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SHORT NOTE [NOTA CORTA]

CHEMICAL COMPOSITION AND *in vitro* GAS PRODUCTION OF SOME LEGUME BROWSE SPECIES IN SUBTROPICAL AREAS OF MEXICO

[COMPOSICIÓN QUÍMICA Y PRODUCCIÓN DE GAS *in vitro* DE VAINAS DE LEGUMINOSAS ARBÓREAS EN AREAS SUBTROPICALES DE MEXICO]

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SUMMARY

The objective of the present study was to determine the chemical composition and *in vitro* gas production of different legume and wild arboreal pods. Seven seeds of legume browse species, Mexican calabash (*Crescentia alata*), esculent leadtree (*Leucaena esculenta*), guamuchil (*Phitecellobium dulce*), bastard cedar (*Guazuma ulmifolia*), needle bush (*Acacia farnesiana*), mimosa (*Mimosa sp*.) and elephant ear tree (*Enterolobium cyclocarpum*). Were evaluated for their chemical composition (g/kg DM) and *in vitro* gas production pattern. Crude Protein was higher for *L. esculenta* (220) and lower for *G. ulmifolia* (70). Neutral and acid detergent fiber were higher for *G. ulmifolia* (687 and 554) and lower for *A. farnesiana (*267 and 176). Lignin was higher for *Mimosa sp*. (219) and lower for *P. dulce* (81). Total gas production (ml gas/g DM) of *P. dulce* (187) and *E. cyclocarpum* (164) were higher (P<0.001) than *Mimosa sp*. The lowest values were for *C. alata* (108), *G. ulmifolia* (102), *L. esculenta* (99) and *A. farnesiana* (90). The nutritional characteristics of *L. esculenta* and *A. farnesiana* might be used as supplements in ruminant diets, due to their major content in CP and *in vitro* digestibility, representing an alternative protein supplement during dry season.

Key words: *Acacia farnesiana; Enterolobium cyclocarpum; Guazuma ulmifolia*; *Crescentia alata*; *Leucaena esculenta*; *Mimosa sp*; *Phitecellobium dulce*; *in vitro* gas production; leguminous.

RESUMEN

El objetivo del presente estudio fue determinar la composición química y la producción de gas *in vitro* de diferentes vainas de leguminosas arbóreas. Siete especies de leguminosas arbóreas fueron estudiadas: Cuatecomate (*Crescentia alata)*, Guaje (*Leucaena esculenta*), Guamuchil (*Phitecellobium dulce*), Guazuma (*Guazuma ulmifolia*), Huizache (*Acacia farnesiana*), Mimosa (*Mimosa sp*.) y Parota (*Enterolobium cyclocarpum*). Fueron evaluadas en su composición química (g/kg MS) y producción de gas *in vitro*. La proteína cruda fue superior para *L. esculenta* (220) y menor para *G. ulmifolia* (70). El contenido de fibra neutro detergente y ácido detergente fueron superiores para *G. ulmifolia* (687 y 554) y menor para *A. farnesiana (*267 y 176). El contenido de lignina fue superior para *Mimosa sp*. (219) y menor para *P. dulce* (81). La producción total de gas (ml gas/g MS) para *P. dulce* (187) y *E. cyclocarpum* (164) fueron superiores (P<0.001) a *Mimosa sp*. Los menores valores fueron para *C. alata* (108), *G. ulmifolia* (102), *L. esculenta* (99) y *A. farnesiana* (90). Las características nutricionales de *L. esculenta* y *A. farnesiana* pueden ser utilizadas como suplementos en dietas para rumiantes, debido a su mayor contenido de PC y digestibilidad *in vitro*, representando una alternativa como suplemento proteico en las épocas secas.

Palabras clave: *Acacia farnesiana; Enterolobium cyclocarpum; Guazuma ulmifolia*; *Crescentia alata*; *Leucaena esculenta*; *Mimosa sp*; *Phitecellobium dulce*; producción de gas *in vitro*; leguminosas.

INTRODUCTION

Mexico as an edaphically and topographic country and its climatic characteristics presents an important abundance of natural resources, mainly by the diversity of vegetal species. Nonetheless, in tropical and sub-tropical zones many of the native forages exhibit low quality; nevertheless, several fruits and seeds represent an alternative to supplement food to ruminants as protein sources, which might be utilized as a supplementing source in dry season.

