DIGESTION OF ALKALI-TREATED ALFALFA SILAGE BY GOATS

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Summary

First crop of alfalfa (*Medicago sativa* L.) was harvested, wilted and ensiled with or without NaOH or NH_3, and fed to three rumen fistulated goats in a 3 × 3 Latin-square design. Each alkali treatment (2.44% of alfalfa dry matter) was made by spraying its solution prior to ensiling. Silage pH, NH_3-N and butyric acid concentration were increased with each alkali addition, and NaOH-treated silage showed the lowest chemical quality. Compared with untreated silage, digestibilities of organic matter, ADF and cellulose were depressed by both alkali treatments, and the reductions in NaOH-treated silage were significant. Crude protein digestibility was also significantly decreased in NaOH-treated silage, but the goats receiving the silage excreted less nitrogen in urine than those on the other two silages. Nitrogen retention of goats was not different among the treatments. Ruminal solubility and potential degradability of dry matter and nitrogen determined with the *in situ* bag technique were reduced, and rate of degradation of the two components were increased by the NaOH treatment. Addition of NH_3 provided ruminal soluble nitrogen to the silage, but the rate of degradation was similar to that of untreated silage. These results suggest that NaOH treatment would denature the protein and reduce the susceptibility to microbial degradation in the rumen, while no positive effect of alkali treatment on fiber digestion and nitrogen utilization was observed in this study.
(**Key Words**: Alfalfa, Alkali Treatment, Goat, Ruminal Degradability, Silage)

Introduction

Upgrading of low quality forage with alkali treatment has received a great deal of attention. Sodium hydroxide and ammonia are two representative chemicals, which increase digestibility of lignocellulosic components (Wanapat et al., 1985). In addition, ammonia treatment provides a nitrogen source for animals, decreases moldy loss of hay (Knapp et al., 1975; Yahara and Numakawa, 1978) and inhibits proteolysis during ensilage (Johnson et al., 1982; Kung et al., 1984).

Fiber content of alfalfa is normally less than that of ryegrass. Low fiber content of forages means less effective rumen fill and leads to an increase of voluntary intake of high forage diets (Mertens, 1987). However, digestibility of fibrous components of legumes is not so high because the stem has highly lignified cellulose microfibrils (Smith et al., 1972). The facts suggest that energy release from the components for rumen microbes is relatively slow and does not balance with nitrogen which is provided from degradation of protein. When the rate and extent of fiber digestion are increased by alkali treatment, nitrogen retention is also expected to be increased.

The objective of this experiment was to study the effects of alkali treatment of alfalfa at ensiling on its digestibility and degradation characteristics in the rumen.

Materials and Methods

Preparation of silage

First crop of alfalfa (*Medicago sativa* L.) grown at the Farm of Nagoya University was harvested at early blooming stage on April 24, 1990, and wilted for about 24 hours with a target of 70% moisture content. The wilted herbage was packed into polyethylene bags at a rate of 15 kg per bag directly or after being sprayed with NaOH or NH_3 solution. Both NaOH and NH_3 were added at a level of 3% (w/w) of dry matter (DM) which was assumed 30% in the wilted material. The pH values of the solution were 13.10 and 13.08, respectively. All bags were preserved in a dark room at ambient temparature

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for about 6 months until the start of animal trial.

