INFLUENCE OF THE SHRUBS *CELTIS PALLIDA* AND *ZIZIPHUS OBTUSIFOLIA* ON INTAKE, DIGESTION AND N BALANCE BY SHEEP

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Abstract. The objective of the study was to evaluate the influence of two shrub leaves on digestion, nitrogen retention and ruminal digestion characteristics of sheep. Twelve rumen-fistulated castrated male sheep (Pelibuey x Rambouillet) of 32.0 ± 2.3 kg live weight were randomly assigned to three diets (four sheep/diet): diet 1 or control, consisted of 49.2% *Medicago sativa* hay and 50.8% of *Cynodon plectostachyus* straw dry matter basis (DM), diet 2 consisted of 36.2% *Celtis pallida* leaves and 63.8% *Cynodon plectostachyus* straw, diet 3 consisted of 43.9% *Ziziphus obtusifolia* leaves and 56.1% *Cynodon plectostachyus* straw. The diets were added with 0.5% of a mineral premix and 5.0% molasses. *Medicago sativa* hay was used as control feed of good nutritional quality. Sheep intakes of dry matter (DM), organic matter (OM), crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), cellulose and hemicellulose were not significantly different among treatment diets. However, the digestion coefficients of DM, OM, CP, NDF, ADF, cellulose and hemicellulose resulted similar (P>0.05) between sheep fed the *M. sativa hay* and the *C. pallida* diets, but both were higher (P<0.05) than sheep consuming the *Z. obtusifolia* diet. The N intake, fecal N and urinary N were not different (P>0.05) among the three diets, but numerically sheep fed *C. pallida*, excreted more N in urine than the sheep on the other diets. Leaves of evaluated shrubs may have potential to be used as alternative browse supplement for sheep fed diets based on roughages of low nutritional quality.
Keywords *In vivo* digestion, nitrogen retention, ruminal digestion, sheep

Introduction. In the past, shrubs (browse) species were considered poor animal feeds and considerations were done to remove or destroy them from prairies (Devendra and Sevilla, 2002). However, at the present days, this attitude has modified and considerable investigation has been developed to demonstrate that shrub species are significant forage sources around the world (Merry et al., 2001). Nonetheless, the feeding value of shrubs is widely variable, depending on species or cultivars, plant parts, phenological stage, environmental conditions and management (Ventura *et al*., 2002; Ogunbosoye and Babayemi, 2010). Moreover, its digestibility is low due to the high presence of secondary compounds, especially lignin and tannins (Egea et al., 2016). Nevertheless, deciduous species maintain their nutritional value during harsh seasons and can be used as feed supplements for ruminants (Papanastasis and Nefzaoui, 2000). Moreover, Research studies confirm that leaves of palatable shrub species can be an effective protein supplement for goats and sheep, when they fed roughages of poor nutritional quality (Patra, 2010); in addition, some shrub species appear to match the *M. sativa* hay as a source of protein for goats and sheep (Ramirez, 1999).

Integration of browse species in ruminant nutrition systems may be a feasible substitute to expand the use of soil and at the same time advance the food resources for animals (Patra, 2010). Some browse species are easily proliferated and do not need a high level of management requirements. Moreover, some species have levels of crude protein (CP) that are superior than other foods that are usually utilized in animal nutrition that upgrades ingestion of low quality forages by animals (Foroughbakhch et al., 2013). Browse plants have good access to water that percolates through the topsoil and infiltrates into the subsoil (30–150 cm) and have the ability to yield high quality and quantity foliage in places with extended drought times. Some browse plants are longstanding (Hanley et al., 2007).

In rangelands of northeastern Mexico, *Celtis pallida* grows as a perennial evergreen shrub species. It belongs to the Ulmaceae family and wildlife and domestic animals such as cattle, goats and sheep consume its leaves. In addition, *Ziziphus obtusifolia* is a spiny shrub that belongs to the family Rhamnaceae. Goats, cattle, sheep and white-tailed deer browse the foliage (Ramirez, 1999). This study was carried out to determine the nutrient intake, digestion coefficients and N retention by sheep fed leaves from *Celtis pallida* and *Ziziphus obtusifolia* in combination with grass straw in comparison to *Medicago sativa* hay.

