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

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Abstract

The effects of nature of nitrogen supplementation on silage DM intake (SDMI), total DM intake (TDMI), rumen parameters and ruminal degradation of DM of silage and wheat straw were studied using four adult *Noire de Thibar* rams. Silage was made from predominantly oats harvested at milk stage and ensiled without preservative. The silage was offered ad libitum as the sole diet (OS) or supplemented with 450 g DM of *iso*-nitrogen (25.6 g/kg DM) and *iso*-energy (0.8 Unité Fourragère Lait: UFL/kg DM) concentrate containing soybean meal (SBM), urea (UR) or poultry litter (PL) as nitrogen supplements. SDMI (g/kg W^{0.75} × day) and TDMI (g/kg W^{0.75} × day) were affected (P < 0.001) by the type of supplement and averaged 63.9, 60.1, 54.9, 51.1 and 63.9, 81.4, 74.8, 72.2 for OS, SBM, PL and UR diets, respectively. In comparison with other diets, SBM diet systematically decreased (P < 0.05) the pH of the rumen after the morning feeding and reduced the rumen concentration of total VFA at 1 h after feeding. SBM diet decreased DM disappearance of the oat silage incubated in nylon bags for 72 h (P < 0.05), but not of wheat straw. The three supplements increased the ammonia-N concentrations in the rumen for several hours after feeding in comparison with the OS diet. However, only the SBM diet kept this difference just before the next meal. It is concluded that despite its low nutritive value (0.62 UFL and 108 g crude protein/kg DM), the oat silage is well consumed by the local ruminants accustomed to use poor forages for a long time. In Tunisia, poultry litter may be used as a nitrogen source to supplement the oat silage for sheep.

Keywords: Oat silage; Nitrogen supplementation; Intake; Ruminal degradation; Poultry litter; Urea; Soybean meal

1. Introduction

Oat hay and oat silage are the most available forages used in ruminant feeding in Tunisia. For different reasons, their nutritive value is generally poor (Nefzaoui and Chermiti, 1989). CP content of oat silage is too low (8-10%) to support an optimal microbial growth in the rumen and an adequate intestinal supply of digestible proteins. Therefore low performances were generally obtained in animals fed unsupplemented oat and other silages (Harrison et al., 1994). Consequently, a large part of requirements is satisfied by imported feedstuff which reached 850,000 tons in 1997 (Kayouli and Belloin, 1997).

Nitrogen supplementation corrects nitrogen deficiency in the rumen and improves the fermentation and microbial activity, hence enhancing the utilisation of silage-based diets. Nevertheless, results depend upon the nature of nitrogen in the concentrate as there are different mechanisms involved in the microbial and animal response to supplementation (Seoane et al., 1993). Nitrogen supplementation is generally based on soybean meal (SBM) which is costly. On the other hand, Tunisia annually generates important quantities of crop and industrial by-products which present a large diversity of nutritional characteristics (Moujahed-Raach et al., 2000). Some of these by-products are nutritionally poor (straw, olive residues, raisin kernels), while others have greater nutritional values (sugar beet, date residues, brewers grain, poultry waste). Poultry litter (600,000 tons per year) has a high level of N (Deshck et al., 1998) and contains a great proportion of uric acid (Bhattacharya and Fontenot, 1965) that is well utilized by rumen microbes (Oltjen et al., 1968). This by-product can partially replace the imported SBM traditionally used to supplement oat silage in Tunisia. Urea is also generally available

in Tunisia at a low cost and may therefore be used to substitute SBM. The objective of this study was to compare effects of poultry litter, urea or SBM as nitrogen supplements on voluntary intake, rumen parameters and degradation of DM in sheep fed oat silage.

2. Material and methods

2.1. Animals

Four adult Noire de Thibar rams (initial live weight 55.7 ± 4.7 kg) each with a rumen cannula and a simple Tcannula inserted in the proximal duodenum were used in this experiment designed as a 4×4 Latin square. They were housed in individual metabolism cages or pens with free access to fresh water. Pens were used during changeover and cages during measurement periods.

