THE EFFECT OF RICE STRAW-POULTRY MANURE SILAGE AND BARLEY ON THE NITROGEN DIGESTION AND MICROBIAL PROTEIN SYNTHESIS IN THE RUMEN OF SHEEP

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Summary

Three sheep fitted with rumen cannulae and abomasal cannulae were given daily 750 g (DM) of three diets consisting of straw-manure silage and barley mixture in the ratios of 75:25, 50:50 and 25:75. As the proportion of barley in the diet increased, there was an increase in the amount of OM apparently digested in the rumen and whole tract ($p < .01$). But ADF digestion was decreased. For the 25:75 diet the NH_3-N content in the rumen showed the highest value, but the total VFA was the lowest. The rumen volume and dilution rate increased with increasing ratio of silage in diets. There were no significant differences between diets in abomasal NAN flow, and the bacterial-N for the 25:75 diet was 7.3 g N as compared with 9.2-9.6 g N for the other diets ($p < .01$). Rates of bacterial nitrogen synthesis in the rumen were 30.5, 24.1 and 14.9 g N per Kg OM apparently digested in the rumen for the 75:25, 50:50 and 25:75 diets, respectively.

(Key Words: Straw-Manure Silage, Barley, Bacterial Protein Synthesis)

Introduction

Fermentation of dietary carbohydrates in the rumen provides carbon-chains and energy for microbial protein synthesis, which serves as the main source of amino acids in the digesta passing to the small intestine. There is considerable evidences that rumen microbial capacity of protein synthesis depends largely upon the amount of substrate fermented (Stern and Hoover, 1979).

The nature of the energy source may be the limiting factor in determining the rate of microbial synthesis in the rumen (Hogan and Weston, 1970). Also, changing the ratio between type of roughage and concentrates in the diet may change rumen fermentation, including degradation and synthesis of protein, rate of passage of digesta and other rumen parameters, in various ways. Rate of carbohydrate degradation is higher with concentrate-rich diets than with roughage-rich diets, which probably influences microbial protein synthesis. Supplementary concentrate feeds also alter microbial protein production in the rumen, partly through effects on the amount of organic matter (OM) fermented to produce ATP. In experiments with sheep given a constant weight of diet varying in the proportion of forage to cereal concentrate, Chamberlain and Thomas (1979), and Mathers and Miller (1981) observed that the efficiency of microbial protein synthesis increased slightly with the inclusion of small portion of concentrate in the diet but was reduced by 25 to 50% when the concentrate proportion was above 0.6 to 0.7. Studies with sheep fed diets containing a large portion of barley or maize have show that bacterial protein synthesis are lower and more variable, 16-33.6 g N per Kg OM apparently digested in the rumen (OMADR) (Chamberlain et al., 1976; Lee et al., 1986). In contrast to this, Hogan and Weston (1967) reported that the rate of bacterial protein synthesis was relatively constant at approximately 30.4 to 36.8 g N per OMADR Kg, for sheep given chopped forages. The above results suggest that at low or moderate levels of concentrate inclusion, the fermentation of forage cell wall carbohydrates in the rumen was substantially suppressed but the increased

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Received August 5, 1989
Accepted October 16, 1989

availability of fermentable carbohydrate in concentrate led a significant improvement in the amount of microbial protein produced. Meanwhile, straw-manure silage, which was developed at the KAIST, was recently given to a large number of Korean native cattle and dairy cows at rural community. At appropriate level of formula feed inclusion on this silage, the improved animal performances were reported by Yoon et al. (1985). With the view of these animal performance, the experiment reported here was conducted to investigate the ruminal synthesis of bacterial protein and its influence on the abomasal flow of nitrogen in sheep given a range of diets varying in the ratio of silage and concentrates. As the concentrate feed-stuff only barley was fed, and protein source was not included.

