CELL-WALL COMPOSITION AND DIGESTIBILITY OF FIVE NATIVE SHRUBS OF THE TAMAULIPAN THORNSCRUB IN NORTHEASTERN MEXICO †

[COMPOSICIÓN DE LA PARED CELULAR Y DIGESTIBILIDAD DE CINCO ARBUSTIVAS NATIVAS DEL MATORRAL ESPINOSO TAMAULIPECO EN EL NORESTE DE MÉXICO]

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SUMMARY

Background: Many trees and shrubs from different parts of the world have forage potential, because they are inexpensive local products used for small ruminants as feed resource, some of these native shrubs are currently already an important fodder resources in arid and semi-arid regions of Northeastern México. **Objective:** The aim of this study was to determine the cell-wall composition and *in vitro* leaf digestibility of five shrub species: *Celtis pallida*, *Croton suaveolens*, *Forestiera angustifolia*, *Guaiacum angustifolium* and *Parkinsonia aculeata*. **Methodology:** Plant material was collected monthly from July 2018 to June 2019 at two sampling sites in Nuevo León, México: Linares and Los Ramones Counties. *In vitro* dry matter digestibility (IVDMD) was calculated using the Daisy^{II} incubator. **Results:** Non-structural carbohydrates (NSC) (total mean = 22.4% dry matter) varied significantly among species, sites, and months ($p<0.001$, $p<0.05$ and $p<0.001$, respectively). Conversely, neither acid detergent lignin (ADL) (8.4%), crude protein (CP) (21.9%), cellulose (12.5%), hemicellulose (20.8%), nor IVDMD (75.5%) varied significantly between sites. In general, NSC values were higher in Linares. The highest and lowest IVDMD values as per the Daisy^{II} incubator were recorded in March and September, respectively. *C. pallida* showed the highest IVDMD associated with a high hemicellulose and low ADL values, whereas *P. aculeata* showed a lower IVDMD value and high cellulose content. **Implications:** All species maintained considerably high levels of digestibility, which might indicate an availability of high CP levels for consumption by ruminants in the semi-arid regions of northeastern México. **Conclusion:** It is concluded that the species under study can be considered as emergency feed resources for small ruminants throughout the year.

Key words: Cellulose; digestibility; fodder resources; hemicellulose; leaves; protein.

RESUMEN

Antecedentes: Muchos árboles y arbustos de diferentes partes del mundo tienen potencial forrajero, ya que son productos locales de bajo costo, utilizados por los pequeños rumiantes como recurso alimenticio, algunos de estos arbustos nativos ya son importantes recursos forrajeros en regiones áridas y semiáridas del Noreste de México. **Objetivo:** El objetivo de este estudio fue determinar la composición de la pared celular y la digestibilidad *in vitro* de las hojas de cinco especies arbustivas: *Celtis pallida*, *Croton suaveolens*, *Forestiera angustifolia*, *Guaiacum angustifolium* y *Parkinsonia aculeata*. **Metodología:** El material vegetal se recolectó mensualmente de julio 2018 a junio 2019 en dos sitios de muestreo en Nuevo León, México: municipios de Linares y Los Ramones. La digestibilidad *in vitro* de la materia seca (DIVMS) se calculó utilizando el incubador Daisy^{II}. **Resultados:** Los carbohidratos no estructurales (CNE) (media total = 22.4% materia seca) variaron significativamente entre especies, sitios y meses

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(p<0.001, p<0.05 and p<0.001, respectivamente). Por el contrario, ni lignina detergente ácida (LDA) (8.4%), proteína cruda (PC) (21.9%), celulosa (12.5%), hemicelulosa (20.8%), ni la DIVMS (75.5%) variaron significativamente entre los sitios. En general, los valores de CNE fueron mayores en Linares. Los valores de DIVMS más altos y bajos según el incubador Daisy^{II} se registraron en marzo y septiembre, respectivamente. *C. pallida* mostró la mayor DIVMS asociado con un contenido alto de hemicelulosa y bajo de LDA, mientras que *P. aculeata* mostró un menor valor de DIVMS y un alto contenido de celulosa. **Implicaciones:** Todas las especies mantuvieron niveles de digestibilidad considerablemente altos, lo que podría indicar una disponibilidad de altos niveles de PC para el consumo de rumiantes en las regiones semiáridas del noreste de México. **Conclusión:** Se concluye que las especies en estudio pueden considerarse como un recurso de alimentación de emergencia para pequeños rumiantes durante todo el año.
Palabras clave: Celulosa; digestibilidad; hemicelulosa; hojas; proteína; recursos forrajeros.

