# Effect of *Samanea saman* (Jacq.) Merr., *Albizia lebbeck* Benth and *Tithonia diversifolia* (Hemsl.) Gray (plant material 23) on the methanogen population and on the ruminal microbial ecology

Juana Galindo, Niurca González, Idania Scull, Yoandra Marrero, Areadne Sosa, Ana I. Aldana, Onidia Moreira, Denia Delgado, T. Ruiz, G. Febles, Verena Torres, O. La O, Lucía Sarduy, Aida Noda and O. Achang

*Instituto de Ciencia Animal, Apartado Postal 24, San José de las Lajas, Mayabeque, Cuba*
*Email: jgalindo@ica.co.cu*

An experiment with completely randomized design in factorial arragement (4 x 5) and under *in vitro* conditions was conducted to assess the effect of *Samanea saman*, *Albizia lebbeck* and *Tithonia diversifolia*, plant material 23, on the methanogen population and on the ruminal microbial ecology. Four treatments were compared: A) *Cynodon nlemfuensis* (star grass, control), B) *Samanea saman* (carob tree), C) *Albizia lebbeck* (albizia) and D) *Tithonia diversifolia*, plant material 23 (tithonia). The samplings were conducted before incubation (hour 0), at 4, 8, 12 and 24 h after the beginning of fermentation. They were replicated four times in time. There was no effect of the foliage trees on the population of total viable bacteria of the rumen. The methanogen populations, in the fermentation up to 8 h, were 70, 34, 19 and 18.5 x $10^9$ ufc $mL^{-1}$ for star grass, carob tree, and tithonia, respectively. The highest population of cellulolytic bacteria was found in albizia, while the cellulolytic fungi, at 8 h of fermentation, had the highest population with this same plant. The protozoa population was 8.9, 7.2, 6.0 and 6.5 x $10^5$ cells $mL^{-1}$ for the treatments A, B, C and D, respectively. No effects on the pH or on the ammonium concentration in the rumen were found. It is concluded that carob tree, albizia and tithonia reduce the methanogen population and have beneficial effects on the ruminal microbial ecology when modifying the populations of protozoa, bacteria and cellulolytic fungi.

Key words: *rumen, bacteria, protozoa, methanogen, trees ecology*.

One of the most important goals on the world cattle production is reducing the methane production in the rumen, hence different strategies are developed. Some of them are for reducing methanogens and others for modifying the metabolic routes for producing this gas (Hegarty 1999, Agarwal *et al*. 2008 and Beauchemin *et al*. 2008). Since the 90's, the advantages of the cattle systems with trees, legumes or not, are being studied to achieve the methane reduction in the rumen.

Galindo (2004) and Galindo *et al*. (2005 and 2006) have demonstrated that the use of these systems modify the ruminal microbial population and the final products of their fermentation, but their effect on the methanogen population and on the ruminal methanogenesis has not been studied yet.

Methanogens co-live endosymbiotically with the rumen protozoa, producing hydrogen equivalents which are, later, reducer agents for methane production. Likewise, condensed tannins and total polyphenols, among other metabolites of these plants, can reduce the protozoa population (Joblin 2004).

The objective of this study was to assess the effect of *Samanea saman* (carob tree), *Albizia lebbeck* (albizia) and *Tithonia diversifolia* (tithonia) on the methanogen population and on the ruminal microbial ecology.

## Materials and Methods

The experiment was cnducted under *in vitro* conditions, therefore the technique of Theodorou *et al*. (1994) was applied, using sealed bottles of 100 mL for incubating the feed samples in ruminal liquid and buffer culture.

Each bottle had 30 mL of the mixture with ruminal liquid and buffer solution, in a ratio of one part ruminal liquid and two of buffer solution. The feeds were included at a rate of 0.3 g $treatment^{-1}$. Out of that amount, 30 % corresponded to the plant to be assessed (carob tree, albizia, tithonia).

*Experimental treatment*. A) star grass as control, B) carob tree, C) albizia and D) tithonia.