Mexico has around 65% of arid and semiarid zones, among which one finds dry topic, in this zone it is common to observe native tree species which remain foliaged and fruited in dry season; they might be used as a strategy in the alimentation of the animals in this season (Alvarez et al., 2003). In addition to feed animals, the use of brows and shrubby species of natural vegetation can be as diverse, live fences, shade, medicinal, ornamental, etc. (Otarola, 1985; Negreros, 1993; Sosa, 2000). Likewise, they contribute to the sustainability of agricultural systems as they increase the recycling of nutrients, control erosion, improve the physical and biological conditions of the soil and are considered as elements of reforestation of the system (Moechiutti et al., 1995). Mexican browse species differ in their nutritional content depending on the time of the year and the zone where they grow and their utilization may be increased in the case of a multipurpose forage for the small producers, either for human consumption, livestock feeding, fuel, wood, etc; besides. Many producers use these species to build fences and provide shelter for the livestock.

There is very little information on their nutritional value and degradation at ruminal level for this last, the technique of *in vitro* gas production (Menke and Steingass, 1988; Theodorou et al., 1994) allows us to predict fermentation and degradation with very little material, which is easy to reproduce and has a high correlation with the *in vivo* methods (Getachew et al., 1998). The objective of the present study was to determine the chemical composition and *in vitro* gas production of different legume and wild arboreal pods.

MATERIALS AND METHODS

Sampling zones and recollection

Legume pods were collected from San Andres Nicolas Bravo, Municipality of Valle de Bravo, State of Mexico, Mexico; which is located at the southern edge of the occidental part of the State of Mexico, at 18º 45' 75" north latitude and 99º 27' 50" west longitude, at 1200 m above sea level, with sub-humid warm climate, mean annual temperature 34.8 ºC, rains in summer and an annual precipitation of 1.177mm^3 (Schneider, 1999). The criterion used to select the plants was by means of questions to the smallholder of the region on which they used as a feed supplement for their animals in dry season. The pods were collected from September to December, being gathered ten plants (1.0 kg as fresh matter per plant) per species, which were classified as: Mexican calabash (*Crescentia alata*), esculent leadtree (*Leucaena esculenta*), guamuchil (*Phitecellobium dulce*), bastard cedar (*Guazuma ulmifolia*), needle bush (*Acacia farnesiana*), mimosa (*Mimosa sp*.) and elephant ear tree (*Enterolobium cyclocarpum*). Plant samples were mixed, and sub sampled (1.0kg DM) for latter analyses.

Chemical composition

Samples were dried (60ºC, 24 h) and ground (cyclonic mill, 2 mm Ø), in order to determine their organic matter (OM) content, to do so the samples were incinerated at 550ºC for 3 h; the content of nitrogen was calculated (N) by means of the Kjeldalh (Büchi K-370) method using Cu as catalyst, and the content of crude protein (CP) multiplying N × 6.25 (AOAC, 1990, procedure 975.06), the content of neutral detergent fiber (NDF), acid detergent fiber (ADF) and lignin was carried out according to Van Soest et al. (1991) without correction by ashes.

In vitro gas production

The technique of *in vitro* gas production described by Theodorou et al. (1994) was performed, using two cannulated sheep in rumen as ruminal liquid donors. The animals were feed with alfalfa and oath hay (50:50) and 2% of a vitamin-mineral supplement (08:00 and 16:00 h), with water *ad libitum*. Ruminal liquor was collected from the two sheep, being filtered through four layers of cheese cloth gauze, and was gassed with CO_2. The solution was prepared according to the technique described by Menke and Steingass (1988), 0.800 g of DM were threefold weighed in 125ml flasks and incubated with 90 ml of solution and 10ml of ruminal liquid; two flasks with barley hay and two without a sample (blanks) were included as correction factors. Gas production was registered at 3, 6, 9, 12, 24, 36 48 and 72 hours in four series of inoculations. Once the inoculation period concluded, the remainders of fermentation were recovered from the flasks and were dried (60ºC, 48h) in order to calculate the portion disappearance from DM (dDM; mg/g initial DM) and determine relative gas production (RGP, ml gas/g dDM) (Gonzalez Ronquillo et al., 1998). Once the results of ruminal degradation were calculated, the curve of gas production of the feed against time was established using the following equation proposed by Krishnamoorthy et al. (1991) $GP = b\ (1\text{-}e^{-ct})$, where GP represents the accumulative gas production (ml/g DM), b is the total gas production

(ml gas), c is the degradation rate in respect to time (h) and t is incubation time (h).

