STUDIES ON THE UTILIZATION OF RICE STRAW BY SHEEP III. EFFECT OF SOYBEAN MEAL AND BARLEY SUPPLEMENTATION ON VOLUNTARY INTAKE, DIGESTIBILITY AND RUMINAL FERMENTATION

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Summary

The effect of soybean meal and barley supplementation on the utilization of rice straw was investigated. Balance trials were conducted with three Japanese Corriedale wethers fed rice straw supplemented with soybean meal and barley at three different levels of protein: low (40 g CP/d, LCP), medium (67 g/d, MCP) and high (94 g/d, HCP). In addition, all the supplements were formulated to contain the same amount of TDN (275 g/d). Voluntary intake of rice straw was not affected by any supplementation, while digestibility of organic matter in sheep given HCP diet was significantly higher ($p < 0.05$) than those on LCP diet. Crude protein, neutral detergent fiber (NDF) and acid detergent fiber (ADF) digestibilities of MCP and HCP diets were significantly improved ($p < 0.05$) over the LCP diet. Average daily gain of the animals under MCP and HCP diets were significantly higher ($p < 0.05$) than those under LCP diet. Differences of rumen pH among the treatments were not significant, while concentration of rumen NH_3-N was significantly higher ($p < 0.05$) for HCP diet than for LCP and MCP diets. Total volatile fatty acids (VFA_s) and blood urea nitrogen (BUN) concentrations were significantly higher ($p < 0.05$) in sheep fed MCP and HCP diets than those fed LCP diet, while plasma total protein concentration was not affected by any supplementation. Sheep fed MCP diet had a higher nitrogen retention than those fed LCP and HCP diets. It was concluded that rice straw was utilized better by sheep when SBM and barley were supplemented at the medium level of protein.

(**Key Words**: Rice Straw, Soybean Meal, Barley, Digestibility, Rumen Fermentation, Sheep)

Introduction

The main constraints in the utilization of rice straw (RS) by ruminants are low nitrogen and high cell wall contents. These factors directly affect the voluntary intake and digestibility, and hence limit animal performance. Although this straw contains abundant energy in the form of cellulose and hemicellulose, it is not readily available for digestion by rumen micro-organisms. Therefore, with proper supplementation of protein and/or energy which is rapidly degraded in the rumen, its nutritional limitations could be overcome, thus increasing voluntary intake and digestibility (Hoek et al., 1988; Church and Santos, 1981; Warly et al., 1992a).

In the previous study (Warly et al., 1992a), it was observed that protein (soybean meal, SBM) supplementation of a RS based diet increased (a) the voluntary intake of RS (by 47% over the unsupplemented diet), (b) daily gain, and (c) tended to improve rumen digestibility in sheep. However, when supplementary protein level was increased by increasing the amount of SBM from 75 g/d to 150 g/d, no further effects were observed in terms of voluntary intake and characteristics of ruminal fermentation. Oldham and Smith (1982) pointed out that the voluntary feed intake, digestibility and efficiency of nutrients absorption which are used for production could be affected by energy and protein contents of a diet. Furthermore, they suggested that when energy input is limiting, responses to increments in protein feeding decrease according to the law of diminishing returns. This means that a part of the increment in protein input is oxidized to provide

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the energy allowing only the remainder to be used. And thus, a negative effect may be achieved when protein input far exceeds that needed to match a given energy input. On the other hand, Doyle (1987) stated that when a low quality roughage is supplemented with sufficient readily fermentable carbohydrate, a depression in roughage intake (substitution effect) invariably occurs, but the level of supplementation at which substitution begins is not always the same.

The present study was designed to evaluate the effects of soybean meal and barley supplementation at varying levels of protein, but the same amount of TDN, on the voluntary intake of rice straw digestibility and ruminal fermentation in sheep given rice straw as a basal diet.

