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in

Papachristou T.G. (ed.), Parissi Z.M. (ed.), Ben Salem H. (ed.), Morand-Fehr P. (ed.). Nutritional and foraging ecology of sheep and goats

Zaragoza : CIHEAM / FAO / NAGREF
Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 85

2009
pages 315-322

Goat kid's growth improvement with a lactic probiotic fed on a standard base diet

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Abstract. The objective of the present study was to increase kid's growth fed on alfalfa hay (*Medicago sativa*) and a high protein concentrate, with the addition of a lactic probiotic containing lactobacilli. A 120 day experiment was carried out in Mexico with 80 alpine kids divided in two groups, with two goats fixed with ruminal cannulas in each group. The basal diet was made of 55% alfalfa and 45% high protein concentrate. Kids in treatment 1 (T1) were fed on a basal diet only. Animals in treatment 2 (T2) were fed on a basal diet mixed with a lactic probiotic solution. Daily weight gain were 129 g/d and 169 g/d for T1 and T2 respectively ($P < 0.05$). NH_3 concentration was higher in T2 ($P < 0.05$). Nitrogen digestibility was greater in T2 (80.2%) in comparison with T1 (57.6%). Fibre digestibility was better in T2 ($P < 0.05$). NDF digestibility were improved in T2 ($P < 0.05$). True digestibility was 47.7% in T2 and 38.3% in T1. Half-time disappearance for hemicellulose was higher ($P < 0.05$) for T2 (32.03 h) compared to T1 (17.37 h). *Lactobacillus plantarum*, *L. helvaticus*, *L. delbrueckii*, *Lactoccocus lactis*, *L. cremoris* and *Leuconostoc mesenteroides* were identified. Lactobacilli counts were 1.6 and 4.4 million/ml the first day and increased to 5.5 and 12.5 million/ml in 7 days. Purines were significantly increased with the probiotic diet of T2 ($P < 0.05$). Adding a probiotic to the diet increased body weight gain and digestibility of fibre in the rumen of goat kids in T2.

Keywords. Probiotic – Lactic bacteria – Goat kids – Rumen physiology.

Amélioration de la croissance des chevreaux avec des régimes enrichis en probiotique lactique

Résumé. *L'objectif de la présente étude était d'améliorer la valeur nutritive d'un régime basé sur le foin de luzerne (*Medicago sativa*) et un concentré riche en protéines par l'adjonction de probiotique lactique. Une expérience de 120 jours a été réalisée au Mexique avec 80 chevreaux divisés en deux groupes avec deux chevreaux de chaque groupe munis de canules ruminales. Les chevreaux dans le traitement 1 (T1) ont reçu la ration de base sans supplémentation. Les animaux dans le traitement 2 (T2) ont été alimentés par la même ration de base mais enrichie de probiotique. Le gain de poids quotidien était, respectivement, de 129 g/j et 169 g/j pour T1 et T2 ($P < 0,05$). La concentration de NH_3 était plus élevée avec T2 ($P < 0,05$). La digestibilité de l'azote était plus élevée avec T2 (80,2%) en comparaison avec T1 (57,6%). La digestibilité de la paroi totale était aussi plus élevée avec T2 ($P < 0,05$). La digestibilité réelle était de 47,7% avec T2 et 38,0% avec T1. La disparition d'hémicellulose dans le rumen était plus importante ($P < 0,05$) avec T2 (32,03 h) par comparaison à T1 (17,37 h).* Lactobacillus plantarum, L. helvaticus, L. delbrueckii, Lactoccocus lactis cremoris *et* Leuconostoc mesenteroides *ont été identifiés. Le nombre de lactobacilli a varié de 1,6 à 4,4 millions/ml au premier jour et a augmenté à 5,5 et 12,5 millions/ml en 7 jours. Les concentrations des dérivés des bases puriques ont nettement augmenté avec l'apport du probiotique ($P < 0,05$). En conclusion, il ressort que l'adjonction d'un probiotique permet d'augmenter la digestibilité des fibres dans le rumen et d'améliorer la croissance des chevreaux.*