Statistical analysis

The mean of the three flasks of each sample and incubation series (four) were utilized as experimental unit; in order to study the differences between species and incubation series, they were contrasted by a variance analysis by incubation time, with the interaction of the incubation series by species, $y = m + \text{Plant specie}_i + \text{incubation serie}_j + \text{Plant specie*incubation serie}_k + e_{ijk}$ according to the procedures described by Steel et al. (1997). For all anayses, significance was declared at $P = 0.05$.

RESULTS AND DISCUSSION

Chemical composition

The chemical composition of the forage pods is presented in Table 1, where we observe that *P. dulce* was the pod with the lowest DM. Alvarez et al. (2003) mention that the pod of *E. cyclocarpum* contains 90.5% of DM, being similar to the value obtained in the present study, in respect to most of the pods, Contreras et al. (1995) analyzing the addition of 4% of urea or sodium hydroxide present DM values for the pod of *G. ulmifolia* of 88%. As for the amount of OM, the pod of *G. ulmifolia* presents the highest content in relation to the pods of *P. dulce*, *E. cyclocarpum*, *A. farnesiana*, *Mimosa sp.* and *L. esculenta*, while the pod of *C. alata* presents the lowest content of OM. Alvarez et al. (2003), using diets for ewes, present values of OM similar to those obtained in the present study, likewise Francais (1991) mentions that the dry green fruit of *G. ulmifolia* contains 95% of OM, similar to the one found in the present study. Sosa et al. (2004) present values of OM for the pods of *E. cyclocarpum* of 92.5%, *G. ulmifolia* 89%, *L. esculenta* 88% and *Mimosa sp.* of 93%, which are similar to those obtained in the present study; depending on the region and season of the year the contents of DM and OM will vary (Sosa et al., 2004).

In relation to the amount of CP (g/kg DM), the pods of *G. ulmifolia*, *Mimosa sp.* and *A. farnesiana* contain the lowest concentration, while *C. alata* and *L. esculenta*, and present the highest contents. Alvarez et al. (2003) find CP values for the pod of *E. cyclocarpum* of 172, similar to the ones in the present study but above the findings of Velasco et al. (1996) of 132 for the seed and 150 for the pod, while Velazquez et al. (2005) present values for the pod of *A. farnesiana* of 111 of CP, being similar to that obtained in the present study. Even if we know that in several Mexican states there are numerous species with suitable characteristics to incorporate them into the diet of ruminants, their exploitation is limited (Benavides, 1993; Camero, 1995). Sosa et al. (2004) obtained CP values for the pods of *E. cyclocarpum*, *L. esculenta*, *Mimosa sp.*, and *P. dulce* (19, 30, 12 and 18.5%, respectively), slightly above those in the present study with the exception of the pods of *G. ulmifolia* and *P. dulce* (7 and 14.5%, respectively).

The amounts of NDF and ADF in pods of *G. ulmifolia*, *Mimosa sp.* and *L. esculenta* is greater than *C. alata*, *E. cyclocarpum*, and *P. dulce* and the lowest was presented by *A. farnesiana*; in relation to the amount of Lignin, *Mimosa sp.* is higher in relation to *G. ulmifolia* and *C. alata*, Contreras et al. (1995) obtained values of lignin for the pod of *G. ulcifolia* of 18% treated with 4% of urea, being similar to those obtained in the present study, whilst the amount of ADF is lower than the one obtained in this study. Velazquez et al. (2005) present values of ADL for the pod of *A. farnesiana* at different levels of inclusion with an average of 12.3% being superior to that obtained in the present study; however the content of NDF and ADF is superior in relation to the present study. Sosa et al. (2004) present values of NDF and ADF for the pod of *G. ulmifolia* similar to those obtained in the present study, for the pods of *E. cyclocarpum* and *Leucaena esculenta* the values of the present study are below those obtained by said authors.

Table 1. Chemical composition (g/kg DM) of foraging legume pods

	E. cyclocarpum	*P. dulce*	*L. esculenta*	*A. farnesiana*	*Mimosa sp*	*G. ulmifolia*	*C. alata*
DM[†]	909	883	907	919	913	921	931
OM	957	963	942	948	946	970	925
CP	166	145	220	112	100	70	214
NDF	361	289	505	267	587	687	494
ADF	262	201	389	176	445	554	339
ADL	107	81	107	85	219	187	164

[†] Expressed in fresh matter

DM, dry matter; OM, organic matter; CP, crude protein; NDF, neutral detergent fibre; ADF, acid detergent fibre; ADL, acid detergent lignin.

