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Alternative Feed Resources and Their Effects on the Parameters of Rumen Fermentation, *in situ* Degradability, the Population of Ciliated Protozoa and the *in vitro* Gas Production Profile in Sicilo-Sarde Sheep

Research Article

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ABSTRACT

The effect of the substitution of imported raw materials (corn and soyabeans) by local food resources (barley, white sorghum, triticale and horse bean) on the parameters of facies fermentation and digestibility in the rumen of sheep was evaluated. Four Sicilo-Sarde rams 4.8±0.5 years of age with an average live weight of 45.25±3.5 kg, permanently canulated in the rumen and housed in individual cages were used. Rams received a daily ration in two equal meals. The diet contained 1.5 kg DM of oat hay, complemented by one of four concentrates. During the test, 50 mL of rumen fluid were collected from each animal before and 2, 5, and 8 hours after the morning meal to measure the pH and ammonia nitrogen. Determining the total gas (CO2 and CH4) was performed on filtered rumen contents, collected before the distribution of the morning meal. Counting and classifying different types of ciliates were carried out on unfiltered rumen juice, collected two hours after the morning meal distribution. The dry matter digestibility of the basal diet was determined by nylon bags calibrated during fixed hours (3, 6, 12, 24, 36 and 48). Results showed that the rumen pH was statistically different ($P<0.05$) before and 2 hours after the morning meal distribution among different types of concentrates, but remained constant at the end of the day ($P>0.05$). The rate of ammonia nitrogen was in favor ($P<0.05$) of CCbf and CCms concentrates 5 and 8 hours postprandially. The amount of ammonia in the rumen decreased significantly without a significant difference among diets ($P>0.05$). The population of ciliates for the concentrate CCbf was significantly higher ($P<0.05$) than those for CCms, CCsf and CCtf concentrates while different genus of these protozoa were comparable among diets. The total volume of gas produced was lower for the CCbf regimen ($P<0.05$) compared to other diets. The degradation of DM evolves for different schemes with significant difference ($P<0.05$) for concentrates CCbf and CCtf.

KEY WORDS local feed resources, microbial activity, rumen, Sicilo-Sarde rams.

INTRODUCTION

Better nutrition is considered the most effective way to improve animal performance. In ruminants availability of high quality diets is a major constraint in livestock in Tunisia as in most Mediterranean countries, given the climatic conditions are harsh and the quality of conserved forage is poor (Rouissi *et al*. 2008).

Therefore concentrates based on corn and soybean meal are a supplement throughout the year. Today, the pitfalls of mass production are evident. Economic constraints include new components such as the sustainability of production

systems, traceability and product quality (Selmi *et al*. 2009a; Hammami *et al*. 2009; Chikagwa-Malunga *et al*. 2009). The context is favorable to promote an alternative to soyabean meal and corn imported raw materials at reasonable prices has not been a stimulating factor for high production (Poncet *et al*. 2003).

Indeed, the use of cereals and protein crops produced on the farm (barley, triticale, sorghum, white, horse bean) is now a common practice in animal production (Michalet-Doreau and Sauvant, 1989; Dehority and Tirabasso, 2006) and the limits of their incorporation are determined by the constraints of the ration's energy and protein balance and not by inherent limitations related to anti-nutritional factors (Michalet-Doreau *et al*. 1987).

In addition, corn and soyabean meal, as other grains, contain non-starch polysaccharides (NSP) (Choct, 1997). In cereals such as wheat, barley and triticale, a part of the polysaccharides is soluble in water (Choct, 1997).

Only this fraction has long been regarded as anti-nutritional because of its power viscosity (Maisonnier *et al*. 2005), their effects on ruminal conditions are minimal or even comparable (Hammami *et al*. 2009). However in recent years it has been shown that the problems of digestion of cereal grains are not only related to increased viscosity of gut contents (Maisonnier *et al*. 2005).

A second hypothesized antinutritional mode of action of polysaccharides, this time linked to the insolubility in water has been. The network of fibres that constitutes the cell wall of the grain would limit the access of endogenous enzymes to nutrients thereby reducing digestion.

The destruction of this network could increase accessibility to nutrients and improve digestion of raw materials. On the other hand, soyabean meal compared with fava beans, the most available High protein, also contains oligasaccharides (raffinose and stachyose) known to reduce the digestion of diets.

MATERIALS AND METHODS

Animals and diets

Four rams, Sicilo-Sarde breed, with an average (±sd) live weight at the beginning of the trial of 45.25 ± 3.5 kg and aged 4.8±0.5 years, fitted with a permanent canula in the rumen were used in this experiment. They were housed in individual wire boxes (length 1.6 m and width 1 m) in a building belonging to the School of Higher Education in Agriculture of Mateur, Tunisia.