INTRODUCTION

It has been reported that many trees and shrubs from different parts of the world have forage potential, due to their protein and mineral contents, and *in vitro* digestibility analyzes have shown that they may be a valuable supplementary feed for ruminants in extensive grazing systems (Ammar *et al*., 2004; Guerrero *et al*., 2012; Habib *et al*., 2016; Luske and van Eekeren, 2018). An important limitation in the performance of grazing ruminants in arid and semi-arid regions is the shortage of high-quality pastures, a situation that becomes critical during the dry season, when the digestibility of shrub foliar tissues and feed intake by cattle and small ruminants decreases. Furthermore, this reduction in biomass and nutrient availability during the dry season affects goats, sheep, and wild ruminants to a lesser extent than larger cattle species in these areas (Assouma *et al*., 2018) due to the browsing behavior of small ruminants, which is more frequently performed by goats and, in an opportunistic manner in sheep when pastures are of poor quality or not available due to the effect of the dry conditions over this season of the year (Sanon *et al*., 2007). Browsing behaviors are directed towards the consumption of leguminous and non-leguminous shrub/herb plant species that remain green and show a relative nutrient content throughout the year (Guerrero *et al*., 2008). Some of these trees and shrubs are currently already important fodder resources for ruminants, as suggested by several studies and evaluations. This is the case of some of the native plants from the Tamaulipan Thornscrub, a semiarid ecosystem in northeastern México, in which to date, no low-palatability or toxic substances, such as tannins have been detected by *in vitro* gas production or by *in situ* methods in the leaves of the shrub species *Celtis pallida* Torr. (Ulmaceae), *Croton suaveolens* Torr. (Euphorbiaceae), *Forestiera angustifolia* Torr. (Oleaceae), *Guaiacum angustifolium* Engelm. (Zygophyllaceae), and *Parkinsonia aculeata* L. (Fabaceae), in quantities that may limit their use as fodder (Domínguez *et al*., 2011; Ramírez *et al*., 2000). Also, their preference for consumption by small ruminants has been demonstrated by Ramírez *et al.* (1997) and Foroughbakhch *et al*. (2013). Indeed, many trees and shrubs are used for ruminant feeding because they are inexpensive local products (Seidavi *et al*., 2018). Further, owing to their high nutritional value, the indigenous browse species in pastoral and agro-pastoral areas need to be restored and conserved for a sustainable livestock production (Derero and Kitaw, 2018) because, although fodder trees and shrubs are perennial, grasses disappear from grasslands under high-intensity grazing regimes due to their carbohydrate content, especially non-structural carbohydrates (NSC), whereby their rapid regrowth after browsing by ruminants requires careful management (Heyden and Stock, 2016).

In vitro techniques are commonly used to assess the nutritional value of feeds due to their convenience, adaptability, and efficiency, especially, the Daisy^{II} incubator technique has been used for digestibility assessment of different types of forages including grasses and shrubs (Holden, 1999). This technique is used on different types of forages from conventional to shrubs. Nonetheless, as has been mentioned, the technique requires adjustments according to chemical properties of the plant material (Norman *et al*., 2010); however, its reliability with forages has also been proven with different amounts of fibers and its use has been recommended due to its feasibility, repeatability, and minimal requirement to use animals, as reliable results are obtained when compared with *in vivo* tests, with very little variation among techniques (Trujillo *et al*., 2010).

Therefore, the aim of this study was to determine the crude protein (CP), cell-wall composition including, cellulose, hemicellulose, and acid detergent lignin, (ADL), non-structural carbohydrates (NSC) and *in vitro* dry matter digestibility (IVDMD) of five shrub species, namely, *Celtis pallida*, *Croton suaveolens*, *Forestiera angustifolia*, *Guaiacum angustifolium*, and *Parkinsonia aculeata*, which might be fed to ruminants in the semi-arid regions of northeastern México.

MATERIALS AND METHODS

Location of the study sites

This research was carried out at two sites in the state of Nuevo León, México, namely, in Linares and Los Ramones Counties. The following is a brief description of the physical characteristics of each study site: Site

1, Linares; located at the Experimental Campus of the School of Forest Sciences of Universidad Autónoma de Nuevo León in Linares County (24°47´45´´ N; 99°32´31´´ W; at an elevation of 350 m above sea level). The climate at this site is subtropical and semi-arid with a warm summer (González *et al*., 2004). The average air temperature registered during the experimental period, from July 2018 to June 2019, was 14.1 °C in January to 30.4 °C in August and rainfall accounted 554 mm. Site 2, Los Ramones; located in "El Abuelo Ranch" in Los Ramones County. The geographical location is 25°39´46´´ N; 99°27´51´´ W; with an area of 100 ha and an elevation of 200 m above sea level. The climate at this site is semi-arid with warm summer (González *et al*., 2004). The average monthly air temperature during the study varied from 14.3 °C in January to 31.5 °C in August. Total registered rainfall for the experimental period was 667 mm.

Sampling and collection frequency

Experimental plots (50 m x 50 m) were marked in an area without disturbance and representative of each study site, in which leaf samples were collected at a height of 1.0 to 1.5 m from the ground, from three randomly selected individuals, representative of each of the five shrub species selected for study; *C. pallida*, *C. suaveolens*, *F. angustifolia*, *G. angustifolium* and *P. aculeata*. The sampling frequency of foliar plant material was carried out at monthly intervals from July 2018 to June 2019, being the seasonal periods in the zone; summer (July, August and June), autumn (September, October and November), winter (December, January and February) and spring (March, April and May).

Sample preparation

The sampled foliar material was transferred to the Laboratory of Chemistry and Plant Physiology of the School of Forest Sciences. Samples where first dried in a forced-air oven (Felisa®, Model FE-292AD, México) at 55 °C for 24 h, prior to separating leaves from the branches. Once the leaves were grouped by month, species, replication, and site, they were processed in a Thomas Willey mill (Thomas Scientific Apparatus, Model 3383) using a No. 60 mesh (1 mm x 1 mm) and stored in Reynolds® zipper bags (20.3 cm x 17.7 cm) at a room temperature of 24 °C until analysis.

Determination of the chemical composition of the leaf samples

Leaf dry matter (DM, g) was determined in triplicate by weighing 1.0 g of each leaf sample previously dried at 100 °C for 24 h in a forced-air oven (Felisa®, Model FE-292AD, México). Organic matter (OM, %) and ash (%) contents (no.942.05, AOAC 2012) were measured by incineration in an electric muffle furnace (Thermo scientific, Model F48010, USA) at 550 °C for 3.5 h. Carbon and Nitrogen contents were analyzed based on Dumas principle by a total combustion method (no.990.03, AOAC 2012) using a CHNSO analyzer (2400 series II, Perkin-Elmer, USA) Crude protein (CP, %) was determined by multiplying N (%, content) x 6.25. Neutral detergent fiber (NDF, %) and acid detergent fiber (ADF, %) were analyzed using an ANKOM^{200} Fiber Analyzer (ANKOM Technologies, Fairport, NY, USA); in turn, acid detergent lignin (ADL, %) was determined by solubilization of cellulose with sulfuric acid, following the procedures described by Van Soest *et al*. (1991). Estimations of hemicellulose (NDF-ADF, %) and cellulose (ADF-ADL,%) were obtained by difference (Lanzas *et al*., 2007). Ether extract (EE) was obtained with petroleum ether using an Ankom^{XT15} extractor (AOCS AM 5-04). Non-structural carbohydrate (NSC) content was calculated by the difference method based on chemical analysis of individual feeds according to the following equation (Van Soest *et al.,* 1991):