Materials and Methods

Leaves from *Celtis pallida* and *Ziziphus obtusifolia* were collected in an area of Cienega de Flores county, of the state of Nuevo Leon, Mexico. *Medicago sativa* hay and *Cynodon plectostachyus* straw were obtained from a commercial store at the city of Monterrey of the state of Nuevo Leon, Mexico. The collection area was located at 24° 20' north latitude and 100° 21' east longitude. It has an average elevation of 600 meters above sea level. The climate is semiarid with an average annual temperature of 21 °C and annual precipitation of 600 mm. After collection of shrubs branches of each shrub were allowed to air dry for a 2-week period on the branches and then leaves were removed by carefully beating the branches with sticks. Immediately after, leaves were ground in a hammer mill and further mixed to diets. *Medicago sativa* hay was used as a legume control of high nutritional value.

Forages were analysed (AOAC, 2012) to determine dry matter (DM), ash, and crude protein (CP). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were also determined (Van Soest et al., 1991). Acid detergent lignin (ADL) was also measured (AOAC, 2012). Hemicellulose (NDF – ADF) and cellulose (ADF – ADL) were calculated by difference. Condensed tannins were determined by the butanol-HCl technique and were expressed as leucocyanidin equivalents (Makkar, 2003).

Three diets were administered to lambs: diet 1 or control, consisted of 49.2% *Medicago sativa* hay and 50.8% of *Cynodon plectostachyus* straw dry matter basis (DM), diet 2 consisted of 36.2% *Celtis pallida* leaves and 63.8% of *Cynodon plectostachyus* straw and diet 3 consisted of 43.9% *Ziziphus obtusifolia* and 56.1% of *Cynodon plectostachyus* straw. The diets were additional added with 0.5% of a mineral premix and 5.0% molasses. All diets were adjusted to contain approximately 13% of CP. Twelve male castrated sheep (Pelibuey x Rambouillet) weighing approximately 32.0 kg BW were housed in metabolism stalls and assigned randomly to the four treatment diets (4 sheep/treatment). Then, diets were offered in two equal portions daily at 08:00 and 16:00 h. Sheep were adjusted to diets for 15 days, and then total feces and urine were collected for 7 days. Subsamples (10%) of urine and feces were taken twice daily and frozen until analyzed. Ten millilitres of a 50% HCl (v/v) solution was added to urine collection buckets daily to prevent NH loss. Feed offered and refusals were sampled (5%) daily with subsamples bulked over collection period for subsequent analysis.

Samples of feed offered and refused were ground in a Wiley mill to pass a 2 mm screen. Feces were thawed at room temperature and partial DM determined by oven drying (55° C) for 76 h. Dry matter (105° C), ash (550° C) and N were analysed on separate samples (AOAC, 2012). The NDF and ADF were also determined (Van Soest et al., 1991). The ADL was also determined (AOAC, 2012). Hemicellulose (NDF – ADF) and hemicellulose (ADF – ADL) were estimated by difference. Nitrogen in urine samples was determined by the Kjeldhal method (AOAC, 2012).

The significance of diet effects on nutrients intakes, digestion coefficients of DM, CP, NDF, ADF, cellulose and hemicellulose, and N retention by sheep was determined by one-way analysis of variance using the GLM procedure of SAS. Means were separated by the least square method ($P<0.05$) (SAS, 2000).

Results

Celtis pallida leaves had more crude protein than *Z. obtusifolia* or *M. sativa* hay or *C. plectostachyus* straw. The ash content was also higher in *C. pallida* than the other forages. The NDF and its components (ADF, ADL, cellulose and hemicellulose) was higher in *Z. obtusifolia* leaves. The concentration of condensed tannins was higher in the *Z. obtusifolia* than *M. sativa* hay or *C. pallida* (Table 1). The CP in diets was similar. The ash content was higher in diet containing *C. pallida* leaves compared to other diets. The NDF, ADF and cellulose were higher in the diet containing *M. sativa* hay followed by *C. pallida* and *Z. obtusifolia* that was lowest. However, the hemicellulose content was higher in the diet with *C. pallida* followed by the diet with *Z. obtusifolia* and *M. sativa* hay was lowest. The diet with leaves of *Z. obtusifolia* was the only that had condensed tannins (Table 1).

