フォルムアルデヒドおよびギ酸を添加したラジノクローバ緑葉蛋白抽出残渣サイレージのヒツジにおける利用性について

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The Effect of Formaldehyde and Formic Acid Treatment on the Utilization of Fibrous Residue Silage made from Ladino Clover in Sheep

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Synopsis

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The fibrous residue obtained after extraction of leaf protein concentrate from ladino clover was ensiled with or without formaldehyde (0.85%/D.M.) and formic acid (2.8%D.M.) mixture and the effect of the additive treatment on the quality and the nutritive value of the silages were studied. For the purpose, wethers were fed the treated or untreated silages at 1.8-2.0% levels of dry matter per kg body weight as the sole ration. The results obtained were as follows;

1. Silages of good quality were obtained from both the treated and untreated fibrous residues. The treatment lowered the pH value and the fermentation acids and ammonia formation.
2. Digestibilities of crude protein and crude fat were significantly lower in feeding of the treated silage than in feeding of the untreated silage.
3. Urinary nitrogen excretion was significantly depressed by the treatment. Consequently, the nitrogen retention significantly increased in feeding of the treated silage.
4. The concentrations of ammonia and VFAs in the rumen fluid were significantly lower in feeding of the treated silage than in feeding of the untreated silage.
5. The concentration of blood urea-nitrogen was significantly lowered with feeding of the treated silage, but plasma total protein level was significantly higher in treated silage feeding than in untreated silage feeding.
6. The concentrations of most of amino acids in the blood plasma were significantly increased with feeding of the treated silage, especially plasma lysine level was about 3.5 times higher in treated silage feeding than in untreated silage feeding.

From the results mentioned above, it is concluded that the utilization of high moisture silage made from a fibrous residue of ladino clover was efficiently improved by addition of formaldehyde and formic acid before ensiling.

Introduction

In order to regulate the degree of fermentation during ensilage and improve the utilization coefficient of silage, a technique using additives, such as formaldehyde and/or formic acid, has been developed by many workers[7,31,35]. The addition of acids is very effective on protecting protein breakdown during ensilage by depressing proteolytic activities of plant and microbial enzymes. The early experiment has shown that neither plants nor the anaerobic

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microorganisms contain proteolytic enzymes capable of action below pH 4 and protein and amino acids can be preserved intactly by adding mineral acids[34].

Recently, the use of formic acid has spread because of its easiness of handling and increasing of supply. Formic acid can be used to reduce the degree of fermentation during ensiling, and will prevent clostridial activity when added to a herbage of low water-soluble carbohydrate content before ensiling[31,35]. Likewise, formaldehyde may be a suitable additive because it has bacteriostatic properties. In addition, formaldehyde may protect plant protein from microbial degradation in the rumen[7,18], and lead to increase total flow of amino nitrogen to the small intestine[3,15].

According to WILKINSON *et al.*[35], the addition of formaldehyde to fresh herbage before ensiling showed to restrict protein degradation and fermentation of soluble carbohydrate, but when an addition of formaldehyde was small quantity, a clostridial fermentation was induced considerably. They, therefore, suggested that the inclusion of a small quantity of acid reduces the risk of producing a clostridial fermentation, and for this reason formaldehyde/acid mixtures are preferred to the use of formaldehyde alone. VALENTINE and BROWN[30] reported that when formic acid was applied with formaldehyde, the ensiling fermentation was inhibited and dry matter intake and wool production of sheep increased compared to the only application of formaldehyde. WALDO *et al.*[32] obtained nearly complete protein protection during ensilage and significant increase of nitrogen retention, digestible-energy intake and weight gain in dairy heifers by treating forage with a mixture of formic acid and formaldehyde at ensiling.

Recently, FUJIHARA and OHSHIMA[10] reported that the ensiled fibrous residue obtained after the extraction of ladino clover leaf protein concentrate was utilized as a roughage as same as orchard grass hay in sheep. However, they also indicated that the utilizability of nitrogen was lower in feeding of the fibrous residue silage than in hay feeding, and this may be attributed to the production of ammonia during ensilage. The purpose of the present investigation was to compare the digestibilities and nitrogen balance of sheep offered an ensiled fibrous residue and a fibrous residue which had been ensiled with formaldehyde and formic acid.

