Effects of Supplementary Chinese Milk Vetch Silage and Rapeseed Meal on the Performance and Rumen Fermentation of Lambs Given Ammoniated Rice Straw Based Diet

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ABSTRACT : This study was proposed to investigate effects of inclusion of Chinese milk vetch silage (MVS) and rapeseed meal (RSM) on the growth and rumen fermentation of Hu-sheep. Fifty weanling lambs were randomly divided into five equal groups and offered ammoniated rice straw (ABRS) *ad libitum* along with 100 g concentrate (Trial 1). The animals in T_0, T_1, T_2, T_3 and T_4 group were respectively supplemented with MVS at levels of 0, 0, 7, 14 or 21% and with RSM at levels of 0, 15, 10, 5 or 0%. Daily gain of lambs was significantly ($p < 0.05$) higher in T_1, T_2 and T_3 group than that in T_0 and T_4 group. Feed conversion ratio was greatly reduced in supplemented groups as compared with T_0 group. In Trial 2, five sheep with rumen cannulae were used in a 5×5 Latin square design. The experimental treatments were as described in Trial 1, but without concentrate. The intake of ABRS was significantly ($p < 0.05$) lower in T_4 group than that in T_0 group, and also significantly ($p < 0.05$) lower than those in T_1 and T_2 group. Little difference among all treatments was found in 48h DM degradability of ABRS, MVS and RSM, and in rumen pH value and microbial protein concentration. Rumen concentrations of individual and total VFA tended to be higher in supplemented groups than those in T_0 group. These results suggest that supplementation with RSM or RSM plus MVS can effectively improve the performance of lambs, and may fail to influence markedly the rumen digestion of ABRS and rumen environments.

(**Key Words**: Chinese Milk Vetch Silage; Rapeseed Meal; Ammoniated Rice Straw; Growth Rate; Rumen Degradation; Rumen Parameter; Lamb)

INTRODUCTION

Ammonia bicarbonate (AB) treatment is an effective way to upgrade the nutritive value of rice straw (RS) (Liu et al., 1992; Liu et al., 1995). However, the nutrients available only from ammonia bicarbonate treated RS (ABRS) are not enough to meet the requirements for optimal performance of ruminants, and additive nutrients are required to optimize the productivity of ruminants. In previous study, we observed that supplementation with formaldehyde-treated rapeseed meal can improve the nutrients utilization, feed conversion efficiency and performance of heifers offered an ABRS-based diet (Wu et al., 1993; Liu et al., 1993).

It is well demonstrated that a small quantity of green forage can improve the utilization of straw diets (Preston and Leng, 1984; Prasad et al., 1993). Thus the introduction of various forage supplements is likely to increase the nutrient intake and improve performance of ruminants in developing countries. A great amount of Chinese milk vetch (*Astragalus sinicus* L.) is cultivated mainly as green manure in South China, surplus of which is fed traditionally to swine after ensiled. However, little information is available on effects of supplementation with Chinese milk vetch silage (MVS) in ruminants. The results obtained at this college suggested that MVS-supplementation can improve growth rate of heifers offered an ABRS-based diet (Liu et al., 1996).

The objective of the present study was to investigate the effects of combined supplementation with MVS and rapeseed meal (RSM) on the performance and rumen function in sheep given an ABRS-based diet.

MATERIALS AND METHODS

Experiment 1

Experimental feeds

The ABRS used was prepared by the 'stack method'. Fertilizer grade AB (17% N) was used as a source of NH_3 to treat the RS at the concentration of 100 g AB kg^{-1} RS and water was added according to the amount of 300 ml kg^{-1} RS (Liu et al., 1992). After treatment was carried

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out for 30 days under the ambient temperature of 15-20℃, ammoniated straw was exposed to the air for 24 h at least before feeding to animals to allow free ammonia to escape.

Chinese milk vetch was mowed at flowing stage and put through a silage chopped into a bunker silo. The silo was opened and made available for feeding to animals 40 days after ensiling.

