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Seasonal nutritional value of *Leucophyllum frutescens* (Berl.) I.M. Jhonst. (Scrophulariaceae) and *Zanthoxylum fagara* (L.) Sarg (Rutaceae), natives of north-eastern Mexico

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Abstract Shrubs are an important source of nutrients for small ruminants in the Tamaulipan thornscrub of north-east ern Mexico. The objective of this study was to evaluate the nutritional value of *Zanthoxylum fagara* and *Leucophyll um frutescens* using *in vitro* procedures. Collection was carried out from three sites from Nuevo León, Mexico, for o ne year, and the CT content, IVGP, IVOMTD, determination of ME and MPS were analyzed in triplicate. Statistical analysis was performed using a general linear model for a completely randomized design with multifactorial arrange ment (4 seasons, 3 sites, 2 species). There were differences ($P < 0.001$) among species, sites and seasons and also in the interactions between these factors on the nutritional attributes of shrub species ($P < 0.001$). Both species had low condensed tannins. For *Z. fagara*, the IVGP parameters and the variables studied indicate it is a valuable species be cause of its nutritional potential (b = 245 ml, c = 0.072 h^{-1}, L= 1.55 h, Gas24 = 194 ml, Gas48 = 231 ml, ME = 2 Mc al kg^{-1} MS, IVOMTD = 88% and MPS = 7 µmol). During the autumn of 2005 and the winter of 2006 a greater volu me of gas, greater energy input and microbial protein synthesis occurred. Both species were considered to be a good source of energy and protein complement of high quality for grazing of small ruminants.
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Key words: North-eastern Mexico • Shrubs • Small ruminants• Nutritional value • *In vitro* gas production.

Abbreviations

ADF	Acid Detergent Fiber
NDF	Neutral Detergent Fiber
LIG	Lignin
CEL	Cellulose
HEMI	Hemicellulose
CP	Crude Protein
CT	Condensed Tannins
DM	Dry Matter
IVGP	*In vitro* gas production
IVOMTD	*In vitro* organic matter true digestibility
EE	Ether extract
ME	Metabolizable Energy
Gas24	Volume of gas produced at 24 h
Gas48	Volume of gas produced at 24 h
MPS	Microbial protein synthesis

Introduction

The Tamaulipan thornscrub is recognized in Mexico as a major provider of medium quality forage through native species of northeast Mexico. These species are adapted to changing environmental conditions; however, seasonal changes affect their availability and the nutritional value for small ruminants (Holecheck, 1984). According to Ramírez et al. (2004), *Zanthoxylum fagara* and *Leucophyllum frutescens* complement the diet of small domestic and wild ruminants. However, the information on their nutritional value through their chemical composition and degree of digestion is scarce. *In vitro* techniques therefore represent a significant potential for understanding the dynamics of the process of rumen fermentation of soluble and insoluble fractions of the food consumed by the ruminant (Makkar 2005), and the degree of removal of these fractions (Van Soest 1994). The objective of this study was to know the nutritional value of these two native shrub species through *in vitro* procedures.

Materials and Methods

Area of Study

This study was conducted at three sites located in the state of Nuevo León, Mexico: 1) "El Abuelo" Ranch in the municipality of Los Ramones (25° 40' N, 99° 27' W); 2) "Zaragoza" Ranch in the municipality of China (25° 31' N, 99° 16' W) and 3) the experimental station of the Faculty of Forest Sciences, Universidad Autónoma de Nuevo León (24° 47' N, 99° 32' W) located in the municipality of Linares. The sites are at altitudes between 200 and 350 meters above sea level, with the type of climate in the region being ACx and BS1 (h), mild temperate sub-humid with annual average precipitation of 805 mm (May, June and September). The main type of vegetation of the area is the Tamaulipan thornscrub and soils are lithosols and lime-clay vertisols with montmorillionite (González et al. 2004). The records of temperature and precipitation that occurred during the duration of the study are shown in Figure 1.

Foliage collection

At each sampling site, five plants per species were chosen randomly within a 50 m x 50 m representative plot of the thornscrub undisturbed by the study site. Approximately 800 grams of terminal buds with fully expanded leaves (Cochran 1977) at browsing level (1.2 m) were taken. Collection dates were conducted in summer 2005 (August 28); autumn 2005 (November 28); winter 2006 (February 28) and spring 2006 (May 28). The samples were dried and then ground and sieved (1 mm). For the corresponding analyses samples were used in triplicate.

Condensed Tannins (CT)

CT were determined according to Makkar (2003a) using a butanol/HCl (95:5 v/v) solution and ferric ammonium sulphate (2.0 g/l00 ml 2N HCl). The results were expressed as the leucocyanidin equivalent.

