Tropical and Subtropical Agroecosystems, 10 (2009): 497 - 503

Tropical and

Subtropical

Agroecosystems

IN VITRO FERMENTATION EFFICIENCY OF MIXTURES OF *Cynodon nlemfuensis*, *Leucaena leucocephala* AND TWO ENERGY SOURCES (MAIZE OR SUGAR CANE MOLASSES)

[EFICIENCIA DE FERMENTACIÓN IN VITRO DE MEZCLAS DE *Cynodon nlemfuensis*, *Leucaena leucocephala* Y DOS FUENTES DE ENERGÍA (MAIZ O MELAZA DE CAÑA)]

Juan M. Estrada-Liévano, Carlos A. Sandoval-Castro*, Luis Ramírez-Avilés and Concepción M. Capetillo-Leal

Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma de Yucatán, Apdo. 4-116, Itzimná, Mérida, Yucatán, México.Email: ccastro@uady.mx
**Corresponding author*

SUMMARY

The *in vitro* fermentation efficiency of *Cynodon nlemfuensis* forage (star grass) and *Leucaena leucocephala* foliage (leucaena) and two energy sources (*i.e.* maize and sugar cane molasses) mixture was evaluated. Mixture samples (1 g DM) were incubated for 24 h. All the mixtures were added with 500 mg of polyetilenglycol (PEG). Adding molasses to star grass increased dry matter true digestibility and carbohydrate fermentation ($P<0.01$), while having a quadratic effect on cell walls digestibility ($P<0.01$). When an energy source was added to the mixture it has a quadratic effect on gas production ($P<0.01$). Mixtures of grass or tree with maize resulted in an increase on the total VFA´s concentration. The grass-leucaena and leucaena-maize mixtures reduced the microbial protein biomass (MPB), while the grass-Leucaena-energy sources mixtures tended to increase MPB production. It is concluded that, the use of molasses, under *in vitro* conditions, improves the fiber digestibility and MPB while, the use of maize increases the VFA´s production.

Key words: Mixtures; *in vitro;* forage tree; microbial protein; ATP.

RESUMEN

Se evaluó la eficiencia de fermentación in vitro de mezclas de Cynodon nlemfuensis (pasto estrella), Leucaena leucocephala y dos fuentes de energía (grano de maíz y melaza de caña). Las mezclas (1g MS) fueron incubadas por 24h y fueron adicionadas con 500 mg PEG. La adición de melaza al pasto estrella incremento la digestibilidad de la MS y la fermentación de carbohidratos ($P<0.01$), mientras que se observó un efecto cuadrático en la digestión de las paredes celulares ($P<0.01$). La adición de una fuente energética resultó en un efecto cuadratico en la producción de gas in vitro ($P<0.01$). La mezclas de pasto con maíz resultaron en un incremento en la concentración total de AGV's. Las mezclas pasto-leucaena y leucaena-maíz resultaron en una reducción de la producción de biomasa microbial, mientras que lass mezclas pasto-leucaena-energía incrementaron la producción de biomasa microbial. Se concluye que el uso de melaza (en condiciones in vitro), mejora la digestibilidad de la fibra y producción de biomasa microbial, mientras que el uso de maíz incrementa la producción de AGV's.

Palabras clave: Mezclas; *in vitro*; árboles forrajeros; proteína microbial; ATP.

INTRODUCTION

In the tropics, cattle feeding is based on grass, despite the fact that they have high fiber (>60% FDN) and low protein (<10%) contents, and limited grow during the dry season (Juarez, 2004). Therefore, tropical production systems should have strategies that allow them to face the nutritional deficiencies, such as the use of molasses-urea blocks, poultry manure and the use of tree foliages. They seek to improve rumen environment and hence microbial activity which could result in two main effects: 1. Increase microbial biomass production; 2. Modify the proportion and production of VFA's. Traditional studies on animal nutrition focused on the evaluation of individual feeds, and assume that there are only additive effects amongst them. Most of studies undertaken to understand the associative effects deal with the effect of a source of ready fermentable carbohydrates (*e.g.* maize silage, barley) on the digestibility of a forage with high fiber content (Moss *et al*., 1992). However, up to date there is no enough evidence that allow understanding the grass-tree-energy interactions. The objective of the present study was to evaluate the fermentation efficiency of mixtures of star grass, leucaena and two sources of energy.