In vitro gas production

The total *in vitro* gas production (ml/g DM) (figures 1 and 2) of the pod of *P. dulce* and *E. cyclocarpum* were superior (P<0.001) in relation to the rest.

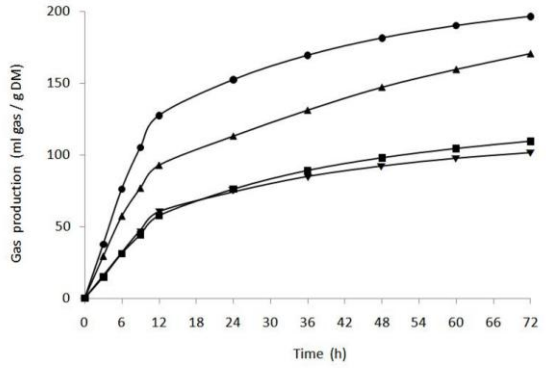

Figure 1. *In vitro* gas production profile of the pods (ml gas/ gDM): *Enterolobium cyclocarpum* (▲), *Phitecollobium dulce* (●), *Crescentia alata* (▼) and *Leucaena esculenta* (■)

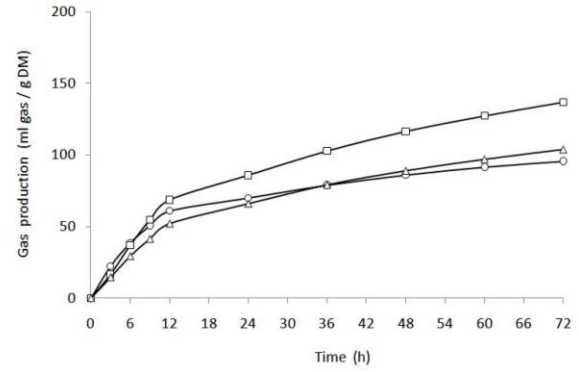

Figure 2. *In vitro* gas production profile of the pods (ml gas/ gDM): *Guazuma ulmifolia* (○), *Acacia farnesiana farnesiana* (□) and *Mimosa sp* (Δ).

The dDM (mg/gDM) (Table 2) presents differences between species (P<0.001), being higher for the pods of *A. farnesiana*, *G. ulmifolia* and *L. esculenta*, in relation to the pods of *E. cyclocarpum*, *P. dulce* (375±36); on the contrary, RGP (ml gas/g dDM) was superior for the pod of *P. dulce* followed by *E. cyclocarpum* and *Mimosa sp.*, in relation to the pods of *C. alata*, *G. ulmifolia*, *L. esculenta* and *A. farnesiana*. This shows an inverse relation between dDM and RGP, which would make us think that the lower the RGP the higher degradability, nonetheless some other anti-nutritional factors of these plants have to be considered, such as the content of tannins (Sosa et al., 2004). Macias and Garcia (2004) present *in vitro* digestibility values of 25% for the pod of *G. ulmifolia* being lower than those of the present study; this may be due to the fact that said authors found a higher percentage of NDF in this species, which decrease its digestibility. Contreras et al. (1995) show digestibility values of 48.5% for *G. ulmifolia*, being lower than the results obtained in the present study. On the contrary, Ortiz et al. (1989) find a digestibility value of 69.5% for the pod of *E. cyclocarpum*, which is superior to the obtained in the present study, these results show there is variability of the results found in the different studies, mainly due to the state of ripeness of the plants, which makes us suggest that the obtained results should be taken cautiously and extrapolated them to every zone of study and season of the year in order to provide a better recommendation to the producers, nevertheless the toxic and anti-nutritional factors presented by some of these species must be considered (Sosa et al., 2004).