Mots-clés. *Probiotic – Bactéries lactiques – Chevreaux – Physiologie du rumen.*

I – Introduction

Animals supplemented with certain probiotic bacteria can significantly increase feed efficiency, live weight gain and disease resistance in ruminants (Gilliland *et al*., 1980; Gusils *et al*., 1999). The

probiotics could contain yeasts (Wallace, 1994), lactic acid bacteria (LAB) (Cruywagen *et al.*, 1996), fungi (Kung, 1990), *Bacillus subtilis* (Jenny *et al.*, 1991), some streptococcus (Higginbotham and Bath, 1993) and enterococcus and/or their mixture. LAB, which include the genus *Lactobacillus*, are the most prevalently administered probiotic bacteria (Brashears *et al*., 2003). Lactic acid bacteria are normal residents of the gastrointestinal tract and they are often considered as natural substitutes for feed antibiotics (Reid and Friendship, 2002).

The rumen is an open, self-contained ecosystem in which feed consumed by the ruminant is fermented to volatile fatty acids and microbial biomass that serve the animal as sources of energy and protein, respectively (Krause *et al*., 2003). Individual microbial species that have developed in the rumen interact in a complex manner and provide some of nature's best examples of microbial symbioses (Domínguez-Bello and Escobar, 1997). However, modern feeding practices geared toward high production have presented some novel challenges to ruminal microflora (Caja *et al*., 2003). The objective of the present study was to increase the nutritive value of diet for goat kids using a lactic probiotic on a diet of alfalfa (*Medicago sativa*) and high protein concentrate.

II – Materials and methods

A 120 days experiment was carried out in Mexico with 80 alpine kids (20.4 ± 0.420 kg) divided in two treatments. The basal diet for both groups was made of 55% alfalfa and 45% high protein concentrate (18% CP). Kids in treatment 1 (T1) were fed on a basal diet only. Animals in treatment 2 (T2) were fed on a basal diet, mixed with a lactic probiotic solution 50 ml/kg DM sprayed daily. Animals were weighed monthly. Two adult goats fixed with ruminal cannulas were allocated in each of both treatments to study ruminal physiology and microbiological counts.

Chemical analyses were performed on the diets (Table 1) according to the procedures of the AOAC (1995). Determination of fibre contents was conducted by the van Soest and Wine (1967) method. Samples (200 g) of the forages offered and the uneaten were collected daily the last five days of each experimental period and stored frozen in plastic bags. The frozen samples were thawed, pooled and thoroughly mixed for individual animals on a weekly basis and their DM determined. Dry matter, organic matter and nitrogen content were determined according to AOAC (1995). Neutral-detergent fibre and acid-detergent fibre content were measured with the technique suggested by Goering and van Soest (1970). The estimation of metabolizable energy content in the forages was based an average of their 48 h degradability in nylon bags in the rumen of the steers at optimal level of nitrogen. The estimation was made using the following equation (Ørskov *et al*., 1988; based on respiration chambers trials):

$$ME\ (MJ/kg\ DM) = 0.09\ X + 4.02 \quad (1)$$

where X = dry matter degradability after 48 h incubation.

Table 1. Chemical composition (% DM) of alfalfa (A) and high protein concentrate (HPC) offered to goat kids

	Alfalfa hay	High protein concentrate
Dry matter (%)	89.6	89.2
Ash	9.7	21.9
Ether extract	2.7	9.8
Crude protein (N × 6.25)	16.2	18.0
Neutral detergen fibre	55.1	36.5
Acid detergen fibre	39.7	13.4
Hemicellulose	27.2	20.4
Nitrogen free extract	40.1	58.3
Metabolizable energy (Mcal/kg DM)	2.4	2.1