In vitro gas production

Two hundred milligrams of sample were incubated in 100 ml calibrated glass syringes (240 syringes in total) according to the methodology proposed by Menke and Steingass (1988). Rumen fluid of three rumen-cannulated Criollo sheep, which were fed on alfalfa hay and commercial feed (75:25) was collected. The procedures were approved by the animal use and care committee of the Universidad Juárez del Estado of Durango. To prepare the inoculum, the ruminal fluid was mixed with a buffer solution containing a reducing agent at a proportion of 1:2. The solution was saturated with CO_2 at 39°C. Each syringe was inoculated with 30 ml of solution. Readings for IVGP were recorded at 0,

3, 6, 9, 12, 24, 48, 72 and 96 h after the onset of incubation. Total gas values were corrected using values from blanks. For a more accurate estimation of the IVGP over the period of *in vitro* fermentation, a non-linear equation was used to analyze data from kinetics (France et al. 2000). The data were adjusted to the equation:

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

where *A* is the volume of gas produced at time *t*, *b* is the asymptote of gas production (ml g^{-1} MS), *c* is the rate constant of gas production *in vitro* (/h), and L (h) is the discrete time delay to the production of gas.

The metabolizable energy content (Mcal kg^{-1} MS) was estimated using the equations of Menke and Steingass (1988) based on the production of gas at 24 h (ml) and contents of crude protein (g kg^{-1} DM): ME = 2.20 + 0.136 $GP_{24\,h}$ + 0.057 CP + 0.0029 EE^2/ 4.184.

To estimate the microbial protein synthesis, another group of 204 syringes was incubated. After 24 h, the contents were transferred to centrifuge tubes and centrifuged at 20,000 x g for 30 minutes at 4°C, and the supernatant was discarded. The pellet was washed with distilled water, centrifuged again and lyophilized. Purine content was analyzed according to the procedure described by Makkar (2003a).

In vitro organic matter true digestibility (IVOMTD)

IVOMTD determinations were made according to the proposed procedure for the equipment used for incubation, Daisy^{II} (ANKOM Technology, Corp., Macedon, NY, USA). Approximately 250 mg DM were deposited in triplicate in F57 filter bags (Ankom Technology, Corp. Macedon, NY, USA). Once sealed, filter bags were placed in digestion jars (25 bags per jar), which in turn contained ruminal fluid with a buffer solution at a 1:4 ratio and saturated with CO_2. Filter bags were incubated for 48 h at 39°C, after which they were taken from the medium, washed with distilled water and processed on the fiber analyzer (ANKOM Technology, Corp., Macedon, NY, USA) with neutral fiber detergent solution.

The data relating to *in vitro* gas parameters (CT, IVOMTD, MPS, EE and ME) were subjected to an analysis of variance (ANOVA) using a general linear model with a completely random multifactorial arrangement design, where the factors were the two species (S), the four seasons of the year (C) and each of the three sampling sites (M):

$$Y_{ijklm} = \mu+S_j+C_k+M_l+(SxC)_{jk}+(SxM)_{jl}+(MxC)_{lk}+(SxMxC)_{jkl}+E_{ijklm}$$

where Y_{ijkl} is the observation of the i-th species (S_i: *L. frutescens* and *Z. fagara*) when it was evaluated in each i-th estimation (C_j: summer to spring) and i-th site (M_j: China, Linares, and Los Ramones); μ is the overall mean; S_j (i=1-2) is the effect of season on the species; C_j is the effect of season (j=1-4); M_j is the site effect (j=1-3), $(SxC)_{ij}$ is the interaction between species and seasons; $(SxM)_{ij}$ is the interaction between species and the site; (MxC) is the interaction between seasons and sites; (SxMxC) is the interaction between the three factors of study (species, seasons and sites); and E_{ijklm} is the experimental error. Pearson correlations were carried out between the chemical composition reported previously (Alvarado et al. 2012) and the variables studied. Statistical analyses were performed with the SPSS package for Windows Version 13 (SPSS 2004).

Results

Condensed Tannins and *in vitro* Gas Production

Variations in the content of CT and parameters of IVGP were observed between species (S), seasons (C) and sites (M) ($P < 0.001$; Table 1). Similar significance ($P < 0.001$) was observed in all double interactions (SxC; SxM; CxM) and in the triple interaction (SxCxM).

The concentration of CT of both species was lower than 1% ($P < 0.001$). *L frutescens* showed higher CT values than *Z. fagara* ($P < 0.001$). The highest CT concentrations were recorded during spring ($P < 0.001$) while at the Linares site a greater effect of the site was encountered ($P < 0.001$).

The asymptotic gas production (b) was 18% higher ($P < 0.001$) in *Z. fagara* than in *L. frutescens*, whereas the effect of season and site was observed through an increase in the potential gas production ($P<0.001$) during the winter and on the Linares site, respectively. The constant rate of gas production *c* was 8% higher ($P<0.001$) in *L. frutescens*; the effect of site was greater ($P<0.001$) in Los Ramones, but was very close to the constant rate of gas production at the China site. Regarding lag phase, *Z. Fagara* substrate presented a 37% greater lag time ($P<0.001$), while this adaptation period of the microorganisms previous to gas production increased during the fall for both species ($P<0.001$). The effect of the site factor was observed in Los Ramones through a greater delay of fermentation time ($P < 0.001$). The differences between species and seasons ($P < 0.01$) in the production of accumulated gas to 6, 12, 24, 48 and 72 h are shown in Figure 2.

