EFFECT OF SUPPLEMENTING RUMEN-PROTECTED LYSINE ON GROWTH PERFORMANCE AND PLASMA AMINO ACID CONCENTRATIONS IN SHEEP

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Summary

This experiment was carried out to investigate the effect of rumen-protected lysine (RPLys) on growth rate, feed efficiency and plasma amino acid concentrations in sheep. RPLys was supplemented at the level of 0% (T_1), 0.2% (T_2) and 0.4% (T_3) of total DMI with 24 sheep in a 56 day feeding trial.

The results are summarized as follows:

1. Live weight gain of sheep in groups T_1, T_2 and T_3 was 219, 216 and 244 g/d, and was significantly ($p < 0.05$) higher for T_3 through the entire experiment.
2. Feed intake was not affected by RPLys supplementation.
3. The group fed T_3 had a significantly ($p < 0.05$) better feed efficiency than the groups fed T_1 and T_3. The response of T_3 was higher in growing period Ⅱ of feeding low protein basal diet than in period Ⅰ.
4. Plasma lysine concentrations tended to be higher with supplementing RPLys, but there were no differences between T_2 and T_3.
5. Supplementing RPLys in the diets increased plasma concentrations of arginine, asparagine, threonine, serine, valine and leucine compared with sheep receiving no RPLys. In contrast, plasma histidine was lower in sheep fed the supplementing RPLys than fed the diet T_1 with significant ($p < 0.05$) difference.

(**Key Words** : Rumen Protected Lysine, Live Weight, Feed Efficiency, Plasma Amino Acids, Sheep)

Introduction

Increases in N retention by post-ruminal infusion of limiting amino acids demonstrate the potential for improving ruminant performance (Schelling et al., 1973; Chalupa, 1975; Burris et al., 1976; Hill et al., 1980). Lysine (Lys) and methionine (Met) have been identified as limiting amino acids for growth of ruminants (Nimrick et al., 1970a,b; Fenderson and Bergen, 1975; Richardson et al., 1978).

For limiting amino acid to be effective in improving performance of ruminants, it must be provided in a form that will be resistant to microbial degradation and yet be available for absorption in the lower gut. Therefore, attempts have been made to supply these amino acids by means of administrating them directly into abomasum so as to be efficiently utilized by a host ruminant. Recently various amino acid preparations that could escape from degradation to ammonia in the rumen, referred to as rumen-protected amino acids (RPAA), have become available, although their biological efficacies are not well clarified.

Variable responses in growth performance and plasma amino acid concentrations have been reported with oral supplementation of various RPAA (Deetz et al., 1985; Oke et al., 1986; Wright and Loerch, 1988). Several other experiments (Kirby et al., 1983; Gill and England, 1984; Veira et al., 1991) have shown that RPAA supplementations improve growth of ruminants with no any effect on feed intake.

The objectives of this research were to determine; 1) the effects of RPLys on growing sheep performance using diets based on corn and 2) the effects of supplemental RPLys on plasma amino acid concentrations in sheep fed corn grain-based diets.

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Materials and Methods

Animals and experimental design

Twenty-four cross-bred (Corriedale × Polworth) sheep, with a mean initial body weight of 20 kg, were used in a randomized complete block design to determine the effects of supplemental RPLys on growth performance and plasma amino acid concentrations.

Sheep were randomly assigned to three treatments with 8 sheep per treatment: 1) T_1; 0% RPLys, 2) T_2; 0.2% RPLys and 3) T_3; 0.4% RPLys were alloted to 4 replicates, with 2 sheep in each pen. The experiment started with a 14-day adaptation period and a 56-day experimental period.

Diets and feeding

The daily allowance of experimental diets containing 30:70 roughage and concentrate was offered in equal portion twice daily (09:00 and 17:00 h) at about 3.5% of body weight. A mineralized salt block and water were provided *ad libitum*. Roughage used in this experiment was orchard grass hay, the chemical compositions of hay were as such: 89.0% DM, 12.1% CP, 31.0% CF, 64.0% NDF and 40.0% ADF.

Composition of the concentrates is presented in table 1 for growing Ⅰ and Ⅱ phases.

