

Effect of the nature of energy and nitrogen sources on the population of ciliates in the rumen of sheep

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Abstract

The effect of the nature of raw materials in concentrate on the population of ciliated protozoa was evaluated in the rumen of Sicilo-Sarde sheep. Four rams with permanent rumen canulas, 4.8 ± 0.5 years with an average live weight of 45.25 ± 3.5 kg were used. Rams were fed a common base ration of hay oats (1.5 kg DM/ head/day). Animals were supplemented with 500g/head/day of one of four iso-energetic and iso-proteic concentrates. The four concentrates were: A concentrate (10% barley, 43.3% corn, 25% wheat bran, 17.7% soybean meal and 4% CMV), B concentrate (71.5% barley, 17.5% horse bean, soybean meal, 7% and 4% CMV), C concentrate (66% white sorghum, 30% fava, 4% CMV) and D concentrate (71% triticale, 18% horse bean, soybean meal, 7%, 4% CMV). The determination of ciliates was performed on unfiltered rumen fluid, collected two hours after the morning meal. The enumeration of protozoa and determination of various genuses were carried out with a HAWSKLEY counting room after several dilutions, using a microscope. The population of ciliates for, the concentrate B was significantly higher ($P < 0.05$) than those for A, C and D concentrates while different genus of these protozoa were comparable among diets. It can be concluded from this study that the use of local resources in the diet of sheep increased micro organisms in the rumen.

Keywords: Ciliates, Local resources, Raw materials imported, Rumen, Sheep

Introduction

Digestive function in ruminants is characterized by the existence of a micro population residing in the pre stomach, especially in the rumen (Krause and Russel, 1996) which can be regarded as a vast ecosystem, within which changes in environmental conditions depending on diet and its ingredients (Krause and Oetzel, 2006; Selmi et al., 2009). Protozoa are most important by their numbers and their influence on digestion. Indeed, the amount of protozoa and specifically Entodiniomorphes varies rapidly with the meal (Jouany and Ushida, 1999; Ben Salah et al., 2004). These Entodiniomorphes are very sensitive to nutrition and do not disappear in 2 to 3 days diet. Moreover, the number and different genus present in the rumen of sheep is related to several factors such as geographic region, the nutritional quality of food resources and adaptation of the animal. For example, in Australia Holotriches are not often found in the rumen of sheep (Calabro et al., 2005). However, the main factor is the diet, because the protozoa population is higher with high-energy diets (Rouissi and Guesmi, 2004; Dayani et al., 2007). Whereas a diet rich in starch

such as concentrated promotes gender Entodinium (Jouany, 1996; Jouany and Ushida, 1999).

The objective of this work was to test the effect of nitrogen source (soybean or scotch bean) and the energy source (corn, sorghum white, triticale and barley) in the feed concentrate on the population of ciliates in the rumen of Sicilo-Sarde sheep.

Materials and Methods

Four Sicilian-Sarde rams with an average live weight of 45.25 ± 3.5 kg and age of 4.8 ± 0.5 years, fitted with permanent canulas in the rumen were used in this experiment. They were housed in individual boxes (1.6 m length by 1 m width) in a building belonging to the farm of the School of Higher Education in Agriculture in Mateur, Tunisie. Animals had a common basal diet of 1.5kg DM/head/day of oat hay supplemented by 500g/head/ day of one of four concentrates (A, B, C and D). Concentrates differ by the nature of protein and energy ingredients they contained. The ration was distributed twice a day at fixed times throughout the trial (at 9.00 and 17.00 hours). The physio-chemical composition of different

concentrates is given in table 1. They were formulated to have comparable protein and energy contents to meet design requirements (AOAC, 1990).

Table 1: Chemical composition of aliments (% DM)

	Concentrate				Oat hay
	A	C	D	B	
DM (%)	94.7	94.7	95.2	95	92
CP (%)	16.3	14.65	15.2	15.26	4.9
CF (%)	12.7	3.7	4.7	9.1	35.6
OM (%)	91.0	88.3	83.8	90.8	92.1

DM: Dry matter, CP: Crude protein, CF: Crude fiber, OM: Organic matter

Different protozoa genus counting was performed on unfiltered content of rumen, collected two hours after the morning meal. A volume of 5 ml of unfiltered juice using a pipette previously sawed and 5 ml of fixative (for 1 liter: 500 ml glycerol + 20 ml + 480 ml formaldehyde distilled water) was sampled. The enumeration of protozoa and determination of various kinds were carried out with a HAWSKLEY counting room after several dilutions, using a microscope with a lens 100X. At the time of counting, protozoa were diluted several times until they were easily distinguishable in the field of the microscope and the counting became easier. Protozoa were identified from photographs and descriptions given by (Ogimoto and Imai, 1981).