MATERIAL AND METHODS

L. leucocephala foliage (leucaena), 65 days old, and *C. nlemfuensis* forage (star grass), 35 days old, were harvested in summer 2004 and chemically analyzed to quantify dry matter (DM), crude protein (CP), lipids (L) and ash contents (A.O.A.C., 1980). Also, contents of neutral detergent fiber (NDF), acid detergent fiber (ADF) (Van Soest *et al*., 1991), total phenols (Price and Butler, 1977) and tannins (Price and Butler, 1978) were quantified. Star grass paddock was fertilized with water from a pig farm.

The elaboration of the *in vitro* medium was carried out following the Menke and Staingass (1988) methods. Gas production technique published by Theodorou *et al*. (1994) was used. Rumen content (both solid and liquid) was collected from two canulated heifers, which were fed with about 16 kg FM/an/d of chopped *Pennisetum purpureum* grass (Taiwan) and supplemented with 3 kg/an/d of commercial concentrate (16 % of CP). Soon after collecting, rumen content sample was placed in a plastic bag, which was sealed to avoid air contamination. Rumen liquid was filtered with a mesh, located inside a bottle, under a CO_2 flux, until 250 ml was obtained. Rumen solid was blended and buffer was added (v/v) to obtain 250 ml. The blended rumen solid was filtered again and mixed with liquid rumen to obtain 500 ml of inoculate. Each mixture (*i.e.* treatment) was placed in a bottle (of 100 ml) and added 6 ml of inoculate and 54 ml of *in vitro* medium. All mixtures were added with 0.500 g of polyetilenglycol (PEG)/g of incubated sample, to avoid the tannins activity and the formation of protein-tannin complex, which could affect availability and utilization of nitrogen (N), from the forages, for the microbial biomass production (MBP).

All treatment mixtures have a total weigh of 1 g of DM, and were incubated with 54 ml of the *in vitro* medium and 6 ml of the inoculate. The gas volume and pressure were recorded at 0, 3, 6, 9, 12, 15, 18, 21 and 24 h post-incubation. After this period, all treatment mixtures were refrigerated to allow sedimentation of particles and to obtain 4 ml of the liquor, which was added with 1 ml of the standard to carry the Volatile Fatty Acids (VFA´s) estimation, using a gas chromatographer. The remaining mixture in each bottle was added with 100 ml of sodium lauril sulphate, boiled for an hour, filtered, washed with hot water and acetone and dried at 60 ºC, to quantify the residual NDF (NDFR) and true digestibility, estimated from the difference between the DM incubated and the residual, and corrected for the control (without the forage sample). The estimation of the MBP, ATP´s and gas production (using an estequiometric method) was undertaken following the technique of Blümmel *et al*. (1997b).

Experimental design

The ingredients of the mixture were: Grass (G), Leucanena (L) and two energy sources (*i.e.* maize and molasses). The proportion of each ingredient on the treatments (mixtures) is presented in the Table 1. All the mixtures weight 1 g of DM.

Table 1. Composition (%) of the treatmens (mixtures).

Treatment	Grass (G)	Tree foliage (L)	Energy source
1	45	45	10
2	60	30	10
3	75	15	10
4	50	50	0
5	0	50	50
6	50	0	50
7	0	100	0
8	100	0	0
9	0	0	100

G: Star grass; L: Leucaena; Energy source : Maize or Molasses.

A mixure design was used (Mead, 1998). Lineal, quadratic and cubic regression analysis were carried out, and only the significant terms were included in the final model. The analysis was undertaken using the statistical program Minitab 12 was used (Minitab, 1997). The statistical model used was:

$$Y = B_t{*}A + B_g{*}G + B_e{*}E + B_{tg}{*}AG + B_{te}{*}AE + B_{ge}{*}GE + B_{teg}{*}AEG + Error_{gte}$$

Whre:

Y = Digestibility, total Gas, Fermentation efficiency, and VFT, Microbial biomass, ATP produced.

B1…n = Partial coefficients of the regression for the individual components and their interactions.