Materials and Methods

Animal and diets

Three Corriedale rams (average body weight: 43.2 Kg) fitted with rumen cannulae and "T" type abomasal cannulae were housed in a temperature-controlled room (15°C + 3). Equal portions of the daily feed allotment were offered to animals at 2 hour intervals per day under 24 hour continuous lighting. And there was free access to drinking water and mineralized salt blocks. Experimental diets were given at 1.75% (DM weight) of body weight daily and were a three mixed diets, consisting of straw-manure silage and barley (table 1). Straw-manure silage was prepared after mixing of 4% sodium hydroxide treated rice straw, wet poultry manure, and wheat bran.

TABLE 1. THE CHEMICAL COMPOSITION OF DIETS CONTAINING THREE RATIO OF STRAW-MANURE SILAGE TO BARLEY

	Silage: Concentrate		
	75:25	50:50	25:75
Crude protein (DM %)	12.02	12.60	13.18
Organic matter	82.56	87.37	92.17
Crude fiber	21.84	16.47	11.09
N D F	52.38	47.38	42.39
A D F	35.60	26.41	17.23
Cellulose	24.52	18.30	12.07
T-A.A*	9.70	11.44	13.19
E-A.A	4.23	4.68	5.12
NE-A.A	5.47	6.77	8.06
ME (Mcal/DM kg)**	2.39	2.65	2.92
DM intake (g/day)	754.4	753.0	753.5

*Total, essential and nonessential amino acid.
**Calculated value.

TABLE 2. SAMPLING SCHEDULE

Day	Time (h)	Action	Location
3	From 0900	Solid marker dose	Feeder
13	From 0900	Liquid marker dose	Rumen infusion
17-18	Four hour interval From 0900	Sampled	Abomasum: flow rate calculation
19	Before 0900	Sampled	Rumen: dilution rate and rumen volume calculation
	After marker stop, 2hr-interval for 24hr	Sampled	
20	Four hours intervals	Sampled	Rumen: isolation of bacteria

Experimental design and procedures

Each experimental periods lasted 20 days with four change-over feeding and dosing and sampling were presented in table 2. Days 1-10 were used for dietary adaptation phase, and then 6 day collection of faeces and urine.

A dual-phase marker system was used to measure abomasal digesta flow. One part of chromic oxide as the solid marker was mixed with four parts of wheat flour and water. This was dried at 100°C oven and then ground at hammer mill. 0.5 g of this marker was mixed with each meal.

The CO-EDTA (Uden et al., 1980) as the liquid phase marker was infused into rumen continuously.

Analytical procedures

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 and whole abomasal digesta were lyophilized, and ground to pass a 1 mm screen and kept for OM, N, DAPA(diaminopimelic acid), amino acids and marker analysis at the P_2O_5 dessicator. The rumen fluid was thawed and strained through four layers of cheesecloth, and isolated bacterial portion was obtained by double centrifugation after washing with 0.9% NaCl (W/V) and deionized water. Separated rumen bacteria was also lyophilized. Proximate analysis was conducted by the procedures of A.O.A.C. (1975), and rumen fluid 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 component was crotonic acid. Ammonia-nitrogen was analyzed by the MgO distillation (A.O.A.C., 1975). The content of DAPA was determined according to the procedures of streamlined method with performic acid oxidation (Mason et al., 1980). Chromic oxide was estimated as described by Fenton and Fenton (1979) on a Perkin Elmer 2380 atomic absorption spectrophotometer. Flow of abomasal digesta was calculated by the matrix equation (Armentano and Russell, 1985). Abomasal digesta bacterial N content was calculated from the N: DAPA ratio in rumen bacteria and DAPA concentration of abomasal digesta. Data were analyzed by analysis of variance using randomized block design and significant differences between treatments were tested according to the multiple range test of Duncan.