On day 14 of each period, samples of rumen fluid (50 ml) of the experimental goats were withdrawn at 0.00, 2.00, 4.00, 6.00, 8.00, 12.00, 16.00 and 22.00 hours through rumen cannula. The rumen fluid sample was strained through four layers of cheesecloth and the filtrate was collected in 50 ml screw-top plastic bottles. Two to three drops of 1 N H_2SO_4 were added after pH determination to decrease the pH below 2. The samples were stored in a deep freezer at –20°C for later analyses. Volatile fatty acids were determined by High Performance Liquid Chromatography. Ten ml of each sample of the rumen liquor were centrifuged once at 5000 g for 15 minutes. The supernatants were ultra-centrifuged twice at 15,000 g for 15 minutes, the supernatants were micro-filtered once using resin filter and an acro-disc (Millipore, Massachusetts, USA, Catalogue No. 9004-70-0). One ml of the final liquid was injected into the chromatography equipment (Waters HPLC, Louisiana, USA). The pH was determined using a portable pH meter (ORION 250, California, USA), within 2-3 minutes of the sample was obtained. Rumen ammonia concentration was determined with a portable ion selective electrode for ammonia (ORION model 250, California, USA) inserted into 10 ml of rumen fluid. The gross energy (Mcal/kg) value of diets and faecal samples was determined using a bomb calorimeter (Adiabatic bomb, Parr instrument Co., Moline, IL). All analyses were completed in triplicate.

The *in situ* DM degradability was determined by incubating 1.5 g of the dry forage in Dacron polyester monofilament bags (53 ± 10 µm pore sizes; 5 × 10 cm diameter) in four rumen fistulated goats. Two experimental animals were fed basal diet and the other two were fed with basal diet plus a lactic probiotic solution 50 ml/kg DM. After the first sampling period, goats were changed to another diet in order to have four experimental units. Water was provided freely. 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, which were not incubated in the rumen in the same way.

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 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 of each period, five goats fed each diet were used to measure diet digestibility. Apparent digestibility was calculated over a 5 d faecal collection period and a daily 25% aliquot was collected for processing. Faeces were dried in a forced air oven at 70°C for 36 h and stored in airtight bottles until required for analyses. In both treatments, total urine excretion was collected daily over 10% sulphuric acid to keep pH 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. Rumen fluid was diluted (10^6 to 10^8) and 0.5 ml of each of the three dilutions was inoculated and incubated anaerobically before colonies were counted, following the technique described by Hungate (1969).

Daily urine production was collected from the animals during the last five days of the feeding period. Microbial nitrogen supply was calculated from purine derivative excretion (PDe) in urine according with the methodology of Chen and Gomes (1992), following the equation:

$$Y = 0.85X + (0.147\ W^{0.75}) \quad (2)$$

where Y and X represents the excretion of PD in urine (Y mmol/d) and absorption of microbial purines (X mmol/d), $W^{0.75}$ = the metabolic body weight (kg) of the steer. The slope 0.85 represents

the recovery of absorbed purines as PD in urine. The component within parentheses represents the net endogenous contribution of PD to total excretion after correction for the utilization of microbial purines by the goats (Chen and Ørskov, 2004). Calculations of intestinal flow of microbial nitrogen were using the following equation:

$$\text{Microbial N (g N/d)} = \frac{X\,(\text{mmol/d}) \times 70}{0.116 \times 0.83 \times 1000} = 0.727\,X \qquad (3)$$

where, digestibility of microbial purines is assumed to be 0.83. This is taken as the mean digestibility value for microbial nucleic acids (Smith and McAllan, 1971; Roth and Kirchgessner, 1979; Storm *et al*., 1983). The nitrogen content of purines is 70 mg/mmol and the ratio of purine-N:total N in mixed rumen microbes is taken as 11.6:100 (Chen and Gomes, 1992; Chen and Ørskov, 2004). Samples of the probiotic bacteria were identified in anaerobic chambers as detailed before (Galina *et al.*, 2008).

Data on voluntary dry matter intake (DMI), body weight gains (BGW) and apparent nutrient digestibility were analysed by ANOVA using individual goats as replicate (Steel and Torrie, 1980). Means differences were compared by Duncan's multiple range tests using the program of SAS (1996).