Animal trial

Three Japanese pygmy castrated mature goats weighing about 23 kg were arranged in a 3 × 3 Latin-square design. They were equipped with permanent rumen fistulae and individually housed in metabolism cages. Each goat was given the untreated, NaOH-treated or NH_3-treated alfalfa silage at 2% (DM basis) of the body weight daily in two equal meals at 08:00 and 20:00 hours. Sodium chloride was offered with the morning meal at a rate of 66 mg per kg of body weight. Water was freely accessible at all times. Each experimental period consisted of 15 days. The first 7 days were assigned for diet adaptation and the following 5 days for fecal and urinary collection. On day 13, 12 ml of Cr-EDTA solution containing 33.2 mg of chromium were introduced into the rumen through the fistula just before the morning meal to estimate the outflow rate of liquid phase from the rumen. Samples of rumen fluid were taken just before (0 hour) and at 1, 2, 4, 8, 12 and 24 hours after the morning meal. On day 14 and 15, ruminal degradability of DM and nitrogen was determined using the *in situ* bag technique. Nylon bags (10 × 6 cm) with a pore size of 42 μm were placed in the rumen of goats. Each bag contained 3 g of sample which was freeze dried and ground through 5 mm screen. Bags were removed from the rumen at 3, 6, 12, 24 and 48 hours after incubation. After rinsing in cold running tap water and squeezing by hand, bags were dried in a draft oven at 60℃ for 16 hours.

Analyses

Dry matter content was determined by freeze drying for silage samples and by oven drying at 60℃ for fecal samples. Analyses of nitrogen, ash, NDF, ADF and ADL were made with dried samples as described previously (Nishino et al., 1992). The pH value and NH_3-N, lactic acid and volatile fatty acid contents of silage and rumen fluid were determined by the methods reported previously (Nishino et al., 1992). Chromium concentration in the rumen fluid was determined by atomic absorption spectroscopy (Shimadzu, AA-646). Liquid outflow rate from the rumen was estimated as the slope of the natural logarithm of Cr concentration on time postdosing. Degradation characteristics of DM and nitrogen were described by the following exponential equation (Ørskov and McDonald, 1979) $p = a + b\,(1-e^{-ct})$, where p is proportion of DM or nitrogen disappeared at time t, a is soluble or rapidly degradable fraction, b is slowly degradable fraction and c is the rate of degradation of fraction b. Estimation of parameters was carried out by fitting the data to the above equation using non-linear regression analysis (NLIN) procedure of SAS (1985). Results were subjected to analysis of variance and statistical significance among treatment means was determined by Duncan's multiple range test.

Results and Discussion

As shown in table 1, dry matter content of untreated silage was 36.9%, which shows the level of the alkali application was actually 2.44% on DM basis. The additon of NaOH decreased organic matter content and NH_3 addition increased crude protein content of the silages. Compared to untreated silage, NaOH treated one showed significantly higher contents of NDF, ADF, ADL and cellulose. The NH_3-treated silage also showed higher contents of those components, but the differences were not significant. There were some reports that alkali treatment increased hemicellulose solubility and decreased cell wall constituents (Klopfenstein et al., 1972; Itoh et al., 1975; Braman and Abe, 1977), while the data obtained in this study were different from those results. These facts could be ascribed to changes of nitrogen distribution in silage by alkali treatments. Both neutral and acid detergent insoluble nitrogen (NDIN and ADIN) were increased by the treatments. Although these components of NH_3-treated silage were less than those of untreated one when expressed as proportion to total nitrogen (table 1), the values based on DM (data are not shown) indicated significantly higher contents in NH_3-treated silage. It is considered that a nitrogen insoluble in neutral detergent but soluble in acid detergent (NDIN-ADIN) represents fiber bound protein and ADIN is lignified nitrogen (Van Soest, 1982).

The untreated silage could be classified as a moderate quality with a dominance of lactic acid in organic acids, only a bit of butyric acid and slightly high NH_3-N (13.8% TN) concentrations.

TABLE 1. CHEMICAL COMPOSITION AND FERMENTATION QUALITY OF UNTREATED, NaOH-TREATED AND NH_3-TREATED ALFALFA SILAGES[1]