Experimental

Animals and silages

Two Japanese Corriedale wethers, weighing about 25 kg, were used repeatedly. The silages were made from the fibrous residue obtained from ladino clover by the method of OHSHIMA and OOUCHI[24]. The fibrous residue only and the one sprayed with formaldehyde (0.85%/D.M.) and formic acid (2.8%/D.M.) were 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 of the silages is shown in **Table 1.**

Experimental procedure

The experimental animals were kept in metabolism cages throughout the experimental period. Five-day sampling period was preceded by 7-day preliminary period. Each wether was fed 1.8–2.0% as dry matter of diet per kg body weight per day, and water was avail-

able *ad libitum*. One-half of the daily ration was given at 9:00 hr and another half at 17:00 hr. Feces and urine were collected just before the morning feeding during sampling period. On the final day of each trial, about 50 ml of rumen contents were sampled using a stomach tube at 9:00, 10:00, 11:00, 12:00, 14:00 and 14:00 and 16:00 hr, and the pH value and the concentrations of ammonia and VFAs of rumen fluid were measured. Similarly, about 5 ml of Jugular blood was collected at 9:00, 12:00, 14:00 and 16:00 hr on the final day of each trial and urea-nitrogen, plasma total protein and plasma free amino acid were determined.

Chemical analysis

Nitrogen in the diet, feces and urine were analyzed by the Kjeldahl method, and the contents of crude fat, crude fiber and crude ash in the diet and feces were determined according to AOAC method[16]. Analyses of pH, lactic acid, volatile fatty acid and ammonia were carried out using cold water extracts of fresh silage samples as described in an earlier publication[23]. The water soluble nitrogen of the residue or silages was obtained by extracting the material with boiling water. Ammonia in the rumen fluid was analyzed by the method of HAWK *et al.*[14]. Ruminal VFAs were determined using a gas chromatography. Blood urea-nitrogen and plasma total protein were analyzed using the Unitest System (Model 300, Biodynamics, Inc., USA). The plasma was deproteinized by treating with picric acid according to the procedure described by STEIN and MOORE[29], and the free amino acids were determined by the ion exchange chromatography on automatic amino acid analyzer (Model KLA-5, Hitachi Co. Ltd., Tokyo).

Results

As shown in Table 1, by being treated with formaldehyde and formic acid, the general components of the residue were well preserved during ensiling. While in the untreated silage, contents of organic matter, crude protein, crude fat and crude fiber increased and NFE content decreased compared with the fresh residue on dry matter basis. The pH value was markedly reduced by the treatment with formaldehyde and formic acid.

Apparent digestibility and nitrogen balance of sheep fed the two silages are shown in **Table 2**. The digestibilities of crude protein and crude fat were significantly decreased in feeding of the treated silage. The urinary nitrogen excretion was signicantly reduced with feeding of the treated silage, and consequently, the nitrogen retention was significantly increased in feeding of the treated silage. The net protein utilization of silage was 32.6% in feeding of the treated silage, whereas it was −7.1% in feeding of the untreated silage.

Table 3 shows the ruminal pH and the concentrations of ammonia and VFAs in rumen fluid of sheep fed the silages. The concentration of ruminal ammonia markedly lowered by the treated silage feeding. Ruminal VFAs concentration was also significantly lower in feeding of the treated silage than in feeding of the untreated silage.

Table 4 shows the concentrations of some blood constituents of sheep after feeding of the two silages. Haematocrit value, although not significant, was slightly lower in feeding of the treated silage. The concentration of blood urea-nitrogen was significantly higher in untreated silage feeding than in treated silage feeding. Concentration of the plasma total protein was

Table 1. Chemical composition of fresh and ensiled residues[a]

	pH	Moisture	Organic matter	Crude protein	Crude fat	Crude fiber	Nitrogen free extract
Fresh residue	—	85.3	91.3[b]	19.7[b]	3.9[b]	26.8[b]	40.9[b]
Treated Silage	3.75	84.8	91.1	19.1	4.0	27.7	40.3
Untreated silage	4.30	85.6	89.8	21.8	4.7	31.4	31.9

Lactic acid	Formic acid	Acetic acid	Water soluble nitrogen	Ammonia nitrogen
—[b]	—[b]	—[b]	18.5[c]	0.2[c]
0.3	1.6	0.4	24.0	3.0
4.6	Trace	5.0	53.9	12.6

a Only trace amounts of propionic and butyric acids were found in both silages but the values were greater in the untreated one.
b % of dry matter.
c % of total nitrogen.