The star grass was obtained from an un-grazed area. Leaves with their petioles were collected for its preparation, simulating the animal bite. The sample was dried in an oven at 60 ºC, for 48 h.

The trees are part of the Arboretum of the Institute of Animal Science, located in San José de las Lajas municipality, Mayabeque province. This facility is at 92 m a.s.l., 22º 53' north latitude and 82º 02' west longitude. The soil is fersialitic, waved, with 4.84 % of organic matter, 0.26 of total nitrogen, 40.59 ppm of phosphorous, 4.60 of calcium, 0.46 of magnesium and pH of 6.34. The trees had been planted for at about seven years.

From the trees selected, the fractions leaves-petioles-green pods were collected, simulating the animals browsing. Once they were picked up, they were spread on a cement plate to sun-dry them for three consecutive days. Later, they were milled at a particle size of 1mm.

The chemical composition of the plants was determined with the methodology proposed by AOAC (1995). The fibrous fraction was quantified according to the protocol described by van Soest *et al*. (1991). The content of secondary metabolites was qualitatively determined with the phytochemical sieving, according Rondina and Cussio (1969, described by Alfonso *et al*. 2000). The crossing system was used for the description of the tests to specify the presence or absence of metabolites in the analysis. Table 1 shows the results.

All the plants have notable and moderate amounts of secondary metabolites. Table 2 shows their phytochemical seiving.

medium was described by Anderson and Horn (1987). The fungi were obtained according to the methodology of Joblin (1981). The protozoa were counted in the optical microscope in Neubauer chamber. They were dyed with a solution of gentian violet at 0.01 % in glacial acetic acid at 1%.

*Experimental design and statistical analysis*. A completely randomized design was used, in factorial arrangement 4 x 5 (four treatments and five sampling times). The statistical treatment of the experimental results was conducted according to experimental designed used for identifying the interaction between treatments (diets) and the fermentation times (sampling times). When necessary, Duncan's

Table. 1. Chemical composition in the foliage of the plants assessed, g kg DM-1

| Plant | CP | NDF | ADF | Ash | Ca | P |
|---|---|---|---|---|---|---|
| *Samanea saman* | 181.5 | 414.5 | 295.1 | 69.2 | 12.6 | 3.2 |
| *Albizia lebbeck* | 171.8 | 498.6 | 346.9 | 65.4 | 11.7 | 2.0 |
| *Tithonia diversifolia* green material 23 | 239.5 | 334.3 | 295.4 | 58.1 | 21.4 | 3.5 |
| *Cynodon nlemfuensis* | 119.0 | 910.6 | 399.5 | 128.7 | 5.4 | 0.2 |

Table 2. Phytochemical seiving of the foliage in the plants assessed

| Plant | Positivity degree | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | tannins | flavonoides | saponins | triterpenos | steroides | antocianidins | reducers | alkalorids |
| *Samanea saman* | ++ | + | + | ++ | ++ | + | + | +++ |
| *Albizia lebbeck* | + | + | ++ | + | + | + | ++ | +++ |
| *Tithonia diversifolia* mv 23 | ++ | ++ | + | + | ++ | + | +++ | ++ |

(+++) high; (++) moderate; (+) low; (-) no presence; ND- Not detected

*Samplings*. The samplings were conducted at 0, 4, 8, 12 and 24 h of incubation, to determine the pH and ammonium concentration. A sampling was conducted at 0, 4 and 8 h for calculating the microbial populations. Four replications were conducted in only one running.

*Determinations*. The pH was measured in a digital pH-meter and the ammonium concentration was determined according to Conway (1957).

The populations of total viable, methanogenic and cellulolitic bacteria and protozoa were determined through the culture technique of Hungate (1970) in rolling tubes and under strict anaerobic conditions. The culture of total viable and cellulolytic bacteria was conducted in the culture medium of Caldwell y Bryant (1966), modified by Elías (1971) and Galindo (1988). The methanogenic bacteria were counted with this same method, but using a mixture of hydrogen and carbonate dioxide (60:40) in the gas stage. The culture

test (1955) was used for $P < 0.05$. The analyses were carried out with the statistical software SPSS+ (Visauta 1998).