$$NSC = 100 - (\%\ NDF + \%\ Crude\ Protein + \%\ EE + \%\ Ash)$$

***In vitro* true digestibility (Daisy ^{II})**

This study was completed in the Laboratory of Animal Nutrition and Feed Quality at the Agronomy School of Universidad Autónoma de Nuevo León, México. *In vitro* true digestibility was calculated using the Daisy^{II} procedure (ANKOM, 2000). Briefly, for each shrub species, the IVDMD was determined in five replicate runs using triplicate foliar samples of approximately 0.25 g of DM each, were placed in multilayer polyester filter bags (F57; 5.0 cm x 5.5 cm, ANKOM Technology Corp., Macedon, NY) previously washed with pure acetone and dried in a forced-air oven at 60 °C for 2 h. The bags were sealed and placed in digestion jars (25 bags per jar). The jars were placed in a Daisy^{II} incubator (ANKOM Technology Corp., Macedon, NY). An inoculum was prepared by diluting ruminal fluid obtained from two sheep of the Saint Croix breed provided with a ruminal cannula and fed with a ration with a forage: concentrate ratio: 80%:20% (this ratio did not contain foliar material from the studied shrubs) and a buffer solution in a ratio of 1:4 according to manufacturer specifications and guidelines. The inoculum was incorporated into the jars previously purged with CO_2. After an incubation period of 48 h at 39 °C, the jars were removed from the incubation chamber and the bags were washed with distilled water. Thereafter, the bags were placed in the Ankom^{200} fiber analyzer (ANKOM Technology Corp., Macedon, NY) and treated with neutral detergent solution for 75 min. The bags were rinsed with hot water and acetone, and then dried at 55 °C. *In vitro* digestibility was calculated as the difference between the DM at incubation start and the residue after NDF treatment.

Environmental variables

To measure the influence of temperature and precipitation at each site, automated HOBO sensors (HOBO Pro Temp / RH Series, Forestry Suppliers, Inc., Jackson, MS, USA) were installed to register environmental variables such as air temperature and relative humidity. These sensors were programmed for data collection at one-hour intervals. The daily amount of rainfall (mm) was quantified using an automated rain gauge (Davis, CA, USA) connected to an Event Onset recorder (HOBO, Bourne, MA, USA).

Statistical analysis

Statistical analysis (ANOVA and comparison of means) of the data on chemical composition and *in vitro* digestibility was analyzed according to a completely randomized design with a factorial arrangement, being the factors species (5), sites (2), and months (12). Double and triple interactions were also considered (Montgomery, 2004). Assumptions of normality and homogeneity of variance were verified using the Shapiro–Wilk and Levene's tests (CP, NSC and IVDMD). When normal distribution or homogeneity of variances were not met, the non-parametric Kruskal–Wallis test were used (ash, cellulose, hemicellulose, and ADL) and means were compared using the Mann-Whitney U test ($p<0.05$). Pearson's correlation analysis was performed between chemical composition, IVDMD, air temperature, relative humidity, and rainfall recorded during the experimental period. The Tukey HSD test was used for mean comparison at $p<0.05$. Statistical analyses were performed using the SPSS statistical analysis software (SPSS for Windows, Version 22.0, Chicago, IL, USA).

Table 1. Monthly foliar ash content (% dry matter) in five shrubs species from northeastern México sampled at two study sites from July 2018 to June 2019. The Kruskal-Wallis statistics for month, site and species are shown.

Site	Linares					Los Ramones						
Month	CPT	CS	FA	GA	PA	CPT	CS	FA	GA	PA	Mean	SEM
July	22.3	7.2	9.3	16.7	10.7	19.4	9.0	6.5	11.4	7.3	12.0	0.2
August	17.1	5.7	6.8	22.2	9.4	22.1	7.4	7.2	15.8	8.5	12.2	0.2
September	22.0	6.0	6.0	17.4	9.8	20.9	8.1	6.3	12.7	8.3	11.7	0.2
October	19.4	10.1	6.8	18.6	8.8	18.9	8.5	6.8	15.3	6.7	11.9	0.2
November	21.3	8.9	7.2	21.1	10.1	23.3	8.3	7.3	16.4	6.5	13.1	0.2
December	21.3	8.1	7.5	18.3	9.5	21.6	7.5	6.8	16.6	6.3	12.4	0.2
January	24.7	7.8	9.1	16.9	8.3	21.2	7.9	8.0	17.7	6.5	12.8	0.2
February	23.8	8.6	6.7	17.3	8.2	21.0	7.7	10.6	18.4	6.4	12.9	0.2
March	26.2	7.3	6.6	13.5	4.8	20.3	7.6	7.2	9.4	5.2	10.8	0.2
April	18.1	9.0	6.4	9.9	6.3	17.3	8.5	6.8	9.8	5.3	9.7	0.2
May	21.0	8.2	5.5	9.3	6.9	19.0	7.8	5.0	11.0	6.2	9.9	0.2
June	20.0	8.0	7.2	11.9	8.7	20.2	8.3	6.4	13.9	7.0	11.8	0.2
Mean	21.4	7.9	7.1	16.1	8.5	20.4	8.1	7.1	14.0	6.7	11.73	
SEM	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2		
Site mean			12.2					11.3				
SEM			0.1					0.1				

Statistic	Month	Site	Species
χ^2	14.3	3.7	267.5
df	11	1	4
p-value	0.191	0.053	<0.001

CPT; *Celtis pallida*, CS; *Croton suaveolens*, FA; *Forestiera angustifolia*, GA; *Guaiacum angustifolium*, PA; *Parkinsonia aculeata*.
Data represent the means (n=3). SEM, standard error of the mean. df; degree of freedom.

