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Effects of supplementing malate and yeast culture *(Saccharomyces cerevisiae)* on the rumen enzyme profile and growth performance of lambs*

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ABSTRACT

The objectives of this study were to determine the effects of adding a mixture of malate and yeast culture *(Saccharomyces cerevisiae)* on cellulolytic, amylolytic, proteolytic and ureolytic activity in the rumen and on the growth performance of lambs. Eight native breed (Lory-Bakhtiary) lambs aged approximately 7 months (four males and four females) were divided into two groups by sex. They were kept in individual pens and fed a total mixed ration (TMR) based on lucerne hay-wheat straw and concentrate. The two groups of lambs were randomly assigned to each of the dietary treatments in a cross-over design: control (without supplementation) and supplemented (with malate and yeast included in the TMR at a level of 10 $g \cdot kg^{-1}$). The experiment was divided into 2 periods, in which the lambs received one diet in the first three-week period, after which they were transferred to the other diet for another three weeks. Urease, cellulase, protease and amylase activities were determined in rumen fluid and rumen microbial biomass. Rumen samples were taken 3-4 h after feeding at the end of each period. The weights of lambs were recorded at the beginning and end of each period. RNA equivalents (RNA-e) in rumen fluid and urine were measured for determination of microbial status in different samples. Supplementation with the malate-yeast mixture increased daily weight gain (from 196 in controls to 259 g/d) but did not affect ($P>0.05$) feed intake (0.970 vs 0.929 kg DM/d for the control vs the supplemented lambs, respectively). Higher cellulase activity was observed in lambs on the malate-yeast diet compared with the control lamb diet. A significant but moderately positive correlation was found between cellulase and protease activities ($r=0.5188$; $P=0.0395$). No significant differences were observed between the two treatments for dry matter, neutral detergent fibre and crude protein apparent digestibilities ($P>0.05$). Neither was microbial biomass (based on RNA-e concentrations) affected by the malate-yeast treatments ($P>0.05$).

KEY WORDS: malate-yeast mixture, enzymes, rumen, growth, lambs

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INTRODUCTION

The ruminal microbes include a large variety of bacterial, protozoal and fungal species making the rumen habitat very complex. Numerous interactions have been observed between the groups of microorganisms that exist in the rumen (Dehority, 1998). Organic acids (aspartate, fumarate, malate) have been proposed as an alternative to currently used antibiotic compounds in ruminant animals (Castillo et al., 2004) and most of the research conducted on this topic has been focused on malate. Several papers (Carro and Ranilla, 2003; Martin, 2004; Gomez et al., 2005) have shown that adding malate to *in vitro* fermentations of mixed rumen microorganisms resulted in changes in final pH and production of methane (CH_4) and volatile fatty acids (VFA) that are analogous to effects obtained using ionophores. Moreover, malate reduced the drop in rumen pH usually observed 2 to 4 h after feeding in animals given high-concentrate diets (Martin et al., 1999; Montano et al., 1999).

Research has shown that malate can stimulate the growth of *Selenomonas ruminantium* in pure cultures (Nisbet and Martin, 1990). Lactate can be fermented in the rumen by *Selenomonas ruminantium*, which accounts for more than 50% of the total viable bacteria within the rumen forming propionate. Malate stimulates lactate uptake by *S. ruminantium* and thus improves the rumen environment and increases propionate production (Martin et al., 1999; Montano et al., 1999). However, results from recent studies (Carro and Ranilla, 2003; Martin, 2004; Gomez et al., 2005) indicate that malate utilization *in vitro* could depend on the nature of the fermented substrate. In this manner, all strains of yeast significantly stimulated amylolytic and proteolytic activity of the digesta in the duodenum, small intestine and ileum but not in the abomasum (Strzetelski et al., 1995a). Yeast cultures, mainly *Saccharomyces cerevisiae*, may also improve ruminal fermentation (Newbold et al., 1996) and therefore provide another enhancer for microbial growth. The persistence of milk yield after the peak of lactation was greater in cows receiving a yeast supplement than in the cows of the control group (Strzetelski et al., 1996). However, the results on the use of *S. cerevisiae* in dairy cows (Dann et al., 2000) and dairy goats (Salama et al., 2002) are also contradictory. Dietary factors such as forage-to-concentrate ratio and forage type are important in determining the response to malate and yeast culture supplementation (Piva et al., 1993), which may explain the contradiction found in the results of the previous studies. In addition, there is little information about the effects of malate-yeast mixture on the growth of mixed rumen microorganisms.

