Nutritional value of foliage meal from four species of tropical trees for feeding ruminants

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In order to evaluate the nutritive value of foliage meals of moringa (*Moringa oleifeira* cv. Super Genius), mulberry (*Morus alba* Linn cv. Cubana), trichanthera (*Trichanthera gigantea*) and leucaena (*Leucaena leucocephala* cv. Perú) for feeding ruminants, their chemical composition was determined, the *in vitro* gas production was measured up to 96 h of incubation, and the *in vitro* degradability of dry matter, neutral detergent fiber and nitrogen was estimated. The gas production was measured up to 96 h, to estimate the parameters of fermentation kinetics, according to the model of Gompertz. A random block design was applied, with four incubations as repetitions and the tree species as treatments. The organic matter of moringa and leucaena surpassed 90 % and trichanthera showed low values (72 %). The crude protein was superior to 20 % in every treatment. The neutral detergent fiber varied between 30 and 50 % and the mulberry showed the lowest values. There was interaction between sampling time and treatment for gas production ($P<0.001$). The highest values were observed in the initial stage for moringa and mulberry, at 16 h, while the highest productions of the final stage corresponded to mulberry (96 h). The potential of mulberry fermentation tended to be higher (189.5 mL g^{-1} incOM), like the values of microbial efficiency factor for moringa (0.149 h^{-1}). Mulberry and moringa showed high values of maximum speed and gas production (V_{max}: 8.36 and 8.08 mL g^{-1} incOM h^{-1}, respectively) and trichanthera showed the lowest value (1.29). *In vitro* degradability of dry matter and neutral detergent fiber were high for mulberry and low for trichanthera ($P<0.001$). Leucaena showed higher *in vitro* digestibility of nitrogen (79 %), followed by mulberry moringa and trichanthera ($P<0.05$). It can be concluded that, under experimental conditions, trichanthera had low potential as food, while mulberry and moringa showed higher nutritional value than leucaena, although this last demonstrated superior potential as supplement protein degraded within the rumen.

Key words: *Moringa oleifeira*, *Trichanthera gigantea*, *Morus alba*, *Leucaena leucocephala*, *fermentation*

Food is, from the financial point of view, the most important element of a cattle rearing system, because it contributes up to 70 % of production costs (Makkar 2014). In tropical areas, the supplementation of diets based on pastures is necessary for assuring a proper supply of nutrients, mainly for ruminants of high productive potential (Jayanegara and Sofyan 2009).

However, the use of concentrates as conventional supplements is almost "prohibited" for small and medium-sized producers. Therefore, shrubs are every time more commonly used as supplements in the feeding systems of ruminants in tropical and subtropical areas (Melesse 2012). The tree species are important due to their high protein content, their contribution with easy fermentation carbohydrates and fiber of better degradability, as well as their positive effect on the use of nitrogen (N) within the rumen. These elements allow to increase the productivity of animals fed with pastures (Rubanza *et al.* 2007). Although *Leucaena leucocephala* is the shrub species more used for cattle rearing in Cuba and many other countries of tropical or subtropical climate (Shelton 1996), there has been, in the last years, a growing interest in using other multipurpose shrub species, like *Moringa oleifeira*, *Morus alba* and *Trichanthera gigantea*.

Combining the analysis of chemical composition with the estimation of parameters of fermentation and *in vitro* degradability kinetics is very important for predicting the nutritional value of a food (Mould 2003 and Mtui *et al.* 2009). The objective of this study was to evaluate the nutritional value of foliage meals of *M. oleifeira*, *M. albus*, *T. gigantea* and *L. leucocephala*, through the analysis of their chemical composition, gas production and *in vitro* degradability.

Materials and Methods

Plant material. The foliage meals of four tropical tree species were used: moringa (*M. olifeira* L, cv. Super Genius), mulberry (*M. alba* Linn cv. Cubana), trichanthera (*T. gigantea* Humbolt et Bonpland, Ness) and leucaena (*L. leucocephala* cv. Perú). These species were collected during October, 2012, in experimental areas from the Instituto de Ciencia Animal, located in San José de las Lajas, Cuba. Mainly young stems and leaves were taken from random plants. Every plant used in sampling had several years of establishment in a typical red ferrallitic soil, without irrigation and fertilization, except those of *M. oleifeira* that had only ten months of establishment. The plant material was collected at a rate of around 200 g of fresh matter from each tree, until obtaining around 2 kg per each species. All the collected material was dried for 72 h inside a forced air oven, with a regulated temperature (60 ºC). Later, it was grounded in a hammer mill, until reaching a particle size of 1 mm, and it was properly stored in sealed nylon bags.

