EFFECT OF SUPPLEMENTING RUMEN-PROTECTED LYSINE AND METHIONINE ON RUMINAL CHARACTERISTICS AND NUTRIENT DIGESTIBILITY IN SHEEP

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Summary

This experiment was conducted to investigate the protein sparing effect of rumen protected lysine (RPLys) and methionine hydroxy analogue (MHA) in sheep. The treatments were T_1 (CP 15% + RPLys 0%), T_2 (CP 12% + RPLys 0%), T_3 (CP 12% + RPLys 0.4%) and T_4 (CP 12% + RPLys 0.4% + MHA 0.3%). Ruminal characteristics, *in situ* and *in vitro* digestibility and nitrogen retention rate were measured in sheep receiving different combinations of dietary supplement.

The results are summarized as follows:

1. Ruminal pH and VFA concentrations were not affected by the treatments. Ruminal ammonia-N concentration was high in sheep fed diets T_2, T_3 and T_4 with the highest value in the T_4 treatment ($p < 0.05$).
2. The digestibilities of dry matter and organic matter were not affected by the treatments.
3. Nitrogen losses through feces and urine were the highest with T_1 ($p < 0.05$). and nitrogen retention rates of groups T_1, T_2, T_3 and T_4 were 18.6, 32.4, 35.5 and 27.5% of nitrogen intake, respectively, indicating that RPLys supplementation improved nitrogen retention in sheep.

(**Key Words**: Rumen Protected Lysine, Methionine Hydroxy Analogue, Nitrogen Retention, Rumen Fluid Characteristics, Sheep)

Introduction

Postruminal supply of amino acids (AA) depends on microbial protein synthesized in the rumen and dietary proteins which escapes ruminal degradation. Thus, the amounts and balance of AA absorbed are influenced by the type of dietary protein and by the efficiency of microbial protein synthesis (Veira et al., 1990).

When grass or legume silage is the main feed consumed, ruminal microbial protein synthesis is relatively low and little dietary protein passes undegraded from the rumen (ARC, 1984). Therefore, it is not surprising that growing cattle fed grass silage respond with increased growth rate and improved feed efficiency when their diets are supplemented with ruminally undegradable protein sources, such as fish meal (Kirby et al., 1983; England and Gill, 1985; Veira et al., 1985, 1988).

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At present, relatively little information on AA requirements of ruminants is available, but from theoretical calculations, Thomas and Chamberlain (1982) suggested that a postruminal deficiency of lysine (Lys) and methionine (Met) may limit productivity of ruminants consuming grass silage. On the other hand, Chow et al. (1990) reported that Lys was more limiting than Met in both high fat or high concentrates diets. Deetz et al. (1985) also observed a significant response in live weight gain in steers fed rumen-protected lysine (RPLys) when given corn based diets containing 12% crude protein. But there are few reports on the effect of feeding rumen-protected AA (RPAA) in ruminants fed corn based diets.

Free amino acids (FAA) are rapidly broken down in the rumen; thus, their addition to the diets does not necessarily increase the amounts of AA absorbed. The development of methods of protecting AA from microbial degradation has made it possible to supplement diets with specific AA, which will become available for absorption at the intestinal level.

Komarek and Jandzinskii (1978a,b) reported that N retention by sheep increased when polymer-encapsulated

rumen-protected Met (RPMet) was supplemented to the diet. Oke et al. (1986) also showed similar increase in N retention in lambs supplemented with RPMet and RPLys.

We have developed a rumen-protected lysine by using various coating materials such as zein, shellac, ethylcellulose, stearic acid and acrylonitrile.

The objectives of this research are; 1) to verify the protection efficiency and availability of RPLys developed by our research team, and 2) to determine the effects on ruminal characteristics and nutrients metabolism of sheep fed corn grain-based diets supplemented with RPLys and/or methionine hydroxy analogue (MHA).

Materials and Methods

Animal and experimental design

Twelve rumen-fistulated cross-bred (Corriedale × Polworth) sheep with an average body weight (BW) of 50 kg were used in a completely randomized design to evaluate the effects of RPLys and MHA on ruminal characteristics and nutrient digestion. Animals were fed individually and housed in metabolism cages for the total collection of feces and urine.

Twelve sheep were randomly assigned to four treatments with 3 sheep per treatment : 1) T_1 (CP 15% without supplemental amino acids), 2) T_2 (CP 12% without supplemental amino acids), T_3 (CP 12% + 0.65% RPLys) and T_4 (CP 12% + 0.65% RPLys + 0.45% MHA) as shown in table 1.