Therefore, the aim of this study was to investigate if addition of a malate-yeast mixture to a basal diet for fattening lambs could improve microbial growth, lamb performance, and rumen enzyme activity. The basal diet was composed of forage and concentrate, which are normal ingredients used for fattening lambs and were selected to be representative of those given to ruminants in practice.

MATERIAL AND METHODS

Animals

Four male and four female lambs (of a native breed, Lory-Bakhtiary) aged approximately 7 months with body weight of 26±2.4 and 25±2.4 kg (males and females, respectively) were selected for the experiment. Lambs were divided into two groups by sex, housed in individual pens and were fed twice daily.

Experimental feeding and design

This experiment was designed as 2×2 cross-over with two treatments in two periods. Sun-dried good quality lucerne was chopped to about five to six centimeters in length. Lucerne hay and wheat straw formed 30% (at a ratio of 2:1) of the rations. The ingredients used in the preparation of the total mixed ration (TMR) are shown in Table 1. At the beginning of the experiment, the two groups of lambs were randomly assigned to one of the dietary treatments: control (without supplementation) and supplemented. For the supplemented group, a mixture of malate (75%) and *Saccharomyces cerevisiae* 1026 culture (25%) (Fariman®, Iranmellas Co., Fariman, Iran) was included in the concentrate at a level of 10 g/kg. The experiment was divided into two periods of 21 days (14 days for adaptation to each diet and 7 days for samplings). By the end of the first period, the groups were changed to the other diet in a cross-over design.

The diets were fed twice daily at 07.00 and 16.00 h, half of the ration being given at each feeding. Clean water was offered *ad libitum*. On the last day of each period the water was removed 1/2 h after feeding to avoid any dilution of the rumen content before sampling.

Daily feed intake was recorded by collection of any feed refusals before each morning feeding.

Table 1. Ingredients and nutrient composition of experimental diet

	Dietary components, %
Ingredients	
lucerne hay	20
wheat straw	10
barley grain	50
maize grain	10
soyabean meal	2
cotton seed meal	4
wheat bran	3
limestone	0.4
vitamin and mineral mixture	0.6
Nutrient composition, % DM	
dry matter[2]	88.30
ME, Mcal/kg[1]	2.68
CP[2]	14.92
NDF[2]	31.20
ADF[2]	17.88
Ca[1]	0.50
P[1]	0.38

DM - dry matter; ME - metabolizable energy; CP - crude protein; NDF - neutral detergent fibre; ADF - acid detergent fibre; [1]calculated according to NRC (2007) for diet component; [2] analysed in laboratory

Sample and data collection

Rations and refusals were sampled daily for determination of dry matter (DM) offered and DM refusal, respectively. Feed and faeces samples were oven dried at 60°C. All samples were ground on a 1-mm screen prior to determining chemical composition. In the last seven days of each period, the urine from each lamb was collected in polythene bags (the bag was fitted to the animal with a type of harness) and sampled twice daily at 06.30 and 15.30 h. The amount of urine was measured by volume and 10% of that was taken and placed in a separate bottle for each animal and stored at 4°C until (maximum 24 h) analysed. Total faeces for each lamb was weighed during the last seven days of each period and 10% of that was taken and dried at 60°C. On the last day of each period, rumen samples from each animal were taken by ruminocentesis, 3 to 4 h after the morning feeding. Samples (15 ml) were kept in an ice bath after collection and transferred immediately to the laboratory. Rumen fluid was centrifuged once at 1000 *g* for 10 min. The supernatant fluid was carefully decanted and stored at -196°C in liquid nitrogen for future analysis (Moharrery et al., 1998).

Chemical analysis

Feed and faeces samples were analysed for crude protein (copper catalyst Kjeldahl method ID 984.13), fat (solvent extraction method ID 991.36) and ash (ID 923.03) content (AOAC, 1991). Neutral detergent fibre (NDF) was determined as ash-free NDF using a Fibertec according to the method of Mertens (2002). Ash in fibre residues was determined by ignition at 525°C and acid detergent fibre (ADF) according to Van Soest et al. (1991).