Table 2. Apparent digestibility and nitrogen balance

Silage	Untreated silage	Treated silage	Significance#
Apparent digestibility, %			
Organic matter	71.7±1.6[a]	69.6±0.4	NS
Crude protein	69.9±1.8	56.3±0.8	**
Crude fat	62.8±1.7	56.9±0.5	*
Crude fiber	71.6±1.4	70.6±1.3	NS
Nitrogen balance (g/kg B. $W^{0.75}$)			
Intake	1.51±0.14	1.44±0.03	
Fecal nitrogen	0.44±0.04	0.63±0.02	%
Urinary nitrogen	1.11±0.05	0.55±0.01	**
Retention	−0.04±0.11	0.26±0.01	**
	(−7.1±11.9)[c]	(32.6±1.4)	

Significance of difference between means within treatment. (* $P<0.05$, ** $P<0.01$)
a Mean of 2×2 trials±S. E.
b Nitrogen free extract.
c Values in parentheses are percent of digested nitrogen.

Table 3. Ruminal pH and the concentrations of ammonia and VFA in ruminal fluid of sheep fed the silage

	Untreated silage	Treated silage	Significance#
PH	6.95±0.05[a]	6.97±0.04	NS
Ammonia (mg/100 ml)	26.39±2.93	9.50±0.96	**
VFA (mM/100 ml)	6.48±0.41	5.23±0.23	*

Significance of difference between means within treatment. (* $P<0.05$, ** $P<0.01$)
a Mean±S. E. of 24 samples.

Table 4. Concentrations of blood urea-nitrogen and plasma total protein in sheep fed the silage

	Untreated silage	Treated silage	Significance#
Ht	30.05±2.17[a]	28.78±2.69	NS
Blood urea-nitrogen (mg/100 ml)	25.18±1.54	17.78±2.54	*
Plasma total protein (g/100 ml)	6.01±0.15	6.37±0.08	*

Significance of difference between means within treatment. (* $P<0.05$, ** $P<0.01$)
a Mean±S.E. of 16 samples.

Table 5. Plasma amino acid concentrations in sheep fed the silage (μm/100 ml)

Amino acid	Untreated silage	Treated silage	Significance#
Thr	12.3±0.8[a]	19.1±1.1	**
Val	24.3±1.5	28.1±1.6	NS
Met	0.6±0.1	0.9±0.1	NS
Ile	8.1±0.5	9.3±0.6	NS
Leu	11.3±0.6	14.3±0.9	**
Phe	4.2±0.3	4.8±0.5	NS
Lys	17.8±1.4	60.7±4.8	**
His	5.2±0.3	4.8±0.7	NS
Arg	10.3±0.6	6.9±0.5	**
Trp	Trace	Trace	
Asp+Asn	3.2±0.3	4.4±0.4	*
Ser	25.9±1.4	33.9±2.2	**
Glu+Gln	26.4±1.7	19.8±1.7	**
Gly	86.3±5.1	105.2±4.7	*
Ala	21.5±1.3	20.9±1.1	NS
Tyr	3.9±0.3	4.0±1.5	NS
Orn	6.3±0.6	5.9±0.5	NS
TAA	267.1±9.7	342.1±12.0	**
EAA	93.8±4.7	147.9±8.2	**
EAA/NEAA	0.55±0.02	0.77±0.04	**

Significance of difference between means within treatment. (* $P<0.05$, ** $P<0.01$)
a Mean±S.E. of 16 samples.

significantly increased with feeding of the treated silage.

