EFFECTS OF BLOOD-MIXED AND HEAT TREATMENT OF PROTEIN FEEDS ON NITROGEN DIGESTION IN THE RUMEN AND HINDGUT OF SHEEP[1]

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Summary

This experiment was conducted to study the effects of blood-mixed and heat-treated protein feeds on protein degradation in the rumen, flow of protein to the abomasum and availability of undegraded protein in the intestine of sheep in a 4x4 Latin square design. Soybean oil meal, rapeseed meal, and whole soybean were mixed with fresh swine blood and dried at 140 °C for 2 h. Proportionate disappearance of apparently digested OM in the postrumen for the blood and heat treated protein group was ranged from 43.2 to 50.0% as compared with 28.0% for the unheated soybean oil meal diet. The treated protein supplements were resulted in greater total N and NAN flow passing at the abomasum than untreated soybean oil meal diet was fed. The quantities of undegraded feed N passing at the abomasum for the treated protein diets was approximately twice as high as that of the untreated soybean oil meal diet and the estimated amount of undegraded N of the protein supplement itself was 79.1 to 84.2% as compared with 15% of soybean oil meal.

(Key Words: Blood and Heat Treatment, Protein Degradation, Microbial N Synthesis, Sheep)

Introduction

Protein required for maintenance and production in ruminants are derived mainly from dietary amino acids that escape ruminal degradation and microbial synthesis. In many conditions the amino acid supply from microbial protein alone is considered sufficient to meet requirements (Chalupa, 1975). But highly productive animals, such as young fast growing animals and high yielding dairy cows, may have protein requirements that exceed the amount provided by bacterial protein, and could benefit from supplementation of a rumen protected protein.

Thus, increasing undegraded protein of feed origin while maintaining optimal microbial protein synthesis in the rumen becomes an alternative way to improve the protein nutrition in high producing animals. Many approaches have been developed and used to enhance the resistant portion of dietary protein to proteolysis and deamination by rumen microbes (Rock et al., 1982; Stern et al., 1985; Pena et al., 1986).

Whole blood from slaughter house is a reusable by-product due to its high protein contents. However, limited research has shown the possibility of mixing blood with other protein feedstuffs to decrease protein breakdown in the rumen (Ørskov et al., 1980). The objectives of this study was to evaluate the effects of blood-mixed and heat-treated protein feeds on protein degradation in the rumen, flow of protein to the abomasum and availability of undegraded nitrogen in the intestine of sheep.

Materials and Methods

Animals and diets

Four mature Corriedale rams averaging 45 kg in weight and fitted with permanent rumen cannulae and T-type cannulae at the abomasum were used as the experimental animals in a 4x4 Latin square arrangement with four change-over feeding. Sheeps were randomly allocated to four treat-

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ments; untreated soybean oil meal (SBM), and blood-mixed and heat treated soybean oil meal (B-SBM), rapeseed meal (B-RSM) and whole soybean (B-SB). And whole soybean was milled in a hammer mill with 3 mm screen before blood-mixing. The blended whole swine blood was added to each protein sources at levels of 1.25 kg/kg DM of protein feedstuffs.

Blood-treated samples were placed on a 10-mm diameter wire screen and spread evenly in a layer 1.5 cm deep. This mixture was dried in a forced-draft oven at 140°C for 2 hours. After blood supplementation, the protein content of treated protein sources increased to average 7.5%. The daily ration and chemical composition of experimental diets are shown in table 1. And each protein source supplied approximately 43% of the total dietary protein. Sheep were fed equal portions of diet at 2 h intervals by automatic feeders. Water was available at all times.

Determination of digesta flow

A solid and liquid phase marker system of digesta defined by physical separation process was used to measure abomasal digesta flow and ruminal passage rates of liquid phase digesta. Chromic oxide as the solid marker and ^{14}C-Cr EDTA as the liquid marker were used. The used chromic oxide was the hammer-milled marker after 1 kg of chromic oxide was pasted with 4 kg of wheat flour and then dried at 100°C oven for 24 h. This chromic oxide of the 1% amount of daily feed intake was mixed each grain meal and fed at 2 h intervals.

Each experiment period was consisted of 28 days. Sheeps were allowed to a 10 days adaptation period, followed by a 6 days faeces and urine collection phase. From day 17 to 22, ^{14}C-Cr EDTA (10.5 μCi/100ml/day) was infused into the rumen. Samples of rumen liquid and abomasal digesta were taken at 6 h intervals for 48 h from day 21. On day 23, samples of rumen liquid for determination of dilution rate were acquired at 2 h intervals over the next 24 h after infusion stop of liquid marker.