Piri et al. (2007), using *A. farnesiana* and *L. esculenta* silage with maize (50:50) to fed sheep, find higher intake and digestibility of protein for *L. esculenta*, being this the best option to use it; our results present a higher content of protein in relation to *A. farnesiana*, even though they present similar dDM and RGP, we might use both *A. farnesiana* and *L. esculenta* as supplementation sources; however, Piri et al. (2007) recommend the use of *A. farnesiana* as a source of supplementation, but they consider that *L. esculenta* presents a lower content of CP, thereby the animals present lower intake of CP, we consider both are alternatives as supplements, being superior *A. farnesiana*, but we have to bear in mind that leguminous pods are an alternative where there is not another source of alimentation. In this case Saha et al. (2008) supplemented goats with *L. esculenta* and *P. dulce* and find protein values of 23 and 25% respectively, for the case of *L. esculenta* our values in CP and digestibility are similar to Saha et al. (2008), not so for *P. dulce* which are inferior, possibly due to the fact that we dehydrated the whole pod with seeds, which increases the content of NDF and OM but decreases its content of CP and digestibility, unlike Saha et al. (2008) where goats could select the pulp and seeds, and with it increase their intake of CP and its digestibility.

In figure 3, the results of total gas production (ml gas/ gDM) are shown on an hourly basis, having the highest production by *P. dulce*, at 12 hours, followed by *E. cyclocarpum*, and *A. farnesiana*, which were superior (P<0.05) to the rest.

Table 2. *In vitro* gas production parameters for foraging legume pods

	E. cyclocarpum	*P. dulce*	*L. esculenta*	*A. farnesiana*	*Mimosa sp.*	*G. ulmifolia*	*C. alata*	SEM
b	164.0^{de}	187.3^{d}	99.1^{f}	89.5^{f}	134.8^{ef}	102.1^{f}	107.7^{f}	6.9
c	0.06^{def}	0.08^{d}	0.06^{def}	0.08^{de}	0.04^{f}	0.04^{f}	0.05^{ef}	0.001
Lag	-0.68^{e}	0.10^{d}	0.05^{d}	-0.39^{de}	-0.59^{e}	-0.71^{e}	-0.14^{de}	0.13
dDM	398.2^{f}	333.7^{f}	618.0^{de}	701.6^{d}	395.4^{f}	649.0^{de}	530.8^{ef}	11.9
RGP	431.9^{e}	591.9^{d}	166.7^{g}	137.4^{g}	346.2^{ef}	167.2^{g}	207.2^{fg}	13.0

b: fermentation rate (h^{-1}); c: fermentation rate ($h^{-1/2}$); Lag: incubation delay time;
dDM: Disappeared dry matter (mg/g); RGP: gas relative production (ml gas/ gDM).
SEM: Standard error Mean
Values expressed in means; different letters indicate significant differences (d < e < f < g, $P < 0.001$).

The results of total gas production (ml gas/g DM) expressed by the hour in function of incubation time are shown in table 3, we observe that at the 3rd hour (H3) there are no significant differences for any of the analyzed legume pods; while at H6 there is a higher production of gas for *P. dulce*, followed by *E. cyclocarpum* and *G. ulmifolia*, being different (P<0.05) to the rest of the pods analyzed in the present study. At H9 we observe *P. dulce* and *E. cyclocarpum* present the highest gas productions, being different (P<0.05) from the rest of pods; while at H12, the highest production (P<0.05) was for *P. dulce*, followed by *E. cyclocarpum* and *A. farnesiana*. The highest production of gas was for *P. dulce* and *E. cyclocarpum*, being different from the rest of the pods used in the present study. Since *leucaena* has a lower effective degradability and slower degradation speed, Delgado et al. (2001) it might be better utilized as it allows part of the protein to pass towards the lower parts of the tract to be used by the animal; it seems as if with *leucaena* protein is more efficiently used (Ørskov, 1992). In figure 3 we observe an initial phase which is of slow or null production of gas, as it is seen at the 3rd hour, during this phase the hydration and colonization of the insoluble substrate by the ruminal microorganisms takes place (Cheng et al., 1980). When the substrate is saturated with microorganisms and enzymes, it enters into a phase where the most degradable part of the potentially soluble substrate is firstly degraded, as it occurs in the 9, 12, and 24 hours in the present study, and the least digestible substrate needs more time to be degraded; when finally the substrate is degraded the production begins to decrease, as it is observed as of the 36 until the 72 hour (Beuvink et al., 1993). Because of this the beginning of the fermentation of microbial population becomes a limiting factor, and by the end of fermentation it is the availability of fermentable substrate what limits the rate of gas production (Schofield et al., 1994).

