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Nutritional Value of two Native Shrub Species That Grown in the Tamaulipan Thornscrub from Mexico

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Introduction

Rangeland owners use native foliage of trees and shrubs species during dry periods, for feeding livestock as green forage, fruits and litter fall. Shrub species of the Fabaceae family, particularly *A. amentacea* and *P. texana* are part of the dominant species at northeastern Mexico (Estrada-Castillón and Jurado, 2005). These species are characterized by having foliage throughout the year, with enough contents of CP and DM for facing the demands of small ruminants grazing in different physiological conditions (Ramírez and Gonzalez, 2010); moreover, these tannins-rich species have an agronomic advantage over the non tannin-containing plants in their adaptation to biotic and environmental stresses (Getachew *et al.*, 2000). Studies performed with the gas production technique are of special interest since they provide kinetic information of the fermentation and are more efficient than other procedures in evaluating the effects of tannins 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 and as an alternative to improve the quality of fodder; then, an increase of *in vitro* gas production indicate 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 on the nutritive value of shrubby species grown in the Tamaulipan scrubland. The objectives of this paper were to estimate the chemical composition and fermentation kinetics of the two shrubby species and in what extent these species can meet the nutritional requirements of ruminants managed in extensive systems.

Material and Methods

Study site and sampling

This study was carried out at county sites Los Ramones, China and Linares, located in the state of Nuevo Leon in northeastern Mexico. The sites have been previously described by González-Rodríguez *et al.* (2010). Monthly collection (2009) of mature leaves was undertaken (800 g), at browsing height (1.0-1.5 m) from the 5 most representative individual plants randomly selected of the shrub species *A. amentacea*, and *P. texana*. Samples were collected from 3 experimental plots (50 m x 50 m) established in each site. Once the samples were dried, at room temperature, leaves were grounded using 1 mm x 1 mm mesh, and stored in labeled plastic containers.

Chemical composition

The crude protein (CP#954.01), ether extract (EE; #929.29) and organic matter (OM#942.05), contents were determined as described by AOAC (1997). The neutral detergent fiber (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 at 550 nm (Makkar, 2003).

In vitro fermentation

Five incubation runs were conducted for each sample within each shrub species with three repetitions. The rate and extent of *in vitro* gas production (GP) from shrub foliage were obtained from a 96 h incubation

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(Menke and Steingass, 1988) in which triplicate 500 mg samples were incubated in 100 mL calibrated glass syringes. The culture medium contained a buffer solution comprising mainly Na₂HPO₄ and NaHCO₃, with smaller amounts of MnCl₂, CaCl₂, CoCl₂ and FeCl₃ and saturated with CO₂ at 39°C. A mixture of 30 milliliters of rumen fluid:buffer solution in a 1:2 ratio was added to each syringe. Inoculum was obtained from three criollo fistulated sheep (60 ± 3.7 kg live weight) fed with alfalfa hay and a concentrate (750:250). A total of 432 syringes with 2 blanks into each run. Samples were treated as follow: one with 1 g PEG 6000 and other without PEG and incubated in 3, 6, 9, 12, 24, 48, 72 and 96 h periods. Three syringes of each sample with each of the 2 shrub species, 3 sites and 12 months, were used. Results of kinetic parameters of GP, 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*; *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 gas production. The ME was calculated as: ME (Mcal/kg DM) = 2.20 + 0.136 GP_{24h} + 0.057 CP + 0.0029 EE², Where GP_{24h} is the gas production after 24h of incubation (ml gas/0.2 g DM); CP= Crude Protein (g/kg DM); EE= Ether Extract (g/kg DM) (Menke and Steingass, 1988).

In vitro true organic matter digestibility (IVTOMD)

Triplicate samples (250 mg OM) from each shrub species were weighed and placed in Ankom filter bags and incubated 48h in a Daisy^{II} incubator (Ankom Technology, Fairport, NY, USA) with a combination of buffer solution and pre-feeding rumen inoculum. Thus, samples were treated with a ND solution, burned and weighed to determine OM losses. The partitioning factor (PF) was calculated as the ratio between mg of OM truly degraded after 48h/ml of gas produced at 48h of incubation (Guerrero *et al.*, 2012).

Purines determination

Another set of syringes was incubated to estimate the microbial protein synthesis (purines). After 24h of incubation, contents of another set 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 lyophilized overnight. Purines in the residue were estimated as Makkar (2003).