T = tree (*L. leucocephala*).

G = grass (*C. nlemfuensis*).

E = Energy (maize or sugar cane molasses).

RESULTS

Chemical composition of the different ingredients used are presented in the Table 2. CP content of leucaena was within the range reported by other authors; however, star grass has higher CP content than that reported in the literature.

Table 2. Chemical composition of the ingredients (DM % except where indicated).

Item	L	PE	Ma	Mo
DM	96.9	94.43	93.5	78.09
CP	24.0	14.7	7.9	4.7
NDF	33.4	65.94	0.1	ND
ADF	16.5	34.1	ND	ND
EE	4.9	1.93	2.5	ND
TP	2.8	0.11	0.0	ND
CT	1.0	0.00	0.0	ND
LIG[1]	7.5	4.04	ND	ND
LIG[2]	7.7	2.9	ND	ND
ASH	8.6	9.53	1.3	13.3

L: Leucaena; PE: Star grass; Ma:Maize; Mo: Molasses. ND (not quantified). TP: total polyphenols. CT: condensed tannins. Lig[1]: lignin without ash correction, Lig[2]: lignin ash corrected.

The individual ingredients have a significant effect on almost all the evaluated variables except on the ATP´s and gas production, estimated by estequiometric method. *In vitro* true digestibility of the dry matter (IVDDM) and the carbohydrate fermentation percentage have an additive performance ($P<0.01$) (Table 3). Digestibility of the NFD has a quadratic response ($P<0.01$); when leucaena and star grass mixtures were added with molasses resulted in an increased on *in vitro* digestibility of the NFD.

The addition of a source of energy to either Leucaena or star grass increased ($P<0.01$) gas production and the fermentation efficiency (Table 4). Gas production, estimated by estequiometric method, has not ($P>0.01$) lineal performance, but the quadratic effect indicated that the addition of maize (as a source of energy) increased ($P>0.01$) gas production higher that with the addition of molasses. Nevertheless, the mix grass-tree-energy resulted in a reduction of gas production.

Total production of VFA´s, ATP´s and Microbial biomass did not have a lineal trend (Cuadro 5). Nevertheless, the addition of any of the two sources of energy to the forage have a quadratic effect ($P<0.01$) on the AGV´s and ATP´s production. However, the addition of molasses to star grass did not ($P>0.01$) influenced the ATP´s production. The mixture star grass-leucaena-energy resulted in a reduction ($P<0.01$) on the production of AGV´s and ATP´s.

Fermentation of star grass and leucaena resulted in a high proportion of acetic acid, while fermentation of both sources of energy gave high proportion of propionic and butyric acids. Acetic acid concentration was increased ($P<0.01$) by the mixture of star grass and leucaena and by the addition of maize to either of them. Production of propionic acid was increased ($P<0.01$) by the addition of any source of energy to leucaena, and by the mixture of the grass and the legume. On the other hand, acetic acid concentration was reduced ($P<0.01$) by the mixture star grass-leucaena-energy (this data is not presented).

The addition of any source of energy had a lineal effect on the microbial biomass production (MBP), but the mixture star grass-leucaena and leucaena-energy have a quadratic effect resulting in a reduction ($P<0.01$) of MBP. The mixture star grass-leucaena-energy promoted an increment on MBP ($P<0.01$).

Table 3. Partial regression coefficient of *in vitro* digestibility of the DM (IVDDM)[1], amount of fermented carbohydrate (FCAR)[1] and *in vitro* digestibility of the NFD (IVDNFD)[1] of the individual ingredients and their mixtures (Mean ± EE).