RESULTS

Chemical composition

The content of ash, cellulose, hemicellulose, and ADL are shown in Table 1, 2, 3, and 4, respectively. The overall mean ash content at Linares and Los Ramones was 12.2% and 11.3%, respectively (Table 1). Among species, *C. pallida* (23.3%, Los Ramones) showed the highest ash content, while *P. aculeata* (4.8%, Linares) showed the lowest content in November and March, respectively (Table 1). With respect to cellulose, higher and lower contents were observed in *P. aculeata* (22.6%) in December at Los Ramones and in *C. pallida* (7.1%) in May at Linares (Table 2). The overall cellulose mean content in foliar tissue was 12.45% and did not differ (p>0.05) between sites. The overall hemicellulose mean content was 21.7% and 19.9% for Los Ramones and Linares, respectively (Table 3). The highest (44.0%) and lowest (8.0%) hemicellulose contents were found in March at the Los Ramones for *C. suaveolens* and *G. angustifolium*, respectively (Table 3). Meanwhile, the overall ADL mean content (8.35%) was similar (p>0.05) at both Linares and Los Ramones sites (Table 4). At both sites, the highest (16.6%) and lowest (1.1%) mean values were observed during May in *G. angustifolium* (Linares) and in *C. pallida* (Los Ramones), respectively (Table 4).

According to the ANOVA, crude protein (Table 5), and non-structural carbohydrates (Table 6) contents, were significantly different among species, months, and sites (except crude protein). The double interactions month*site, month*species, site*species (except CP) and the triple interaction month*site*species were all significant (p<0.001).

Table 2. Monthly foliar cellulose content (% dry matter) in five shrubs species from northeastern México sampled at two study sites from July 2018 to June 2019. The Kruskal-Wallis statistics for month, site and species are shown.

Site	Linares					Los Ramones						
Month	CPT	CS	FA	GA	PA	CPT	CS	FA	GA	PA	Mean	SEM
July	8.4	18.7	9.8	11.0	17.4	10.1	18.5	7.8	9.6	22.1	13.3	0.3
August	9.2	19.7	7.8	10.4	18.8	9.7	16.8	6.7	9.2	21.0	12.9	0.3
September	9.8	19.5	8.8	10.2	17.0	8.6	19.8	8.1	13.5	15.9	13.1	0.3
October	9.9	14.8	10.0	11.1	15.1	8.8	15.7	8.2	9.5	19.7	12.3	0.3
November	8.7	17.5	8.7	8.7	17.3	8.3	16.0	8.5	8.6	19.5	12.2	0.3
December	8.1	15.0	8.6	9.8	18.1	8.1	14.2	9.8	9.5	22.6	12.4	0.3
January	8.0	14.1	10.2	11.2	18.4	8.7	15.7	7.6	8.7	20.5	12.3	0.3
February	7.2	13.3	7.2	9.9	18.0	8.0	14.4	8.1	8.4	20.8	11.5	0.3
March	7.4	17.5	9.1	8.6	17.9	8.9	13.9	7.9	7.5	18.6	11.7	0.3
April	8.8	16.3	9.2	14.6	16.2	9.0	13.8	8.8	12.6	22.2	13.2	0.3
May	7.1	15.3	6.9	14.6	16.5	8.3	13.4	7.2	11.4	20.9	12.2	0.3
June	8.7	14.8	6.9	13.8	17.2	8.5	15.3	7.8	11.8	18.8	12.4	0.3
Mean	8.4	16.4	8.6	11.2	17.3	8.7	15.6	8.0	10.0	20.2	12.5	
SEM	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2		
Site mean			12.4					12.5				
SEM			0.1					0.1				

Statistic	Month	Site	Species
χ^2	9.3	0.2	274.8
df	11	1	4
p-value	0.593	0.681	<0.001

CPT; *Celtis pallida*, CS; *Croton suaveolens*, FA; *Forestiera angustifolia*, GA; *Guaiacum angustifolium*, PA; *Parkinsonia aculeata*.
Data represent the means (n=3). SEM, standard error of the mean. df; degree of freedom.

Table 3. Monthly foliar hemicellulose content (% dry matter) in five shrubs species from northeastern México sampled at two study sites from July 2018 to June 2019. The Kruskal-Wallis statistics for month, site and species are shown.

Site	Linares					Los Ramones						
Month	CPT	CS	FA	GA	PA	CPT	CS	FA	GA	PA	Mean	SEM
July	36.0	19.0	15.4	12.2	17.1	37.7	16.4	17.9	13.9	18.6	20.4	0.5
August	37.9	18.8	12.7	14.7	19.2	40.3	17.2	15.8	12.8	18.8	20.8	0.5
September	39.2	20.5	19.3	12.6	19.9	41.9	20.9	25.4	20.0	22.4	24.2	0.5
October	38.5	31.4	22.6	12.6	17.6	34.7	19.0	18.5	12.7	18.9	22.7	0.5
November	27.0	20.1	17.4	10.2	18.6	43.5	17.8	17.8	16.9	20.5	20.99	0.5
December	27.7	18.5	17.3	14.1	22.7	42.4	18.6	14.9	13.7	19.4	20.93	0.5
January	18.5	19.5	18.2	15.2	19.4	40.7	15.4	14.8	9.1	16.4	18.7	0.5
February	20.0	14.7	15.0	12.1	15.5	27.4	18.4	17.6	16.2	19.7	17.7	0.5
March	33.0	20.9	15.7	10.0	19.8	36.7	44.0	15.5	8.8	30.1	23.5	0.5
April	30.4	20.4	15.3	16.6	27.5	41.1	16.9	13.2	14.0	17.5	21.3	0.5
May	22.9	18.0	16.7	15.7	18.6	32.2	16.2	17.2	14.1	20.2	19.2	0.5
June	32.6	16.1	14.0	13.9	17.3	39.4	16.1	12.1	14.9	19.3	19.6	0.5
Mean	30.3	19.8	16.6	13.3	19.4	38.2	19.7	16.7	13.9	20.1	20.8	
SEM	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5		
Site mean			19.9					21.7				
SEM			0.2					0.2				

