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Effect of replacing Soya by broad beans on fermentation parameters in the rumen of Sicilo-Sarde rams

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Abstract

The objective of this work was to study the effects of replacing Soybean meal by broad bean (*Vicia faba L.*) in the formulation of concentrates on fermentation parameters in the rumen of Sicilo-Sarde rams. Parameters were pH, N-NH3 concentrations, protozoa, total gas and methane productions and in sacco digestibility of oat hay for different protein sources. Four Sicilo-Sarde rams with permanent canulas were used in this trial. Rams (mean age = 4.75±0.5; mean live weight = 53.3±6.6 kg) were kept in individual boxes and received a ration distributed in two meals. The ration included 1.5 kg dry matter of oat hay and 500 g /ram/day of Soybean (S) during one month and there after the broad bean (V) during another one month period. Each of the trial periods was preceded by a two weeks adaptation period. Water was *ad libitum*. The S concentrate was 82.5% barley, 13.5% Soya, and 4% mineral mixture, while the F concentrate included 71.5% barley, 17.5% *Vicia faba*, 7% Soya and 4% mineral mixture. The energy and protein contents were 0.54, 0.96 and 0.96 UFV/kg DM and 5.20, 16.8 and 16.2 % for hay, S and V concentrates, respectively. Samples of 50 ml of rumen juice were taken before the morning meal and after 2, 5 and 8 hours to determine pH and ammonia nitrogen concentration. Types and counts of protozoa were determined on unfiltered rumen juice taken 2 hours after the morning meal and kept in a 100 ml fixing mixture. Protozoa types were counted by means of a HAWSKLEY counter. To determine gas production (CO_2 and CH_4), rumen content was collected in 100 ml plastic syringes before the morning meal and was filtered through four surgical gas layers. Rams were deprived from drinking water during the night before sampling. The DM degradability was determined by *in sacco* method using nylon bags with 50 micron diameter. Each bag contained 3 g of crushed hay and was incubated in the rumen for 48 hours. The pH of ruminal juice was comparable (P>0.05) for the S and V concentrates. Total counts of protozoa types showed that animals fed the V concentrate had higher protozoa population in the rumen (6.07 vs 5.24×10^5/ml) than other animals. However, proportions of protozoa types *(Entodinium, Isotricha, Ophryoscolex, Polyplastron)* were similar for both regimen (P<0.05).The proportion of ammonia nitrogen tended to be higher for the V compared to the S regimen (P>0.05). Total gas production was comparable for the V and S concentrates (mean = 53.5 ml, STD = 6.31 ml and mean = 51.8 ml, STD =11.5 ml, respectively), whereas potential gas production was significantly higher (p<0.05) for the V (65.4) compared to that for the S concentrate (26.8). Equations modeling the kinetic of gas production were Yt = 65.4 (1 - exp (-0.002t)) and Yt = 26.8 (1 - exp (-0.006t)) for V and S concentrates, respectively. *In sacco,* degradability was significantly more important (P<0.05) for the S compared to the V diet.

Key words: Broad Bean, Protozoa, Protein Source, Ammonia Nitrogen, Methane, Sicilo-Sarde Rams

Introduction

Rumen micoflora is dense and diversified. Polysaccharides and proteins are energy and nitrogen sources for the growth of the rumen micro-organisms. Nitrogen nutrition of ruminants requires the study of microbien system and the rumen. Several authors (Journet et al., 1983; Chenost, 1987) showed that ration valorisation by animals depends upon an efficient nitrogen supplementation. In fact, rumen ciliated protozoa are metabolically very active, able to influence fermentation of feeds and other rumen microbial populations and, consequently, to affect the amount and proportion of the end products from rumen fermentation including methane (Eugène et al., 2004). In most trials, the principal objectives were to study the effects of increasing nitrogen content in the ration on animal performances. Production of proteins by ruminants may be limited by insufficiency in some essential amino acid (AA) administrations. The

composition of digested AA by ruminants is not constant. However, even if microbial proteins and their content in AA are constant and affect variations of AA composition in intestinal contents, they depend on the ration content in AA and in lowly degradable proteins (Rulquin et al., 2001).

In Tunisia, ovine population that includes around 8 000 ewes (E.S.E.A, 2005) is solely managed by the organised sector and to lower extent by farmers. The Sicilo-Sarde is the unique dairy breed. This breed is principally found in the Beja and Bizerte regions in the North of the country. Milk, production of the Sicilo-Sarde breed is low and varies from 60 to 120 kg/an (Moujahed et al., 2009). The nutrition of this dairy ewe is based on pasture and forages supplemented with concentrate during critical physiological phases (Rouissi et al., 2008). During last years, there was a continuous increase in feed resources mainly concentrates rich in proteins. That is, the search for feed resources alternatives (Barely, *Vicia faba*, etc.) is compulsory. The objective of this trial was to study the effect of incorporating *Vicia faba* beans in the ration in replacement of Soya beans on microbial activity in the rumen of Sicilo-Sarde sheep.