As shown in **Table 5**, the concentrations of threonine, leucine, lysine, aspartic acid+asparagine, serine and glycine in the blood plasma were significantly increased with feeding of the treated silage. Especially, the plasma lysine level in treated silage feeding was about 3.5 times higher than that in untreated silage feeding. As a result, the concentrations of total amino acids and total essential amino acids were significantly high in feeding of the treated silage. On the other hand, the concentrations of arginine and glutamic acid+glutamine were significantly low in the blood plasma of sheep fed the treated silage. The ratio of essential to non-essential amino acid concentrations was also significantly improved with feeding of the treated silage.

Discussion

It is well known that the treatment of fresh herbage with formaldehyde and formic acid restricts the degradation of plant protein during ensiling[3,35]. In the present experiment, the fermentation during ensilage was extremely reduced by the treatment with formic acid and formaldehyde as indicated in Table 1. The low pH value of the treated silage can be attributed to the residual formic acid and the low ammonia content. The pH value of untreated silage used in the present study was very in agreement with that of our previous result[10]. The NFE content of untreated silage was much lower than those of the fresh residue and treated silage, and this might show a vigorous carbohydrate fermentation during ensiling of the untreated fibrous residue. During the fermentation, some loss of dry matter might occur because the contents of the other general components than NFE calculated on dry matter basis increased considerably. Gaseous loss during ensilage has been reported elsewhere[23,27].

The crude protein digestibility was significantly reduced by the additions of formaldehyde and formic acid in the present study. Yu Yu[37] reported that the digestibility of crude protein was lower in alfalfa silage treated with formaldehyde (7.7 g/100 g C. P.) than in the frozen alfalfa leaves when they were given to sheep. Although the amount of formaldehyde in the present study was 4.3 g/100 g C. P. which was lower than that of Yu Yu[37], the digestibility of crude protein showed the same tendency as described by Yu Yu[37]. Brown and Valentine[5] also reported that nitrogen digestibility was significantly lower for the lucerne silage treated with formaldehyde (1.0 g/100 g C. P.) than for the untreated silage when they were given to sheep *ad libitum*. On the other hand, according to Waldo *et al.*[33], the addition of 0.5% formaldehyde and 2.24% formic acid per dry matter, 0.85 and 2.5% respectively as same levels as in the present experiment, did not influence on the digestibility of crude protein of silage in dairy heifers.

In our previous paper[10], it is reported that a positive nitrogen balance was observed in sheep fed the fibrous-residue silage as the only feed, and suggested that the fibrous-silage would has similar nutritive value as a roughage as same as orchard grass hay. On the other hand, the low nitrogen retention has been reported in sheep on silage ration and is caused by the lower energy to nitrogen ratio of silage[5]. The negative nitrogen retention recorded for sheep fed the untreated silage in the present study was probably due to the submaintenance energy intakes and a relatively wide variation of silage intake among animals. Brown and Velentine[5] also reported that the nitrogen retention was −0.05 g/day per kg of metabolic body size in sheep fed the untreated lucerne silage *ad libitum*. As indicated in Table 2, the retention of nitrogen by sheep offered the silage treated with formaldehyde and formic acid was significantly higher than that of sheep offered the untreated silage. This might show that the protection of plant protein from microbial degradation during ensiling and ruminal digestion increases the nitrogen retention. Pike[28] also reported greater nitrogen retention in sheep fed the formaldehyde treated silage than in those fed the untreated silage.

The reduction of ruminal ammonia concentration in feeding of the treated silage was probably the result of reduced degradation of plant protein in the rumen. Barry and Fennessy[1] reported that the addition of formaldehyde (2.2–4.4% D. M.) to clover-dominant

herbage decreased nitrogen breakdown in the silage and reduced ammonia production *in vitro*. According to YU YU[37], the solubility of nitrogenous compounds in the rumen linearly decreased with an increase of formaldehyde added to silage from 0 to 20 g per 100 g of crude protein *in vitro*. It is known that some reduction of herbage protein solubility improves its utilization in ruminants by yielding a greater net absorption of amino acids at the intestine[12]. According to WILKINSON *et al.*[35], the addition of formaldehyde (4–6 g/100 g C. P.) to a low moisture silage increased the absorption of non-ammonia nitrogen and amino acids from the small intestine. Similarly, in the present experiment, it is probable from the ammonia concentration in the rumen that the nitrogen escaped from ruminal degradation and entered to the post-ruminal alimentary tract was more in feeding of the treated silage than in feeding of the untreated silage. This might reflect the low digestibility of dietary nitrogen in the treated silage feeding and the greater amino acid absorption in the lower gut as indicated in Table 2. The ruminal ammonia level in the treated silage feeding of this study was lower than that of FUJIHARA[11], in which the sheep was fed a hay at level of 1.4 g N/day per kg of metabolic body size. This might indicate that in feeding of the treated silage the microbial degradation of plant protein in the rumen was more reduced than that of hay feeding.

