アルファルファサイレージの消化性およびルーメン内分解性に及ぼす水酸化ナトリウム処理あるいはアンモニア処理の影響

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Effect of Sodium Hydroxide or Ammonia Treatment on Digestion and Rumen Degradation of Alfalfa Silage in Goats

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Synopsis

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Three rumen cannulated goats were used to investigate the effect of NaOH and NH_3 treatment (2.74% DM) at ensiling on digestion, nitrogen utilization and *in situ* protein degradation of low moisture alfalfa silage. Wilted alfalfa was sprayed with water (control), NaOH or NH_3 solution, and fed to goats after 6 months storage in a 3×3 Latin square design. Both alkali treatments increased the pH value, NH_3-N and butyric acid of the silage, but NH_3-treated silage preserved as much amino acids as control silage. The two alkali treatments could not improve digestibilities of fibrous components (NDF, ADF and cellulose), and NaOH addition significantly reduced the digestibility of crude protein. The NaOH treatment decreased the rate of N degradation in the rumen, while solubility and potential degradability was not affected by the treatment. The NH_3 addition increased ruminal soluble nitrogen in the silage, but rate of N degradation was similar to that of control silage. These results suggest that NaOH treatment would make protein less susceptible to microbial degradation in the rumen despite of deteriorating the fermentation quality of the silage.

Key words: Alfalfa, Alkali treatment, Digestion, Goat, Ruminal degradability, Silage.

Introduction

Sodium hydroxide and ammonia treatment have practically been made with an intention of improving the nutritive value of low quality forage such as cereal straw[2], late cut grass[1] and legume crop[7,10]. In addition to the primary benefits, NH_3 treatment could give a greater stability of preservation by reducing fungal growth of hay[12,23] and proteolysis during ensilage[9,13]. Addition of NaOH to barley grain was also reported to make more stable storage[18].

Recent researches suggested that these treatments can be applicable to manipulate the degradation of DM and N in the rumen. ROBINSON and KENNELLY[21] found that ammoniation of barley grain reduced the rate of starch degradation in the rumen. MIR *et al.*[15] and WALTZ and LOERCH[22] reported that NaOH treatment of soybean meal lowered both the rate and extent of ruminal protein degradation and improved N retention of ruminants. Since legume protein

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was found to be degraded at a faster rate and a greater extent than grass protein[20], alkali treatment could improve the nutritive value of legume crop by reducing protein degradation and enhancing fiber digestion in the rumen. Our previous study[17] indicated that the NaOH treatment of alfalfa at ensiling depressed ruminal solubility and potential degradability of protein were decreased in spite of increasing the NH_3-N content of the silage. Silage quality would possibly be improved when used low moisture material. Therefore, this experiment was carried out to investigate the effect of NaOH and NH_3 treatment at ensiling on fermentation quality, digestibility and *in situ* degradation of low moisture alfalfa silage using goats.

Materials and Methods

1. Silage preparation

Second cutting of alfalfa (*Medicago sativa* L) grown at the Farm of Nagoya University was harvested at full blooming stage on June 26, 1991. It was wilted for about 24 h with a target of 60 % moisture content. The wilted herbage was packed into polyethylene bags after spraying water (control), NaOH or NH_3 solution. The amount of additional water was almost the same among the treatments. Both NaOH and NH_3 were added at 3 % (DM basis) of wilted material, assuming its DM content was 40%. Bags were stored in a dark room at ambient temperature for about 6 months until animal trial has been started.

