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PAPER

Nutritional value of *Acacia amentacea* and *Parkinsonia texana* grown in semiarid conditions

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Abstract

In order to evaluate the nutritional value of *Parkinsonia texana* and *Acacia amentacea*, two leguminosae species of the Tamaulipan scrubland, Northeastern Mexico, two experiments were carried out: the first tested the effects of season and browse species on chemical composition as nutritional variable to small ruminants; the second tested the effect of the addition of polyethylene glycol (PEG) on fermentation parameters. Foliage samples were collected from three sites. Data of chemical composition were analysed using analysis of variance for a bi-factorial arrangement, whereas the effect of PEG was analysed by a strip plot design. Results of chemical composition were affected by interacting factors season*species as individually they were significantly different (P<0.001). Addition of PEG affected (P<0.001) fermentation parameters. Significantly higher values of neutral detergent fibre (42%), condensed tannins (19%), purines (9 μmol), partitioning factor (PF) (6.1) and gross energy losses (GEL=6.7%) were found in *A. amentacea*, while *P. texana* gave higher crude protein (18%), *in vitro* true organic matter digestibility (82%), metabolisable energy (ME) [2.1 Mcal/kg dry matter (DM)], *A* (183 mL), *c* (0.07/h) and *L* (0.86 h). Addition of PEG increased ME, and affected (P<0.001) fermentation parameters *A* and *c*, while purines and PF decreased. Results indicate that chemical composition and fermentation parameters vary according to seasons and species. PEG addition increases the fermentation parameters, which indicates that PEG counteracts the detrimental effects of secondary components of samples. Data suggest that using both species combined could supply necessary nutritional requirements to small ruminants in the Tamaulipan scrubland.

Introduction

Native species are characterised by a wide range of growth patterns, diversity in leaf longevity, dynamics and contrasting phenological development (Reid *et al*., 1990). Rangeland owners frequently use foliage from native trees and shrubs species during dry periods as green forage to livestock, in addition to fruit and litter fall (González *et al.,* 2010). The knowledge of native vegetation allows the establishment of management programmes for increasing biodiversity, higher biomass production, to improve nutritional quality and promote sustainability (Rosales and Gill, 1997). The cattle's ranching was practiced for 350 years in some areas of Tamaulipan scrubland from Northeastern Mexico. Long-term consequences of grazing were quality and quantity losses in forage species (Foroughbakhch *et al*., 2009). Currently, shrub species of the Fabaceae family, particularly *A. amentacea* and *P. texana*, are part of the dominant species of this area in Northeastern Mexico (Estrada and Jurado, 2005). These species have foliage throughout the year, with enough contents of crude protein (CP) and dry matter (DM) for facing the demands of small ruminants grazing in different physiological conditions (Ramírez and Gonzalez, 2010). Those tannins-rich species have an agronomic advantage of adaptation to biotic and environmental stresses (Getachew *et al*., 2000) over the non tannin-containing plants; therefore, the use of these species for livestock could ensure animal production. However, shrubs quality can be affected by the maturity of plant, soil management and secondary metabolites, such as condensed tannins, which form different complexes with plant compounds including protein and decreasing digestibility (Waghorn, 2008). When foliage of shrubby species combines, high organic matter (OM) digestibility and low CH_4 production are potentially able to reduce the enteric CH_4 and increase ruminant productivity. The knowledge of the CH_4 levels produced by the two native shrubby species should allow producers to adapt their production systems in order to reduce the emissions of this compound; for this reason, decreasing losses of CH_4 have become a research priority (Alexander *et al*., 2008). The main sources of metabolisable protein are undegraded protein and microbial protein reaching the duodenum, being the microbial protein the most important part. Thereby, purine content represents the net microbial protein synthesis which reflects the efficiency of microbes to transform carbon and nitrogen into microbial mass (Getachew et al., 2000). Studies performed with gas production (GP) technique are of special interest as they provide kinetic information with small amount of samples. Moreover, *in vitro* gas technique is more efficient than other procedures in the evaluation of the effects of tan-

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nins and other anti-nutritive factors (Makkar, 2003). The addition of binders such as polyethylene glycol (PEG) has been used in order to decrease the tannin activity in plants, as an alternative to improve the quality of fodder; then, an increase of *in vitro* GP indicates the effect on the activity of tannins in feeds (Getachew *et al*., 2000). Despite the diversity of floristic, ecological, biological and physiological studies developed about native plants in arid and semiarid regions, only sparse information exists about the nutritive value of shrubby species grown in the Tamaulipan scrubland (López *et al*., 2014). Thus, the objectives of this paper were: i) to know the chemical composition and fermentation kinetics of *A. amentacea* and *P. texana* treated with and without PEG; and ii) to know if these species are able to meet the nutritional requirements of small ruminants managed in extensive systems.

