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Nutrients, Mineral and Volatile Fatty Acids Content in Four *Leucaena* Species and the Hybrid K743

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Abstract: In the semiarid and subtropical regions of northeastern Mexico, leaves and pods of *Leucaena* sp. are important forage complements into ruminant feeding. In order to determine the effectiveness of the use of *Leucaena* as fodder, samples of leaves of four species *L. diversifolia*, *L. leucocephala*, *L. pulverulenta*, *L. shannoni* and the hybrid *L. leucocephala* K743 were collected for estimation of some of their chemical characteristics, cell wall components, mineral composition, Volatile Fatty Acid (VFA) concentration and tannin and mimosine content. Results from chemical analysis revealed significant ($p<0.05$) differences in nutrient values between species and hybrid of *Leucaena.* The highest value of crude protein (27.4%) and lowest ash content (7.9%) were exhibited by *L. leucocephala* K 743, while the highest NDF (38.5%) and ADF (26.8%) content were observed in *L. pulverulenta* and *L. diversifolia*. Low level of hemicellulose and soluble ash was observed in *L. pulverulenta* (7.3% and 0.11% respectively). The maximum mimosine content was observed in *L. leucocephala* (3.02%) and *L. pulverulenta* (2.91%). The VFA was higher in *L. diversifolia* (55.6mM) and *L. leucocephala* (51.5 mM) compared to *L. pulverulenta* (42.0 mM) and *L. shannoni* (48.3 mM). *L. leucocephala* had higher content of iron (115.5 ppm), lead (2.02 ppm) and aluminum (133.2 ppm) compared to others species, whereas, *L. diversifolia* showed the higher values in zinc (14.3 ppm), cobalt (0.06 ppm) and manganese (36.8 ppm). The leaves of *Leucaena* sp. should be considered as different entities than other ingredients of the traditional diets when characterizing nutrient quality.

Key words: Nutrient content, volatile fatty acids, mineral content, forage, *Leucaena*

INTRODUCTION

The study on the improvement of ruminant production in rural zones during the winter season is an economical issue of interest. Most of the works about the chemical composition of forage consumed by ruminants are essentially based on the relationship between the nature of the vegetal components and their degradation in the digestive apparatus (Jarrige, 1988; Genin, 1990; Martin *et al*., 1995; Ramirez *et al*., 2001). However, the available information about this kind of relationship is still insufficient for the case of forage from trees and shrubs. To this day a few woody forage species have been subjected to agronomical and nutritional studies which have resulted in a lack of knowledge on the alimentary use of trees and shrubs.

Many shrubs and trees in northeastern Mexico can provide feed material but only a few of them have been actually incorporated into ruminant feeding systems. The scattered information on the nutritive value of forages from shrubs and trees suggests that, in general, leaves from shrub and trees contains higher levels of crude protein than many common forages despite some browse forages are high in deleterious components (Devendra, 1990). Legume forages are advantageous for their high-quality protein and energy content. Among them the *Leucaena* sp. have become popular for incorporation into ruminant feeding systems (Blair, 1990). *Leucaena* sp. has shown good potential in northeastern Mexico as a high-protein fodder that could substitute conventional concentrated feeds for cattle under special circunstances.

The acetic, propionic and butyric acids are the main Volatile Fatty Acids (VFAs) produced during the microbial fermentation of dietary carbohydrates in the rumen and, in fact, VFAs are practically the only source of energy for the ruminant. Thus, those forages that produce high concentrations of VFA during fermentation are

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consequently good energy-producing forages (Van Soest, 1982). The genus *Leucaena* is considered as an alimentary supplement owing to their rich content in protein. Nevertheless, although their nutritive value is noticeably superior to native vegetation, it is still inferior to the nutritive value of concentrated foods so it becomes necessary to supplement the diet with other concentrated feed.

MATERIALS AND METHODS

Leaves of four *Leucaena* sp.: *L. divesifolia, L. shannoni, L. pulverulenta, L. leucocephala* and *L. leucocephala* hybrid K743 were collected at the Experiment Station of Forestry Sciences in Linares, Nuevo Leon, Mexico (24°27´N, 99° 32´W; elevation 400 m). Original seeds for planting of the *Leucaena* sp. and the hybrid K743 (L. *leucocephala*×*esculenta*) were obtained from the Oxford Forestry Institute, Oxford, U.K. The climate of Linares is warm-subhumid with two rainy seasons and a dry winter period. Average annual rainfall is 750 mm. The mean annual temperature is 22.3°C with peaks above 40°C during summer and a cool period from December to March when frosts may occur (Foroughbakhch *et al.*, 2006).