Statistic	Month	Site	Species
χ^2	23.3	0.5	222.1
df	11	1	4
p-value	0.016	0.458	<0.001

CPT; *Celtis pallida*, CS; *Croton suaveolens*, FA; *Forestiera angustifolia*, GA; *Guaiacum angustifolium*, PA; *Parkinsonia aculeata*.
Data represent the means (n=3). SEM, standard error of the mean.
df; degree of freedom.

With respect to CP, maximum (36.7% in November at Linares) and minimum (10.3% in June at Linares) contents were observed in *C. pallida* and *F. angustifolia*, respectively (Table 5). CP content was similar (p>0.05) at both sites (22%). Linares and Los Ramones registered an overall mean value for non-structural carbohydrates (NSC) of 22.5% and 21.5%, respectively (Table 6). Maximum NSC values were observed for *F. angustifolia* in Linares (49.9%) and Los Ramones (48.9%) in June, while minimum values were detected in *C. pallida* with 0.1% (October) and 0.1% (August, September and November) at Linares and Los Ramones, respectively (Table 6).

In vitro dry matter digestibility

Values of *In vitro* dry matter digestibility (IVDMD) was found higher in March (79.0%) and lower in September (74.1%) is shown in Table 7. Average IVDMD for both sites were approximately 75.5%. The lowest and highest IVDMD values in the species were found at Los Ramones for *C. suaveolens* (56.5%) and *C. pallida* (88.5%) in September and December, respectively (Table 7).

DISCUSSION

Chemical composition

Shrubs from arid and semi-arid regions are reportedly be used as feed resources by small ruminants (Guerrero *et al*., 2008). Consistently, our findings herein, suggest that these plant species have a high CP content that remains at satisfactory levels throughout the year, unlike CP of grasses (Ramírez *et al*., 2009). The overall highest and lowest CP values were recorded in

October and February, respectively. This observation contrasts with results reported by Ammar *et al*. (2004) for shrubs in the Mediterranean, in which case, maximum CP content was recorded at the beginning of the spring, during the initial growth of the leaves, likely due to a high mitotic activity and a strong demand for nutrients, particularly nitrogen, while thereafter, CP content decreased during the growing season in response to tissue aging, particularly during the fall, when nutrients are transferred to perennial tissues before abscission. Consistently, Alvarado *et al*. (2012) observed the highest CP values in the summer months. In addition, our results indicate that, regardless of month and site, all studied species remain at satisfactory levels for ruminant nutrition, since they exceed the 7% level of CP recommended for optimal growth of microorganisms in the rumen (Yousef and Rouzbehan, 2008). Likewise, the percentage of CP for *C. pallida*, *G. angustifolium*, and *F. angustifolia* were higher than reported by Ramírez *et al*. (2000), Guerrero *et al*. (2008), and Domínguez *et al*. (2011).

Cell wall components showed the same trend as CP. Thus, the highest levels were observed during the summer months, while the lowest were observed during the spring months. These results agree with Ammar *et al*. (2004), who found that the increase in cell wall components was associated with plant maturity processes. Cellulose content differed significantly (p<0.001) among species and months. Additionally, the overall mean cellulose content (12.5%) was lower than that of hemicellulose (20.8%). Finally, the cellulose content estimated in this study for foliar tissue of *C. pallida* and *G. angustifolium* was different than that reported by Ramírez *et al*. (2000). In general, all shrub species in this study showed

Table 4. Monthly foliar acid detergent lignin content (% dry matter) in five shrubs species from northeastern México sampled at two study sites from July 2018 to June 2019. The Kruskal-Wallis statistics for month, site and species are shown.

Site	Linares					Los Ramones						
Month	CPT	CS	FA	GA	PA	CPT	CS	FA	GA	PA	Mean	SEM
July	2.0	5.5	7.1	13.2	8.9	3.6	5.9	7.4	15.7	9.4	7.9	0.2
August	2.5	6.8	6.3	14.3	9.3	3.2	5.0	7.9	15.7	9.4	8.1	0.2
September	2.9	6.7	6.6	14.7	9.3	2.9	14.1	7.6	13.6	11.4	9.0	0.2
October	2.5	9.4	7.8	14.4	9.4	2.0	8.2	8.3	13.8	10.6	8.6	0.2
November	2.3	5.8	11.3	15.1	8.6	1.7	5.2	14.3	14.8	10.0	8.9	0.2
December	2.4	9.6	12.3	13.7	9.9	1.7	7.7	8.2	12.8	13.0	9.1	0.2
January	2.5	9.2	8.3	14.2	10.0	2.1	7.1	7.3	11.9	10.3	8.3	0.2
February	1.7	7.4	7.0	13.3	10.5	2.0	7.2	8.0	13.5	11.8	8.2	0.2
March	1.2	5.1	12.9	11.7	5.1	2.2	5.8	10.8	9.2	6.0	7.0	0.2
April	1.9	3.5	9.7	14.0	7.0	1.6	3.5	10.9	14.0	9.4	7.6	0.2
May	2.8	7.7	13.3	16.6	10.0	1.1	4.1	12.1	14.4	10.2	9.2	0.2
June	2.3	5.0	9.4	15.3	10.6	1.4	5.5	10.6	14.2	9.5	8.4	0.2
Mean	2.3	6.8	9.3	14.2	9.1	2.1	6.6	9.5	13.6	10.1	8.4	
SEM	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2		
Site mean			8.3					8.4				
SEM			0.1					0.1				