Methane conversion factor

To calculate this factor, first the equation $dE(\%) = 0.975 * OMD(\%) - 0.07$ (Andrieu and Demarquilly, 1987) was utilized to estimate the apparent GE digestibility. After that, the $dE(\%)$ was incorporated to the polynomial equation of Cambra-Lopez *et al.* (2008): $Y_m = -0.0038x + 0.3501x - 0.8111$. $R^2 = 0.8653$, Where: Y_m = fraction of GE transformed into CH₄ (%); $x = dE(\%)$.

Statistical analyses

Data were analyzed using a one way ANOVA with a tri-factorial arrangement being the factors sites (3), months (12) and shrub species (2). Statistical analysis data was performed using the Computer statistical software for Windows SPSS (2009, Version 17).

Results

Chemical composition of substrates

The OM content (mean 82.4%) was not different except in the months*sites interaction ($P < 0.01$). For the remaining variables, results showed differences between months, sites and species (Table 3). The double and triple interactions were also significant ($P < 0.001$). The average content of NDF was 40.8% in *P. texana* and higher by 3.5% in *A. amentácea*, whereas the CT content was 9.6% in *P. texana* and it increased by 48% in *A. amentácea*. The CP content varied throughout the year in a range of 14 to 22%, although the species' annual average (17.7%) ranged in narrow margin (17.4% to 18.1%). The EE content (mean 1.3%) was higher by 1.6% while IVTOMD (mean 66.1%) was higher by 38.6% in *P. texana* compared with its counterpart. ME without the addition of PEG (average 1.9 Mcal/kg DM), showed a value of 2.1 Mcal/kg DM in *P. texana* while in *A. amentácea* it was 20% lower. Gross energy losses (% as CH₄ production) were calculated as 3.0% for *P. texana* and 6.7% for *A. amentácea*.

Substrates fermentation

The fermentation parameters (Table 2) were different between the studied species (Table 3; $P < 0.001$). Values in *P. texana* for *A*, *c*, *L* and IVTOMD (annual mean = 183 mL/g DM, 0.0715 /h; 0.8679 h; 82 %, respectively) were higher than in *A. amentácea*, whereas values of *P* and PF were higher (annual mean = 8.142 μ mol; 6.1 mg microbial protein/ml of gas) in *A. amentácea*.

PEG effect

The addition of PEG affected the fermentation characteristics ($P < 0.001$), except for the interaction sites*species for lag phase *L*. The PEG addition increased the total gas volume *A* by 58% in *P. texana*, the rate of gas production *c* by 41%, but the *L* decreased by 24% for *A. amentácea* and decreased in *P. texana*

by 8%. The addition of PEG in *P. texana*, increased the total gas volume *A* by 9%, the rate of gas production *c* by 21%, but the lag phase *L* decreased by 53% and the PF by 42%.

Table 1. Chemical composition; *In Vitro* True Organic Matter Digestibility (IVTOMD); Metabolizable Energy and Gross Energy Losses (GEL) of leaves of two native shrub species in northeastern Nuevo Leon, Mexico.

Sites	Species	OM	NDF	CT	CP	EE	IVTOMD	ME		GEL
								-PEG	+PEG	
1	a.a	81.2	47.3	19.0	14.6	1.1	52.4	1.6	1.9	6.5
2	a.a	80.1	49.2	19.6	14.9	1.0	47.7	1.8	1.9	6.8
3	a.a	85.0	50.3	18.3	15.0	1.0	50.9	1.7	1.9	6.7
1	p.t	82.1	31.2	10.0	20.3	1.6	83.3	2.1	2.2	2.5
2	p.t	82.3	32.9	9.6	22.0	1.7	82.5	2.1	2.1	2.7
3	p.t	83.7	34.2	9.5	20.1	1.6	79.9	2.1	2.2	3.3
mean		82.4	40.9	14.3	17.8	1.3	66.1	1.9	2.0	4.8
SEM		4.3	1.1	0.4	0.6	0.1	0.7	0.1	0.04	0.15

Site 1= Los Ramones; Site 2= China; Site3= Linares; a.a= *Acacia amentacea*; p.t= *Parkinsonia texana*; OM= Organic Matter (% DM); NDF= Neutral Detergent Fiber (% DM); CT= Condensed Tannins (% DM); CP= Crude Protein (% DM); EE= Ether Extract (% DM); IVTOMD= *In Vitro* True Organic Matter Digestibility (%); ME= Metabolizable Energy (Mcal ME/kg DM); GEL= Gross Energy Losses (% , as methane production); SEM =Standard Error of Mean; * (P<0.05); ** (P<0.01); *** (P<0.001).