The animals had a common basal diet of 1.5 kg dry matter/head/day of oat hay supplemented by four concentrates (CCms), (CCsf), (CCtf) and (CCbf) at a rate of 500 g/head/day by the different nature of protein and energy ingredients they contain. In a 4 x 4 Latin square experimental model, the four rams received successively four concentrates during a measurement period of 30 days separated by an adjustment period of fourteen days.

The ration was distributed twice daily at fixed times throughout the trial (9 and 17 h). Samples of different materials tested were analyzed for their mineral content (OM) and total nitrogenous matter (CB) according to AOAC (1990).

The percentage composition of feed concentrates and chemical composition of the various constituents of the diet are illustrated in Table 1. It is noted that the share of horse bean could not exceed 30% in the formulation of concentrated feed given its high tannin and anti nutritional factors.

Rumen fermentation

The sampling for the determination of various parameters of rumen fermentation took place just before serving meals in the morning and 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 sample collection 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 of pH 4 and pH 7, the electrode tip in a solution of KCl. Before each measurement of pH, the electrode was rinsed with distilled water and wiped dry.

The concentration of ammonia nitrogen (NH3-N) was measured using the method of Conway (1962). 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 (K2CO3) and captured with a solution of boric acid (H3BO4) 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 at 2500 revolutions per minute for 15 minutes was deposited on one side and 1 mL of K2CO3 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:

NH3-N mg/mL of juice= (V(HCl E)-V(HCl T)) x N (HCl) x 14 g

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

Ingredient %	Type of concentrate				Oat hay
	CCms	CCsf	CCtf	CCbf	
Barley	10	-	-	71.5	-
corn	43.3	-	-	-	-
white sorghum	-	66	-	-	-
Wheat bran	25	-	-	-	-
Triticale	-	-	71	-	-
horse bean meal	-	30	18	17.5	-
soybean meal	17.7	-	7	7	-
VMC sheep	4	4	4	4	-
Chemical composition					
DM (%)	94.7	94.7	95.2	95	92
Organic matter	91.0	88.3	83.8	90.8	92.1
Crude protein	16.3	14.65	15.2	15.26	4.9
Crude fiber	12.7	3.7	4.7	9.1	35.6

CCms: 10% barley; 43.3% corn; 25% wheat bran; 17.7% soybean meal and 4% CMV; CCsf: 66% white sorghum; 30% horse bean and 4% of CMV;
CCtf: 71% triticale; 18% horse bean; 7% soybean meal; and 4% of CMV.
CCbf: 71.5% barley; 17.5% horse bean; 7% soybean meal and 4% of CMV.
NB: it is noted that the share of horse bean could not exceed 30% in the formulation of concentrated feed given its high tannin and anti-nutritional factors.

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

Counting different protozoa genera 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).

In vitro gas production

Determining the total gas (CO2 and CH4) was performed on the filtered rumen contents, collected before the distribution of the morning meal.

The animals were fed a liquid diet the day before sampling. 0.5 g of substrate (oat hay milled at 1 mm), 10 mL of rumen fluid and 40 mL of artificial saliva (Menke and Steingass, 1988) in syringes vertically in a water bath at 39 ˚C Readings were taken every 2 hours until total gas volume produced plateaued. At the end of each incubation were injected 5 mL of NAOH (10 N) in each syringe, the piston moves back, the difference in volume reflects the amount of methane produced.

This technique applied to model Orskov and Mc. Donald (1979) to determine the value of "plateau" of production and total gas production rate. The mathematical model is as follows:

$$\text{Gas (mL)} = b\,(1-e^{-ct}),$$

Where:
b: potential gas production.
c: velocity of gas production.
t: time.

In situ degradability

We used the technique of nylon mesh bags calibrated, which tracks the kinetics of digestion of food in the rumen degradability of measuring foods and their constituents in the rumen. The bags are 15 cm long and 5 cm wide and having pores of 50 µ in diameter, the weight of each bag is determined before each use. After introducing each bag approximately 3 g of oat hay ground through a grid of 1 mm, the bag is sealed on two levels with a non-biodegradable thread.

The first attachment is located at 9 cm from the bottom of the bag, and 1 cm above, a second fastener is designed to allow the fixing of the bag to a plastic rod of about 20 cm long bearing at its ends a counter weights about 100 g so it does not float in the rumen.