The concentration of ruminal VFAs was significantly lower in feeding of the treated silage than in feeding of the untreated silage, and this result shows that the carbohydrate fermentation in the rumen was reduced by addition of formaldehyde and formic acid to the silage. But the reduction rate of VFAs was much lower than that of ammonia. This difference can be attributed to both different concentration of ammonia between the two silages and the insolubility of residual protein in the treated silage, because the partial depression of microbial activity by formaldehyde may equally affect to VFAs formation and ammonia formation from natural protein. BARRY and FENNESSY[1] reported with sheep than in post-feeding samples the formaldehyde treatment before ensiling had no effect on VFAs concentration in the rumen fluid. From this, it is obvious that the rate of digestion of formaldehyde-treated silage was more stable than that of the untreated silage.

The blood urea-nitrogen concentration of sheep fed the untreated silage was significantly higher than that fed the treated. silage. It is known that the level of blood urrea-nitrogen concentration is dependent on ruminal ammonia adsorption which relate to the solubility of feed protein and on the amino acid balance of absorbed nitrogen from intestine. According to FUJIHARA and TASAKI[8,9], the ruminal ammonia concentration has become high when readily soluble protein was introduced into the rumen and it resulted in an increase of urinary nitrogen excretion. Similar tendency was reported by many workers[19,36]. While in experiments with growing rats and pigs, MUNCHOW and BERGNER[20] found a very high negative correlation between the biological value of feed and the blood urea level. This may also be true for proteins passed through rumen digestion. It has been shown in ruminant animals that the quality of absorbed nitrogenous compounds is reflected on the concentration of circulating blood urea-nitrogen[17]. The results obtained in the present study can be explained from the facts mentioned above. The first explanation is that the untreated silage contained more water soluble nitrogen than the treated silage as shown in Table 1 and the remaining proteins in the treated silage must have been reduced ruminal degradation by being reduced

their solubility and being increased their resistance to enzymatic attack[4]. The second explanation is that the amino acid composition of the hydrolysates which is not shown in this paper but was similar to those reported in a previous paper[26] was better in the treated silage than in the untreated silage. But which of the two reasons was more influential was not clear.

The level of plasma total protein after feeding of the untreated silage was very in agreement with that of our previous result[10], and was slightly lower than that generally accepted in mammals[13]. The significant increase in the level of plasma total protein after feeding of the treated silage probably related to the significant increase of plasma free amino acid concentration as shown in Table 5. According to MUNRO[21], the plasma protein might represent a vehicle of limited capacity for amino acid transport between the liver and other tissues. Therefore, it is considered that the high level of plasma protein after feeding of the treated silage would reflect the increase of concentration of free amino acids in plasma.