III – Results and discussion

In this study, we have investigated how the addition of probiotics to the rations of goats kids affect the feeding performance, microbial protein production and other ruminal parameters. The probiotics are used in animal nutrition either by direct addition to feed or water (Caja *et al.*, 2003). The animals with probiotic (T2) grew better indicated by a higher BWG over the experimental period (Table 2). Our results support the findings from other research carried out on growing Maltese goat that received lactobacilli, which had higher BWG than control group (Chiofalo *et al*., 2004). DMI and N intake were higher in T2 compared with T1 ($P < 0.05$).

Table 2. Ruminal pH and NH_3, dry matter intake, apparent *in vivo* nitrogen digestibility and nitrogen metabolism in goat kids fed a basal diet of alfalfa and concentrate without (T1) or with (T2) probiotics

	T1	T2
N intake (g/day)	118.6 ± 3.31[b]	146.0 ± 1.63[a]
Fecal N (g/day)	22.4 ± 1.03[b]	33.7 ± 2.65[a]
Urinary N (g/day)	52.3 ± 2.32[b]	82.5 ± 2.69[a]
N retention (g/day)	50.3 ± 1.84[a]	59.8 ± 1.52[a]
Apparent *in vivo* N digestibility (%)	57.6 ± 1.50[b]	80.2 ± 1.51[a]
NH_3 (mg/100 ml)	14.9 ± 2.42[b]	17.9 ± 2.91[a]
pH	6.40 ± 0.29[a]	6.54 ± 0.30[a]
DMI (kg DM/d)	1.120 ± 0.150[a]	1.312 ± 0.156[a]
Daily body gain (kg)	0.129 ± 0.022[b]	0.169 ± 0.018[a]

[a,b]Means with different letters differs ($P < 0.05$) between treatments.

Stabilisation of pH is generally associated with decreased levels of lactic acid in rumen. The stimulation of lactic acid-utilising bacteria decreases lactic acid concentrations and the corresponding moderation of ruminal pH. Average ruminal pH in the present study (Table 2) did not differ between treatments ($P > 0.05$). Results in the present study are in the range mentioned by Mould and Ørskov (1983) for optimal fibre degradation by rumen microorganism. Treatment with

probiotic (T2) had the highest concentration of ruminal ammonia (Table 2) in comparison to T1 ($P < 0.05$). Differences observed in ammonia nitrogen in the rumen fluid could be associated with a stimulation of proteolytic bacteria (Newbold *et al.*, 1995). Satter and Slyter (1974) suggested a concentration of 3 to 5 mg of NH_3/100 ml of ruminal liquid as optimal to increase ruminal microorganism growth.

Apparent *in vivo* digestibility (%) was higher but not significant in T2 in dry matter and superior in T1 in organic matter as it is shown in Table 3. *In vivo* digestibility of DM and OM did not differ between diets (Table 3). True digestibility of NDF was higher ($P < 0.05$) in T2 (47.73%) compared with T1 (38.31%) (Table 4).

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

Apparent *in vivo* digestibility (%)	T1	T2
Dry matter	64.5 ± 2.8[a]	67.41 ± 1.8[a]
Organic matter	93.2 ± 0.4[a]	92.0 ± 0.8[a]

[a]Means with different letters differs ($P < 0.05$) between columns.

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) (%)	62.45 ± 3.1[a]	66.03 ± 1.9[a]
Soluble fraction (*a*) (%)	6.06 ± 0.5[a]	6.07 ± 0.3[a]
Indigestible fraction [100 – (*a* + *b*)] (%)	31.49 ± 3.25[a]	27.90 ± 3.7[a]
Passage rate (k_p/h)	0.061 ± 0.004[b]	0.082 ± 0.015[a]
Digestion rate (k_d/h)	0.035 ± 0.003[a]	0.039 ± 0.034[a]
True digestibility ($k_d/k_d + k_p$) (%)	38.31 ± 3.12[b]	47.73 ± 2.62[a]
Half-time disappearance $t_{1/2}$ (h)	23.16 ± 2.97[a]	20.16 ± 1.68[a]

[a,b]Means with different letters ($P < 0.05$) between treatments.