The bags attached to stems and bags witnesses (to estimate physical losses) are washed thoroughly for five minutes, then the rods are inserted into the rumen through the openings of fistulas and know witnesses were placed in an oven at 60 ˚C.

The incubation time of the bags was 3, 6, 12, 24, 36 and 48 hours. It takes a bag of nylon per pet, per stay time.

These were rinsed with tap water until they become clear. The bags are washed and placed in an oven at 50 ˚C for 24 h to constant weight. Digestibility was determined as follows:

Dg DM (%)= DMC - DMR / DMC x 100

Where:
DMC: Dry matter corrected (g)= DM x (1-loss rate)
DMR: Dry residue (g) of the bag incubated in the rumen.

Statistical analysis

The results of the effects of diets on the parameters measured were subjected to analysis of variance by GLM procedure of SAS (1989) and compared by Duncan's test (1955). The model used to compare the rumen pH, concentration of ammonia nitrogen, the number of ciliated protozoa and different genuses 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.
Eij: random residuals.

The kinetics of gas production and kinetics of degradation of DM of hay used were analyzed using the nonlinear regression model by Orskov and MacDonald (1979):

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

RESULTS AND DISCUSSION

Rumen fermentation parameters

The pH of the rumen before the morning meal distribution was statistically comparable (P>0.05) for diets CCms, CCtf and CCbf 6.67±0.34; 6.60±0.27 and 6.71±0.27 and significantly lower (P<0.05) for the CCsf system 6.28±0.22. This result is similar to those of Rouissi (1994) and Hammami *et al*. (2009) and below the range of pH in the rumen of sheep receiving hay alone (Giger *et al*. 1988).

Two hours postprandial, pH decreased for the four diets, but the decrease was larger for diets CCbf and CCtf (0.44 and 0.49 points respectively), than the minimal decrease observed in the other two systems (0.19 to 0.1 for CCms and CCsf). After 5 hours the pH continued to decrease, it was (6.22±0.42; 5.97±0.22; 6.06±0.29 and 6.01±0.12) respectively concentrates CCms, CCsf, CCbf and CCtf.

Statistical analysis reveals that there is no difference between the pH of the different diets (P>0.05). This trend agrees with those of Santra *et al*. (2007) and Hammami *et al*. (2009). At the end of the day, the pH increased significantly (P<0.05) and is more stable and buffered diets CCtf and CCbf over plans CCms and CCsf.

The general trend of the change in pH in the rumen of Sicilian Sarde rams is along the same lines 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 diets leads to changes in the flow of digesta leaving the rumen on the one hand, the quantity and nature of the products absorbed in ruminal 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 ($HCO3^-$) and phosphate ($HPO4^{2-}$) in saliva that occurs in a massive way during rumination (Sauvant *et al*. 2006).

Concentrates with cereal energy sources (triticale and barley) have the highest values with significant difference (P<0.05) compared to concentrates with white sorghum as their energy source. This can be explained by the intense production of saliva during rapid digestion of cereals compared to white sorghum and maize (Michalet-Doreau and Sauvant, 1989). The significant difference (P<0.05) between CCms and CCsf groups before meal distribution may be due to the fact that the concentrate contains CCms except maize, the proportion of barley and wheat that are cereals on the one hand and secondly because the size difference is concentrated CCms type cap while CCsf is mealy.

This parallels the conclusion of Sauvant (2000) 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.

The pH drop two hours after the meal distribution is highly significant (P<0.05) for concentrates where the energy source is a cereal.

These values are within the ranges noted in the work developed by Giger *et al*. (1988) and Sauvant *et al*. (2006) who reported that the pH is lower for the concentrate-rich cereals (barley, triticale), which is explained by the amount of rapidly fermentable starch they contain and the increased production of volatile fatty acid (VFA) which in turn promote stability in pH after absorption through the rumen wall.

In this same context, Sauvant and Van Milgen, (1995) 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 acetate to propionate ratio (A/P), which is an index of energy status of specific microbes and rumen pH. 5 hours post-prandial, the pH continued to decrease without statistical difference between the different diets (P>0.05) and the fall was most notable for groups CCms and CCsf (-0.26 and -0.21).

This is attributed to the slow degradation of corn and sorghum starch-white. So that by the end of the day (after 8 hours of the morning meal distribution), the rumen pH stabilizes again with significant differences between diets (P<0.05). The highest values were found for cereal containing concentrates.

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 NH3-N concentration before food intake was 10.85±2.54 mg/100 mL of rumen fluid for the regime barley-fava (CCbf), which differedsignificantly (P<0.05) from other regimes as shown in Table 2. The NH3-N concentration increases to reach its peak two hours after the meal for the four diets with a statistically signficantly lower amount (P<0.05) produced by the regime Triticale-fava (CCtf).

