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INFLUENCE OF THE NATURE OF THE ENERGY SOURCE IN THE CONCENTRATE ON THE CONCENTRATION AND MOLAR PROPORTIONS OF VOLATILE FATTY ACIDS IN THE RUMEN OF THE SICILO-SARDE SHEEP BREED

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ABSTRACT: *The effect of the nature of the source of energy supplementation on ruminal pH, concentration of volatile fatty acids (VFA) and the proportions of the main acids in the rumen of the dairy Sicilo-Sarde breed were evaluated. Four rams with an average live weight at the beginning of the experience of 45.25 ± 3.5 kg and aged 4.8 ± 0.5 years, fitted with permanent cannulas in the rumen were used in this experiment. The animals had a common basal diet at 1.5 kg DM / head / day of oat hay supplemented in turn by four concentrate at 500 g / head / d. Concentrates differed by the nature of energy ingredients they contain. The concentrate A: included 10% barley, 43.3% corn, 25% wheat bran, 17.7 % soybean meal and 4% CMV; The concentrate B was made of 66% white sorghum, 30 % beans and 4% CMV; the concentrate C had 71% triticale, 18% horse bean, 7% soybean meal and 4% CMV; and finally the D concentrate included 71.5% barley, 17.5% field bean, 7% soybean meal, and 4% CMV. 50 ml samples were taken before, 2, 5 and 8 hours after the distribution of the morning meal, and were filtered through four layers of surgical gaze. These samples were used for the analysis of volatile fatty acids (VFA) concentrations by gas chromatography. Results showed that the rumen pH was statistically different (P<0.05) before and 2 hours after the morning meal distribution among concentrates. It was in favour of C and D (P<0.05) concentrates but it has stabilized at the end of the day (P>0.05). The concentration of total VFA was significantly higher (P<0.05) for diets C and D just after the distribution of the meal before it became comparable (P>0.05) among concentrates after 5 and 8 hours post prandial. The proportion of acetate and butyrate (C2 and C4) acids evolved in the same way during the day regardless of the regimen but were in a reversed manner for the propionic acid (C3).*

Keywords: *Acetate, butyrate, supplements, energy source, pH, propionate*

ORIGINAL ARTICLE

INTRODUCTION

Rumen microbial population and network convert all carbohydrates into monosaccharides (hexoses or pentoses) with special enzymatic equipment. The soluble carbohydrates are hydrolyzed very rapidly and completely. Cereal starch is degraded from 90 to 95% in the rumen by bacterial amylase (Grinari et al., 2000; Russell and Gahr, 2000). Microbial population degrades carbohydrates walls (cellulose, hemicellulose and pectin) into monosaccharides that are then fermented anaerobically in ways known as metabolism (Cuvelier et al., 2005). The products of this fermentation is a mixture of short chain organic acids, known as volatile fatty acids, mainly acetic, propionic and butyric acids, and carbon dioxide and methane (Fonty et al., 1995). The ruminant gets most of the energy it needs from volatile fatty acids (VFA) by