The experimental period lasted for 10 days and consisted of 5d during which urine and feces samples were collected and 5d for the collection of rumen fluid. Animals were allowed an 8d adjustment period to the new diet before the experimental period was begun.

TABLE 1. DESCRIPTION OF EXPERIMENTAL DESIGN

Items	Treatments			
	T_1	T_2	T_3	T_4
Protein concentration (%)	15	12	12	12
Protected lysine (%)	0	0	0.65	0.65
MHA (%)	0	0	0	0.45

Diets and Feeding

Concentrates were composed of ingredients shown in table 2. The daily allowances of experimental diets containing 35:65 roughage to concentrates were offered in two equal portions twice daily (09:00 and 17:00 h) at 2.5% of body weight. A mineralized salt block and water were provided *ad libitum*. The roughage used in this experiment was 3% NaOH treated rice straw provided in a cube form. The rice straw contained 3.8% crude protein.

The concentration of vitamin and mineral contents and supplements such as molasses, limestone and salt were of same amounts in all concentrates. Chemical compositions of concentrates are also presented in table 2.

RPLys was manufactured by our research team and other coworkers. It contains 65% lysine and 35% of protected materials.

Concentrates were formulated to contain 15% and 12% CP (DM basis) for high protein (HP, T_1) and low

TABLE 2. INGREDIENT AND CHEMICAL COMPOSITIONS OF CONCENTRATES FOR SHEEP

Items	Treatments[1]			
	T_1	T_2	T_3	T_4
Ingredient composition (%)				
Corn grain	72.65	72.30	76.05	77.15
Soybean meal (44%)	6.40	10.60	9.40	9.80
Corn gluten meal	8.60	–	–	–
Wheat bran (pellet)	–	7.80	6.50	5.50
Sun-cured alfalfa (pellet)	8.00	4.90	3.00	2.50
Molasses	2.50	2.50	2.50	2.50
Limestone	1.40	1.45	1.45	1.45
Salt	0.20	0.20	0.20	0.20
Vitamin mixture[2]	0.05	0.05	0.05	0.05
Mineral mixture[3]	0.20	0.20	0.20	0.20
RPLys[4]	–	–	0.65	0.65
MHA[5]	–	–	–	0.45
Total	100.00	100.00	100.00	100.00
Chemical composition (%)				
Moisture	11.90	12.47	12.53	12.55
Crude protein	15.38	12.46	12.28	12.32
Ether extracts	3.11	3.35	3.38	3.37
Crude fiber	4.46	4.33	3.72	3.55
Crude ash	3.67	3.96	3.72	3.67
Ca	0.69	0.68	0.66	0.65
P	0.28	0.33	0.31	0.30
Lysine	0.50	0.55	0.91	0.91
Methionine	0.31	0.22	0.21	0.21

[1] T_1 : CP 15%, T_2 : CP 12%, T_3 : CP 12% + RPLys 0.65%, T_4 : CP 12% + RPLys 0.65% + MHA 0.45%.

[2] Vitamin mixture contains following amounts of micro nutrients per kg: Vitamin A, 1.6×10^7 IU; Vitamin D_3, 3.2×10^6 IU; Tocopherol, 6×10^4 IU.

[3] Mineral mixture contains following amounts of micro nutrients per kg: Cu, 5 g; Fe, 20 g; Mn, 25 g; I, 0.25 g; Co, 0.15 g; Se, 0.1 g.

[4] RPLys : Rumen-protected lysine.

[5] MHA : Methionine hydroxy analogue.

protein (LP, $T_2 \sim T_4$) treatments, respectively by using different ratio of ingredients. Diet 3 and diet 4 were formulated to contain similar concentrations of lysine and methionine with 0.65% of RPLys or/and 0.45% MHA together with different ingredients.

Ruminal characteristics

Samples of ruminal contents were obtained via the rumen cannulae at 0, 2, 4, 6 and 8 h after the morning feeding. Samples were immediately strained through four layers of cheese cloth and pH was measured. The concentration of volatile fatty acid (VFA) in rumen fluid was determined by gas liquid chromatography (Packard, 439-GLC) according to Erwin et al. (1962). A 12 ml aliquot of the filtrate was centrifuged at 475 × g for 15 min, and ammonia-N on the supernatant.

In vivo digestibility and nitrogen retention

Offered feeds were recorded at each feeding, and feed samples were collected daily and bulked throughout the experimental periods for later analysis.

Refusals were weighed and sampled daily before morning feeding, and DM content was determined.

Feces were weighed daily after the morning feeding, a 10% subsample of which was bulked for each sheep and frozen for future analysis.

