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Short note [Nota corta]

NUTRITIONAL VALUE AND IN VITRO DIGESTIBILITY OF LEGUME PODS FROM SEVEN TREES SPECIES PRESENT IN THE TROPICAL DECIDUOUS FOREST[1]

[VALOR NUTRICIONAL Y DIGESTIBILIDAD IN VITRO DE LAS VAINAS LEGUMINOSAS DE SIETE ESPECIES DE ÁRBOLES PRESENTES EN EL BOSQUE CADUCIFOLIO TROPICAL]

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SUMMARY

The pods of trees from tropical deciduous forest are relevant in the dry season due to their high availability. Whole pods from seven plant species *Acacia pennatula* (Schltdl. & Cham.) Benth., *Caesalpinia gaumeri* (Britton & Rose) Greenm., *Leucaena leucocephala* (Lam.) de Wit, *Lysiloma latisiliquum* (L.) Benth., *Mimosa bahamensis* Benth., *Piscidia piscipula* (L.) Sarg. and *Senegalia gaumeri* (S. F. Blake) Britton & Rose which are reported as consumed by small ruminants were evaluated. Their nutritional value, phenolic compounds, *in vitro* dry matter (IVDMD) and organic matter digestibility (IVOMD) and metabolizable energy (ME) with and without the inclusion of polyethylene glycol (PEG) were evaluated. The pods from *P. piscipula* showed the highest crude protein content. The pods of *L. leucocephala*, *C. gaumeri,* and *A. pennatula* resulted with the highest IVDMD (46.3 and 44.5%), IVOMD (44.8 and 45.4%) and ME (6.8 and 6.7 MJ) (P<0.05). The phenolic compounds were not detected in *C. gaumeri*, *L. latisiliquum* and *S. gaumeri* pods. The IVDMD, IVOMD and ME on pods of *L latisiliquum* and *P. piscipula* (P<0.05) was lower when PEG was added. Pods from *A. pennatula, C. gaumeri* and *L. leucocephala* showed acceptable nutritional value for their use as supplement for ruminants.
Keywords: Digestibility; polyphenols; ruminants; tree pods; tropical forest.

RESUMEN

Las vainas de los árboles del bosque tropical caducifolio son relevantes en la estación seca debido a su alta disponibilidad. Se evaluaron vainas enteras de siete especies de plantas; *Acacia pennatula* (Schltdl. & Cham.) Benth., *Caesalpinia gaumeri* (Britton & Rose) Greenm., *Leucaena leucocephala* (Lam.) De Wit, *Lysiloma latisiliquum* (L.) Benth., *Mimosa bahamensis* Benth., *Piscidia piscipula* (L.) Sarg. y *Senegalia gaumeri* (S. F. Blake) Britton & Rose, que según estudios previos son consumidos por pequeños rumiantes. Se evaluó su valor nutricional, compuestos fenólicos, digestibilidad *in vitro* de materia seca (IVDMD) y materia orgánica (IVOMD) y energía metabolizable (ME) con y sin la inclusión de polietilenglicol (PEG). Las vainas de *P. piscipula* mostraron el mayor contenido de proteína cruda. Las vainas de *L. leucocephala*, *C. gaumeri* y *A. pennatula* resultaron con los valores más altos de IVDMD (46.3 y 44.5%), IVOMD (44.8 y 45.4%) y ME (6.8 y 6.7 MJ) (P <0.05). No se detecto compuestos fenólicos en *C. gaumeri*, *L. latisiliquum* y *S. gaumeri.* La IVDMD, IVOMD y ME fue menor en las vainas de *L. latisiliquum* y *P. piscipula* (P <0.05) con la inclusión de PEG. Las vainas de *A. pennatula*, *C. gaumeri* y *L. leucocephala* mostraron un valor nutricional aceptable para su uso como suplemento para los rumiantes.
Palabras clave: Digestibilidad; polifenoles; rumiantes; ramas de árboles; bosque tropical.

