Revista Brasileira de Zootecnia
© 2014 Sociedade Brasileira de Zootecnia
ISSN 1806-9290
www.sbz.org.br

R. Bras. Zootec., 43(8):436-439, 2014

Short Communication

Abundance and diversity of rumen protozoa in lambs fed *Gliricidia sepium* silage

Isabel Martinele[1], Lucas Feitosa Silva[2], Marta D'Agosto[1], Evandro Neves Muniz[3], José Luiz de Sá[4], Gladston Rafael de Arruda Santos[2]

[1] Universidade Federal de Juiz de Fora, Juiz de Fora, MG, Brasil.
[2] Universidade Federal de Sergipe, Aracaju, SE, Brasil.
[3] Embrapa Tabuleiros Costeiros, Aracaju, SE, Brasil.
[4] Embrapa Semiárido, Petrolina, PE, Brasil.

ABSTRACT - The objective of this study was to evaluate changes in ruminal protozoa in lambs after partial replacement of feed concentrates in their diets with *Gliricidia sepium* silage. Twenty-four male Santa Ines lambs with an average initial weight of 14.5 kg were used. The experimental design was completely randomized, with four treatments and six replications. Treatments (given as a percentage of dry matter) were as follows: control - corn silage (600 g kg^{-1} as fed) + concentrate (400 g kg^{-1} as fed); GS133 - corn silage (600 g kg^{-1} as fed) + *G. sepium* silage (133 g kg^{-1} as fed) + concentrate (267 g kg^{-1} as fed); GS267 - corn silage (600 g kg^{-1} as fed) + *G. sepium* silage (267g kg^{-1} as fed) + concentrate (133 g kg^{-1} as fed); and GS400 - corn silage (600 g kg^{-1} as fed) + *G. sepium* silage (400 g kg^{-1} as fed). Samples of rumen contents were obtained at slaughter, and analysis revealed the presence of nine genera of rumen protozoa that were present in all animals, with the exception of *Enoploplastron* and *Eremoplastron*. There were no significant differences in the average total numbers of rumen ciliates or in the composition of species between lambs. Inclusion of up to 400 g kg^{-1} (as fed) *G. sepium* silage in the diet of lambs does not affect the diversity or density of rumen protozoa.

Key Words: protein supplementation, rumen microorganism, ruminant

Introduction

In tropical and arid areas, the demand for lower cost, readily available alternative protein sources in ruminant feed has stimulated experimentation with different legumes. In addition to their protein value, some legumes have secondary compounds that can potentially act as defaunating agents. Defaunating agents reduce the density of or even eliminate protozoa in the rumen and have been considered an alternative strategy for reducing ruminant methane emissions (Delgado et al., 2012; Goel and Makkar, 2012).

Gliricidia sepium, provided in the form of hay or silage, has been used as a ruminant feed in many tropical countries because it can be stored for months or even years, provides dietary protein and solves the problem of feeding ruminants in the dry season (Avilés-Nieto et al., 2013; Oduguwa et al., 2013).

Received January 20, 2014 and accepted June 3, 2014.
Corresponding author: isabelmartinele@yahoo.com.br
http://dx.doi.org/10.1590/S1516-35982014000800006

Several studies have examined the effects of *G. sepium* on different parameters of ruminant physiology and productivity (Avilés-Nieto et al., 2013; Oduguwa et al., 2013). However, studies evaluating the effects of *G. sepium* on ruminal microorganisms are preliminary and contradictory (Espinosa et al., 2006; Delgado et al., 2010). Therefore, the present study evaluates the effects of replacing a portion of concentrate feed with *G. sepium* silage on rumen protozoa in lambs.

Material and Methods

The experiment was conducted at the Embrapa Semi-Arid Experimental Station (10°13'06"N latitude, 37°25'13"W longitude) in the state of Sergipe, Brazil. Twenty-four Santa Ines lambs with an average initial weight of 14.6±2.9 kg were used. The lambs were uncastrated and approximately four months old. The animals were housed in individual pens and distributed in a completely randomized design with four treatments and six replicates, with the lamb as the experimental unit. The animals were divided into four treatments (Table 1) which were provided at 08.00 h and 16.00 h, and the amounts were adjusted daily. Animals

were given between 100 g kg^{-1} day^{-1} and 150 g kg^{-1} day^{-1} scraps and received water *ad libitum* and mineral salt.