Table 2. Fermentation characteristics, Purines and Partitioning Factor of leaves of two native shrub species in northeastern Nuevo Leon, Mexico.

Sites	Species	<i>A</i>		<i>c</i>		<i>L</i>		Purines		PF	
		-PEG	+PEG	-PEG	+PEG	-PEG	+PEG	-PEG	+PEG	-PEG	+PEG
1	a.a	109	164	0.04	0.06	0.63	0.54	12.8	12.3	5.9	3.5
2	a.a	96	156	0.04	0.06	0.68	0.55	5.7	4.7	6.0	3.3
3	a.a	97	154	0.04	0.06	0.57	0.42	8.0	7.4	6.4	3.7
1	p.t	187	207	0.07	0.09	0.93	1.50	12.0	11.7	4.7	4.2
2	p.t	187	202	0.08	0.09	1.20	1.37	3.6	4.1	4.6	4.2
3	p.t	176	191	0.06	0.08	0.47	1.12	5.1	4.8	4.9	4.4
Mean		142	179	0.06	0.07	0.75	0.92	7.8	7.5	5.4	3.9
SEM		4.2	4.5	0.003	0.004	0.22	0.23	0.7	0.61	0.10	0.06

Site 1= Los Ramones; Site 2= China; Site 3= Linares; a.a= *Acacia amentacea*; p.t= *Parkinsonia texana*; *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 (μ mol); PF= Partitioning Factor; SEM= Standard Error of Mean; * (P<0.05); ** (P<0.01); *** (P<0.001).

Table 3. P-values, from one-way ANOVA results for chemical composition and fermentation parameters.

Factors	Months (A)	Sites(B)	Species (C)	A*B	A*C	B*C	A*B*C
Probability	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Discussion

Chemical composition of substrates

Higher structural and secondary compounds were recorded in the periods from March to May when the shrubs regrew and from June to November in presence of rain, as observed by Alvarado *et al.* (2013). Differences in CP content between species may be due to inherent characteristics of each species related to their ability to extract and accumulate nutrients. The wide variation in IVTOMD in native forages is primarily associated to their NDF and CP content; our results are controversial with this statement because these variables had a non-significant relation with IVTOMD ($r = -0.088$ and $r = 0.025$; $P > 0.05$). However, the IVTOMD was positively associated with their content of NSC and the *A* fraction ($r = 0.478$; $r = 0.818$; $P < 0.01$) and negatively with the CT content ($r = -0.702$; $P < 0.01$). Values of IVTOMD and ME are positively related ($r = 0.575$; $P < 0.01$); thus, the differences among studied factors reflect the variation in NSC between the forage species. It has been observed that the maximum of foliage in shrubby species is in autumn (90%) while the lowest amount is recorded in the spring (61%); indeed, shrubs can regrow in any season after moderate heavy rains (Rzedowski, 2006), as in the present study from August up to November (summer to autumn). This fact is reflected in higher contents of IVTOMD and ME during the summer and autumn. Livestock is a major source of greenhouse gases that go into the atmosphere, with enteric fermentation being one of them in sources of CH_4 . At the same time, CH_4 production means a

waste of the energy provided by forages. The GEL, as CH₄ production, 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 IVTOMD and CH₄ emission, as in this study ($r = -0.923$; $P < 0.01$). It is often claimed that forage-based diets result in higher enteric CH₄. However, 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 ($r = -0.477$; $P < 0.01$). In grazing animals consuming low quality forage, the GEL can be from 7.7 to 8.4%, while in those consuming high digestibility forages, the GEL are reduced to 1.9 to 2.2%. The higher GEL recorded in the studied species (range from 2.9 to 6.7% GE) was lower than the upper limit reported as normal for feedstuffs (Cambra-López *et al.*, 2008).

Substrates fermentation

Anti-nutritive factors such as tannins may contribute to reduce the microbial activity and consequently the *A* fraction, constant rate of gas production *c* and *L* fraction. In fact, content of NDF was not related to ruminal gas production ($r = -0.062$; $P > 0.05$); instead, CT and GEL negatively affected the *A*, *c* and *L* fractions (Table 3). Fixation of C and N in microbial biomass is important because it reduces C losses as CO₂ and CH₄. Values of Purines in our study were higher (7.5 μ mol) than those previously reported in shrubby species by Guerrero *et al.* (2012). The PF of the studied species are close to the range 2.7 to 4.4 theoretically possible proposed by Blümmel *et al.* (1997). The wide variation among studied factors in fermentation parameters may be due to their variable nutrient content and secondary compounds that interact with the nutrients, which provide energy and nitrogen to ruminal microorganisms.

