EFFECTS ON EATING AND RUMINATION BEHAVIOUR IN SHEEP OF FORMIC ACID AND FORMALDEHYDE TREATMENT AND METHIONINE-SUPPLEMENTATION TO LADINO CLOVER FIBROUS RESIDUE SILAGE

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Summary

The effects of formic acid and formaldehyde treatment and methionine supplementation to ladino clover fibrous residue silage on eating and rumination behaviour were studied in sheep. From the ladino clover fibrous residue, two silage were prepared, either untreated or treated with formic acid and formaldehyde. Four experimental diets: untreated silage, treated silage, untreated silage with supplementation of methionine and treated silage with supplementation of methionine, were offered to four sheep at a restricted level of DM intake (2% of BW/d) twice daily in a two-way layout design. Methionine supplementation with the treated silage significantly ($p < 0.05$) reduced daily time spent eating, and consequently, markedly increased rate of eating. However, there was little effect of methionine supplementation on the daily time spent eating and eating rate for sheep offered untreated silage. Methionine supplementation with the treated silage reduced daily time spent ruminating, although the same effect was not observed for untreated silage. The rumination index (time spent ruminating/100 g DM eaten) was remarkably smaller ($p < 0.05$) with methionine supplement in feeding treated silage, although it did not differ for sheep offered untreated silage. There were no clear effect of methionine supplementation on the rumination efficiency (i.e. number of chews/bolus, bolus time and rumination chewing rate) both feeding untreated silage and treated silage.

(**Key Words** : Fibrous Residue Silage, Methionine Supplementation, Eating and Rumination Behaviour, Sheep)

Introduction

It has been reported that the ensiled fibrous residues of legume plants can be utilized as a roughage in the same way as cocksfoot and/or timothy hays in sheep, and their nutritive value can be markedly improved by treatment with formic acid and formaldehyde before ensiling the materials (Fujihara and Ohshima, 1980a,b; 1982). From the result of changes in the concentrations of free amino acids in blood plasma of sheep after feeding the both silages, furthermore, it has been suggested that the supplementation of methionine, which is one of the limiting amino acids of rumen bacteria for ruminants, should efficiently improve the protein utilization of the silages in sheep (Fujihara and Ohshima, 1980b). Lee and Tasaki (1977) reported the positive effect of methionine supplemented to a semi-purified diet containing urea as nitrogen source in goats. Barry and Fenessy (1973) have also described that an orally supplementation of DL-methionine clearly promoted the nitrogen retention in sheep fed the silage treated with formaldehyde. Kaufmann and Lupping (1982), however, discussed the beneficial effects of methionine under practical feeding condition by a protection from bacterial degradation in the rumen.

On the other hand, the eating and rumination behaviour in sheep fed the fibrous residue silages of legumes has also been investigated in detail, and it was suggested that the silages will require rather less comminution through chewing during eating and ruminating than hay after feeding them in similar amounts of dry matter in sheep (Fujihara, 1981; Fujihara and Nakao, 1982). These findings would indicate that the fibrous residue of legume plants was relatively more digestible than hay. Thus, it has also been concluded that the food value of the silages as roughage feed in sheep will be slightly superior to that of hays, such as suncured cocksfoot, timothy or Italian-ryegrass hay.

In this paper, the eating and ruminating behaviour in sheep fed the ensiled fibrous residue of ladino clover with or without an oral supplement of methionine are discussed

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in relation to the utilization of the silage as a roughage feed, and also compared with the results obtained previously using the same silage(Fujihara, 1981). Preliminary results of some of the present work have been reported orally (Fujihara et al., 1983).

Materials and Methods

Two silages were prepared from the fibrous residue left after the extraction of ladino clover (*Trifolium repense* L. Var. giganteum) leaf protein by the method of Ohshima and Oouchi (1976). One part of the fibrous residue was ensiled without additive and the other part was sprayed with formaldehyde (0.85 %/DM) and formic acid (2.8 %/DM) for regulating the degree of fermentation and/or protecting protein breakdown during ensilage. Each fibrous residue was packed in polyethylene bags and then air in the bags was removed by a vacuum pump, thereafter they were stored in a dark room for 3-4 months. The chemical composition (as % of DM) of treated and untreated silages sampled at just before feeding, determined by the method of AOAC (1960), was: organic matter, 89.2 and 89.3; crude protein 20.2 and 23.8; crude fat, 3.7 and 5.3; crude fibre, 26.7 and 25.0; nitrogen free-extract (NFE), 38.6 and 35.2; crude ash, 10.8 and 10.7, respectively.