Experimental procedure. The *in vitro* technique of gas production in glass bottles, described by Theodorou *et al.* (1994), was applied. An amount of 1.0 g of dry

matter (DM) of each treatment was incubated in bottles of 100 mL, in a culture medium (Menke and Steingass 1988) and an inoculum of ruminal microorganisms, in a proportion of 0.20 of the total volume of incubation (80 mL).

The inoculum used was the ruminal content of two cows with cannula in the rumen, fed *ad libitum* with forage of grasses and free access to water and mineral salts. The ruminal content of each animal was collected before offering the morning food. Later, it was kept in closed thermos until getting to the lab, where it was filtered with several layers of gauze. The two inocula were mixed in equal proportions. During the process, the temperature of the inoculum was 39 ± 1 ºC, and the conditions of anaerobiosis were kept through the continuous flow of CO_2. The bottles were sealed and incubated in a bath, at a controlled temperature (39 ºC). That moment was considered as the starting time of incubation.

Two experiments were carried out, each one with four repetitions in time, and in each repetition, four bottles per treatment were used as well as four bottles with control, without substratum, to know the gas contribution of the microbial inoculum.

Experiment 1. Gas production was measured at 2, 4, 6, 8, 10, 12, 16, 20, 24, 36, 48, 72 and 96 h using a manometer HD8804, connected to a pressure calibrator TP804 (DELTA OHM, Italy). After each measuring, the gas was released until the external pressures were equal to the internal pressure of the bottles. The volume of gas was estimated using the pressure data of a previously established equation of linear regression:

Gas (mL)= (pressure [10^3 Pa]+4.95)/2.5858), n= 132; r= 0.991) (Rodríguez *et al.* 2013).

The volume of gas was expressed by grams of incubated organic matter (incOM). In order to estimate the kinetics of gas production, the single-phased model of Gompertz was used:

$Y = A*Exp (-B*Exp(-C*t))$

Where:

Y- gas production in time t (mL g^{-1} incOM)

A- potential of gas production (asymptote when t= ∞; mL g^{-1} incOM).

B- relative rate of gas production

C- constant factor of microbial efficiency (h^{-1})

t- time of incubation (h)

Besides, the time, in which the maximum speed (Tv_{max}) of gas production was reached, was estimated according to the second derivative from the model of Gompertz, evaluated in zero (inflexion point of the sigmoidal model). The V_{max} (mL g^{-1} incOM h^{-1}) was also estimated, when T_{Vmax} was replaced the first derivative of the model.

At the end of incubation (hour 96), the bottles were opened and its content was filtered through nylon bags. The bags with the fermentation residues were dried for 72 h in a forced air oven, with a regulated temperature (60 ºC). Using the gravimeter method, the *in vitro* degradability of dry matter (IVDDM) and the *in vitro* degradability of neutral detergent fiber (IVDNDF) were determined as the difference between DM or NDF within the incubated substrate and within the solid residue of fermentation (96 h), divided by the DM or NDF incubated in each bottle, respectively (Blümmel *et al.* 1997).

Metabolizable energy (ME) and digestibility of organic matter (DOM) of forage meal from four shrubs, at 24 h, were estimated through the equations proposed by Menke *et al.* (1979):

ME (MJ kg^{-1} DM) = 2.20 + 0.136 GP_{24h} + 0.057 CP

DOM (%) = 14.88 + 0.889 GP_{24h} + 0.45 CP

Where:

GP_{24h} is the volume of gas produced at 24 h (mL• 200 mg^{-1} incubated DM) and the CP is expressed in percent.

Experiment 2. The amount of solubized N was estimated according to the procedure of Raab *et al.* (1983), modified by Mota *et al.* (2005). This procedure is based on the determination of N-NH_3 and gas production. To estimate the relation between gas production and the inclusion of N-NH_3 to the microbial protein, treatments were incubated by themselves and with two levels of soluble starch (150 and 300 mg, respectively), at a rate of three bottles per treatment and level of starch. The dissolved N was calculated after 24 h of incubation, through the linear regression of the amount of N-NH_3 ("Y" axis, mg N-NH_3) versus gas production ("X" axis, mL gas). The intercept of "Y" axis was considered as the amount of N-NH_3 released to the environment when there was no available energy (zero microbial synthesis). The difference between this value and the amount of N-NH_3, determined in the controls, indicated the amount of N dissolved during the degradation of nitrogen compounds. The *in vitro* degradability of nitrogen (IVDN) was estimated as the quotient between dissolved N and total N content of the treatments.