This may be due to the high microbial activity and rumen pH which is favorable to the proliferation of ciliated protozoa (Kayouli *et al.* 1991). These results are consistent with those found by Rouissi and Guesmi, (2004) and Castillejos *et al.* (2007).

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 Hammami *et al.* (2009). This is attributed to the wealth of fava bean digestible protein compared to soybean meal (Poncet *et al.* 2003).

The optimal concentration observed after two hours post meals for the four concentrates could be explained by more intense degradation of proteins and the deamination of their amino acids (Mahouachi *et al.* 2003) and secondly the significant correlation observed between the concentration of NH3-N and the total number of ciliates (Jouany and Senaud, 1982). After 5 and 8 hours postprandially, the amount of ammonia in the rumen decreased significantly, without a significant difference between diets (P>0.05). This result resembles that of Hammami *et al.* (2009) and could be explained by absorption through the rumen wall and use by bacteria to synthesize their own proteins.

Number of ciliated protozoa and different genuses

The majority of protozoa found in the rumen of sheep belong to the phylum of ciliates. Their numbers varied rapidly with the meal.

Furthermore, protozoal species vary with the geographic area, nutritional quality of food resources and adaptation of the animal (Yanagita *et al.* 2000). This study focused on 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 Williams and Withers (1993); Jouany and Ushida (1999) and Selmi *et al.* (2009a). The CCbf concentrate, made from barley and fav,a was associated with the highest numbers of protozoa (6.40±0.15 105/mL) compared to other diets (P<0.05), while the CCtf concentrate resulted in the lowest number of protozoa.

Moreover, results revealed that there were no significant differences (P>0.05) between CCms and CCsf 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 Polyplastron* genera. The Entodinium protozoa were 55.64±6.21, 54.86±15.00, 50.97±3.10 and 56±4.09% for the CCms, CCsf, CCtf and CCbf diets, respectively, without statistical differences (P>0.05), which is consistent with the results found by Selmi *et al.* (2009a) 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 CCbf diet. This can be explained by the nature of starch granules in barley that rapidly ferment in the rumen, resulting in a higher concentration of protozoa and more intense production of butyrate, the end product of the metabolism of protozoa (Jouany, 1994; Demeyer and Fievez, 2000), and protein quality in terms of fava beans from those of soybean meal (Selmi *et al.* 2009a). This result further explains what was 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.

Table 2 Rumen fermentation parameters

Regimen	Hours after the meal			
	0	2	5	8
	pH ruminal			
CCms	6.67^{a} ± 0.34	6.48^{a}±0.38	6.22^{a}±0.42	6.25^{b}±0.34
CCsf	6.28^{b} ± 0.22	6.18^{b}±0.13	5.97^{a}±0.22	5.99^{b}±0.31
CCtf	6.60^{a} ± 0.27	6.16^{b}±0.21	6.06^{a}±0.29	6.40^{a}±0.35
CCbf	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
N-NH3 (mg/100 mL)				
CCms	9.21^{ab}±2.63	12.83^{a}±3.76	6.40^{a}±2.47	5.23^{a}±3.08
CCsf	8.05^{b}±3.76	11.66^{a}±3.00	7.46^{a}±2.88	6.3^{a}±3.01
CCtf	8.98^{ab}±1.73	9.45^{b}±3.22	5.71^{a}±3.07	4.55^{a}±1.35
CCbf	10.85^{a}±2.54	14.13^{a}±3.21	6.41^{a}±2.34	5.71^{a}±2.42
SME	0.72	0.95	0.78	0.73

The means within the same row with at least one common letter, do not have significant difference (P>0.05).

Table 3 Effect of dieton the population and genera of ciliates in the rumen of sheep

	population (10^5/mL)	Genuses of ciliates (%)			
		Entodinium	Isotricha	Ophryoscolex	Polyplastron
CCms	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
CCsf	6.06^{b}±0.22	54.86^{a}±15	29.7^{a}±15.29	8.06^{b}±2.62	5.73^{a}±3.93
CCtf	5.66^{c}±0.09	50.97^{a}±3.10	30.48^{a}±1.57	11.24^{a}±2.35	6.55^{a}±1.35
CCbf	6.40^{a}±0.15	56^{a}±4.09	30.37^{a}±3.92	8.32^{b}±1.83	5.29^{a}±1.83

The means within the same row with at least one common letter, do not have significant difference (P>0.05).

