Nutritional value and kinetics of the ruminal fermentation of flowers, tree fruits and shrubs in the Cauto Valley, Cuba

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The nutritional value and ruminal fermentation kinetics was studied in flower samples (*Baryxylum inerme* Roxb, *Tabebula maestrensis* Urb. and *Hibicus rosasinensis*) and fruit trees and shrubs (*Samanea saman* Merr, *Licania campestre*, *Prosopis chilensis*, *Lysiloma bahamensis*, *Eugenia psiloclados* Urb. and *Moringa oleifera* Lam) of the Cauto Valley in Cuba. The chemical constituents, the gas production at different incubation times, the non-degraded apparent residue, the concentration of volatile fatty acids and the *in vitro* true digestibility of OM and DM were determined. The DM, OM, ash and EE differed ($P < 0.05$) between species, while the CP and NDF reached values of up to 26 and 25 %, respectively, without differences. The production of gases derived from the fermentation of the degradable fraction b differed ($P < 0.05$) in the flowers and pods with seeds. In the flowers, the superior values for this variable were found in *H. rosasinensis*, while, in the group of pods with seeds were in *L. brahamensis*. In the flowers, the constant rate of gas production c was higher in *T. maestrensis* (0.081/h), being superior (0.115 /h) in the pods with seeds *E. psiloclado*. There were differences ($P < 0.05$) in the true digestibility of DM and OM in flowers and pods with seeds. The concentration of volatile fatty acids in pods with seeds varied ($P < 0.05$), while in the flowers, only the acetic acid was different ($P < 0.05$). The flowers of *T. maestrensis* and *H. rosasinensis*, as well as the pods with seeds of *S. saman*, *P. chilensis* and *E. psiloclados* could be alternatives for cattle supplementation. Researches to assess the productive response of the animals through the use of these species and their performance under different management conditions and edaphoclimatic regions are recommended.

Key words: *in vitro, gas production, chemical composition, digestibility.*

In the Eastern part of Cuba, the productive efficiency of cattle rearing has decreased as consequence of extreme natural events, such as draught and hurricanes. The 54.7 % of the soils are salinized, 78 % are poorly productive and 42 % of the areas have strong erosive processes. Compactness and deforestation are always present as other degradation ways. Specifically, there is high salinity and soil erosion in Cauto Valley. However, some trees and legumes are highly persistant and can develop on these hostile environments (Benítez *et al*. 2009). Certain plants like *Pithecellobium dulce*, *Clitoria ternatea*, *Tamarindos indica* and *Cordia alba*, are well consumed by grazing animals in Cauto Valley. Nevertheless, the nutritional contribution of other plants should be assessed.

The nutritional value of ruminants' feeding is commonly estimated from the characteristics of their chemical components, as well as from their fermentation pattern (Hamid *et al*. 2007). The *in vitro* procedures are valuable tools for estimating the association of the constant degradation rate or gas production with the feeds characteristics and how microorganisms obtain nutrients to favor their growth (Sanon *et al*. 2008).

The objective of this study was to determine the nutritional value and ruminal fermentation kinetics of flowers and fruits of some trees and shrubs in the Cauto Valley.

Materials and Methods

Localization. The study was conducted in the Cattle Experimental Station of the Agricultural Research Institution "Jorge Dimitrov", located at 11 km of Bayamo city, Cauto Valley, Granma province, Cuba. The soil of this region is vertisol type (Hernández *et al*. 1999), of mid salinity. The accumulated rainfall do not surpass 700 mm.

Samples collection. The experimental units were 15 samples, randomly collected from different parts of the plant: flowers of white oak (*Baryxylum inerme* Roxb), framboyán amarillo (*Tabebula maestrensis* Urb) and mar pacífico amarillo (*Hibicus rosasinensis*). Samples were also taken from pods with seeds of algarrobo del país (*Samanea saman* Merr), carbonero (*Licania campestre*), algarroba china (*Prosopis chilensis*), soplillo (*Lysiloma bahamensis*), yarúa (*Eugenia psiloclados* Urb) and paraíso francés (*Moringa oleifera* Lam). The samples were dried at 55 ºC for 48 h, in an air-forced oven. They were ground at 1 mm and placed in cristal flasks hermetically closed for their analysis.

Chemical analysis. The contents of DM, ash, OM, EE and CP were determined according to AOAC (1997). The NDF was quantified according to Goering and van Soest (1970).