Chemical analysis. The DM, OM and CP of foliage meals were determined according to AOAC (1995), and the NDF was obtained by the procedure described by van Soest *et al.* (1991). In addition, the content of DM and NDF was determined in the solid residues of shrub fermentation. The concentration of ammonia was quantified according to Conway (1957). The analyses were performed three times.

Statistical and experimental design. A random blocks experimental design was used in both experiments. The incubations performed were considered as replications (4), the foliage meals of the evaluated tree species as treatments, and the averages of variables, measured per treatment in each replication, were considered as an experimental unit.

Results of gas production, being repeated measures in the same experimental unit, were analyzed by

MANOVA, in order to prove if there was an interaction between treatments and sampling time. To agree the number of times in which the gas production was measured, according to MANOVA, the analysis of fermentation was divided a priori into two stages with four sampling times each. The first stage was stated from the beginning of fermentation to 16 h, and the second from that moment up to 96 h. When the interaction between treatments and times was confirmed, an analysis of variance of split plots was performed to the means of these interactions.

The remaining studied variables were analyzed with ANOVA. The statistical package InfoStat (Di Rienzo *et al.* 2010) was used for both analyses. When differences ($P < 0.05$) were found, treatment means were compared using the multiple range test of Duncan (1955).

Results and Discussion

Table 1 shows the chemical composition of the four tree species. Values of OM varied between 72 and 92 %, with moringa and leucaena higher than 90 %, mulberry had intermediate values and trichanthera showed the lowest values. The low OM contents of trichanthera coincide with those reported by other authors (Delgado *et al.* 2007 and García *et al.* 2008). The levels of OM in mulberry were similar to those obtained by Delgado *et al.* (2007), but lower than those found by García *et al.* (2008).

Regarding the CP, all foliage meals had values over 20 % of the DM, with leucaena as the species of the highest protein content (27 %). These results coincide with the tendency observed in other studies with shrubs (García *et al.* 2008, Edwards *et al.* 2012 and Aye and Adegun 2013), although there is a wide variation in the values found in the literature, probably due to the effect of the parts of collected plants, their phenological state, season in which it was collected, cut frequency and environment where collected material was developed, on the results (Edwards *et al.* 2012).

The values of NDF varied between 30 and 50 %. The lowest values corresponded to mulberry. This performance coincided with the tendency informed by García *et al.* (2008), but, overall, the results were superior to those informed for moringa, trichanthera and leucaena (Mtui *et al.* 2009 and Asaolu *et al.* 2011).

The analysis of MANOVA of *in vitro* gas production showed that, in both stages of fermentation, the interaction occurred between treatments and times in which the gas was measured (table 2). In the initial stage of fermentation, all legumes showed high values of accumulated production of gas, while incubation time increased. The lowest values of gas production belonged to trichanthera, at 4 h of incubation, while the highest values appeared at 16 h for moringa and mulberry, which had no differences between each other ($P < 0.001$). In the final stage of fermentation, the same performance of incubation time was confirmed. Mulberry produced higher amount of gas at 96 h of incubation, followed by moringa, leucaena and trichanthera, which again showed the lowest gas productions, regarding the remaining evaluated meals ($P< 0.001$).

A high gas production indicates higher availability of OM of the substratum to be fermented by ruminal microorganisms. This is related to quality, digestibility and energy value of the evaluated food (Menke *et al.* 1979 and Mtui *et al.* 2009). The high gas production of mulberry foliage meal may be attributed to its low content of NDF and its high content of easy fermentation carbohydrates. Meanwhile, the low gas productions of trichanthera may be related with its high fiber contents (Delgado *et al.* 2007). Besides, it is known that there is a negative correlation between the content of NDF of a substratum and the extent of the production of gas obtained from its fermentation (Njidda and Nasiru 2010).

Table 3 shows the kinetic parameters of *in vitro* fermentation of the four evaluated foliage meals. Mulberry had the highest potential value of gas production (parameter A) and trichanthera and leucaena had the lowest. However, no significant differences in the potential of gas production between Mulberry and Leucaena were reported by Mtui *et al.* (2009). The lowest potential of gas production of leucaena may be related to its high levels of CP, because the protein contributes less to gas production than carbohydrates. It is known that the level of CP in a substratum is negatively correlated to gas production resulting from its *in vitro* fermentation (Getachew *et al.* 2004).