In addition to, samples of abomasal digesta without radioisotope were taken at 6 h intervals from 24 to 25 day. Dose of solid marker started at day 5. On the remaining days, samples of rumen digesta were sampled at 6 h intervals for the deter-

TABLE 1. DAILY FEED AMOUNTS AND CHEMICAL COMPOSITION OF EXPERIMENTAL DIETS GIVEN TO RAMS (AS FED G)

Feed ingredients	Protein sources			
	Soybean oil meal	B-soybean oil meal	B-rapeseed meal	B-whole soybean
Grass, hay, sun-cured (g)	159.89	175.88	171.24	170.97
Cracked corn	519.64	519.64	519.64	519.64
Soybean oil meal	103.93	–	–	–
B-soybean oil meal	–	87.94	–	–
B-rapeseed meal	–	–	97.85	–
B-whole soybean	–	–	–	91.45
Vit. + Mineral mix[1]	15.99	15.99	16.31	15.90
ME of diet DM[2] (kcal/day)	2550	2530	2520	2580
Crude protein (%)	15.50	15.57	15.36	15.43
Organic matter (%)	89.90	89.96	89.86	90.03
TAA-N (%)	2.124	2.336	2.177	2.179
EAA-N (%)	1.229	1.333	1.283	1.309

B-: blood-mixed and heat treatment.
TAA-N: total amino acid-nitrogen, EAA-N: essential amino acid-nitrogen
[1]Contains Ca 150 mg, P 100 mg, Na 30 mg, Fe 5 mg, Zn 2.5 mg, Mn 2 mg, Mg 2 mg, K 300 mg, Co 6 mg, I 6 mg, NaCl 380 mg, Vit-A 2,500,000 IU, Vit-D_3 50,000 IU and molasses 50 mg/Kg.
[2]Calculated value

mination of bacterial marker and chemical analysis. 1 ml of the saturated mercuric chloride solution was added to 100 g of rumen sample and then frozen. The rumen digesta was thawed and strained through four layers of cheesecloth, and separation of rumen bacteria was conducted according to Siddons et al. (1985). The NH_3 concentration and VFA analysis of rumen liquid was checked at each sampling time. Each abomasal samples were composited and homogenized in a blender after thawing, centrifuged at 100 x g for 15 min. Each liquid and solid phase was lyophilized and ground to pass a 1 mm screen and kept for organic matter, nitrogen, marker and diaminopimelic acid determination at the P_2O_5 dessicator.

For the determination of rumen liquid dilution rate and abomasal flow rate, each sample was centrifuged at 100 x g for 15 min and counted by liquid scintillation counter. And the remained solid fraction of abomasal digesta was extracted with distilled water at five times, and then counted. This counting recovery was 98%.

Analytical procedures

All sample was ground through a 1-mm screen in a Wiley mill before the following analysis. Organic mater and N were determined by the method of A.O.A.C. (1975), and ammonia nitrogen of rumen and abomasal digesta were analyzed by the MgO distillation (A.O.A.C., 1975) with the Kjeltec system (Tecator Co.). Rumen liquid was analyzed for VFA concentration by gas chromatography on a Packard 439 with 10% SP-1200/1% phosphoric acid on 80- to 100-mesh chromosorb W AW and the internal standard was crotonic acid. The concentration of diaminopimelic acid (DAPA) as a bacterial marker and amino acids were determined according to the streamlined methods with performic acid oxidation (Mason et al., 1980) on a Biotronic 5001 amino acid analyzer. Chromium was estimated as described in the method of Fenton and Fenton (1979) on a Perkin Elmer 2380 atomic absorption spectrophotometer. The radioactivity of ^{14}C was determined according to the method of Harrison (1974) in a Packard 3255-Tri-Carb Liquid Scintillation Counter using an external standard channel ratio for quenching correction.

Calculations and statistical methods

The method for calculating digesta flow was the mathematical equation according to the method of Armentano and Ruesell (1985). The ratio DAPA: N in rumen bacteria and digesta was used to calculate the bacterial N in abomasal digesta. Apprarent ruminal degradability was calculated as [abomasal N (g/day)-Bacterial N (g/day)] /N intake (g/day). Apparrent disappearance in the intestine was calculated as [abomasal N (g/day)-fecal N (g/day)]/abomasal N (g/day). Data were analyzed by analysis of variance using Latin square design and significant differences between treatment were according to the multiple range test of Duncan (Steel and Torrie, 1980).