Results

The chemical composition of experimental diets are given in table 1. Crude protein, OM and total amino acid content were increased with decreasing level of silage in the diet and fibrous component was vice versa. DM intake was identical among the treatments, but ME intake for silage 75: barley 25, silage 50: barley 50 and silage 25: barley 75 was estimated for 1.12, 1.21 and 1.39 times higher than that of maintenance ME intake, respectively. As the proportion of barley in the diet increased, there was an increase in the amount of OM intake (table 3). OM digestion in the whole tract and apparent ruminal OM digestion were increased significantly ($p < 0.01$) in higher barley diet. The bacterial OM passing the abomasum for three treatments was determined as 113.02, 109.62, and 73.77g per day, respectively. There was shown significantly lower OM flow in silage 25: barley 75 ($p < 0.01$). ADF passing the abomasum were also significantly lower ($p < 0.05$) in treatment of silage 25: barley 75 than that in silage 75: barley 25.

Mean values for rumen pH and the concentrations of VFAs are given in table 4. As the proportion of barley in the diet increased there was a reduction in rumen pH and total VFA concentration. For the molar percentage of individual VFA, there were significantly increased values of isobutyric, butyric and iso-valeric acid with the increased proportion of barley in the diets. And there were great differences in NH_3-N concentrations between experimental diets. Associated with the high barley diet (25:75), mean ammonia content in the rumen fluid was shown the highest levels of 12.79 mg/100 ml as compared with 4-6.07 mg/100 ml for the other diets. Dietary silage level did affect rumen volume and dilution rate of liquid. Dilution rate and ruminal liquid outflow was decreased significantly ($p < .01$) with decreasing silage level.

The flow rate, nitrogen digestibility, and bacterial N synthesis in sheep received diets containing three different ratios of straw-manure silage and barley are shown in table 5. No significant differences between diets were observed in the mean values obtained for nitrogen passing the abomasum, faecal N and urinal N, N retention, and apparently digestion of N. However, abomasal N flow per intake for the silage 75: barley 25 diet was significantly increased ($p < 0.01$) than abomasal N flow for the 25:75 diet. It was non-significant net gain in the quantity of total N in the passage of the digesta through the stomach for all diets. No significant differences between diets in the quantities of non-ammonia nitrogen (NAN) passing the abomasum ($p > 0.05$) was observed.

It was also observed that there was significant differences between diets in the flow of bacterial N at the abomasum ($p < 0.01$). Daily flow of bacterial N for silage 75: barley 25, silage 50: barley 50, and silage 25: barley 75 was measured for 9.18, 9.60, and 7.28 g, respectively. The flow of NH_3-N in the abomasal digesta was significantly

TABLE 3. THE FLOW RATE AND DIGESTION OF ORGANIC MATTER IN SHEEP RECEIVED DIETS CONTAINING THREE DIFFERENT RATIOS OF STRAW-MANURE SILAGE TO BARLEY

	Silage: Concentrate			SE
	75:25	50:50	25:75	
Organic matter (g/day)				
Intake	607.64	647.65	713.64	–
Abomasum,	299.20^{b}	245.57^{a}	231.92^{a}	8.19
Faeces,	176.30	163.41	158.20	7.93
Bacterial OM flow at the abomasum	113.02^{b}	109.62^{b}	73.77^{a}	3.84
Digested OM amount (g/day) in the:				
Rumen (apparently)	308.44	402.08	481.72	29.34
Rumen (truly)	421.47^{a}	511.70^{b}	555.49^{c}	6.92
Whole tract	431.35^{a}	484.24^{b}	555.44^{c}	4.35
Proportion digested OM (%) in the				
Rumen (apparently)	50.53^{a}	62.10^{b}	67.86^{c}	1.03
Rumen (truly)	69.50^{a}	79.02^{b}	78.14^{b}	0.98
Whole tract	70.52^{A}	74.72^{AB}	78.33^{B}	1.34
Acid detergent fiber (g/day)				
Intake	270.20	200.24	132.22	
Abomasum	132.96^{c}	89.56^{b}	60.31^{a}	4.43
Faeces	108.47^{b}	93.77^{ab}	76.99^{a}	3.88
Proportion digested ADF in the:				
Rumen	50.67	55.55	54.67	2.34
Whole tract	59.15^{B}	53.01^{AB}	42.98^{A}	2.63

Values with the different superscripts in the same line are significantly different (A,B: $p < .05$ and a,b,c: $p < .01$).

increased ($p < 0.01$) at high level of barley diet.