Materials and methods

Four Sicilo-Sarde rams with permanent canulas in the rumen were used in the trial. Rams had an average 4.75 (standard deviation = 0.5 years) years of age and a 53.2 (standard deviation = 6.6 kg) mean live body weight. They were lodged in individual boxes throughout the essay. Rams were each fed 1.5 kg DM per day distributed twice a day. They were supplemented with 500g of one of two concentrates. The first concentrate (S) included 82.5% barley (*Hordeum Vulgare L.*), 13.5% Soya and 4% of mineral mixture, while the second concentrate (V) included 71.5% barley, 17.5% broad beans (*Vicia faba L.*), 7% Soya and 4% mineral mixture. Nutritional values of feeds were 0.54, 0.96 and 0.96 UFV/kg DM and 5.20, 16.8 and 16.2% crude protein for the hay and the S and V concentrates, respectively. Water was at will. Each regimen was used during one month and was preceded by a tow weeks adaptation period.

The ammonia nitrogen and pH were measured on 50 ml of rumen juice before and 2, 5 and 8 hours after the morning meal. Ciliate protozoa were searched for on unfiltered rumen juice sampled two hours after the morning meal and kept in a 100 ml fixing mixture at 4°c. Protozoa counts and genus were determined by means of a HAWSKLEY counter after multiple dilutions and using an X100 microscope. Total gas (CO_2 and CH_4) was determined on rumen juice taken before the morning meal and filtered through four layers of surgical gas .Rams were deprived from drinking water the night of sampling. In plastic syringes of 100 ml each, were put 0.5 g of substrate (oat hay crushed at 1 mm), 10 ml of rumen fluid and 40 ml of artificial saliva. Then, the content of each syringe was bubbled with CO_2 to eliminate oxygen and were then locked up by a piston lubricated with Vaseline. Syringes were then placed vertically in a water bath at 39°C. Gas production was recorded each 2 hours until a plateau was reached.

The dry matter degradability was determined (*in sacco*) using 15×5cm nylon bags. Incubation times were 3, 6, 12, 24, and 48 hours. The regimen effect was tested by a one way analysis of variance (SAS, 1989) and means were compared by the student t test. The Orscov and Mc Donald (1979) model was fitted to the degradability of oat hay:

$$Dg = a + b*(1 - e^{-ct})$$

Where Dg is DM degradability of oat hay at time t, is immediately soluble fraction, b is slowly degradable fraction and c is fraction b speed of degradation.

Results and discussion

The pH mean values of the rumen content for the V and S regimens recorded before and 2, 5 and 8 hours after the morning meal are given in table 1. These pH levels were comparable for the V and S concentrates and are favorable for microbe proliferation. There is a similar tendency in both regimens that the pH decreases during the five hours following the morning meal (mean values were 6.02 and 6.0 for the V and S concentrates, respectively). Thereafter, the pH tended to increase 8 hours following the morning meal to reach 6.08 and 6.06 for the V and S concentrates, respect-tively, probably because of a stabilizing effect of ciliate protozoa digesting starch (Santra et al., 2007). Using Vicia faba beans in formulating concentrates seemed not to affect pH in the rumen of Sicilo-Srade rams.

Identified protozoa in this study were mainly Entidiniomorphes and rarely Holotriches. Counts of total protozoa were in favor ($P<0.05$) of the V concentrate compared to the S concentrate (6.07 vs 5.24 $\times 10^5$/ml). However, Proportions of various types (*Entodinium, Isotricha, Ophryoscolex, Polyplastron*) were comparable for both concentrates (table 2). Rouissi (1994) observed similar results on ovine fed concentrates but Dayani et al. (2007) found lower protozoa total counts. Vicia Faba beans are rich in amino acids mainly methionine and lysine. These amino acids are good quality feeds for the rumen protozoa which may enhance their proliferation (development and count). The Entodinium genus count was the highest among protozoa types for both concentrates (table 2).

Table 1: Evolution of pH in the rumen juice

	Time (hours)			
	0	2	5	8
Regimen (V)	6.38(0.15)	6.19(0.12)	6.02(0.18)	6.08(0.14)
Regimen (S)	6.39(0.12)	6.15(0.13)	6.0(0.12)	6.06(0.12)
Statistical significance	NS	NS	NS	NS

( ): Standard deviation; NS: Not significant at α = 5%; Concentrate S: 82.5% barley, 13.5% Soya, and 4% mineral mixture; Concentrate V: 71.5% barley, 17.5% *Vicia faba*, 7% Soya and 4% mineral mixture.