Results and Discussion

The experimental result in table 2 was shown that organic matter (OM) digestion was affected by the compound treatment with addition of blood and heat. Daily OM digestion in the rumen for untreated soybean oil meal was significantly higher ($p < .05$) that in the treated protein group, for which there was increased OM flow to the abomasum. OM flow to the abomasum for the treated protein group was ranged from 354 g to 396 g as compared with 263 g for the soybean oil meal diet. Higher abomasal OM flow was reported in heat treated soybean oil meal (Hudson et al., 1970), extruded whole soybean (Stern et al., 1985), and cottonseed meal (Pena et al., 1986).

The approximately 65% more increased OM flow than that of untreated soybean oil meal diet to the abomasum was greatly higher value compared with the results (15-30%) of Hudson et al. (1970) and Pena et al. (1986) with only roasting or heat treatments. Plegge et al. (1985) and Stern et al. (1983) did not increase the OM flow in the duodenum, but there was a significantly increased non-ammonia nitrogen flow. Even though this experiment does not differentiate between the effects of blood and heat treatment, this trend may be attributed to the compound effects of blood and heat. OM digestion in the soybean oil meal, rapeseed meal and whole soybean was estimated as 81%, 78%, 78% and 79%, respectively. This result indicates that the blood and heat treatment, as used in the present experiment, did not cause adverse effects on the digestion of OM in the intestine. Proportionate disappearance of apparently digested OM in the rumen for the treated protein group was ranged from 50% to

TABLE 2. EFFECT OF BLOOD-MIXED AND HEAT-TREATED PROTEIN SOURCES ON FLOW RATE AND DIGESTION OF ORGANIC MATTER IN THE RUMEN AND POSTRUMEN OF RAMS

Organic matter	Protein source				SE
	Soybean oil meal	B-soybean oil meal	B-rapeseed meal	B-whole soybean	
Intake (g/day)	626.39	633.75	646.94	634.32	
Flow to:					
abomasum	263.08[a]	353.97[b]	395.91[b]	375.31[b]	32.40
faeces	121.97	141.60	144.65	130.63	5.93
Digestion in:					
rumen	363.31[b]	279.78[ab]	251.03[a]	259.01[a]	26.37
postrumen	141.12[a]	212.37[b]	251.26[b]	244.74[b]	28.57
Microbial OM flow at abomasum	117.42	135.76	141.07	131.10	11.63
Truly digested in the rumen	480.72	415.54	392.10	390.11	30.10
Digestion of intake (%)					
rumen (apparently)	58.01[b]	44.15[ab]	38.79[a]	40.82[a]	4.32
rumen (truly)	76.80[b]	65.57[a]	60.60[a]	61.49[a]	4.58
whole GI tract	80.61	77.78	77.69	79.37	0.62
Proportionate disappearance of apparently digested OM					
rumen	71.97[b]	56.85[a]	49.98[a]	51.41[a]	5.30
postrumen	28.03[a]	43.15[b]	50.02[b]	48.59[b]	5.33

B- : blood-mixed and heat treatment.
Means that different superscripts in the same line are significantly different (a, b, $p < .05$).

57% as compared with 72% for the untreated soybean oil meal diet.

It was observed that ruminal pH, ammonia N, liquid dilution rate in table 3 were not influenced by supplying of the treated protein. However, total VFA concentration in the rumen liquid for the untreated soybean oil meal was significantly higher ($p < .05$) than that for the treated protein group. It was suggested that the total VFA concentration was decreased by blood and heat treatment due to the tendency toward a lower OM disappearance in the rumen for the treated protein group (table 2). Acetic acid and propionic acid were dominant VFA in all treatment but there was no significant difference between treatment. However, iso-butyric acid and butyric acid in soybean oil meal and treated soybean oil meal diet were significantly increased than those in treated rapeseed meal and whole soybean diet.

Glipm et al. (1967), Mielk and Schingoethe (1981), and Pena et al. (1986) reported that iso-acids and valeric acid were significantly decreased when the undegradable proteins were fed to ruminants. The decline in concentration of iso-acids suggests that blood treatment reduced the degradability of their precursors, the branched chain amino acids (Hungate, 1966). Indeed, the leucine and valine contents of blood meal was very high (11.5% and 8.5%) as compared with 2.5-3.5% and 1.8-2.3% for soybean oil meal and rapeseed meal, respectively (NRC, 1975). And the leucine, isoleucine, and valine contents of the treated protein sources were 5.9-4.6%, 2.6-1.8% and 4.0-3.2% after blood treatment, respectively. The flow rate of dietary and endogenous branched chain amino acids to the abomasum for the treated protein