Materials and Methods

Animal management and experimental diets

This experiment was conducted with three Japanese Corriedale wethers with an average initial body weight (BW) of 53.1 ± 1.5 kg in a 3 × 3 Latin square design. All the sheep were fitted with a permanent fistula (40 mm diameter) in the dorsal sac of the rumen and were kept in metabolism crates throughout the experimental periods. Each sheep was fed with one of three experimental diets as follows: (1) rice straw + 320 g barley (low crude protein, LCP), (2) rice straw + 235 g barley + 85 g SBM (medium, MCP), and (3) rice straw + 150 g barley + 170 g SBM (high, HCP). The supplements were formulated to be of the same amount of TDN (275 g/d) but varying levels of protein (40, 67 and 94 g/d CP for LCP, MCP and HCP, respectively). These levels were chosen to supply approximately 40, 70 and 100% of CP and about 50% of the TDN requirements for maintenance of sheep weighing 50 kg (NRC, 1975).

The RS was chopped into 1~2 cm length and was given *ad libitum*, i.e. 25% greater than the amount consumed on the previous day. The daily allowance of the straw and supplements were offered in two equal portions twice daily at 09:00 and 17:00 hrs, while water and a mineralized salt block were freely available. A vitamin premix was given at a dose of 1 g/d/head. Each experimental period was of 13 days, in which the first 7 d were the preliminary period, 5 d for urine and feces collection and the last day for collection of rumen fluid and blood samples. Total feces from each sheep was collected daily, dried in a forced air oven at 60℃ for 24 hr. An aliquot sample was taken from the 5-d collection period and ground through a 1 mm screen. Daily urine excretion was also collected and 50 ml of 10% H_2SO_4 were added to prevent the loss of nitrogen. On the final day of each experimental period, approximately 50 ml of ruminal fluid was sampled from each sheep just before and at 1, 2, 3, 5 and 7 hrs after morning feeding. While blood samples were collected just before and at 3, 5 and 7 hrs after morning feeding. The samples of urine and ruminal fluid were then stored at −20℃ for later analyses.

Laboratory analysis

Chemical composition of feeds and feces were analyzed according to the Association of Official Agricultural Chemists (AOAC, 1984). Neutral detergent fiber (NDF), acid detergent fiber (ADF), lignin and silica contents were determined according to the methods of Goering and Van Soest (1970). Urinary nitrogen concentration was analyzed by the Kjeldahl method (AOAC, 1984) and concentration of rumen NH_3-N was determined by the method of Oser (1965). Total and individual volatile fatty acids (VFA_s) concentrations in the rumen fluid were determined by gas chromatography (Erwin et al., 1961). Blood urea-nitrogen (BUN) concentration was analyzed using the Unitest System (Model 300, Biodynamics, Inc. USA) and plasma total protein concentration was measured according to the method of Lowry et al. (1951). The chemical composition of RS was 82.3% organic matter (OM), 3.6% CP, 73.8% neutral detergent fiber (NDF) and 55.5% acid detergent fiber (ADF). Soybean meal used in this study contained 93.1% OM and 44.3% CP; while barley contained 98.1% OM and 12.6% CP. The TDN content of SBM and barley was 86.8% and 86.4%, respectively (The standard chemical composition of Japanese feedstuff, 1980).

Statistical analysis

All data were subjected to analysis of variance for a 3 × 3 latin square, and differences among the treatment means were determined by the least significant difference method (Steel and Torrie, 1981), at the significance level of 1 and 5%.

Results and Discussion

Average intake of CP were 62, 89.5 and 116.2 g/d in sheep fed LCP, MCP and HCP diets, respectively. When expressed as percentage of the entire diet (as fed), CP content of the experimental diets were 6.7, 9.5 and 12.4% for LCP, MCP and HCP diets, respectively. Table 1 shows voluntary intake of RS, daily gain and apparent digestibility. The voluntary intake of RS was not affected by increasing protein supplementation. This finding is in agreement with the result obtained by Martin et al. (1981) that the voluntary intake of hay made from low quality roughages was not increased by supplementation of nitrogen (urea) when the diets contained a rapidly rumen degradable energy source (160 g molasses). In a review of Doyle (1987), Crabtee and Williams (1971) found that increasing amounts of mixtures of barley and SBM in the diet depressed hay intake, but the higher protein content in the supplement tended to increase forage intake. Doyle et al. (1988) found that offering 173 g of supplement (a mixture of oat grain and sunflower meal) did not significantly affect the voluntary intake of oat hay by lambs. However, when supplied at higher rates (330-370 g and 510-550 g DM) the voluntary intake of the oat hay was depressed at a mean rate of 92 g/100 g of supplement. In the present study, the average intake of RS was 32 g/kg $BW^{0.75}$, which was lower than in a previous study (46 g/kg $BW^{0.75}$) when sheep were fed with rice straw supplemented with either 75 or 150 g of SBM per day (Warly et al., 1992a). Therefore, it is suggested that the failure of increasing level of protein supplementation to stimulate intake of RS has probably been caused by a large amount of supplement offered in the present study (320 g/d).