Item	Untreated	NaOH treated	NH_3-treated	S.E.[2]
Chemical composition				
Dry matter (%)	36.9	31.9	33.6	1.77
Organic matter (% DM)	85.8^{a}	82.2^{b}	85.7^{a}	0.62
Crude protein (% DM)	22.4^{b}	22.4^{b}	28.5^{a}	0.43
NDF (% DM)	30.7^{b}	34.2^{a}	32.2^{b}	0.69
ADF (% DM)	29.2^{b}	31.5^{a}	30.0^{b}	0.45
ADL (% DM)	7.00^{b}	7.93^{a}	7.50^{ab}	0.09
Cellulose (% DM)	22.2^{b}	23.6^{a}	22.5^{ab}	0.39
NDIN[3] (% Total-N)	12.3^{b}	15.8^{a}	10.9^{c}	0.42
ADIN[4] (% Total-N)	7.13^{b}	9.00^{a}	6.78^{b}	0.12
Silage quality				
pH	4.63^{b}	6.32^{a}	5.54^{ab}	0.39
Lactic acid (% DM)	6.77^{a}	3.03^{b}	5.76^{ab}	1.18
Acetic acid (% DM)	3.06^{a}	3.52^{a}	4.18^{a}	0.44
Propionic acid (% DM)	0.07^{b}	1.02^{a}	0.25^{b}	0.26
iso-Butyric acid (% DM)	n.d[5]	0.28	0.04	0.09
n-Butyric acid (% DM)	0.03^{b}	5.07^{a}	1.26^{ab}	1.48
NH_3-N (% Total-N)	13.8^{b}	29.7^{a}	33.5^{a}	4.35

[1] Means of 4 observations. Values in the same row with different superscript letters are significantly different ($p < 0.05$).

[2] Pooled standard error.

[3] Neutral detergent insoluble nitrogen.

[4] Acid detergent insoluble nitrogen.

[5] Not detected.

Addition of NaOH significantly decreased lactic acid, and increased butyric acid and NH_3-N concentrations of the silage. Similar effects were observed in NH_3-treated silage, but the extents were relatively small and the silage still showed a lactate type fermentation. These results suggest that although both NaOH and NH_3 added to alfalfa acted as buffering components, the capacity was greater in NaOH than NH_3. Some reports indicated that NH_3 addition could modify a silage fermentation through enhancing lactic acid production and depressing clostridial growth (Johnson et al., 1982; Kung et al. 1984, 1989). The different results may be attributable to a higher rate of NH_3 applied in this study (2.44% DM) than those done in other experiments (around 1% DM).

Results of digestion and nitrogen balance trial are given in table 2. Digestibility of dry matter was not significantly different for all the silages, whereas it tended to be decreased by both alkali treatments. Compared with untreated silage, significantly lower digestibilities of organic matter, crude protein, ADF and cellulose were observed with NaOH treated silage. Similarly, NH_3 treatment decreased organic matter, ADF and cellulose digestibilities of the silage, but the differences were not significant except for cellulose digestibility. Digestibility of NDF was not affected by each alkali treatment.

As far as the authors know, there is no report on the reduction of crude protein digestibility by NaOH treatment of forage. The increases of NDIN and ADIN as mentioned above could partly explain the changes of nitrogen digestibility by the NaOH treatment. Van Soest (1982) stated that NDIN was less digestible than soluble N in the detergent and ADIN was indigestible nitrogen. Negative effects of NaOH treatment on ADF and cellulose digestibilities were also specifically observed in this experiment. Numerous researches showed that alkali treatment increased digestibility of cell wall constituents of cereal straw (Braman and Abe, 1977; Garrett et al., 1979), grass (Brown 1988; Ben-Ghedalia et al. 1988) and legume (Klopfenstein et al., 1972; Canale et al., 1988), but a lesser effect on digestibility could be expected for legume crops (Klopfenstein et al., 1972). Gordon (1975) and Kondo et al. (1990) reported different characteristics of alkali labile lignin-carbohydrate complexes between grass and legume, and found a greater interferance with enzymatic degradation in grass lignin. Besides the above facts, the lower fermentation quality of NaOH- and NH_3-treated silages may have influence on the negative effects of the alkali treatments on ruminal fiber digestion.