2.2. Diets

The four experimental diets consisted of oat silage alone (sole diet, OS) or supplemented with one of three following concentrates in which the proteins were mainly (50-60%) based on SBM, PL or UR, respectively. All supplements provided a similar amount of energy (0.8 UFL/kg DM) and CP (162 g/kg DM). Formulation of the supplements and their chemical composition are presented in Table 1. The silage was prepared from oats (*Avena sativa* crème). At harvesting (milk stage), the forage contained a low proportion of vetch (7%) and barley (5%). It was ensiled without wilting or preservative and had a 10 cm chop length. Before each experimental period, sufficient amounts of silage were removed from the silo, mixed and preserved at 4°C. Samples were collected daily during the experiment and the DM content was determined on the aliquot. Poultry litter was recovered from the waste of 2000 birds bedded on straw. After collection, poultry litter was sun dried, screened through a locally devised sieve and preserved dry. The bacteriological analysis carried out at Institut de Recherches Vétérinaires (Tunis, Tunisia) did not show any presence of pathogenic organisms.

	Concentrates	8	
	SBM	PL	UR
Ingredients			
Barley	23.2	47.0	60.2
Wheat bran	17.0	3.0	-
Olive pulp	33.5	-	32.0
Soybean meal	21.3	-	-
Poultry litter	-	45.0	-
Urea	-	-	2.8
Minerals and vitamins ^a	5.0	5.0	5.0
Chemical composition			
Organic matter	919	875	933
Crude protein	161	162	162
Crude fiber	198	84	182
Energy value	0.81	0.85	0.79

Table 1. Ingredients (%DM), chemical composition (g/kg DM) and energy value (UFL/kg DM) of the concentrates used to supplement oat silage fed to Tunisian sheep

UFL: Unité Fourragère Lait (French system; Vermorel, 1988); SBM: soybean meal; PL: poultry litter; UR: urea. ^aCa (100 g/kg); P (35 g/kg); Na (80 g/kg); Vitamin A (400,000 UI); Vitamin D₃ (80,000 UI); Vitamin E (160 mg); Vitamin K (40 mg).

2.3. Measurements and analyses

Each period of the Latin square lasted 43 days. After 3 weeks of adaptation to the diets, silage intake was measured for a 10 day period. Silage was fed twice a day at 115% of the previous day's consumption. Rams were fed 225 g of supplement according to their diet program at 0830 and 1630. The supplements were offered separately before the silage. Prior to the morning feeding, orts of silage and concentrate were collected and weighed. They were dried at 60 °C in a forced-air oven until constant weight was achieved. Representative samples of feeds were collected at regular intervals throughout each period and pooled for subsequent analysis.

For the rest of the period, the intake levels corresponded to 90% of the average amount of silage consumed on a metabolic weight basis during the previous 10 days. The supplements represented 24% of the total DM ingested. The concentrate was adjusted to the weight of the rams at the beginning of each experimental period. After a 7-day adaptation period, runnial fluid was sampled prior and 1, 2, 4, 6 and 8 h after the morning feeding during 2 successive days. The pH was determined, for each sample, with a combination electrode immediately after collection. Samples were strained through four layers of surgical gauze and 11 ml of preservative were added (5 ml of orthophosphoric acid plus 1 g of HgCl₂)/100 ml of strained fluid and stored frozen (-18 ° C) pending VFA and ammonia-N quantifications. For protozoal counts, 5 ml of runnen fluid sampled 2 h after morning feeding were mixed with 5 ml of 48% glycerol (v/v), 2% formol (v/v) solution and stored at 4 °C.

The in situ procedure (Orskov and Mc Donald, 1979) was used to determine the extent of oat silage and wheat straw DM disappearance in the rumen. Wheat straw was used to study cellulolytic activity in the rumen. Nylon bags (13 cm \times 5 cm, pore size 40 μ m) containing 3 g of forage ground in a mill to pass a 2-mm screen were incubated for 72 h in the rumen of the rams (2 x 3 bags per animal). After removal, bags were immediately rinsed lightly with cold water, then they were machine-washed (3 x 5 min) and subsequently oven dried at 60 °C to constant weight.