The low concentration ofciliates for the regimen CCtf 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 CCms and CCsf 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; Sauvant, 2004).

In vitro gas production

In the rumen, any biological reaction is accompanied by a loss of energy as heat or gas production. Digestion of various dietary ingredients is accompanied by gas production from food; carbon dioxide (CO2) is removed by eructation by direct diffusion through the wall of the rumen and methane (CH4) whose route of elimination is exclusively belching. This production depends mainly on the degradation rate and the nature of starch and carbohydrates in the diet parietal characteristics.

Gas production in syringes starts rapidly after incubation without latency since the microorganisms are already adapted to the substrates. The total volume of gas after 36 hours incubation was statistically different (P<0.05) between diets and the concentrate CCbf displays the highest value (87.00±17.29 mL), which is similar to the results of Selmi *et al.* (2009b). This could be explained by the rich grain of barley and horse bean starch being rapidly degradable in the rumen thereby fostering an environment rich in VFA and NH3-N used by bacteria and protozoa to their development and proliferation (Michalet-Doreau and Sauvant, 1989). The lowest volume of gas is observed in the CCtf concentrate (56.58±13.06 mL). This low gas production can be explained by the fact that the grains of triticale contain trypsin inhibitors minimizing their digestibility compared to other cereals (Grela, 1996) while the concentrates CCms and CCsf occupy an intermediate position without a significant difference between them (P>0.05) and this is because they contain the same type of starch that is slowly degradable. Taking into account the influence of the nature of the nitrogen source on the total volume of gas, their energy sources are comparable (white sorghum and maize), replacing soybean field bean does not affect the total gas volume (P>0.05). This finding is consistent with Selmi *et al.* (2009b) and explains the nutritional value of horse beans (May *et al.* 1993).

The proportion of methane (CH4) produced for the four concentrates is in the range of 25 to 35% of the total gas volume, as shown in Table 4. CH4 production in the rumen was 24.91 (±5.69 mL) representing 28.6% for the CCbf concentrate and 19.75±3.88 mL or 34.9% for the concentrate with CCtf, showing a significant difference between them (P<0.05), which is similar to the work of Sauvant (2000). This trend is attributed to the close of CH4 and acetate in the rumen (Demeyer and Fievez, 2000).

The quantities produced by the CCms and CCsf concentrates occupy an intermediate position given the nature of their starches that are slowly degradable in the rumen. The potential gas production, represented in the model Orskov and Macdonald (1979) by the constant "b", is highest (P<0.05) for the regime CCms (58.7) and lowest for CCbf (34.5). The statistical difference between the different regimes can be explained by the nature of their raw materials.

Indeed, the complex Corn-Soybean has the characteristics of protein and energy the most important and most digestible in the rumen.

In comparing the two concentrates CCms and CCsf whose energy sources are botanically similar, the replacement of soybean meal by horse beans did not affect the production potential of gas. This result supports the findings of Selmi *et al.* (2009b).

While both concentrates were based on cereals and horse beans, which have anti-nutritional factors like tannins and beta-glucan (Larbier and Lerclercq, 1992), this production is occurring in the rumen with the same speed (P<0.05) therefore substitution and the substitution of imported raw materials from local raw materials has no effect on the rate of gas production in the rumen.

In situ degradability

The *in situ* degradability of food is a test commonly used to characterize the feed for ruminants. It is usually done with animals fed a standard diet based on hay (70%) and concentrate feed (Andrade *et al.* 2004).

The value of the digestibility of the basal diet depends largely on the nature and technological treatment of food concentrates and therefore the parameters of facies fermentation in the rumen.

The results of DM degradability of oat hay in nylon bags are presented in Table 5. After 3 h of incubation in the rumen, digestibility was 18.19 (2.25) and 18.9 (2.75) % DM for concentrates from cereals compared to other diets (9.81 (4.6) for CCms and 14.55 (3.13) for CCsf). This result is higher than those found by Rouissi (1994) with a coarse diet without supplementation. This difference (P<0.05) between the four diets may be due to the change in rumen pH, which significantly decreased for concentrated CCbf and CCtf, cellulosic activity thereby promoting more intense. The degradation of DM demonstrated a significant difference (P<0.05) for the CCtf and concentrated CCbf which is similar to results from Andrade *et al.* (2004).