TABLE 1. VOLUNTARY INTAKE OF RICE STRAW, DAILY GAIN AND APPARENT DIGESTIBILITY IN SHEEP FED RICE STRAW SUPPLEMENTED WITH BARLEY AND SOYBEAN MEAL AT VARIOUS LEVELS OF PROTEIN

Item	LCP	MCP	HCP	SEM[1]
Rice straw intake:				
(g DM/day)	603.1	619.1	622.9	70.3
(g DM/kg $BW^{0.75}$)	31.0	32.2	32.0	3.2
TDN intake (g/d)	552.3	584.3	581.1	35.4
Weight gain (g/d)	41.1[a]	83.3[b]	85.6[b]	12.4
Digestibility (%):				
Organic matter	66.8[a]	69.1[ab]	70.1[b]	0.9
Crude protein	53.4[a]	68.5[b]	74.7[b]	2.0
Crude fiber	63.6	68.9	68.9	2.4
NDF	50.6[a]	57.4[b]	56.1[b]	1.5
ADF	42.9[a]	50.5[b]	48.9[b]	0.1

[1] Standard error of the mean.

[a,b] Values in the same row with different superscripts differ significantly ($p < 0.05$).

Total digestible nutrients (TDN) intake in sheep fed MCP and HCP diets were slightly higher than those fed LCP diet, and all of these values met the requirement of TDN for maintenance of sheep weighing 50 kg, as recommended by NRC (1975). Daily gain of the sheep were positive for all the treatment diets. Supplementation of protein at medium and high levels resulted in higher ($p < 0.05$) weight gains compared to the low level. However, no significant differences were observed between the MCP and HCP diets. It was probably caused by increasing protein supply, particularly with increased undegraded supplementary protein arriving at the duodenum as well as the quantity of microbial protein.

Digestibility of OM in sheep fed the HCP diet was significantly higher ($p < 0.05$) than those on LCP diet. A similar finding was also reported by Silva et al. (1989) in their study on barley straw, that digestibility of DM and OM were markedly improved by supplementation of fish meal, sugar-beet pulp or a combination of them. Crude protein, NDF and ADF digestibilities were significantly higher ($p < 0.05$) when sheep were fed MCP and HCP diets than when they were fed LCP diet; and they were relatively higher compared with the results obtained in the previous study (Warly et al., 1992a). These results suggest that replacement of a predominantly carbohydrate supplement with a high protein supplement increased digestibility of fibrous components presumably by increasing microbial digestive capability.

Effects of treatments on rumen fluid pH, ammonia concentration, total and individual volatile fatty acids (VFA_s) production are presented in table 2. Rumen fluid pH was not significantly affected by any level of protein supplementation at all sampling times, although it was slightly higher in sheep fed LCP diet. However, the concentration of rumen ammonia was increased ($p<0.05$) with increasing levels of dietary protein. The average ammonia concentrations based on the 6 sampling times were 6.3, 14.1 and 24.1 mg/100 ml for sheep fed LCP, MCP and HCP diets, respectively. These values were higher than the concentration of 5 mg/100 ml reported by Satter and Slyter (1974) as being necessary for maximal microbial protein synthesis. However, maximal rumen fermentation was achieved when rumen ammonia reached 19 to 23 mg/100 ml (Mehrez et al., 1977). Milne et al. (1979) reported a relationship between ruminal ammonia concentration and voluntary intake of low quality roughage by sheep. Above a concentration of 6 mM/l (10.22 mg/100 ml) of rumen ammonia, there was no further increase in the intake. In the present study, the increase in rumen ammonia concentration from 6.3 to 14.1 and 24.1 mg/100 ml only resulted in a very small increase in RS intake, but no increase in intake was observed between the 14.1 and 24.1 mg/100 ml of rumen ammonia concentrations. According to Satter and Rofler (1981) the amount of ammonia that can be utilized by rumen bacteria will depend on the number of rumen bacteria, how rapidly they are growing and the availability of fermentable energy. Improvements in digestibility may be associated with increases in rumen microbial activity and thus microbial protein synthesis, as has been previously suggested (Warly et al., 1992b). A higher concentration of total VFA_s ($p < 0.05$) in sheep fed MCP and HCP diets also suggests increased rumen microbial activity. The average total VFA_s concentrations based on the 6 sampling times were 6.26, 6.94 and 7.81 mmol/100 ml for sheep fed LCP, MCP and HCP diets, respectively. Acetic, propionic and valeric acid concentrations were also significantly increased ($p < 0.05$) by increasing levels of dietary protein, while butyric acid concentration was not different ($p > 0.05$) among the treatments. Effects of treatments on the pattern of rumen fluid pH, rumen ammonia and total VFA_s concentrations are graphically presented in figures 1, 2 and 3, respectively.

