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Effect of slow nitrogen intake supplementation with or without a lactic probiotic on Pelibuey lamb growth

M.A. Ortiz-Rubio*,, M.A. Galina* and L.J. Pineda****

*Facultad de Estudios Superiores Cuautitlán-Universidad Nacional Autónoma de México, Ctra. Cuautitlán-Teoloyucan km 2.5, San Sebastián Xhala, Cuautitlán izc., Estado de México 54700 (Mexico)
**Universidad de Colima, Av. Universidad #333, Colonia Las Víboras, CP 28040, Colima, Col. (Mexico)

Abstract. This study was conducted to assess the effect of a slow nitrogen intake supplement (SNIS) with or without a lactic probiotic on the nutritive value of a diet based on *Zea mays* (stover). One hundred hair lambs (15 ± 2.4 kg) were used during 120. Lambs were randomly assigned to two treatments. Animals in treatment 1 (T1) were fed on a basal diet (70% maize stover, 10% alfalfa, 20% SNIS). SNIS was a mixture of ammonium sulphate (1.8%), animal fat (4.0%), cement kiln dust (1.6%), corn (11.2%), cottonseed meal (16.4%), fish meal (4.2%), limestone (3.2%), mineral salts (1.0%), molasses (18.2%), orthophosphate (3.0%), poultry manure (11.6%), rice polishing (16.0%), NaCl (4.0%) and urea (3.8%). Lambs in group 2 (T2) were fed on the basal diet sprayed with 50 ml/kg DM of lactic probiotic. Daily weight gain was 0.281 and 0.341 kg/d for T1 and T2 respectively ($P < 0.05$). Total DM intake was 0.72 and 0.94 kg for T1 and T2, respectively ($P < 0.05$). The pH average levels were 6.2 (T1) and 6.5 (T2). Rumen NH_3 concentration was 6.7 mg/100 ml (T1) and 10.4 mg/100 ml (T2) ($P < 0.05$). Lactobacilli counts were 1.3 and 4.8 million/ml the first day and increased to 10.5 and 12.8 million/ml in 7 days. *Lactobacillus plantarum*, *L. helvaticus*, *L. delbrueckii*, *Lactoccocus lactis cremoris* and *Leuconostoc mesenteroides* were identified. Excretion of purine derivatives was higher in T2 in comparison with T1 ($P < 0.05$). The combination of SNIS and lactic probiotic increased bacterial protein synthesis and body weight gain as consequence of a better use of forage.

Keywords. Probiotic – Lactic bacteria – Lamb – Rumen physiology.

Effet d'une lente supplémentation azotée avec ou sans probiotique lactique sur la croissance du mouton

Résumé. *L'objectif de cette étude est d'étudier l'effet d'un supplément d'azote à consommation lente (SNIS) avec ou sans probiotique lactique sur la valeur alimentaire d'un régime à base de cannes de maïs. Cent agneaux (15 ± 2,4 kg) ont été répartis en deux groupes et ont été soumis pendant 120 jours à deux traitements. Les animaux dans le traitement 1 (T1) ont reçu un régime de base composé de maïs (70%), luzerne (10%) et SNIS (20%). SNIS est un mélange de sulfate d'ammonium (1,8%), de graisse animale (4,0%), poudre de cimentérie (1,6%), maïs (11,2%), farine de coton (16,4%), farine de poisson (4,2%), chaux (3,2%), sels minéraux (1,0%), mélasse (18,2%), orthophosphate (3,0%), fientes de volailles (11,6%), farine de riz (16,0%), NaCl (4,0%) et urée (3,8%). Les agneaux dans le groupe 2 (T2) ont reçu la ration de base susmentionnée après son arrosage avec 50 ml/kg MS de probiotique. Le gain de poids quotidien était de 0,281 et 0,341 kg/j pour T1 et T2 respectivement ($P < 0,05$). La consommation totale de MS était de 0,72 et 0,94 kg pour T1 et T2 respectivement ($P < 0,05$). Le pH s'élevait à 6,2 (T1) et 6,5 (T2). La concentration de NH_3 était de 6,7 mg/100 ml (T1) et 10,4 mg/100 ml (T2) ($P < 0,05$). Le nombre de lactobacilli était de 1,3 et 4,8 millions/ml du premier jour et a augmenté à 10,5 et 12,8 millions/ml en 7 jours.* Lactobacillus plantarum, L. helvaticus, L. delbrueckii, Lactoccocus lactis cremoris *et* Leuconostoc mesenteroides *ont été identifiés. L'excrétion des dérivés des purines était plus élevée avec T2 ($P < 0,05$). L'apport simultané de SNIS et du probiotique lactique a augmenté la synthèse des protéines microbiennes et le gain de poids à travers une meilleure utilisation du fourrage.*