The concentrations of ribonucleic acid equivalents (RNA-e) were determined in rumen fluid and urine. Purine in yeast RNA was used as a standard for calculating total microbial production (Moharrery and Das, 2001). The procedure combines standard literature methods for hydrolysis of nucleotides by perchloric acid followed by precipitation of free purines with silver nitrate to separate the purines from interfering compounds. Acid resolubilized purines in samples were quantified spectrophotometrically at 260 nm as a yeast RNA equivalent, according to the procedure of Zinn and Owens (1986).

Urease (urea amidohydrolase; EC 3.5.1.5). The assay procedure for urease activity was performed according to the method of Pathak et al. (1996). Enzyme activity was determined by measuring the amount of ammonia produced during incubation of the enzyme sample with urea. Enzyme activity was defined as mg ammonia nitrogen released per min per ml sample.

Cellulase. Filter paper activity (FPase) represents total cellulase activity. The activity of the enzyme was determined colorimeterically by measuring the amount of reducing sugar released during incubation of the enzyme sample with filter paper (Whatman No. 1). For this purpose, 1 ml sample, 1 ml phosphate buffer and 1 ml distilled water were mixed in a tube that contained a 50 mg strip of filter paper and were incubated for 1 h at 39°C. Next, to develop colour, a dinitrosalicylic (DNS) solution was used as described by Moharrery and Das (2001). Enzyme activity was expressed as mg reducing sugars (glucose) released per h per ml sample.

Protease. The assay procedure was based on the Blackburn (1968) method. A unit of proteolytic activity was defined as the amount of enzyme that would solubilize the equivalent of 1.0 mg tyrosine in 1 min.

Amylase (1,4, α -D-glucanohydrolase; EC 3.2.1.1). The activity of α-amylase was determined by measuring the rate of release of reducing sugars during incubation of the enzyme with starch. For this purpose, 0.25 ml sample, 0.25 ml starch solution (1 g starch in 100 ml distilled water) and 0.5 ml phosphate buffer, were mixed in a tube and incubated for 15 min at 39°C. Next, to develop colour, a DNS solution was used as described by Moharrery and Das (2001). Enzyme activity was expressed as μg reducing sugars (glucose) released per min per g DM sample.

Statistical analysis

This experiment was designed as 2×2 cross-over with two treatments in two periods. All measurements were performed in at least duplicate. All data from each factor were analysed with the GLM procedure of SAS (2003). The correlation among enzymes was determined and correlation coefficients were tested using a *t*-test (SAS, 2003).

RESULTS

The effect of adding a malate-yeast supplement to a basal diet on lamb performance is presented in Table 2. The addition of malate-yeast resulted in a numerically 32% higher daily weight gain and 21% better feed conversion,

Table 2. Lamb performance on different treatment under growing period

Item	Malate-yeast		Lamb sex			Probability		
	with	without	male	female	SE	D	S	D×S
Weight, kg								
initial	26.2	26.9	27.6	25.4	2.28	0.55	0.09	0.05
final	31.6	31.0	33.2	29.4	2.83	0.95	0.06	0.17
Daily gain, g	259	196	268	188	61.9	0.07	0.03	0.20
Daily feed intake, kg	956	943	1039	860	206	0.70	0.11	0.90
Feed conversion	4.02	4.87	4.25	4.64	0.900	0.08	0.41	0.25
Water intake, l	1.90	1.90	1.96	1.83	0.451	0.82	0.26	0.75
Water/feed	2.03	2.08	1.95	2.19	0.402	0.97	0.53	0.96

D - malate-yeast application; S - lamb sex; D×S - interaction; SE - standard error

which almost reached significance (P=0.07 and P=0.08, respectively) compared with the lambs on the control diet. Supplementing malate-yeast did not influence daily feed intake or water consumption (P>0.05) compared with lambs on the basal diet. Male lambs had 43% higher daily weight gains than females (P=0.03), the sex of the lambs did not influence any of the other production parameters (P>0.05).