TABLE 1. FORMULA AND CHEMICAL COMPOSITIONS OF THE CONCENTRATES FED TO SHEEP (FROM 20 TO 30 kg)

Items	Treatment[1)]					
	Growing Ⅰ (20-25 kg)			Growing Ⅱ (25-30 kg)		
	T_1	T_2	T_3	T_1	T_2	T_3
Ingredients (%)						
Corn grain	69.45	69.63	70.00	85.25	85.43	85.60
Soybean meal (44%)	20.80	20.30	19.60	8.00	7.50	7.00
Molasses	8.00	8.00	8.00	5.00	5.00	5.00
Limestone	1.00	1.00	1.00	1.00	1.00	1.00
Salt	0.50	0.50	0.50	0.50	0.50	0.50
Vitamin mixture[2)]	0.05	0.05	0.05	0.05	0.05	0.05
Mineral mixture[3)]	0.20	0.20	0.20	0.20	0.20	0.20
RPLys	0.00	0.32	0.65	0.00	0.32	0.65
Chemical composition (%)						
CP	15.10	15.20	15.10	10.58	10.62	10.68
EE	2.90	2.90	2.88	2.48	2.45	2.42
Ca	0.52	0.52	0.52	0.46	0.46	0.46
P	0.30	0.30	0.30	0.26	0.26	0.26
Lysine	0.74	0.93	1.11	0.43	0.62	0.81

1) T_1; RPLys 0%, T_2; RPLys 0.2% and T_3; RPLys 0.4%.

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

3) Mineral mixture contains the following amounts of micro nutrients per kg: Cu, 5 g; Fe, 20 g; Mn, 425 g; I, 0.25 g; Co, 0.15 g and Se, 0.1 g.

Concentrates were formulated to contain about 15% and 11% CP (DM basis) for sheep of growing Ⅰ and Ⅱ phase, respectively, by changing the ratio of corn grain to soybean meal. In consideration of animal growth stages and the length of the experiments, two different concentrates were used; concentrate formulated for growing sheep was used for the first 4 weeks, and that for fattening for the remaining 4 weeks.

Rumen-protected lysine (RPLys) developed by our research team was added at 0%, 0.32% and 0.65% to T_1, T_2 and T_3 concentrate diets, resulting in 0% RPLys, 0.2% RPLys and 0.4% RPLys in the total ration. The RPLys used in these trials consisted of a lysine core coated with a pH-sensitive co-polymers to maintain structural integrity at typically encountered ruminal pH. Low abomasal pH triggers breakdown of the coating, allowing release of lysine for absorption. Used coating materials were such as zein, shellac, ethylcellulose, stearic acid and acrylonitrile.

Measurements and sampling procedures

The rations offered and refused (separating concentrate from roughage) were measured daily throughout the whole 56 d experimental period. Body weight of sheep was also determined on three consecutive days at the beginning and the completion of experiment, and no two consecutive days weekly throughout the experiment.

Jugular blood samples were collected from randomly selected animals (5 sheep per treatment) into vacutainers containing EDTA (1.0 mg/ml blood) at 3 h post feeding in the last day of experiment. Plasma was separated by centrifugation at 3,000 × g for 15 min at 4℃. The supernatant fractions was decanted, and stored at −20℃ for amino acid analysis.

Chemical analysis

Feeds and refusals were dried at 100℃, then ground through an 1 mm screen, and analyzed for DM, crude fiber and Kjeldahl N according to AOAC (1984) procedures. The content of Ca was analyzed by atomic absorption spectrophotometer after nitric/perchloric acid digestion (AOAC, 1984). Phosphorus was determined by the meta-vanadate method (AOAC, 1984). Plasma amino acids were determined according to Spackman et al. (1958) using an automatic AA analyzer (LKB, Model 4150-alpha) after deproteinization of samples with sulfosalicylic acid (25 mg/ml of plasma).

Statistical analysis

Data were analyzed as a completely randomized block design, and mean were compared by Duncan's multiple range test using GLM (general linear model) procedures of SAS (1985).