	IVDDM (g)		FCAR (g)		IVDNFD (g)	
	L-Ma	L-Mo	L-Ma	L-Mo	L-Ma	L-Mo
G	522±0.80**	521±1.01**	485±0.01**	480±0.01**	26.1±1.91**	26.1±3.92**
L	782±0.95**	785±1.22**	757±0.01**	756±0.01**	32.9±2.25**	34.6±4.24**
E	930±1.02**	995±1.27**	870±0.01**	786±0.01**	43.6±2.45**	-0.2±4.25ns
PA	ns	Ns	ns	ns	ns	4.3±16.55ns
PE	ns	ns	ns	ns	ns	55.4±20.34**
LE	ns	ns	ns	ns	ns	63.8±20.32**
R^2	96.9	96.6	97.1	92.5	47.3	57.6
S.D	2.32	3	0	0	5.54	8.4

G: Star grass; L: Leucaena; E: Energy source (*i.e.* Ma: Maíze; Mo: Molasses) **$P<0.01$ denotes significan effect for individual components. PA, PE, AE **$P<0.01$ denotes significan quadratic effects, ns: no significant effect ($P>0.05$). Values corrected for the blank[1].

Table 4. Partial regression coefficient of fermentation efficiency (FERE), gas production (GAP) and gas production by estioquiometric method (GAPES) for the individual ingredients and their mixes (Mean ± EE).

	FERE (ml gas/g DM)		GAP (ml)		GAPES (ml)	
	L-Ma	L-Me	L-Ma	L-Me	L-Ma	L-Me
G	137.0±7.52**	137.1±7.26**	65.1±5.4**	66.2±4.9**	88.0±8.97ns	91.0±6.88ns
L	107.6±7.84**	108.1±7.64**	81.3±5.6**	81.4±5.2**	79.0±9.42ns	79.0±7.25ns
E	138.4±7.82**	130.8±7.67**	120.6±5.6**	102.7±5.2**	88.0±9.44ns	80.0±7.27ns
PA	46.5±33.47ns	19.5±30.60ns	36.1±23.8ns	20.7±20.7ns	269.0±50.94**	264.0±39.21**
PE	123.7±59.88**	116.6±37.52**	91.6±43.8**	76.7±25.5**	172.0±78.05**	16.0±35.63ns
AE	120.3±37.51**	96.2±37.54**	90.0±27.5**	72.8±25.5**	503.0±46.37**	65±35.63ns
PAE	Ns	Ns	ns	Ns	-3620±599.24**	-2835±436.01**
R^2	40.4	44.8	72.8	59.0	82.7	60.4
S.D	15.8	15.4	11.4	10.6	18.9	14.6

G: Star grass; L: Leucaena; E: Energy source (*i.e*. Ma: Maíze; Mo: Molasses) **P<0.01 denotes significan effect for individual components. PA, PE, AE **P<0.01 denotes significan quadratic effects, ns: no significant effect (P>0.05). Values corrected for the blank[1].

.

Table 5. Regression coefficient of total concentration of VFA (CTVFA´s), ATP (PATP´s) production and microbial biomass (PMB) for the individual ingredients and their mix (Mean ± EE).

	CTVFA´s (m*M*/L)		PMB (g/g DM incubated)		PATP´s (mols/g DM incubated)	
	L-Ma	L-Mo	L-Ma	L-Mo	L-Ma	L-Mo
G	36±3.25ns	38±2.63ns	246±25.44**	241.6±21.94**	5.9±0.55ns	6.1±0.45ns
L	33±3.41ns	33±2.88ns	552±26.71**	552.9±23.13**	5.3±0.58ns	5.2±0.48ns
E	37±3.42ns	33±2.88ns	636±26.78**	577.2±23.19**	6.2±0.58ns	5.4±0.48ns
GL	97±18.46**	95±15.03**	-626±144.55**	-619.7±125.04**	15.1±3.15**	14.8±2.69**
GE	69±28.38**	13±13.76ns	-328±221.33ns	-38.2±113.62ns	10.9±4.83**	1.7±2.46ns
LE	177±16.77**	37±13.76**	-1181±131.22**	-192.8±113.62ns	29.1±2.86**	5.9±2.46**
GLE	-1362±217.26**	-1070±167.14**	9203±1669.15**	8216.2±1390.18**	-218.1±37.11**	-170.4±28.99**
R^2	82.3	58.3	53.6	46.4	81.1	53.1
S.D	6.9	5.6	85.7	86	1.2	0.9

G: Star grass; L: Leucaena; E: Energy source (*i.e*. Ma: Maíze; Mo: Molasses) **P<0.01 denotes significan effect for individual components. PA, PE, AE **P<0.01 denotes significan quadratic effects, ns: no significant effect (P>0.05). Values corrected for the blank[1].