From 12 h of incubation, the digestibility for CCsf is statistically comparable to those of CCbf and CCtf. This pattern resembles that of Hammami *et al.* (2009) and is attributed to the characteristics of horse beans (protein source of food) for their richness in both protein and energy with 40 to 50% DM starch, 24 MS 32% protein, 5-10% dry cellulose and 1-3% DM fat, compared to soy protein and rich in oils and low in starch (Moss and Givens, 2002). While the low digestibility values displayed by the regime CCms could be explained by the richness of soybean meal protein and the large number of protozoa and increasing predation of cellulolytic bacteria (Jouany, 1994; Santra *et al.* 2007). In addition the presence of starch in high quantity and slowly degradable forms promotes the development of amylolytic flora at the expense of cellulolytic bacteria resulting in decreases in fiber digestion.

Digestion potential (a+b) is statistically higher (P<0.05) for the local commercial concentrate. This result explains the advantage of results of the digestion of DM (%) is in favor of these concentrates.

Table 4 Gas volume and methane (mL) in the rumen

	Type of concentrate				SME
	CCms	CCsf	CCtf	CCbf	
Gas_{24} (mL)	66.41±11.53^{a}	64.33±16.37^{a}	44.33±12.83^{b}	70.91±14.79^{a}	2.78
Total gas (mL)	77.66±11.65^{a}	78.41±16.61^{a}	56.58±13.06^{b}	87.00 ±17.29^{a}	1.07
CH_4 (mL)	22.08±4.18^{ab}	21.16±3.21^{ab}	19.75 ±3.88^{b}	24.91 ± 5.69^{a}	1.25
b	58.7^{a}	50.3^{ab}	48.1^{b}	34.5^{c}	0.84
c	0.001^{a}	0.002^{a}	0.002^{a}	0.003^{a}	0.03

The means within the same row with at least one common letter, do not have significant difference (P>0.05).
G24: gas to 24 h of incubation; CH4: methane; b: potential gas production and c: velocity of gas production.

Table 5 Degradation kinetics of DM (%) of oat hay in the rumen

	Time of incubation in the rumen					
	3 h	6 h	12 h	24 h	36h	48h
CC ms	9.8^{c}±4.6	15.2^{c}±4.3	20.1^{b}±3.7	25.2^{b}±6	29.7^{b} ± 3.85	35.6^{b} ± 5.9
CCsf	14.6^{b}±3.1	18.9^{b}±2.1	24.5^{a}±2.4	29.5^{a}±3.1	35.3^{a} ± 4.2	38.9^{b} ± 4.4
CCtf	18.9^{a}±2.7	24.2^{a}±5.5	27.3^{a}±6.9	32.1^{a}±6.8	36^{a} ± 5.7	43.3^{a} ± 4.4
CCbf	18.2^{a}±2.3	21.5^{a}±1.8	25.7^{a}±2	31.9^{a}±3.3	34.7^{a} ± 2.4	43.1^{a} ± 2.2
SME	0.96	1.09	1.21	1.46	1.21	1.27

The means within the same row with at least one common letter, do not have significant difference (P>0.05).

This trend partly explains the difference in digestion due to the rapid degradation of starch in grains of barley and triticale (85%) and corn starch (50%) while the fermentation of starch fava beans occupies an intermediate position (Michalet-Doreau and Sauvant, 1989). The rate of degradation is also in favor of local concentration (CCtf and CCbf) ($P<0.05$).

This can be explained by the rate of fermentation of starches and proteins of each raw material. While the difference in rate of degradation of concentrates based on corn and sorghum white, which have the same kind of starch, may be due to the nature of the constituents of horse beans compared to soybean meal. In conclusion, 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 maintain or improve some parameters of rumen and in *situ* digestibility of the basic ration.

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 concentrate feed can maintain or improve some parameters of rumen and *in situ* digestibility of the basic ration. Indeed, the rumen pH varies with concentrates based on cereals as their starches are rapidly degradable. This trend explains well the general shape of the concentration of ammonia nitrogen during the day. Gas production is increased for the concentrate with barley compared with other diets so that the production of methane represents a loss of up to 10% of the digestible energy of the ration and this increases with digestion of the ration and production of acetate and thus the nature of the ingredients. Various methods are available to reduce CH4 production by ruminants by inhibition of methanogenesis in the rumen by acting on the constitutions of the diet either by increasing the ingredients containing starch to slow degradation or incorporation of halogenated methane analogues.

REFERENCES

Andrade P.V.D., Giger-Reverdin S., Chapoutot P. and Pierres J.L. (2004). Liaison entre la dégradabilité « *in sacco* » des aliments et les paramètres fermentaires ruminaux des animaux fistulés. *Rencontre Recherche Ruminants*. **11,** 203-207.

AOAC. (1990). Official Methods of Analysis. Vol. I. 15th Ed. Association of Official Analytical Chemists, Arlington, VA.