Urine samples were collected through 2 layers of surgical gauze into 200 ml of 2.8% HCl solution (w/w) and volume was measured daily. A 10% subsample was stored at −20℃ for future analysis.

In situ disappearance rates

To determine the extent of ruminal degradation of RPLys and MHA in the rumen, an *in situ* trial was carried out. DM and Lys disappearance rates were measured by incubating samples in nylon bags (25 μ pore size; 80 × 130 mm i.d) which were suspended in the rumen. Bags were washed, dried at 55℃ for 48 h, and weighed before and after filling with samples (5 g of DM). The bags were soaked in warm water for 10 min and placed in the rumen at 30 min after the morning feeding. Duplicate bags were removed at 0, 0.5, 1, 3, 9 and 24 h of incubation. Upon removal, bags were washed until the wash water was clear, and then dried at 55℃ for 48 h. Bags were weighed, and the residues were analyzed for Lys content.

Chemical analysis

Feed and refusals were dried at 100℃. Fecal samples were dried at 55℃ for 48 h, ground through a 1 mm screen, and analyzed for DM, crude fiber and Kjeldahl N according to AOAC (1984) procedures. The content of Ca in basal diets was analyzed by atomic absorption spectrophotometry after nitric/perchloric acid digestion (AOAC, 1984). Phosphorus was determined by the meta-vanadate method (AOAC, 1984).

The urine concentrations of total N, ammonia-N and urea were analyzed according to AOAC (1984), Chaney and Marbach (1962), and using a urea-analysis kit (Asan Chem. Co. Ltd), respectively.

Statistical analysis

Analysis of variance was carried out and means were compared by Duncan's multiple range test using GLM (general linear model) procedures of SAS (1985).

Results and Discussion

Ruminal characteristics

Ruminal pH and concentrations of ammonia-N and VFA in rumen fluid are shown in table 3, 4 and 5, respectively.

Ruminal pH was significantly higher at 2 and 8 h postfeeding for sheep fed diet T_1 than those fed diets T_2 to T_4. Ruminal pH was similar for sheep fed diets T_2 to T_4, and tended to increase as the postfeeding time increased (table 3).

TABLE 3. RUMINAL FLUID pH OF SHEEP FED EXPERIMENTAL DIETS

Time after feeding	Treatment[1]			
	T_1	T_2	T_3	T_4
2 hr	6.0±0.24[2a]	5.8±0.09[b]	5.6±0.05[b]	5.6±0.03[b]
4 hr	5.7±0.07	5.8±0.05	5.6±0.07	5.6±0.05
6 hr	5.9±0.08[b]	6.0±0.10[a]	5.7±0.11[b]	5.7±0.06[b]
8 hr	6.2±0.15[a]	6.1±0.07[ab]	5.7±0.10[b]	5.7±0.05[b]

[1] T_1: CP 15%, T_2: CP 12%, T_3: CP 12% + RPLys 0.65%, T_4: CP 12% + RPLys 0.65% − MHA 0.45%.

[2] Mean ± SE.

[a,b] Mean values with different superscripts within the same row are significantly different ($p < 0.05$).

The results indicated that ruminal pH was not affected by the supplementation of RPLys and MHA but was affected by dietary protein level.

Wanapat et al. (1988) and Yang et al. (1986) reported that ruminal pH, VFA and ammonia-N are generally unaffected by Lys and Met supplementation of RPAA.

The range in pH values of 5.6~6.2 was lower than the opitmum pH values for both proteolysis and deamination reviewed by Tamminga (1979). This is due to feeding of

high concentrate diets.

As shown in table 4, ruminal ammonia-N concentrations were affected by both CP level and supplementation with RPLys and/or MHA in the diet. Sheep fed diet T_1 and T_2 had significantly ($p < 0.05$) lower ammonia-N concentrations than those fed diet T_3 and T_4.

This finding may suggest that the protein in diet T_1 was degraded at a slower rate than in diet T_2 to T_4 or that the diet resulted in improved microbial utilization of ammonia-N. This is probably due to the difference in feed ingredient. Diet 1 contained 8.6% corn gluten meal, which is well known for its low degradability.