The animals were adapted to the experimental diets for 15 days, and the total experimental period was 87 days. Weekly samples of the provided rations were collected to determine the dry matter (DM), crude protein (CP), neutral and acid detergent fiber (NDF and ADF, respectively) contents (Table 1). Dry matter and CP were analyzed according to Silva & Queiroz (2002). The cell-wall fractions of neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined as described by Van Soest et al. (1991).

At the end of the experimental period, the animals were subjected to a period of fasting for 14 hours and were subsequently slaughtered. To analyze rumen protozoa, samples of rumen contents were collected from the rumen mass center during slaughter. Each sample consisted of 50 mL of rumen contents fixed in a 6.16 mol/m³ formol (v/v) solution (Dehority, 1984). The density of rumen protozoa per milliliter was obtained by using a Sedgewick-Rafter counting chamber (Dehority, 1984) with the modifications proposed by D'Agosto and Carneiro (1999), and the protozoa present in each sample were identified based on the criteria described by Ogimoto and Imai (1981).

Table 1 - Composition of treatments and chemical composition of treatments as a percentage of dry matter

	Composition of treatments (g kg^{-1} as fed)			
	Control	GS133	GS267	GS400
Corn silage	600	600	600	600
Concentrate[1]	400	267	133	0
G. sepium silage	0	133	267	400
	Chemical composition (g kg^{-1} as fed)			
CP	116.3	120.7	123.0	125.3
NDF	362.4	377.8	413.4	438.8
ADF	206.5	237.6	269.0	300.1

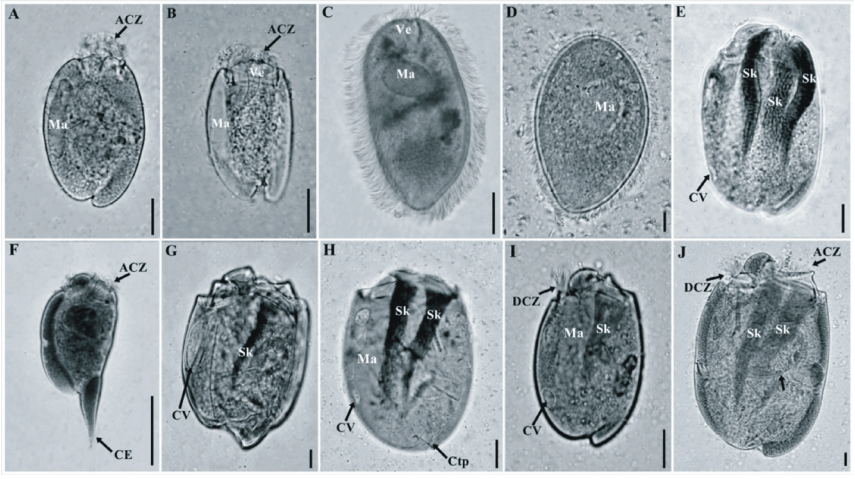
Control - 600 g kg^{-1} corn silage (CS) + 400 g kg^{-1} concentrate (C); GS133 - 600 g kg^{-1} CS + 267 g kg^{-1} C + 133g kg^{-1} *G. sepium* silage (GS); GS267 - 600 g kg^{-1} CS + 133 g kg^{-1} C + 267 g kg^{-1} GS; GS400 - 600 g kg^{-1} CS + 400 g kg^{-1} GS.
CP - crude protein, NDF - neutral detergent fiber; ADF - acid detergent fiber.
[1] Composition: milled corn (777 g kg^{-1}), soybean meal (211 g kg^{-1}) and calcitic limestone (20 g kg^{-1}).

The total and average densities of several protozoa genera were assessed. Data were tested for normality and were log base 10 (log10)-transformed when necessary. One-way ANOVA was performed on the data using the statistical analysis program R (R Development Core Team, 2010). Significant differences were declared at $P<0.05$.