.

DISCUSSION

The range on forage chemical composition was associated with the differences between the plant species and also is associated with irrigation with water from a pig farm, which provided high levels of macronutrients and organic matter to the soil. Leucaena CP content was within the range of previously reported values (Rosales, 2005; Monforte 2004; Rodríguez *et al.*, 2006; Sandoval-Castro *et al.*, 2002; Llamas *et al*., 2001; Pinto *et al.*, 2000).

The associative effects on the cell wall digestibility indicated that molasses increased the fiber digestibility of leucaena, which could be due to a high microbial degradation of the cell wall and their components increasing, consequently, the nutrient availability and the growth rate of the cellulolytic microorganisms and, therefore the cell wall digestibility was increased. Goodchild and McMeniman (1994) indicated that rumen ammoniac concentration is high in soon after the ingestion of tree foliage, which could be appropriate to maximize the microbial growth rate and the colonization of the cell walls. In the present study, the mixture star grass-molasses increased the fiber digestibility due to the high CP content (14 %), which promoted an increment on the growth rate of the cellulolytic microorganisms and, consequently, increased the digestibility of the cell components. This finding is in agreement with that reported by Ibrahim *et al.* (2001), who indicated that the molasses supplementation aids to increased microbial activity during *in vitro* digestion, which explains the high microbial activity with the use of molasses.

The increment of gas production when leucaena was added with any of the two sources of energy could be associated with the fact that the soluble components provided an associative effect in a short period of incubation. It is important to point out that, the microbial efficiency plays an important role since part of the consumed substrate that is fermented is incorporated in the cells; also this fermented substrate is associated with the production of other important substances, such as VFA´s, etc. (Fahey and Hussein, 1999). As it has been reported by Blümmel *et al.* (1997), the fermentation efficiency plays an important role on the fermentation process of the mixtures. The differences on the ingredients of the mixtures could result on variations on the availability of nutrients, which in turn could cause either differences on the microbial biomass growth and production or changes on gas production. A high proportion of propionic acid production will lead to a higher partition factor than that obtained with a high acetic acid production (Blümmel *et al.*, 1997). There is a negative relationship between the production of VFA´s and MBP.

The rate of VFA´s production is closely related with the rate of substrate fermentation, whereas the molar proportion is associated with the microbial species present in the rumen. In the present study, both star grass and leucaena resulted in a high proportion of acetic acid. It has been shown that (Sutton *et al.*, 1993), an increment on fiber content on the diet cause an increase the proportion of acetic acid and n-butyrate, and a reduction on the molar proportion of propionic and butyric acids. The total concentration of VFA´s was increased when leucaena was mixed with either star grass or any of the sources of energy, which is in agreement with the reports by Bonsi *et al.* (1995), who found that the rumen microbial organisms produce more VFA´s when tree foliage is included in the diet. Similarly, O´Mara *et al.* (1997) report that the VFA´s concentrations are increased when grass basal diets are supplemented with molasses.

ATP´s production, estimated by estequiometric method, is closely related with VFA´s production and with the microbial biomass. In the current study, it was found that ATP´s production was similar from both star grass and leucaena, while the energy sources resulted in an increase, although light, of both ATP´s and VFA´s production, which could be associated with the high molar proportion of propionic acid. It is worth note taking that, a mol of acetic, propionic and butyric acids allows the production of 2, 3, and 3 mmol of ATP´s, respectively.

The mixture star grass-leucaena had a quadratic negative effect (P<0.01) on MBP. A likely reason for this is the lack of a source of energy that allowed less N lost. Ammoniac increment, from the mixture mentioned above, increased the maintenance cost of the microbial population, as it has been found by Umunna *et al.* (1995); the rumen microbes degraded the protein to ammoniac, but its assimilation was inhibited by the lack of a source of energy. Therefore, the consumption of energy and the availability of carbohydrates are the primary factors that regulate the microbial protein synthesis in the rumen (Hristov and Ropp, 2003). The increment of the MBP obtained from the mixture star grass-leucaena-energy could be associated with ammoniac concentration due to the use of tree foliage and energy sources that allowed maximization of microbial growth. The advantage of supply a source of energy and protein when feeding ruminants is the likely reduction on N loss. The fermentation and synthesis of microbial protein in the rumen are increased with energy and amino acid supplementation. Therefore, a high efficiency on MBP is negatively related with N and carbohydrate availability. The lack of any of this components increase the maintenance cost of the microbial population resulting on a low efficiency on the MBP. Kolver *et al*. (1998) suggested that, the poor efficiency in which the ruminants use N could be due to the different patterns of protein and energy absorption. Obara y Dellow (1993) reported that, the synthesis of microbial protein is closely related to the total VFA´s production. In this way, a high efficiency on the synthesis on microbial protein is associated with a higher proportion of propionic acid.