2. Animal trial

Three Japanese pigmy castrated growing goats weighing about 14.7 kg were used. They were fitted with permanent rumen cannulae and kept individually in metabolism crates throughout the experiment. Diets used were control, NaOH-treated and NH_3-treated silages and fed to goats at a level of 45 gDM/$BW^{0.75}$/day in two equal meals at 08 : 00 and 20 : 00 h. Water and mineral block were freely accessible at all times. The experiment was designed in a 3 ×3 Latin square and lasted 48 days with three experimental periods. The first 7 days were employed as diet adaptation and following 5 days as fecal and urinary collection. On day 13, samples of rumen fluid were taken by vacuum extraction through the cannula just before (0 h) and at 1, 2, 4, 12 and 24 h after morning meal. Samples were strained through two layers of cheesecloth and a portion was acidified with 25% metaphosphoric acid, centrifuged at 3000 rpm and frozen for NH_3-N analysis. At the time of first sampling, 7 ml Cr-EDTA solution containing 19.4 mg Cr was administered through the cannula to measure the outflow rate of rumen liquid. On day 14, degradability of DM and N was determined by *in situ* bag technique. Nylon bags, each having 42 μm pore size and containing 3 g freeze dried sample, were inserted into the rumen of goats at 08 : 00 h and removed after 3, 6, 12, 24 and 48 h incubation. The bags removed from the rumen were washed for 5 minutes, dehydrated using a domestic washing machine, and then dried to a constant weight in a draft oven at 60 ℃.

3. Analyses

Dry matter was determined by freeze drying for silage and by oven drying at 60℃ for feces. Chemical composition and fermentation quality of silage were determined in the same manner described previously[16]. Amino acids analysis was performed on an acid hydrolysate of freeze dried sample using an automated amino acid analyzer (JEOL, JLC-300). Hydrolysis was made in a sealed tube with 6 N HCl at 110℃ for 22 h. Allantoin in urine was determined

by the method of CHRISTMAN *et al.*[5]. Chromium concentration in rumen fluid was determined by atomic absorption spectrophotometry (Shimadzu, AA-646), and liquid outflow rate was calculated as the slope of the natural logarithm of Cr concentration on time postdosing.

The data from degradation of DM and N in the nylon bags were fitted to the exponential equation $p=a+b\ (1-e^{-ct})$, recommended by ØRSKOV and MCDONALD[19] using non-linear regression analysis (NLIN) procedure of SAS[24], where p is the percentage of DM or N disapearance, t is time and a, b and c are constants of the equation. Results from silage quality, digestibility, nitrogen balance and parameters describing the degradation characteristics were subjected to analysis of variance using the general linear model (GLM) procedure of SAS. Statistical significance among treament means was detected by DUNCAN's multiple range test.

Results

1. Chemical composition and fermentation quality of silage

Dry matter content of the wilted material was 43.8%, which was close to the initial target value of 40 %. The fact indicated that the actual level of alkali application was 2.74% on DM basis. Addition of NaOH decreased organic matter and that of NH_3 increased crude protein of the silage (Table 1), while the NDF, ADF, ADL and cellulose contents were not affected by the treatment.

The quality of the control silage was not so good as being expected. It contained much more acetic acid (16.0 % DM) than lactic acid (1.11 % DM), and the percent of NH_3-N to Total-N was above 20% (Table 1). Addition of NaOH at ensiling greatly enhanced clostridial fermentation in the silo. The silage showed an alkaline pH and contained nearly 9 % (DM basis) of

Table 1. Chemical composition and fermentation quality of control, NaOH-treated and NH_3-treated silages.

Item	Control	NaOH-treated	NH_3-treated	S.E.
Chemical composition				
Dry matter (%)	42.2	41.8	42.9	0.78
Organic matter (% DM)	87.4^{a}	83.9^{b}	87.4^{a}	0.46
Crude protein (% DM)	20.9^{b}	20.5^{b}	24.9^{a}	0.78
NDF (% DM)	44.0	43.9	44.3	0.44
ADF (% DM)	39.1	39.1	39.2	0.33
ADL (% DM)	8.97	8.59	8.95	0.24
Cellulose (% DM)	30.1	30.6	30.3	0.32
Silage quality				
pH	5.33^{c}	8.30^{a}	6.95^{b}	0.27
Lactic acid (% DM)	1.11^{a}	0.02^{b}	0.62^{ab}	0.20
Acetic acid (% DM)	16.0^{a}	5.21^{b}	6.26^{b}	1.80
Propionic acid (% DM)	1.29	1.26	0.74	0.21
iso-Butyric acid (% DM)	0.11^{b}	0.68^{a}	0.20^{b}	0.08
n-Butyric acid (% DM)	0.60^{b}	8.92^{a}	4.06^{b}	1.28
NH_3-N (% Total-N)	20.7^{c}	31.4^{a}	28.2^{b}	0.93

Means of 3 observations. Values in the same row with different superscript letters are significantly different ($P<0.05$).