Results and Discussion

The genera of protozoa present (Figure 1), the average density of protozoa and the relative abundance (%) in the rumen were not affected by the replacement of concentrate with *G. sepium* silage ($P>0.05$). The exception was *Enoploplastron*, which tended to have the highest density in the 267 g kg^{-1} *G. sepium* silage treatment ($P = 0.06$) (Table 2).

In an *in vitro* study, Delgado et al. (2010) demonstrated that the use of dried leaves of *G. sepium* containing 19.2 g kg^{-1} total polyphenols and 3 g kg^{-1} condensed tannins has a defaunating effect on ruminal protozoa. However, this effect was not observed in the present study. The effect was also not observed in another study of pastured sheep (Espinosa et al., 2006) in which feed was supplemented with *G. Sepium* flour, resulting in total protozoa densities of



A-B - *Entodinium* sp.; C - *Dasytricha* sp.; D - *Isotricha* sp.; E - *Enoplastron* sp.; F - *Eremoplastron* sp.; G - *Eudiplodinium* sp.; H - *Metadinium* sp.; I - *Diploplastron* sp.; J - *Polyplastron* sp. (arrow - predatory habit).
ACZ - adoral ciliary zone; DCZ - dorsal ciliary zone; Ma - macronucleus; CV - contractile vacuole; CE - caudal spines; Sk - skeletal plate; Ctp - cytoproct.
Scale: 20 µm.

Figure 1 - Rumen ciliates prepared with Lugol's solution.

Table 2 - Mean density and standard error of the mean (SEM) of rumen protozoa/mL (x10^4) of ruminal content in lambs fed different levels of G. *sepium* silage

Rumen protozoa	Control	GS133	GS267	GS400	SEM	P-value
Entodinium	240.42 (96.9)	277.81 (94.80)	227.68 (92.48)	272.64 (96.19)	40.01	0.80
Dasytricha	0.21 (0.08)	1.22 (0.42)	2.66 (1.08)	1.01 (0.35)	0.50	0.76
Isotricha	0.32 (0.13)	1.33 (0.45)	0.42 (0.17)	1.28 (0.45)	0.51	0.45
Enoploplastron	0	0.85 (0.29)	6.56 (2.66)	2.08 (0.73)	1.85	0.06
Eremoplastron	0.08 (0.03)	0.05 (0.02)	0	0.10 (0.03)	0.05	0.49
Eudiplodinium	0.34 (0.14)	1.17 (0.40)	1.65 (0.67)	1.28 (0.45)	0.63	0.34
Metadinium	0.48 (0.19)	0.26 (0.09)	0.16 (0.06)	0.37 (0.13)	0.21	0.72
Diploplastron	0.90 (0.36)	2.82 (0.96)	3.52 (1.42)	1.33 (0.46)	1.01	0.52
Polyplastron	5.33 (2.15)	7.52 (2.57)	3.52 (1.42)	3.30 (1.16)	1.97	0.49
Total	248.10	293.06	246.18	283.41	41.92	0.82

Control - 600 g kg^{-1} corn silage (CS) + 400 g kg^{-1} concentrate (C); GS133 - 600 g kg^{-1} CS + 267 g kg^{-1} C + 133 g kg^{-1} G. *sepium* silage (GS); GS267 - 600 g kg^{-1} CS + 133 g kg^{-1} C + 267 g kg^{-1} GS; GS400 - 600 g kg^{-1} CS + 400 g kg^{-1} GS.
Values in parentheses are the abundances relative to total rumen protozoa (%).

17.28 and 20.73 × 10^4 protozoa/mL of rumen contents in supplemented and unsupplemented animals, respectively. According to Espinosa et al. (2006), the effect of defaunatory *G. sepium* may be dependent on the associated feed, which may lead to an increase in ruminal protozoa populations. The higher density of protozoa observed in this study compared with that reported in Espinosa et al. (2006) corroborates this information, likely because the use of corn silage in the feed composition provides sufficient nutrients to stimulate growth and maintenance of protozoa.