Regarding the parameter C, moringa had the highest values of this constant factor of microbial efficiency, and trichanthera had the lowest ones. When estimating V_{max}, it was confirmed that mulberry and moringa had the highest values and trichanthera, the lowest values. However, the inflexion point of the sigmoidal curve, which describes speed changes of *in vitro* gas production within time, indicated that V_{max} was reached in lower times by moringa (9 h), mulberry (11 h) and leucaena

Table 1. Chemical composition of the evaluated tree foliage meals (%).

Specie	Residual DM	OM	CP	NDF
Moringa	90.2	91.8	22.2	40.5
Morera	93.3	82.0	22.5	30.2
Trichanthera	94.1	72.0	20.3	49.9
Leucaena	91.3	90.7	27.0	45.5

Table 2. Profile of *in vitro* gas production (mL g^{-1} inc OM) of the four evaluated tree foliage meals.

Stage	Treatment	Times				Sign.
		4	8	12	16	
Initial	Moringa	19.34^{cd}	52.21^{g}	90.82^{j}	108.68^{k}	P < 0.0001
	Morera	16.68^{c}	48.03^{f}	86.15^{i}	111.45^{k}	
	Trichantera	6.27^{a}	12.14^{b}	18.12^{c}	22.77^{d}	
	Leucaena	12.43^{b}	28.70^{e}	47.27^{f}	60.30^{h}	

SE of times at the same level of treatments ± 2.96
SE of treatments at the same or different level of times ± 0.77
^{abcdefghijk}Different letters indicate significant differences at P < 0.05 (Duncan 1955)

Stage	Treatment	Times				Sign.
		24	48	72	96	
Final	Moringa	135.31^{f}	151.5^{g}	158.41^{h}	162.43^{i}	P < 0.0001
	Morera	147.36^{g}	176.32^{J}	189.49^{k}	197.31^{k}	
	Trichantera	34.20^{a}	67.26^{b}	95.20^{d}	110.90^{e}	
	Leucaena	84.2^{c}	111.62^{e}	126.67^{f}	136.11^{f}	

SE of times at the same level of treatments ± 6.50
SE of treatments at the same or different level of times ± 1.60
^{abcdefghijk}Different letters indicate significant differences at P < 0.05 (Duncan 1955)

Table 3. Kinetic parameters of *in vitro* fermentation of foliage meals of evaluated tropical shrubs

Foliage meal	Parameter A (mL g^{-1} incOM) (±SE)	Parameter B (±SE)	Parameter C (h^{-1}) (±SE)	SE Curve	R^2 Curve	V_{max} (mL g^{-1} incOM h^{-1})	TV_{max} (h)
Moringa	147.46 (1.397)	3.72 (0.204)	0.149 (0.0059)	9.210	0.983	8.08	8.82
Morera	189.48 (2.540)	3.72 (0.247)	0.120 (0.0060)	5.530	0.989	8.36	10.95
Trichanthera	113.15 (3.125)	3.00 (0.065)	0.031 (0.0014)	2.735	0.992	1.29	35.44
Leucaena	118.97 (2.039)	2.78 (0.142)	0.074 (0.0041)	5.890	0.979	3.24	13.82

(14 h), regarding trichanthera, which reached it at a four times higher time than moringa.

The parameter C, as the V_{max} in which a food is fermented, shows the growth of ruminal microorganisms and the accessibility of the microbial enzymes to nutrients of this food (Getachew *et al.* 2000). The performance of these parameters, in the case of moringa and mulberry, may be related to their high content of easy fermentation carbohydrates, while the high levels of NDF in leucaena and trichanthera make the nutrients less accessible to the action of ruminal microorganisms (Akinfemi *et al.* 2009).

Figure 1 shows time changes of gas production speed of evaluated treatments. Profiles of moringa and mulberry had a similar performance, with a linear and accelerate increase until reaching the V_{max}, and an also fast drop of speed, after surpassing the inflexion point, although both species reached the minimum speed values in different moments (48 and 60 h for moringa and mulberry, respectively). Leucaena showed a less well-defined curve due to a low acceleration and deceleration in the growing and decreasing phases of the curve, respectively. It also reached the minimum speed values, at around 72 h. Finally, trichanthera had a discrete speed curve regarding the others, and at 96 h, the fermentation was still in process.

