GP during digestion of the substrate.

The effect of PEG on gas production, during digestion of the *G. sepium* and *C. coriaria* fruits was due to the fact that the TP and CT have high biological activity to form complexes with nutrients, making them poorly digestible. Mbugua et al. (2008) reported those food-rich secondary compounds (mainly TP and CT) reduce GP; and that the addition of PEG in these cases significantly increases the amount of gas produced. Tiemann et al. (2008), Acamovic and Brooker (2005) and Salem et al. (2007), report that the addition of PEG had no effect on the fermentation parameters in the non-taniniferous legumes, but in taniniferous plants; which means that PEG binds strongly to tannins and reduces their toxic or antinutritional effects in vitro and in vivo and favours the fermentation and digestion of feed by bacteria in the rumen (Mbugua et al. 2008; Salem et al. 2017).

In vitro fermentation parameters of fruits with and without PEG

The effect of the fruits on fermentation parameters (OMD, ME, GY_{24h} and SCFA) were higher ($P < 0.0001$) in the fruits of *A. farnesiana*, *P. dulce*, *E. cyclocarpum* and *C. coriaria*, while MMP was lower in these fruits; these results were related with higher GP and low NDF and ADF of the fruits. Kumara et al. (Kumara et al. 2009) report that high levels of ADF, NDF and lignin, limit gas production, which affects the GY_{24h} and SCFA production from the substrate, since Makkar (2005) reports that the measurement of in vitro gas reflects the SCFA production.

There are reports where the use of PEG ensures greater availability of digestible nutrients (Salem et al. 2007; Hatew et al. 2014; Salem et al. 2006). The addition of PEG increased GP, the OMD and ME content in *A. angustissima* (Rubanza et al. 2005). Elahi et al. (2014), correlates the increased gas production in the presence of PEG, with the capacity of total phenols and tannins to precipitate proteins, this is because that the secondary compounds in high feed concentration (> 6%) can bind to proteins and form protein-tannin complexing and decrease nutrient digestibility, when the PEG binds to the CT, the nutrients diet no are precipitated and are exposed to rumen bacteria for digestion (Hatew et al. 2014; Lorenz et al. 2014). Arhab et al. (2009) reported that the addition of PEG resulted in an increase in gas production (20.2%),

OMD (30.7%) and ME (1.8 units of MJ/kg DM), and similarly the effect was accentuated at the substrate with the higher content of tannins (Calabrò et al. 2012; Hatew et al. 2014). Guimarães-Beelen et al. (2006) reported the capacity of the PEG to bind to polyphenolic compounds and reduce astringency on approximately 70%, which promotes the action of enzymes and bacteria for the degradation of the feed in the rumen (Salem et al. 2017; Waghorn 2008).

The effect of PEG on the GY_{24h} and SCFA production, derived from the fermentation of the fruit ($P < 0.0001$) (Table 3), has been reported in several studies that have shown that the addition of PEG ensuring greater availability of digestible nutrients and SCFA, by an increase on in vitro gas production, this is due the fact that one can estimate the molar ratio of SCFA from the produced gas (Elahi et al. 2014; Salem et al. 2014; Makkar 2005).

The negative effect of PEG on the MMP in the fruit is similar to that reported by Arhab et al. (2009) in *Aristida plumosa*. Getachew et al. (2004), Elahi et al. (2014), Guerrero et al. (2012) and Makkar (2005) reported that the tannin-containing substrates incubated with PEG reduced microbial protein synthesis in relation to those incubated without PEG; and is attributed to an increased degradation of the substrate with a smaller increase in gas production. In addition, Makkar (2005) and Salem et al. (2007) reported that the relation between SCFA production and microbial mass is not constant, and it attaches to the variation in production of microbial mass per unit ATP generated during the digestion of the substrate.

Fermentation kinetic of fruits

The largest gas volume (b) in the fruits of *A. farnesiana*, *P. dulce* and *E. cyclocarpum* in the absence of PEG indicates that they are more digestible. The effect of PEG in the b in the fruits of *G. sepium* and *C. coriaria* indicates that the fruits of these trees had secondary compounds that bind to the nutrients and reduce digestibility, similar to what was reported by Rojas-Hernández et al. (2015).

The differences in c in the fruits of *P. dulce*, *E. cyclocarpum*, and *C. coriaria*; and t_{Lag} in *L. divaricate* corroborate with the report of Calabrò et al. 2012. They reported that the rate of degradation and colonization time depend on the substrates used. The addition of PEG and interaction fruit x PEG has no

effect on the c and t_{Lag} , indicating that the fermentation of the fruit is not accelerated. This result does not agree with the report of Calabrò et al. 2012 who reported that PEG is capable of accelerating the fermentation rate and reducing colonization time in substrates with representative contents of CT.

Conclusions

Arboreal fruit can be considered as a source of N to supplement ruminants' diet in the dry season when the trees produce their fruit and the fodder used for feeding animals is scarce in quantity and poor quality. The high content of ADF and NDF, as well as the content of TP and CT in some of them boost their digestibility. This nutritional composition makes them a suitable supplement for animal feed. The addition of PEG showed that the TP and CT containing the fruits evaluated (*C. coriaria*, *G. sepium* and *L. esculenta*) have high biological activity to link protein diet. Additionally, the PEG increased the availability of nutrients and fermentation parameters.

Compliance with ethical standards

Conflict of interest All authors declare that there are no present or potential conflicts of interest among the authors and other people or organizations that could inappropriately bias their work.

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