The dry matter (DM), organic matter (OM), CP, NDF, ADF, cellulose and hemicellulose intakes of sheep were not significantly different among treatment diets (Table 2). However, the digestion coefficients of DM, OM, CP, NDF, ADF, cellulose and hemicellulose resulted similar ($P>0.05$) in sheep fed the *M. sativa hay* and the *C. pallida* diets, but both were higher ($P<0.05$) than sheep consuming the *Z. obtusifolia* diet. The N intake was not different ($P>0.05$) among the three diets, but numerically the diet containing *C. pallida* was higher (Table 2), followed by the diet with *M. sativa* hay and the lowest value was for the diet containing *Z. obtusifolia*. The fecal N was also not different ($P>0.05$) among diets, but sheep fed *Z. obtusifolia* excreted more fecal N than sheep fed *M. sativa* hay or *C. pallida* leaves. Urinary N excretion was also not different ($P>0.05$) between diets, but numerically sheep fed *C. pallida*, excreted more N in urine than the sheep on the other diets. Likewise, urinary N as percent of N intake was not different ($P>0.05$) among diets, even though numerically the sheep fed *C. pallida* excreted more N in urine. The N retained was lower in sheep fed *M. sativa* hay than sheep fed the other diets. The N balance as a percent of N intake was also not different ($P>0.05$) between diets, but the sheep fed *Z. obtusifolia* had the highest value, followed by sheep on *C. pallida* and the lowest was in sheep on *M. sativa* hay.

Discussion and Conclusions

Sheep fed the diets containing *M. sativa* hay or *C. pallida* leaves digested similar amounts of DM, OM, CP, NDF, ADF cellulose and hemicellulose than sheep fed the *Z. obtusifolia* diet that had high levels of condensed tannins. (Table 2). Moreover, Barnes (1988) found that white-tailed deer dramatically reduced dry matter intake, during all seasons of the year, when fed *Acacia berlandieri* with high condensed tannins content (16.0%). Conversely, Nuñez-Hernandez *et al.* (1989) reported that Angora goats that were fed diets with *Juniperus monosperma,* with moderate levels of condensed tannins, had a high intake of dry matter compared with those fed *M. sativa* hay diets. Moreover, Boutouba *et al*. (1990)

found that Angora goats increased dry matter intake when they were fed diets with low levels of *Cercocapus montanus* a tanniniferous plant (15.8%).

Table 1. **Chemical composition (dry matter basis) of *Medicago sativa* hay, *Cynodon plectostachyus* straw and the shrubs *Celtis pallida* and *Ziziphus obtusifolia* and diets offered to sheep**

Concept	*M. sativa*	*C. pallida*	*Z. obtusifolia*	*C. plectostachyus*
Forages, %[1]				
Crude protein	17.0	21.2	18.4	5.2
Ashes	13.9	21.6	8.3	8.7
Neutral detergent fiber	30.8	24.9	33.0	80.4
Acid detergent fiber	24.3	17.5	27.0	49.5
Cellulose	15.1	9.6	15.0	33.9
Hemicellulose	15.7	15.3	18.6	30.9
Acid detergent lignin	8.0	5.3	11.4	13.6
Condensed tannins	0.0	0.0	14.0	0.0
	M. sativa	*C. pallida*	*Z. obtusifolia*	
Diets, %[2]				
Crude protein	13.7	13.8	12.9	
Ashes	11.0	14.8	9.6	
Neutral detergent fiber	56.8	54.9	53.4	
Acid detergent fiber	35.1	32.0	34.7	
Cellulose	24.5	18.5	20.6	
Hemicellulose	21.7	22.9	18.7	
Acid detergent lignin	8.6	9.3	12.3	
Condensed tannins	0.0	0.0	6.6	

[1]Dry matter basis.
[2]In each diet contained 0.5% of a premix (8.0% of P; 7.0% of Ca; 0.8% of Mg; 400 ppm of Fe; 2800 ppm of Mn; 2400 ppm of Zn; 1450 ppm of I; 8 ppm of Co and 6500 IU of vitamin A) and 5% of liquid molasses.

Table 2. **Live body weight, nutrient intakes, digestion coefficients and nitrogen utilization of sheep fed diet containing different levels of *Medicago sativa* hay, *Celtis pallida* or *Ziziphus obtusifolia* leaves**