Table 2: Counts (10^5/ml) and genus (%) of ciliate protozoa

		Genus			
Regimens	Total count	*Ento-dinium*	*Isotricha*	*Ophry-oscolex*	*Poly-plastron*
Regimen (V)	6.07	59.31	26.01	9.66	5.02
Regimen (S)	5.24	58.73	26.20	9.13	5.94
Statistical significance	*	NS	NS	NS	NS

*: Values in the same column are significantly different (P<0.05); NS: Not significant at α = 5%; Concentrate S: 82.5% barley, 13.5% Soya, and 4% mineral mixture; Concentrate V: 71.5% barley, 17.5% *Vicia faba*, 7% Soya and 4% mineral mixture.

Table 3: Ammonia nitrogen concentration (mg/ 100ml) in the rumen juice of Sicilo-Sarde rams fed two regimens

	Time (hours)			
	0	2	5	8
Regimen(V)	11.25(2.19)	15.28(4.76)	13.33(4.2)	11.05(2.4)
Regimen(S)	12.01(1.49)	14.6(3.28)	12.32(2.27)	10.16(1.6)
Significance	NS	NS	NS	NS

( ): Standard deviation; NS: Not significant at α = 5%; Concentrate S: 82.5% barley, 13.5% Soya, and 4% mineral mixture; Concentrate V: 71.5% barley, 17.5% *Vicia faba*, 7% Soya and 4% mineral mixture.

Rumen juice concentration on ammonia nitrogen reflects the activity of micro-organisms digesting proteins and synthesis of microbial amino-acids. The N-NH_3 concentration in the rumen depends upon various factors such as the: rate of N-NH_3 absorption through the rumen wall, proteolic activity of micro-organisms and the rate of utilization of N-NH_3 by micro-organisms. The concentration in N-NH_3 tended to be relatively higher (mg/100 ml of rumen juice) for the V regimen compared to the S regimen although the difference was not significant (P>0.05) (table 3). This result may be explained by the nature of proteins in Faba beans and the important number of protozoa in the rumen of rams fed the regimen V (Rouissi and Guesmi, 2004; Castillejos et al., 2007). The evolution of N-NH_3 content in the rumen juice (figure 1) showed that a pick

Table 4: Degradability of hay DM in the rumen

	Incubation time (hours) of nylon bags					
	3	6	12	24	36	48
V	4.35	6.44	11.64	19.85	26.75	32.87
S	5.4	9.01	14.02	27.43	31.17	34.05
Pr	0.6	0.001	0.048	0.009	0.019	0.35
R^2	7%	93%	64%	84%	78%	21%
Significance Level	NS	*	*	*	*	*

*: Means in the same row are significantly different (P<0.05), NS: Not significant at α = 5%

Table 5: Constants of hay degradation in the rumen of Sicilo-Sarde ewes: Estimated from Dg = a + b (1 – e-ct)

Regimens	a	B	C
Regimen (V)	1.80	49.40	0.020
MSE[1]	1.45	12.52	0.008
Regimen (S)	0.13	41.00	0.039
MSE[1]	1.98	4.39	0.011

MSE[1]: Mean standard error.

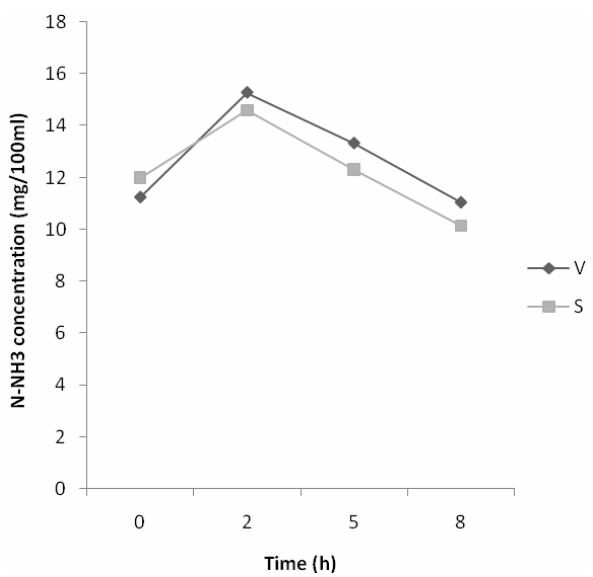

Fig. 1: Evolution of ammonia concentration (N-NH_3 in mg/100ml) in function of the nitrogen source (V: broad bean; S: Soybean)

occurred 2 hours following meal for both regimens probably because of a high microbial activity and a favorable pH for ciliate protozoa proliferation (Kayouli, 1992). Pronounced declines in N-NH3 probably because of a reduced drop in amino- acids of VFA and a change in enzymatic activity in the use of recycled N-NH_3 in the rumen. The production of N-NH_3 was the lowest (11.05 ± 2.4 and 10.16 ± 1.6 for the V and S regimens, respectively) 8 hours following the morning