The rate of synthesis of bacterial nitrogen per kg OMADR and OMTDR was shown significantly differences between the 75:25, 50:50 and 25:75 diets. The efficiency of bacterial protein synthesis was decreased with decreasing proportion of barley in the diet, reaching the minimum with the 25:75 diet.

The flow of amino acids in the abomasal digesta are shown in table 6. There were no significant differences between diets for the quantities of total AA-N, essential AA-N and nonessential AA-N passing at the abomasum ($p > 0.05$). No significant differences between diets in the quantities of individual amino acid of abomasal digesta except lysine and cysteic acid were observed.

Discussions

The results for the increase in OM digestion with increasing concentrate in the diets are generally in line with those of other workers (Chamberlain and Thomas, 1979; Tamminga, 1981; Rode et al., 1985; Brink and Steele, 1985). The postruminal OM digestion decreased as level of barley was increased. This tendency may be associated with the dilution rate, rumen pH and total VFA concentration in the rumen. Brink and Steele (1985) reported postruminal starch digestion decreased as level of corn was increased. Also Slyter (1976) have shown decreased rumen pH and reduced rumen motility in ruminants fed high concentrate diets. However, Goetsch and Owens (1985) reported that with 90% concentrate diet postruminal digestion of OM was greater than that of 65% concentrate diet.

Increasing the proportion of barley in the diet

TABLE 4. THE RUMEN FERMENTATION PARAMETERS AND DILUTION RATE IN SHEEP RECEIVED DIETS CONTAINING THREE DIFFERENT RATIOS OF STRAW-MANURE SILAGE AND BARLEY

	Silage: Concentrate			SE
	75:25	50:50	25:75	
pH	6.27	6.31	6.10	0.06
NH_3 N (mg/dl)	4.06[a]	6.07[a]	12.79[b]	0.86
Total VFA (mM/l)	80.58	74.56	45.91	10.17
Individual VFA (molar %)				
Acetic acid	65.78	61.00	59.03	1.62
Propionic acid	19.76	20.18	23.27	1.70
Iso-Butyric acid	0.59[a]	1.23[b]	1.34[b]	0.07
Butyric acid	11.96[a]	15.15[b]	12.49[a]	0.41
Iso-Valeric acid	0.38[a]	0.80[a]	2.69[b]	0.09
Valeric acid	1.53	1.64	1.17	0.23
Rumen liquid				
Volume (l)	3.33	2.94	2.43	0.27
Dilution rate (hr^{-1})	0.1157[b]	0.0908[a]	0.0881[a]	0.0017
Rumne liquid outflow (ml/hr)	375[b]	290[b]	126[a]	22.71

Values with the different superscripts in the same line are significantly different (a, b: $p < .01$).

TABLE 5. THE FLOW RATE, DIGESTION OF NITROGEN, AND BACTERIAL NITROGEN SYNTHESIS IN SHEEP RECEIVED DIETS CONTAINING THREE DIFFERENT RATIOS OF STRAW MANURE SILAGE AND BARLEY

	Silage: Concentrate			SEM
	75:25	50:50	25:75	
Nitrogen				
Intake (g/day)	14.48	15.24	16.58	
Abomasum,	12.89	12.82	11.54	0.43
Faeces,	4.51	4.71	4.54	0.21
Urine,	5.79	6.97	5.69	0.82
Absorption,	9.97[a]	10.53[a]	12.05[b]	0.16
Retention,	4.18	3.56	6.36	0.84
Apparent digestion (%)	68.33	68.98	73.03	1.48
Abomasum flow/intake,	90.47[b]	85.37[ab]	69.39[a]	3.29
NH_3 at abomasum,	0.35[a]	0.31[a]	0.52[b]	0.03
NAN flow	12.55	12.51	11.02	0.43
Bacterial	9.18[b]	9.60[b]	7.28[a]	0.25
Non-bacterial	3.37	2.91	3.74	0.30
Bacterial/NAN	73.15[b]	77.03[b]	65.71[a]	1.82
Bacterial N synthesis:				
g N/kg OMADR	30.47[b]	24.05[b]	14.93[a]	1.24
g N/kg OMTDR	22.05[b]	18.90[b]	12.95[a]	0.71

Values with the different superscripts in the same line are significantly different (a, b: $p < .01$).