The effects of *G. sepium* and other leguminous feeds on ruminal protozoa, and consequently on rumen physiology, may be related to the nature and concentration of secondary metabolic compounds. Secondary metabolites are influenced by various factors including local environmental and cultivation characteristics, plant maturity and form of processing and supply, among others (Goel and Makkar, 2012). Thus, understanding the factors previously mentioned and the chemical properties of *G. sepium* and other legumes used in animal feeding can aid in the understanding of their effects on the ruminal microbiota.

Conclusions

Inclusion of up to 400 g kg^{-1} of *G. sepium* silage in the diet of lambs replacing concentrate does not affect the protozoan communities, which show significant generic diversity and a high population density.

Acknowledgments

The authors thank Fundação de Apoio à Pesquisa e à Inovação Tecnológica do Estado de Sergipe (FAPITEC/SE) for the Master's Fellowship granted to L. F. Silva, Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for the Postdoctoral Scholarship granted to I. Martinele, Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for the Productivity Fellowship granted to M. D'Agosto, Embrapa Tabuleiros Costeiros (CPATC/SE) and Embrapa Semiárido (CPATSA/PE) for the assignment of facilities, and Franciane Cedrola for her assistance in preparing the figure.

References

Avilés-Nieto, J. N.; Valle-Cerdán, J. L.; Castrejón-Pineda, F.; Angeles-Campos, S. and Vargas-Bello-Pérez, E. 2013. Digestibility of Buffel grass (*Cenchrus ciliaris*)-based diets supplemented with four levels of *Gliricidia sepium* hay in hair sheep lambs. Tropical Animal Health and Production 45:1357-1362.

D'Agosto, M. and Carneiro, M. E. 1999. Evaluation of lugol solution used for counting rumen ciliates. Revista Brasileira de Zoologia 16:725-729.

Dehority, B. A. 1984. Evaluation of subsampling and fixation procedures used for counting rumen protozoa. Applied and Environmental Microbiology 48:182-185.

Delgado, D. C.; Galindo, J.; González, R.; Savón, L.; Scull, I.; González, N. and Marrero, Y. 2010. p.49-54. In: Sustainable improve of animal production and health. Odongo, N. E.; García, M. and Viljoen, G. J., eds. Food and Agriculture Organization of the United Nations, Rome.

Delgado, D. C.; Galindo, J.; González, R.; González, N.; Scull, I.; Dihigo, L.; Cairo, J.; Aldama, A. I. and Moreira, O. 2012. Feeding of tropical trees and shrub foliages as a strategy to reduce ruminal methanogenesis: studies conducted in Cuba. Tropical Animal Health and Production 44:1097-1104.

Espinosa, M. A. M.; Sánchez, D. H.; Peralta, M. A. C.; Cerrilla, M. E. O.; Martínez, G. D. and Arcos-Garcia, J. L. 2006. Comportamiento productivo y fermentación ruminal de corderos suplementados con harina de cocoíte (*Gliricidia sepium*), morera (*Morus alba*) y tulipán (*Hibiscus rosa-sinensis)*. Revista Científica FCV-LUZ 16:249-256.

Goel, G. and Makkar, H. P. S. 2012. Methane mitigation from ruminants using tannins and saponins. Tropical Animal Health and Production 44:729-739.

Oduguwa, B. O.; Oni, A. O.; Arigbede, O. M.; Adesunbola, J. O. and Sudekum, K. H. 2013. Feeding potential of cassava (*Manihot esculenta* Crantz) peels ensiled with *Leucaena leucocephala* and *Gliricidia sepium* assessed with West African dwarf goats. Tropical Animal Health and Production 45:1363-1368.

Ogimoto, K. and Imai, S. 1981. Atlas of Rrmen microbiology. Japan Scientific Societies, Tokyo.

R Development Core Team. 2010. R: A language and environment for statistical computing, version 2.13.2. R Foundation for Statistical Computing, Vienna, Austria.

Silva, D. J. and Queiroz, A. C. 2002. Análise de alimentos: métodos químicos e biológicos. 3.ed. UFV, Viçosa, MG.

Van Soest, P. J.; Robertson, J. B. and Lewis, B. A. 1991. Methods for dietary fiber, neutral detergent fiber and non-starch polysacharides in relation to animal nutrition. Journal of Dairy Science 74:3583-3597.