TABLE 2. EFFECTS OF SUPPLEMENTING RUMEN-PROTECTED LYSINE ON BODY WEIGHT, FEED INTAKE AND FEED EFFICIENCY OF SHEEP

Items	Treatments[1]		
	T_1	T_2	T_3
Growing Period Ⅰ (1-4 weeks)			
Initial BW (kg)	19.53	20.64	20.01
Final BW (kg)	25.00	25.84	25.74
ADG (g)	195^{b}	186^{c}	205^{a}
Feed intake (g/d)			
Total	788	794	794
Concentrate	615	615	621
Roughage	173	178	173
Feed efficiency	4.0^{b}	4.3^{a}	3.9^{c}
Growing Period Ⅱ (5-8 weeks)			
Initial BW (kg)	25.00	25.84	25.74
Final BW (kg)	31.78^{b}	32.73^{ab}	33.67^{a}
ADG (g)	242^{b}	246^{b}	283^{a}
Feed intake (g/d)			
Total	979	986	986
Concentrate	789	801	799
Roughage	190	185	187
Feed efficiency	4.0^{a}	4.0^{a}	3.5^{b}
Whole Period (1-8 weeks)			
ADG (g)	219^{b}	216^{b}	244^{a}
Feed intake (g/d)			
Total	883	890	890
Concentrate	702	708	710
Roughage	181	182	180
Feed efficiency	4.0^{a}	4.1^{a}	3.7^{b}

[1] T_1; RPLys 0%, T_2; RPLys 0.2% and T_3; RPLys 0.4%.

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

Results and Discussion

Feed intake, growth performance and feed efficiency

Effects of dietary RPLys levels on feed intake, growth performance and feed efficiency of growing Ⅰ and Ⅱ period are presented in table 2.

Average daily gain (ADG) was the highest ($p < 0.05$) in T_3 treatment, and significantly lower in T_2 treatment than T_1 and T_3 during growing period Ⅰ (1-4 wks). No differences in feed intake existed between treatments, therefore the feed efficiency significantly ($p < 0.05$) improved when diet T_3 was fed to sheep.

In the growing period Ⅱ (5-8 wks), ADG was also increased by 16.9% ($p < 0.05$) in T_3 treatment compared to T_1 but, no differences ($p > 0.05$) were observed between T_1 and T_2. As was in period Ⅰ total feed intakes were not different between treatments. Therefore, this resulted in a significant ($p < 0.05$) improvement of the feed efficiency in T_3.

During the entire period, the daily feed intakes were not also affected ($p > 0.05$) by RPLys supplementation. Live weight gains were greater ($p < 0.05$) for sheep fed the diet T_3 than those fed the diet T_1 or T_2 (244 vs 219 or 216 g/d, respectively). Feed efficiency followed the opposite trend to ADG, with sheep fed the diet T_3 being more efficient ($p < 0.05$) in converting feed to gain than those fed T_1 or T_2 (3.7 vs 4.0 or 4.1, respectively).

Although the ADG of growing sheep was the highest in sheep fed diet T_3, especially, in period of feeding 11% crude protein (CP) diet the ADG was affected to a greater extent by RPLys treatment than in 15% CP diet. Also, the effect of feed efficiency was obviously observed high in growing period Ⅱ with low protein diet. This result was simillar to the report of Deetz et al. (1985) who found a significant response of supplementing RPAA in steers when the corn-based diet contained 11.2% CP, but not when it contained 14.2% CP. However, several other researchers (Papas et al., 1984b; Schwab and Bozak, 1988a,b) reported that supplementation of RPAA in the diet of dairy cows did not influence feed intake.

Plasma amino acid concentrations

Amino acid concentrations in plasma collected from sheep are shown in table 3.

The total AA concentration in plasma collected from sheep fed T_2, but not T_3, was significantly ($p < 0.05$) higher than that of T_1, whereas the ratio of essential AA (EAA) to nonessential AA (NEAA) in plasma was not affected. Seymour et al. (1990) reported that Met-Lys infusion into duodenum increased the ratio of EAA to NEAA in plasma as well as plasma Lys and taurine, and suggested that the demand for NEAA appeared to have been increased by Met-Lys infusion.