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the degradation of cytoplasmic and cell wall carbohydrates by rumen microorganisms. They can provide 65 to 75% of the energy absorbed (Jouany, 2000; Moujahed et al., 2003). With the usual diet forage-based used by ruminants, the relative proportions of VFA in % molecules are: Acetic acid (C 2) from 60 to 70%, Propionic acid (C3) from 15 to 20%, butyric acid (C4) from 10 to 15% and other VFA from 2 to 5% (Bergman, 1990). The VFA are then absorbed through the rumen epithelium, with more efficiency for longer carbon chains, presumably by passive diffusion of undissociated acid on the one hand, and mainly in anionic form in the other hand (Russel and Gahr, 2000). The VFA are in fact weak acids (pK ≤ 4.8) and with rumen pH approaching neutrality, they are mainly present as anions acetate, propionate and butyrate rather than as acetic, propionic and butyric acid (Bergman, 1990). The proportions of different VFA products are mainly dependent on the nature of the regimen. Indeed, rumen microorganisms depend upon the substrates they are capable of degrading and / or fermenting (Cuvelier et al., 2004). Nutrients in the diet therefore determine the nature of the rumen microorganisms, which guide the production of VFA metabolism by their respective companies. Diets rich in forage promote the production of acetate propionate. Butyric acid production is, in turn, increased in diets containing ingredients high in soluble sugars, such as beets (sucrose) or whey (lactose) (Jouany et al., 1995). Rouissi (1994) reported that the concentration of VFA in the rumen was significantly higher for a diet based on hay + concentrate compared to a diet made of hay whatever the species and sampling time during the day were. In the same context, Peyraud (1993) showed that the addition of concentrate to forage composed of rapidly fermentable carbohydrates such as barley and wheat compared to maize and sorghum fermentation causes a deviation towards a higher proportion of propionic acid and / or butyric acid at the expense of acetic acid.

Moreover, a decrease in the level of ingestion causes a decrease in the amount of organic matter fermented in the rumen, and thus a significant reduction in the production of VFA and especially propionate (Doreau et al., 2000). This trend is more pronounced with diets rich in concentrate (Merchen et al., 1986) while a decrease in particle size or increasing the particle density by grinding increases the VFA content of rumen fluid and the molar proportion of propionic acid at the expense of that of acetic acid (Sauvant, 2000).

The effect of the nature of the ingredients of the concentrate on the production of VFA and the proportions of the main acids in the rumen of Sicilo–Sarde sheep was the subject of this study when replacing maize (imported feed resource) by different local energy feed resources (white sorghum, triticale and barley).

MATERIALS AND METHODS

Animals, diet and feeding regimen

Four Sicilo - Sarde rams with an average live weight at the beginning of the experience of 45.25 ± 3.5 kg and aged 4.8 ± 0.5 years, fitted with permanent cannulas in the rumen were used in this experiment. The animals had a common basal diet at 1.5 kg DM / head / day of oat hay supplemented in turn by four concentrated feed at 500 g / head / day. Concentrates were made of different energy sources, maize, white sorghum, triticale and barley. The experiment was conducted in a Latin Square design. The ration was distributed twice a day at fixed times throughout the test (9am and 17 pm). The chemical composition (AOAC, 1995) and food values (Sauvant, 1981) concentrated feed used are summarized in Table 1.

Table 1. Centesimal composition and nutritive value of fed concentrates

Ingredients (%)	Concentrates			
	A	B	C	D
Corn	43.3	-	-	-
Wheat bran	25	-	-	-
Barley	10			71.5
white sorghum	-	66	-	-
Triticale			71	
Faba bean meal	-	30	18	17.5
Soybean meal	17.7	-	7	7
VMC	4	4	4	4
UFL	0.98	0.99	0.98	1.1
PDIE (g/kg of DM)	104.9	95	103	103
PDIN(g/kg of DM)	99	96	102	100

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Sampling procedure

The samples for the determination of various parameters of rumen fermentation (pH, total VFA and VFA molar proportion) were taken just before the distribution of the morning meal (0:00), 2, 5 and 8 hours after the same meal. The inoculum (a mixture of the solid phase and the liquid phase of rumen) was collected using a plastic rod of length 35 cm and internal diameter of 2.5 cm. The pH of the inoculum was measured immediately after each sampling to avoid changes in air using a digital pH meter (Hanna, HI 9024/HI 9025). Before each measurement, the instrument was calibrated using two buffer solutions pH 4 and pH 7, the electrode tip in a solution of KCl. 50 ml samples filtered through four layers of surgical gauze were used for analysis of VFA concentrations by gas chromatography. The samples were centrifuged for 20 minutes at a rate of 4000 revolutions / min in a centrifuge Hettich centrifuges type EBA 21. The device used is a chromatograph type GC - FID (Agilent 6890 N) equipped with a flame ionization detector and using a column headed HP-removable type INNOWX stationary phase polyethylene glycol and having a length of 30 m and an inner diameter of 250 µm and the thickness of the wire is 0.25 µm.