Table 3 shows the effects of treatments on

TABLE 2. RUMEN PH AND CONCENTRATION OF RUMEN AMMONIA AND TOTAL VOLATILE FATTY ACIDS (VFAs) IN SHEEP FED RICE STRAW SUPPLEMENTED WITH BARLEY AND SOYBEAN MEAL AT VARIOUS LEVELS OF PROTEIN[*]

Item	LCP	MCP	HCP	SEM
Rumen pH	6.56	6.47	6.49	0.09
Rumen ammonia (mg/100 ml)	6.27[a]	14.07[a]	24.10[b]	2.35
VFA_s (mmol/100 ml):				
Total	6.26[a]	6.94[b]	7.81[c]	0.16
Acetic acid	4.42[a]	5.07[b]	5.63[c]	0.11
Propionic acid	0.93[a]	1.10[b]	1.15[c]	0.03
Butyric acid	0.77	0.81	0.86	0.04
Valeric acid	0.11[a]	0.12[a]	0.17[b]	0.01

[*] Each value is the mean of 6 sampling times.

[a,b,c] Values in the same row with different superscripts differ significantly ($p < 0.05$).

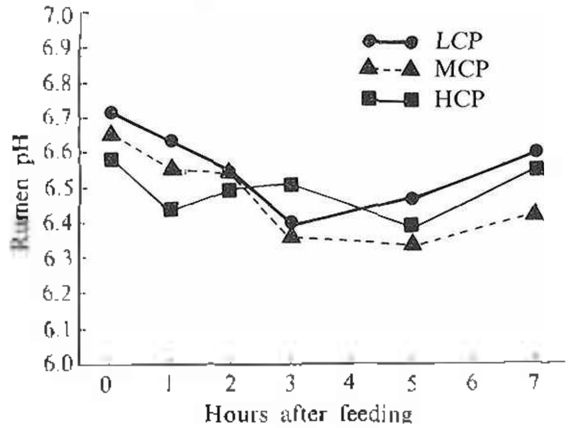

Figure 1. Effect of varying levels of barley and soybean meal supplementation on the pattern of rumen pH.

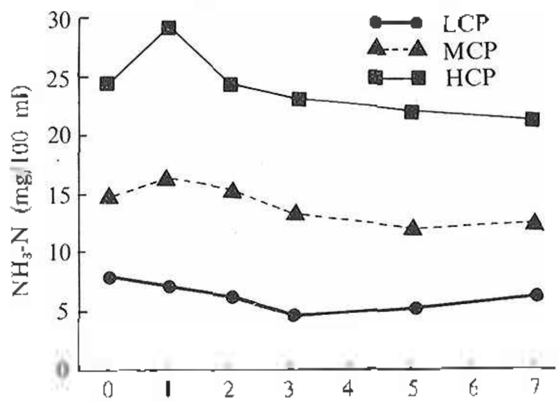

Figure 2. Effect of varying levels of barley and soybean meal supplementation on the pattern of rumen ammonia (NH_3-N) concentration.