Animals and design

Fifty weanling Hu-lambs, 55 days of mean age at the beginning of the experiment, were randomly divided into five equal groups. Each group was allocated randomly to receive one of five treatments in a single factorial design. In all five treatments, each lamb were given ABRS *ad libitum* along with 100 g concentrate mixture per head per day, and supplemented with the MVS and RSM at different levels. The levels of supplementary MVS were 0, 7, 14 or 21% of dietary dry matter (DM) intake, and the levels of supplementary RSM were 15, 10, 5, or 0% of DM intake in treatment T_1, T_2, T_3 or T_4, respectively. Treatment T_0 (Control) contained no supplements. Drinking water and mineralized salt block were freely available for each animal at all times to ensure the supply of minerals.

The experimental period was of 60 days duration.

Feeding trial

Lambs for each treatment were fed in groups. Care was taken to see that the supplements were well distributed along the trough to ensure that all animals had an equal share. The daily feed intake of each group was measured, and the lambs were weighed before morning feeding at intervals of 20 days over the trial.

Statistical analysis

The results were analyzed by one way analysis of variance. The difference of means for the five treatments was tested by using Duncan's new multiple range test (Steel and Torrie, 1980).

Experiment 2

Animals and their management

Five adult Hu-sheep equipped with permanent rumen cannulae were kept individually in metabolism crates. Feed was offered in two equal meals per day at 08:00 and 16:00 h, the amount given was calculated as experimental design. The sheep had *ad libitum* access to drinking water and mineralized salt block.

Experimental design

The experiment was conducted according to a 5×5 Latin square design. Animals in all five treatments were given ABRS *ad libitum*, and also supplemented with the MVS and RSM at the same levels as described in experiment 1. But all experimental diet contained no concentrate mixture. Each experimental period consisted of 20 days, the first 10 days of which were for adaptation followed by 10 days of determinations. The daily feed intake was measured and recorded over 7 days (from day 11 to day 17). On day 18, rumen fluid samples were taken for determination of pH, concentrations of ammonia nitrogen (NH_3-N) and volatile fatty acids (VFA), and microbial protein (MP). Rumen degradations of feeds were determined over the last 2 days of each period (from day 19 to day 20).

Dry matter intake

Feeds were offered twice per day in two equal meals. The ABRS was chopped to about 3-4 cm before feeding. The daily amount of ABRS given to each animal was calculated to exceed that eaten on the previous day by about 10% in order to avoid selective feed intake, and was weighed accurately before feeding every time. Daily subsamples of the diets offered were taken, and feed residues were recovered, weighed accurately and sampled before every morning feeding for late analysis of DM, and for calculation of DM intake.

Rumen degradation studies

The rumen degradation of DM and nitrogen (N) for ABRS, MVS and RSM was determined with the *in sacco* technique (Ørskov, 1985). Duplicate 3 g samples of each feed ground through a screen of 2 mm were placed in nylon bags and suspended in the rumen of sheep. The bags were removed from the rumen at 48 h incubation. Determinations of DM and crude protein (CP) were made on samples of feeds and residues in the bags with the methods described by AOAC (1990). The 48 h rumen degradability (D_{48}) was then calculated for each component.

Measurement of rumen parameters

Sampling

On day 18, rumen fluid samples were withdrawn by a tube through the rumen cannulae at 0, 3 and 6 h after the morning feeding, and 50 ml subsamples were immediately frozen at −20℃ for late MP determination. The remainder of the samples was strained through four layers of surgical gauze and 1 ml of 30% H_2O_2 was added to suppress microbial activity. Twenty ml subsamples of the strained rumen fluid were mixed with an equal volume of

20% formaldehyde solution and stored at −4℃ for protozoa counts. Further samples were stored at 4℃ for analysis of pH, NH_3-N and VFA concentration.

Rumen pH

The pH values of rumen fluid were determined immediately after removal by using a precise pH meter (PHS-3c Model).

Rumen NH_3-N concentration

Five ml samples of rumen fluid with duplicates were placed in the Kjeldahl semi-microdistiller, and then 5 ml of KOH solution (2 mol L^{-1}) was added. The rumen NH_3-N concentration was then determined by steam distillation into 2% boric acid solution and titration with dilute hydrochloric acid (10 mmol L^{-1}).