Mots-clés. *Probiotique – Bactéries lactiques – Agneaux – Physiologie du rumen.*

I – Introduction

In the developing countries, ruminants have an important role in the sustainability of village

communities, and in many cases form the major source of income. Smallholders rely on subsistence farming, with few or no purchased inputs, but forages are usually available on farm and generally provide the sole source of nutrition to the animal (Galina *et al*., 2003). Over the last years, significant improvements in our understanding of digestion in the rumen have been ameliorated and much of this information has been translated into practical nutritional management strategies. For example, an understanding of the importance of nitrogen to the degradation of fibre by fibrolytic microorganisms has led to the inclusion of urea supplements in ruminant diets (Ortiz-Rubio *et al*., 2007), and mechanical and chemical treatments of forages has improved their digestibility. A variety of feed additives have been developed to achieve this objective (Caja *et al*., 2003). It has been reported that the addition of direct-feed microbials to the ration of sheep resulted in decreased numbers of harmful microorganisms in the intestines, improved fattening performance, and feed conversion rate (Lema *et al*., 2001).

The probiotics contain generally yeasts (Wallace, 1994), lactic acid bacteria (Cruywagen *et al*., 1996) and fungi (Kung, 1990) and/or mixtures. Lactic acid bacteria are normal residents of the gastrointestinal tract and include the genus *Lactobacillus*. They are the most prevalently administered probiotic bacteria (Reid and Friendship, 2002; Brashears *et al*., 2003) to increase de value of diet in ruminants. The objective of the present study was to increase the nutritive value of a diet for lambs based on maize stover and a slow nitrogen intake supplement with or without a lactic probiotic.

II – Materials and methods

A 120 days experiment was carried out in Queretaro, Mexico with 100 hair lambs (initial body weight 15.4 ± 2.4 kg) randomly assigned to two treatments. Animals in treatment 1 (T1) were fed on a basal diet (70% maize stover, 10% alfalfa, 20% SNIS, see Table 1). SNIS was mixture of ammonium sulphate (1.8%), animal fat (4.0%), cement kiln dust (1.6%), corn (11.2%), cottonseed meal (16.4%), fish meal (4.2%), limestone (3.2%), mineral salts (1.0%), molasses (18.2%), orthophosphate (3.0%), poultry manure (11.6%), rice polishing (16.0%), NaCl (4.0%) and urea (3.8%). The animals consume the supplement in seven hours average. Lambs in group 2 (T2) were fed on the basal diet sprayed with 50 ml/kg DM of a lactic probiotic sprayed daily. Water was provided freely. Four sheep fixed with ruminal cannulas were used in order to analyse ruminal fermentation parameters and bacterial presence. Chemical analyses were performed on diets according to the procedures of AOAC (1995). Determination of fibre contents was conducted by the van Soest and Wine (1967) method. The gross energy (Mcal/kg) value of diets and faecal samples was determined using a calorimeter bomb.