Ruminal ammonia-N concentrations increased gradually with dietary RPAA (ruminal protected amino acids, RPLys + MHA) level in groups T_2 to T_4, and was highest

TABLE 4. AMMONIA-N (mg/100 ml) CONCENTRATION IN THE RUMEN FLUID OF SHEEP FED DIFFERENT DIETS

Time after feeding	Treatment[1]			
	T_1	T_2	T_3	T_4
2 hr	17.0 ± 1.11^{2b}	15.9 ± 1.10^{b}	17.3 ± 0.78^{a}	20.0 ± 0.75^{a}
4 hr	11.4 ± 0.84^{c}	13.1 ± 0.47^{b}	13.8 ± 0.54^{bc}	15.3 ± 0.23^{a}
6 hr	13.9 ± 0.64^{c}	15.8 ± 0.52^{bc}	16.5 ± 0.30^{b}	21.5 ± 0.72^{a}

[1] T_1 : CP 15%, T_2 : CP 12%, T_3 : CP 12% + RPLys 0.65%, T_4 : CP 12% + RPLys 0.65% + MHA 0.45%.
[2] Mean ± SE.
[a,b,c] Mean values with different superscripts within the same row are significantly different ($p < 0.05$).

($p < 0.05$) with treatment T_4. The supplemented RPLys and MHA may have been partially degraded in the rumen, supplying some of RPLys and MHA for microorganisms compared with control diet (T_2 treatment).

Total VFA concentrations in ruminal fluid were not significantly different between treatment. The total VFA concentration was similar for treatments (T_2 to T_4). The result indicated that RPAA did not affect total VFA concentrations when compared with T_2 treatments as control. Yang et al. (1986) also reported that total VFA concentrations in ruminal fluids were similar for cows fed heat treated-soybean meal (HSBM) and HSBM + RPMet.

Of the individual VFA concentrations, acetate, propionate and butyrate, as well as ratio of acetate to propionate were unaffected by RPLys and MHA supplementation.

In vivo digestibility

Results on nutrient digestibility of experimental diets are presented in table 5.

Sheep fed diet T_1 had lower digestibilities of DM (DMD) and OM(OMD) than those fed diet T_2 to T_4. DMD and OMD were not affected by supplementation with RPLys and MHA which is in agreement with results reported by Oke et al. (1986), and Wright and Loerch (1988).

TABLE 5. *IN VIVO* NUTRIENT DIGESTIBILITY (%) OF EXPERIMENTAL DIETS FOR SHEEP

Nutrient	Treatments[1]			
	T_1	T_2	T_3	T_4
Dry matter	69.4 ± 1.75^{2b}	72.9 ± 0.51^{a}	72.6 ± 0.46^{a}	72.6 ± 2.20^{a}
Organic matter	72.6 ± 1.88^{b}	76.1 ± 0.57^{a}	75.7 ± 0.66^{a}	76.8 ± 1.65^{a}

[1] T_1 : CP 15%, T_2 : CP 12%, T_3 : CP 12% + RPLys 0.65%, T_4 : CP 12% + RPLys 0.65% + MHA 0.45%.
[2] Mean ± SE.
[a,b] Mean values with different superscripts within the same row are significantly different ($p < 0.05$).

In situ digestibility

The effect of rumen incubation time on *in situ* DM and Lys disappearance of RPLys and MHA is presented in table 6.

The values in table 6 are calculated by substracting 0 hr values, which was obtained by washing representative bags without incubation. The disappearance rate of DM and lysine in protected lysine, and MHA of 0 hr was 22.7 ± 2.99, 32.6 ± 0.96, and 54.9 ± 4.69%, respectively.

TABLE 6. *IN SITU* RUMINAL DRY MATTER AND LYSINE DISAPPEARANCE RATE (%) OF PROTECTED LYSINE AND MHA IN SHEEP

Incubation time	Protected lysine		MHA[2]
	DMD[1]	Lysine	DMD
0.5h	4.8±0.46[3]	19.4±0.30	30.3±0.59
1h	5.6±2.44	14.4±1.81	28.6±1.45
3h	9.8±3.41	19.0±2.44	31.0±0.53
9h	6.8±3.78	29.2±1.74	38.4±0.21
24h	21.0±0.81	36.7±2.73	41.2±0.18

[1] DMD : Dry matter disappearance rate (%).
[2] MHA : Methionine hydroxy analogue.
[3] Mean ± SE.

In situ DM disappearance (DMD) of RPLys was less than 10% up to 9 h of incubation and were not affected ($p > 0.05$) by incubation time.

The sample RPLys used in this work has shown to be also stable in *in vitro* study (Ko, 1994). And ruminal lysine disappearance of the product was 12% when incubated in the rumen of Holstein dairy cows. Ko (1994) also reported that the postruminal lysine availability of the RPLys was more than 96%.