Concept	Diets			SEM[2]
	M. sativa	*C. pallida*	*Z. obtusifolia*	
Live body weight, kg	29.8	31.6	35.2	2.6
Nutrient intakes, g/day[1]				
Dry matter	825.4	849.7	841.0	20.1
Crude protein	99.5	107.3	100.2	2.7
Neutral Detergent Fiber	462.0	466.3	451.4	5.2
Acid Detergent Fiber	284.4	272.1	292.0	14.4
Cellulose	177.5	167.4	173.3	11.3
Hemicellulose	157.6	154.2	157.2	10.2
Digestion coefficients, %[1]				
Dry matter	58.0 [a]	55.4 [a]	47.8 [b]	1.2
Organic Matter	60.1 [a]	56.7 [a]	49.1 [c]	1.2
Crude Protein	68.2 [a]	64.9 [a]	50.3 [b]	1.2
Neutral Detergent Fiber	52.3 [a]	49.3 [a]	36.7 [b]	1.5
Acid Detergent Fiber	46.4 [a]	44.5 [a]	26.8 [c]	1.5
Cellulose	59.2 [a]	55.7 [a]	47.4 [b]	3.2
Hemicellulose	61.7 [a]	60.9 [a]	54.0 [b]	1.5
Nitrogen balance, g/day[1]				
Nitrogen intake	15.5	18.8	16.0	2.1
Fecal nitrogen	5.1	6.6	7.5	1.5
Urinary nitrogen	9.4	10.9	7.1	1.4
Nitrogen balance	1.0	1.3	1.4	1.3
Percent of nitrogen intake	6.4	6.9	8.8	1.4

[1]Dry matter basis; [2]standard error of the mean.
[abc]Means in a row with different letter superscripts are different (P<0.05).

The condensed tannins (antinutritional factor) could contribute to limit the use of shrubs in animal feeding systems because they produce astringency that increase salivation and decreases the palatability (Copani et al., 2013). They also reduce the digestibility of nutrients of cell wall components (Min et al., 2003; Mlambo et al., 2015). It seems that in this study, *Z. obtusifolia* formed indigestible complexes with the nutrients that did not allow the use of its components in the digestive tract of sheep. However, *C. pallida* leaves, compared with *M. sativa* hay, may be a good choice in the diet of in grazing small ruminants and in general could be considered to be part of the range systems as a protein source in this region (Ramirez, 1999). Ramirez and Lara (1998) reported similar responses in an *in vivo* digestibility trial using sheep that was fed bean straw as a basal diet and supplemented with different levels of native shrubs such as *Acacia rigidula*, *Cercidium macrum* or *Acacia farnesiana* and *M. sativa* hay as a control forage.

In this study, all sheep, in all diets, resulted with positive N balance. Similarly, Ramirez and Lara (1998) also found that sheep had positive N balance, with the exception of sheep fed *A. rigidula* that contained high levels of condensed tannins (15%). Waghorn (2008) argued that ruminants fed diets high in shrubs that contained condensed tannins have high concentrations of fecal N, but such increases may be offset to reduce urinary N losses (Ogunbosoye and Babayemi, 2010). In addition, diets high in condensed tannins can also increase post-ruminal absorption of N (Egea et al., 2016) and increase N retention (Foroughbakhch et al*.,* 2013).

Many browsers species contain condensed tannins and phenols in amounts that reduce ruminal availability of protein (Aganga and Tshwenyane, 2003) and fiber (Babayemi and Bamikole, 2006) and diminish palatability (Al-Masri, 2013). Results of this study showed that leaves of *C. pallida* were digested in the rumen in a very similar manner than *M. sativa* hay, and because its high levels of condensed tannins the leaves of *Z. obtusifolia* were poorly digested. Ramirez and García (1996) who evaluated browse plants such as *Leucaena leucocephala* or *Acacia berlandieri*, and compared with *M. sativa* hay, reported that because of its high level of condensed tannins, *Acacia berlandieri* had an *in situ* digestibility of dry matter and crude protein lower than *Leucaena leucocephala* or *M. sativa* hay.

On the other hand, Ramirez and Lara (1998) using the nylon bag technique in sheep, evaluated three native woody plants in this region such as *Acacia rigidula, Cercidium macrum* or *Acacia farnesiana* and compared to *M. sativa* hay. These authors found that *A. rigidula* had lower values *in situ* digestibility of dry matter, crude protein and neutral detergent fiber, compared with other plants. This fact was due to the *A. rigidula* contained high levels of condensed tannins, which may have influenced a decrease in the rumen microbial activity to degrade the dry matter, crude protein and neutral detergent fiber of *A. rigidula*. Similar responses were reported by Holecheck *et al*. (1990) when fed goats with diets containing *Juniperus monosperma* leaves.

It seemed that condensed tannins in leaves of the shrub *Ziziphus obtusifolia* affected the ruminal digestion of sheep. However, leaves of the shrub *Celtis pallida* may have potential as an economical supplementary feed for sheep fed diets based on roughages of low nutritional quality, when diets are compared to those containing *M. sativa* hay, a legume of high nutritional quality.

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