Rumen volatile fatty acids

Duplicate 2 ml samples of rumen fluid were mixed uniformly with 8 ml of distilled water (approximately 4℃) in graduated tubes. One ml of 25% meta-phosphoric acid was added, and after thorough mixing the samples were put in a refrigerator (4℃) for 30 min. Then they were centrifugalized at 3,000 rpm for 10 min. The supernatants of the samples were obtained for the VFA determination which was conducted on a gas chromatograph (Model GC-9A). The supernatants of 2.0 μl were injected into a 2 m ×6 mm glass column packed with Porapak Q (80 mesh). The temperature of the column, oven and detector were regulated at 220℃, 240℃ and 210℃ respetively. Nitrogen gas was used as a carrier at a flow rate of 63 ml min^{-1}. The concentrations of the individual acids were determined by comparing the peak heights of the rumen acids with the corresponding standards.

Rumen protozoa counts

Ciliate protozoa were counted by the method of Ogimoto and Imai (1981). Two ml of formaldehyde-preserved samples were mixed with 3 ml methylgreen-formaldehyde-saline solution. The mixture was held overnight and then pipetted into a counting chamber (haemocytometer) 0.4 mm deep. The protozoa were counted in 4 microscopic fields.

Rumen MP concentration

On the day of analysis the rumen fluid was thawed, and then the samples taken at three sampling times were mixed thoroughly and strained through four layers of surgical gauze. Microbial protein were then determined by the method of zinn and Owens (1986) based on purine measurement. Yeast RNA was used as a standard.

Statistical analysis

The results were analyzed as a 5 × 5 Latin square design. The difference of the five treatment means was tested by using Duncan's new multiple range test (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

The results of the DM intake are presented in table 1. The measured levels of supplementation were 0, 0, 6.8, 14.1 and 23.9% of the dietary DM intake for MVS, and 0, 15.2, 9.1, 4.7 and 0% for RSM in T_0, T_1, T_2, T_3 and T_4 group respectively, very similar to those in initial experimental design. The DM intake of ABRS by lambs was lower in the supplemented groups than that in T_0 group with no supplements in trial 1, and tended to decrease with increasing level of supplementary MVS in diets. The ABRS intake was the lowest in T_4 group with MVS alone (highest level of MVS) and 32.5% lower than that in T_0 group, whereas the ABRS intake increased when RSM was supplemented alone or along with MVS as compared with T_4 group. A similar tendency was also found in trial 2 (table 1). The ABRS intake was also the lowest in T_4 group and significantly ($p < 0.05$) lower than that in T_0 group. However, the ABRS intake did not significantly reduce when RSM was supplemented alone or along with MVS in comparison with T_0 group, and was significantly ($p < 0.05$) higher than that in T_4 group. This indicates that the high level of supplementary MVS (23.9%) would influence the ABRS intake adversely and hence result in a high substitute rate, whereas supplementation with RSM may have positive effect on the ABRS intake. Liu et al. (1996) observed that the ABRS intake decreased when heifers were supplemented with MVS in excess of 10% of DM intake. Tharmaraj et al. (1989) also reported a similar result on supplementation with Gliricidia. Total DM intake, however, increased slightly in T_1, T_2 and T_3 except T_4 group in comparison with T_0 group.

The data of feeding trial are shown in table 2. Daily liveweight gain of lambs was significantly higher ($p < 0.05$) in T_1, T_2, T_3 group than that in T_0 group, and also significantly higher ($p < 0.05$) than that in T_4 group. Furthermore, T_4 group also had significantly ($p < 0.05$) higher gain than did T_0 group (table 2). This indicates that MVS supplementation may enhance the growth rate of lambs when given an ABRS-based diet, moreover, the growth rate can be improved further and more effectively when RSM was supplemented along with MVS. The enhanced growth rate could be associated with the improved feed conversion ratio (FCR). The FCR was

Table 1. Dry matter intake of ammoniated rice straw (ABRS), Chinese milk vetch silage (MVS), rapeseed meal (RSM) by lambs given different experimental diets

Group	T_1	T_2	T_3	T_4	T_0	sem
Trial 1						
DM intake (g kg^{-1} $W^{0.75}$)						
ABRS	58.8	65.7	60.8	48.4	71.4	8.86
RSM	12.4	8.2	4.1	0	0	0.21
MVS	0	6.2	12.3	18.5	0	0.35
Concentrate	10.5	10.5	10.5	10.5	10.5	0
Total	81.6	90.5	87.7	77.4	81.8	8.79
Substitution rate[#]	1.1	0.4	0.7	1.3	–	0.53
Measured supplementary level (%)						
MVS	0	6.8	14.1	23.9	0	1.76
RSM	15.2	9.1	4.7	0	0	1.01
Trial 2						
DM intake (g kg^{-1} $W^{0.75}$)						
ABRS	48.2^{a}	44.1^{ab}	43.1^{bc}	37.7^{c}	46.4^{ab}	1.65
RSM	4.2	2.8	1.5	0	0	0.40
MVS	0	2.5	4.8	7.1	0	0.69
Total	52.4^{a}	49.4^{ab}	49.4^{ab}	44.8^{b}	46.4^{b}	1.50
Substitution rate	–	0.11	0.21	0.98	–	0.26