Materials and methods

Study site and sampling

This study was carried out in three sampling plots located in Northeastern Mexico, where the main type of vegetation of the area is known as the Tamaulipan thornscrub or subtropical thornscrub woodlands (INEGI, 2002). The first plot was located in Los Ramones county (25º 40' N; 99º 27' W) with an elevation of 200 m and a total surface of 100 ha. Historically, the annual mean temperature is 22°C and annual mean rainfall is 700 mm. The second plot was located in China county (25º 31' N; 99º 16' W), which is 200 m asl and has a total surface of 300 ha. The annual mean temperature and rainfall are 22°C and 500 mm, respectively. The third plot was located in the Campus of Forest Sciences Faculty of Universidad Autonoma de Nuevo Leon located in Linares county (24º 47' N; 99º 32' W), with and elevation of 370 m and a total surface of 500 ha. The annual rainfall is 800 mm and the annual mean temperature is 22.3°C (Reid *et al*., 1990). In general, the three sampling plots are under a similar semi-arid climatic pattern with a bimodal rainfall pattern (May to June and August to October). Dominant soils are deep, dark-gray, lime-gray, lime-clay vertisols, with montmorillonite, which shrink and swell noticeably in response to changes in soil moisture content (INEGI, 2002). Monthly rainfall and mean temperatures registered during the period of study are showed in Figure 1.

Seasonal collection (Spring to Winter 2009) of mature leaves and twigs were undertaken (800 g) at browsing height (1.0 to 1.5 m) from the five most representative individual plants randomly selected (Montgomery, 2004) of the two shrub species *A. Amentacea* and *P. texana*. Fifty-four samples per season were collected from three experimental plots (50×50 m) which were established randomly at each site. Leaves were separated from twigs, kept in paper bags and air-dried prior to oven-drying at 60°C for 48 h (Pardo *et al*., 2007), then ground in a Willey mill (Model 3383; Thomas Scientific, Swedesboro, NJ, USA) using a Nº 60 (1×1 mm) mesh and stored in labeled plastic containers, until the analyses were performed.

Chemical composition

Samples by triplicate of each plant species were subjected to chemical analyses. The DM (#934.01), OM (#942.05), CP (#954.01) and ether extract (EE; #929.29) contents were determined as described by AOAC (1997). The neutral detergent fibre (NDFom) was completed following Van Soest *et al*. (1991). The condensed tannins (CT) were determined using butanol/HCl (95:5 v/v) and ferric ammonium sulfate (20 g/L 2N HCl) as reagents and leucocyanidin (1 mg/mL aqueous acetone, 700 mL/L) as standard. Absorbance was measured against a blank at 550 nm (Makkar, 2003).

In vitro fermentation

Inoculum was obtained from three criollo fistulated sheep (60±3.7 kg live weight) fed with (750:250) alfalfa hay [CP=18% DM; NDF=50%; metabolizable energy (ME)=2.1 Mcal/kg DM] and a concentrate (CP=18% DM; NDF=18%; ME=2.3 Mcal/kg DM). The rumen fluid was flushed with CO_2 and filtered through three layers of cheese cloth and mixed (1:2, v/v) with mineral buffer solution under anaerobic condition (Makkar, 2003). Approval of the procedures was gained from The Animal Care and Use Committee of the Universidad Juárez del Estado de Durango, Mexico.

Mineral buffer solution used to measure tannins effects with PEG was prepared according to Menke and Steingass (1988), and it contained per L: $NaHCO_3$, 35.0 g; NH_4HCO_3, 4.00 g; Na_2HPO_4, 5.7 g; KH_2PO_4, 6.2 g; $MgSO_4 \bullet 7H_2O$, 0.6 g; $CaCl_2 \bullet H_2O$, 13.2 g; $MnCl_2 \bullet 4H_2O$, 10.00 g; $CoCl_2 \bullet 6H_2O$, 1.00 g; $FeCl_2 \bullet 6H_20$, 0.8 g; resazurin, 0.01 g; and 49 mL of freshly prepared reduction solution containing 580 mg $Na_2S \bullet 9H_2O$ and 3.7 mL 1NNaOH solution. The mixture was kept stirred, under CO_2 flushing at 39°C, using a magnetic stirrer fitted on a hot plate.

A total of 432 syringes was incubated by five groups of 96 syringes each (one group per week). Two treatments: one with 1 g PEG 6000 and another without PEG were incubated in 3, 6, 9, 12, 24, 48, 72 and 96 h periods using three syringes of each sample of the two shrub species (*A. amentacea* and *P. texana*) and four seasons. In each incubation run, two syringes containing no substrate were used as blanks and two syringes containing alfalfa (*Medicago sativa*) in order to control the fermentation. The rate and extent of *in vitro* GP from shrub foliage were obtained from 96 h incubation according to the method proposed by Menke and Steingass (1988) in which 500 mg samples in triplicate were incubated in 100 mL calibrated glass syringes. Of a mixture of rumen fluid:buffer solution, 30 mL were added to each syringe which were vertically positioned in a water bath at 39°C.