All dried samples were ground with a mill to pass a 1-mm screen. Feeds and orts samples were analysed for DM, ash crude fiber (CF) and N (AOAC, 1980). The energy value of feeds is determined according to the French system (Unité Fourragère Lait) based on equations established by Vermorel (1988) and in vitro organic matter digestibility (Tilly and Terry, 1963). Ruminal ammonia-N and VFA were determined on thawed and centrifuged samples ($3000 \times g$ for 15 min) using the Conway (1962) technique and by gas chromatography as described by Jouany (1982), respectively. Protozoa were enumerated in a THOMA cell at a magnification of 100 and after 1/10 dilution. The genus was determined from photos and descriptions established by Ogimoto and Imai (1981). Silage samples were extracted with distilled water (50/450 g water) and their pH was measured. Water extracts acidified with H₂SO₄ 98% were analysed for ammonia N by the Conway (1962) technique.

2.4. Statistical analyses

The experiment was initially performed according to a Latin square design. Before periods 2 and 3, one sheep had to be withdrawn from the experiment due to some problems and was replaced with another one. Thus, data were analysed statistically by the GLM procedure of SAS (1985) for randomised factorial design with dietary treatment and period in the statistical model. Differences between means were compared using the Neuwman Keuls test (Dagnelie, 1980). Significance differences between means were P < 0.05 unless otherwise stated.

3. Results and discussion

3.1. Feed composition

The concentrations of DM, OM, CP and CF of the oat silage were 248, 879, 108 and 349 g/kg DM, respectively. The pH and ammonia-N were 4.22 and 84.3g/kgN, respectively. The energy value of the oat silage was low (0.62UFL/kg DM) confirming the findings of other Tunisian workers (Nefzaoui and Chermiti, 1989). This can be explained by the practices of farmers who harvest grass at a late stage of growth looking for quantity rather than quality. Therefore any improvement in animal performance requires supplementation of such forages with energy and protein sources. SBM had a high nutritive value (470 g CP and 1.25UFL/kg DM) as is expected (INRA, 1988). The poultry litter contained high levels (g/kg DM) of CP, ash and CF and consequently had a low energy content (Table 2). These values were similar to those reported by numerous authors (Deshck et al., 1998; Al-Rokayan et al., 1998; Moujahed-Raach et al., 2000).

Table 2. Chemical composition (g/kg DM) and energy value (UFL/kg DM) of main feeds used in the three supplements

	Feeds					
	Oat silage	Barley	Wheat bran	Olive pulp	Soybean meal	Poultry litter
ОМ	879	968	950	961	920	838
CP	108	131	190	87	470	205
CF	349	61	96	451	72	113
Energy value	0.62	1.15	1.04	0.3	1.25	0.62

3.2. Intake

Silage DM intake (SDMI) averaged 1.38 kg (S.D. = 0.22) for sheep fed OS diet (Table 3) which was equivalent to $63.9 \text{g/kg W}^{0.75}$ (S.D. = 9.8). This level intake was either higher than in sheep receiving grass (40-55 g/kg W^{0.75} (Offer et al., 1998) mean of 88 different grass silages), maize silages (Dulphy et al., 1990) or was similar to that reported by other workers (Van Os et al., 1995), even when their silage was better preserved than in the current experiment. Expressed on LW basis, the level intake observed in the present experiment (2.3%) was similar to some values obtained (1.9 and 2.3% of LW) in cattle (Veira et al., 1995). The level of forage intake supports observed palatability (time separating distribution and start of eating) and can be explained by (i) the fact that prior to feeding the waste silage was removed, thus reducing the impact of quality conservation on intake (Dulphy et al., 1990); (ii) sheep were less sensitive than cattle to fermented products (Offer et al., 1998); (iii) the high level of production of sheep used in this trial as they were selected for rapid growth; and (iv) possibly that the local breeds of sheep which are accustomed to graze poor forages for generations, developed an intake capacity so as to consume a sufficient quantity of these feed resources to meet their minimum requirements for maintenance and production. This last hypothesis seems to find confirmation in the data obtained in Tunisia with another breed of local sheep (Chermiti et al., 1996) or the local cross-bred heifers (Chermiti et al., 2000). Thus, the study of intake behaviour of local ruminants, which might present some particularities relative to European breeds, should stir interest of researchers.