Table 2. Total amino acid contents (mg/g DM) of control, NaOH-treated and NH_3-treated silages.

Amino acids	Control	NaOH-treated	NH_3-treated	S.E.
Asp	12.1	11.5	11.8	0.40
Thr	5.09	4.72	5.27	0.18
Ser	4.88[b]	4.92[b]	5.64[a]	0.12
Glu	11.6[b]	11.4[b]	14.9[a]	0.51
Pro	8.12[a]	5.72[b]	7.96[a]	0.25
Gly	10.1[a]	6.59[b]	10.5[a]	0.17
Ala	18.3[b]	7.49[c]	31.6[a]	2.33
Val	11.6[a]	7.35[b]	11.4[a]	0.22
Ileu	8.64[a]	5.80[b]	8.35[a]	0.19
Leu	13.0[b]	10.2[c]	14.2[a]	0.24
Tyr	3.23[b]	3.67[a]	3.88[a]	0.07
Phe	6.87	6.81	9.16	0.69
His	2.76[a]	2.06[c]	2.37[b]	0.09
Lys	10.3[a]	6.77[b]	9.92[a]	0.19
Arg	3.94	4.14	4.50	0.16

Means of 3 determinations. Values in the same row with different superscript letters are significantly different ($P<0.05$).

butyric acid, and about 1.5 times as much NH_3-N as control silage. Ammonia addition also increased butyric acid, but the extent was relatively small so that the silage showed an acetate type fermentation. The NaOH addition significantly reduced glycine, alanine, valine, isoleucine, leucine, histidine, lysine and proline contents of the silage (Table 2). In contrast, NH_3 addition caused less amino acids breakdown than control silage in spite of the enhanced clostridial fermentation. The silage showed the highest value on serine, glutamic acid, alanine, leucine and tyrosine contents.

2. Digestion trial

Digestibilities of DM and OM were not changed by any alkali treatments (Table 3). However, digestibility of crude protein significantly decreased from 73.5% for control to 69.5% for NaOH-treated silage. The NH_3-treated silage showed similar protein digestibility to control silage. Neither NaOH nor NH_3 treatment could improve digestibilities of fibrous components (NDF, ADF and cellulose).

When goats fed NaOH-treated silage, they excreted more N than goats on control silage and showed negative N retention (Table 3). The goats given NH_3-treated silage excreted more N in feces and urine than those given the other silages. Nevertheless, no significant difference was observed in N retention because urinary N excretion were somewhat fluctuated during the experimental periods. Allantoin excretion in urine, determined as an indicator of microbial protein production in the rumen, was not different among the treatments. All goats excreted around 65 mg/kg $BW^{0.75}$/day.

Considerably high ruminal NH_3-N concentrations were observed in all the tretments (Fig. 1), whose peak values found at 1 h after feeding were above 50 mgN/dl. Although goats fed NaOH-treated silage received more NH_3-N than those fed control silage, they expressed lower

Table 3. Digestibilities of nutrients, nitrogen balance and urinary allantoin excretion by goats given control, NaOH-treated and NH_3-treated silages.

Item	Control	NaOH-treated	NH_3-treated	S.E.
Digestibility (%)				
Dry matter	56.4	56.1	56.0	0.61
Organic matter	58.7	57.1	57.9	1.04
Crude protein	73.5[a]	69.5[b]	74.8[a]	0.84
NDF	42.9	44.7	43.6	1.34
ADF	48.3	47.7	48.5	1.36
Cellulose	60.2	60.3	59.7	1.10
Nitrogen balance (g/kg $BW^{0.75}$/day)				
Intake N	1.52[b]	1.48[b]	1.80[a]	0.07
Fecal N	0.40[b]	0.45[a]	0.45[a]	0.01
Urinary N	1.02[b]	1.05[b]	1.31[a]	0.06
Retained N	0.10	−0.02	0.04	0.07
Allantoin (mg/kg $BW^{0.75}$/day)	61.8	63.7	69.9	7.81

Means of 3 goats. Values in the same row with different superscript letters are significantly different ($P<0.05$).