The effect of the malate-yeast treatment on enzyme activities in rumen fluid is presented in Table 3. The malate-yeast supplement, as compared with the control, increased cellulase activity by 24% (P<0.01) and amylase activity by 19% (P=0.09), indicating increased activities of cellulolytic as well as amylolytic bacteria, simultaneously. Other enzymes were not affected by the treatment (P>0.05). A significant difference was observed between males

and females in cellulolytic, proteolytic and amylolytic activities (P<0.05). Male lambs had higher amylolytic activity (P<0.01) but lower cellulolytic and proteolytic activities compared with female lambs (P<0.05). With the exception

Table 3. Parameters in rumen fluid and urine, mean

Item	Malate-yeast		Lamb sex		SE	Probability		
	with	without	male	female		D	S	D×S
Rumen pH	5.85	6.00	5.93	5.92	0.123	0.03	0.84	0.91
Urine pH	8.65	8.76	8.82	8.59	0.251	0.42	0.09	0.02
Enzyme activity[1]								
cellulase	64.7	52.2	52.0	65.0	7.02	0.01	<0.01	0.07
protease	1.93	1.84	1.51	2.26	0.668	0.78	0.05	0.60
amylase	59.5	49.9	64.8	44.6	10.62	0.09	<0.01	0.84
urease	19.1	18.2	17.7	19.6	4.10	0.67	0.38	0.74

D - malate-yeast application; S - lamb sex; D×S - interaction; SE - standard error

[1] units of enzyme activity are: urease (mg ammonia N/min/ml), cellulase (mg gluc./h/ml), protease (Unit/ml), amylase (mg gluc./min/ml), lipase (unit/ml)

of urine pH, there was no difference (P>0.05) in the interaction between ration treatment and sex in all parameters related to the rumen fluid and urine. Although the enzyme profile in the rumen fluid differed significantly between two treatments, total apparent digestibility was not affected by malate-yeast treatment, sex or interaction between ration and sex for any of the nutrient components analysed (Table 4; P>0.05). Data related to rumen microbial biomass are presented in Table 5. Malate-yeast supplementation had no effect on the microbial nitrogen content in rumen fluid or total microbial biomass (P>0.05). The latter in male lambs was 74% higher compared with female lambs

Table 4. Digestibility coefficent in gastrointestinal tract, mean

Item	Malate-yeast		Lamb sex		SE	Probability		
	with	without	male	female		D	S	D×S
Dry matter	81.1	78.5	80.0	82.3	4.82	0.31	0.40	0.95
NDF	62.5	58.6	60.8	65.0	9.04	0.24	0.31	0.91
Crude protein	75.2	74.3	76.1	76.3	5.35	0.31	0.76	0.69

D - malate-yeast application; S - lamb sex; D×S - interaction; NDF - neutral detergent fibre; SE - standard error

(P<0.01), but when microbial biomass production was calculated per unit of metabolic body weight, it seemed that female lambs had 68% more biomass compared with male lambs (P<0.01). There was no interaction (P>0.05) between ration treatment and sex with respect to microbial production.

Table 5. Microbial biomass in different treatments and sex

Item	Malate-yeast		Lamb sex		SE	Probability		
	with	without	male	female		D	S	D×S
In rumen fluid								
RNA-e, mg/ml	6.65	6.87	6.28	7.24	0.501	0.74	0.17	0.10
MN, mg/ml	2.28	2.35	2.15	2.48	0.446	0.74	0.17	0.10
Totally								
urine RNA-e, mg/ml	17.92	15.95	17.36	16.51	2.643	0.62	0.83	0.68
MP, g CP/d	60.06	59.62	75.98	43.70	22.55	0.97	0.02	0.34
MB/$W^{0.75}$, g/kg	4.72	4.52	5.50	3.49	1.71	0.95	0.04	0.36

D - malate-yeast application; S - lamb sex; D×S - interaction; MN - microbial nitrogen; MP - microbial protein; $W^{0.75}$ - metabolic weight; SE - standard error

Correlation coefficients related to rumen fluid enzymes and to cellulase and amylase activities and their intermediate products (i.e. reducing sugar) in the rumen fluid are shown in Table 6. A positive correlation was found between cellulase and protease activity, but no significant correlations were observed between any of the other rumen fluid enzymes ($P>0.05$). High and significant correlations were observed between cellulase and reducing sugar ($r=0.84$; $P\leq0.01$) and between protease activity and rumen ammonia content ($r=0.58$; $P=0.02$).