The counting of the microorganisms was transformed according to Log N for guaranteeing the normality conditions in the growth curve. For the analysis, the formula (K+ N).10x was applied, where:

- K is the constant representing the dilution logarithm where the microorganism was inoculated.
- N is the logarithm of the colonies counting, determined as cfu x $mL^{-1}$, tfu x $mL^{-1}$, or cells x $mL^{-1}$.
- 10 X is the dilution inoculated.

## Results and Discussion

Under the experimental conditions of this study, there was no significant interaction between the fermentation time and the plants in the population of total viable bacteria, cellulolytic, methanogens and protozoa.

Table 3 presents the effect of the different plants

on the population of total viable bacteria in the rumen. There was no effect of the use of carob tree, albizia and tithonia in respect to the control with star grass.

It has been proved that the inclusion of trees and shrubs foliage in ruminants feeding has defaunating effects as it reduces the protozoa population in the rumen. On this respect, Galindo *et al*. (2000) and

Table 3. Effect of the foliage of three trees on the population of some integrants of the ruminal microbial population

| Microbial group | *C. nlemfuensis* | *S. saman* | *A. lebbeck* | *T. diversifolia* | SE |
|---|---|---|---|---|---|
| Total viable bacteria, $10^{11}$ cfu $mL^{-1}$ | 3.89 (36.00) | 3.69 (40.00) | 3.74 (42.00) | 3.56 (35.00) | 0.23 |
| Cellulolytic bacteria, $10^{5}$ cfu $mL^{-1}$ | $2.72^{b}$ (15.20) | $2.28^{c}$ (9.80) | $3.14^{a}$ (23.10) | $2.85^{ab}$ (17.40) | 0.31 P < 0.001 |
| Methanogens, $10^{9}$ cfu $mL^{-1}$ | $4.26^{a}$ (70.00) | $3.66^{b}$ (34.00) | $3.04^{c}$ (19.00) | $2.99^{c}$ (18.50) | 0.35 P < 0.001 |
| Protozoa, $10^{5}$ cell. $mL^{-1}$ | $2.18^{a}$ (8.90) | $1.92^{b}$ (7.20) | $1.79^{c}$ (6.00) | $1.87^{c}$ (6.50) | 0.13 P < 0.05 |

[a,b,c] Means with different letters within the same row differ at P < 0.05 (Duncan 1955)
Data transformed according to Logn., original means between parentheses

The fact that the population of total viable bacteria keeps in the range of $10^{11}$ cfu.$mL^{-1}$ is of great ecological importance in the rumen. This coincides with the reports of Hungate (1966), Hoover and Miller (1991) and González (2010) in respect to the total number of bacteria living in the rumen of animals fed fibrous diets. For these reasons, the use of the foliage of the cited plants is demonstrated without diminishing the total bacteria in the rumen. This guarantees a proper balance between the different populations in the organ.

The population of cellulolytic bacteria varies considerably, depending on the plant assessed (table 3). As shown, the highest population of cellulolytic bacteria was found when supplementing with albizia, followed by tithonia. The use of carob tree reduced (P < 0.001) the la representation of this microbial group. This leads to search other inclusion levels of this plant in the diet of ruminants, mainly, because in feeding systems based on fiber, the principal response to be expected is the increase of digester microorganisms of the fiber. They, consequently, are responsible of the cellulose degradation in the rumen.

The effect of the plants foliage on the group Archaea or methanogens of the rumen is of great importance. All the plants reduce the population of methanogenic bacteria in respect to star grass. However, albizia and tithonia diminish, in great extent, these microbial groups. The populations of these microorganisms were 70, 34, 19 and 18 cfu $ml^{-1}$ for star grass, carob tree, albizia and tithonia, respectively.

One of the most outstanding results of this study is referred to the effect of trees foliage on the protozoa population in the rumen. The carob tree, albizia and tithonia reduce the protozoa population. Albizia and tithonia are the most promising species for these purposes.

