

EFFECTS OF LYSINE OR RUMINALLY PROTECTED LYSINE ADMINISTRATION ON NITROGEN UTILIZATION IN GOATS FED A DIET SUPPLEMENTED WITH RUMINALLY PROTECTED METHIONINE

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Summary

The objectives of the present study were to investigate whether or not dietary lysine addition could improve N balance of female Japanese Saanen goats at 15 to 32 months of age, weighing 31 to 40 kg, fed on a wheat bran-hay cube diet supplemented with methionine, and whether or not ruminally protected lysine supplementation could give as good an N balance performance as lysine in the presence of ruminally protected methionine when given orally to the goats. It was considered from changes in N balance and N utilization that the first and second-limiting amino acids in the diet were methionine and lysine respectively, under the present experimental conditions. The ruminally protected lysine in addition to the ruminally protected methionine gave no improvement in N balance and N utilization compared with the ruminally protected methionine alone, suggesting that the ruminally protected lysine used in the present study was not effectively utilized by the goats.

(Key Words: Ruminally Protected Methionine, Ruminally Protected Lysine, Goats, Nitrogen Utilization)

Introduction

Although the needs for limiting amino acids vary depending on dietary conditions and expected performance in a given animal species, methionine (Met) may frequently be the most limiting amino acid in ruminants. Bacterial protein, which is the major protein source reaching the small intestine, accounting for 60 to 85% of the total protein absorbed (Buttery and Foulds, 1988), is deficient in Met (Storm and Orskov, 1984).

One of the methods to alleviate methionine deficiency in ruminants is to infuse Met alone or in combination with other amino acids such as lysine (Lys) directly into the abomasum as shown in growing lambs (Nimrick et al., 1970), growing steers (Richardson and Hatfield, 1978), and in lactating cows (Rulquin, 1987; Seymour et al., 1990). However, animal performance with abomasal infusion can hardly reach a level usually found in intact animals due to reduced feed

intake. A more practical approach for delivering Met postruminally, therefore, would be to supplement a diet with ruminally protected methionine (RPMet), which is known to be resistant to ruminal degradation (Kaufmann and Luppig, 1982).

Amino acids have been protected from degradation in the rumen by use of amino acid analogues, amino acid polymers and encapsulation. With products such as these, some beneficial effects of RPMet alone or in combination with ruminally protected Lys (RPLys) have been demonstrated in a number of studies with lambs (Oke et al., 1986), and lactating cows (Papas et al., 1984; Illg et al., 1987; Rogers et al., 1989; Donkin et al., 1989), except for one study (Wright and Loecher, 1988) where no improvement in performance was shown with lambs and cows.

In the goat, however, the present subject has scarcely been documented in the literature. Supplementing a diet with RPMet enhanced whole-body protein synthesis and improved N balance in goats (Muramatsu et al., 1989, 1991). The present study was conducted, therefore, to investigate whether or not (a) dietary Lys addition could improve N balance of goats fed on a wheat bran-hay cube diet supplemented with Met, and

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(b) RPLys supplementation could give as good an N balance performance as Lys when given orally to goats.

Materials and Methods

Altogether three experiments were conducted with female Japanese Saanen goats. In the first experiment, the effect of supplementing with RPMet (Lactet[®] containing 30% DL-methionine, Nihon Soda, Co. Ltd., Tokyo, Japan) on N balance in goats was investigated. In experiment 2, the effect of combined addition of DL-methionine and L-lysine on N balance in goats was tested to determine an optimal level of the assumed second-limiting amino acid, lysine. In experiment 3, the effect of RPLys on N balance was compared with that of Lys.

In experiment 1, eight goats at 15 to 17 months of age, weighing 31.8 ± 0.5 (SE) kg, were used, and given a wheat bran-hay cube diet once a day at 10:00 hrs for 7 days. The composition of experimental diet is given in table 1. For a 30 kg goat, daily meal consisted of 350 g wheat bran, 338 g dehydrated alfalfa hay cube, and 22 g corn oil to give 11.4% digestible crude protein (DCP) and 2.56 Mcal ME/kg or as daily amounts of 6.3 g DCP/kg⁷⁵ and 0.15 Mcal DE/kg⁷⁵ to meet the requirements for goats, assuming to support daily weight gain of 50 to 100 g (National Research Council, 1981). Extra mineral was provided with a mineral block (Koen, Nihon Zenyaku Co., Fukushima, Japan) which contained (g/kg): NaCl, 995.5 g; ZnSO₄, 1,235 mg; MnCl₂, 1,146 mg; Fe₂O₃, 715 mg; CuSO₄, 377 mg; KI, 65.5 mg; CoSO₃, 6.6 mg. The goats were then transferred to metabolism cages, and distributed into two experimental groups of four. Each goat was fed on the same diet or the same diet supplemented with RPMet at an amount of 5 g to supply 1.5 g DL-methionine/day per goat for 2 weeks. The supplemental level of RPMet was chosen to give a beneficial effect on N balance almost equivalent to 8.0 g DL-Met according to our previous findings that RPMet was 4.6 times as efficient as Met (Muramatsu et al., 1991). Unlike the DCP and DE intake, the level of RPMet supplement was not adjusted for metabolic body weight, i.e. kg^{0.75}. However, because of a small variation in metabolic body weights among goats, the effect of unadjustment was judged to