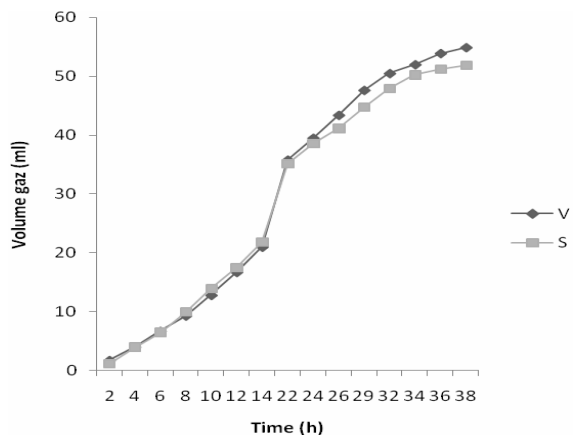

Fig. 2: Kinetics of gas production (0.5 g of oat hay) in function with the nitrogen source (V: broad bean; S: Soybean).

meal. This tendency is explained by a reduction in the microbial activity (Jouany and Senaud, 1982).

Gas production was comparable for both regimens (figure 2). This production was 53.5 ± 6.31 ml and 51.8 ± 11.5 ml for the V and S regimens, respectively. A strong relation exists between VFA measured in vivo and in sheep fed hay and maize (Rymer and Givens, 1998). The addition of concentrates in the ration favors the amylolytic fermentation and limits the cellulotic fermentation and consequently the acetic acid production necessary for CH_4 production (Vermorel, 1995). The gas production was comparable for both regimens at the beginning of the incubation (figure 2) reaching 20 ml during the first 14 hours. This slow phase corresponds to fixation of the microbial flora to particles and the adaptation of this flora to their environment. Then, the volume of produced gas increased at 22 hours of incubation corresponding to the optimal microbial fermentation. Differences in gas produced by both regimens increased thereafter until plateaus at 32 hours. The V regimen had relatively higher gas production compared to the S regimen probably because of the content of Faba beans in energy from starch (38 to 45 %). Equations describing gas production for both models were:

Regimen (V): gas (ml) = 65.4 x (1- $e^{-0.002xt}$)
Regimen (S): gas (ml) = 26.8 x (1-$e^{-0.006xt}$)

Potential gas production (b) for the V regimen was greater (P<0.05) than that obtained for the S regimen. These maximum gas productions in favorable rumen conditions were 65.4 and 26.8 ml for V and S regimens, respectively. Inversely, the rate of gas production (c) was greater for the S regimen.

Mean values of (*in sacco*) degradability of the DM of oat hay at 3, 6, 12, 24, 36 and 48 hours in the rumen are given in table 4. There were significant differences ($p < 0.05$) in DM used in the rumen at 6, 12, 24, 36 and 48 hours. In addition, the regimen seemed to affect degradability. This degradability was higher for the S regimen compared to the V regimen, essentially after 4 hours of incubation where mean values of degradability were 27.43 and 19.85 for the S and V regimens, respectively. Differences in DM degradability may be explained by the nature of the nitrogen source. That is, the Faba beans include tannins which may reduce digestibility of hydro carbohydrates (Pearson correlation coefficient = -0.84). And, the content of Faba beans in starch favors activity and limits the cellulosic activity. Digestibilities of DM after 48 hours of incubation were 34.05 and 32.87% for the S and V regimens, respectively.

The, b and c constants (table 5) estimated by Dg = a+b (1–e^{-ct}) show significant differences between both regimens for the fraction (a). The value of (a) varied from 0.13 to 1.80% of DM for the S and V respectively. These values are lower than those reported in the literature (Carro et al., 1991; Khazaal et al., 1993). Likewise significant differences were observed for the fraction (b). The fraction « b » was 49.4% for the regimen V and 41% for the regimen S. These values are comparable to those reported by Khazaal et al. (1993) for dried roughages (15.9 to 49.3%). The rate of disappearance (c) of the fraction (b) differed also between regimens (P<0.05) and was higher for the regimen S.

Conclusion

Results of this study showed that the incorporation of Faba beans in replacement of Soya beans in the ration of Sicilo-Sarde sheep maintained optimal bacterial activity in the rumen. These promoting results may be complemented by assessing the effect of the use of Faba beans in the ration on milk production of ewes.

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