Leaves were removed from branches by hand and grounded to pass a 2 mm screen.

Dry matter (105°C), ash (550°C), Crude Protein (CP), Ether Extract (EE) and Crude Fiber (CF) were determined (AOAC, 1990). Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF) (Goering and Van Soest, 1970) and Acid Detergent Lignin (ADL) (AOAC, 1990) were determined. Leaves were also analyzed for condensed tannins using the vanillin-HCL procedure (Burns, 1971) as modified by Price *et al.* (1978).

Mimosine analyses of leaf samples were conducted following the method of Matsumoto and Sherman (1951).

For VFA analysis, a batch culture *in vitro* procedure was used with Mc Dougall´s artificial saliva (Galyean, 1988) as buffer. Rumen fluid inoculums (8 mL) from 3 rumen sheep fed with alfalfa hay was added to 0.5 g of each leaf sample in 50 mL *in vitro* plastic tubes, which then were flushed with Co_2 and incubated in a 39°C water bath.

Triplicate tubes from each species and triplicate blank culture tubes (buffer plus inoculum) were incubated for 48 h. Afterwards, tubes were centrifuged at 10,000×g for 10 min. Supernatant fluid (5 mL) was mixed with 1 mL of 25% (w v^{-1}) metaphosphoric acid containing approximately 2 g L^{-1} of 2-ethylbutyric acid (internal standard) and centrifuged at 10,000×g for 10 min. The resulting supernatant fraction was analyzed for VFA by chromatography (Goetsch and Galyean, 1983). Total concentrations (mM) of acetate, propionate and butyrate were calculated by adjusting forage cultures for the VFA concentration from the appropriate blank culture.

The significance of plant effects on nutrient content and VFA concentration was determined by one-way analysis of variance using the GLM procedure of SAS (1988).

Means were separated by the Least Significant Difference (LSD) method ($p<0.05$) (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

A wide range of variation was observed for quality parameters amongst *Leucaena* sp. (Table 1). The Organic Matter (OM) content was higher ($p<0.05$) in K743 (92.1%) and *L. diversifolia* (90.4%) than in *L. leucocephala* (84.5%), *L. pulverulenta* (87.0%) and

Table 1: Chemical composition of *Leucaena* sp. (percent dry matter basis)

Analysis	*L. diversifolia*	*L. shannoni*	*L. pulverulenta*	*L. leucocephala*	*L. leucocephala K743*	Standard error
Organic matter	90.45a	88.12b	87.01b	84.55c	92.10a	0.78***
Crude protein	22.00bc	19.80cd	16.30d	24.60b	27.40a	1.25***
Total ash	9.54c	11.43b	12.96b	15.45a	7.90d	1.12**
Ether extract	4.35c	6.73a	4.94b	3.55c	2.86d	0.53***
Crude fiber	13.82b	11.95c	16.78a	11.12c	13.65b	0.82**
Nitrogen-free extract	50.21a	51.36a	48.67b	49.30b	43.25c	1.98**

* Within rows, means with different letters differ significantly ($p<0.05$); **($p = 0.01$); ***($p\leq 0.001$)

Table 2: Cell wall components, condensed tannins (% DM) and mimosine content of *Leucaena* sp.

Analysis	*L. diversifolia*	*L. shannoni*	*L.pulverulenta*	*L. leucocephala*	*L.leucocephala K743*	Standard error
Neutral detergent fiber	35.5ab	32.16c	38.50a*	31.40c	28.5d	2.06**
Acid detergent fiber	21.0bc	23.5b	26.8a	18.3cd	17.5cd	1.65***
Cellular content	64.5b	67.84ab	61.50c	68.6ab	71.5a	0.86***
Cellulose	10.2c	12.3ab	13.1ab	9.8d	11.9.b	1.24***
Hemicellulose	15.3a	14.1ab	7.3d	15.1a	12.8c	0.97***
Lignin	8.2b	7.5b	9.1a	6.4d	7.1c	1.11**
Insoluble ash	0.13b	0.20a	0.11b	0.10b	0.05c	0.06*
Tannins	6.8c	7.0c	10.9a	8.7b	6.1cd	0.76**
Mimosine	1.23c	2.14b	2.91a	3.02a	2.10b	0.24**

* Means within rows followed by different letters differ significantly at the 5% level,* ($p\leq 0.05$), **($p = 0.01$); ***($p\leq 0.001$)

Table 3: Mineral composition of *Leucaena* sp.