be small. Water was provided daily at 2 to 3 liters per goat. The urine volume and feces weight for the last 7 days of the experimental period were measured every day, and the last 3-day samples were used to determine N concentration.

TABLE 1. COMPOSITION AND DAILY INTAKE LEVEL OF BASAL DIET

Ingredients	Intake	
	(g/kg diet)	(g/day) ¹
Dehydrated alfalfa hay cube	474	338
Wheat bran	495	350
Maize oil	31	22
Calculated:		
DCP (g/kg diet)		114
(g/day) ¹		81
DE (Mcal/kg diet)		2.56
(Mcal/day) ¹		1.92
Methionine (g/kg diet)		1.8
(g/day) ¹		1.2
Lysine (g/kg)		6.7
(g/day) ¹		4.6

Extra mineral was provided with a mineral block (Koen, Nihon Zenyaku Co., Japan), which contained (g/kg): NaCl, 995.5 g; ZnSO₄, 1,235 mg; MnCl₂, 1,146 mg; Fe₂O₃, 715 mg; CuSO₄, 377 mg; KI, 65.5 mg; CoSO₃, 6.6 mg.

¹ For a goat weighing 30 kg. The daily amounts given were changed proportionally to metabolic body sizes, kg^{0.75}.

In experiment 2, four goats at 27 to 29 months of age, weighing 41.9 ± 0.3 (SE) kg, were used, and given the same wheat bran-hay cube (basal) diet as in experiment 1 once a day at 10:00 hrs for 7 days. The goats were then transferred to metabolism cages, and fed on either the basal diet (Control), the basal diet with 9.0 g DL-Met (M), DL-Met with 10.0 g L-Lys (ML10), DL-Met with 20.0 g L-Lys (ML20), or Met with 30.0 g L-Lys (ML30) for 35 days at 7-day intervals according to the 5 × 5 Latin-Square design. As in experiment 1, urine volume and feces weight were measured every day for 7 days, and the last 3-day samples were used to determine N concentration.

In experiment 3, essentially the same protocols and the basal diet as in experiment 2 were used with goats at 30 to 32 months of age, weighing

40.3 \pm 0.3 (SE) kg except for the number of goats and treatments being reduced to four to be analyzed as the 4 \times 4 Latin-Square design. The treatments were the basal diet, the basal diet with 5.0 g RPMet, RPMet with 20.0 g Lys (RPMet+Lys), or RPMet with 20.0 g RPLys (containing 25% Lys, Nihon Soda Co. Ltd., Tokyo, Japan) (RPMet+RPLys). Tentatively, the RPLys supplement used was assumed to be 4 times as efficient as Lys. The urine and feces collection were done as in the previous experiments.

The N excretion in urine and feces was calculated by multiplying N concentration (mg/ml urine or g feces) determined from the last 3-day samples, by the average urine volume (ml/day) and feces weight (g/day) over 7 days. The method of collection and sample processing for N analysis were described previously (Muramatsu et al., 1991). The N contents in the diets, feces and urine were determined by the Kjeldahl method (AOAC, 1990), and the N recovery was validated using a standard of ammonium sulfate.

For statistical treatment of data, analyses of

variance were conducted according to a completely randomized block design (experiment 1), and a Latin-Square design (experiments 2 and 3), and significance of differences between treatment means was assessed by Duncan's multiple range test by using the General Linear Model procedure of Statistical Analysis System (1985).

Results

The values for N intake, N excretion in feces, and urine, N digestibility, N balance and N utilization in experiment 1 are given in table 2. There were no significant differences in N intake, fecal N excretion and N digestibility with or without RPMet supplementation. Urinary N excretion was lower ($p < .05$) in goats fed the RPMet added diet as compared with those fed the basal diet. Because of this lower urinary N excretion, both N balance and N utilization were significantly improved in goats fed the basal diet supplemented with RPMet compared with those fed the basal diet.