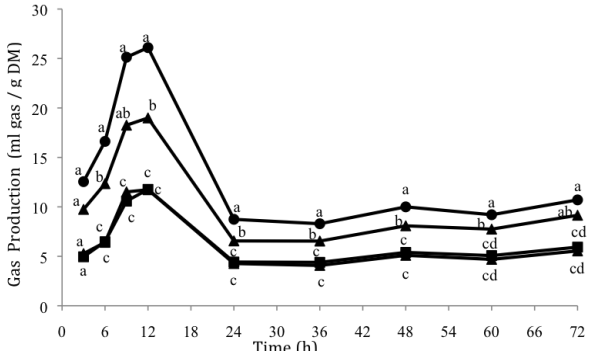

Figure 3. Ruminal fermentation rates for the legume pods (ml gas/ gDM): *Enterolobium cyclocarpum* (▲), *Pithecellobium dulce* (●), *Crescentia alata* (▼), *Leucaena esculenta* (■).

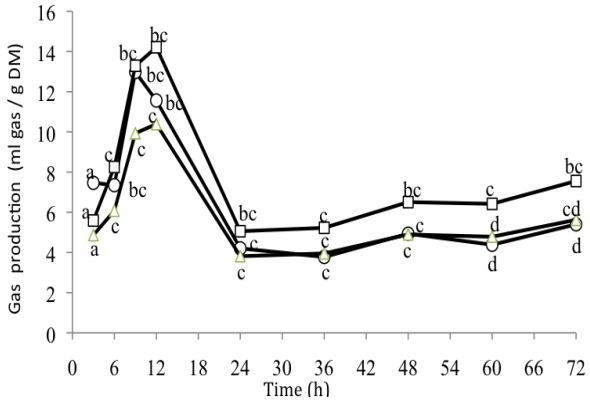

Figure 4. Ruminal fermentation rates for the legume pods (ml gas/ gDM): *Guazuma ulmifolia* (○), *Mimosa sp.* (Δ), *Acacia farnesiana* (□).

Table 3. Parameters of *in vitro* gas production (ml/g DM) of foraging legume pods in function of fermentation time

Time (h)	*E. ciclocarpum*	*P. dulce*	*L. esculenta*	*A. farnesiana*	*Mimosa sp*	*G. ulmifolia*	*C. alata*	SEM	P *Value*
3	2.50^{a}	2.50^{a}	2.50^{a}	2.50^{a}	2.50^{a}	2.50^{a}	2.50^{a}	0.01	0.0001
6	9.74^{b}	12.5^{a}	4.95^{c}	5.59^{c}	4.86^{c}	7.46^{bc}	5.27^{c}	0.56	0.0001
9	12.39^{ab}	16.61^{a}	6.47^{c}	8.25^{bc}	6.07^{c}	7.35^{bc}	6.41^{c}	1.07	0.0001
12	18.25^{b}	25.13^{a}	10.58^{c}	13.29^{bc}	9.93^{c}	12.98^{bc}	11.51^{c}	1.14	0.0001
24	18.98^{b}	26.09^{a}	11.76^{c}	14.21^{bc}	10.38^{c}	11.56^{c}	11.73^{c}	1.42	0.0001
36	6.55^{b}	8.74^{a}	4.42^{c}	5.05^{c}	3.81^{c}	4.20^{c}	4.27^{c}	0.30	0.0001
48	6.54^{b}	8.29^{a}	4.39^{c}	5.22^{bc}	3.95^{c}	3.77^{c}	4.07^{c}	0.34	0.0001
60	8.08^{b}	10.00^{a}	5.40^{cd}	6.50^{c}	4.89^{d}	4.93^{d}	5.10^{cd}	0.31	0.0001
72	7.75^{ab}	9.20^{a}	5.07^{cd}	6.41^{bc}	4.78^{cd}	4.38^{d}	4.69^{cd}	0.38	0.0001

SEM, standard error mean.
Values expressed in means, different letter indicate significant differences (a < b < c < d, $P < 0.0001$).

CONCLUSION

The nutritional characteristics of some species of arboreal pods, such as *L. esculenta* and *A. farnesiana* allow them to be used as a complement in the diet of ruminants because of their higher content of protein and *in vitro* gas production, representing an option as a protein supplement in dry season. It is necessary however to broaden the knowledge on the identification of other species with potential to feed ruminants, their nutritional value and anti-nutritional values, conditions of handling and use by the producers for the benefit of the animals.

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