To estimate kinetic parameters of GP, results (mL/g DM) were fitted to the PROC NLIN procedure according to France *et al*. (2000) as:

$$A=b\times(1-e^{-c\,(t-L)})$$

where *A* is the total volume of GP (mL/g DM) at time t (h); *b* is the asymptotic GP (mL/g DM); *c* is the rate of GP (/h); and *L* (h) is the discrete lag time prior to GP.

The ME was calculated from *in vitro* GP in accordance with the equation:

$$ME\ (Mcal/kg\ DM)=2.20+0.136\ GP_{24h}+0.057\ CP+0.0029\ EE^2$$

where GP_{24} h is the GP after 24 h of incubation (mL gas/0.5 g DM); CP is the crude protein (g/kg DM); EE is the ether extract (g/kg DM) (Menke and Steingass, 1988).

In vitro true organic matter digestibility

To estimate the *in vitro* true organic matter digestibility (IVTOMD), samples (250 mg DM) in triplicate from each shrub species were weighed and placed in Ankom F57 filter bags and incubated during 48 h in a Daisy^{II} incubator (Ankom Technology, Macedon, NY, USA). This method offers more precise predictions than conventionally determined digestibility estimates (Adesogan, 2005). To complete the analysis of 216 samples for IVTOMD, three runs of incubations were performed according to procedures described by Anassori *et al*. (2012). A salivary buffer solution was prepared according to the procedure recommended by Ankom Technology. The A solution was made by dissolving, in distilled water, 10 g/L of KH_2PO_4, 0.5 $MgSO_47H_2O$, 0.5 NaCl, 0.1 $CaCl_22H_2O$, and 0.5 urea. B solution was prepared by dissolving in distilled water, 15 g/L of

Na_2CO_3 and 1.0 Na_2S $9H_2O$. After that, a combined buffer solution was prepared by combining ~266 mL of B solution and 1330 mL of A solution (1:5 ratio). Finally, before starting to incubate samples, 400 mL of rumen fluid were added to each jar containing the 1600 mL of combined A/B mixture, pre-warmed up to 39°C, and purged with CO_2 gas for 30 sec (1 filter bag per jar was used as blank). Rumen fluid was obtained as before. Samples were then treated with neutral detergent solution, washed, dried overnight at 55°C, burned and weighed to determine OM losses. The partitioning factor (PF) was calculated as the ratio between truly degraded OM (mg) and gas produced (mL) after 48 h of incubation (Thirumalesh and Krishnamoorthy, 2013).

Purines determination

Another set of syringes was incubated to estimate the microbial protein synthesis as purines. After 24 h of incubation, the contents of syringes were transferred to centrifuge tubes and centrifuged at 20,000×g for 30 min at 4°C and the supernatant was saved. The pellet was washed with distilled water, centrifuged again and lyophilised overnight. Purines in the residue were estimated according to Makkar (2003).

Methane conversion factor

To calculate this factor, the equation dE (%)=0.975*OMD(%)-0.07 (Andrieu and Demarquilly, 1987) was first utilised to estimate the apparent energy digestibility. The dE (%) was then incorporated into the polynomial equation of Cambra-López *et al*. (2008):

$$Ym=-0.0038x+0.3501x-0.8111.\ R^2=0.8653$$

where, Ym is the fraction of gross energy of the substrates that is transformed into methane (%), and x is dE (%).

Statistical analyses

Experiment 1 considered two factors: seasons (4) and shrub species (2) with 27 replications. Data were analysed using the computer statistical software for Windows SPSS (2009) with the statistical model:

$$Yijk=\mu+\tau i+\varepsilon(a)ij+\gamma k+\varepsilon(b)ik+(\tau\gamma)ik+\varepsilon(c)jik \text{ (experiment 1)}$$

where: Yijk is the parameter measured of the ijk experimental unit, μ is the overall mean, τi is the effect of species, ε(a)ij is the experimental error to evaluate species, γk is the effect of season, ε(b)ik is the experimental error to evaluate seasons, (τγ)ik is the effect of interaction among species and seasons, and ε(c)jik is the experimental error to evaluate interaction effects. Data from experiment 2 were evaluated using the statistical model:

$$Yijkl=\mu+\tau i+\gamma j+(\tau\gamma)ij+\varepsilon(a)ijk+\beta l+\varepsilon(b)kl+(\tau\beta)il+(\gamma\beta)kl+(\tau\gamma\beta)ijl+\varepsilon(c)jikl$$

where Yijkl is the measured parameter of the ijkl experimental unit; μ is the overall mean; τi is the effect of species; γk is the effect of PEG; (τγ)ij is the effect of interaction species×PEG; ε(a)ijk is the experimental error for species and PEG; βl is the effect of season; ε(b)kl is the experimental error to evaluate season; (τβ)il is the effect of interaction species×season; (γβ)kl is the effect of PEG×season; (τγβ)ijl is the interaction of species×PEG×seasons; and ε(c)ijkl is the experimental error to evaluate interaction effects. Pearson correlation coefficients between the chemical composition of shrub foliage and the rumen fermentation parameters were also performed (Table 1).