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INTRODUCTION

The tropical deciduous forests (TDF) plant biodiversity has been undervalued and quality information is needed to revalorize this resource (Torres-Acosta et al. 2016). It possess many leguminous trees and shrubs and the foliage and pods of many species are consumed by browsing sheep and goats (González-Pech et al. 2015). In addition, these legume pods are abundant during the dry season and are readily consumed by sheep and goats when falling to the ground (González-Pech et al. 2015). Nevertheless, most of the research on tropical forages describe the nutritional composition of plant foliage (*i.e.* Landa-Becerra et al. 2016) but only a few reports are related to their pods (*i.e.* Sotelo et al. 1995; Pinto-Ruiz et al. 2009; Loyra-Tzab et al. 2013).

Nutritional quality of forages and pods could be affected by their content of secondary compounds. For example, condensed tannins consumed in high concentrations can cause a reduction of the voluntary intake, digestibility and growth rates in grazing ruminants (Torres-Acosta et al. 2008; Ojeda et al. 2015). Therefore, it is important to determine the levels of these compounds in the different species and parts of plants as well as their biological activity.

Therefore, to explore suitability of pods from legume trees as a feed for ruminants it is necessary to assess not only their chemical composition but also the nutritional value (digestibility and secondary compounds). Thus, the objective of this study was to quantify the nutritional value, the levels of polyphenolic compounds and the *in vitro* digestibility of the dry and organic matter of pods belonging to seven plant species previously reported as consumed by small ruminants grazing the TDF. The hypothesis of this study was that the pods from the trees and shrub evaluated in the present study have a nutritional quality (crude protein, acid and neutral detergent fiber, polyphenolic compounds and dry matter and organic matter digestibility) that would allow them to be included in the diets of ruminants.

MATERIALS AND METHODS

Collection site and species evaluated

The study was carried out at the Campus of Biological and Agricultural Sciences – Universidad Autónoma de Yucatán (20 ° 52 '7.14 "N and 89 ° 37' 24.04" W). The predominant climate in this area is classified as sub-humid, warm tropical with summer precipitation (AW_0), with average temperature of 26 °C (maximum 36 °C and minimum of 16 °C) (INEGI, 2012). The pods of seven different plant species present in the TDF were evaluated: *Acacia pennatula* (Schltdl. & Cham.) Benth. (Chimay), *Caesalpinia gaumeri* (Britton & Rose) Greenm. (Kitin-che), *Leucaena leucocephala* (Lam. de Wit) (Huaxin), *Lysiloma latisiliquum* (L.) Benth. (Tzalam), *Mimosa bahamensis* Benth. (Sak-katzin), *Piscidia piscipula* (L.) Sarg. (Jabin) and *Senegalia gaumeri* (S.F. Blake) Britton & Rose (Box-katzin). Pods of ten trees were collected for each plant species, these samples were weighed, dried in a forced air oven at 50 °C, milled using a 1 mm screen and finally stored until further laboratory analysis.

Chemical composition

The chemical composition was determined in the Animal Nutrition Laboratory, using for each plant species a pooled sample from ten individual trees. The analysis were: dry matter (DM), ash and crude protein (CP) (AOAC 2002). Neutral detergent fiber (NDF), acid detergent fiber (ADF) and lignin (Lig) (Van Soest et al. 1991). Total phenols (TP) content (Price and Butler 1977), total tannins (TT) (Folin-Ciocalteu + PVPP method, Makkar et al. 1993) and condensed tannins (CT) (Vainillin method, Price et al. 1978).