TABLE 2. EFFECT OF SUPPLEMENTING WITH RUMINALLY PROTECTED METHIONINE (RPMET) ON NITROGEN BALANCE AND NITROGEN UTILIZATION IN GROWING FEMALE GOATS FED A WHEAT BRAN-HAY CUBE DIET (EXPERIMENT 1)

Diet	Control	RPMet	Pooled SE (6 df)
N intake (g/kg ^{0.75} /day)	1.27	1.26	0.01
N excreted (g/kg ^{0.75} /day)			
In feces	0.32	0.32	0.03
In urine	0.78 ^a	0.58 ^b	0.05
N digestibility (%)	74.7	74.9	1.9
N balance (g/kg ^{0.75} /day)	0.17 ^a	0.36 ^b	0.05
N utilization (%) ¹	13.4 ^a	28.6 ^b	4.0

^{a,b} Means ($n=4$) not sharing a common superscript letter within the same row are significantly different at $p < .05$.

¹ N retained \times 100/N intake.

The effects of Lys supplementation in combination with Met were examined to see whether or not Lys was the second-limiting amino acid for goats under the present experimental conditions (table 3). Because unprotected amino acid supplements were used, the amount of Met added to the diet was increased to give almost an equivalent response to feeding 7.0 g/day of RPMet according to our previous findings (Muramatsu et al., 1991). N intake was increased

($p < .05$) as both Met and Lys were added to the diet. At the highest level of Lys supplementation, 30 g/day, the value for fecal N excretion was higher ($p < .05$) than those of the control, M and ML10 diets, implying that a part of dietary Lys was not absorbed, and excreted directly into the feces. Higher ($p < .05$) N digestibility was found in all three Lys-added diets compared with that in the basal and M diets. N balance was improved, though not significantly,

TABLE 3. EFFECT OF SUPPLEMENTING WITH GRADED LEVELS OF LYSINE (LYS) IN ADDITION TO METHIONINE (MET) ON NITROGEN BALANCE AND NITROGEN UTILIZATION IN GROWING FEMALE GOATS FED A WHEAT-HAY CUBE DIET (EXPERIMENT 2)

Diet	Control	Met	ML10	ML20	ML30	Pooled	
Supplements ^a	Met	9	9	9	9	SE	
(g/goat per day)	Lys	None	None	10	20	30	
						(12 df)	
N intake (g/kg ^{0.75} /day)		1.07 ^a	1.13 ^b	1.25 ^c	1.36 ^d	1.49 ^e	0.01
N excreted (g/kg ^{0.75} /day)							
In feces		0.21 ^a	0.22 ^a	0.21 ^a	0.23 ^{ab}	0.25 ^b	0.01
In urine		0.69	0.54	0.71	0.53	0.81	0.09
N digestibility (%)		80.0 ^a	80.7 ^a	83.1 ^b	83.1 ^b	83.5 ^b	0.5
N balance (g/kg ^{0.75} /day)		0.17 ^a	0.31 ^{ab}	0.33 ^{ab}	0.49 ^b	0.43 ^{ab}	0.08
N utilization (%) ²		16.3	27.5	26.6	36.0	28.9	6.0

^{a,b,c,d,e} Means (n=5) not sharing a common superscript letter within the same row are significantly different at $p < .05$.

¹ Supplemented as DL-methionine, and L-lysine at an amount as indicated.

² N retained $\times 100$ /N intake.

by supplementing with Met. The concomitant addition of Met and Lys tended to give better N balance value than the addition of Met alone, and the greatest improvement ($p < .05$) in N balance was observed with the ML20 diet compared with the control diet. The values for N utilization followed a similar trend to N balance, but the difference failed to reach statistical significance.