Castillejos L., Calsamiglia S., Ferret A. and Losa R. (2007). Effects of dose and adaptation time of a specific blend of essential oil compound on rumen fermentation. *Anim. Feed Sci. Technol*. **132,** 186-201.

Chikagwa-Malunga S.K., Adesogan A.T., Szabo N.J., Littell R.C., Phatak S.C., Kim S.C., Arriola K.G., Huisden C.M., Dean D.B. and Krueger N.A. (2009). Nutritional characterization of *Mucuna pruriens* 3. Effect of replacing soybean meal with *Mucuna* on intake, digestibility, N balance and microbial protein synthesis in sheep. *Anim. Feed Sci. Technol*. **148,** 107-123.

Choct M. (1997). Feed Non-Starch Polysaccharides: Chemical Structures and Nutritional Significance. Feed Milling International, June Issue.

Conway E.J. (1962). Microdiffusion Analysis and Volumetric Error 5th Ed., Crosby Lock Wood and Sons. London.

Dehority B.A. and Tirabasso P.A. (2001). Effect of feeding frequency on bacterial and fungal concentrations, pH, and other parameters in the rumen. *J. Anim. Sci*. **79,** 2908-2912.

Demeyer D. and Fievez V. (2000). Ruminants et environnement: la méthanogenèse. *Ann. Zootech*. **49,** 95-112.

Duncan D.B. (1955). Multiple ranges and multiple F test. *Biometrics*. **11,** 1-42.

Eugene M., Archimède H., Weisbecker J.L., Periacarpin F., Saminadin G. and Sauvant D. (2004). Effects of defaunation on digestion and growth, in sheep receiving a mixed diet (fresh digitaria decumbens grass and concentrate) at four proteins to energy ratios. *Anim. Res*. **53,** 111-125.

Giger S., Sauvant D., Durand M. and Hervieu J. (1988). Influence de la nature de l'aliment concentré sur quelques paramètres de la digestion dans le rumen. *Reprod. Nutr. Déve*. **28(1),** 117-118.

Grela E.A. (1996). Nutrient composition and content of antinutritional factors in spelt (triticum spelta L) cultivars. *J. Sci. Food Agric*. **71(3),** 399-404.

Hammami M., Rouissi H., Selmi H., Rekik B. and Ben Gara A. (2009). Effects of replacing Soya with Vicia faba beans on fermentation in the rumen of the ovine Sicilo- Sarde breed. 6th International Symposium on Ruminant Physiology. Clermont-Ferrand. France.

Jouany J.P. (1991). Defaunation of the rumen. Pp. 245 in Rumen Microbial Metabolism and Ruminant Digestion. J.P. Jouany, Ed. Paris, France.

Jouany J.P. (1994). Les fermentations dans le rumen et leur optimisation. *INRA Prod. Anim*. **7(3),** 207-225.

Jouany J.P. and Ushida K. (1999). The role of protozoa in feed digestion. *Asian-Aus. J. Anim. Sci*. **12,** 113-128.

Jouany J.P. and Senaud J. (1982). Influence des ciliés du rumen sur la digestion des différents glucides chez le mouton. I. Utilisation des glucides pariétaux (cellulose et hémicellulose) et de l'amidon. *Reprod. Nutr. Dev*. **22,** 735-752.

Kayouli C., Jouany J.P. and Ben Amor J. (1991). Comparison of microbial activity in the forestomachs of the dromedaries and the sheep measured in vitro and in Sacco on mediterranean roughages. *Anita. Feed Sci. Technol*. **33,** 237-245.

Larbier M. and Lerclercq B. (1992). Nutrition et Alimentation des Volailles. Editions Quae. INRA, Paris.

Mahouachi M., Haddad L., Kayouli C., Théwis A. and Beckers Y. (2003). Effects of the nature of nitrogen supplementation on voluntary intake, rumen parameters and ruminal degradation of dry matter in sheep fed Oat silage- based diets. *Small Rumin. Res*. **48,** 181-187.

Maisonnier-Grenier S., Dalibard P. and Geraert P.A. (2005). Inter-

et d'une préparation multienzymatique sur des régimes à base de Maïs-Soja chez le Poulet. Sixièmes Journées de la Recherche Avicole, St Malo.

May M.G., Otterby D.E., Linn J.G. Hansen W.P. Johnson D.G. and Putnam D.H. (1993). Lupins (*Lupinus albus*) as a protein supplement for lactating Holstein dairy cows. *J. Dairy Sci*. **76,** 2682-2691.