Results

Effect of season and plant species on chemical composition of substrates (experiment 1)

The effect of seasons and plant species on DM, OM, NDF, CT, CP, IVTOMD and gross energy losses (GEL) are shown in Table 2. The interaction season*species affected (P<0.001) DM, CT, CP, IVTOMD and GEL, but there was no effect of this interaction on OM and NDF (P>0.05). Seasons affected the entire chemical components (P<0.001), while species did not affect the OM content (P>0.05). The content of OM and NDF decreased in the studied species from spring to summer and increased their content through autumn and winter. The CT content did not change across the spring to summer, although decreased from summer to autumn; after that, only slightly variations were observed from autumn to winter. In both species, the CP increased throughout spring to winter, mainly in *P. texana*. The IVTOMD progressively increased in *P. texana* from spring to winter, whereas *A. amentacea* had similar

Table 1. Pearson correlation coefficients among chemical composition, *in vitro* fermentation parameters, digestibility and gross energy losses of leaves of two native shrub species in Northeastern Nuevo Leon, Mexico.

	OM	NDF	CT	CP	NSC	ME	IVTOMD	GEL	*A*	*c*	*L*	P	PF
DM	-0.147*	0.105	-0.132	-0.294**	0.213**	0.141*	0.197**	-0.217**	0.173*	0.189**	0.099	0.055	-0.116
OM		0.073	0.022	0.006	-0.098	-0.210**	-0.089	0.053	-0.103	-0.096	-0.001	-0.131	0.075
NDF			0.139*	-0.606**	0.016	0.093	-0.088	0.040	-0.062	0.002	0.084	-0.104	-0.145*
CT				-0.092	-0.342**	-0.359**	-0.702**	0.707**	-0.731**	-0.630**	-0.308**	0.151*	0.065
CP					-0.224**	0.055	0.025	0.004	-0.017	-0.046	0.045	-0.013	0.078
NSC						0.199**	0.478**	-0.477**	0.443**	0.347**	-0.022	-0.050	-0.056
ME							0.575**	-0.540**	0.361**	0.328**	0.366**	0.226**	-0.336**
IVTOMD								-0.923**	0.818**	0.626**	0.246**	-0.054	-0.217**
GEL									-0.868**	-0.684**	-0.261**	0.068	0.246**
A										0.751**	0.235**	-0.135*	-0.194**
c											0.420**	-0.140*	-0.137*
L												0.091	-0.028
P													-0.233**
PF													1

OM, organic matter (% DM); NDF, neutral detergent fibre (% DM); CT, condensed tannins (% DM); CP, crude protein (% DM); NSC, non-structural carbohydrates (% DM); ME, metabolisable energy (Mcal ME/kg DM); IVTOMD, *in vitro* true organic matter digestibility (%); GEL, gross energy losses (%, as methane production); *A*, total gas production (mL/g DM); *c*, the rate of gas production (/h); *L*, the initial delay before gas production begins (h); P, purines (μmol); PF, partitioning factor; DM, dry matter (%). *P<0.05; **P<0.01.

digestibility throughout summer to winter. The GEL decreased for both species throughout the seasons. Higher CP content and IVTOMD were registered in *P. texana*, while the higher values of NDF, CT content and GEL were observed in *A. amentacea*.

Effect of polyethylene glycol addition on fermentation parameters (experiment 2)

The effect of PEG on total GP (*A*), fractional rate of GP (*c*), lag phase (*L*), PF and ME throughout the seasons is shown in Table 3. The interaction species*PEG affected (P<0.001) the *A*, *L*, PF and ME values in all seasons, but no effect (P>0.05) was observed on *c* values and purine content. The studied species are significantly different (P<0.001) with respect to their fermentability. *P. texana* have higher values of total GP, fractional rate of GP and metabolisable energy. The interaction species*PEG*season affected (P<0.001) the values of purines, PF and ME; contrarily, the *A*, *c* and *L* parameters were not affected (P>0.05). The interaction season*PEG affected significantly (P<0.001) total gas produced, lag phase, PF and ME. The interaction species*season affected (P<0.001) overall fermentation parameters, except the lag phase. Season also affected (P<0.001) the fermentation parameters, except total GP (P>0.05).

Discussion

Chemical composition of substrates (experiment 1)

Significant differences in chemical composition among seasons in native browse plants have been reported by others (Lovett *et al.*, 2006). Environmental changes alter the nutritional quality of plants; in this way, high temperatures and the development of its water transport system (xylem) increased the NDF content (Hoffman *et al.*, 2007). Wide variations of NDF content in the studied species throughout the seasons (33 to 49%) could result from chemical modifications of leaf tissue due to foliage advancing in phenological stage (Ramírez-Lozano, 2004).