Energy content, digestibility and microbial protein synthesis

The EE, ME and IVOMTD contents varied among species (S), seasons (C) and sites (M) (Table 2). Similarly significant double and triple interactions were recorded ($P<0.001$). The microbial protein synthesis was different ($P<0.001$) except in the species factor (S) and in the interaction SxM.

The EE, ME and IVOMTD contents were 40%, 15% and 18% greater respectively ($P<0.001$) in *Z. fagara* compared to *L. frutescens* (Table 2); however, the effect of season was not the same for these variables as

during winter, higher values of EE and ME were observed (P<0.001). However, the largest IVOMTD occurred in the autumn for both species (P<0.001), although L. *frutescens* also showed a similar increase in spring and *Z. fagara* a similar increase during the summer. The site effect manifested itself more intensely in China (P<0.001) for the two first variables, while the greater IVOMTD occurred at the Linares site (P<0.001).

With respect to microbial protein synthesis, no effect of species was observed (P<0.001), with both obtaining an average of 7 µmol. During the spring, there was an increase (P<0.001) per season. In addition, at the Linares site, greater microbial protein synthesis was observed as an effect of season and site, respectively. Despite this, the interaction of species * site was reflected in a greater MPS for each species during the winter for *Z. fagara* and during the spring for *L. frutescens*.

Discussion

Condensed Tannins (CT)

Condensed tannins have positive or negative effects depending on their concentration and nature (Makkar 2003b). These compounds have the ability to form complexes with proteins linked to the cell wall, and may cause adverse effects in the ruminal digestion at concentrations of 6-12% (Frutos 2002), explaining the negative correlation with all IVGP parameters (Table 3).The low concentration of CT found in this study on *L. frutescens* and *Z. fagara* can have a positive effect of shielding the proteins of rumen fermentation, allowing them to reach the small intestine as by-pass protein and in turn control microorganism populations that are adverse to fermentation (Reed 1995; Makkar 2003b).

In vitro gas production potential

The average content of ADF (27%), NDF (46%) and LIG (14%) of the assessed species has been previously documented by Alvarado et al. (2012) and Ramírez (2004). The nutritional value of the forage depends on the content of soluble fiber which helps the fermentation and the insoluble fiber that impairs it (Van Soest et al. 1991). From the species tested here, *Z. fagara* produced 18% more gas volume than *L. frutescens*. Likewise, the Pearson correlation revealed that gas production from the slowly degradable fraction of the cell wall is negatively associated (P<0.01) with LIG, ADF, CEL and the CT content (Table 3). The gas volumes were similar to those obtained from the fermentation of *Acacia saligna* (Salem 2005) where the author attributed this positive result to both the low content of condensed tannins and fiber. In the present case, the CT do not limit fermentation; however, fiber fractions can become more important in limiting *in vitro* fermentation (Ndlovu and Nherera 1997).

Van Soest et al. (1991) differentiate between soluble fiber fractions (cellular content fully available for rumen fermentation) from the insoluble fiber in neutral detergent (insoluble cell wall whose availability is controlled by characteristics that bind to cellulose, hemicellulose and lignin). For this reason, its characterization is important to predict and explain their nutritional value. In this study, *Z. fagara* presents a greater production of gas from the slowly-fermentable fraction (asymptote) to that reported in *P. domestica* (Salem 2012) and multipurpose woody species (Larby et al. 1998). However its fermentation potential is less than some shrub forage of Tunisia (Ammar et al. 2005).

The seasonal oscillations in asymptotic gas production (*b*) of the studied species may reflect the behavior of the shrub species of the Tamaulipas thornscrub, as these do not adhere to the marked temperate phenological patterns. They instead maintain leaves with replacement and repeated bloom events during the winter months (Alvarado 2003). Furthermore, it has been reported that the nutritional value of winter plants, such as the ones studied in this work, may be at its highest point during the winter or early spring in the geographical area of South Texas and northeast Mexico (Fullbright and Ortega 2007).

Constant rate of gas production (*c*)

Under an analysis system based on detergents to differentiate the types of fibers and their effect on the nutrition of ruminants, Van Soest classifies the fractions thereof, being the cellular content, the fraction that is fully available for fermentation in the rumen, which promotes a higher rate. The opposite occurs when the main volume is of structural carbohydrates as the NDF (Ramírez et al. 2002).

The constant rate of gas production reflects the speed at which the microbiota ferments food components (France et al. 2000). When the rate constant of gas production increases, so too does the potential volume of gas produced. This reflects the increase in accessibility of microorganisms to the substrate due to the hydration of the particles, microbial adhesion and to an increase in microbial population after the initial delay in gas production (France et al. 2005). The species evaluated in this study present a greater *c* rate than the diet consumed by small ruminants such as goats and white-tailed deer in the north and northeast of Mexico, as reported by Domínguez et al. 2011 and Guerrero et al. 2012. This may reflect a higher concentration of highly soluble and available cellular compounds for the ruminal microbiota and therefore a more relevant nutritional value of the studied species. As mentioned previously, this parameter reflects the accessibility of microorganisms to insoluble but degradable cellulose and hemicellulose fractions,

which was confirmed in substrates of both species by a positive association with the NDF content (Table 3). The higher rate of gas production for *L. frutescens* over *Z. fagara*, considering an overall average, suggests that more carbohydrates and proteins are available in this species compared to its counterpart (Edwards et al. 2012). However, during the summer, *Z. fagara* presented the highest value of rate constant of gas production, suggesting an increase in its nutritional potential during this season under the temperature and humidity conditions recorded in this study.

