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Imapact of replacing corn and soya beans with sorghum and faba beans on ruminal fermentation and milk quality in sicilo sarde dairy ewes

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Abstract:

In the first study: Four rams bred Sicilo - Sarde aged of 4.8 ± 0.5 years with an average live weight of 45.25 ± 3.5 kg, permanently canulated in the rumen. Rams received a daily ration in two equal meals. The diet contained 1.5 kg DM of oat hay, complemented by two concentrates: The control concentrate (A) included 10 % barley, 43.3 % corn, 25 % wheat bran, 17.7 % soybean meal, and 4 % of sheep mineral mixture (VMC) and the experimental concentrate (B) included 66 % white sorghum, 30 % faba bean, and 4% of VMC. The results showed that ruminal pH is statistically different ($p < 0.05$) before and 2 hours after the morning meal distribution between the two types of concentrates but stabilizes at the end of the day ($p > 0.05$). The rate of ammonia nitrogen is in favor of the control diet ($p < 0.05$) before the distribution of the morning meal. The population of ciliated protozoa is similar for both diets (p> 0.05. Different genres are statistically comparable for the two regimes. They were (55.64 ± 6.21%) Entodinium, (27.31 ± 6.46%) Isotricha, (10.95 ± 1.32%) Ophryoscolex , (7.82 ± 2.82%) for the concentrate Polyplastron witness against (56 ± 4.09%, 30.37 ± 3.92%, 8.32 ± 1.83%, 29.05 ± 1.83%) for the experimental concentrate. In the second test, twenty (20) ewes were divided into two groups homogenous for age (5.3 ± 1.25 vs. 5.7 ± 1.15 year), litter size (1.1 ± 0.31), the rank of lactation (4.3 ± 1.25 vs. 4.6 ± 0.96) and live weight (33.83 ± 5.63 vs. 33.95 ± 5.58 kg). Animals were lodged in similar boxes (1.01 m^2/ewe) and received 1.5 kg DM/ewe/day of oat hay. The level of average production was 0.4 l for the control group (A) and the experimental group (B). Regarding the quality of milk, replacing corn and soybean to white sorghum and faba affected the dry matter content and density ($p < 0.05$) without altering the fat content (TB), the protein content (TP) and pH ($p > 0.05$). As the urea content is highly elevated in favor of the regime A ($p < 0.01$). The concentrations of various fatty acids in milk were statistically comparable ($p > 0.05$).

Key Words: Dairy sheep, imported raw materials, local raw materials, milk performances, ruminal fermentation.

1. Introduction

Today, production is not enough even if the products are of high quality. The difficulty lies in marketing and the reduction of production costs. For this, it is imperative to have a comprehensive understanding of the economic environment and its stakeholders: consumer, intermediate and breeder. In addition, we must not forget that economic considerations do not stop at our borders.

For years, corn-soybean diets were considered staple foods for ruminants whatever their production (milk, meat) [1], because of their richness in protein and energy [2] and are generally imported prices. Today, in the context of a growing shortage of raw materials, the use of search alternatives for livestock nutrition is imperative [3] and [4]. Indeed, the use of grasses and protein produced at the farm (white sorghum, faba bean) remains a common practice in animal production [5]. In addition, to meet the nutritional needs of animals, it is necessary to establish rationing plans adapted to the intake capacity and tastes of animals at lower cost [6]. In addition, the choice of a food concentrate is made from technical and economic criteria [7] and from the technical criteria, it is necessary to take into account not only the nutritional characteristics of the food but also of the reaction vis-à-vis the microorganisms in the rumen and the profile of the animal.

It is within this frame work that fits our work focuses on the effects of replacing imported raw materials (corn and soybean) by local resources (white sorghum and faba) on fermentation parameters (pH, concentration of nitrogen ammonia NH_3-N, and Entodiniomorphes population) in the first place and evaluation

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performance dairy and physico-chemical quality of milk of Sicilo-Sarde sheep in a second.

2. Materials and Methods

2.1 . Study 1. Ruminal fermentation

2.1.1. Animals and diets

Four rams breed Sicilo - Sarde with an average live weight at the beginning of trial 45.25 ± 3.5 kg and aged 4.8 ± 0.5 years, fitted with a permanent canulas in the rumen were used in this experiment. They were housed in individual boxes with wire length 1.6 m and width 1 m in a building belonging to the farm from the School of Higher Education in Agriculture of Mateur, Tunisia. The animals had a common basal diet at 1.5 kg Dry Matter / head / day of oat hay supplemented by two concentrates (A) and (B) at a rate of 500 g / head / day by the different nature of protein and energy ingredients they contain. The four rams have received successively four concentrates during a measurement period of 30 days separated by an adjustment period of two weeks). The ration was distributed twice daily at fixed times throughout the trial (9 am and 17h after noon). Samples of different materials tested were analyzed for their mineral content (OM) and total nitrogenous matter (CB) according to [8]. The percentage composition of feed concentrates and chemical composition of the various constituents of the diet are illustrated in Table 1.