The concentration of total free amino acids in blood plasma was significantly higher after feeding of the treated silage than after feeding of the untreated silage. The value in feeding of the untreated silage was slightly higher than that reported by FUJIHARA and OHSHIMA[10] in which the sheep received the fibrous-residue silage at level of 1.8% as dry matter per kg of body weight per day. A significant increase of total free amino acids in blood plasma after feeding of the treated silage might reflect an increase of amino acid absorption in the small intestine. WILKINSON *et al.*[35] also reported that the amino acid absorption in the small intestine was increased by the treatment formaldehyde (6 g/100 g C. P.) in sheep fed a ryegrass silage. The plasma concentrations of lysine, threonine, leucine, glycine, serine and aspartic acid+asparagine were significantly higher and that of arginine and glutamic acid+glutamine were significantly lower in the treated silage feeding than in the untreated silage feeding. Of them, the largest difference was observed in lysine concentration which was 18 μmole/100 ml in the untreated silage feeding and 60 μmole/100 ml in the treated silage feeding. OHSHIMA and OOUCHI[24] studied on the nutritive value of ladino clover leaf protein for rats and reported that plasma concentrations of lysine, threonine and serine were significantly reduced by being added with methionine which was the first limiting amino acid of the protein. This fact means that the deficiency of methionine in diets accompanies the increase of lysine, threonine and serine in plasma. Furthermore, it is known that a great increase of the concentration of lysine accompanies a decrease of the concentration of arginine in plasma[38]. The 1st, 2nd and 3rd limiting amino acids of herbage protein for rats are methionine, lysine and threonine, respectively[24], and the tissue requirements for essential amino acids are qualitatively the same in ruminants as in mono-gastric animals[6], and it is also known that the first 3 limiting amino acids of rumen bacteria for ruminants are also methionine, lysine and threonine which were determined by feeding urea as the sole source of nitrogen to growing lambs[22]. During ensilage of untreated herbage, the degradation of some amino acids occurs and the rate is greater in lysine and threonine than in methionine[25], while the formaldehyde-formic acid treatment depresses the protein degradation during ensiling as is evident in Table 1. These facts suggest that the rate of methionine to lysine and threonine of amino compounds arrived in the gastro-intestinal tract might be greater in the untreated silage feeding than in

the treated silage feeding, which should be resulted in the different plasma concentrations of lysine, threonine, arginine and serine. In the sheep experiment intraperitoneal supplementation with methionine increased liveweight gain and wool growth on both untreated and formaldehyde-treated silages and the best performance was obtained in the sheep fed the treated silage and injected methionine[2]. The plasma concentrations of both the total essential non-essential amino acids were significantly higher after feeding of the treated silage than after feeding of the untreated silage. This result might be attributed to the different quality and volume of nitrogen arrived into the intestine. The ratio of essential to non-essential amino acids was greater in sheep fed the treated silage than in those fed the untreated silage reflecting the extremely higher concentration of lysine of the former.

From the results obtained in the present experiment, it is concluded that the addition of formaldehyde and formic acid to high-moisture fibrous-residue at ensiling is very effective on preserving plant components and controlling the microbial protein breakdown in the rumen, and improves the protein utilization of silage in sheep.

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フオルムアルデヒドおよびギ酸を添加したラジノクローバ緑葉蛋白抽出残渣サイレージのヒツジにおける利用性について

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要 約

ラジノクローバ緑葉蛋白の抽出後，得られた残渣にフオルムアルデヒドとギ酸をそれぞれ乾物当たり 0.85%，2.8% 添加してサイレージを調製し，それらの添加物処理がサイレージの品質および栄養価に及ぼす影響について検討するため，去勢ヒツジを用いて代謝試験を行い，次の様な結果を得た。なお，同一材料を用いて無添加のサイレージを調製し，対照とした。

1. 添加・無添加サイレージ共良質のものであったが，特にフオルムアルデヒド・ギ酸添加によってアンモニアの生成が抑制され，pH 値は低くなった。

2. 粗蛋白質および粗脂肪の消化率は，添加サイレージ給与時では，無添加サイレージ給与時よりも有意に低下した。

3. 尿中窒素排出量は添加サイレージ給与の場合有意に減少し，その結果，窒素の体内蓄積量は有意に増加した。

4. 第1胃内液中のアンモニアおよび VFA 濃度は，添加サイレージ給与時では，無添加サイレージ給与時よりも有意に低下した。

5. 血中尿素態窒素濃度は添加サイレージ給与時では有意に低下したが，一方血漿中総蛋白濃度は無添加サイレージ給与時に比して有意に高くなった。

6. 血漿中遊離アミノ酸は添加サイレージ給与時に増加したが，特にリジンの増加が著しく，その値は無添加サイレージ給与時の約 3.5 倍であった。

これらの結果から，ラジノクローバ緑葉蛋白抽出残渣を用いた高水分サイレージの利用性は，調製前のフオルムアルデヒドおよびギ酸処理によって有意に改善されることが明らかになった。

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