Four dietary treatments were prepared: the silage without an additive (untreated silage), the silage with formic acid and formaldehyde (treated silage), untreated silage with supplementation of methionine, and treated silage with supplementation of methionine. Methionine was supplemented with each silage at a level of 10% of silage-nitrogen intake. Four Japanese Corridale sheep were used. The experimental diets were offered to the animals according to a factorial design (two way layout design). The animals were kept in metabolism cages throughout the experimental period, and 5-day sampling period were preceded by 7 day adaptation period. Each animal was fed the diet in which the DM was 2.0% of BW per day. Methionine supplementation was conducted orally by means of topdressing just before feeding the silage. Water and salt licks containing trace minerals were accessible at all times. Half of the daily ration supplemented with methionine were given at 09:00 h and the other half at 17:00 h. During the 5-day sampling period, the time spent chewing during eating and ruminating was counted daily by the method of Fujihara (1980) using a wire strain gauge on the lower jaw (Harumoto and Kato, 1979). The terms used for indicating eating and rumination behaviour are the same as in a previous report of Fujihara (1980) based on the work of Gordon (1955).

Results were subjected to a two-way layout design with the silage-treatment and methionine-supplementation as factors. Statistical analyses were conducted by analysis of variance and the differences in mean value between each treatment were compared using Duncan's multiple range test.

Results and Discussion

The quality and the chemical composition of the silages used in the present study were almost the same as the silages made from the fibrous residue of ladino clover reported previously (Fujihara and Ohshima, 1980a,b).

As shown in table 1, the sheep offered treated silage spent less time eating ($p < 0.05$) and increased rate of eating with methionine supplement. Contrarily, the time spent eating untreated silage did not change with or without methionine. As a result, rate of eating the treated silage was also significantly ($p < 0.05$) faster with methionine supplement than that without metnionine, and that of untreated silage was similar in both the treatments with or without methionine supplement. As mentioned earlier (Fujihara, 1980), the bulkiness of ration is thought to be an important factor which could influence eating rate by sheep or cattle fed the same amounts of dry matter. In the present study, a similar silage made from ladino clover residue was used, so there could be no difference in bulk density and ease of bolus-formation between the two treatments, i.e., with or without methionine supplementation. Thus, it can be considered that the difference in time spent eating between the methionine supplementation and no supplementation could be mainly due to differences in ruminal fermentation by supplemented methionine in the treated silage. It may be, therefore, clear that the energy cost of eating (Osuji et al., 1975) the treated silage in the present study had been saved by methionine supplementation. Methionine supplementation greatly reduced the rumination appearance (lag time after eating) in the feeding of treated silage, but not significantly ($p > 0.05$), although there was rather a negative effect of methionine supplement in the feeding of untreated silage. This would show that ruminal supplement of methionine has little effect on fermentation as a stimulus to raise rumination to a receptor on the rumen wall (see Fujihara and Nakao, 1990).

Daily time spent ruminating after feeding the treated silage was significantly ($p < 0.05$) reduced with methionine supplement, though there was no difference observed after feeding untreated silage with or without methionine. This clearly show that the added methionine may have stimulated an increase in one factor, i.e.,

TABLE 1. EATING AND RUMINATION BEHAVIOUR IN SHEEP FED THE LADINO CLOVER FIBROUS RESIDUE SILAGE WITH OR WITHOUT METHIONINE-SUPPLEMENTATION