These season and site effects affected *L. frutescens* differently, with greater values of the rate constant of production observed in different seasons of the year. It was notable that its value was higher than that of *Z. fagara* in the Los Ramones site, which suggests seasonal changes of this species that are additional to the influence of the sites on this parameter. The seasonal fluctuations, between the sites and species, reflect changes in the nutritional quality of the plants due to the effect of environmental conditions or the fertility of the soil. These modify the chemical composition of plants, mainly the structural carbohydrate concentration such as cellulose and hemicellulose (NRC 2007, Longo et al. 2012). The differences between sites regarding the constant rate of gas production during the study, may be due to variations in terms of soil fertility, sun exposure, environmental conditions of precipitation and temperature, as some authors have mentioned before (Foroughbakhch et al. 2007).

Lag phase

The *Lag* phase is the period in which the rumen microorganisms develop to allow digestion of the substrates (Mauricio et al. 2001). It is usually associated with the microbial adhesion process and the hydration of the particles to the NDF fraction of food (Tedeschi et al. 2008). The factors that affect the presence and extent of *Lag* include the nature of the incubated substrate, the inoculated microbial species and the amount of inoculum added (France et al. 2000). *Z fagara* presented a higher *L* phase in terms of annual average. This can be explained by the characteristic chemical constituents of *Z. fagara* with a higher content of HEMI and CEL (Ramírez et al., 2002), which hinder access of micro-organisms and initiate enzymatic action. The above was corroborated at least partially by the negative correlation that occurred between the fiber fractions and the *Lag* parameter (Table 3).

Even with this potential disadvantage with respect to other forage shrubs of the north region of Mexico (Guerrero et al. 2012), the *L* time that the studied species presented is lower than in diets with corn stover and weeds (Martínez et al. 2011), hybrid varieties of Triticale (Aguilar et al. 2013), oaks (Camacho et al. 2010) and some trees and shrubs of the central region of Mexico (Salem 2012).

Volume of gas produced at 24 h

The production of gas at 24 hours and the chemical composition of foods are associated with measured values of metabolizable energy *in vivo* (Menke and Steingass 1988; Ginger-Riverdan et al. 2000). The differences in the production of gas with energetic potential were affected by changes in the content of ADF, LIG, CEL and CT of the studied shrub species. The negative influence of the insoluble components of the cell wall and the CT on the production of gas at 24 hours (Table 3) have previously been documented by Ammar et al. (2005), who mentioned that *Phillyrea angustifolia* (197 ml g^{-1} DM) and *Calicotomevillosa* (211 ml g^{-1} DM) are considered as forage resources of medium and high digestibility, due to the content of lignin and condensed tannins. The production of Gas24 in the present study was greater in *Z. fagara* and is similar to *Acacia constricta* (173 ml g^{-1} DM) and *Cordiaparvifolia* (171 ml g^{-1} DM) reported for the north of Mexico (Guerrero et al. 2012) during three seasons (summer, autumn and winter).

Volume of gas produced at 48 h

The *in vitro* production of gas at 48 h is associated with digestibility (Van Soest 1994). In this study the contents of ADF, LIG and CEL were correlated negatively with the production of gas at 48 hours (Table 3). Similar results have been reported in shrubs of Zimbabwe (Ndlovu and Nherera 1997), Egypt (Salem et al. 2007), Mexico (Domínguez et al. 2011; Guerrero et al. 2012) tropical grasses of Mexico (Juárez et al. 2009) and in some fruits and flowers in Cuba (Juárez et al. 2013). The drier season was during winter (Fig. 1) and coincided with the increased production of Gas48, similar to that found by Larby et al. (1998).

In the present study, results for accumulated *in vitro* gas production (Figure 2) reflect seasonal changes in the species studied resulting in estimates on the energy potential and digestibility of both species. Cumulative gas production was higher than what was reported for some hybrid varieties of *Triticale* and barley (Aguilar et al. 2013) as grasses have higher lignin contents. Greatest fermentation potential were found in those species that were distributed in vertisol-type soils in a study by Tefera el al. (2008), which is in agreement with the results obtained in this study in that the Linares site has this type of soil (González et al. 2004) and was the site that registered a greater potential production of gas, and gas at 48 hours.