In vitro dry matter digestibility

The *in vitro* dry matter digestibility (IVDMD) and organic matter digestibility (IVOMD) were estimated following the methodology described by Barros-Rodríguez et al. (2012). This procedure consisted on the incubation of 0.5 g of each pod species (substrate) in 100 ml glass bottles with 40 ml of culture media (Menke and Steingass 1988) and 20 ml of ruminal inoculum. Two bottles were used as control containing only the culture media and the ruminal inoculum. Incubation of all samples was performed in a 39 °C for 48 hours (Theodorou et al. 1994). Four replicates of each substrate were made using ruminal liquid and day of incubation as the replication criteria. To evaluate the role of the polyphenols, additional samples of each substrate (0.5 g DM) were incubated by adding 0.5 g (fresh base) of polyethylene glycol (PEG) (Pluracol®, E-3350). The IVDMD was calculated by difference between the weight before and after incubation and corrected for the weight of the bottles without substrate. The IVOMD was calculated in a similar way to the IVDMD, but using the organic matter content (OM) of the pods. The metabolizable energy was calculated by the following equation: ME (MJ / kg DM) = 0.016 * (%) Digestible organic matter (AFRC 1993).

Statistical analysis

A completely random block design was used, with 4 replicates. The factors analyzed were: the presence of

PEG, tree pod species and interaction between the factors (PEG * pod species). The GLM of the statistical program Minitab (2007) was used. The differences between the means with P value <0.05 were considered as statistically significant. The Pearson correlation analysis was used to evaluate relationship between the chemical composition of the pod species and their IVDMD, IVOMD and ME. In addition, the stepwise method was used to determine the best predictor between chemical composition and IVDMD, IVOMD and ME, with and without PEG.

RESULTS AND DISCUSSION

Chemical composition

The results of the chemical analysis can be observed in the Table 1. The CP content ranged from 7.58% in *C. gaumeri* pods to 19.91% in *P. piscipula* pods. The CP content of the pods (7 to 20%) was consistent with previous report for legume pods such as *Acacia spp*., *Caesalpinia spp*., *L. leucocephala*, *E. cyclocarpum* and *Mucuna pruriens* (L.) DC. Var *pruriens* (Pinto-Ruiz et al. 2009; García Galván et al. 2012). Considering that the amount of CP required by the rumen microorganisms is around 8% (Van Soest 1994), the consumption of the any of pod species under study will not represent a constraint on rumen function but would likely contribute to an adequate rumen function. The NDF and ADF, the values were within the range for forage legume pods such as *A. pennatula*, *Caesalpinia vesicaria* L., *Caesalpinia yucatanensis* subsp. *yucatanensis*, *L. leucocephala* and *S. gaumeri* (Sotelo et al. 1995; Pinto et al. 2002). *A. pennatula* presented the highest CT value (9.5%) and three species of pods showed no content of condensed tannins (*C. gaumeri*, *L. latisiliquum* and *S. gaumeri*). Their CT content (up to 9.5% CT) is similar to previous reports of the forages *Acacia milleriana* (sinom. *Acacia cochliacantha* Humb. & Bonpl. Ex Willd.), *E. cyclocarpum*, *Leucaena collinsi* Britton & Rose and *Ficus glabrata* Kunth (Pinto-Ruíz et al. 2009). The results obtained after PEG addition indicate that the tannins contained in these pods had little effect on *in vitro* digestibility, thus supporting their use for feeding small ruminants.

In vitro dry matter digestibility

Table 2 shows the estimation of IVDMD, IVOMD and ME with and without addition of PEG. *A. pennatula, C. Gaumeri* and *L. leucocephala* had the highest (P <0.05) IVDMD, IVOMD and ME content (with and without PEG), while *L. latisiliquum* had the lowest IVDMD, IVOMD and ME (P <0.05) (with and without PEG), which are similar to those reported by Sotelo et al. (1995) for pods of *Senna atomaria* (L.) H. S. Irwin & Barneby, *Albizia lebbeck* (L.) Benth., *S. gaumeri*, *A. pennatula* and *L. leucocephala*.