Slight variations in CT content in *A. amentacea* were registered through seasons (18 to 20%); conversely, CT content in *P. texana* was clearly affected by the seasons, decreasing from 12.4% in spring when the regrowth of shrubs to 6.6% in autumn and winter in the presence of rain (Figure 1). Content of CT is also affected by the phenological stage. This

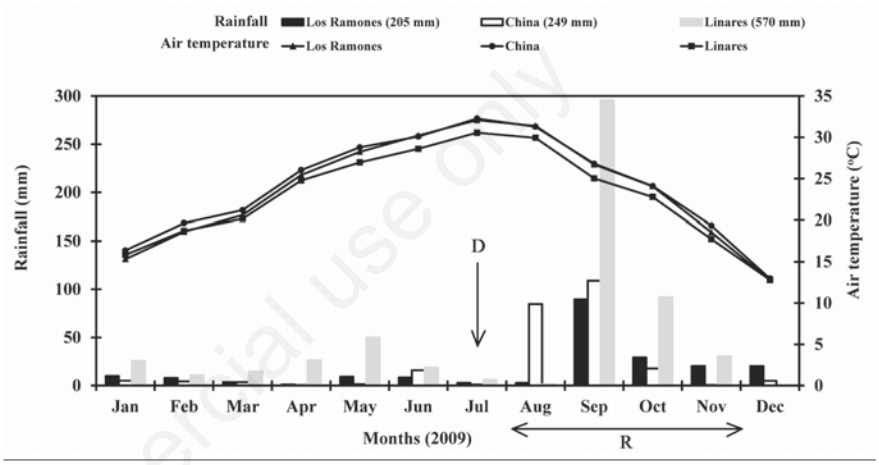

Figure 1. Air temperature mean and monthly rainfall recorded during the course of the study. The arrows indicate the drought (D) and rainfall (R) periods at the study sites.

Table 2. Effect of seasons and plant species on chemical composition, *in vitro* true organic matter digestibility and gross energy losses of leaves of two native shrub species in Northeastern Nuevo Leon, Mexico.

	DM, %	OM, % DM	NDF, % DM	CT, % DM	CP, % DM	EE, % DM	IVTOMD, %	GEL, % (as methane production)
A. amentacea								
Spring	95.9	85.9	52.8	18.5	15.0	0.6	42.6	7.1
Summer	96.9	80.1	45.4	20.3	13.6	1.0	46.8	6.9
Autumn	95.2	80.7	49.6	18.4	15.4	0.8	53.2	6.5
Winter	92.9	81.6	48.0	18.8	15.2	1.6	58.7	6.2
Mean	95.2	82.1	49.0	19.0	14.8	1.0	50.3	6.7
P. texana								
Spring	94.7	84.2	36.8	12.4	19.2	1.7	76.7	4.1
Summer	96.1	80.4	28.8	12.3	20.1	1.5	84.2	2.3
Autumn	93.6	81.9	32.8	6.6	22.2	1.5	84.2	2.3
Winter	91.6	84.2	32.6	7.4	21.6	1.6	82.5	2.7
Mean	94.0	82.7	32.8	9.7	20.8	1.6	81.9	2.9
Probability								
A	***	ns	***	***	***	***	***	***
B	***	***	***	***	***	***	***	***
A*B	***	ns	ns	***	***	***	***	***
SEM								
A	0.14	0.90	0.16	0.19	0.19	0.03	0.21	0.06
B	0.27	1.13	0.80	0.41	0.25	0.06	0.14	0.18
A*B	0.32	1.39	0.61	0.50	0.39	0.06	1.14	0.16

DM, dry matter; OM, organic matter; NDF, neutral detergent fibre; CT, condensed tannins; CP, crude protein; EE, ether extract; IVTOMD, *in vitro* true organic matter digestibility; GEL, gross energy losses; A, species; B, season. ***P<0.001; ns, not significant.

effect is a well-known phenomenon previously described by Verdecia *et al*. (2013). High concentrations of CT are considered as one of the main factors of low nutritional value of native legumes, as it occurred with values of CT in this work because they cause a reduction of ME (r=-0.359; P<0.01) and IVTOMD (r=-0.702; P<0.01). These findings are in accordance with those reported by Ramírez-Lozano (2004), for different types of forages. The suppressing effect of CT on nutrients utilisation probably resulted from a reduction in microbial attachment to feed particles, as well as on their effects on the microbial population and on its enzymatic activity (Calabrò *et al*., 2012).

Wide variations in CP content throughout seasons, as observed in this study (13 to 22%), are in accordance with other studies (Safari *et al*., 2011). Content of CP in *A. amentacea* and *P. texana* was lower at the beginning of spring (higher by almost 30% in *P. texana*), when the initial leaf growth is accompanied by a strong demand for nutrients, particularly N (Bahamonde, 2011). On the contrary, CP content increased from summer to winter when there is a new regrowth.