	Treated silage[1]		Untreated silage	
	No methionine	+ Methionine	No methionine	+ Methionine
Time spent eating (min/day)	79.1±30.1[a,2]	34.1± 0.8[b]	51.3± 0.3	51.8± 5.8
Rate of eating (g DM/min)	7.5± 2.7[a]	16.2± 0.6[b]	16.1± 0.4	16.3± 2.2
Rumination appearance (min)	418.3±99.2	364.0±39.5	406.4±35.5	437.9±90.8
Ruminating time (min/day)	326.8±17.0[a]	249.9±22.3[b]	278.9± 6.4	279.2±13.3
No. of boli regurgitated/day	274.9± 9.2	236.9±53.2	268.6±31.9	262.9±46.5
No. of rumination periods/day	19.6± 1.4[a]	24.2± 0.5[b]	23.0± 0.4	25.4± 1.0
Cyclic rate (sec)[3]	71.1± 1.3	64.3± 9.0	63.1± 6.1	65.2± 8.5
Length of rumination period (min)	17.5± 1.1[a]	10.4± 0.7[b]	12.2± 0.5	11.0± 1.0
No. of boli per rumination period	14.5± 1.0[a]	9.9± 1.9[b]	11.7± 1.6	10.4± 2.2
Rumination index[4]	63.0± 4.5[a]	45.7± 5.0[b]	34.0± 0.2	34.1± 1.0
No. of chews per bolus	62.2± 5.6	54.7±13.3	47.4± 6.9	45.7± 5.6
Bolus time (sec)	59.0± 5.5	48.2±10.0	41.7± 6.9	42.4± 5.6
Rumination chewing rate/min	63.3± 0.3	69.0± 3.0	59.7±11.0	60.6± 6.7

[1] The silage was sprayed with formaldehyde (0.85 %/DM) and formic acid (2.8 %/DM).
[2] Mean ± S.E. for four sheep.
[3] Total rumination time (sec)/no. of boli regurgitated (see Gordon, 1961).
[4] Time spent ruminating/100 g DM eaten (see Fujihara, 1980).
[a,b] Means different superscripts in a same row are significantly different ($p < 0.05$). Figures without superscripts in the same row do not differ ($p > 0.05$).

microbial degradation, to decrease particle size of ingesta in the rumen, rather than the other factor, i.e., chewing activity during rumination, and consequently, sheep could have saved the time required for comminuting the ingesta regurgitated.

Daily number of rumination periods after feeding of the treated silage was markedly ($p < 0.05$) increased with methionine supplement, and was also increased, but not significantly ($p > 0.05$), by methionine supplement in the feeding of untreated silage. Consequently, time spent ruminating and the number of boli per rumination period were significantly ($p < 0.05$) smaller with methionine supplement than without methionine in the feeding of treated silage. In our previous reports (Fujihara and Harumoto, 1990; Fujihara and Nakao, 1990), it has been also suggested that the changes in the dietary treatment (in particular physical treatment) will be able to lead to some changes in daily number of rumination periods in sheep fed roughage feed alone. Likewise, in this study, the daily number of rumination periods was also changed with dietary treatment, in which methionine was supplemented orally to sheep fed fibrous residue-silage alone. These findings will suggest that dietary chemical treatment can lead some changes in daily number of rumination periods of sheep fed on roughage feed as well as a physical treatment of diet as mentioned above. Cyclic rate defined by Gordon (1961) did not change with or without methionine supplement in feeding both silages, and this supports an opinion that cyclic rate seems to be a relatively stable parameter in rumination behaviour in sheep (Gordon, 1965). The rumination index (time spent ruminating per 100 gDM eaten) indicating the work done by sheep comminuting a diet (Fujihara, 1980) was significantly ($p < 0.05$) smaller with methionine supplement in the feeding of treated silage, though it did not change in the feeding of untreated silage supplemented with methionine.

The average number of chews per bolus tended to be lower with methionine supplementation than without methionine in feeding of the treated silage, and it was almost the same with or without methionine in feeding of untreated silage. The bolus time also tended to be small in the feeding of treated silage with methionine compared with that in feeding of treated silage without methionine, though it was similar in feeding untreated silage with or without methionine. As a result, subsequently, rumination

chewing rate was faster with methionine than without methionine in feeding of the treated silage. In the feeding of untreated silage, rumination chewing rate did not change much with or without methionine supplement. These facts obviously show that in feeding the treated silage with methionine, rumination by these sheep was not intensive. As a result, there were no clear differences in the parameters indicating the rumination efficiency, i.e., the number of chews per bolus, bolus time and rumination chewing rate, after the feedings of both silages with or without methionine supplement.

From the results obtained in the present study, it can be concluded that the methionine supplemented ruminally to improve the protein nutrition of sheep fed on the silage treated with formic acid formaldehyde for controlling fermentation during ensiling, obviously contributed to reduce energy expenditure on chewing during eating and rumination. This contribution is thought to be mainly caused by a stimulation of microbial fermentation in the rumen.