Table 4 gives the N balance performance in experiment 3 where the efficacy of RPLys relative to unprotected Lys was determined. N intake was

higher ($p < .05$) on the RPMet + Lys diet than on the other diets. No significant difference was detected in fecal N, urinary N excretion or N digestibility. In contrast, the values for N balance and N utilization were higher ($p < .05$) in the RPMet than in the control group. By supplementing the RPMet diet with unprotected L-Lys, a modest, but not significant, increase was observed in N balance or N utilization compared with the RPMet diet. The addition of RPLys in combination with RPMet gave slightly lower, though not significantly, values for N balance

TABLE 4. EFFECT OF LYSINE (LYS) OR RUMINALLY PROTECTED LYSINE (RPLYS) SUPPLEMENTATION IN THE PRESENCE OF RUMINALLY PROTECTED METHIONINE (RPMET) ON NITROGEN BALANCE AND NITROGEN UTILIZATION IN GROWING FEMALE GOATS FED A WHEAT-HAY CUBE DIET (EXPERIMENT 3)

Diet	Control	RPMet	RPMet + Lys	RPMet + RPLys	Pooled
					SE (6 df)
N intake (g/kg ^{0.75} /day)	1.07 ^a	1.14 ^a	1.27 ^b	1.11 ^a	0.03
N excreted (g/kg ^{0.75} /day)					
In feces	0.19	0.19	0.21	0.19	0.01
In urine	0.70	0.60	0.67	0.68	0.03
N digestibility (%)	82.1	82.9	83.8	83.3	0.8
N balance (g/kg ^{0.75} /day)	0.17 ^a	0.34 ^{bc}	0.40 ^c	0.25 ^{ab}	0.03
N utilization (%) ¹	16.3 ^a	28.4 ^{bc}	31.1 ^c	22.0 ^{ab}	2.5

^{a,b,c} Means (n=4) not sharing a common superscript letter within the same row are significantly different at $p < .05$.

¹ N retained $\times 100$ /N intake.

and N utilization than did the addition of RPMet alone, and had lower ($p < .05$) values than did the addition of RPMet + Lys.

Discussion

In the present study, dietary protein and energy intakes were set according to the metabolic body sizes, i.e. $\text{kg}^{.75}$, whereas the amino acid supplements were given at a fixed amount per goat per day. In a strict sense, this would certainly introduce an error to the N balance results. However, the difference in metabolic body weights in each goat was small, and therefore, it was believed that the manner of supplementation with unprotected amino acids or protected amino acids did not seriously disturb the conclusion reached.

In goats fed on the basal diet and reared under the present experimental conditions, it was considered that Met was the first-limiting amino acid as shown by improved N balance obtained by the addition of RPMet or Met alone to the diet (tables 2, 3 and 4). These results are in good agreement with the previous findings with goats fed on a similar diet (Muramatsu et al., 1989, 1990, 1991).

The improved response of N balance by raising Lys supplementation in the presence of Met, having the best N balance obtained at 20 g of Lys per goat per day, implied that Lys was the second-limiting amino acid under the present conditions. This is in line with the findings with other ruminant species that demonstrate beneficial effects of supplementing with Met alone or in combination with Lys in a ruminally protected form (Papaz et al., 1984; Oke et al., 1986; Illg et al., 1987; Donkin et al., 1989; Rogers et al., 1989). The level of Met was chosen to give an equivalent N balance response to that expected from feeding 7.0 g of RPMet (containing 2.1 g DL-Met) according to our previous findings (Muramatsu et al., 1991). Because Met and Lys were not added in the protected form in experiment 2, a portion of Met and Lys might be excreted directly into feces without being absorbed as shown by significantly higher fecal N on the ML30 diet than on the basal diet.

Since no information was available about the ruminal protection of RPLys compared with unprotected Lys, the bioefficacy of RPLys was

arbitrarily set to have slightly lower efficiency than RPMet relative to Met (i.e. 4 times as efficient as Lys according to the information from the manufacturer on characteristics of coating materials). The supplementation with Lys in addition to RPMet showed a higher, though not significantly, N balance than the single supplementation with RPMet. To our surprise, however, N balance obtained by the further addition of RPLys in combination with RPMet showed a poorer, though not significantly, value than that of found by the addition of RPMet alone, resulting in no significant difference from the basal diet.

The reason for this inferior N balance on the RPMet + RPLys diet is unclear. At least, however, overestimation of the assumed bioefficacy could not entirely be responsible, since even with no efficiency of RPLys, the N balance value should have remained at the same level as that found by the addition of RPMet alone. It is possible that the coating material becomes toxic for the growth of bacteria and/or host goats when given in the diet in excess. The daily amounts of the coating material in the RPMet diet and RPMet/RPLys diets in experiment 3 were 3.5 and 18.5 g per goat, respectively. In a preliminary experiment, it was found that supplementing a diet with the coating material of RPMet in a large excess such as 140 g per day resulted in reduced microbial N in the rumen of goats (unpublished data). The true reason for poor N balance on the RPMet + RPLys diet as well as the accurate estimation of bioefficacy of the RPLys supplement remains to be investigated.

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