The IVTOMD progressively increased from spring to autumn in *A. amentacea* (43 to 59%), whereas *P. texana* had similar digestibility throughout spring to winter (76 to 84%). Wide differences in IVTOMD between species were identified, being *P. texana* higher in digestibility (mean=82%) as compared to *A. amentacea* (mean=50%). Seasonal differences in IVTOMD are closely related to NDF and CP content (Basha *et al*., 2014). Our results are at odds with this statement because these variables had non-significant (P>0.05) relationships with IVTOMD (NDF: r=-0.088 and CP: r=0.025). McSweeney *et al*. (1999) also observed that anti-nutritional factors were poorly correlated with DM digestibility; conversely, the IVTOMD was positively associated with the content of non-structural carbohydrates (NSC) and the *A* fraction (r=0.478; r=0.818; P<0.01) and negatively associated with the CT content (r=-0.702; P<0.01).

Gross energy losses as methane production decreased for both species throughout seasons, while the NSC contents increased (r=-0.477; P<0.01). The GEL remained in narrow limits through seasons in *A. amentacea*, whereas in *P. texana* varied within a range of 2.3 to 4.1%. For both species, higher values were found in spring when the CT is higher, which contribute to the high positive relationship between these variables (r=0.707). The GEL, varies from 2 to 12%, depending on the IVTOMD of forages (Cambra-López *et al*., 2008), therefore a negative correlation is usually established between forage digestibility and CH_4 emission, as in this study (r=-0.923; P<0.01). It is often claimed that forage-based diets generally result in higher enteric CH_4 (Klevenhusen *et al*., 2011); nevertheless, a negative correlation albeit no significant (r=-0.932; P=0.068) was found between the tannin content and CH_4 production (Guglielmelli *et al*., 2011), indicating that CH_4 production consistently declined as the structural compounds content increased. Nevertheless, in this study GEL values were not related to the NDF (r=0.040; P>0.01), whereas the relationship with the NSC (data not shown) was negative (r=-0.477; P<0.01) as also found by Lovett *et al*. (2006).

Nutritional implications

of substrates

The NDF contents of the species *A. amentacea* and *P. texana* (annual mean=40%) indicate high availability of nutrients. High concentrations of CT, such as 19% in *A. amentacea*, are one of the main factors of low nutritional value of forage legumes; however, CT has also has favourable effects such as increasing the by-pass protein. Results of Juárez *et al*. (2004) indicate that 60% of the metabolizable protein in the range goat's diets comes from by-pass protein. Although CT content as high as 13% can reduce the population of substrate degrading bacteria, the effect on rumen microbial metabolism could be insufficient to alter the efficiency of microbial protein synthesis. Additionally, the antiparasitic

Table 3. Effect of polyethylene glycol on fermentation characteristics, purines, partitioning factor and metabolisable energy of leaves of two native shrub species in northeastern Nuevo Leon, Mexico.

	PEG	A, mL/g DM	c, /h	L, h	P, μmol	PF	ME, Mcal kg DM
A. amentacea							
Spring	-	109	0.04	0.42	6.1	4.9	1.2
	+	164	0.06	0.14	6.9	2.8	1.6
Summer	-	97	0.02	0.40	7.1	5.9	1.9
	+	163	0.06	0.59	5.5	3.0	2.0
Autumn	-	89	0.04	1.03	15.2	7.2	2.2
	+	157	0.06	0.93	13.2	3.7	2.1
Winter	-	105	0.04	0.66	7.0	6.5	1.5
	+	147	0.06	0.36	6.9	4.3	1.8
Mean		129	0.05	0.56	8.5	4.8	1.8
P. texana							
Spring	-	171	0.07	0.52	5.7	4.8	1.7
	+	193	0.08	1.13	4.2	4.1	1.9
Summer	-	186	0.06	0.49	7.3	4.8	2.2
	+	208	0.08	1.28	6.4	4.1	2.2
Autumn	-	188	0.07	1.45	9.6	4.7	2.4
	+	202	0.08	1.88	11.5	4.3	2.4
Winter	-	187	0.08	1.01	4.9	4.7	2.0
	+	195	0.09	1.03	5.3	4.4	2.2
Mean		190	0.08	1.10	6.9	4.5	2.1
Probability							
A		***	***	***	***	***	***
PEG		***	***	***	ns	***	***
A*PEG		***	ns	***	ns	***	***
B		ns	***	***	***	***	***
A*B		***	***	ns	***	***	***
B*PEG		***	ns	***	ns	***	***
A*PEG*B		ns	ns	ns	***	***	***
SEM							
A		1.08	0.0008	0.042	0.218	0.048	0.011
PEG		1.08	0.0008	0.042	0.218	0.048	0.011
A*PEG		1.53	0.0011	0.060	0.308	0.069	0.016
B		2.42	0.0018	0.063	0.780	0.107	0.056
A*B		2.43	0.0018	0.073	0.384	0.128	0.022
B*PEG		2.43	0.0018	0.073	0.384	0.128	0.022
A*PEG*B		3.44	0.0025	0.104	0.544	0.182	0.032

PEG, polyethylene glycol; A, total gas production; c, the rate of gas production; L, the initial delay before gas production begins; P, purines; PF, partitioning factor; ME, metabolizable energy; A, species; B, season. ***P<0.001; ns, not significant.

effect of tannins is far from being negligible, as demonstrated by Sandoval-Castro *et al.* (2012).