Table 6. Correlation coefficients between enzyme activities and some metabolite in rumen fluid

	Cellulase	Protease	Amylase	Urease	Reducing sugar	Ammonia
Cellulase	1.00 [a] (<0.00 [b])	0.52 (0.04)	-0.14 (0.60)	0.46 (0.07)	0.84 (<0.00)	-
Protease		1.00 (<0.00)	-0.37 (0.16)	0.29 (0.27)	-	0.58 (0.02)
Amylase			1.00 (<0.00)	0.02 (0.95)	-0.08 (0.78)	-
Urease				1.00 (<0.00)	-	-0.05 (0.86)

[a] coefficient of correlation; [b] significant level

DISCUSSION

Lambs on the malate-yeast-supplemented diet showed a 32% higher average daily gain ($P=0.07$; Table 2) than control animals. This result is in agreement with Martin et al. (1999) who reported that the average daily weight gain in crossbred

steers increased linearly with more DL-malate and was 8.6% greater for DL-malate than for the control (1.86 vs 2.02 kg/d). After 98 days of fattening, average daily gain was linearly increased by DL-malate, with the greatest increase occurring with 80 g of DL-malate (1.76 vs 1.96 kg/d). In addition, better feed conversion in lambs on the malate-yeast diet (17% lower feed conversion compared to the control) in the present study shows consistency with reports on steers, whose gain efficiency increased with more DL-malate and was 4.9% greater for DL-malate than for the control (171 vs 163 g/d). There were no differences in feed ($P>0.05$) intake (Table 2), which agrees with previously reported results (Martin et al., 1999; Montano et al., 1999) and indicates that no negative effects of malate-yeast application on feed intake should be expected when it is included at levels up to 1% of dietary DM. Sex significantly ($P=0.03$) affected average daily gain and most of the enzymes in the rumen. Male lambs grew faster (+80 g/day), showed higher feed intake (+0.179 kg/ day) and better feed conversion (-0.39) than female lambs. Our results are in agreement with El Fadili et al. (2001) who found that male lambs grew faster, with higher feed intake and better feed conversion than female lambs.

Lambs on the malate-yeast diet showed 24% higher cellulolytic activity in the rumen ($P<0.01$; Table 3) compared with control animals. The use of malate-yeast is coupled with cellulolytic activity, which also needs NH_3 as a component for cellulolytic bacteria growth. On the other hand, isoacid is an extraordinary factor for cellulolytic bacteria in relation to growth promotion and cellulase production (Moharrery and Das, 2001). In agreement with our results, Strzetelski et al. (1995b) reported that all yeast supplements caused changes in yeast cell density, protozoa number, pH, ammonia concentration and VFA proportion in the rumen of bulls.

The effect of sex was also observed for cellulase, protease, and amylase activity ($P<0.05$). Female lambs showed higher cellulase and protease activities and lower amylase activity in the rumen compared with male lambs. It seems that lower feed intake in female lambs (Table 2) results from a higher retention time in the rumen. With increasing retention time in the rumen, microorganisms have enough time for more activity on feed, which results in higher enzyme activity in this group of lambs. In this regard, female lambs also produced 68% more microbial biomass per unit of metabolic weight (Table 5), which was attributed to more microbial action on digesta in the rumen.

Significant differences were detected between treatments for ruminal pH (Table 3) but no difference was observed in the concentrations of ammonia-N (values not shown). Correspondingly, increases of VFA production caused by malate supplementation have been consistently found when different substrates were incubated in batch cultures of mixed ruminal microorganisms (Carro and Ranilla, 2003) or in Rusitec fermenters (Gomez et al., 2005). In contrast, in the

studies of Martin et al. (1999) and Montano et al. (1999), malate did not affect total VFA concentration in the rumen or molar proportions of acetate, propionate, and butyrate. These inconsistent responses could be related to the different experimental conditions.

Daily urinary excretion of RNA-equivalent and, consequently, microbial biomass production (Table 5) were not affected by malate-yeast supplementation and, therefore, the estimated microbial N flow at the duodenum did not differ between treatments. Few studies have investigated the effects of malate on rumen microbial growth. Montano et al. (1999) used purine bases as a microbial marker to estimate microbial N duodenal flow in steers supplemented daily with 80 g of malate (2.6% of diet), and reported no differences from the control group. Nisbet and Martin (1990) showed that malate (4 to 12 m*M*) stimulated the growth of *S. ruminantium* in pure cultures, but *in vivo* results seemed to indicate that malate does not stimulate growth of mixed ruminal microorganisms under the conditions of the cited experiments.