Metabolizable energy (ME)

The metabolizable energy content represents the portion of food energy that is left available to the

metabolic processes of the animal. Therefore, the metabolizable energy provides an adequate measure of the nutritive value of food (Jung and Allen 1995). *L frutescens* and *Z. fagara* showed similar values and higher, respectively, to the most valued shrubs of the chaparral in California, USA and Spain (Frutos et al. 2002; Nevarez et al. 2010). Furthermore, both species showed values of metabolizable energy within the range of 0.88-2.83 Mcal kg^{-1} DM, which has been reported for shrubs and trees in Mexico (Calderón 2012; Salem 2012) and Iranian oaks and shrubs (Yousef et al. 2012; Mancilla et al. 2013), and which are considered as good sources of energy for small ruminants. During the winter season the contribution of ME was greater in both species. The energy potential expressed by the species analyzed here may be associated with periods of early bloom in *Z. fagara* from October until the beginning of spring in March, as well as to up to five events of flowering in *L. frutescens*, mainly in spring and autumn. These result in greater movement of solutes, increased photosynthetic activity and leaf and flower bud growth, and could explain a low formation of cell wall (Alvarado 2003; Azcón-Bieto and Talón 2008) that is their relatively low NDF content (35%) and higher content of highly soluble compounds.

In vitro organic matter true digestibility (IVOMTD)

The cellular content of the shrub species that grow in northeastern Mexico and southern Texas, USA, varies from 37-81% (Ramírez 2009), which are overall in accordance to the values reported herein. Nonetheless, *Z. fagara* had the largest values ranging from 86 to 91%, whereas *L. frutescens* showed digestibility values ranging from 70% to 74%. The values of digestibility obtained in this study are larger than those reported in legumes and hay (Karabulut et al. 2007) and also those of other shrubs of the north of Mexico (Guerrero et al. 2012). The greater disappearance of respective substrates was correlated negatively with the ADF, LIG and CEL of the fiber fractions and with the CT, while the CP and HEMI showed a positive association with the IVOMTD. Various authors state that the variation in the IVOMTD is associated with chemical composition. On one hand, the negative association is based on the fact that fractions as lignin hinder the microbial attack on cellulose fibers, and therefore decrease the digestibility (Jung and Allen 1995). On the other hand, the high content of protein and NDF are positive. Results reported in this study may therefore be explained by *Z. fagaraes* having reported values of NDF between 35% and 47%, and of crude protein between 18 and 22%. Together, with a low concentration of condensed tannins and a lower proportion of ADF/NDF, a high potential of fermentation is expected, i.e. to produce gas and to be digested by ruminant (Ammar et al. 2005; Camacho et al. 2010).

Seasonally, the highest values of IVOMTD occurred in autumn (82%), while lower values occurred during the winter season (78%); the shrubs of the northeastern region of Mexico may have shoot re-growth throughout the year, with quick responses to any rain event (Alvarado 2003). This coincides with the extraordinary rains that occurred during the period of study by Hurricane Emily in late July (Figure 1) which could positively affect the digestibility values during these seasons. In the case of *Z. fagara*, the highest values of IVOMTD might reflect its ability to generate leaves in cycles of up to 116 days (Nelson et al., 2002). Likewise, it has been documented that *Z. fagara* and *L. frutescens* have the ability to generate new leaf tissue (90% and 30% respectively) during the spring and autumn seasons. However *L. frutescens* tends to lose leaves slowly throughout the year, mainly in the winter and summer (Alvarado 2003). This physiological response may be associated with a low digestibility of this species in these seasons due to plant translocation of nutrients to storage organs and a decrease in the photosynthetic activity during the loss of leaves (Azcón-Bieto and Talón 2008).

Microbial protein synthesis (MPS)

The content of purines in forages is an indicator of microbial protein production (Getachew et al. 2000) through the fixation of C and N in microbial biomass. At the same time, it reduces carbon losses as CO_2 and methane (Blümmel et al. 1997). This protein provides more than half the amino acids absorbed by ruminants but its synthesis is affected by anti-nutritional factors, sources of carbohydrates and protein, and by the synchronization of the rumen functions (Rodríguez et al. 2007). In this study, no difference was found between average annual values between species (7 µmol), which were lower than those reported for forage shrub species in Egypt and Mexico (Salem et al. 2007; Guerrero et al. 2012). There were differences in the effect of season and site, with the microbial synthesis being higher for *Z. fagara* during winter and for *L. frutescens* in the spring. The effect of the site is manifested by a higher MPS in Linares (Table 2).

The MPS was apparently affected by the LIG and the ADF since as shown in Table 3, there is a negative correlation with ADF and LIG. Despite a good CP content in both species, the MPS is low. According to Pathak (2008) poor degradation of carbohydrates may be due to the availability of nitrogen compounds to meet the requirements of microbial growth (NRC 2007). The increased production of microbial protein during the winter for *Z. fagara* coincides with a greater volume potential of gas produced (*b*) and lower NDF content, which makes us infer that the greatest content

of soluble compounds of *Z. fagara* confer it greater energy efficiency and protein during the colder and drier season of the year. On the other hand, *L. frutescens* produced one of its largest gas volumes during the spring, also coinciding with a high content of NDF and IVGP. Lower rates of microbial growth occur when cellulose is used as the sole source of energy, but the degradation of carbohydrates also depends on the amounts of lignin contained in the food (Rodríguez et al., 2007). This may indicate that the slow fermentation of structural carbohydrates and protein trapped in the cell wall allows synchrony between energy and nitrogen compounds for the synthesis of microbial protein during the spring for this second species when the lignin content is reported as its lowest in the year.