[a,b,c] Means with different superscripts within rows are different significantly ($p < 0.05$).
[#] Means ratio of decrease in DM intake of ABRS to the amount of supplementary MVS plus RSM.

Table 2. Liveweight gain and feed conversion ratio in growing lambs given ammoniated rice straw *ad libitum* and different supplements

Group	T_1	T_2	T_3	T_4	T_0	sem
Number of animals	10	10	10	10	10	
Initial body weight (kg)	17.6	17.7	17.7	17.7	17.8	0.63
Daily gain (g d^{-1})	93^{a}	70^{b}	70^{b}	51^{c}	32^{d}	8.83
Feed conversion ratio (kgDM kg^{-1})	7.54	11.15	10.81	13.10	23.73	1.77
Concentrate consumption/gain (kgDM kg^{-1})	2.11	2.30	1.79	1.76	3.00	0.73
Feed cost/gain (Yuan kg^{-1})	4.32	5.74	5.47	6.90	8.93	0.84

[a,b,c,d] Means with different superscripts within rows are different significantly ($p < 0.05$). 1 USD = 8.3 Yuan.

greatly improved in supplemented groups even when supplemented with MVS alone as compared with T_0 group, and it was improved further with increasing level of supplementary RSM in diets. (table 2). And also inclusion of RSM or/and MVS saved concentrate consumption per kg weight gain, 3.0, 2.1, 2.3, 1.8 and 1.8 kg kg^{-1} in T_0, T_1, T_2, T_3 and T_4 group, respectively. Therefore, the feed cost per kg weight gain was greatly reduced in supplemented groups in comparison with T_0 group, and decreased further when RSM was supplemented alone or along with MVS. Liu et al. (1996) and Wu et al. (1993) observed that the growth rate and FCR were improved in heifers receiving an ABRS-based diet when supplemented with MVS or RSM.

Results in rumen degradation were summarized in table 3. The differences among all treatments didn't reveal statistical significance in the 48 h DM degradability (D_{48}) of ABRS, MVS and RSM ($p > 0.05$), although the DM (D_{48}) of ABRS tended to be slightly lower in supplemented groups than that in T_0 group (table 3). This indicates that inclusion of RSM or/and MVS may fail to influence markedly the rumen degradation of

straw, MVS and RSM. The lack of response to supplementation in the rumen degradation may be due to the fact that the conditions for cellulolysis of straw were adequate in all treatments, as indicated by the rumen parameters (table 4).

Table 3. The rumen degradation of DM and CP of ABRS, MVS and RSM at 48 h incubation in sheep given different experimental diets

Group	T_1	T_2	T_3	T_4	T_0	sem
DM degradation (%)						
ABRS	62.8	64.7	64.6	63.5	67.5	0.53
MVS	78.5	79.5	79.6	77.4	79.0	0.47
RSM	57.9	59.9	60.1	55.9	57.1	0.91
CP degradation (%)						
ABRS	77.9	78.3	79.8	78.8	80.5	0.46
MVS	88.8	89.7	90.3	88.8	89.5	0.47
RSM	58.3	61.0	61.6	56.2	58.5	1.37

[a,b,c] Means with different superscripts within rows are different significantly ($p < 0.05$).

The mean rumen pH values at all three sampling times revealed little difference among all treatments and ranged from 7.1 to 7.3 (table 4), which accorded with the optimal pH for the growth of cellulolytic bacteria and degradation of straw as suggested by Mould et al. (1983). Rumen NH_3-N concentration at all sampling times was lower in T_0 group than that in supplemented groups, and tended to increase with increasing level of RSM in diets. The NH_3-N concentrations in all groups, however, reached the critical level of NH_3-N ranging from 50 to 280 mg per litre of rumen fluid as reported by Durand (1987). It is suggested that all diets were sufficient in NH_3-N for optimal fermentation and microbial growth. These results would account for our failure to detect differences in the DM degradation of ABRS, RSM and MVS among all treatments.