Statistical analyses

The results of the effects of diets on ruminal pH, total VFA concentration and proportions of different acids were subjected to analysis of variance using the GLM procedure of SAS (1989) and compared by a Duncan test (1955). $Yij = \mu + R + Eij$

Where Yij: measured parameter
µ: overall mean
R: effect of the ith diet (1, .., 4)
Eij: residual error for the jth replicate

RESULTS AND DISCUSSION

The pH of the rumen before the morning meal distribution was statistically comparable ($P>0.05$) for plans A, C and D, respectively. Measured mean values were 6.67 (STD = 0.34) 6.60 (STD= 0.27) and 6 71 (STD= 0.27) and low ($P<0.05$) for the regimen B (6.28 (STD = 0.22)). This result is similar to those of Rouissi (1994) and Hammami (2009) and below the range of pH in the rumen of sheep receiving hay alone (Giger et al., 1988). Two hours postprandial, the pH decreases for the four feeding schemes, but the decrease was greater for diets C and D (0.44 and 0.49 points respectively), while the other two systems the decrease was minimal (0.19 to 0.1 for A and B). After 5 hours, the pH continues to decline, it was 6.22 (STD = 0.42) 5.97 (STD = 0.22) 6.06 (STD = 0.29) and 6.01 (STD =0.12) for the A, B, C and D concentrates, respectively. The statistical analysis revealed that there is no difference between rumen pH among the four regimens ($P>0.05$). This trend reached those reported by Santra et al. (2007) and Hammami et al. (2009). At the end of the day, the pH increased significantly ($P<0.05$) and was more stable and buffered diets C and D compared to diets A and B.

The general trend of the change in pH in the rumen of Sicilo- Sarde rams is in the same direction as those of Giger et al. (1988); Rouissi (1994) and Hammami et al. (2009). This variation during the day is explained by the fact that the addition of different types of concentrates in the diet causes changes in the flow of digesta leaving the rumen on the one hand, the amount and nature of the products absorbed in ruminal walls on the other hand (Oetzel et al., 1999). Just before the distribution of the morning meal, the pH is at its maximum value explained by the role of bicarbonate ions (HCO-3) and phosphate (HPO^{-2} 4) in the saliva that occurs in a massive way in rumination (Sauvant et al., 2006). Concentrate feed with their energy source are grains of cereals (triticale and barley) have the highest values with significant differences ($P<0.05$) compared to the concentrated energy source which is the white sorghum. This can be explained by intense production of saliva and the rapid digestion of sorghum grain compared to white corn (Michalet-Doreau and Sauvant, 1989). The significant difference ($P<0.05$) between the A and B concentrates before the distribution of the meal may be due to the fact that the concentrate A contains besides the corn, a proportion of barley and wheat that are grains with large sizes and that the size of type A is concentrated cap while the B concentrate is starchy. This joins the conclusion of Sauvant (2000) who showed that the decrease in pH is almost routine when the size of particles from the feeding plan or any of its components is reduced which may explain the decrease in the daily duration of rumination and therefore the decrease in saliva production. The decrease in pH two hours after the meal distribution is highly significant ($P<0.05$) for concentrated energy based on cereals (barley, triticale). These values are within the ranges noted in the results found by Giger et al. (1988) and Sauvant et al. (2006). The latter other reported that the pH is lower for the concentrate rich in cereals (barley, triticale), which is explained by the amount of rapidly fermentable starch in it and the high production of VFA which in turn promote the stability of the pH after absorption through the rumen wall. In