Evaluated leguminosae species supply enough CP to meet the maintenance requirements (7 to 9%) and body weight gain (17%) for adult range small ruminants (National Research Council, 2007). Levels of 15% of CP in diets consumed by small ruminants provide 74 g/d of metabolisable protein, which ensure an adequate supply of N for maintenance of an adult range goat (Juárez *et al*., 2004). The values of IVTOMD of studied species were of medium quality in spring and summer (60%), whereas in autumn and winter (70%) they might be considered to be forages of good quality. A value of 1.2 Mcal/kg of ME in shrub species is low, whereas the ME requirement of maintenance for free ranging small ruminants is 2.1 Mcal/kg (National Research Council, 2007). Accordingly, the ME of studied species (mean value=1.9) would appear to be sufficient to satisfy the small ruminants maintenance requirements in late summer and autumn, whereas ME content of *A. amentacea* could not satisfy these requirements in winter and spring.

Plants with high PF values, as observed in this study, have a higher efficiency in microbial protein synthesis. This has positive implications in ruminant nutrition since it suggests that feedstuffs with high PF values can be used more efficiently. Nevertheless, high values of PF that are biologically deviated from conventional values (2.1 to 4.4) cannot be, by themselves, a sufficient measurement to evaluate the nutritive value of forages (Makkar *et al.*, 1998). Data of purines suggest that negative effects of tannins on microbial growth may be overcome if high NSC and CP content are present (20-30% and 14-20% in this study, respectively) which provide adequate levels of nutriments to the microbes, in spite of the presence of tannin-dietary protein complexes (Mbugua *et al*., 2008).

The CH_4 production means a waste of the energy provided by forages. In grazing animals consuming low quality forage, the GEL can range from 7.7 to 8.4%, while in those consuming high digestibility forages, the GEL is reduced to 1.9 to 2.2% (Cambra-López *et al*., 2008). In this study, GEL in *P. texana* (IVTOMD=82%) was calculated as 2.9%, whereas in *A. amentacea* (IVTOMD=54%) it was 6.7%, which represents a value about 2.3 times higher. Thus, values of GEL in this study indicate that chemical composition of *A. amentacea* leads to higher losses of gross energy as compared to *P. texana*.

Effect of polyethylene glycol addition on fermentation parameters (experiment 2)

The total GP was not affected by seasons (P>0.05). This is not in agreement with other studies (Lovett *et al*., 2005) which indicate that season of harvest leads to significant differences in cumulative GP; however, a significant effect (P<0.001) of species was registered in total GP, with leaves of *P. texana* having the highest increment (+58%) observed after the addition of PEG (P<0.001), as compared to *A. amentacea* (+8.7%). Gas production can be regarded as an indicator of carbohydrate degradation and the low increase observed in *A. amentacea* may be explained by the presence of condensed tannins binding to the structural compounds (r=-0.731) and then preventing the microbial digestion (Sallam *et al*., 2010). Normally, low GP would indicate low degradability, but feedstuffs containing high CP (as 15% in *A. amentacea* and 21% in *P. texana*) might produce less gas during fermentation, even if their extent of degradation is high, because protein fermentation produces ammonia, which influences the carbonate buffer equilibrium without release of CO_2 (Cone and Van Gelder, 1999).

Addition of PEG to fermentations of tannin-containing legumes significantly improves the amount and rate of GP (Mbugua *et al*., 2008). An increase (mean=+11%) of rate of GP was observed throughout the seasons in studied samples. As expected, the rate of gas production *c* was faster after the addition of PEG (P<0.001) throughout the seasons in *P. texana* (0.08/h; increase of 42%) as compared to *A. amentacea* (0.06/h; increase of 21%). As expected, increase in the rate of GP after the addition of PEG is probably due to an increase in cellulolytic activity of microbial enzymes, by reducing negative effects of secondary compounds. The low values of *c* in *A. amentacea* may indicate that the substrate structure exhibits physical barriers that prevent its hydrolysis (Mbugua *et al*., 2008). In fact, values of this fraction are positively related to the amount of NSC (r=0.347; P<0.01) Guerrero *et al*. (2012) found values of 0.05/h in shrub species consumed by small ruminants, which are similar to those obtained in *A. amentacea*.