The correlations between the chemical composition of the pods and IVDMD, IVOMD and ME is presented in Table 3. The best predictor for their nutritional value was lignin with values of -0.95, -0.96 and -0.95 for IVDMD, IVOMD and ME, respectively (P <0.05). The second-best predictor was the ADF for all three variables with a -0.90 to -0.93 correlation (P <0.05). Total phenols and total tannins showed a lower correlation with the IVDMD, IVOMD and ME (P<0.05) but were not selected as the best predictors.

It has been reported that pods with a digestibility of 45.5% could be considered an appropriate resource for animal production (Sotelo et al. 1995). Higher digestibility values have been found for some pods such as *A. pennatula* and *E. cyclocarpum* (66.8% and 72.3% respectively, Briceño-Poot et al. 2012). The differences between the IVDMD of the pods may be due to varying levels of NDF, ADF and lignin which is associated with digestibility (Pinto et al. 2002). Thus, in agreement with the negative association between the fibrous fractions and the IVDMD, IVOMD and ME found (Table 3), the lowest IVOMD values were for pods from *L. latisiliquum*, *M. bahamensis* and *S. gaumeri* (P <0.05). These species also had higher levels of ADF, which may suggest a lower proportion of soluble compounds available for the animal, hence reflecting its low digestibility (Sarnklong et al. 2010).

Table 1. Chemical composition (%) of the pods from seven forage tree species of tropical deciduous forest.

Pods species	DM	CP	EE	Ash	NDF	ADF	Lig	TP	TT	CT[1]
Acacia pennatula	88.90	8.94	0.73	3.33	55.29	43.15	15.89	5.13	2.22	9.50
Caesalpinia gaumeri	93.13	7.58	1.38	3.14	59.56	38.34	8.16	6.39	3.18	0.0
Leucaena leucocephala	71.65	19.02	2.71	5.32	49.70	37.11	11.58	3.72	1.65	2.31
Lysiloma latisiliquum	86.43	9.38	0.51	5.05	69.93	60.93	29.29	1.5	0.9	0.0
Mimosa bahamensis	76.54	13.64	1.19	4.45	68.87	51.45	23.34	2.2	1.0	1.7
Piscidia piscipula	37.06	19.91	4.38	9.14	46.34	33.12	12.31	4.4	1.8	4.4
Senegalia gaumeri	92.42	10.42	0.92	4.23	69.68	49.95	24.28	1.36	0.95	0.0

DM (Dry matter), CP (Crude protein), EE (Ethereal extract), Ash, NDF (Neutral detergent fiber), ADF (Acid detergent fiber), Lig (Lignin), TP (Total phenols), TT (Total tannins), CT (Condensed tannins). [1]Equivalent to catequins).

Table 2. *In vitro* dry matter digestibility (IVDMD), *in vitro* organic matter digestibility (IVOMD) and metabolizable energy (ME) with or without the inclusion of polyethylene glycol on the pods from seven tree species of tropical deciduous forest.

	IVDMD (%)		IVOMD (%)		ME [1]	
	-PEG	+PEG	-PEG	+PEG	-PEG	+PEG
Acacia pennatula	44.47 [a]	45.81 [a]	43.56 [a]	45.39 [a]	6.74 [a]	7.02 [a]
Caesalpinia gaumeri	46.02 [a]	47.85 [a]	45.32 [a]	47.30 [a]	7.02 [a]	7.33 [a]
Lysiloma latisiliquum	21.41 [c]	6.61 [d]	21.22 [c]	7.61 [d]	3.23 [b]	1.16 [c]
Leucaena leucocephala	46.38 [a]	48.89 [a]	44.85 [a]	47.34 [a]	6.79 [a]	7.17 [a]
Mimosa bahamensis	26.17 [b]	20.95 [c]	25.87 [c]	20.48 [c]	3.96 [b]	3.13 [b]
Piscidia piscipula	42.15 [a]	36.92 [b]	43.01 [a]	38.17 [b]	6.25 [a]	5.55 [b]
Senegalia gaumeri	29.45 [b]	30.66 [b]	28.60 [c]	29.87 [c]	4.38 [b]	4.58 [b]
SE[2]	1.73		1.74		0.26	

[1]ME (MJ/kgDM) = 0.016 * Digestible Organic Matter;
[2] SE: Standard error.
[(a,b)] Means with different literals in the variable differ significantly ($P<0.05$).