CONCLUSION

VFA´s and gas production were increased when maize was added to the mixture. Also maize tended to increase the butyric acid, compared to molasses addition.

The mixture star grass-leucaena-energy causes an increment in the MBP due to a higher N and energy utilization. This resulted in a negative relationship between the production of VFA´s and ATP´s.

ACKNOWLEDGEMENT

J.M. Estrada received a scholarship from CONACYT-Mexico to undergo MSc. Studies. This work was financed by project 31661-B – CONACYT-México.

RERERENCES

A.O.A.C. 1980. Oficial Methods of Analysis, 13th Ed. Washington, DC, USA. 1018 pp.

Blümmel, M., Makkar, S.H.P., and Becker, K. 1997b. *In vitro* gas production: a technique revisited. Journal Animal Physiology Animal Nutrition, 77: 24-34.

Bonsi, M.L.K., Osuji, P.O., and Tuah, A.K. 1995. Effects of supplementation teff straw with diferent levels of Leucaena or Sesbania leaves on the degradabilities of teff straw, Sesbania, Leucaena, Tagastes and Vernonia and certain rumen and blood metabolites in Ethiopian Menz Sheep. Animal Feed Science Technology, 52: 101-129.

Fahey, C.G., and Hussein, S.H. 1999. Forty years of forage quality research: accomplishments and impact from an animal nutrition perspective. Crop Science, 39: 4–12.

Goodchild, A.V and McMeniman, N.P. 1994. Intake and digestibility of low quality roughages when supplemented with leguminous browse. Journal Agricultural Science, Cambridge, 122: 151-160.

Hristov. N.A., and Ropp, K.J. 2003. Effect of Dietary Carbohydrate Composition and Availability on Utilization of Ruminal Ammonia Nitrogen for Milk Protein Synthesis in Dairy Cows. Journal Dairy Science, 86: 2416–2427.

Ibrahim, M., Franco, M., Pezo, D.A., Camero, A. and Araya, J.L. 2001. Promoting intake of *Cratylia argentea* as a dry season supplement for cattle grazing *Hyparrhenia rufa* in the subhumid tropics. Agroforestry Systems, 51:167-175.

Juárez, L.F.I., Lagunes, M.M., García, S.C., y Canudas, L.E.G. 2004. Evaluación nutricional de gramíneas forrajeras tropicales para bovinos. Curso: Evaluación Nutricional de Forrajes en la Región del Golfo de México. 13-16 septiembre FMVZ-UADY. Mérida, Yucatán. México.

Kolver, S. E., Muller, E.L.D., Varga, G.A., and Cassidy, T.J. 1998. Synchronization of ruminal degradation of supplemental carbohydrate with pasture nitrogen in lactating dairy cows. Journal Dairy Science, 81:2017–2028.

Llamas, E., Castillo, J.B., Sandoval, C., y Bautista, F. 2001. Trees forage production and quality analyses with forage potentiality on a quarry soil in Merida. Yucatan, Mexico. In: International symposium on silvopastoral systems. Second congress on agroforestry and livestock production in Latin America. Theme: Silvopastoral systems for restoration of degraded tropical pasture ecosystems. Compiler M. Ibrahim. San José, Costa Rica. April. Pp. 2-9.

Mead, R. 1998. The Design of Experiments. Cambridge University Press, UK. 567 pp.

Menke, K.H., and Steingass, H. 1988. Estimation of the energetic feed value obtained from chemical analysis and *in vitro* gas production using rumen fluid. Animal Research and Development, 28: 7-55.