Statistic	Month	Site	Species
χ^2	9.0	0.006	279.2
df	11	1	4
p-value	0.619	0.937	<0.001

CPT; *Celtis pallida*, CS; *Croton suaveolens*, FA; *Forestiera angustifolia*, GA; *Guaiacum angustifolium*, PA; *Parkinsonia aculeata*.
Data represent the means (n=3). SEM, standard error of the mean.
df; degree of freedom.

lower contents of cellulose than those reported by Okunade *et al*. (2014) for browse plant species in the rangelands of Nigeria. The hemicellulose content also varied significantly among species. Furthermore, the hemicellulose contents registered for *C. pallida* and *G. angustifolium* were different than those reported by Ramírez *et al*. (2000). The results reported herein were also higher than those reported by Derero and Kitaw (2018) for browse species in eastern Ethiopia, in which case, hemicellulose content ranged from 4.5% to 29.0% DM. The differences in hemicellulose content for all shrubs among months varied from those reported for *Acacia* leaves by Salem (2005), who did not find any significant differences among growing seasons. Acid detergent lignin content has been related to plant developmental stage (Raffrenato *et al*., 2017). Therefore, we believe that the lowest lignin percentage should be observed in March, thus coinciding with seasonal growth. A higher content in ADL is associated with a lower fiber digestibility for ruminants (Anele *et al*., 2009). These authors reported that an ADL content between 8.0% and 9.1% DM can be considered low to moderate. In this study, *C. pallida* and *F. angustifolia* showed similar to low levels of lignin (2.1 and 9.3% DM, respectively) compared to those observed by Alvarado *et al*. (2012), who found between 8 and 12% DM, respectively.

With respect to NSC, the lowest levels were recorded in the autumn months. This finding might be a consequence of a transfer of nutrients to the root for plant survival and adaptation to cold weather over the winter months (Oppong *et al*., 2008), followed by an increase during the spring months as was observed in *F. angustifolia*, which showed a high content. The remaining shrub species showed a lower content, particularly *C. pallida*. This finding contrasts with Alvarado *et al*. (2012), who pointed that shrub species from northeastern México are rich in NSC.

Table 5. Monthly foliar crude protein content (% dry matter) in five shrubs species from northeastern México sampled at two study sites from July 2018 to June 2019.

Site	Linares					Los Ramones						
Month	CPT	CS	FA	GA	PA	CPT	CS	FA	GA	PA	Mean	SEM
July	22.1	14.7	13.5	20.5	20.1	25.3	19.5	16.1	23.4	19.1	19.4	0.5
August	20.6	18.7	16.5	25.5	20.8	29.5	20.1	19.9	24.9	20.4	21.7	0.5
September	21.2	19.2	22.2	20.8	18.9	27.4	26.3	20.8	23.6	24.5	22.5	0.5
October	33.3	25.9	24.5	24.0	31.5	29.9	29.3	27.1	31.2	33.4	29.0	0.5
November	36.7	32.1	22.1	26.2	24.3	30.3	22.2	22.7	25.2	24.9	26.7	0.5
December	28.1	24.2	20.2	24.1	23.8	26.0	24.5	16.1	25.9	25.6	23.8	0.5
January	24.2	25.3	22.7	24.7	24.1	20.2	20.3	20.4	24.1	22.9	22.9	0.5
February	21.3	20.9	18.9	20.5	17.1	16.3	18.3	17.8	17.8	22.9	19.2	0.5
March	19.6	27.9	15.0	23.7	28.2	19.6	24.6	16.0	28.8	22.5	22.6	0.5
April	30.1	26.3	12.2	23.4	29.7	28.7	25.4	13.7	19.5	18.6	22.8	0.5
May	20.5	19.3	11.1	18.6	21.1	17.9	20.6	11.0	16.4	17.3	17.4	0.5
June	20.0	15.4	10.3	16.7	13.7	18.7	13.8	12.6	15.6	16.8	15.4	0.5
Mean	24.8	22.5	17.4	22.4	22.8	24.1	22.1	17.9	23.0	22.4	21.9	
SEM	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4		
Site mean			22.0					21.9				
SEM			0.2					0.2				

Factors	Month (A)	Site (B)	Species (C)	A × B	A x C	B x C	A x B x C
p	***	NS	***	***	***	NS	***

CPT; *Celtis pallida*, CS; *Croton suaveolens*, FA; *Forestiera angustifolia*, GA; *Guaiacum angustifolium*, PA; *Parkinsonia aculeata*.
Data represent the means (n=3). SEM, standard error of the mean.
*** Significant at 0.001 level, NS; Not significant.

Table 6. Monthly foliar non-structural carbohydrates content (% dry matter) in five shrubs species from northeastern México sampled at two study sites from July 2018 to June 2019.