Table 3. Correlation coefficients between the chemical composition and *in vitro* dry matter and organic matter digestibility (IVDMD, IVOMD) and metabolizable energy (ME) without the addition of polyethylene glycol on pods from seven plant species of tropical deciduous forest.

	IVDMD		IVOMD		ME	
	Coefficient	P value	Coefficient	P value	Coefficient	P value
Without PEG						
CP	0.256	0.579	0.279	0.545	0.204	0.661
EE	0.491	0.263	0.532	0.219	0.456	0.304
NDF	-0.848	0.016	-0.868	0.011	-0.828	0.021
ADF	-0.915	0.004	-0.932	0.002	-0.900	0.006
Lig	-0.957	0.001	-0.964	0.000	-0.957	0.001
Ash	0.046	0.921	0.099	0.832	0.012	0.979
TP	0.876	0.010	0.886	0.008	0.902	0.006
TT	0.814	0.026	0.818	0.024	0.847	0.016
CT	0.476	0.280	0.485	0.270	0.480	0.275
With PEG						
CP	0.183	0.695	0.192	0.680	0.144	0.757
EE	0.372	0.411	0.400	0.373	0.351	0.440
NDF	-0.722	0.067	-0.747	0.054	-0.715	0.071
ADF	-0.870	0.011	-0.887	0.008	-0.863	0.012
Lig	-0.906	0.005	-0.919	0.003	-0.908	0.005
Ash	-0.106	0.821	0.071	0.880	-0.125	0.790
TP	0.793	0.034	0.813	0.026	0.816	0.025
TT	0.758	0.048	0.773	0.041	0.785	0.037
CT	0.421	0.347	0.438	0.325	0.433	0.332

CP (Crude protein), EE (Ether extract), Ash, NDF (Neutral fiber detergent), ADF (Acid detergent fiber), Lig (Lignin), TP (Total phenols), TT (Total tannins), CT (Condensed tannins).

It was expected that PEG inclusion would improve digestibility due to its tannin binding capacity. However, its use did not improve IVDMD and IVOMD of pods in five of the seven plant species evaluated. This lack of response indicate both, that fiber fractions were responsible for the digestibility values and a low biological activity of tannins contained in pods. Nevertheless, an additive effect of both fraction was recently proposed by Mengistu et al. (2017) who found that proanthocyanidin could be bound to the fibrous portions thus reducing their digestion and the PEG binding. Thus, any positive effect of PEG due to their tannin binding capacity could be reduced, masked or even result on apparently contrary effects. For example, addition PEG reduced the IVDMD, IVOMD and ME (P <0.05) of pods from *P. piscipula* and *L. latisiliquum.* There are two possible explanations: 1) For the case of *P. piscipula* pods maybe the PEG-tannin complexes resulted insoluble in the detergent solutions and appear in the NDF and ADF fractions as an NDF-ADF-lignin model, consequently a low digestibility was displayed (Makkar et al. 1995). 2) For *L. latisilquum* pods, which showed the highest values of fibrous fractions and very low tannin content, a polymer such as PEG could bind to the fibrous fractions and result on a decrease in the estimation of the IVDMD and IVOMD (Makkar et al. 1995; Monforte-Briceño et al. 2005; Mengistu et al. 2017). These aspects warrant further research.

CONCLUSION

The chemical composition and the *in vitro* digestibility of the pods species under study showed that these resources have a nutritional quality compatible with the nutritional requirements of small ruminants. Although the pods from some plant species studied showed medium to high CT levels, no negative effect was found on their IVDMD, IVOMD and ME.

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Conflict of interest Statement
None.

Ethical standards
The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional guides

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