In situ DMD of MHA was more than 35% following 9 h of incubation, and this DMD value appeared to be higher compared to the result of Wright and Loerch (1988) who reported that *in situ* DMD of RPMet was less than 4% following 48 h of incubation and was not affected by ruminal characteristics. One possible reason for the higher DMD of MHA used in current study might be due to physical escape from nylon bags.

In situ DMD of concentrate incubated in the rumen of sheep fed each treated diet is shown in table 7. *In situ* DMD when incubated in the rumen of sheep fed LP diets ($T_2 \sim T_4$ treatment) was significantly ($p < 0.05$) higher compared to incubating in the rumen of sheep fed HP diet (T_1 treatment). The same trend was obtained in an *in vivo* digestion study (table 5). There is no clear explanation on the differences in digestion between treatments. However, digestion of diet components may have affected the total

TABLE 7. *IN SITU* RUMINAL DRY MATTER DISAPPEARANCE RATE (%) OF CONCENTRATES INCUBATED IN THE RUMEN OF SHEEP

Treatment[1]	Rumen incubation time (h)				
	0	2	6	12	24
T_1	40.7±1.70[2]	47.2±0.78[b]	48.4±0.16[c]	60.6±0.89[c]	67.5±1.28[c]
T_2	41.7±0.16	51.9±0.67[a]	54.8±1.25[b]	71.8±2.43[a]	78.2±2.14[a]
T_3	40.3±0.62	47.0±2.01[b]	52.0±1.11[bc]	65.3±2.30[b]	72.0±2.21[b]
T_4	42.0±0.91	51.7±0.95[a]	69.6±1.35[a]	69.0±3.87[a]	78.2±0.94[a]

[1] T_1 : CP 15%, T_2 : CP 12%, T_3 : CP 12% + RPLys 0.65%, T_4 : CP 12% + RPLys 0.65% + MHA 0.45%.
[2] Mean ± SE.
[a,b,c] Mean values with different superscripts within the same column are significantly different ($p < 0.05$).

digestion of each diet (table 2).

Nitrogen retention rate

The effects of supplemental RPLys and MHA on sheep nitrogen metabolism are presented in table 8.

There were no difference in N intake among sheep fed LP treatments ($T_2 \sim T_4$), but those fed HP treatment (T_1) consumed significantly ($p < 0.05$) more N than those of the LP treatments.

Fecal and urinary N (g/d) was higher ($p < 0.05$) for sheep fed diet (T_1) compared with sheep fed diet (T_2 to T_4).

Compared with sheep fed T_2, there was no increase in N retention in T_4 sheep, while diet T_3 resulted in an improvement in N retention.

Schelling et al. (1973) reported that abomasal infusion of amino acids primarily reduced urinary N excretion and not fecal N excretion. Oke et al. (1986) also reported urinary N was lower for sheep fed RPLys and RPMet compared with sheep fed no supplemental amino acids, including a more efficient metabolism of nitrogenous compounds.

In our experiments, this trend was also observed by showing significantly lower urinary N excretion for sheep fed diet T_3. However, combined with MHA (T_4), N retention was not increased. It is not clear why N retention of sheep in our experiment did not increase in response to added MHA, as did those of Oke et al. (1986).

TABLE 8. NITROGEN RETENTION IN SHEEP FED DIFFERENT DIETS

Items	Treatments[1]			
	T_1	T_2	T_3	T_4
Total N intake (g/d)	30.7[a]	28.2[b]	28.2[b]	28.2[b]
Excretion (g/d)				
Feces	12.1 ± 1.10[2a]	9.6 ± 0.05[b]	10.4 ± 0.19[b]	10.1 ± 0.47[b]
Urine	12.9 ± 0.93[a]	9.4 ± 0.63[b]	7.8 ± 0.47[c]	10.3 ± 0.36[b]
Total	25.0 ± 0.43[a]	19.0 ± 0.58[bc]	18.2 ± 0.31[c]	20.4 ± 0.48[b]
Retention (g/d)	5.7 ± 0.43[c]	9.1 ± 0.58[ab]	10.0 ± 0.31[a]	7.7 ± 0.48[b]
Retention (%)	18.6 ± 1.31[d]	32.4 ± 1.94[b]	35.5 ± 1.05[a]	27.5 ± 1.61[c]

[1] T_1 : CP 15%, T_2 : CP 12%, T_3 : CP 12% + RPLys 0.65%, T_4 : CP 12% + RPLys 0.65% + MHA 0.45%.
[2] Mean ± SE.
[a,b,c,d] Mean values with different superscripts within the same row are significantly different ($p < 0.05$).

These results suggest that Met was either a non-limiting AA for these sheep or the MHA did not have a sufficient rumen protection.

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