Galindo *et al*. (2001a and 2001b) indicated that protozoa and fungi in the rumen keep a relation presented in the equation Y = 0.8210 -3.415 X +4.311 X2. Therefore, it is logic to think that the effect of the secondary metabolites on the population of fungi and cellulolytic bacteria is indirect, as the protozoa engulf huge amounts of these microbial groups during the day. Defaunation, as strategy for managing the ruminal microbial fermentation, has been used in fibrous diets of low quality.

Data of Hegarty (1999) demonstrated that the methanogens live inside or adhered to the surface of the ciliated protozoa of the rumen, and are responsible of more than 37 % of methane emissions. When the protozoa populations are minimized, the rumen methane emissions are reduced at about 13%. This effect varies with the diet of the animals. So, the use of the plants assessed, as they diminish the protozoa population, is a promising way for diminishing methanogens (Mc Allister *et al*. 1996¸Tokura *et al*. 1997 and Kobayashi 2010).

Significant interaction was found between treatments (plants) and the fermentation time in the cellulolytic fungi of the rumen. Figure 1 shows the effect on both factors under study. The use of the different plants reduced the cellulolytic fungi population compared with the control treatment with star grass.

The increase of fungi, when supplementing with carob tree, from the beginning of fermentation up to four hours, was light and increased later to eight hours.

Carob tree kept diminished fungi population, without differences between the fermentation times. This indicates that the presence of factors contained in this plant hinders the development of these microbial

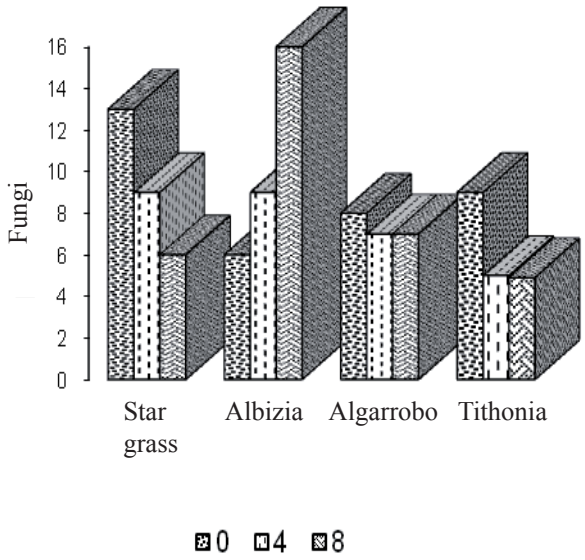


Figure 1. Effect of the foliage of three trees on the population of cellulolytic fungi in the rumen, $10^4$ cfu x $mL^{-1}$, SE ± 0.45, P < 0.05

groups.

When assessing the performance of tithonia in time, a reduction of the fungi population was observed, with differences between four and eight hours after the beginning of fermentation.

These results suggest the study of intrinsic factors in these plants, which may influence on the diminishing of cellulolytic fungi. Further studies about the effect of secondary metabolites of these plants on the microbial populations, and specifically, on those of cellulolytic fungi, are necessary.

The total viable and methanogenic bacteria showed modifications in time. The total number of bacteria increased from 31.08, before the fermentation began, to 47.96 x $10^{11}$ cfu $mL^{-1}$, eight after it began. The methanogenic bacteria could duplicate their initial population in the same time (figure 2).

Even when using techniques to reduce the undesired microbial population, its increase in time should be expected, unless the toxic effect on the populations impedes these effects.

As relevant aspect, the use of carob tree, albizia and tithonia did not modify the pH and the ammonium concentration in the rumen compared with the treatment with star grass (table 4). This response does not seem to be related with the protein contents of these plants, which were 18.15, 17.18 and 23.95 %, respectively. Meanwhile, the protein content of star grass was of 11.90 %. The ruminal fermentation of proteins is very variable and, as consequence, certain amounts of ammonia (NH3) are produced. The solubility and degradability of proteins are among the factors modifying their ruminal fermentation.