Site	Linares					Los Ramones						
Month	CPT	CS	FA	GA	PA	CPT	CS	FA	GA	PA	Mean	SEM
July	6.1	31.4	42.7	22.2	22.8	0.8	28.2	42.0	20.7	21.2	23.8	0.8
August	9.9	26.9	47.7	8.8	19.2	0.1	30.8	40.0	14.4	19.0	20.8	0.8
September	0.2	25.9	35.0	18.5	21.6	0.1	11.2	29.1	9.8	14.6	15.9	0.8
October	0.1	6.9	25.9	15.2	16.2	4.5	18.8	29.5	12.3	9.5	13.4	0.8
November	2.6	14.8	30.0	14.1	18.8	0.1	29.7	27.7	12.0	17.0	15.8	0.8
December	10.6	23.1	32.3	15.6	13.5	0.1	25.7	42.6	17.7	10.8	19.0	0.8
January	20.4	22.7	29.8	12.1	17.5	5.1	32.0	40.4	22.6	21.1	22.4	0.8
February	24.2	33.7	43.2	21.8	28.5	23.5	32.2	35.6	20.0	16.1	27.9	0.8
March	10.4	19.0	38.7	29.7	21.4	9.1	1.9	40.3	33.5	15.3	21.9	0.8
April	8.6	22.0	45.6	18.9	10.5	0.9	29.4	44.5	27.5	24.9	23.3	0.8
May	23.4	28.2	44.1	22.5	24.0	19.5	35.0	45.5	28.9	22.6	29.4	0.8
June	14.1	38.2	49.9	25.6	29.6	10.0	39.5	48.9	25.3	25.8	30.7	0.8
Mean	10.5	24.4	38.8	18.7	20.3	3.9	26.2	38.8	20.4	18.2	22.4	
SEM	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7		
Site mean			22.5					21.5				
SEM			0.3					0.3				

Factors	Month (A)	Site (B)	Species (C)	A × B	A x C	B x C	A x B x C
p	***	**	***	***	***	***	***

CPT; *Celtis pallida*, CS; *Croton suaveolens*, FA; *Forestiera angustifolia*, GA; *Guaiacum angustifolium*, PA; *Parkinsonia aculeata*.
Data represent the means (n=3). SEM, standard error of the mean.
*** Significant at 0.001 level, ** Significant at 0.01 level.

In vitro dry matter digestibility

The IVDMD assay was performed using rumen fluid inoculum of two canulated sheep and the Daisy^{II} incubator. According to Ammar *et al*. (2008), for *in vitro* studies, when animals are fed with same diet, differences between sheep and goat rumen fluid used as source of inoculum can be considered of little nutritional significance. The values obtained showed a trend opposite to that shown by CP content and fiber fractions, with the highest levels of digestibility recorded for samples obtained during the spring months and the lowest during the summer months. These observations might be explained by the process of plant development (Ammar *et al*., 2008). Furthermore, ADL (r = -0.55; p<0.001) and cellulose (r = -0.71; p<0.001) contents were negatively correlated with IVDMD, as the species *C. pallida* and *F. angustifolia* showed the highest IVDMD values concomitantly with the lowest levels of ADL and cellulose. In contrast, *P. aculeata* was characterized by the highest levels of cellulose and the lowest levels of IVDMD.

Table 7. Monthly foliar *in vitro* dry matter digestibility (% dry matter) in five shrubs species from northeastern México sampled at two study sites from July 2018 to June 2019.

Site	Linares					Los Ramones						
Month	CPT	CS	FA	GA	PA	CPT	CS	FA	GA	PA	Mean	SEM
July	86.0	70.4	76.8	75.5	72.5	82.4	68.2	77.8	72.1	71.4	75.3	0.3
August	81.8	66.3	79.4	76.5	71.9	82.2	72.2	79.3	74.3	68.4	75.2	0.3
September	80.9	68.2	79.6	76.5	74.0	79.6	56.5	80.4	75.8	69.7	74.1	0.3
October	78.7	69.0	76.0	76.6	74.3	81.9	69.6	79.4	77.0	69.7	75.2	0.3
November	86.4	68.0	72.8	75.8	68.3	87.3	73.0	76.4	77.6	67.5	75.3	0.3
December	85.3	71.3	76.0	76.1	69.9	88.5	70.7	76.9	75.3	62.1	75.2	0.3
January	86.1	68.3	74.5	74.8	70.8	85.9	71.0	77.3	78.4	67.7	75.5	0.3
February	87.2	74.8	76.1	76.4	69.2	88.2	70.5	75.4	76.9	65.8	76.0	0.3
March	83.5	72.6	74.0	82.3	79.0	83.6	76.6	73.7	86.8	77.9	79.0	0.3
April	82.0	74.3	75.6	70.3	79.6	82.3	77.5	74.4	71.1	66.2	75.3	0.3
May	83.8	73.3	75.0	67.7	74.8	83.1	74.5	75.3	72.3	66.2	74.6	0.3
June	81.3	76.6	77.0	68.0	69.8	81.6	78.5	77.9	71.8	70.9	75.4	0.3
Mean	83.6	71.1	76.1	74.7	72.8	83.9	71.6	77.0	75.8	68.6	75.5	
SEM	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3		
Site mean			75.7					75.4				
SEM			0.1					0.1				

Factors	Month (A)	Site (B)	Species (C)	A × B	A x C	B x C	A x B x C
p	***	NS	***	***	***	***	***

CPT.; *Celtis pallida,* CS; *Croton suaveolens,* FA; *Forestiera angustifolia,* GA; *Guaiacum angustifolium,* PA; *Parkinsonia aculeata.*
Data represents the mean (n=3). SEM, standard error of the mean.
*** Significant at 0.001 level, NS; Not significant.

We did not detect a relationship between NSC and degree of digestibility. This is likely owing to the fact that the latter is related to cell-wall contents, which determine a lower digestibility with increasing cell-wall lignification (Gámez *et al*., 2018), as in *C. pallida*, *C. suaveolens*, *G. angustifolium*, and *P. aculeata*. According to our findings, although NSC comprises simple sugars as well as starch, they are associated with the major energy sources for rumen microorganisms, this fraction might be fermented by ruminal bacteria (Sniffen *et al*., 1992), which can better use the lower CP levels found in the leaves of *F. angustifolia* and thus, partially suggesting the second highest IVDMD observed in this study. The comparison of our results with those reported for other forage species should be regarded with caution, as differences in species digestibility may be due to variations in chemical composition.