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this context, Sauvant and Van Milgen (1995); Claps et al. (2000) reported that the close relationship between rumen pH and rumen VFA profile may be an indicator of the nature of the rumen fermentation, especially the ratio acetate / propionate (A / P). This ratio is an index of energy status of specific microbes and rumen pH. 5 hours post-meal, the pH continued to decrease with no statistical differences among the different regimes (P>0.05) and the fall is most notable for plans A and B (- 0.26 and - 0.21). This is attributed to the slow degradation of corn starch and sorghum-white. At the end of the day (after 8 hours of the morning meal distribution), the ruminal pH stabilizes again with significant differences between diets (p <0.05), the highest values are displayed Plans for the grain.

Volatile fatty acids are the end products of rumen digestion of carbohydrate foods that include various compounds which are derived from either plant cell walls such as cellulose, hemicellulose and pectin, or the cell contents, such as the starch and soluble sugars (Jarrige et al. 1995; Sauvant, 1997), their concentration depends on the amount of energy provided by the food and the quality of starch degradation in slow or fast (Sauvant et al. , 1994; Cuvelier et al., 2005).

The study of the effect of the nature of the energy source at the complementation showed that the concentration of total VFA in the rumen just before the distribution of the morning meal is low compared to other periods of control during the day with a minimum value observed for the concentrate B (P<0.05). This can be explained by the absorption of VFA across the rumen wall used by the bacteria to produce their own proteins. Two hours after the distribution of the morning meal, the concentration increases with an intense speed (P<0.05) for diets C and D (86.5 ± 1.76 and 85.45 ± 0.69 mmol / l respectively) compared to diets A and B. This result is consistent with that of Chikagwa-Malunga et al. (2009). This trend can be explained by the quality of the starch found in the seeds of barley and triticale.

After five hours of the morning meal, the concentration of VFA from the schemes A and B reached the peak (89.03 ± 0.82 and 87.28 ± 1.05 mmol / l respectively), this would be attributed to the degradation of starch grains of white maize and sorghum. This corroborates with the results of Russell and Gahr (2000), no statistical difference among the means of four concentrates (P>0.05). It is also noted that the high concentration of VFA for concentrate C is correlated with the low gas production especially at the beginning of incubation. At the end of the day, the VFA concentration is stabilized (P>0.05) for the different regimes, this decrease in concentration can be explained by the rate of absorption and activity of microorganisms in the rumen (Rouissi, 1994) (Table 2).

Table 2. Effect of the nature of energy sources on the ruminal pH and Total VFA (mmol/l)

	Hours after the morning feeding			
	0	2	5	8
Ph				
A	6.67 ^{a} ± 0.34	648 ^{a} ± 0.38	6.22 ^{a} ± 0.42	6.25 ^{b} ± 0.34
B	6.28 ^{b} ± 0.22	6.18 ^{b} ± 0.13	5.97 ^{a} ± 0.22	5.99 ^{b} ± 0.31
C	6.60 ^{a} ± 0.27	6.16 ^{b} ± 0.21	6.06 ^{a} ± 0.29	6.40 ^{a} ± 0.35
D	6.71 ^{a} ± 0.27	6.16 ^{b} ± 0.12	6.01 ^{a} ± 0.12	6.34 ^{a} ± 0.24
SME	0.084	0.069	0.083	0.091
Total VFA (mmol/l)				
A	76.85^{a} ±1.1	81.4^{b} ± 1.46	89.03^{a} ± 0.82	86.3^{a} ± 0.83
B	70.33^{b} ±1.58	78.36^{b} ± 1.3	87.28^{a} ± 1.05	83.9^{a} ± 1.22
C	75.45^{a} ± 0.59	86.5^{a} ± 1.76.	88.55^{a} ± 07	83.3^{a} ± 1.24
D	74.68^{a} ±1.49	85.45^{a} ± 0.69	87.58^{a} ± 0.74	84.66^{a} ± 1.03
SME	1.96	2.74	1.09	1.87

$^{a, b \text{ and } c}$: Means with different superscripts within a row differ significantly (P <0.05).