Table 1. Ingredient proportions and chemical composition (% DM) of concentrate and oat hay**.**

	Type of Concentrate		**Oat hay**
	A	**B**	
Barley	10	-	-
Corn	43.3	-	-
White sorghum	-	66	-
Wheat bran	25	-	-
faba bean meal	-	30	-
soybean meal	17.7	-	-
VCM sheep	4	4	-
DM (%)	94.7	94.7	92
Organic matter	91.0	88.3	92.1
Crude protein	16.3	14.65	4.9
Crude fiber	12.7	3.7	35.6

2.1.2. Analytical Methods

The samples for the determination of various parameters of rumen fermentation took place just before serving meals in the morning (before, 2, 5 and 8 hours after the meal). Inoculum (a mixture of the solid phase and liquid phase rumen contents) was collected using a plastic rod of length 35 cm and internal diameter of 2.5 cm. The pH of the inoculum was measured just after each sampling to avoid changes in air using a digital pH meter (Hanna, HI 9024/HI 9025). Before each measurement, the instrument is calibrated using two buffer solutions pH 4 and pH 7, the electrode tip in a solution of KCl. Before each measurement of pH, the electrode is rinsed with distilled water and wiped dry.

The content of ammonia nitrogen (NH_3-N) was conducted using the method of [9]. The principle of this method is based on a simple gaseous diffusion on the substance evaporated. The ammonia was removed by a solution of potassium carbonate (K_2CO_3) and captured with a solution of boric acid (H_3BO_4) 1%. To do this, 1 ml of boric acid was deposited in the center of a disc of Conway. In the outer chamber of the same disk, 1 ml of rumen fluid centrifuged to 2500 revolutions per minute for 15 minutes was deposited on one side and 1 ml of K_2CO_3 on the other side. The juice was then mixed thoroughly with potassium carbonate by rotating the disc gently closed. The release of ammonia by potassium carbonate leads after receipt by the boric acid, the color changes from red to light green. The titration was done 4 hours after the closure of the cells with hydrochloric acid (HCl: 0.01 N). The samples were analyzed in duplicate, controls (without rumen fluid) were also prepared in the same way and therefore the concentration of ammonia nitrogen was determined using the following formula:

NH_3-N mg /ml of juice = (V (HCl E) - V (HCl T)) * N (HCl) * 14 g

Where: V (E HCl): volume of the sample, V (T HCl): volume of the witness.

N (HCl): HCl normality.

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 [10].

2.1.3. Statistical analysis

The results of the effects of diets on the parameters measured were subjected to analysis of variance by GLM procedure of [11] and compared by Duncan's test.

The model used to compare the rumen Ph, concentration of ammonia nitrogen, the number of ciliated protozoa and different geniuses and digestibility of hay depending on the experimental diets.

Equation model: $Yij = \mu + R + Eij$

Where Yij: parameter measured

μ: average, R: effect of the regimen, and Eij: random residuals

2.2. Study 2. Milk yield and quality

2.2.1. Animals and Diets

Twenty (20) Sicilo-Sarde ewes were divided into two homogeneous batches according to age (5.3±1.25 years as against 5.7±1.15 years), the litter size (1.1±0,31), lactation number (4.3±1.25 vs. 4.6±0.96) and weight (33.83±5.63 vs. 33.95± 5.58 kg) received a ration common base (oat hay) at 1.5 kg DM/ewe/day supplemented by a 500g/ewe/day standard concentrate (A): (10% barley, 43.3% corn, 25% wheat bran, 17.7% soybean meal, 4% sheep Vitamin and Mineral Mixture (VMC)), experimental group (B): (66% white sorghum, 30% faba, 4% sheep VMC). The percentage composition of feed concentrates and chemical composition of the various constituents of the diet are illustrated in Table 1 (Study 1).

2.2.2. Milk analysis

Throughout the period of the experiment, it was a milk recording quantitatively with a daily rate of 2 milking per day manual: around 10 am and the second at 15:30. The study of milk quality was performed once a week on a sample of bulk milk batch to measure pH and determine the physico-chemical composition of milk by Lactoscan (Milkotronic LTD, serial No. 4696, Hungary), content milk urea is determined by the method DMAB [12]. Then, we adjust the spectrophotometer to zero through the reagent blank and read the absorbance at 420 nm. Before the analysis of milk samples by chromatography, extraction of lipids took place following the method of [13]. The device used is a gas chromatograph FID type of fatty acid methyl esters, with a column OMEGAWAX 250. The temperature at the detector and the injector was 220° C while the level of the column was programmed from 45 ° C to 190 ° C.