Results obtained from measuring *in vitro* gas production indicated the high nutritive value of the varieties of moringa and mulberry, regarding those of leucaena and trichanthera. Moringa, like mulberry, are considered as alternative supplements, with a fermentation pattern, similar to that of concentrated feeds and a nutritional value that widely surpasses that of traditional forages (González 2010 and Jayanegara *et al.* 2010).

Table 4 shows data related to degradability of estimated ME, DM, NDF and OM. The IVDDM, at 96 h, showed the highest values for mulberry (80 %)

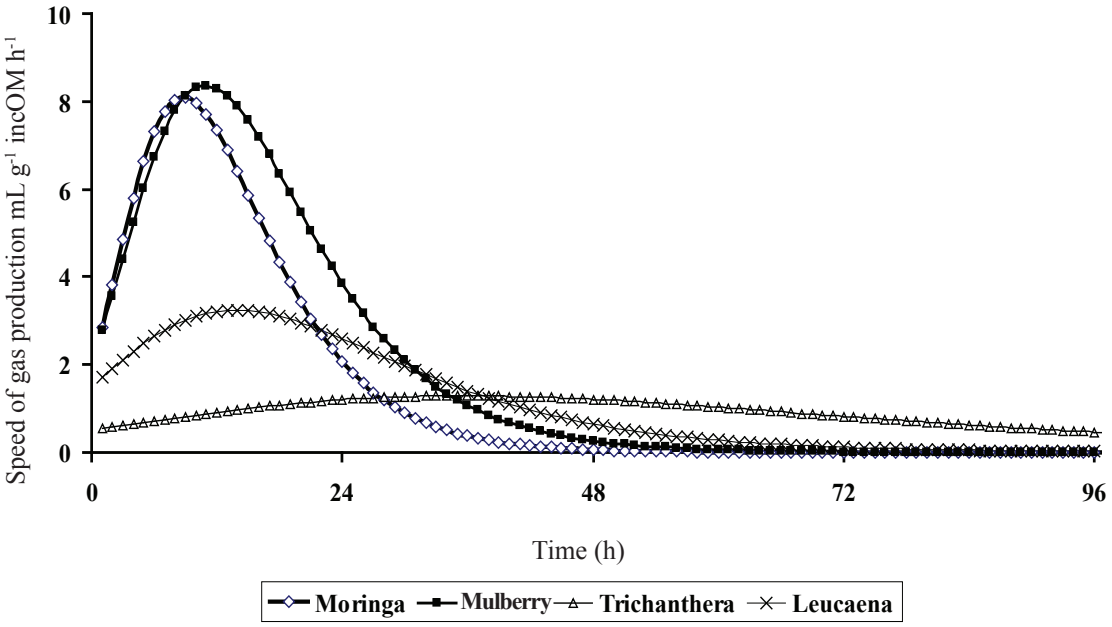

Figure 1. Speed performance of *in vitro* gas production within time, for each evaluated tree species

and the lowest for trichanthera (37 %), while moringa and leucaena had intermediate values, and there was no difference among them (P < 0.001). The IVDNDF had a similar performance but differences were found between moringa and leucaena (P < 0.001). Likewise, DOM estimated at 24 h of incubation followed a similar pattern than the case of IVDNDF (P< 0.05), but, in the case of ME had a particular performance: leucaena> mulberry> moringa> trichanthera (P < 0.001).

The high degradability of DM of foliage meals of tropical shrubs is precisely what turns them into proper supplements for ruminants, fed with diets based on low quality forages (Ndemanisho *et al.* 1998). The high degradability of mulberry and moringa coincided with the high values of gas production and the potential of gas production, informed for these species, which are even superior to those of forages of more traditional use, like leucaena (García *et al.* 2006 and Njidda and Nasiru 2010). However, other studies, in which these four species were compared, confirmed the highest degradation of DM and OM of moringa, over mulberry and leucaena (García *et al.* 2008). In the case of trichanthera meal, other authors agree that the use of this shrub in livestock systems may be limited due to its low digestibility (Edwards *et al.* 2012).

Generally, differences in degradability of substrates are related to their fiber and CP contents. The evaluated species have low representation of toxic metabolites and low concentration of possible non nutritional factors (García *et al.* 2006), so, everything seems to indicate that the level of NDF influenced on the differences, because the content of CP of the four forage meals was superior to 20 %. Besides, Njidda and Nasiru (2010) reported a negative correlation between the NDFcontent of substrates and its DM degradability.