	Diet				
	OS	SBM	PL	UR	S.E.M.
Silage DM intal	ke				
kg per day	1.38a	1.28b	1.15c	1.06d	0.01
g/kgW ^{0.75}	63.9a	60.1b	54.9c	51.4d	0.51
Total DM intak	e				
kg per day	1.38d	1.73a	1.57b	1.50c	0.01
g/kgW ^{0.75}	63.9d	81.4a	74.8b	72.2c	0.55

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Different letters in the same line is different (P < 0.05). OS: oat silage; S.E.M.: standard error mean.

Supplementation decreased SDMI (Table 3) as a result of interaction between forage and concentrate (Dulphy et al., 1990). Substitution rates were 0.72, 0.54 and 0.35 for UR, PL and SBM concentrates, respectively. They were within the level (from 0.2 to 0.6) given by Jarrige (1988) for SBM and poultry litter, but it was greater for urea. Our results confirmed those comparing SBM to urea (Church and Santos, 1981) and SBM to poultry litter (Matejovsky and Sanson, 1995) as well as the three supplements (Huber and Kung, 1981). Variable effects of concentrates on forage intake may be explained by the smell of poultry litter or the taste of urea which are known to depress the palatability of diets (Baumont, 1996). Moreover, UR and PL concentrates contained more barley than SBM (Table 1). Starch from barley is rapidly degraded in the rumen (32.2%/h according to Sauvant et al., 1995) leading to a rapid satiety state (Baumont, 1989 cited by Jarrige et al., 1995) for UR and PL diets. It is interesting to note that concentrates distributed before the silage were consumed at different rhythms. The SBM concentrate was always rapidly consumed by the sheep while the two others required longer time. The tendency towards slower ingestion rate with PL and UR concentrate probably reflects reduced palatability of these concentrates when supplementing the oat silage in our experiment.

Finally, SDMI was significantly affected by the period (1.44, 1.30, 1.20 and 0.94 kg DM per day for periods 1, 2, 3 and 4, respectively). Thus, at period 4 the SDMI was particularly reduced when the silage was supplemented with UR (0.51 kg per day) or with SBM concentrate (0.83 kg per day).

3.3. Rumen parameters and ruminal degradation

After the morning feeding, the pH in the rumen of sheep fed SBM diet was the lowest among the diets (Table 4) probably reflecting a greater amount of fermentable carbohydrates in the rumen or the rate of intake. The pH measured in sheep fed the UR and PL diets was lower than that of the OS diet only at 2 h after feeding. For each diet, the range of variation was between 0.3 and 0.4 units of pH (Table 4).

The ammonia-N concentrations induced by the OS diet were often higher than a minimum of 50mg/l N-NH_3 in the rumen required to optimise the microbial synthesis (Satter and Slyter, 1974). This result, which was unexpected, implied either sufficient degradable N in the oat silage or lack of fermentable carbohydrates for

micro-organisms. The former hypothesis seems unlikely because CP concentration in silage equalled 108 g/kg DM and only 8.4% of silage N was present as N-NH₃.

All supplements increased ammonia-N concentrations in the rumen, particularly at 1-2 h after the morning feeding (Table 4). This rise was pronounced with the UR diet and moderate with the SBM and PL diets. Following the peak, ammonia-N concentrations declined more rapidly with the UR and PL diets than with SBM (Table 4). The evolution of ammonia-N concentrations in the rumen agreed well with the degradability of nitrogen of supplements (INRA, 1988). Ammonia-N concentration in the rumen is also balanced by the availability of fermentable carbohydrates for micro-organisms. The UR and PL diets contained less silage and more barley than the SBM diet. Therefore, such differences may explain variation of ammonia-N concentrations between diets after the morning feeding.