2.2.3. Statistical analysis

The results of the effects of diets on the parameters measured were subjected to analysis of variance by GLM procedure of [11]. Equation model: $Yij = \mu + R + Eij$

3. Results and discussion

3.1. Rumen fermentation parameters

The pH of the rumen before the morning meal distribution (Table 2), is significantly lower ($p < 0.05$) for diets A, (6.67 ± 0.34) for B (6.28 ± 0.22). This result is similar to those of [14] and [15] and below the range of pH in the rumen of sheep receiving hay alone [16]. Just before the distribution of the morning meal, the pH is at its maximum value explained by the role of bicarbonate ions ($HCO3^-$) and phosphate ($HPO4^{2-}$) in saliva that occurs in a massive way during rumination [17]. The significant difference ($p < 0.05$) between the regime A (6.48 ± 0.38) and the regime B (6.18 ± 0.13) before meal distribution may be due to the fact that the concentrate contains A except maize, the proportion of barley and wheat that are cereals on the one hand and secondly because the size difference is concentrated A type cap while B is mealy, this parallels the conclusion of [18], which showed that the pH drop is almost routine when the size particles of the system or one of these components is reduced and explained by the decrease in the daily duration of rumination and consequently the decreased production of saliva. Five hours post-prandial, the pH continued to decrease without statistical difference between the different diets ($p > 0.05$) and that the fall is most notable for plans A and B (- 0.26 and - 0.21). This is attributed to the slow degradation of corn and White sorghum. So that by the end of the day (after 8 hours of the morning meal distribution), the rumen pH stabilizes again with no differences between diets ($p > 0.05$).

Ammonia is an essential precursor for microbial growth of most species of bacteria and protozoa in the rumen ciliates. It is even regarded as the main source of nitrogen for several bacterial strains, particularly those involved in the digestion of cellulose and starch.

The concentration of ammonia nitrogen from the rumen fluid is the result of three key factors whose effects are additive: The rate of absorption of ammonia through the rumen wall, the proteolytic activity of microorganisms in the rumen and the rate of use of ammonia nitrogen by rumen microorganisms which is itself proportional to the amount of energy (ATP and VFA) in this compartment. The NH_3-N concentration before food intake was 9.21 ± 2.63 mg/100 ml of rumen fluid for the regime A with significant difference ($p < 0.05$) with B (8.05 ± 3.76)

Taking into account the nitrogen source, there is no significant difference between the soybean meal and field bean ($p > 0.05$), which is similar to the results of [15]. This is attributed to the wealth of fava bean digestible protein compared to those of soybean meal [19]. The optimal concentration observed after two hours of serving meals for the two concentrates on the one hand explains more intense degradation of proteins and the desamination of their amino acids [20] and secondly the correlation significant between the concentration of NH_3-N and the total number of ciliates protozoa [21]. After 5 and 8 hours postprandial, the amount of ammonia in the rumen decreased significantly without a significant difference between diets ($p > 0.05$). This appearance resembles that of [15] and can be explained by absorption through the rumen wall and used by bacteria to synthesize their own proteins.

Table 2. Rumen parameters fermentation

Regimen	Hours after the meal			
	0	2	5	8
pH				
A	6.67 ^{a} ± 0.34	6.48 ^{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
SME	0.084	0.069	0.083	0.091
N-NH_3 (mg/100ml)				
A	9.21 ^{a} ± 2.63	12.83 ^{a} ± 3.7	6.40 ^{a} ± 2.47	5.23 ^{a} ± 3.08
B	8.05 ^{b} ± 3.76	11.66 ^{a} ± 3	7.46 ^{a} ± 2.88	5.71 ^{a} ± 2.42
SME	0.72	0.95	0.78	0.73

^{ab}: Means with different superscripts within a row differ significantly ($p < 0.05$).

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 [22]. In our study we were interested in counting Entodiniomorphes (Entodinium, and Ophryoscolex Polyplastron) and the main kind of Holotriches (Isotricha). From table 3, the total number of protozoa in the rumen regardless of the nature of the raw material making the food concentrate was similar to that reported by [23] and [24]. Regarding the types of ciliates, they were dominated by the Entodinium genus regardless of the regimen. Entodinium genus is then followed in numbers by *Isotricha, Ophryoscolex and Polyplastron* geniuses. The Entodinium protozoa were 55.64 ± 6.21, 54.86 ± 15 % for the A and B diets respectively, without statistical differences ($P > 0.05$), which is consistent with the results found by [3] who showed that the nitrogen source affect the total number of Entodinium and the proportion of *Isotricha*, *polyplastron.* This result further explains what is found by [25] and [26] 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 and 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-NH_3) and the number of protozoa [27].