The proportion of acetic acid changes the same way for concentrated feed C and D, the minimum value was observed after two hours of the distribution of meals (65.4 and 66.06% respectively). Then increases after 5 hours and stabilized at the end of the day with no statistical difference among the regimes (P>0.05). This trend is similar to that demonstrated by Chikagwa-Malunga et al. (2009) and can be explained by the orientation of the fermentation of starch grains of barley and triticale with a strong and rapid degradation thereby reducing the synthesis of acetate and promoting that of propionate increase after the circulation of morning meal and the maximum value was displayed after two hours (P>0.05) (17, 08 and 16.83% for the C and D diets, respectively) (Jouany et al., 1995). This can partly explains what is reported by Giger et al (1988) that the concentration of acetate and propionate in the rumen are reversed during the day. For diets of slowly degradable starch resources, minimum values are reached after 5 hours (65.63 and 65.58%) as shown in Figure 1, while propionate is highest at this time (Figure 2). The proportion of acetate is stable at the beginning and the end of the day

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(P>0.05). This is mainly due to the rate of absorption of through the rumen wall and its use by bacteria in the presence of ammonia nitrogen for the synthesis of their protein, whereas it is statistically higher (P<0.05) for A and B 2 hours post prandial. On the concentration of butyric acid in the rumen, it has a profile similar to that of acetate as shown in Figure 3; the proportion was 11 to 13% during the day. This is consistent with results reported by Rouissi (1994) and is lower than that determined by Jouany et al. (1995) especially when the plan is based on beet.

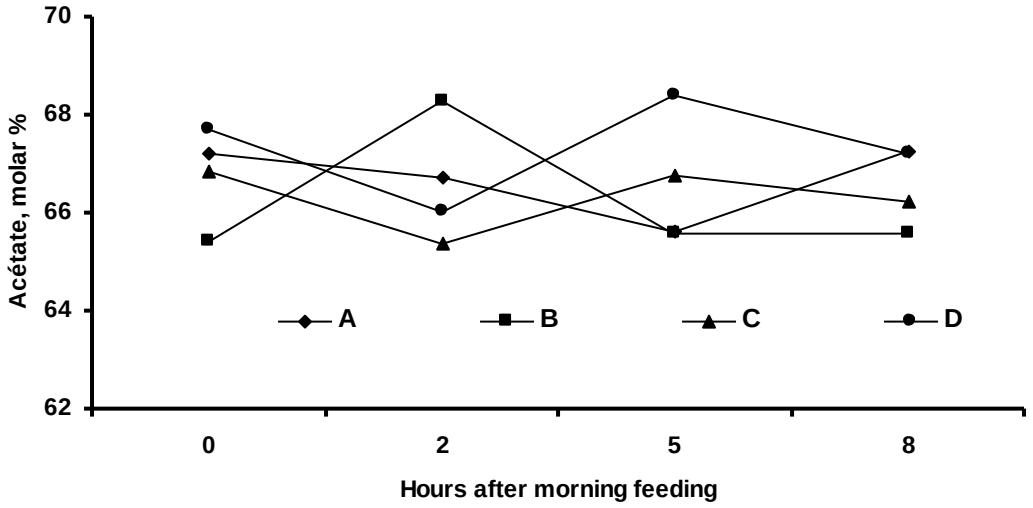

Figure 1. Ruminal acetate proportion (%) in the rumen of Sicilo- Sarde rams fed different concentrate