Finally, leaves of *Z. fagara* and *L. frutescens* did not have a CT content that posed a risk of toxicity for ruminants. The low production of microbial protein in both species could be associated with its chemical composition and the lack of synchrony in the release of energy and nitrogen compounds, or due to the presence of some other secondary compound, which is hinted by the fruits of *Z. fagara* being reported to have anti-fungal properties in agriculture (Prieto et al. 2011).

Conclusion

Through the *in vitro* gas production and digestibility techniques applied to samples of *Z. fagara* and *L. frutescens* it was possible to observe a greater availability of nutrients for both species during winter and autumn. Due to a longer time to the beginning of fermentation, the existence of limiting chemical or physical factors for the development of the greatest total potential observed for *Z. fagara* could be considered. Both species could be valuable sources of carbohydrates for a suitable energy supply and for a complementary supply of good quality protein for small ruminants in the region, especially during the winter season. This information is valuable to promote native species in grazing ruminant production systems, although it is necessary to carry out further investigation at the level of animal physiology to validate these results.

References

[1]. Aguilar, L.E.Y., Bórquez, J.L., Domínguez, I.A., Morales, O.A., Gutiérrez, M.M.G., González, R.M., 2013. Forage yield, chemical composition and *in vitro* gas production of triticale (*X Triticosecale wittmack*) and barley (*Hordeum vulgare*) associated with common vetch (*Vicia sativa*) preserved as hay or silage.Journal of AgriculturalScience, 5, 227-238.

[2]. Alvarado, M.S., González, R.H., Ramírez, L.R.G., Cantú, S.I., Gómez, M.M.V., Cotera, C.M., Jurado, I. E, Domínguez, G.T.G., 2012. Chemical composition and digestion of shrubs browsed by white-tailed deer (*Odocoileusvirginianus*texanus). Journal of Animal and Veterinary Advances, 11, 4428-4434.

[3]. Alvarado, V.M.A., 2003. Análisis fenológico y algunos aspectos reproductivos en especies selectas del matorral xerófilo del noreste de México (Tesis Doctor en Ciencias Biológicas sin publicar, UANL, México)

[4]. Ammar, H., López, S., González, J.S., 2005. Assessment of the digestibility of some Mediterranean shrubs by *in vitro*techniques.Animal Feed Science and Technology, 119, 323-331.

[5]. Azcón-Bieto, J., Talón, M., 2008.Fundamentos de Fisiología Vegetal. 2ª edición.McGraw-Hill/Interamericana de España, S.A.

[6]. Blümmel, M., Steingaß, H., Becker, K., 1997.The relationship between in vitro gas production, *in vitro* microbial mass yield and 15N incorporation and its implications for the prediction of voluntary feed intake of roughages. British Journal of Nutrition, 77, 911-921.

[7]. Calderón, R.J.M., 2012. Valor nutricional de *Parkinsonia aculeata* y *Acacia rigidula* tratadas con Polietilen glicol (Tesis de Maestría sin publicar, Universidad Juárez del Estado de Durango, México)

[8]. Camacho, L.M., Rojo, R., Salem, A.Z.M., Mendoza, G.D., López, D., Tinoco, J.L., Albarrán, B., Montañéz, O.D., 2010. *In vitro*ruminal fermentation kinetics and energy utilization of three Mexican tree fodder species during the rainy and dry period. Animal Feed Science and Technology, 160, 110-120.

[9]. Cochran, W.G., 1977. Sampling techniques.3rd edición. John Wiley &Sons., New York, N.Y., USA.

[10]. Domínguez, G.T.G., González, R.H., Guerrero, C.M., Cerrillo, S.M.A., Juárez, R.A.S., Alvarado, M.S., Ramírez, L.R.G., 2011. Polyethylene glycol influence on *in vitro* gas production parameters in four native forages consumed by White-tailed deer. RevistaChapingoserieCienciasForestales y delAmbiente, XVII, 21-32.

[11]. Edwards, A., Mlambo, V., Lallo, C.H.O., Garcia, G.W., Diptee, M.D., 2012. *In vitro*ruminal fermentation parameters of tanner grass (*Brachiariaarrecta*) supplemented with leaves

from three forage trees. Livestock Research for Rural Development, 24 (6).

[12]. Foroughbakhch, R., Hernández-Piñero, J.L., Ramírez, R., Alvarado, M.A., González de León, O.A. Rocha, A., Badii, M. H. 2007.Seasonal dynamics of the leaf nutrient profile of 20 native shrubs in Northeastern Mexico. Journal of Animal and Veterinary Advances, 6, 1000-1005.

[13]. France, J., Dijkstra, J., Dhanoa, M.S., López, S., Bannink, A., 2000.Estimating the extent of degradation of ruminant feeds from a description of their gas production profiles observed *in vitro*: derivation of models and other mathematical considerations. British Journal of Nutrition, 83, 143-150.

[14]. France, J., Lopez, S., Kebreab, E., Bannink, A., Dhanoa, M.S., Dijkstra, J. 2005. A general compartmental model for interpreting gas production profiles. Animal Feed Science and Technology, 123-124, 473-485.