Figure 2 shows the IVDN of evaluated species, estimated in experiment 2, at 24 h of incubation. The superior values of N degradability corresponded to leucaena (79 %), followed by mulberry, moringa and trichanthera (P < 0.05). The highest N degradability of leucaena meal, despite of its confirmed high content of anti nutritional factors, mainly condensed tannins (García *et al.* 2008), may be caused by the fact that some tannins do not affect CP degradability due to their nature, chemical properties or concentration (Norton 1994).

Although it is known that ruminal degradability

Table 4. Degradability of DM and NDF (96 h), and estimated ME and DOM (24 h) for the four evaluated foliage meals.

Species	IVD DM (%)[1]	IVD FND (%)	DOM (%)	ME (MJ kg^{-1} DM)
Moringa	52.44^{b}	63.75^{c}	59.34^{c}	4.93^{b}
Morera	79.76^{c}	83.47^{d}	60.38^{d}	5.06^{c}
Trichanthera	37.18^{a}	40.08^{a}	43.93^{a}	3.73^{a}
Leucaena	51.99^{b}	56.11^{b}	56.42^{b}	6.30^{d}
SE (±)	2.02	1.77	1.06	0.09
P	< 0.001	< 0.001	< 0.001	< 0.001

^{abcd}Different letter indicate differences among treatments of a same column at P< 0.05 (Duncan 1955)

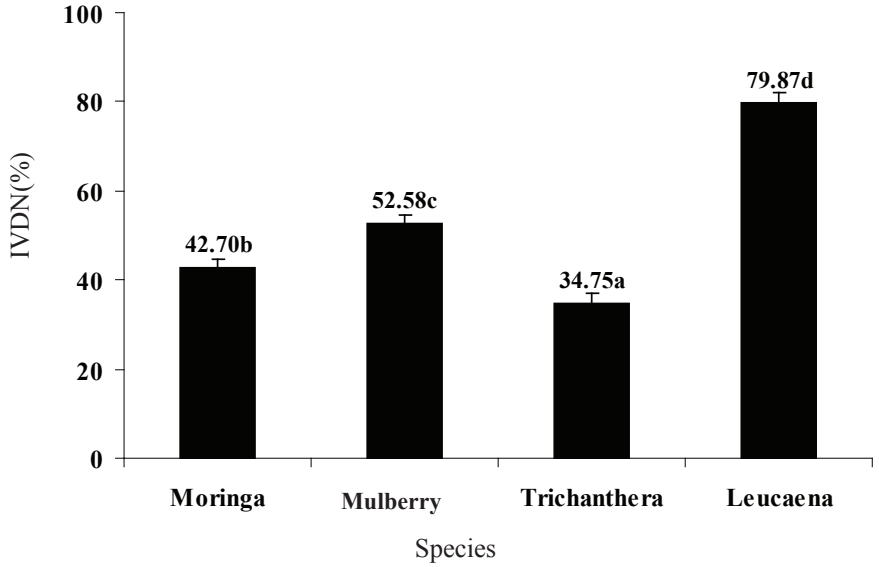

Figure 2. *In vitro* degradability of N at 24 h of incubation of evaluated tree species (P< 0.05).

of CP of leucaena may be high, regarding the other tropical forage species (Cáceres and González 1998, Pinto *et al.* 2002 and Benavides 2003), the IVDN do not coincide with the results previously obtained, in the evaluation of this species under *in vitro* conditions (Mota *et al.* 2005). These authors informed a particular pattern in the use of the nitrogen of this shrub, when between 30- 32 % of all CP is degraded within the rumen and 27- 30 % of CP content is added as a surpassing and digestible protein from the small intestine. This, under production conditions, would probably give a balanced contribution of N for microbial synthesis within the rumen and for meeting the needs of surpassing protein of animals (Rodríguez *et al.* 2009 and Naranjo 2014).

It is important to highlight that values of N degradability had a similar performance to that of the CP level of evaluated meals. These aspects do not have to be related but they indicate that leucaena, among the evaluated meals, from the N point of view, has the highest potential for providing nitrogen compounds to the rumen. However, Paengkoum *et al.* (2013) observed a higher *in situ* degradability of CP in moringa, with regard to leucaena.

Results of chemical composition, gas production kinetics and *in vitro* degradability allow to conclude that trichanthera has low potential as feed for improving the nutritive value of ruminant diet, while the evaluated varieties of mulberry and moringa have higher nutritional value than leucaena. However, from the point of view of N content and degradability of this fraction, this variety of leucaena showed the highest potential as supplement of degradable protein within the rumen.

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