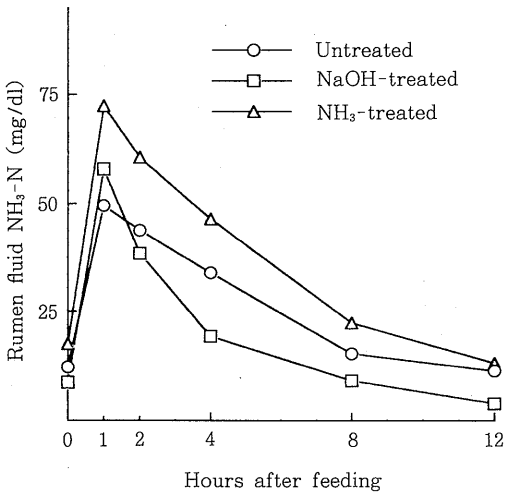

Fig. 1. Changes in rumen fluid NH_3-N concentrations of goats fed untreated, NaOH-treated and NH_3-treated alfalfa silages.

ruminal NH_3-N concentration except at 1 h after feeding. When goats were given NH_3-treated silage, they consistently showed the highest concentrations at every sampling time. Mean values for liquid outflow rate from the rumen in goats fed control, NaOH-treated and NH_3-treated silages were, 4.66, 6.65 and 7.24 %/h, respectively, and the latter two values were significantly greater than the former value.

Degradation characteristics of DM and N in the rumen are summarized in Table 4. The

soluble fraction of DM, which was 30.9% in control silage, slightly increased when treated with NaOH and NH_3. The NaOH addition tended to decrease the rate of DM degradation in the rumen, but NH_3 addition had little influence on it. Although the badly preserved NaOH-treated silage contained much more NH_3-N than control silage, the soluble N fraction in the rumen of the two silages were not different. The fraction increased with NH_3 treatment because some parts of NH_3 added to alfalfa would be easily released in the rumen. Although the NaOH-treated silage was significantly lower in protein digestibility than the other two silages (Table 3), they showed similar potential N degradability. Rate of N degradation greatly decreased by the NaOH treatment but the difference was not significant. The NH_3 treatment had no effect on the rate of N degradation.

Discussion

Alkali treatment have been made primarily to improve the digestibility of fibrous components, but this study indicated that the treatment could not improve the digestibility of fibrous components of alfalfa silage. Some evidence showed beneficial effects of alkali treatment on digestibility of alfalfa[7,10], whereas in our previous experiment[17] both NaOH and NH_3 treatment decreased the ADF and cellulose digestibility. CHESSON *et al.*[4] and KONDO *et al.*[11] reported that the alkali labile lignin-carbohydrate complexes are different in their characteristics between grass and legume crop, and the complexes of grass greatly interfered with enzymatic digestion compared with that of legume crop.

Both NaOH and NH_3 added to alfalfa buffered the pH fall and enhanced clostridial fermentation in the silo. Similar results were obtained in our previous study[17] using alfalfa ensiled at 36.9 % DM and treated with 2.44 % NaOH and NH_3. FLIPOT *et al.*[6] found an increase of lactic acid and a decrease of NH_3-N when alfalfa was ensiled at 41 % DM and treated with 3% NaOH, but an inverse effect was observed when ensiled at 34 % DM. Although high DM (43.8%) alfalfa was used in this experiment, the silage failed to achieve good fermentation.

The enhanced amino acids breakdown in NaOH-treated silage indicated a high activity of

Table 4. Parameters for the exponential equation $P=a+b\ (1-e^{-ct})$ used to describe the degradation characteristics of dry matter and nitrogen of control, NaOH-treated and NH_3-treated silages[1].