In vitro gas production. A total of 200 mg of samples

were incubated in glass calibrated syringes of 100 mL (Häberle Labortechnik, Alemania). As inoculum, 30 mL of the buffer solution was used: ruminal liquid (2:1). This last was obtained from three goats fed alfalfa hay and commercial concentrate (75:25). The gas production was recorded at 0, 3, 6, 9, 12, 24, 48, 72 and 96 h (Menke *et al*. 1979).

The data of *in vitro* gas production (mL/g DM) were adjusted to the model $G=b \times (1-e^{-k\,(t-L)})$ (France *et al*. 2000), where:

G is gas production in time t

b is the gas volumen (mL) corresponding to the complete digestion of the substrate (asymptote)

k is the constant gas production rate (/h) of the potentially degradable material

L is the colonization time, previous to the beginning of the gas production

The ME content was estimated in agreement with Grings *et al*. (2005) from the *in vitro* gas production:

$ME\ (MJ\ kg\ DM^{-1}) = (2.20+0.136GP_{24h}+0.057CP+0.0029SE^2)$, where:

GP is gas production at 24 h

Non-degraded apparent residue. A total of 500 mg of sample per triplicate were used. They were incubated in glass syringes, calibrated with 40 mL of inoculum. After 24 h of incubation, the syringes content was transferred to plastic tubes and centrifugated at 13 500 rpm for 30 min. The floating material was thrown away. The granulate was washed with distilled water and centrifugated again. The precipitate, formed by non-digested apparent substrate and microbial mass, was lyophilized. The non-degraded apparent residue was estimated by difference between the incubated sample and the lyophilized residue.

Determination of in vitro volatile fatty acids concentration. The samples of flowers and pods with seeds were incubated by triplicate for the *in vitro* gas production. After 24 h of incubation, the syringes were cold and the content was centrifugated at 1000 x g for 20 min. In plastic tubes of 10 mL, 5 mL of the floating material were placed, containing 1 mL of metaphosphoric acid at 25 % (W/V). They were centrifugated at 1000 x g for 20 min to determine later the volatile fatty acids concentration (Getachew *et al*. 2004) with a gas chromatographer Perkin Elmer (AutoSystem XL), with column PE-WAX (30 m length and 0.25 mm diameter).

In vitro true digestibility of the DM and OM. The determinations were made according to the DaisyII procedure (Adesogan 2005). At about 250 mg of sample were deposited in filter bags (10 x 5 cm and 40-60 µM; Ankom Technology®) and were incubated at 39°C for 48 h in ruminal liquid buffer solution. The inoculum was obtained from four adult Rambouillet lambs fed forage and concentrate in proportion of 75:25.

Statistical analysis. The gas production parameters b, c and L were estimated through PROCNLIN (Cody and Smith 1997). The data of *in vitro* gas production, ME, chemical composition, digestibility and volatile fatty acids were treated with analysis of variance, according to PROC GLM (Cody and Smith 1997) for a completely randomized design. The differences between means were found with the Tukey test. Correlation analysis was conducted between the chemical composition and the *in vitro* gas production (Steel and Torrie 1980).

Results and Discussion

The flowers of the plant varied ($P < 0.05$) in their DM, OM, ash and EE content (table 1). This could be due to the adaptation degree and the responses to the diverse conditions of draught and saline soils they were developed in (La O *et al*. 2008 and 2009). Besides, under physiological stress conditions, the plants are capable of develop defense mechanisms as to produce secondary metabolites and change some ways of storing and using the chemical components of the plants (Rodríguez 2004). Although this aspect was not subject of study, for the specific case of these plants, further researches to prove this hypothesis and their relation with the ecosystem of high draught and salinity of the soils are needed.

The CP and NDF contents of the flowers reached values of up to 26 and 25 %, respectively, without differences between them. These values are superior to those reported by Ammar *et al*. (2004) in *Cytisus scopanus* flowers in Spain.

The CP concentrations in pods with seeds reached up to 35 % in carob bean ($P < 0.05$), while the NDF values did not surpass 36 % in paraíso francés, with differences between the plants studied ($P < 0.05$). A similar pattern was recorded in the contents of DM, OM, ash and EE ($P < 0.05$). These results were inferior to those reported by La O *et al*. (2008 and 2009) in *Cordia alba*, in pods with the arils of *Pithecellobium dulce* and in leaves and petiols of *Tamarindus indica*, from the same ecosystem. They were also below that referred by Freyre *et al*. (2003) for the *Prosopis ruscifolia* fruit.

Unquestionably, there are differences between the chemical constituents of flowers and pods with seeds, determined mainly by the biochemical specificity of each part of the plant and the physiological development of each of them (Herrera 2006).