Supplementing RPLys resulted in increased plasma concentrations of arginine, asparagine, threonine, serine, valine, leucine and lysine compared with the control. In contrast, plasma concentrations of histidine were lower in sheep fed the RPLys diets than those fed the control diet (T_1) with significant ($p < 0.05$) difference. Concentrations of other plasma amino acids were not significantly affected by treatments.

Although plasma Lys concentration tended to be increased with supplementing RPLys, there was no significant difference between T_2 and T_3 ($p > 0.05$). In contrast, Oke et al. (1986) found that feeding RPLys or RPMet and abomasal infusion of Lys resulted in similar increases in plasma concentrations of these amino acids, whereas feeding unprotected Lys and Met did not increase plasma levels of these amino acids. Other researchers have demonstrated that blood concentrations of limiting AA remain relatively constant until tissue requirements are met (Nimrick et al., 1970a,b; Bergen, 1979).

Oltjen (1969) reported that the plasma concentration of glycine was decreased in steers fed or abomasally infused with a mixture of amino acids, which resulted in greater N retention. In our experiment, however, the same trend was not observed, and glycine concentration was higher in T_2 compared to T_1 and T_3.

TABLE 3. AMINO ACID CONCENTRATIONS (mg/l) OF PLASMA COLLECTED FROM THE JUGULAR VEIN OF SHEEP FED EXPERIMENTAL DIETS ON DAY 70 (MEAN ± SE)

Amino acids	Treatments[1]		
	T_1	T_2	T_3
Essential amino acid (EAA)			
Arginine	3.6 ± 0.01^a	4.0 ± 0.01^c	3.8 ± 0.01^b
Histidine	2.7 ± 0.01^c	2.5 ± 0.01^b	2.3 ± 0.00^a
Iso-leucine	2.0 ± 0.01	2.1 ± 0.01	2.0 ± 0.01
Leucine	6.8 ± 0.02^a	7.4 ± 0.03^b	7.3 ± 0.01^b
Lysine	6.8 ± 0.01^a	7.2 ± 0.02^b	7.0 ± 0.01^{ab}
Phenylalanine	3.7 ± 0.01	3.8 ± 0.01	3.7 ± 0.01
Threonine	4.7 ± 0.02^a	5.4 ± 0.03^b	5.1 ± 0.03^b
Valine	4.6 ± 0.01^a	5.3 ± 0.02^b	5.2 ± 0.02^b
Total	34.9	37.8	36.5
Nonessential amino acid (NEAA)			
Alanine	3.7 ± 0.02	3.9 ± 0.01	3.7 ± 0.01
Asparagine	6.8 ± 0.02^a	7.5 ± 0.02^b	7.3 ± 0.01^b
Glutamine	11.4 ± 0.04	12.7 ± 0.05	12.1 ± 0.02
Glycine	2.4 ± 0.01^a	2.7 ± 0.01^b	2.5 ± 0.02^a
Proline	3.8 ± 0.02	3.9 ± 0.01	3.6 ± 0.03
Serine	4.5 ± 0.02^a	5.6 ± 0.03^b	5.3 ± 0.05^b
Tyrosine	3.6 ± 0.02	3.8 ± 0.01	3.6 ± 0.01
Total	36.3	40.1	38.2
Total AA	71.2 ± 0.25^a	77.9 ± 0.26^b	74.7 ± 0.17^{ab}
EAA/NEAA	0.96	0.94	0.95

[1] T_1; RPLys 0%, T_2; RPLys 0.2% and T_3; RPLys 0.4%.
[a,b,c] Mean values with different superscripts within the same row are significantly different ($p < 0.05$).

In summary, growth rate was not affected by T_2 and consistently improved when sheep were fed T_3 compared to T_2 and T_1. Therefore, the level of 0.2% RPLys in the total diet does not appear to be enough to enhance performance of sheep based on our experiment. Also, the increase in daily gain and plasma Lys data support the assumption that the RPLys was survived reaching the small intestine for absorption by the sheep and influencing the efficiency with which absorbed N was utilized.

Of course, the true reason for lower plasma Lys on sheep on T_3 comparing to T_2 is not clear, and requires more investigation. Studies on the estimation of efficacy of the RPLys combined with other limiting amino-acids are warranted.

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