Table 3. Effect of the nature of energy and nitrogen sources on the population of ciliates in the rumen of sheep (10^5/ml) and genus of protozoa (%).

	population (10^5/ml)	Genres of ciliates (%)			
		Entodinium	Isotricha	Ophryoscolex	Polyplastron
A	6.08 ^{b} ± 0.23	55.64 ^{a} ± 6.21	27.31 ^{a} ± 6.46	10.95 ^{a} ± 1.32	7.82 ^{a} ± 2.82
B	6.06 ^{b} ± 0.22	54.86 ^{a} ± 15	29.7 ^{a} ± 15.29	8.06 ^{b} ± 2.62	5.73 ^{a} ± 3.93
SME	0.82	1.02	1.7	0.9	1.0

^{ab}: Means with different superscripts within a row differ significantly ($p < 0.05$).

3.2.Milk yield and quality

The level of average production was 0.4 l for the control group (A) and the experimental group (B). The fat content of milk was 7.58 ± 0.6% and 7.21 ± 0, 41% for the experimental group (B) and the control group (A). Statistical analysis reveals that there is no difference between the two groups (p> 0.05) as there is an apparent superiority to the fat content of the batch S. This result is consistent with that reported by [6] and [4]. This can be explained by the high energy white sorghum seeds and their fat content compared to corn one hand and by the nature of faba bean seeds which are more energy because of the large amount starch easily degradable in the rumen it contains the other part [27]. The average fat content obtained in this trial is higher compared to that reported by [15] who found an average of 5.34 to 5.83% by working on concentrated food source whose energy is a cereal (barley) during the lactation. This explains the results of [28] who reported that the fat content is influenced by the amount of milk produced (decrease causes an increase in these rates) and length of use (the end of the milking phase is accompanied by an increase in the rate butyric acid).

The protein content of milk was statistically comparable between the two diets (p> 0.05), (6.04 ± 0.57% and 5.86 ± 0.54% respectively for B and A). This result converges with those of [29]. This is attributed to the fact that the milk protein content is positively correlated with the energy balance of the diet since energy intake stimulates the synthesis of microbial protein in the rumen [30]. What is in our case, the plans are iso energy (0.98 and 0.99 UFL / kg DM respectively for A and B).The comparable effect of soybean meal and field bean as protein sources in feed concentrates can be explained by the wealth of faba bean seeds in essential amino acids such as lysine and methionine.

Lactose is a milk sugar specific. It is virtually the only carbohydrate sheep's milk. Statistical analysis showed that the lactose content is not significantly different (p> 0.05) according to the regime. However, we note that the average content of lactose in milk of ewes of experimental group is higher compared to that of the control sheep. This agrees with the result found by [6]. It is about 4.27 ± 0.43% for B and 4.15 ± 0.5% for A, this could be due to the richness of field bean in easily degradable carbohydrate (starch) and cellulose present especially at the seed coat of faba bean by comparing it with that of soybean meal [31].

The content of urea which is correlated with blood urea and considered an indicator of protein utilization [32]. The average values found are 53.5 ± 8.76 for the lot A and 35 .5 ± 3.4 mg/dl for the lot B outcome of the sheep milk fed with significant difference (P<0.01). This result is consistent with that found by [33] and farther from that report by [34] who worked on diets rich in tannins. which is higher than the experimental milk throughout the trial period this may be the ratio of protein/energy in the diet appears to be the factor having the greatest nutritional impact on the rate of urea [35].This trend could be attributed to Soya grain quality rich in protein degradable in the rumen compared with faba beans that contains a large amount of starch but also influences negatively urea concentration. Indeed, the energy level affects the amount of protein and non protein nitrogen to be used by micro-organisms [36]. Thus, an increase in energy intake in the diet will cause a decrease of urea on the one hand and secondly the tannin content in the integument of faba beans may reduce protein degradation and therefore reduce the amount of urea.