Table 4. The pH value, concentrations of NH_3-N, volatile fatty acids and microbial protein and protozoa counts in the rumen fluids of sheep given different experimental diets

Group	T_1	T_2	T_3	T_4	T_0	sem
pH	7.1	7.3	7.1	7.3	7.3	0.08
NH_3-N(mg dl^{-1})	27.3^{a}	26.3^{ab}	24.9^{ab}	24.1^{ab}	22.4^{b}	1.1
Volatile fatty acids (mM)						
Acetate	57.5	59.0	58.5	53.0	53.0	1.2
Propionate	15.0	16.1	16.1	14.1	13.0	0.4
Butyrate	4.2	5.4	5.5	6.0	3.3	0.3
Total	76.7	80.5	80.0	73.1	69.2	1.8
Acetate : Propionate	3.83	3.66	3.75	3.76	4.08	0.18
Acetate : Butyrate	13.69	10.93	10.88	8.83	16.06	1.36
Protozoa counts ($\times 10^5$ ml^{-1})	1.08	1.11	1.12	0.87	1.04	0.06
Microbial protein (mg ml^{-1})	0.68	0.76	0.84	0.72	0.76	0.12

[a,b,c,d,e] Means with different superscripts within rows are different significantly ($p < 0.05$).

Little difference among all treatments was found in the MP concentrations in rumen fluids (table 4). The rumen protozoal biomass was the lowest in T_4 group, and also lower than that in T_0 group, whereas no evident difference was found among other groups. This indicates that protozoal population in the rumen may be inhibited at a high level of MVS. And the inhibition may be associated with some anti-nutritional factors in the MVS. Liu et al. (1996) observed that protozoal population decreased in sheep supplemented with MVS. Navas-Camacho (1994) also observed that protozoal populations in the rumen dramatically decreased when sheep were

supplemented with leguminous tree leaves having high or medium saponin contents. Further studies are indicated to confirm the findings of the present work.

Concentrations of individual and total VFA in the rumen tended to be higher in supplemented groups than those in T_0 group, with slightly higher values in T_1, T_2, T_3 group than in T_4 group (table 4). This suggests that sheep supplemented with RSM or/and MVS could produce more VFA in the rumen and therefore obtain more supply of the metabolizable energy than those without supplements. Furthermore, the ratios of acetate to propionate and to butyrate tended to decrease in supplemented groups as compared with T_0 group. This means that inclusion of MVS or/and RSM may enhance the proportions of propionate and butyrate in total VFA. These differences may partly explain the improved FCR in supplemented groups.

The D_{48} of CP was 79.1, 89.4 and 59.1 for ABRS, MVS and RSM, respectively (table 3). The CP D_{48} of RSM was much lower than that of ABRS and MVS, indicating that the content of undegraded protein was higher in diets supplemented with RSM or RSM plus MVS than in those without RSM. The MVS was more digestible than the ABRS as indicated by D_{48} value of DM in table 3. Therefore the diets supplemented with MVS were of higher digestibility than diets without MVS. These differences and the increased DM intakes indicate that the intakes of digestible energy and by-pass protein were greater from diets with RSM or RSM plus MVS than from those without supplements, which may account for the improved growth rate and FCR in lambs supplemented with RSM or RSM plus MVS.

CONCLUSIONS

The DM intake of ABRS was markedly reduced and a high substitute rate would occur when sheep receiving an ABRS-based diet were supplemented with MVS alone at a high level, however, inclusion of RSM along with MVS can alleviate this response in the intake. Furthermore, supplementation with RSM or RSM plus MVS can more effectively improve the growth rate and feed conversion efficiency of lambs given an ABRS-based diet than that with MVS alone, and save concentrate consumption per kg liveweight gain. The rumen degradations of ABRS, MVS and RSM weren't influenced greatly by supplementation with MVS or/and RSM, which may be explained by the fact that the rumen conditions for fibre digestion were generally good in sheep of all treatments. A tendency of increase in the concentrations of VFA and in the proportions of propionate and butyrate in total VFA was found in animals supplemented with MVS or/and RSM, may partly account for the improved FCR in supplemented groups.

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