Table 1. Chemical composition of maize stover (MS), alfalfa (A) and slow nitrogen intake supplement (SNIS)

	MS	A	SNIS
Dry matter (%)	90.6	89.6	86.2
Ash (% DM)	5.8	9.7	21.9
Ether extract (% DM)	1.2	2.7	9.8
Crude protein (N × 6.25) (% DM)	4.2	17.6	22.0
Neutral detergent fibre (% DM)	71.8	55.1	38.7
Acid detergent fibre (% DM)	43.7	39.7	13.4
Hemicellulose (% DM)	3.7	27.2	21.1
Nitrogen free extract (% DM)	47.9	40.1	61.5
Metabolizable energy (Mcal/kg DM)	1.98	2.39	2.80

The *in situ* DM degradability was determined by incubating about 1.5 g of the dried maize stover in

Dacron polyester monofilament bags (53 ± 10 micron pore sizes; 5 × 10 cm diameter) in four rumen fistulated sheep. The bags were inserted in the rumen prior to feeding and were retrieved at 8, 12, 24, 36, 72 and 96 h post incubation. Immediately after removal, bags were briefly washed under running tap water and thereafter for 12 min with cold water in a domestic washing machine (Cherney *et al*., 1990) and dried for 48 h at 60°C in a forced-air oven. Washing losses were determined by treating four bags per sample. The DM degradation data were fitted to the exponential equation $P_t = a + b\ (1 - e^{-ct})$ using the Neway Excel Programme (Chen, 1995) where P_t is DM degradation (%) at time *t*. The degradation characteristics of the forage defined as *A* is equal to washing loss; $B = (a + b) - A$, representing the insoluble but fermentable material and *c* the rate of degradation of *B*.

During the last 5 days five lambs fed each diet were used to measure diet digestibility. Apparent digestibility was calculated over a 5 d fecal collection period and a daily 25% aliquot was collected for processing. In both treatments, total urine excretion was collected daily over 10% sulphuric acid (final pH of urine was kept below 3). Urine was weighed and urine samples were taken (100 ml) and frozen immediately at –20°C until analysis. Samples of rumen fluid (50 ml) were withdrawn at 0, 2, 4, 6, 8, 12, 16 and 22 hours through a rumen cannula. About 100 ml of rumen fluid was passed through two layers of gauze and kept in a CO_2 pregassed thermos flask. In the laboratory, the filtered rumen fluid was immediately transferred into smaller bottles while gassing with CO_2. The bottles were stored in the incubator at 39°C before dilution and inoculation. Rumen fluid was inoculated, incubated and colonies counted as described by Hungate (1969). Data on voluntary dry matter intake (DMI), body weight gain (BGW) and apparent nutrient digestibility coefficients were analysed with ANOVA using individual lambs as replicate (Steel and Torrie, 1980). Means were compared by Duncan's multiple range tests using SAS program (1996).

III – Results and discussion

The probiotics are used in animal nutrition either by direct addition to feed or to water (Caja *et al*., 2003). The animals with probiotic (T2) performed better than those on the control diet (Table 2). These findings are consistent with other published reports indicating that addition of microbial feed additive (probiotics) to the ration of sheep resulted in increased BWG and feed intake (Emanuelle *et al*., 1992; Lema *et al*., 2001). Treatments with probiotics enlarged ($P < 0.05$) N pool in the rumen (Table 2).

Table 2. Ruminal pH and NH_3, feed intake, apparent *in vivo* nitrogen digestibility and nitrogen balance in lambs fed without (T1) or with (T2) probiotics

	T1	T2
N intake (g/day)	12.3 ± 4.19[b]	16.0 ± 2.35[a]
Fecal N (g/day)	1.8 ± 1.34[b]	2.2 ± 1.59[a]
Urinary N (g/day)	3.5 ± 2.26[b]	4.3 ± 2.34[a]
N retention (g/day)	7.6 ± 1.47[a]	9.6 ± 1.53[a]
Apparent *in vivo* N digestibility (%)	52.4 ± 1.35[b]	69.2 ± 1.16[a]
NH_3 (mg/100 ml)	6.7 ± 1.95[b]	10.4 ± 1.4[a]
pH	6.2 ± 0.2[a]	6.5 ± 0.1[a]
DMI (kg DM/d)	0.72 ± 0.16[a]	0.94 ± 0.11[a]
Daily body gain (kg)	0.281 ± 0.036[b]	0.341 ± 0.020[a]

[a,b]Means within the same line with different letters differ ($P < 0.05$).