TABLE 6. THE FLOW OF AMINO ACIDS IN THE ABOMASAL DIGESTA IN SHEEP RECEIVED DIETS CONTAINING THREE RATIO OF STRAW-MANURE SILAGE TO BARLEY

	Silage: Concentrate			SE
	75:25	50:50	25:75	
Total amino acid				
Intake (g/day)	72.82	85.88	102.98	
Abomasum,	59.67	64.69	56.45	4.44
Abomasum/intake	81.94^{B}	75.33^{B}	54.82^{A}	9.81
Essential amino acid				
Intake (g/day)	31.81	35.14	39.96	–
Abomasum,	30.86	30.56	26.06	1.61
Abomasum/intake	97.01^{b}	86.97^{b}	65.22^{a}	5.07
Nonessential amino acid				
Intake (g/day)	41.01	50.75	63.02	
Abomasum,	28.81	34.13	30.39	2.87
Abomasum/intake	70.23	67.25	48.22	5.85
Amino acid content of abomasal digesta (g/day)				
Lys	4.01^{B}	4.12^{B}	3.29^{A}	0.18
His	4.37	4.50	3.60	0.24
Arg	3.33	3.34	2.99	0.14
Asp	5.77	6.74	5.80	0.32
Thr	2.81	2.96	2.63	0.21
Ser	2.74	3.06	2.74	0.39
Glu	8.54	11.37	10.82	1.03
Pro	3.57	3.97	3.50	0.40
Gly	3.04	2.97	2.63	0.15
Ala	4.11	4.56	3.73	0.30
Val	4.52	3.76	3.22	0.31
Met	1.04	1.20	1.09	0.10
Ileu	3.30	3.21	2.78	0.23
Leu	4.42	4.71	3.91	0.28
Phe	3.06	2.76	2.55	0.22
Cys	1.04^{a}	1.46^{b}	1.17^{a}	0.05

Values with the different superscripts in the same line are significantly different (A,B: $p < .05$ and a,b: $p < .01$).

tends to decrease the digestion of ADF. It has often been observed that with concentrate rich diets the degradation of cell wall components is inhibited. This inhibition may be caused by a specific inhibition of cellulose digesting strains of rumen bacteria (Stewart, 1977). And ADF digestion in the rumen was more than that of total tract digestion. This may have been caused by disproportionate quantities of solid in duodenal samples or accumulation of fibrous artifacts when faeces were ovendried (Combs et al., 1983; Rode et al., 1985). However, this difference of ADF digestion between rumen and whole tract for the 25:75 diet was great compared with other results (Goetsch and Owens, 1983; Rode et al., 1985).

Dietary silage level did effect the rumen fermentation parameters. The higher liquid dilution rate with high silage diets is similar to reports by Rode et al. (1985), Goetsch and Owens (1985), and Snyder et al. (1984). And non-significant higher rumen liquid volume at high-silage diets may be linked with the characteristics of straw-

manure silage. Before the silage making, the process of this silage included the treatment of 4% NaOH to the straw. This would partially indicate that the greater intake of water generally associated with the feeding of diets with a high content of Na may have accelerated the rate of passage and increased rumen volume (Voight and Piatkowski, 1974).

In addition, total VFA concentrations were greater (80.58 vs. 45.91 mM/l) for sheep fed high silage diets rather than high barley diets. Generally diets of high energy and low fiber content are associated with greater concentration of VFA as compared to more fibrous feedstuffs. The large amounts of readily degradable starch provided by the low fiber diet may have promoted the growth of lactic acid-producing bacteria in the rumen. Hence, an increased incorporation of carbon into lactate may account for the unusual differences in VFA concentration (Schwartz and Gilchrist, 1975). These responsible for the decreased rumen pH and higher ammonia content at high barley diet.