TABLE 2. DIGESTIBILITIES OF SOME NUTRIENTS AND NITROGEN BALANCE IN GOATS GIVEN UNTREATED, NaOH-TREATED AND NH_3-TREATED ALFALFA SILAGES[1]

Treatment	Untreated	NaOH-treated	NH_3-treated	S.E.[2]
Digestibility (%)				
Dry matter	67.2	62.3	65.0	1.63
Organic matter	71.6^{a}	65.7^{b}	69.3^{ab}	1.32
Crude protein	76.3^{a}	71.1^{b}	78.3^{a}	1.17
NDF	53.3	53.3	52.5	1.52
ADF	59.4^{a}	55.3^{b}	57.1^{ab}	1.13
Cellulose	73.9^{a}	71.9^{b}	72.2^{b}	0.38
Nitrogen balance (g/day kg $BW^{0.75}$)				
Intake N	1.66^{b}	1.49^{b}	2.15^{a}	0.09
Fecal N	0.39^{b}	0.43^{ab}	0.46^{a}	0.02
Urinary N	1.01^{b}	0.79^{c}	1.18^{a}	0.04
Retained N	0.26	0.27	0.51	0.11

[1] Means of 3 goats. Values in the same row with different superscript letters are significantly different ($p < 0.05$).
[2] Pooled standard error.

Compared with goats receiving untreated silage, those on NaOH-treated one excreted less urinary nitrogen despite higher NH_3-N concentration in the silage. The mean proportions of urinary to digested nitrogen in goats fed untreated, NaOH treated and NH_3-treated silages were 79.5, 74.5 and 69.8%, respectively. These results suggested that the decrease of urinary nitrogen excretion in goats fed NaOH-treated silage may be due to a slight improvement of availability of nitrogen reaching the abomasum, whereas lower protein digestibility of NaOH-treated silage could be also responsible. An enhancement of microbial protein production in the rumen, which could contribute to a reduction of nitrogen loss in urine, was reported in steers fed a NaOH-treated forage (Sriskandarajah and Kellaway, 1984). However, this explanation would be unlikely in this study, bacause the digestibility of organic matter was depressed and amino acid breakdown during ensilage was enhanced by the NaOH treatment.

Rumen fluid characteristics and liquid outflow rate in goats are summarized in table 3. Feeding of each alkali-treated silage raised the ruminal pH of goats reflecting the pH value of the silage. Although treatment of alfalfa with NaOH reduced organic matter digestibility, no significant difference was detected in ruminal total VFA concentrations among treatments at any sampling times. Outflow rate of liquid phase was not significantly affected by feeding of any alkali-treated silages, but goats fed NaOH treated silage tended to show an increase of outflow rate compared with those given the other silages. These facts may be associated with the higher sodium intake (Harrison et al., 1975). Goats fed NaOH-treated silage showed higher molar proportion of butyric acid in the rumen compared with those given the other silages, which may also be a reflection of the quality of ingested silages.

Time course of ruminal NH_3-N concentrations in goats is shown in figure 1. Considerably high concentrations were observed in this study because the goats were fed high protein diets which included large proportion of soluble or rapidly

TABLE 3. MEAN VALUES FOR RUMEN FLUID CHARACTERISTICS AND LIQUID OUTFLOW RATE FROM THE RUMEN IN GOATS GIVEN UNTREATED, NaOH-TREATED AND NH_3 TREATED ALFALFA SILAGES[1]

Treatment	Untreated	NaOH-treated	NH_3-treated	S.E.[2]
pH	6.75^{b}	6.97^{a}	6.82^{ab}	0.06
Total VFA (mmol/dl)	9.50	9.54	9.26	0.78
Molar % of VFA				
Acetic acid	68.9	66.3	69.9	1.35
Propionic acid	18.3	16.5	17.8	0.87
iso-Butyric acid	1.88	2.17	1.92	0.12
n-Butyric acid	7.72^{b}	11.7^{a}	7.23^{b}	0.89
NH_3-N (mg/dl)	33.7^{b}	28.5^{b}	51.3^{a}	5.57
Outflow rate (%/h)	5.45	5.95	5.34	0.60

[1] Over all mean values of 3 goats with 6 sampling times for rumen fluid characteristics (n = 18) and mean values of 3 goats for liquid outflow rate. Values in the same row with different superscript letters are significantly different ($p < 0.05$).