Stabilisation of pH is generally associated with decreased levels of lactic acid in the rumen. The stimulation of lactic acid-utilising bacteria induced decreases in lactic acid concentrations and the

corresponding moderation of ruminal pH. Average ruminal pH in the present study (Table 2) did not differ between treatments (P > 0.05). Our results are in the range reported by Mould and Ørskov (1983) for optimal fibre degradation by rumen microorganism. To optimise fermentative digestion of low-quality forage, an adequate NH_3 supply in the rumen is needed to provide the majority of N for microbial growth. However, a wide range was reported for the level of NH_3 that supports maximum intake, digestibility and N utilization, from 5 mg/dl (Satter and Slyter, 1974) to 20 mg/dl (Perdock *et al*., 1988). A relatively high and more uniform level of ruminal NH_3 was achieved when probiotic was offered to lambs in the present study. Differences observed in ammonia nitrogen could be associated with a stimulation of proteolytic bacteria (Newbold *et al*., 1995).

In vivo digestibility of DM, organic matter (OM) and neutral detergent fibre (NDF) digestion rate (k_d) did not differ (P > 0.05) between diets (Table 3). NDF passage rate (k_p) was higher (P < 0.05) in T1 than T2 (0.059/h for T1 and 0.076/h for T2). Table 4 shows that true digestibility of NDF was higher (P < 0.05) in T2 (45.64%) compared with T1 (37.29%).

Table 3. Apparent *in vivo* digestibility (%) of nutrients in lamb fed without (T1) or with (T2) probiotics

Apparent *in vivo* digestibility (%)	T1	T2
Dry matter	69.5 ± 2.1[a]	72.1 ± 1.5[a]
Organic matter	61.1 ± 0.34[b]	67.6 ± 0.38[a]
NDF	59.8 ± 9.5[a]	68.9 ± 7.5[b]
Cellulose	66.6 ± 13.1[a]	70.7 ± 9.0[a]
Hemicellulose	65.4 ± 9.4[a]	71.5 ± 5.8[a]

[a,b]Means in the same line with different letters differ (P < 0.05).

Table 4. Potential digestible and indigestible fractions, digestion rate, passage rate, true digestibility and in situ half-time disappearance of NDF

	T1	T2
Potential digestible fibre (b) (%)	60.40 ± 3.3[a]	67.12 ± 1.7[a]
Soluble fraction (a) (%)	6.36 ± 0.5[a]	6.45 ± 0.3[a]
Indigestible fraction [100 – (a + b)] (%)	30.49 ± 3.1[a]	28.90 ± 3.2[a]
Passage rate (k_p/h)	0.059 ± 0.003[b]	0.076 ± 0.013[a]
Digestion rate (k_d/h)	0.037 ± 0.001[a]	0.036 ± 0.031[a]
True digestibility ($k_d/k_d + k_p$) (%)	37.29 ± 3.12[b]	45.64 ± 2.62[a]
Half-time disappearance $t_{1/2}$ (h)	24.22 ± 2.97[a]	21.23 ± 1.68[a]

[a,b]Means within the same line with different letters differ (P < 0.05).

Previous studies (Dawson *et al*., 1990; Newbold *et al*., 1995) have shown that treatment with probiotics (lactobacilli and yeast culture) increases the number of cellulolytic bacteria in the rumen and, in some cases, increases cellulose degradation. Digestion rate of hemicellulose was higher in T2 than T1 (P < 0.01). There was no difference in digestion rate of cellulose (Table 5). True digestibility of cellulose and hemicellulose was higher in T2 compared with T1 (P < 0.05). Half-time ($t_{½}$) disappearance for hemicellulose was higher (P < 0.05) for T2 (26.37 h) compared to T1 (19.03 h) (Table 5). Newbold *et al*. (1995) suggested that *Aspergillus oryzae* and *S. cerevisiae* stimulated the rate rather than the extent of fibre digestion by ruminal microorganisms.