Many shrub species are known to contain various levels of tannins. In addition, condensed tannins (CT), which are plant secondary compounds (PSC) from the polyphenols family, could improve rumen fermentation efficiency at low concentrations (lower 4% DM) (Belachew *et al*., 2013). However, high CT concentrations (7.5% to 15.2% DM) may reduce the nutritional value of dietary components and the digestibility of protein and cell-wall components of shrubs from northeastern México (Ramírez *et al*., 2000). Furthermore, higher browsing pressure on rangelands might result in leaves with higher CT content (Mnisi and Mlambo, 2017). This situation did not occur in this study since the analyzed plots remained without disturbance, although the occasional presence of goats and sheep from neighboring rangelands, also white-tailed deer could appear during the study period. The presence of PSC such as CT may explain why some species exceeded the minimum CP requirement, despite having lower digestibility (Camacho *et al*., 2010). On the contrary, *F. angustifolia* showing the lowest CP content and the lowest levels of cellulose, concomitantly it showed higher IVDMD than any other species, except for *C. pallida*, and also acceptable levels of metabolizable energy ranging between 6 to 9 MJ/kg of this shrub species have been reported by Domínguez-Gómez *et al*. (2011). Therefore, it seems more than convenient that the concentration of these compounds be determined before any further animal diet testing. However, these species may be insufficient as a sole feed source for other classes of livestock, and protein requirements may vary depending on the age and reproductive condition of the animals (O'Keeffe *et al*., 2015). *Celtis pallida* showed the highest IVDMD rate of all species under study (78.7%-88.5% DM) and the highest CP content (16.3%-36.7% DM). According to Ramirez *et al*. (2016), *C. pallida* leaves may have potential as an economical supplementary feed for small ruminants diets based on roughages of low quality, compared to those containing *Medicago sativa* hay, a legume of high nutritional quality.

Due to the lack of information regarding the use of the Daisy^{II} method on foliar tissue samples similar to the species under study, the values reported herein might be somewhat overestimated, as the Daisy^{II} method requires standardization according to the forage to be analyzed. Previous studies report that using 0.25 g samples may result in higher digestibility values than if 0.50 g samples are used (Damiran *et al*., 2008). Similarly, the IVDMD values for high CP feedstuff are significantly higher when analyzed with the Daisy^{II} incubator than those obtained by the traditional Tilley and Terry method (Mabjeesh *et al*., 2000). Also, the use of F57 ANKOM bags with a 25 µm pore size could allow the escape of indigestible and digestible fiber fractions as argued by Tassone *et al*. (2020). Therefore, the comparison with finer pore bags (e.g., F58 ANKOM bags 10 µm pore) could be presented as a study opportunity for the standardization of the Daisy^{II} incubator for its use to determine the digestibility of leaves of shrubs plants for semiarid and arid rangelands. The use of 0.25 g samples and the high CP content in the foliar samples analyzed in this study might be the reasons for the high digestibility values observed. A previous study showed that the nutritional potential of non-leguminous Australian shrub species can be overestimated and lead to poor animal productivity, as *in vitro* organic matter digestibility (OMD) values were 6% to 20% higher than *in vivo* OMD values (Norman *et al*., 2010). These differences were attributed to high salt content and other anti-nutritional factors that change the composition of rumen microbial communities.

High salt contents have not been reported for shrubs of northeastern México (Domínguez *et al*., 2014). However, Trujillo *et al*. (2010) demonstrated that the Daisy^{II} method underestimated DM and neutral detergent fiber disappearance values, compared to *in situ* methods. The IVDMD values of the present study were higher than that reported by Avornyo *et al*. (2019) in Ghana, and similar to those reported for Olafadehan and Okunade (2018) in Nigeria. Both studies evaluated the nutritional value of trees and shrubs consumed by goats. Previous *in vitro* studies for digestibility assessment conducted on fodder tree leaves in northern Pakistan with methods different that the Daisy^{II} incubator reported digestibility values above 50% (Habib *et al*., 2016); however, those were still lower than the values reported herein. Furthermore, Revell *et al*. (2013) reported that *in vitro* OMD ranged between 36% and 69% for perennial shrubs in Australia. IVDMD values obtained in the shrubs of the present study (56.4% to 88.4%), indicate readily available nutrients for livestock. Hence, future studies should be aimed at determining the acceptability of these feed resources by ruminants (Derero and Kitaw, 2018).

Despite the advantages for ready *in vitro* determination of digestibility, the use of the ruminal fluid from sheep as an inoculum for the Daisy^{II} incubator requires thorough consideration of the negative side effects of the wide range of anti-nutritional factors that ruminants face when consuming these shrub species to match true-to-life results obtained from *in vivo* methods of digestibility determination. Furthermore, differences between goats and other ruminants as inoculum donors were not significant (Ammar *et al*., 2008). However, managing domestic ruminants to consume a larger portion of shrub material in their diets requires adequate supplementation to maintain satisfactory productivity levels (Perryman *et al*., 2011).

CONCLUSIONS

The results reported herein have shown that the CP content of the shrub species under study was high enough throughout the year to consider the exploration of these species as an alternative protein source for small ruminants in extensive grazing systems. However, the method used to determine CP in this study only estimated total N. Further studies should be focused to determine proteins bound in secondary compounds-protein complexes that may be unavailable for assimilation by the animals. Compared to the previous data reported, our results suggest that the shrubs *F. angustifolia*, *G. angustifolium*, but especially *C. pallida,* can be considered highly digestible; however, further studies with different pore size bags, grinding and sample weights are required to standardize and obtain more accurate results using the Daisy ^{II} incubator. In general, cell-wall components, ADL and CP content tend to increase, while digestibility shows a tendency to decrease with plant physiological maturity, having the highest digestibility levels in the months of spring, a season of active plant growth and development in the Tamaulipan Thornscrub ecosystem of northeastern México. It is concluded that all shrub species studied maintained high digestibility values during the experimental period and may therefore be considered as an alternative feed resource throughout the year.

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Data availability. Data is available with the MC. Miguel Chávez Espinoza, chavezespinoza13@hotmail.com, upon a reasonable request.

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