TABLE 3. EFFECT OF BLOOD-MIXED AND HEAT-TREATED PROTEIN SOURCES ON RUMEN FERMENTATION PARAMETERS AND DILUTION RATES IN THE RUMEN OF RAMS

Item	Protein source				SE
	Soybean oil meal	B-soybean oil meal	B-rapeseed meal	B-whole soybean	
pH value	5.93	6.07	6.04	6.01	0.06
Total VFA (mM/L)	92.73^{b}	78.82^{a}	72.91^{a}	71.18^{a}	3.42
Individual VFA (molar %)					
Acetic acid	53.28	60.07	61.10	61.07	2.84
Propionic acid	21.31	16.52	22.90	23.13	3.06
Iso-Butyric acid	3.16^{C}	2.30^{BC}	0.31^{A}	0.85^{AB}	0.32
Butyric acid	14.60^{b}	14.64^{b}	9.68^{a}	8.79^{a}	1.07
Iso-Valeric acid	5.14	5.01	4.98	5.09	0.46
Valeric acid	2.52^{B}	1.47^{A}	1.03^{A}	1.08^{A}	0.17
NH_3-N (mg/100ml)	15.86	16.22	13.57	11.56	2.52
Liquid dilution rate (h^{-1})	0.075	0.064	0.070	0.075	0.009
Rumen volume (L)	5.67	5.00	5.88	5.10	0.542
Rumen liquid outflow (ml/hr)	374.47	328.90	370.36	378.18	25.44

B- : blood-mixed and heat treatment
Means that different superscripts in the same line are significantly different (a,b, $p < .05$; A,B,C, $p < .01$)

diets was average 4.6 g/day compared with 2.5 g/day of untreated soybean oil meal diet and the rumen degradability of this branched chain amino acids was 32.6% as compared with 61.7% of soybean oil meal diet.

Nitrogen (N) intake, N flow, non-ammonia N (NAN) flow, undegraded N and supplemental N undegraded from rumen, and bacterial N synthesis were shown in table 4. Abomasal total N flow, N retention, and apparent N digestibility were not significantly different between treatments. However, abomasal NAN flow was shown the lowest value ($p < .01$) for sheep fed the untreated soybean oil meal diet, suggesting greater degradation of soybean oil meal than treated protein sources. Bacterial NAN flow was similar between treatment but non-bacterial NAN flow to the abomasum was significantly different ($p < .05$) between treatments. Non-bacterial NAN for the treated protein group was approximately twice as high as that for the soybean oil meal diet. These data are in similar tendency with observations of higher N flow to the duodenum of lambs fed heated soybean meal (149°C for 4 hr, Hudson et al., 1970), dairy cattle fed extruded whole soybean (132°C and 149°C, Stern et al., 1985), steers fed roasted soybean meal (145°C, Plegge et al., 1985) and dairy cow fed extruded or roasted whole cottonseed (Pena et al., 1986).

Apparent absorption in the intestine expressed as a percentage of NAN flow to the abomasum was ranged from 79% to 83% but this for sheep fed soybean oil meal diet was significantly lower ($p < .05$) than that for sheep fed the treated protein diet. Faecal and urinary N excretion and apparent N digestibility in the whole gastrointestinal tract were similar in all treatment, indicating that there was nothing to worry about over-protection under this processing conditions. Over-protection of protein source has been responsible for reduced dry matter digestibility. N digestibility and N retention (Stock et al., 1981; Pena et al., 1986).

The amount of undegraded dietary N and the estimated amount of undegraded N of the supplement itself from rumen for untreated soybean oil meal diet were significantly lower ($p < .01$) than those for the treated protein diets. The undegraded portion of dietary N from rumen for un-

TABLE 4. EFFECT OF BLOOD-MIXED AND HEAT-TREATED PROTEIN SOURCES ON FLOW RATE AND DIGESTION OF NITROGEN IN THE RUMEN AND POSTRUMEN OF RAMS