Table 5. Potential digestible and indigestible fractions, digestion rate, passage rate, true digestibility and *in situ* half-time disappearance of cellulose and hemicellulose of experimental diets

	Cellulose		Hemicellulose	
	T1	T2	T1	T2
Potential digestible fibre (%)	52.8 ± 3.45[b]	67.9 ± 2.87[a]	41.8 ± 5.24[b]	40.18 ± 4.65[a]
Soluble fraction (%)	5.9 ± 1.91[b]	5.32 ± 2.11[a]	4.8 ± 1.63[a]	4.9 ± 1.91[a]
Indigestible fraction (%)	36.14 ± 2.5[a]	24.32 ± 2.5[b]	28.01 ± 2.1[a]	38.92 ± 2.3[b]
Passage rate (k_p/h)	0.055 ± 0.003[a]	0.063 ± 0.011[a]	0.049 ± 0.003[a]	0.037 ± 0.005[b]
Digestion rate (k_d/h)	0.061 ± 0.009[a]	0.066 ± 0.004[a]	0.039 ± 0.002[b]	0.024 ± 0.001[a]
True digestibility ($k_d/k_d + k_p$) (%)	36.65 ± 1.13[b]	48.48 ± 1.23[a]	37.54 ± 2.66[b]	42.51 ± 2.17[a]
Half-time disappearance $t_{1/2}$ (h)	22.45 ± 2.35[b]	17.21 ± 2.34[b]	26.37 ± 3.54[b]	19.03 ± 2.21[a]

[a,b]Means in the same line with different letters differ (P < 0.05).

Very little work has been done on the effect of probiotics on microbial protein synthesis, and the results are conflicting. Jouany *et al*. (1998) found that probiotic (*Saccharomices* spp.) had no effect (P > 0.05) on the duodenal microbial N flow, whereas Erasmus *et al*. (1992) observed a higher (P < 0.05) duodenal supply of microbial proteins in cattle treated with probiotic (*Saccharomices* spp. and *Aspergillus oryzae*). Positive effect on microbial nitrogen supply was found in the present study (Table 6). The type of microorganism and diets composition could explain these differences.

Table 6. Urinary excretion of purine derivatives (PDe, mmol/d) and duodenal microbial N (g/d) in lambs fed without (T1) or with (T2) probiotics

	Allantoin	Uric acid	Hypoxanthine + xanthine	Total PDe	Duodenal microbial N
T1	15.1 ± 1.6[b]	1.8 ± 0.02[a]	1.2 ± 0.05[b]	18.1 ± 2.3[b]	15.6± 2.3[b]
T2	19.2 ± 1.2[a]	2.2 ± 0.03[a]	1.8 ± 0.01[a]	23.2 ± 1.6[a]	19.9± 1.7[a]

[a,b]Means in the same line with different letters differ (P < 0.05).

Lactobacillus plantarum, *L. helvaticus*, *L. delbrueckii*, *Lactoccocus lactis cremoris* and *Leuconostoc mesenteroides* were identified. The mean counts of Lactobacilli were 1.3 (T1) and 4.8 (T2) million/ml the first day and increased to 10.5 (T1) and 12.8 (T2) million/ml in 7 days (P < 0.05), in this respect, our results are similar to those of Newbold *et al*. (1995), who found a significant increase in ruminal bacteria numbers in sheep supplemented with *Saccharomices cerevisiae*.

IV – Conclusions

Results of the present study indicated that addition of a lactic probiotic to a slow nitrogen intake supplement to the rations of lambs increased body weight gain and improve rumen fermentation.

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