[15]. Frutos, P., Hervás, G., Ramos, G., Giráldez, F.J., Mantecón, A.R. 2002. Condensed tannin content of several shrub species from a mountain area in northern Spain, and its relationship to various indicators of nutritive value. Animal Feed Science and Technology, 95, 215-226.

[16]. Fullbright, T.E., Ortega, S.J.A., 2007. Ecología y Manejo de Venado Cola Blanca. Texas A & M University Press EUA.

[17]. Getachew, G., Makkar, H.P.S., Becker, K., 2000. Effect of polyethylene glycol on *in vitro* degradability of nitrogen and microbial protein synthesis from tannin-rich browse and herbaceous legumes. British Journal of Nutrition, 84, 73-83.

[18]. Ginger-Riverdan, S.C., Maarouf, S.C., Bontems, V., Jousse, C., 2000.Interest and limits of the HFT method to evaluate the energy value of compound feeds for small ruminants. CIHEAM Options Méditerranéennes, 52, 39-42.

[19]. González, R.H., Cantú, S.I., Gómez, M.M.V., Ramírez, L.R.G., 2004. Plant water relation of thornscrub shrub species north-eastern Mexico.Journal of AridEnvironments, 58, 483-503.

[20]. Guerrero, M., Cerrillo, S.M.A., Ramírez, R.G., Salem., A.Z.M., González, H., Juárez, R.A.S., 2012. Influence of polyethylene glycol on *in vitro* gas production profiles and microbial protein synthesis of some shrub species. Animal FeedScience and Technology, 176, 32-39.

[21]. Holechek, J.L., 1984. Comparative contribution of grasses, forbs, and shrubs to the nutrition of range ungulates. Rangelands, 6, 261-263.

[22]. Juárez, A.S., Cerrillo, M.A., La, O.O., Herrera, R.S., Scull, I., Guerrero, M., Bernal, H., 2013.Valor nutricional y cinética de la fermentación ruminal de flores y frutos de árboles y arbustos de la cuenca del río Cauto, Cuba. Revista Cubana de Ciencia Agrícola, 47, 37-43.

[23]. Juárez, R.A.S., Cerrillo, S.M.A., Gutiérrez, O.E., Romero, T.E.M., Colín, N.J.C., Bernal, B.H., 2009. Assessment of the nutritional value of tropical grasses obtained from conventional analyses and *in vitro* gas production.TécnicaPecuaria, México, 47, 55-67.

[24]. Jung, H. G., Allen, M. S., 1995. Characteristics of plant cell walls affecting intake and digestibility of forages by ruminants. Journal of Animal Science, 73, 2774-2790.

[25]. Karabulut, A., Canbolat, O., Kalkan, H., Gurbuzol, F., Sucu, E., Filya, I., 2007. Comparison of *in vitro* gas production, metabolizable energy, organic matter digestibility and microbial protein production of some legume hays. Asian-Australasian Journal of Animal Sciences, 20, 517-522

[26]. Larby, A., Smith, J.W., Kurdi, I.O., Adekunle, I.O., Raii, A.M., Ladipo, D.O., 1998.Chemical composition, rumen degradation, and gas production characteristics of some multipurpose fodder trees and shrubs during wet and dry seasons in the humid tropics.AnimalFeedScienceTechnology, 72, 81-96.

[27]. Longo, C., Hummel, J., Liebich, J., Bueno, I.C.S., Burauel, P., Ambrosano, E.J., Abadilla, A.L., Anele, U.Y., Sudekum, K.H. 2012. Chemical characterization and *in vitro* biological activity of four tropical legumes, *Styzolobiumaterrimum* L., *Styzolobiumdeeringianum*, *Leucaenaleucocephala*, and *Mimosa caesalpiniaefolia*, as compared with a tropical grass, *Cynodon* spp. for the use in ruminant diets. Czech Journal of Animal Science, 57 (6), 255-264.

[28]. Makkar, H.P.S., 2003a. Quantification of tannins in tree and shrub foliage. Kluwer Academic Publishers, Dordrecht, The Netherlands.

[29]. Makkar, H.P.S., 2003b. Effects and fate of tannins in ruminant animals, adaptation to tannins, and strategies to overcome detrimental effects of feeding tannin-rich feed. Small Ruminant Research, 49, 241-256.

[30]. Makkar, H.P.S., 2005. *In vitro* gas methods for evaluation of feeds containing phytochemicals. Animal Feed Sciences and Technology, 123-124, 291-302.

[31]. Mancilla, L.J.M., Martín, V. A., Delgado, P.M., 2013. Summer diet selection of

dairy goats grazing in a Mediterranean shrublandand the quality of secreted fat.SmallRuminantResearch, 113, 437-445.

[32]. Martínez, L.R., Castelán, O.O.A., González, R.M., Estrada, F.J.G., 2011. Determinación de la calidad nutritiva, fermentación *in vitro* y metabolitos secundarios en arvenses y rastrojo de maíz utilizados para la alimentación del ganado lechero.Tropical and SuptropicalAgroecosystems, 14, 525-536.