Item	Control	NaOH-treated	NH_3-treated	S.E.
Dry matter				
Soluble fraction (%)	30.9	32.1	34.6	3.27
Degradable fraction (%)	35.9	37.8	34.0	2.91
Rate of degradation (h^{-1})	0.116	0.103	0.123	0.012
Nitrogen				
Soluble fraction (%)	63.4	63.8	66.3	5.38
Degradable fraction (%)	26.6	26.7	24.5	5.21
Rate of degradation (h^{-1})	0.225	0.176	0.221	0.050

1) P=disappearance of dry matter or nitrogen at time t (%), a=soluble or rapidly degradable fraction (%), b=slowly degradable fraction (%), c=rate of degradation of fraction b (h^{-1}).

amino acids catabolism by clostridia under high pH condition. However, the NH_3-treated silage, which also showed an enhanced clostridial fermentation, preserved as much amino acids as control silage. Many evidence have shown that NH_3 treatment could decrease the proteolysis during ensilage[7,9,12], even when butyrate fermentation occurred[8]. The significant increase of alanine by NH_3 treatment agreed with ammoniated corn silage reported by JOHNSON *et al.*[9]. They stated the increase could be ascribed to a decarboxylation of aspartic acid, transamination of pyruvic acid or microbial synthesis from NH_3, while the mechanism was not known in detail.

The NaOH treatment tended to depress the rate of N degradation and significantly increased the outflow rate of rumen liquid, suggesting the increase of dietary protein reached the abomasum. But the low digestibility shown in Table 3 suggests that the NaOH treatment reduced the intestinal digestion of protein. A reduction of protein digestibility caused by NaOH treatment was also found in our previous study[17]. But MIR *et al.*[14] did not find any adverse effects on protein digestibility of soybean treated with 3% NaOH.

WALTZ and LOERCH[22] reported a protection of protein from degradation in the rumen by NaOH treatment, which involved reductions of both solubility and rate of degradation of protein. Although the rate of N degradation was reduced by treating with NaOH at ensiling, N solubility in the rumen was not affected. This result may relate to the postprandial change of ruminal NH_3-N concentration in goats fed NaOH-treated silage. The concentrations were unexpectedly similar to that of goats receiving control silage. BRODERICK *et al.*[3] stated that a possible mechanism of protein protection by NaOH treatment would be crosslinking reactions such as the formation of lysinoalanine. Lysinoalanine was actually found in NaOH-treated silage. But the content was only 74.1 mg in 100 g protein and detected only from one bag silo, so that the amino acid could not have major role on protein degradation in this study.

This experiment indicated many negative effects of NaOH treatment on alfalfa silage. But some other experiments recently made in our laboratory suggested the NaOH treatment could have beneficial effects on both preservation and rumen degradation of protein. There it seems neccessary to succeed such experiments to understand the mechanism of protein modification or deterioration by NaOH treatment.

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アルファルファサイレージの消化性およびルーメン内分解性に及ぼす水酸化ナトリウム処理あるいはアンモニア処理の影響

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要　　約

サイレージ調製時の NaOH 処理および NH_3 処理が低水分アルファルファサイレージの消化性，窒素利用性およびルーメン内分解性に及ぼす影響について，ルーメンフィステルを装着したシバ山羊 3 頭を用いて検討した。予乾したアルファルファに水（対照），NaOH および NH_3 溶液を噴霧してサイレージを調製し，6 ヶ月後に開封して 3×3 ラテン方格法に基づく消化試験に供した。

いずれのアルカリ処理もサイレージの pH を高め，NH_3-N および酪酸を増加させたが，NH_3 処理サイレージでは対照サイレージと同程度のアミノ酸が保存されていた。いずれのアルカリ処理も繊維成分（NDF，ADF およびセルロース）の消化率を高めることはできなかった。また，NaOH の添加は粗蛋白質の消化率を有意に低下させた。

NaOH 処理は，ルーメン内における窒素の溶解性および潜在的分解性には影響しなかったが，分解速度を低下させた。NH_3 の添加は窒素の溶解性を高めたが，分解速度の値は対照サイレージの値と同様であった。これらの結果から，NaOH 処理はサイレージの発酵品質を著しく劣質化させるが，蛋白質のルーメン微生物による分解を抑制することが示された。

キーワード：アルファルファ，アルカリ処理，サイレージ，消化性，ルーメン内分解性，山羊.

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