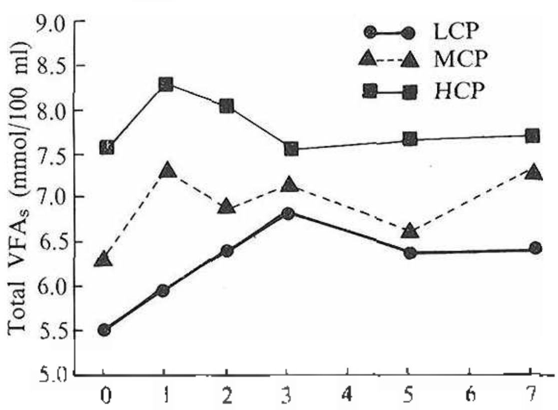

Figure 3. Effect of varying levels of barley and soybean meal supplementation on the pattern of rumen total VFA_s concentration.

nitrogen balance, plasma total protein and blood urea-nitrogen (BUN) concentrations. Nitrogen intake was increased significantly ($p < 0.05$) by increasing the level of supplemental protein. Urinary nitrogen excretion also increased ($p < 0.05$) but fecal nitrogen excretion was unchanged. Nitrogen retention was higher ($p < 0.05$) in sheep fed the MCP diet than those fed LCP and HCP diets. When expressed as a percentage of nitrogen intake, the nitrogen retention was 16.93, 28.21 and 14.53% for LCP, MCP and HCP diets, respectively. These results suggest that when energy supplement was maintained at a constant rate of 50% of the TDN requirement for maintenance, nitrogen supplies from both supplement and microbial protein were utilized better by sheep fed MCP diet than those fed LCP or HCP diets. It is also suggested that when protein is supplied in excess of available energy, efficiency of retention declined as protein is used for gluconeogenesis.

In terms of plasma total protein, no significant differences were observed among the treatments. The average plasma total protein concentrations based on the 4 sampling times were 9.85, 9.29 and 8.84 g/100 ml for sheep given LCP, MCP and HCP diets, respectively. Blood urea nitrogen concentrations in sheep fed MCP and HCP diets were significantly higher ($p < 0.05$) than those fed LCP diet. Average BUN concentrations based on the 4 sampling times were 10.55, 20.70 and 28.18 mg/100 ml in sheep fed LCP, MCP and HCP diets, respectively. These values reflected directly the rumen ammonia concentrations and its absorption through the rumen wall and conversion to blood urea. Annison and Lewis (1959) have proposed that blood urea could be used as a supplementary test for the efficiency of protein utilization. According to Swenson (1977), the normal level of BUN in sheep is 8–20 mg/100 ml. Results of this study show that at the low and medium levels of protein supplementation, the BUN concentrations were in the range of the normal level, while at the high protein supplementation the BUN production was far above the normal level. Martin et al. (1981) suggested that the presence of more readily available energy in the rumen could have resulted in greater incorporation of non protein nitrogen (NPN) into micorbial protein and thus lowered the amount of ammonia absorbed from the rumen and converted to blood urea

In conclusion, the present study indicates that the levels of protein and energy were important factors in the utilization of rice straw as feedstuff for sheep. At 50% supplementation of TDN requirement, the optimum level of protein supplementation which resulted in the highest daily gain, fiber digestibility, nitrogen retention, efficiency of ruminal fermentation and optimum level of BUN concentration was 67 g/d (medium level). However, it was difficult to define a clear relationship between energy and protein levels in this experiment due to differing potential extents and sites of digestion of the supplement used. Therefore to further clarify the effect of energy and protein supplementation on the utilization of rice straw, further study using pure protein and energy sources is required.

TABLE 3. NITROGEN BALANCE AND CONCENTRATION OF BLOOD UREA NITROGEN (BUN) AND PLASMA TOTAL PROTEIN IN SHEEP FED RICE STRAW SUPPLEMENTED WITH BARLEY AND SOYBEAN MEAL AT VARIOUS LEVELS OF PROTEIN

Item	LCP	MCP	HCP	SEM[1]
Nitrogen balance (g/d):				
Intake	9.92[a]	14.32[b]	18.65[c]	0.40
Loss in feces	4.62	4.52	4.75	0.36
Loss in urine	3.62[a]	5.76[b]	11.19[b]	1.51
Retention:				
(g/d)	1.68[a]	4.04[b]	2.71[a]	0.67
(% of N intake)	16.93	28.21	14.53	–
BUN (mg/100 ml)[1]	10.55[a]	20.70[b]	28.18[c]	2.32
Plasma total protein (mg/100 ml)[1]	9.85	9.29	8.84	1.54

[1] Each value is the mean of 4 sampling times.
[a,b,c] Values in the same row with different superscripts differ significantly ($p < 0.05$).

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