The influence of the incorporation of white sorghum as an energy source in the formulation of feed concentrates to replace corn in the diet of ewes was assessed through an analysis of different concentrations of unsaturated fatty acids in milk. The statistical analysis revealed that there were no differences among fatty acids in milk of both ewe groups regardless of the regime as shown in Table 4. The main fatty acid was Palmitic acid C16 (27.75 ± 1.29% vs. 27.77 ± 1.21% of total FA for diets B and A, respectively) and miristic acid C14 (12.06 ± 0.82% for A and 12.47 ± 1.21% for B). This result converges with that advanced by [37] who have shown that milk from sheep fed a concentrate is rich in C16 and C14 compared to other types of fatty acids. White sorghum as an energy source rich in fat maintained the concentrations of key fatty acids compared with corn energy source commonly used as a main ingredient in concentrates made for sheep. The stearic acid (C18: 0) represented respectively 6.9 ± 0.74 and 6.82 ± 0.82% of TFA in milk of the A and B ewe groups. The concentration of the oleic acid C18: 1 n-9 was 20.7 ± 3.2 for milk from sheep receiving the concentrate feed based on corn as an energy source (A) and 22.5 ± 4.6% for milk of ewes fed the B regimen with no statistical difference between oleic acid contents in milk of both groups of ewes (p> 0.05). Capric acid (C10) occupied an intermediate place in concentration in the milk, the capric acid content was 7.17 ± 1.17

and 7.38 ± 1.39% (p> 0.05) in the A and B groups. These contents are high compared to those reported by [38] and [39] who worked on courses at different levels of altitude. Conjugated fatty acids appeared almost exclusively in the milk fat and meat in sheep. The CLA content of sheep milk are dependent on feeding conditions and the nature of the complementation. The concentration of CLA from this study was similar to that reported by [37] for a diet based on concentrate feed but was lower compared with milk from sheep grazing on ryegrass or barley reported by the same authors. This concentration was 0.48 ± 0.03 and 0.36 ± 0.09% comparable (p> 0.05) between both groups of ewes which show that the white sorghum is a good alternative for corn because of the important amount of fat in its seeds.

The percentage of saturated fatty acids is higher in milk whatever diet compared with the polyunsaturated fatty acids. This can be explained by the biohydrogenation of PUFA in the diet. The percentage of monounsaturated fatty acids was 23.9 ± 6.1 and 25.33 ± 4.76 respectively for A and B with no statistical difference (p> 0.05). This can be explained by the quality of fatty acids comparable in grain maize and white sorghum that are rich in linoleic acid (1305 mg), oleic acid (964 mg) and Palmitic acid (407 mg). The ratio PUFA / SFA is similar for both groups (p> 0.05) and higher compared to that advanced by Atti et al (2006) who worked on the sheep on pasture. This can be explained by the significant contribution of polyunsaturated fatty acids in feed concentrates compared to forage that are rich in short chain fatty acids (Table 4).

Table 4: Concentration of Fatty acids profile and CLA in Milk

	A	*B*	*Pr >F*	**SME**
Capric Acid C10	7.17±1.17	7.38±1.39	Ns	0.4
Miristic Acid C14	12,06±0,82	12.47±1.21	Ns	1.03
Palmitic Acid C16	27.75±1.29	27.77±1.21	Ns	1.06
Stearic Acid C18	6.9 ± 0.74	6.82 ± 0.98	Ns	0.87
Conjugated Linoleic Acid (CLA)	0.48 ± 0.11	0.36 ± 0.1	Ns	0.11
SFA	65. 76± 4.04	66.84±4.92	Ns	0.96
MUFA	23.9 ±6.1	25.33±4.76	Ns	0.84
PUFA	3.88±1.1	3.7±0.8	Ns	0.56
CCFA	14.62±4.21	14.54±5.35	Ns	1.1
LCFA	53.17±2.42	53.67±3.63	Ns	0.57
PUFA/SFA	0.59	0.55	Ns	0.73
FA omega-3	0.369	0.364	Ns	0.44
FA omega-6	0.09	0.15	Ns	0.82
Ratio n-6/n-3	0.32	0.46	Ns	0.67

SFA: Saturated fatty acids; MUFA: mono-unsaturated FA, PUFA: poly-unsaturated FA; SCFA: Short chaine FA; SEM: standard error of the mean; Ns: not significant (p>0.05)

4. Conclusion

Following this experiment, it appears that the effect of the incorporation of local raw materials instead of imported raw materials in the formulation of feed concentrate feed can be maintained or improved some parameters of rumen and improved some physico-chemical quality of milk, and fatty acid concentrations in milk similar. The higher content of urea in the control group tells us about the waste of protein used in the diet and the coverage rate of nitrogen needs of the Sicilo- Sarde sheep. Indeed, the use of imported soybean prices important in the diet of ruminants can cause damage not only economically but also the quality of products and their processing and storage. For this, the search for alternatives has shown good results and provides a stimulating factor for significant production and income for farmers.

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