[33]. Mauricio, R.M., Owen, E., Mould, F.L., Givens, I., Theodorou, M.K., France, J., Davies, D.R., Dhanoa, M.S., 2001. Comparison of bovine rumen liquor and bovine faeces as inoculum for an *in vitro* gas production technique for evaluating forages. Animal Feed Science and Technology, 89, 33-48.

[34]. Menke, K.H., Steingass, H., 1988. Estimation of the energetic feed value obtained from chemical analysis and gas production using rumen fluid. Animal Research and Development, 28, 7-55.

[35]. NRC. 2007. Nutrient requirements of small ruminant. Sheep, goats, cervids, and new world camelids.National Academy of Press.National Research Council, Washington, DC. USA.

[36]. Ndlovu, L.R., Nherera, F.V., 1997. Chemical composition and relationship to *in vitro* gas production of Zimbabwean browsable indigenous tree species. Animal Feed Science Technology, 69, 121-129.

[37]. Nelson, J.A., Barnes, P.W., Archer, S., 2002. Leaf demography and growth responses to altered resource availability in Woody plants of contrasting leaf habit in a subtropical savanna. Plant Ecology, 160, 193-205.

[38]. Nevarez, N., Brosh, A., Pittroff, W., 2010. Seasonal dynamics of nutritional quality of California chaparral species. Animal Feed Science and Technology, 158, 44-56.

[39]. Pathak, A.K., 2008. Various factors affecting microbial protein synthesis in the rumen. Veterinary World, 1, 186-189.

[40]. Prieto, A.J., Patiño, O.J., Delgado, W.A., Moreno, J.P., Cuca, L.E., 2011. Chemical composition, insecticidal and antifungal activities of fruit essential oils of three Colombian *Zanthoxylum* species. Chilean Journal of Agricultural Research, 71(1), 73-82.

[41]. Ramírez, R.G., Quintanilla, J.B., Aranda, J., 1997. White-tailed deer food habits in northeastern Mexico. Small RuminantResearch, 25, 141-146.

[42]. Ramírez, O.R., Ramírez, R.G., López, G.F. 2002.Factores estructurales de la pared celular del forraje que afectan su digestibilidad. Ciencia UANL, 2, 180-189.

[43]. Ramírez, L.R.G., 2004. Nutrición del venado cola blanca. Universidad Autónoma de Nuevo León-Unión Ganadera Regional de Nuevo León-Fundación Produce, A.C., Nuevo León, México.

[44]. Ramírez, R.G. 2009. Nutrición de rumiantes. Sistemas Extensivos. 2ª Ed. México. Editorial Trillas UANL.

[45]. Reed, J.D., 1995. Nutritional toxicology of tannins and related polyphenols in forage legumes.Journal. Animal Sciences, 73, 1516-1528.

[46]. Rodríguez, R., Sosa, A., Rodríguez, Y., 2007. La síntesis microbiana en el rumen y su importancia para los rumiantes. Revista Cubana de Ciencia Agrícola, 41 (4), 303-311.

[47]. Salem, A.F.Z.M., 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. Animal Feed Science and Technology, 123-124, 67-79.

[48]. Salem, A.Z.M., 2012. Oral administration of leaf extracts to rumen liquid donor lambs modifies *in vitro* gas production of other tree leaves. Animal Feed Science and Technology, 176, 94-101.

[49]. Salem, A.Z.M., Robinson, P.H., El-Adawy, M.M., Hassan, A.A., 2007.*In vitro* fermentation and microbial protein synthesis of some browse tree leaves with or without addition of polyethylene glycol. Animal Feed Science and Technology, 138, 318-330.

[50]. Soltero, G.S., Ortega, I.M., Bryant, F.C., 1994. Nutrient content of important deer forage plants in the Texas Coastal Bend. Texas Journal of Science, 46 (2), 133-142.

[51]. Statistical Package for the Social Science (SPSS). 2004. Standard released version 13 for Windows, SPSS Inc., Chicago, IL. USA.

[52]. Tedeschi, L.O., Schofield, P., Pell, A.N., 2008. Determining feed quality for ruminants using *in vitro* gas production technique. Evaluating different models to assess gas production measurements.In: EMBRAPA.The 4th Workshop on Modeling in Ruminant Nutrition: Application of the Gas Production Technique, Juiz de Fora, MG, Brazil.

[53]. Tefera, S., Mlambo, V., Dlamini, B.J., Dlamini, A.M., Koralagama, K.D.N., Mould, F.L., 2008.Chemical composition and *in vitro*ruminal fermentation of common tree forages in the semi-

arid rangelands of Swaziland. Animal Feed Science and Technology, 142, 99-110.

[54]. Van Soest, P. J., 1994. Nutritional Ecology of the Ruminant, 2nd.Edición.Cornell University Press, Ithaca, N.Y.

[55]. Van Soest, P.J., Robertson, J.B. y Lewis, B.A. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition.Journal of Dairy Sciences.74, 3583-3597.

[56]. Yousef, E.M., Rouzbehan, Y., Rezaee, A., 2012. Effects of phenolic compounds in three oak species on *in vitro* gas production using inoculums of two breeds of indigenous Iranian goats. Animal Feed Science and Technology, 176, 26-31.

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