Analysis/*Leucaena* sp.	*L. diversifolia*	*L. shannoni*	*L. pulverulenta*	*L. leucocephala*	*L.leucocephala K743*	Standard error
Ca (% DM basis)	2.03b	2.78ab	3.05a*	1.46cd	1.78c	0.54*
Mg (% DM basis)	0.78c	0.97b	1.12a	0.35d	0.15e	0.23***
Cu (ppm)	5.69bc	4.12d	6.35b	5.55bc	7.56a	0.14**
Zn (ppm)	14.39a	9.66d	11.68c	13.18ab	10.34d	0.46***
Fe (ppm)	85.96b	75.63bc	65.12d	115.52a	55.85e	1.34**
Co (ppm)	0.06a	0.03c	0.07a	0.05b	0.01d	0.04*
Mn (ppm)	36.87a	32.97b	30.29c	30.03c	35.93a	3.54*
Pb (ppm)	1.28b	0.65d	0.89c	2.02a	1.16b	0.09**
Al (ppm)	33.35d	42.54c	65.89b	133.25a	13.52e	2.87***
Na (ppm)	112.38bc	98.77d	126.45b	70.15e	182.36a	4.97**
Cr (ppm)	1.96cd	2.08c	2.88a	2.58ab	2.49ab	0.08**

* Within rows, means with different letters differ significantly ($p<0.05$), * ($p\leq 0.05$), **($p = 0.01$); ***($p\leq 0.001$)

Table 4: Volatile Fatty Acid (VFA) concentration (millimolar) in leaves of five *Leucaena* sp.

Analysis	*L. diversifolia*	*L. shannoni*	*L. pulverulenta*	*L. leucocephala*	*L.leucocephala K743*	Standard error
Total VFA	55.6a	48.3bc	42.0d	50.5b	46.2c	3.65**
Acetic acid	32.9d	37.6c	46.8a	36.4c	43.1ab	2.72*
Propionic acid	13.1b	14.3ab	16.4a	10.8c	10.1c	1.68**
Butyric acid	6.4a	5.2b	7.0a	3.1d	4.2c	0.63***

Within rows, means with different letters differ significantly ($p<0.05$), * ($p\leq 0.05$), **($p = 0.01$); ***($p\leq 0.001$)

L. shannoni (88.2%). The CP content was higher ($p<0.05$) in K743 (27.4%) than in *L. pulverulenta* (16.3%), *L. shannoni* (19.8%) and *L. diversifolia* (22.0%). Values of CP found in this study are in agreement to those found by Castillo *et al.* (1994), who reported values of CP from the cross of L. *pallida* K376 and L. *leucocephala K8* between a range of 16 and 33%, with an average of 26.6%. Ramirez and Garcia (1996) also reported values of CP in *L. leucocephala* (25.3%) similar to those we found.

The ash content in *L. leucocephala* (14.4%), *L. pulverulenta* (13.0%) and *L. shannoni* (11.4%) was higher ($p<0.05$) than *L. diversifolia* (9.5%) and K743 (7.9%). The EE content was higher ($p<0.05$) in *L. shannoni* (6.73%), compared to K743 (2.86).

The Crude Fiber (CF) content, however, was higher ($p<0.05$) in *L. pulverulenta* (16.7%), compared to *L. shannoni* (12.0%), *L. leucocephala* (11.1%) and *L. diversifolia* (13.8%). Finally, the nitrogen free extract (NFE) was similar ($p>0.05$) in *L. pulverulenta* (50.2%) and *L. shannoni* (51.3%) and lower in K743 (43.2%). Values of EE, CF and NFE of *Leucaena* sp. reported in this study are comparable to those values reported by Saadullah (1990) in *L. leucocephala* collected in Bangladesh and to those reported by Joshi and Singh (1990) in *Leucaena* sp. collected in Nepal.

L. pulverulenta (38.5%) and *L. diversifolia* (35.5%) had higher ($p<0.05$) NDF content than *L. leucocephala* (28.5%) hybrid K43 (Table 2). *L. leucocephala* (18.3%) and the hybrid K743 (17.5%) had lower ($p<0.05$) ADF. The highest cellular content was found in the *L. leucocephala* hybrid K743 (71.5%) and the lowest in *L. pulverulenta* (61.5%), while the rest of the species showed values between 64 and 69%. The cellulose content varied in the species between 9.8 and 13.1%, being *L. pulverulenta* (13.1%) and *L. shannoni* (12.3%) the species with higher values with 13.1 and 12.3% respectively, in contrast with *L. leucocephala* (9.8%) and *L. pulverulenta* (10.2%).