The *in vitro* gas production technique is a valuable tool for assessing the nutritional quality of feeds used in ruminants' feeding (Williams 2000). In this study, the gas production technique was used to identify the differences of the fermentation kinetics of flowers and pods with seeds of tree species and shrubs growing in Cuba. The profile of these plants is not well known, especially those in areas with stressing factors such as soil quality, high temperatures and intense periods of draught.

The volume of gas produced due to the degradation

Table 1. Chemical composition of flowers and pods with seeds of trees and shrubs in Cauto Valley, Cuba

Species	DM (%)	OM (%)	Ash (%)	EE (%)	CP (%)	NDF (%)
			Flowers			
Baryxylum inerme	90.6[a]	90.6[a]	4.0[c]	5.3[b]	24.3	23.9
Tabebula maestrensis	91.4[ab]	88.3[b]	11.6[b]	6.1[a]	26.0	25.3
Hibicus rosasinensis	89.2[b]	86.3[c]	13.8[a]	3.7[c]	23.3	25.4
SE±	0.5	0.4	0.6	0.2	1.5	0.8
			Pods with seeds			
Samanea saman	88.3[bc]	93.7[a]	2.63[d]	3.40[dc]	34.3[ab]	32.5[b]
Licania campestre	89.2[bc]	90.4[d]	9.60[a]	2.70[d]	29.3[c]	30.1[c]
Prosopis chilensis	87.2[c]	92.1[c]	7.70[b]	3.06[dc]	35.0[a]	35.3[a]
Lysiloma bahamensis	91.5[ab]	97.3[a]	2.63[d]	3.96[c]	31.5[bc]	32.4[b]
Eugenia psiloclados	88.6[bc]	93.9[b]	6.10[c]	5.43[b]	34.2[ab]	30.4[c]
Moringa oleifera	93.7[a]	94.0[b]	5.93[c]	6.90[a]	30.6[c]	36.5[a]
SE±	1.4	0.1	0.2	0.3	1.2	0.6

[a,b c,d] Means in columns with different letters differ ($P < 0.05$)

of substrate b had differences ($P < 0.05$) in the group of flowers and pods with seeds (table 2). Likewise, differences were recorded in the gas production at 24 and 48 h between the groups studied. However, the colonization time did not differ between treatments. According to France *et al*. (2000), the gas production is directly proportional to the degradation rate of the substrate. On this respect, the high values of b, found in species like *Hibicus rosasinensis*, *Tabelula maestrensis*, *Lysiloma bahamensis* and *Prosopis chilensis*, could result in higher use of their energy content by ruminants (Makkar 2004).

Ojeda *et al*. (2012) reported gas production values inferior to those found in this study in the foliage of *Samanea saman*, as well as Guerrero (2009) in samples of flowers and fruits of trees species native of north Mexico. This could be due to the variations on the chemical composition of species, as well as the characteristics that the environment gives to the plants in a certain geographical area (Nelson and Moser 1995).

The colonization time is known as the growth initial period of a bacterial culture, during which the number of cells is static before their exponential development. According to Mertens and Ely (1982), several factors inherent to the substrate (hydration index and chemical or physical changes) like microbial factors, may affect the colonization stage. The zero values, obtained for this variable in *B. inerme* and *S. saman*, would indicate that the microorganisms ferment preferably starch or

Table 2. Parameters of *in vitro* gas production of flowers and pods with seeds of trees and shrubs in the Cauto Valley, Cuba.

Species	b	k	L	Gas 24h	Gas 48h
		Flowers			
Baryxylum inerme	149[b]	0.0683[b]	0.0000	120[b]	144[b]
Tabebula maestrensis	216[a]	0.0818[a]	0.7259	186[a]	212[a]
Hibicus rosasinensis	235[a]	0.0670[b]	0.8531	186[a]	224[a]
SE±	11	0.0097	0.5395	19	15
		Pods with seeds			
Samanea saman	205[ab]	0.0684[b]	0.0000	165[a]	197[ab]
Licania campestre	115c	0.0512[b]	0.0320	81[b]	105[c]
Prosopis chilensis	213[ab]	0.0644[b]	0.4084	64[a]	201[ab]
Lysiloma bahamensis	250[a]	0.0675[b]	0.4367	200[a]	240[a]
Eugenia psiloclados	185[b]	0.1152[a]	0.6287	173[a]	184[b]
Moringa oleifera	88[c]	0.0543[b]	0.3708	62[b]	80[c]
SE±	16	0.0106	0.3836	15	15

[a b c,d e] Means in columns with different letters differ ($P<0.05$).

b = gas volume corresponding to the complete digestion of the substrate (asymptote) (mL/g DM)

c = constant rate of gas production of the potentially degradable material (/h)

L = colonization time

any other carbohydrates of rapid fermentation at the beginning of the incubation period.