The total VFA concentration at 1h after feeding was lower (P < 0.05) in the SBM diet than in other diets (Table 4), while the protozoa concentrations were higher in the SBM diet than in the other ones (8.0, 14.0, 10.4 and 8.8 $\times 10^3$ ml for the OS, SBM, PL and UR diets, respectively). *Entodiniomorphs* were always dominant, whereas *Holotriche* were rare and were represented mostly by the *Dasytricha* type. The DM degradability of wheat straw was not affected by the diet, whereas the SBM diet decreased (P < 0.05) the DM degradability of the oat silage (Table 5).

Table 4. Effects of the diets on pH value, ammonia-N (mg $N-NH_3/I$) and VFA (mmol/1) concentrations in the rumen during post-feeding time in Tunisian sheep

01	Time after fee	eding (h)	1			
	0	1	2	4	6	8
pH						
OS	6.86a	6.48a	6.51a	6.54a	6.56a	6.56b
SBM	6.55b	6.21b	6.22c	6.28b	6.23b	6.38c
PL	6.81a	6.45a	6.38b	6.57a	6.50a	6.54b
UR	6.80a	6.43a	6.42b	6.49a	6.54a	6.65a
S.E.M.	0.01	0.01	0.01	0.01	0.01	0.01
Ammonia-N						
OS	61.8	130.7d	125.4c	92.2c	55.6b	45.1b
SBM	67.3	164.2b	171.9b	121.9a	89.6a	97.9a
PL	58.6	155.7c	164.2b	111.0b	63.7b	42.7b
UR	64.0	188.4a	178.5a	97.3c	82.4a	41.0b
S.E.M.	2.1	1.1	2.1	1.7	2.3	0.9
VFA						
OS	68.9	95.4a	79.4	76.6	69.5	56.6
SBM	66.8	78.3b	85.4	73.7	69.1	61.7
PL	63.8	89.6a	87.9	72.0	66.2	59.7
UR	65.3	87.7a	85.5	68.7	69.5	68.5
S.E.M.	1.0	1.2	1.5	1.3	1.4	1.4

Different letters in the same column is different (P < 0.05).

Table 5. Effect of the diets on DM disappearance (%) of oat silage and wheat straw in the rumen of Tunisian sheep

	Diet				
	OS	SBM	PL	UR	S.E.M.
Wheat straw	64.1	62.5	61.3	61.1	0.5
Oat silage	67.9a	64.5b	69.1a	68.9a	0.2

Different letters in the same line is different (P < 0.05).

The objective of the supplementation undertaken in this experiment was to correct the shortage of ammonia in the rumen resulting from feeding oat silage which may depress microbial synthesis and fermentation of carbohydrates by the micro-organisms. Our results showed that ruminal disappearance of forage DM was higher,

especially for wheat straw, than that obtained (from 40 to 44%) by Rouissi (1994) and Chermiti (1994). This probably reflected intense fibrolytic activity with the oat silage-based diet. However, the SBM diet decreased this activity and this was associated with a greater number of protozoa in the rumen and a lower concentration of VFA in the rumen 1 h after feeding. In contrast, poultry litter did not affect the fermentation in the rumen. Thus, poultry litter-based concentrate is of interest to supplement poor forages as reported by Bhattacharya and Fontenot (1965). It must be kept in mind however, that such cheap supplement decreased the intake of forage (our results) and the range of chemical composition and nutritional value of poultry litter is very wide (Deshck et al., 1998). In the case of urea, our results confirmed the conclusion of Teller and Godeau (1984) that nitrogen supplement based on urea should be used with moderation because it generates ammonia in the rumen rapidly. Moreover, the decrease in the silage intake in the present study was the highest.

4. Conclusion

The present study showed that despite its poor chemical composition, silage was well ingested by breeds of local sheep. Supplementation of oat silage with SBM, UR and PL depressed its intake but total DM intake (TDMI) was increased. The three supplements induced a rise in the ammonia-N concentration in the rumen. However, SBM supplement decreased the fibrolytic activity in the rumen while PL and UR enhanced it. Compared to urea, poultry litter generated a lower ammonia-N peak in the rumen and a higher intake of the oat silage. The results imply that poultry litter, a cheap by-product available in Tunisia, would be of interest in order to replace SBM partially as nitrogen supplement for oat silage-based diets fed to moderately by productive local sheep.

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