Item	Protein source				SE
	Soybean oil meal	B-soybean oil meal	B-rapeseed meal	B-soybean	
Intake (g/day)	17.46	17.63	17.78	17.54	
Intake as protein source	7.49	7.46	7.46	7.37	
Flow to:					
abomasum	16.66	21.05	21.76	21.34	2.16
faeces	3.50	3.85	3.92	3.60	0.22
urine	12.74	11.30	11.15	10.98	0.34
Retention	1.22	2.48	2.71	2.96	0.60
Apparent digestibility (%)	79.95	78.16	78.07	79.04	1.04
Abomasum flow/intake	0.9542^{a}	1.1940^{b}	1.2238^{b}	1.2283^{b}	0.11
NAN flow at abomasum (g/day)	15.98^{A}	19.64^{B}	20.24^{B}	19.35^{B}	2.13
Bacterial NAN	10.32	9.20	9.34	8.66	0.67
Non-bacterial NAN	5.66^{a}	10.44^{b}	10.90^{b}	10.69^{b}	1.91
Digestion in postrumen	13.16	17.20	17.84	17.74	2.13
% of intake	75.61^{a}	97.77^{b}	100.34^{b}	101.14^{b}	11.19
% of abomasal flow	78.99^{a}	81.39^{b}	81.85^{b}	83.34^{b}	2.68
Undegraded N from rumen[1] (%)	32.42^{A}	59.22^{B}	61.30^{B}	60.95^{B}	4.45
Undegraded N of protein source from rumen[2] (%)	14.91^{A}	79.09^{B}	84.21^{B}	83.41^{B}	3.85
Bacterial N synthesis:					
g N/kg OMADR	28.55	32.41	37.21	33.43	1.91
g N/kg OMTDR	21.38	23.41	24.14	24.06	1.54

B-: blood-mixed and heat treatment.
Means that different superscripts in the same line are significantly different (^{a,b} $p < .05$; ^{A,B} $p < .01$)
[1]Non-bacterial NAN ÷ N intake x 100
[2]Calculated on the basis of the result of Zinn and Owens (1983) that the undegraded N of corn protein in the rumen was 60%[nonbacterial NAN-(Nintake as corn x 60%)] ÷ N intake as protein sources x 100

heated soybean oil meal, and treated soybean oil meal, rapeseed meal and whole soybean diets was estimated as 32.4, 59.2, 61.3 and 61.0%, respectively. And the undegraded portion of N from rumen for soybean oil meal, and treated soybean oil meal, rapeseed meal and whole soybean itself were estimated as 14.9%, 79.1%, 84.2% and 83.4%, respectively. These data for the undegraded N are similar with some reports (Zinn et al., 1981; Plegge et al., 1985; Peterson et al., 1985; Siddons et al., 1985), when was conducted with different protein source or treatment procedures. Mercer et al. (1980) reported that the undegraded N of fish meal was 69%. Meanwhile, Waller (1978) and Loerch et al. (1983) reported that the escaping N from blood meal was ranged from 71 to 81.7%.

However, since this estimated amount of undegraded N of the supplemental protein itself was calculated on the basis of the result of Zinn and Owens (1983) that the escaped portion of corn

protein from rumen was 60% and the degraded portion of grass was not corrected, this result would be overestimated. The contributed portion of dietary total N intake as grass N was less than 10% (average 1.7g N/day). If we consider that the undegraded portion of the added blood meal is 75% (Waller, 1978; Loerch et al., 1983), the calculated values of the undegraded N portion for the net protein sources from the rumen is about 85% as compared with 15% of untreated soybean oil meal. This tendency will be probably attributed to the coating effect of blood for protein feed particles.

Efficiency of bacterial protein synthesis, expressed as g bacterial N synthesized per kg OM apparently digested in the rumen of sheep fed treated diets was higher ($p > .05$) than that of untreated diet. Bacterial N synthesis per kg OM truly digested in the rumen for soybean oil meal, and treated soybean oil meal, rapeseed meal and whole soybean was estimated as 21.4, 23.4, 24.1 and 24.1 g, respectively. Similar patterns were observed in the results of Ben-Ghedalia et al. (1978), Stern et al. (1985), Siddons et al. (1985) and McMeniman and Armstrong (1979).

Considering that more bacterial OM flow to the abomasum and efficiency of production of bacterial N was also increased but OM fermentation in the rumen decreased, when the treated protein diets was fed, the cause of the improved efficiency of bacterial growth is not known. However, Ben-Ghedalia et al. (1978) suggested that the release of the particular peptide of corn gluten may stimulated microbial growth when replacing urea-N with corn gluten meal. When the less rapidly degraded protein source was added to the diet, ammonia level may sustained more consistent with the high efficiency of bacterial growth. Possibly none even pattern of ruminal NH_3 concentration from undegraded N source (table 3) may be significant factor for increased efficiency of bacterial N synthesis. And the blood is generally considered as good medium source for microbial growth, it is postulated that preformed factor for stimulation of microbial growth will be presented at the blood meal and released or as rate of passage of OM from the rumen increased, efficiency of bacterial N synthesis increased (Zinn et al., 1981).

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