Previous studies conducted at the Institute of Animal Science have revealed that the foliage of trees and shrubs, leguminous or nor, have tannin concentrations. They, linked with the proteins in the rumen, reduce their

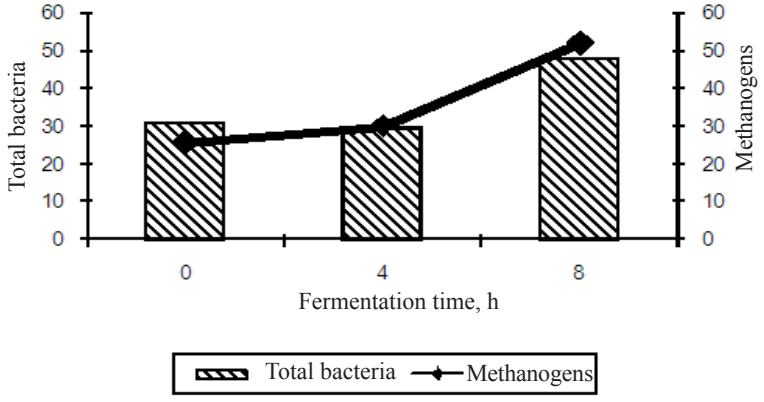


Figure 2. Effect of the fermentation time of the populations of total viable and methanogenic bacteria of the rumen *in vitro* SE ± 0.16** and SE ± 0.25**

Table 4. Effect of the foliage of three trees on the pH and ammonia concentration in the rumen

| Treatments | pH | $NH_3$, mmol. $L^{-1}$ |
|---|---|---|
| *Cynodon nlemfuensis* | 6.4 | 15.76 |
| *Samanea saman* | 6.3 | 14.68 |
| *Albizia lebbeck* | 6.3 | 15.99 |
| *Tithonia diversifolia* material vegetal 23 | 6.3 | 15.71 |
| SE ± Signification | 0.01 | 1.23 |

degradation (La O 2001). Likewise, the feeding protein would be closer to a surpassed protein. It is evident that, if the protein degradability in the rumen is reduced, the ammonia concentration is lower. Nevertheless, further studies involving the plants under study should include the proteins nature.

It is concluded that carob tree, albizia and tithonia reduce the methanogens populations and have beneficial effects on the ruminal microbial ecology, when modifying the populations of protozoa, bacteria and cellulolytic fungi.

## References


Agarwal, N., Kamra, D.E., Chatterjee, P.N., Kumar, R. & Chaudhary, L.C. 2008. *In vitro* methanogenesis, microbial profile and fermentation of green forages with buffalo rumen liquor as influenced by two bromoethanesulphonic acid. Asian Austr. J. Anim. Sci. 21:818

Alfonso, M., Fernández, L.; González, N. & Avilés, R. 2000. La Achira (*Canna edulis* Ker.) y su potencialidad en el control de plagas. Ponencia XII Forum de Ciencia y Técnica. INIFAT. Ciudad de La Habana, Cuba. 11 p.

Anderson, M.A. & Horn, G.M. 1987. Effect of Lasalocic in wheight gain, ruminal fermentation and forage intake of stocker cattle grasing wintwer pasture. J. Animal Sci. 65:865

AOAC. 1995. Official Methods of Analysis.15th Ed. Assoc. Off. Anal. Chem. Arlington, V.A.

Beauchemin, K.A., Kreuzer, M., Mara, F.O. & Mc Allister, T.A. 2008. Nutritional management for enteric methane abatement's review. Australian J. Exp. Agric. 48:21

Caldwell, D.R. & Bryant, M.P. 1966. Medium without fluid for non selective enumeration and isolation of rumen bacteria. Appl. Microbiol. 14:794

Conway, E.J. 1957. Microdiffusion analysis and volumetric error. 4th. Ed. Crosby Lockwood Sons, Ltd. London

Duncan, D.E. 1955. Multiple range and multiple F. test. Biometrics 11:1

Elías, A. 1971. The rumen bacteria of animals fed on a high-molasses urea diet. PhD Thesis. Aberdeen, Scotland, UK