Literature Cited

Barry, T. N. and P. F. Fennessy. 1973. Effect of formaldehyde treatment on the chemical composition and nutritive value of silage. Ⅱ). Digestibility of the silages and the chemical composition of rumen fluid in sheep supplemented with or not supplemented with DL-methionine. N. Z. J. Agr. Res. 16:59-63.

Fujihara, T. 1980. The eating and rumination behaviour in sheep fed only grass diets in either the fresh or dried form. J. Agr. Sci. Camb. 95:729-732.

Fujihara, T. 1981. Eating and rumination behaviour in sheep given silage made from the fibrous residue of ladino clover. J. Agr. Sci. Camb. 97:485-488.

Fujihara, T. and T. Nakao. 1982. Eating and rumination behaviour in sheep given silage made from the fibrous residue of broad bean (*Vicia Faba* L.). J. Agr. Sci. Camb. 98:237-240.

Fujihara, T. and T. Nakao. 1990. The eating and rumination behaviour in sheep fed the diet of ensiled residue of ladino clover (*Trifolium repense* L. var. giganteum) with timothy (*Phleum pratense* L.) hay. Jpn. J. Zootech. Sci. 61:757-761.

Fujihara, T. and T. Harumoto. 1991. The effect of mechanical processing of hay on the eating and rumination behaviour in sheep. AA J. Anim. Sci. 4:7-14.

Fujihara, T. and M. Ohshima. 1980a. Utilization of ensiled fibrous residue obtained after extraction of ladino clover leaf protein concentrate in sheep. J. Japan. Grassl. Sci. 26:94-101.

Fujihara, T. and M. Ohshima. 1980b. The effect of formaldehyde and formic acid treatment on the utilization of fibrous residue silage made from ladino clover in sheep. J. Japan. Grassl. Sci. 26:191-200.

Fujihara, T. and M. Ohshima. 1982. The food value of ensiled fibrous residue left after the extraction of brod bean (*Vicia faba* L.) leaf protein in sheep. J. Japan. Grassl. Sci. 28:209-216.

Fujihara, T., T. Harumoto and M. Ohshima. 1983. The effect of orally supplementation of methionine on eating and rumination behaviour in sheep fed only an ensiled fibrous-residue of ladino clover. Kansai J. Anim. Scid. Technol., 95:19 (Abstract). (in Japanese).

Freer, M., R. C. Campling and C. C. Balch. 1962. Factors affecting the voluntary intake of feed by cows. 4. The behaviour and reticular motility of cows receiving diets of hay, oat straw and oat straw with urea. Brit. J. Nutr. 16:279-295.

Gordon, J. G. 1955. Rumination in the sheep. Ph. D. thesis, University of Aberdeen.

Gordon, J. G. 1961. The relationship between rumination and frequent feeding. Anim. Behav. 9:16-19.

Gordon, J. G. 1965. The relationship between rumination and the amount of roughage eaten by sheep. J. Agr. Sci. Camb. 64:151-155.

Hancock, J. 1953. Grazing behaviour of cattle. Anim. Breed. (Abstr.) 21:13-23.

Harumoto, T. and M. Koto. 1979. Effect of different amounts of hay intake on the rumination behaviour in sheep. Jpn. J. Zootech. Sci. 50:155-160. (in Japanese with English summary).

Hoitz, H. (ed.). 1960. Official Methods of Analysis. 9th ed. A.O.A.C. Washington, D. C. pp. 283-288.

Kaufmann, W. and W. Lupping. 1982. Protected proteins and protected amino acids for ruminants. In: Protein contribution of feedstuffs for ruminants (Ed.: Miller, E. L., I. H. Pike and A. J. H. Van Es). Butterworth Scientific. pp. 36-75.

Lee, K. and I. Tasaki. 1977. Effect of methionine supplementation on plasma free amino acids pattern of goats fed a urea diet. Jap. J. Zootech. Sci. 48:570-572. (in Japanese with English table).

Ohshima, M. and K. Oouchi. 1976. The order of limitation of amino acids in ladino clover leaf protein concentrate for growing rats. Nutr. rep. Int. 14:611-620.

Osuji, P. O., J. G. Gordon and A. J. F. Webster. 1975. Energy exchanges associated with eating and rumination in sheep given grass diets of different physical forms. Brit. J. Nutr. 34:59-71.