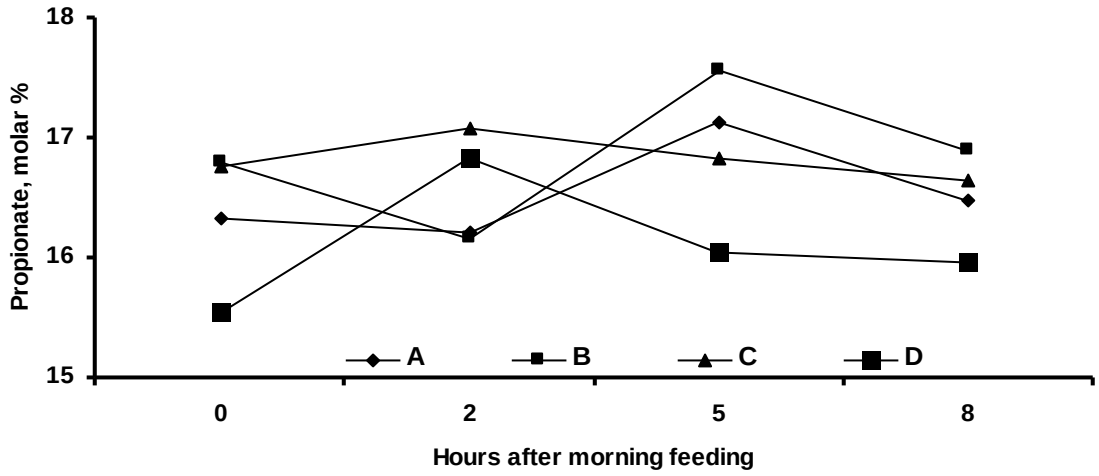

Figure 2. Ruminal propionate proportion (%) in the rumen of Sicilo- Sarde rams fed different concentrate

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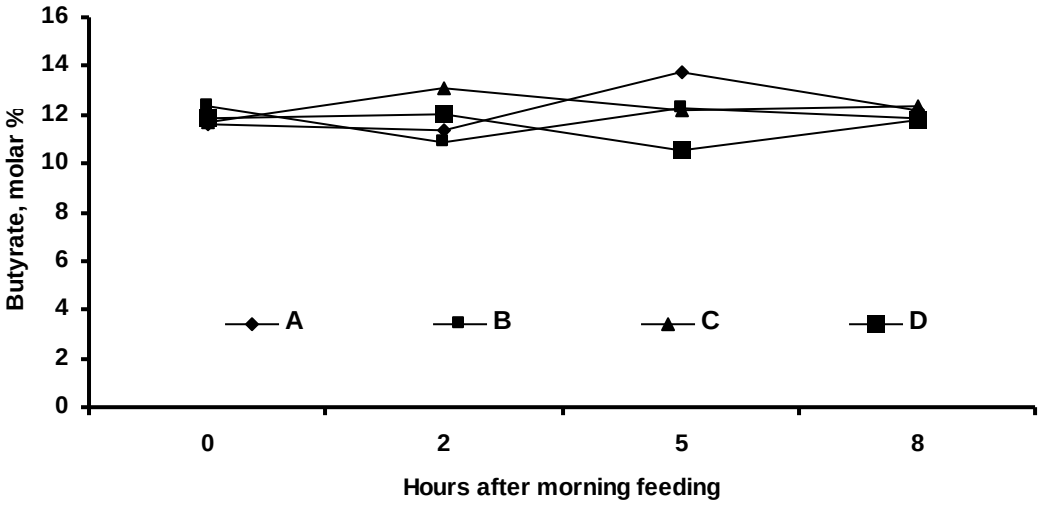

Figure 3. Ruminal Butyrate proportion (%) in the rumen of Sicilo- Sarde rams fed different concentrate

CONCLUSION

Through this experiment, it appears that the effect of the incorporation of local raw materials rich in energy such as white sorghum, triticale and barley to replace corn in the formulation of concentrates for feeding dairy sheep can have a significant concentration of volatile fatty acids with a significant superiority for the diet based on barley and triticale for the nature and amount of starch they contain and results in a change in other parameters such as fermentation, pH, and total gas production, especially of methane, which is closely related to the amount of acetic acid and butyric acid.

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