Values of the initial delay before GP begins (*L*, h) were lower in spring and summer (0.05 h), intermediate in winter (0.083 h), and higher in autumn (1.2 h). In all four seasons, the addition of PEG decreased the *L* fraction by 20% for *A. amentacea*, while *P. texana* showed an increase of 52%. A lower value of *L* suggests an increase in the energy density of the substrates, which favours microbial growth and rapid colonization of the insoluble but potentially degradable fraction. However, this is not always the case, *e.g.* when the capacity of microorganisms that metabolise excess soluble material is at its maximum (mean of both species=23%), the onset of degradation of the insoluble fraction could take longer and the value of *L* would be higher (Dijkstra *et al*., 2002). This could be the case of *P. texana*, whose *L* increased (from 0.868 to 1.2 h) in presence of PEG. Plants having high values of lag phase would reflect the presence of chemical or structural constraints of the substrates (Menke and Steingass, 1988), as in this study (r=-0.308; P<0.01)

Purine values, a measure of microbial protein synthesis in the rumen, showed an increase from 5.7 to 12.3 μmol in all four seasons of the year, after the addition of PEG. Purine content was higher in autumn (12.3 μmol), whereas the lower value was noted in spring, summer and winter (mean=6.3 μmol). Although the species were not affected by the addition of PEG, *A. amentacea*, was numerically higher (8.5 μmol) than *P. texana* (6.9 μmol), which indicates that this legume was more efficient in microbial protein production than *A. amentacea*. Reduced values of purines can be attributed to the presence of CT and other chemical compounds that likely inhibit fermentation, which might affect the degradation of substrates (Singh *et al*., 2012). Both *P. texana* and *A. amentacea* were high in NSC (20 and 30%, respectively) which suggest that negative effects of tannins on microbial growth, may be overcome if readily fermentable carbohydrates are present (Mbugua *et al*., 2008). Additionally, the high level of NSC might promote the rapid growth of microbial groups tolerant to secondary compounds. Furthermore, the energy requirements of ruminal microbes increase in presence of anti-nutritional factors, which could be provided by NSC. In this case, when high levels of dietary protein and NSC are present (20 and 28.5% DM in *P. texana*, respectively), adequate levels of protein may be available to the microbes in spite of the presence of tannin-dietary protein complexes (Mbugua *et al*., 2008).

The PF varied significantly (P<0.001) according to the seasons, the addition of PEG increased the PF from 4.1 to 5.1. The PF of investigated browses decreased after the addition of PEG (P<0.001) from 6.1 to 3.4 in *A. amentacea* and from 4.7 to 4.2 *P. texana*. Values of PF without PEG addition for *A. Amentacea* were higher than the theoretically possible maximum value (4.4); surely, this was because tannins form complexes with proteins, which

are largely insoluble in neutral detergent and may contribute to the undegradable fraction (Makkar *et al*., 1995). The calculated IVTOMD:gas production ratio (PF) indicate that when feed is metabolised by rumen microbes, the degraded component is either incorporated into microbial biomass production or fermented (Mbugua *et al*., 2008). In this way, the increase in GP after PEG addition in both species could simply result in lower partitioning of nutrients to microbial protein synthesis and reduced PF values (Sallam *et al*, 2010). High values of PF that are biologically deviated from conventional values (2.1 to 4.4) cannot be, by itself, a sufficient measurement to evaluate the nutritive value of forages (Makkar *et al.*, 1998).

Metabolizable energy content (Mcal/kg DM) was influenced by seasons and species. According to all seasons of the year, the ME increased from 1.5 to 2.3 after the addition of PEG. Concerning the studied species, ME content increased by 13% in *A. amentacea*, after the addition of PEG, whilst the increased percentage in *P. texana* was not evident. Data indicate that there was an overall increase in ME content for *A. amentacea* and *P. texana* from the summer to fall. Differences in ME among feeds might reflect the variation in NSC ($r=0.199$; $P<0.01$) as reported by Carmona *et al*. (2005). It has been observed (Rzedowski, 2006) that the maximum foliage of *Acacia* species occurs in autumn (90%) while the lowest amount is recorded in spring (61%). This fact is reflected in a higher content of the ME in studied species, during summer and autumn. Values of ME in *P. texana* were consistently higher (1.7 to 2.4) across the seasons as compared to the values observed in *A. amentacea* (1.2 to 2.2). It has been often suggested that the quality of feeds depends primarily on their energy content (Mlay *et al*., 2006), but values of ME in shrub species might be as low as 1.2 Mcal/kg DM (Khanum *et al*., 2007). In this study, similar low values of ME were observed in *A. amentacea*, during the spring.

Conclusions

Overall chemical composition of the studied species varies according to seasons and species. *Parkinsonia texana* seems to be better as forage because it has higher CP, IVTOMD and ME. Conversely, CT and GEL are higher in *A. amentacea*. PEG addition increases the fermentation parameters with *A. amentacea* having the largest overall improvement, while *P. texana* has the lowest. Results of this study suggest that using both species in combination could supply the nutritional requirements to adult Spanish goats late in gestation and at the beginning of lactation (autumn and winter seasons) in the Tamaulipan scrubland.

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