Menke K.H. and Steingass H. (1988). Estimation of the energetic feed value obtained from chemical analysis and in vitro gas production using rumen fluid. *Anim. Res. Dev*. **28,** 7-55.

Michalet-Doreau B. and Sauvant D. (1989). Influence de la nature du concentré ou céréale ou pulpe de betterave, sur la digestion chez les ruminants. *INRA Prod. Anim*. **2,** 235-244.

Michalet-Doreau B., Geay Y., Doreau M. and Poncet C. (1987). Interaction entre espèce animale et nature de l'aliment concentré sur la digestibilité de la ration chez le ruminant. *Reprod. Nutr. Dev*. **27,** 277-278.

Moss A.R. and Givens D.I. (2002). The effect of supplementing grass silage with soya bean meal on digestibility, in Sacco degradability, rumen fermentation and methane production in sheep. *Anim. Feed Sci. Technol*. **97,** 127-143.

Oetzel G.R., Nordlund K.V. and Garret E.F. (1999). Effect of ruminal pH and stage of lactation on ruminal lactate concentrations in dairy cows. *J. Dairy Sci*. **81,** 38-47.

Ogimoto K. and Imai S. (1981). Atlas of Rumen Microbiology. Japanese Science Society Press, Tokyo.

Orskov E.R. and McDonald I. (1979). The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. *J. Agric. Sci. Cambridge*. **92,** 499-503.

Poncet C., Rémond D., Lepage E. and Doreau M. (2003). Comment mieux valoriser les protéagineux et les oléagineux en alimentation des ruminants. *Fourrages*. **174**, 205-229.

Rouissi H. (1994). Etude comparative de l'activité microbienne dans le rumen des dromadaires, des ovins et des caprins. Ph D Thèsis. Université de Gent, Belgique.

Rouissi H. and Guesmi A. (2004). Etude comparée de la population des protozoaires ciliés dans le rumen des ovins et caprins. *Options méditerranéennes*. **1,** 57-59.

Rouissi H., Rekik B., Selmi H., Hammami M. and Ben Gara A. (2008). Performances laitières de la brebis Sicilo-Sarde Tunisienne complémentée par un concentré local. *Livest. Res. Rural. Dev*. **20(7),** 102-108.

Santra A., Karim S.A. and Chaturvedi O.H. (2007). Rumen enzyme profile and fermentation characteristics in sheep as affected by treatment with sodium lauryl sulfate as defaunating agent and presence of ciliate protozoa. *Small Rumin. Res*. **67,** 126-137.

SAS User's Guide (1989). Version 6.10 for Windows, SAS Inst. Inc., Cary, NC.

Sauvant D. (2004). Table de composition et de valeur nutritive des matières premières destinées aux animaux d'élevage. *INRA Prod. Anim*. **8,** 301-117.

Sauvant D. (2000). Granulométrie des rations et nutrition du ruminant. *INRA Prod. Anim.* **13,** 99-108.

Sauvant D., Giger-Reverdin S. and Meschy F. (2006). Le contrôle de l'acidose ruminale latente. *INRA Prod. Anim*. **19,** 69-78.

Sauvant D. and Van Milgen J. (1995). Dynamic aspects of carbohydrate and protein breakdown and the associated microbial matter synthesis. Pp. 71-91 in Ruminant Physiology, W. Engelhardt, S. Vonleanhard, G. Breves and D. Gieseck, Eds. Verlag, Stuttgart.

Selmi H., Hammami M., Rekik B., Ben Gara A. and Rouissi H. (2009b). Effet du remplacement du soja par la féverole sur la production du gaz "in vitro" chez les béliers de race Sicilo-Sarde. *Livest. Res. Rural. Dev*. **21(7),** 33-37.

Selmi H., Hammami M., Rekik R., Salah N., Ben Gara A. and Rouissi H. (2009a). Effet du remplacement du soja par la féverole sur les protozoaires ciliés dans le rumen des béliers de race Sicilo- Sarde. *Lives. Res. Rural. Dev*. **21(9),** 103-109.

Williams A.G. and Withers S.E. (1993). Changes in the rumen microbial population and its activities during the refaunation period after the reintroduction of ciliate protozoa into the rumen of defaunated sheep. *Can. J. Microb*. **39,** 61-69.

Yanagita K., Kamagata Y., Kawaharasaki M., Suzuki T., Nakamura Y. and Minato H. (2000). Phylogenetic analysis of methanogens in sheep rumen ecosystem and detection of methanomicrobium mobile by fluorescence In situ hybridization. *Bioscience. Biotechnol. Biochem*. **64,** 1737-1742.