As the proportion of barley in the 25:75 diet were increased a greater amount of readily fermentable carbohydrate became available to the rumen microorganisms. There was, however, no associated increase in the passage of N at the abomasum. The amount of N at the abomasum did not differ significantly between diets and mean values were in the range between 12.55-11.02g N/day and especially for high barley diets, the efficiency of bacterial protein synthesis was greatly low (14.93g N/kg OMADR). The reduction in bacterial protein synthesis with relation to the decreased ratio of roughage to concentrates is generally similar with other results (Cole et al., 1976; Chamberlain and Thomas, 1976; Mathers and Miller, 1981; Rode et al., 1985). Their findings were probably related to the difference between diets in the rate of bacterial protein synthesis in the rumen and rumen fermentation pattern. In the experiment of Chamberlain and Thomas (1979), diets containing of either pure roughage or diets containing of over 85% concentrates showed a decreased efficiency of microbial protein synthesis (15.04g N/Kg OMADR). For high concentrates and low forage and high concentrate-mixed diets, a low efficiency may be due to a less than optimal supply of nitrogen as reflected in lower rumen content of ammonia.

Optimal concentration of NH_3-N reviewed by Owens and Bergen (1983) range from 0.35 to 29 mg/dl. Under the value of NH_3-N concentration found in this result, the rate of synthesis may be a consequence of the rumen conditions of microbial population in the rumen. Bacterial synthesis is impaired by low pH (Rook, 1975) and low ruminal liquid clearance rates (Kennedy et al., 1976; Harrison et al., 1976). Both conditions were found in sheep consuming high-barley diets.

With diet lacking the amount of fibrousness in sheep receiving high-concentrates diets, fermentation pattern in the rumen is characterised by a low pH, a reduction in the numbers of cellulolytic and fibre-digesting bacteria and there is a substantial fall in acetate and a rise in the ratio of propionate to butyrate in the rumen liquor (Rook 1975). The low efficiency with sheep receiving high-barley diets would be linked with high ammonia content and a large proportion of butyric acid in VFA, both of which are consistent with the presence of substantial protozoal populations (Jackson et al. 1971; Abe et al., 1973).

With high starch diets the rumen protozoa form a large part of the total microbial population (Eadie and Mann, 1970). Because the protoza engulf bacteria and increase the recycling of nitrogen, when the protozoal numbers are high the efficiency of bacterial synthesis is reduced (Weller and Pilgrim. 1974). Chamberlain et al. (1985) reported that the presence of protozoa have lead to high rumen NH_3-N concentration and this is usually associated with butyrate fermentation. Hagemesister et al. (1981) indicated that with extremely high levels of concentrates in the ration, the gain of energy (ATP) per 100g of fermentable OM is reduced because of the production of lactate in the rumen. This leads to a reduced microbial protein synthesis (Russell and Baldwin, 1979). However, most of research on the efficiency of bacterial protein synthesis by sheep given a different ratio of roughage and concentrate diets, was conducted with the some inclusion of natural protein sources, or two or three starch sources. Accordingly, if this value of about 30g N/kg OMADR at 75:25 diets is compared with the general efficiency, 30-36.8g N/OMADR (Hogan and Weston, 1970), there may be the possibility of the promoted bacterial synthesis efficiency by inclusion of other protein sources. Other results (Rooke et al., 1986) indicated that when silage was fed either

alone or with barley the efficiency of bacterial-N synthesis was stimulated by the inclusion of soy bean oil meal or natural protein sources. Orskov and Grubb (1978) indicated that when more fermentation energy was potentially made available by alkali treatment of the straw, its actual availability to the rumen microbes was limited by nitrogen unless additional nitrogen was given.

Acknowledgement

We wish to express our sincere appreciation to GTZ of Federal Republic of Germany and the Ministry of Science and Technology, Korea for the financial support.

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