Consistently with earlier studies (Montano et al., 1999), malate-yeast did not influence (P=0.24 to 0.31) apparent digestibility of DM, CP or NDF (Table 4). In contrast, some *in vitro* studies with batch cultures of mixed ruminal microorganisms (Carro and Ranilla, 2003) or Rusitec fermenters (Gomez et al., 2005) showed that malate increased ruminal degradability of diets of diverse composition. As it seems that all enzyme activities in the rumen show higher activities in lambs on malate-yeast treatment compared with lambs on a control diet, it is possible that malate-yeast stimulated diet ruminal degradation *in vivo*, but digestion in the postruminal tract counteracted this effect. However, Montano et al. (1999) reported that malic acid supplementation (2.6% diet DM) did not affect ruminal digestion of OM or ADF in steers fed an 81% steam-flaked barley-based diet. Differences in microbial concentrations in the rumen compared with the *in vitro* systems, which are much more dilute, should be taken into account when considering these results.

In the rumen fluid, it was best to use the coefficient of determination (r^2) to explain the degree of association between two variables. In this regard, r^2 calculated for cellulase with protease and urease is 0.27 and 0.21, respectively (Table 6). These amounts signify that 27 and 21% of the total variation in cellulase activity can be explained by the relationship between cellulase activity with the magnitude of protease and urease activities, respectively. Some by-product such as isoacid derived from the action of proteolytic enzyme acts as a factor for the growth and promotion of cellulolytic bacteria activity (Moharrery and Das, 2001). The net result of these activities was a strong and positive correlation between cellulase and protease (Table 6). This possibility may show that some species of bacteria have the potential for production of two or more enzymes at one time. The positive significant correlation supports this hypothesis. Although bacteria have this potential, under practical conditions, these types of relationships have not been seen between enzymes.

The use of NH_3 for microbial synthesis caused reduction of the rumen ammonia concentration. On the other hand, ammonia, as an end product of urease activity, has been reported to inhibit urease activity of some anaerobic bacteria (Gibbons and Doetsch, 1959) and there is evidence that another enzyme, glutamine synthetase, can regulate the production of urease in *Selenomonas ruminantium* (Smith and Bryant, 1979) because this enzyme is involved in NH_3 uptake for bacterial protein synthesis. The activities of both these enzymes could be increased many-fold when ammonia is limiting. The net result of this procedure was a moderately positive correlation between urease and cellulase activities in the rumen ($r=0.46$, $P=0.07$). When urea degradation is limited for some reason, protease can provide nitrogenous components from proteins, which may produce ammonia as an end product. It was surprising that ammonia shows a positive and strong correlation with protease activity rather than urease activity in the rumen fluid (Table 6). Ureolytic bacteria used ammonia for their growth and the ammonia concentration should be reduced with their activity when urea is limited. In this regard, none of the anaerobic proteolytic bacteria isolated by Blackburn and Hobson (1960) showed urease activity and it is possible that the higher activity of proteolytic bacteria, resulted in a higher ammonia concentration in the rumen. This means that there is a positive correlation between proteolytic bacteria and ammonia concentration in the rumen fluid (Table 6). A similar, only slightly different, situation was observed in relation to reducing sugar concentration and cellulase activity (Table 6).

Cellulases are a group of enzymes catalysing an enzymatic reaction system in which cellulose is decomposed into glucose, cellobiose, or cellooligosaccharides. Mainly symbiotic bacteria in the rumen produce this type of cellulase. This process is slow and enzyme activity produces chains with two to seven glucose units with beta-linkages. The amylolytic process is relatively rapid and the product that is produced by the enzyme is rapidly converted to volatile fatty acids by microorganisms. It may suggested that lower fermentation rates *via* the cellulolytic vs the amylolytic pathways resulted in accumulation of the reducing sugars in the rumen fluid and correlated positively with cellulolytic action.

IMPLICATIONS

Supplementing the diet of growing lambs with malate-yeast at a level of 10 g/kg of total dietary dry matter affected lamb growth performance and cellulolytic activity in the rumen but did not affect feed intake or total tract digestibility. Malate-yeast treatment changed the pattern of enzyme concentration in the rumen, increasing the correlation between ammonia concentration and protease rather than urease activity. Consequently, the reducing sugar concentration was more highly correlated with cellulase rather than amylase activity in the rumen fluid. It is possible that malate-yeast treatment produced an increase in the molar propor-

tion of isoacids (from protein degradation) to stimulate cellulolytic bacteria. The strong and positive correlation between cellulolytic and proteolytic activities in ruminal fluid may explain this aspect of cooperation among bacteria. However, more studies with diets of variable composition and different doses of malate are required to assess the conditions that influence the effectiveness of malate in ruminant feeding.

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