The higher values ($p<0.05$) of hemicellulose content were found in *L. diversifolia* (15.3%), *L. leucocephala* (15.1%) and *L. shannoni* (14.1%). Lignin content in *L. pulverulenta* (9.1%) was higher ($p<0.05$) than in other plants. Cell wall values found in this study were lower than those reported by Akbar and Gupta (1986) and Castillo *et al.* (1994). They reported values of NDF and ADF in *L. leococephala* around 40 and 21%, respectively for *L. leucocephala* and the hybrid. Condensed tannins in *L. pulverulenta* (10.9%) was higher ($p<0.05$) than *L. diversifolia* (6.8%) and *L. shannoni* (7.0%). The condensed tannins values in K743 (6.1%) was lower than those (6.6%) reported by Castillo *et al.* (1994) and higher than those (1.9-2.5%) reported in *L. leucocephala* by Tagendjaja *et al.* (1986).

It has been reported by Ramirez *et al.* (2001), that high levels of condensed tannins (5-10%) in *Lotus* sp. resulted in detrimental effects on ruminant diets whilst low concentrations (1-3%) are likely to be beneficial in protecting protein from rumen degradation.

The average mimosine content on a dry matter basis ranged between 1.23 mg g^{-1} for *L. diversifolia* to 3.02 mg g^{-1} for *L. leucocephala*, with minimum concentrations observed at the onset of flowering. Mimosine content of *L. pulverulenta* and *L. leucocephala* followed the same pattern as nitrogen concentration. According to Lowry (1982) young leaves usually show higher levels of mimosine than mature leaves.

There were no remarkable differences in the mineral content between species (Table 3). *L. leucocephala* had a higher content of iron, lead and aluminum as compared to others species, whereas, *L. diversifolia* showed the higher values of zinc, cobalt and manganese. Likewise, *L. pulverulenta* had higher content in calcium, magnesium and chrome. Generally, the values of cooper and zinc content observed in all species were lower than those reported elsewhere by Ahmad and Ng (1981), Haag and Mitidier (1980) and Akbar and Gupta (1984, 1986). The resilts on manganese, copper, iron and sodium were also similar to those reported by Mtenga and Laswai (1994).

Total VFA concentration (mM, acetic plus propionic plus butyric acids) was similar ($p>0.05$) in *L. pulverulenta* (42.0%) and *L. leucocephala* K743 (46.2%), but both were lower than *L. diversifolia* (55.6%) and *L. shannoni* (48.3%, Table 4). Low concentration of VFA in *L. pulverulenta* may be related to its high concentration on condensed tannins (10.9%). However, even though *L. leucocephala* also had high level of condensed tannins (8.7%), the level of VFA (50.5%) was higher than its hybrid K743. These plants may have different types of tannins. Tannins in *L. leucocephala* negatively affected the microbial fermentation in the in vitro procedure, resulting in low VFA (energy) production, as confirmed by Moya Rodriguez *et al.* (2003).

Individual concentrations of acetic, propionic and butyric acids (mM) in the leucaenas followed the same pattern as the total VFA concentration (Table 4). In general, higher values were observed in *L. pulverulenta* and *L. shannoni* than in *L. leucocephala* hybrid K743 and thus these species may be better suited for use in ruminant diets.

CONCLUSION

The highest crude protein and crude fiber were exhibited by *L. leucocephala* K 743, while the highest NDF and ADF content were observed in *L. pulverulenta* and *L. diversifolia*. Low level of hemicellulose in most of species indicated a lower digestibility portion of cell wall constituents. The maximum mimosine content was observed in *L. leucocephala* and *L. pulverulenta*.

The results show that low levels of cell wall in all plants may be beneficial to promote higher ruminant intakes. Moreover, high levels of condensed tannins had detrimental effects in the ruminal rate of degradation and effective degradability of DM and CP.

In view of its chemical, mineral constitution and VFA content it can be confirmed that the *Leucaena* leaves may be considered as an appropriate potential forage feed and a protein complement of good nourishment properties worth of interest for the improvement of graze livestock. The leaves would considerably allow the reduction of the feed cost during the Winter period thus improving the animal yield.

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