PEG effect

Mean annual value of ME for the studied species was 2.0 Mcal/kg DM and the observed increase by addition of PEG in *A. amentacea* and *P. texana* was 11% and 10%, respectively. Increased values of ME up to 21% have been reported by Guerrero *et al.* (2012). The gas produced *A* by *A. amentacea* and *P. texana* showed a rise of 58% and 9%, respectively, compared to the treatment without PEG. The total gas *A* was negatively affected by the CT content ($r = -0.731$; $P < 0.01$). The addition of PEG decreased the *L* fraction in 20% for *A. amentacea* while *P. texana* showed an increase of 50% as it was observed by others. A lower value of the *L* suggests an increase in the energy density of the substrates, which favors microbial growth and rapid colonization of the insoluble but potentially degradable fraction. However, this is not always true, e.g. when the maximum capacity of microorganisms that metabolize excess of soluble material, the onset of degradation of the insoluble fraction could take a longer time and the value of the lag phase *L* would be higher; this could be the case of *P. texana*, whose *L* was increased in presence of PEG (Dijkstra *et al.*, 2002). As expected, the *c* fraction increased with the addition of PEG probably due to an increase in cellulolytic activity of microbial enzymes, by reducing negative effects of CT and low values of *c* could indicate that the substrate structure exhibits physical barriers that prevent its hydrolysis. Values of this fraction are positively related to the amount of NSC ($r = 0.347$; $P < 0.01$) and varied from 0.061 (/h) in *A. amentacea* to 0.086 (/h) in *P. texana*. Results of PF without PEG varied from 5.4 in *A. amentacea* to 3.8 in *P. texana* and the addition of PEG decreased these values in 39% and 11%, respectively. Purines concentration were lower after the addition of PEG (general mean = 7.8 decreasing to 7.5 μ mol), and *A. amentacea* was more affected by addition of PEG since the purines content decreased by 8% whereas in *P. texana* no changes were observed. Reduced values of Purines can be attributed to the presence of CT ($r = 0.151$; $P < 0.05$) and other chemical compounds that likely inhibit fermentation, which might affect the degradation of substrates.

Nutritional implications of substrates

The NDF content of species *A. amentacea* and *P. Texana* (annual mean = 40%) indicate high availability of nutrients and increased animal performance. High concentrations of CT, as 19% in *A. amentacea*, are one of the main factors of low nutritional value of forage legumes because they cause a reduction of DM, OM, and N digestibility. However, CT has also favorable effects such as the increase the by-pass protein and antiparasitic effect. Evaluated species supply enough CP to meet the maintenance requirements (7 to 9%) and body weight gain (17%) for adult range small ruminants and wildlife. Levels of 15% of CP in diets consumed by small ruminants provide 74 g/d of metabolizable protein, which insure an adequate supply of N for maintenance of an adult range goat. Forages containing IVTOMD coefficients from 0.45, 0.45 to 0.55 and above 0.55 to 0.70 are of poor, low and medium nutritional quality, respectively. Thus, the shrub species studied were of medium quality in spring and summer (60%), whereas in autumn and winter (70%) they might be considered as forages of good quality. Values of 1.2 Mcal/kg of ME in shrub species are low, whereas the ME requirements of maintenance for free ranging small ruminants are 2.1

Mcal/kg. According to this, the ME of studied species would appear to be sufficient to satisfy the small ruminants and wildlife maintenance requirements in late summer and autumn, whereas ME content could not satisfy these requirements in winter and spring. Plants with high PF values in general have a higher efficiency in microbial protein synthesis; this fact has positive implications in ruminant nutrition since it suggests that feedstuffs with high PF values can be used more efficiently; conversely, forages having low digestibility produce a greater amount of CH₄, which represents a higher GEL. In grazing animals consuming low quality forage, the GEL can be from 7.7 to 8.4%, while those consuming high digestibility forages, the GEL are reduced to 1.9 to 2.2%. 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 an about 2.3 times higher value.

Conclusions

Parkinsonia texana seems to be better forage than *A. amentacea* because it has higher CP, IVTOMD, ME and it is more rapidly fermentable. Although CT and GEL content is higher in *A. amentacea*, their values of purines and PF lead to higher microbial protein synthesis compared with *Parkinsonia texana*. Results of this study suggest that both species could be a good combination to supply the nutritional requirements in autumn and winter seasons to adult Spanish goats at the late gestation and at the beginning of lactation and for wildlife when the mating and gestation occurs, in the Tamaulipan scrubland.

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