Galindo, J. 1988. Efecto de la zeolita en la población de bacterias celulolíticas y su actividad en vacas que consumen ensilaje. PhD Thesis. Instituto de Ciencia Animal. La Habana, Cuba

Galindo, J. 2004. Fermentación microbiana ruminal y pasaje hacia las partes bajas del tracto gastro intestinal de árboles, arbustos y leguminosas. Memorias del Curso "Sistemas Sivopastoriles, una opción sustentable". Tantakín, Mérida. Pp. 132-152

Galindo, J., Aldama, A.I., Marrero, Y. & González, Y. 2000. The effect of Sapindus saponaria on the protozoa genera and ruminal bacteria populations. Cuban J. Agric. Sci. 337:34

Galindo, J., González, N., Aldama, A.I. & Marrero, Y. 2001a. Effect of *Enterolobium cyclocarpum* on rumen microbial population and its activity under *in vitro* conditions. Cuban J. Agric. 35:229

Galindo, J., González, N., Aldama, A.I. & Marrero, Y. 2001b. Effect of *Gliricidia sepium* on rumen protozoa population and cellulolytic organisms. Cuban J. Agric. Sci. 35:223

Galindo, J. & Marrero, Y. 2006. Manipulation of ruminal microbial fermentation. Cuban J. Agric. Sci. 39:439

Galindo, J., Sosa, A., González, N., Delgado, D., González, R., Aldana, A. I., Moreira., O., Cueto, M. & Cairo, J. 2005. Effect of ruminal fermentation activator on the on the population of protozoa, methanogenic and cellulolytic bacteria and fungi in the rumen. Informe AIEA. Institute of Animal Science. La Habana. Cuba

González, N. 2010. Evaluación de morera (*Morus alba* Lin) en la fermentación y control de la metanogénesis ruminal de búfalos de río (*Bubalus bubalis*). PhD Thesis. Instituto de Ciencia Animal. La Habana. Cuba

Hegarty, R.S. 1999. Reducing rumen methane emissions through elimination of rumen protozoa. Aust. J. Agr. Res. 50:1321

Hoover, W.H. & Miller, T.K. 1991. Rumen digestive Physiology and Microbial Ecology. Food Animal Practice.7: 311

Hungate, R.E. 1966. The rumen and its microbes. Academic Press, New York. 3trd Ed. Pp. 3-115

Hungate, R.G. 1970. The anaerobic, mesophilic cellulolitic bacteria. Bacterial Ref. Vol. 14

Joblin, K.N. 1981. Isolation, enumeration and maintenance of rumen anaerobic fungi in roll tubes. Appl. Environ. Microbiol. p. 1119

Joblin, K.N. 2004. Methanogenic Archaea. In: I Planning Meeting of Project Contract Research and Training Workshop "Development and Use of Rumen Molecular Techniques for Predicting and Enhancing Productivity". Brisbane,

Kobayashi, Y. 2010. Abatement of methane production from ruminants: trends in the manipulation of rumen fermentation. Asian Aust. J. Anim. Sci 23: 410

Mc Allister, T.A., Okine, E.K., Mathison, G.W. & Cheng, K.J. 1996. Dietary, environmental and microbiological aspects of methane production in ruminants. Canadian J. Anim. Sci. 76:231

Theodorou, M.K, Williams, B.A., Dhanoa, M.S., McAllan, A.B. & France, J. 1994. A simple gas production method

using a pressure transducer to determine the fermentation kinetics of ruminants feed. Anim. Feed. Sci. Tech. 48:185

Tokura, M., Ushida, K., Miyazaki, K. & Kojima, Y. 1997. Methanogens associated with rumen ciliates. FEMS Microbiol. Ecology 22:137

Van Soest, P.J., & Robertson, L.B. 1991 Carbohydrate, methodology, metabolism and nutritional implications in dairy cattle, methods for dietary fiber, neutral detergent fiber and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583

Visauta, B. 1998. Análisis estadístico con SPSS para Window. Estadística multivariada. Vol II. Mc Graw-Hill. Interamericana de España, S.A.V. p. 358

**Received: January 15, 2011**