Dawson *et al.* (1990) and Newbold *et al.* (1995) have shown previously 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 for hemicellulose was higher in T2 than T1 ($P < 0.01$), although there was no difference in the digestion rate for cellulose (Table 5). True digestibility of cellulose and hemicellulose was higher in T2 compared with T1 ($P < 0.05$). Half life-time ($t_{½}$) for hemicellulose disappearance was higher ($P < 0.05$) for T2 (32.03 h) compared to T1 (17.37 h) (Table 5). Newbold *et al.* (1995) suggested that *A. oryzae* and *Saccharomices cerevisiae* stimulated the rate rather than the extent of fibre digestion by ruminal microorganisms. However more research need to be undertaken on the specific effect of lactic bacteria on fibre digestion in the rumen of goats is necessary.

The excretion of purine derivatives (μmol/$W^{0.75}$) in urine was affected ($P < 0.05$) by the addition of lactic probiotic to the diets (Table 6). Information on the effect of lactic bacteria inoculation on PD excretion in goat kids is scarce. However, Jouany *et al*. (1998) reported no difference in PD excretion in sheep supplemented with *Saccharomyces cerevisiae* and *Aspergillus oryzae.*

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 (%)	67.89 ± 3.45[b]	78.9 ± 2.87[a]	57.17 ± 5.24[b]	66.18 ± 4.65[a]
Soluble fraction (%)	3.97 ± 1.9[b]	6.78 ± 2.1[a]	4.82 ± 1.6[a]	4.9 ± 1.9[a]
Indigestible fraction (%)	28.14 ± 2.54[a]	14.32 ± 2.9[b]	38.01 ± 3.1[a]	28.92 ± 2.4[b]
Passage rate (k_p/h)	0.057 ± 0.003[a]	0.068 ± 0.016[a]	0.049 ± 0.002[a]	0.037 ± 0.002[b]
Digestion rate (k_d/h)	0.063 ± 0.002[a]	0.068 ± 0.003[a]	0.059 ± 0.004[b]	0.064 ± 0.003[a]
True digestibility ($k_d/k_d + k_p$) (%)	48.65 ± 1.23[b]	57.48 ± 1.03[a]	37.54 ± 2.1[b]	42.51 ± 2.2[a]
Half-time disappearance $t_{1/2}$ (h)	21.45 ± 3.15[b]	16.21 ± 2.94[b]	17.37 ± 3.34[b]	32.03 ± 2.01[a]

[a,b]Means with different letters ($P < 0.05$) between treatments.

Table 6. Urinary excretion of purine derivatives (μmol/$W^{0.75}$) in goat kids fed without (T1) or with (T2) probiotics

	Allantoin	Uric acid	Hypoxanthine	Xanthine	Total PDe
T1	413.2 ± 15.4[b]	16.8 ± 3.3[a]	11.8 ± 2.98[a]	61.0 ± 3.1[a]	502.8 ± 9.2[b]
T2	488.1 ± 9.4[a]	15.2 ± 2.4[a]	11.2 ± 2.1[a]	54.7 ± 1.9[b]	569.2 ± 8.6[a]

[a,b]Means with different letters ($P < 0.05$) between treatments.

Lactobacillus plantarum, *L. helvaticus*, *L. Delbrueckii*, *Lactoccocus lactis cremoris* and *Leuconostoc mesenteroides* were identified. The mean counts of *Lactobacilli* were 1.6 (T1) and 4.4 (T2) million/ml the first day and increased to 5.5 (T1) and 12.5 (T2) million/ml in 7 days ($P < 0.05$), our results are similar with those of Dawson *et al.* (1990), who found a significant increase in most probable number counts of bacteria in rumen contents of steers on a forage diet in response to supplementation with a live probiotic.

IV – Conclusions

Addition of a lactic probiotic increased N intake (23%) and microbial protein synthesis, improved fibre degradation, NH_3 concentration, and body weight gain of growing kids.

Acknowledgment

This study was supported by DGAPA IN215706 UNAM, Cátedra FES-C IN2-20.

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