Minitab, 1997. Minitab User´s Guide 2: Data Analysis and Quality Tools. State Collage, PA. USA. Pp 21 (1)-21 (28).

Monforte-Briceño, G.E., Sandoval-Castro, C.A, Ramírez-Avilés, L., Capetillo Leal, C.M. 2005. Defaunating capacity of tropical forage trees. Effect of PEG and its relationship with *in vitro* gas production. Animal Feed Science and Technology, 123-124: 313-327.

Moss, A.R., Givens, D.I., and Phipps, R.H. 1992. Digestibility and energy values of combinations of forage mixtures. Animal Feed Science and Technology, 39:151-172.

O´Mara, P.F., Stakelum, K.G., Dillon, P., Murphy, J.J., and Rath, M. 1997. Rumen fermentation and nutrient flows for cows fed grass and grass supplemented with molassed beet pulp pellets. Journal Dairy Science, 80: 2466-2474.

Obara, Y and Dellow, W.D. 1993. Effects infusion of urea, sucrose or urea plus sucrose on plasma urea and glucose kinetics in sheep fed chopped Lucerne hay. Journal Agricultural Science, Cambridge, 121: 125-130.

Pinto, R., Ramírez. A.L., Ku, V.J.C., Hernández, A., Sánchez. F., y Saucedo, H. 2000. Evaluación químico-nutricional y degradabilidad ruminal de especies arbóreas del centro de Chiapas, México. IV Taller Internacional Silvopastoril. Memorias: “Los árboles y arbustos en la ganadería tropical”. FAO. Tomo 1. Indio Hatuey. Matanzas, Cuba. Pp . 47-50.

Price, M.L., and Butler, L.G. 1977. Rapid visual estimation and spectrophotometric determination of tannin content of sorghum grain. Journal Agricultural Food Chemistry, 25: 1268-1273.

Price, M.L., Van Scoyoc, S., and Butler, L.G. 1978. A critical evaluation of the vanillin reactions as an assay for tannin in sorghum grain. Journal Agricultural Food Chemistry, 26: 1214-1218.

Rodríguez, R., Mota, M., Fondevila, M. and Fuente, G. 2006. *In vitro* fermentation of four tropical browse legumes: estimation of the effect of tannins by gas production. In: Sandoval Castro, C.A., Hovell, FD DeB, Torres Acosta, J.F.J., Ayala Burgos, A. (Eds). Herbivores. The assessment of intake, digestibility and the roles of secondary compounds. BSAS publication No. 34. Nottingham University Press. Notthingham, U.K. Pp . 101-107.

Rosales, M.M. 2005. Efectos asociativos *in vitro* de mezclas de forrajes arbóreos para rumiantes. http:// www. cipav.org.co/redagrofor/ memorias99/ Rosales.htm. Fecha de consulta: 08-05-2005.

Sandoval-Castro, C.A., Capetillo, L.C., Cetina, G.R., y Ramírez, A.L. 2002. A mixture simples design to study associative effects with an *in vitro* gas production technique. Animal Feed Science Technology, 101: 191-200.

Sutton, D.J., Morant, V.S., Bines, A.J., Napper, J.D., and Givens, I.D. 1993. Effect of altering the starch:fibre ratio in the concentrates on hay intake and milk production by Friesian cows. Journal Agricultural Science Cambridge, 120: 379-390.

Theodorou, M.K., Williams, B.A., Dhanoa, M.S., McAllan, A.B., and France, J. 1994. A simple gas production method using a pressure transducer to determine the fermentation kinetics of ruminant feeds. Animal Feed Science and Technology, 48:185-197.

Umunna, N.N., Nsahlai, I.V. and Osuji, P.O. 1995. Degradability of forage protein supplements and their effects on the kinetics of digestion and passage. Small Ruminant Research, 17:145-152.

Van Soest, P.J., Robertson, J.B., and Lewis, B.A. 1991. Methods for dietary fibber, neutral detergent fibber, and nonstarch polysaccharides in relation to animal nutrition. Journal Dairy Science, 10: 3583-3597.

Submitted April 30, 2008 – Accepted November 25, 2008
Revised received January 05, 2009