The constant gas production rate shows how rapid the ruminal microbiota ferments the feeds component. This allows the characterization of nutrients availability of the forage consumed by ruminants (Khazaal *et al*. 1995).

The constant gas production rate c in the flowers was superior in 25 % in *Tabebula maestrensis* compared to *B. inerme*, while in the pods with seeds *E. psiloclados* was superior 121 %, in respect to the lowest value recorded in *L. campestre*. Blümmel and Becker (1997) reported values for the constant gas production rate c, that fluctuated from 3.2 to 6.5%h^{-1} (average = 4.9%h^{-1}) in fiber forages. On the contrary, Ammar *et al*. (2008) stated similar values for the constant rate of production c in flowers (0.052 – 0.108 %h^{-1}) and fruits (0.048 – 0.102 %h^{-1}). In this study, the highest values reached in flowers and pods with seeds could indicate higher amount of nutrients available, in respect to forages which structural compound contents are more important.

The digestibility data are important for the nutritional and economical assessment of forages and support their proper inclusion in formulating the diets for cattle (Weiss 1995). The *in vitro* true digestibility of the flowers species *H. rosasinensis* and *T. maestrensis* was 30 units higher ($P < 0.05$) than that recorded in those of the *B. inerme* (table 3). This difference existed for the DM and OM digestibilities.

In this sense, the values of the DM digestiblity for the flowers of *H. rosasinensis* and *T. maestrensis* (72 %) were similar to those reported by Ammar *et al*. (2008) for the flowers of rosa canina (71.8 %). However, the results informed by these authors in fruits of shrubs (73 %) were superior to those recorded in this study (48 %). These differences in the digestibility could be explained, partially, through variations on the fiber content and lignification of the plant materials (Ammar *et al*. 2005). This characteristic determines finally its use by the animals. In respect to pods with seeds, the species *P. chilensis* and *S. saman* highlight for their high digestibility.

The OM digestibility has direct effect on the energy content of the plants. The ME content of the *B. inerme* flowers was low ($P < 0.05$) in 24 %, compared with *H. rosasinensis* and *T. maestrensis*. Likewise, the pods with seeds of the species *P. chilensis*, *S. saman*, *L. bahamensis* and *E. psiloclados* have high ME content (9.0 MJ kg DM^{-1}), superior ($P < 0.05$) in 57 % to that reported for *M. oleifera* and *L. campestre*.

Guerrero *et al*. (2010) found that the flowers of *Yucca sp*. have similar ME to *H. rosasinensis* and *T. maestrensis*. The same happened when comparing the fruits of *Atriplex canescens* with *M. oleifera* Lam and *L. campestre*. It should be considered that *Yucca sp*. and *A. canescens* grow and develop in the semiarid area of north Mexico.

Babayemi (2006) indicated similar values for fruits of the legume tree *Enterolobium cyclocarpum* that grows in Nigeria. In this study, the flowers of *H. rosasinensis* and *T. maestrensis* and the pods with seeds of *P. chilensis*, *S. saman*, *L. bahamensis* and *E. psiloclados* have enough ME (NRC 2007) to cover the maintainance and mid activity requirements of small ruminants under grazing conditions (8 MJ kg DM^{-1}).

The non-degraded substrate, determined from the *in vitro* gas production, allows the rapid estimation of the forages digestibility (Salem 2005). This variable was higher ($P < 0.05$) in 10 percent units in *T. maestrensis*, in respect to *B. inerme*. In the pods with seeds, the non-

Table 3. Digestibility, ME content and non-degraded residue of flowers and pods with seeds of trees and shrubs in the Cauto Valley, Cuba.