Statistical analysis

The number of ciliated protozoa and different geniuses were subjected to analysis of variance by the GLM procedure in SAS (1989) using the following model:

$Y_{ij} = \mu + R_i + E_{ij}$, where Y_{ij} : is total protozoa or the count of a protozoa genus.

μ : average,

R_i : effect of the i^{th} diet (1, 2, 3, 4),

E_{ij} : random residuals

Means of different diets were compared by the test Duncan.

Results and Discussion

The majority of protozoa found in the rumen of sheep belong to the phylum of ciliates. Their numbers varied rapidly with the meal. Furthermore, protozoa species vary with the geographic area, nutritional quality of food resources and adaptation of the animal (Yanagita et al., 2000). In our study we were interested in counting Entodiniomorphes (Entodinium, and Ophryoscolex Polyplastron) and the main kind of Holotriches (Isotricha). From table 2, the total number of protozoa in the rumen regardless of the nature of the raw material making the food concentrate was similar to that advocated by Williams and Withers (1993), Jouany and Ushida (1999) and Selmi et al. (2009). The B concentrate that was made of barley and fava was associated with the highest numbers of protozoa (6.40 ± 0.15 10⁵/ml) compared to other diets ($P < 0.05$), while the D concentrate resulted in the lowest number of protozoa. Moreover, results revealed that there were no significant differences ($P > 0.05$) between A and C diets. Regarding the types of ciliates, they were dominated by the Entodinium genus regardless of the regimen. This result is in agreement with findings of Jouany and Ushida (1999). Entodinium genus is then followed in numbers by Isotricha, Ophryoscolex and Polyplastrongenuses. The Entodinium protozoa were 55.64 ± 6.21 , 54.86 ± 15 , 50.97 ± 3.10 and $56 \pm 4.09\%$ for the A, C, D and B diets, respectively, without statistical differences ($P > 0.05$), which is consistent with the results found by Selmi et al. (2009) who showed that the nitrogen source affect the total number of Entodinium and the proportion of *Isotricha polyplastron*.

Total number of ciliates in the rumen is more significant ($P < 0.05$) for the B diet. This can be explained by the nature of starch granules in barley that are rapidly fermentable in the rumen, which will result in a higher concentration of protozoa and more intense production of butyrate, end product of the metabolism of protozoa (Jouany, 1994; Demeyer and Fievez, 2000), and protein quality in terms of fava beans from those of

Table 2: Effect of the nature of energy and nitrogen sources on the population of ciliates in the rumen of sheep (10⁵/ml) and genus (%) of protozoa

	population (10 ⁵ /ml)	Genres of ciliates (%)			
		Entodinium	Isotricha	Ophryoscolex	Polyplastron
A	6.08 ± 0.23^b	55.64 ± 6.21^a	27.31 ± 6.46^a	10.95 ± 1.32^a	7.82 ± 2.82^a
B	6.40 ± 0.15^a	56 ± 4.09^a	30.37 ± 3.92^a	8.32 ± 1.83^b	5.29 ± 1.83^a
C	6.06 ± 0.22^b	54.86 ± 15^a	29.7 ± 15.29^a	8.06 ± 2.62^b	5.73 ± 3.93^a
D	5.66 ± 0.09^c	50.97 ± 3.10^a	30.48 ± 1.5^a	11.24 ± 2.35^a	6.55 ± 1.35^a

Means in the same row with different superscripts are significantly different at $p=0.05$; Concentrate A: 10% barley, 43.3% corn, 25% wheat bran, 17.7% soybean meal and 4% CMV; Concentrate B: 71.5% barley, 17.5% horse bean, soybean meal, 7% and 4% CMV; Concentrate C: 66% white sorghum, 30% fava, 4% CMV; Concentrate D: 71% triticale, 18% horse bean, soybean meal, 7%, 4% CMV

soybean meal (Selmi *et al.*, 2009). This result further explains what is found by Jouany (1991) and Eugene *et al.* (2004) who reported that in the rumen of conventional animals, deamination is intense and the ammonia concentration is always higher than that measured in the defaunated animals. While the low concentration of the population of ciliates for the regimen D can be explained by the anti-nutritional factors and triticale seed coat that prevents the degradation of proteins and starch grains even if they are readily biodegradable. The A and C diets occupy an intermediate position relative to other regimens in terms of protozoa counts. This is explained by the nature of the starch of corn and sorghum white and the speed of digestion of nutrients in addition to the close relationship between the concentration of ammonia nitrogen in the rumen (N-NH₃) and the number of protozoa (Jouany, 1994; Jouany and Senaud, 1982; Sauviant, 2004).

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