Species	I*n vitro* true DM digestibility (%)	*In vitro* true OM digestibility (%)	ME (MJ kg DM^{-1})	Non-degraded residue (%)
		Flowers		
Baryxylum inerme	40.6^{b}	40.3^{b}	6.94^{b}	35.8^{b}
Tabebuia maestrensis	72.9^{a}	70.3^{a}	8.36^{a}	46.4^{a}
Hibicus rosasinensis	72.1^{a}	68.0^{a}	8.95^{a}	---
SE±	1.9	1.9	0.25	1.5
		Pods with seeds		
Samanea saman	61.7^{a}	62.7^{a}	9.07^{a}	53.2^{a}
Licania campestre	40.9^{b}	39.2^{cd}	5.94^{b}	33.6^{b}
Prosopis chilensis	59.7^{a}	58.1^{ab}	8.99^{a}	48.9^{a}
Lysiloma bahamensis	47.0^{b}	45.2^{bc}	8.91^{a}	44.1^{a}
Eugenia psiloclados	48.1^{b}	47.5^{bc}	8.87^{a}	45.7^{a}
Moringa oleifera	31.3^{c}	25.4^{d}	5.48^{b}	20.2^{c}
SE±	3.0	5.4	0.3	3.7

$^{a,b\ c\ d}$ Means in columns with different letter differ at ($P<0.05$).

Table 4. *In vitro* gas production of volatile fatty acids (Mmol L^{-1}) of trees and shrubs in the Cauto Valley, Cuba

Species	Acetic	Propionic	Butyric	Isovaleric	Valeric
		Flowers			
Baryxylum inerme	5.25[b]	2.35	0.83	0.28	0.21
Tabebuia maestrensis	9.31[a]	3.00	1.23	0.35	0.26
SE±	1.59	0.51	0.25	0.07	0.03
		Pods with seeds			
Samanea saman	9.26[ab]	3.32[ab]	1.48[a]	0.38[a]	0.35[a]
Licania campestre	5.34[bc]	1.66[cd]	0.85[b]	0.33[ab]	0.18[b]
Prosopis chilensis	8.35[ab]	2.89[bc]	0.94[ab]	0.25[b]	0.17[b]
Lysiloma bahamensis	5.08[bc]	2.04[bcd]	0.92[b]	0.25[b]	0.18[b]
Eugenia psiloclados	11.12[a]	4.53[a]	1.02[ab]	0.31[ab]	0.21[b]
Moringa oleifera	1.83[c]	0.96[d]	0.18[c]	0.14[c]	0.08[c]
SE±	1.71	0.56	0.19	0.03	0.03

[abcd] Means in columns with different letters differ ($P < 0.05$).

degraded substrate was different in the plant species studied ($P < 0.05$).

The variations of the *in vitro* DM true digestibility of the pods with seeds and flowers are explained in 84 and 97 %, respectively. According to the correlation analysis, this could be due to the variations of the degraded substrate in 24 h of incubation.

Differences ($P < 0.05$) were recorded in the concentrations (Mmol L^{-1}) of acetate in the flowers (table 4). The values for this variable were superior in *T. maestrensis* in respect to *B. inerme*. However, there were no differences in the concentrations of propionate, butyrate, isovalerate and valerate in these species. The high concentrations of acetic acid in *T. maestrensis* could correspond with its high degradability, due to the low amount of lignin (Valenciaga *et al*. 2009). On this respect, van Houtert (1993) mentioned the cell wall components (cellulose, hemicellulose, pectin and others) as the main substrate of the fermentation to supply the carbonate chains promoting the acetate synthesis. Ramírez *et al*. (2000) referred that the flowers have high values of soluble carbohydrates, originating the high concentration of propionate, similar performance to that obtained in this study.

Differences ($P < 0.05$) between the plants were found in the pods with seeds. The highest values of acetic and propionic acids were obtained in *E. psiloclados*, while *S. saman* reached higher values of butyric, isovaleric and valeric acids.

In this study, the VFA concentrations were inferior to those found by Guerrero (2009) in shrub pods of the north Mexico. This could be related with the peculiar characteristics of the species, their adapatation to the environmental conditions and the response to the environment they developed in (Nelson and Moser 1995).

The flowers of *T. maestrensis* and *H. rosasinensis* showed values on their chemical compoition, digestibility, energy content, fermentation kinetics and *in vitro* VFA concentration that allow them as an option for ruminants' feeding.

When considering the indicators studied in pods with seed, specially CP, NDF and DM and OM digestibility, the *M. oleífera* recorded the lowest values, while *S. saman*, *L. campestre*, *P. chilensis*, *L. bahamensis* and *E. psiloclados* had contents suggesting their use as cattle supplement, mainly *S. saman*, *P. chilensis* and *E. psiloclados*.

Additional studies are needed to assess the productive response of the animals with the use of the studied species, as well as their performance under different ecosystems and management conditions.

Acknowledgements

Thanks are given to the Program of Professor Improvement-SEP (Mexico) for the financial support to the project "Productive and economic impact of agro-industrial by-products and non-conventional forages in ruminants' feeding".

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Received: November 25, 2012