[2] Pooled standard error.

degradable nitrogen. Although NaOH-treated silage contained about twice as much NH_3-N as untreated silage, concentrations of NH_3-N in the rumen fluid of goats receiving the two silages were almost similar through every sampling time. The results may be related to an increase of microbial protein production or an inhibition of proteolysis within the rumen. As the digestibility of organic matter was decreased by NaOH treatment, the former explanation seems to be impossible. When goats were fed NH_3-treated silage, the highest NH_3-N concentrations were consistently observed. The mean NH_3-N concentration was above 50 mg/dl, but all goats remained their health during the experiment.

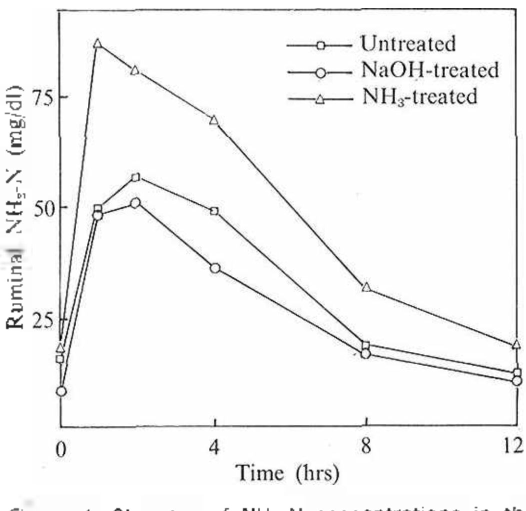

Figure 1. Changes of NH_3-N concentrations in the rumen fluid of goats given untreated, NaOH-treated and NH_3-treated alfalfa silages.

Degradation characteristics of dry matter and nitrogen in the rumen and the constants estimated by fitting the data to the exponential equation are detailed in table 4. Washing loss and the degradability of dry matter at initial 3 hours of incubation were less in alkali-treated than untreated silage, but the differences were diminished after 12 hours of incubation. Treatment with NaOH also decreased washing loss and initial degradability of nitrogen compared to untreated silage in spite of higher NH_3-N concentration in the former silage. Ammonia-treated silage showed the highest nitrogen degradability at all incubation times because some parts of added ammonia would be easily released in the rumen. Dry matter and nitrogen degradabilities at 48 hours of incubation paralleled with those digestibilities described in table 2. From the constants of the exponential equation, it was suggested that the solubility (a) of dry matter and nitrogen in the rumen was decreased and the rate of degradation (c) of the two components tended to be increased by the NaOH treatment, but there was little change in the proportion of degradable fraction (b). Mir et al. (1984) and Waltz and Loerch (1986) demonstrated that when soybean meal was subjected to NaOH treatment, solubility, potential degradability and the rate of degradation of protein in the rumen were reduced without any adverse effects on protein digestion. Although

TABLE 4. DEGRADATION CHARACTERISTICS OF DRY MATTER AND NITROGEN AT DIFFERENT TIMES OF INCUBATION OF NYLON BAGS IN THE RUMEN AND THE CONSTANTS FROM THE EXPONENTIAL EQUATION $p = a + b\ (1-e^{-ct})$[1].

Treatment	Item	Washing loss (%)	Disappearance from nylon bags (%) 3 h	6 h	12 h	24 h	48 h	a	b	c	Residual S.D
Untreated	DM	46.5	51.2	58.1	69.4	76.4	79.8	45.1	35.9	0.08	2.03
	N	63.8	66.8	72.5	81.6	86.4	87.9	62.4	26.6	0.09	2.40
NaOH-treated	DM	42.8	47.3	59.4	70.1	74.7	77.0	40.9	36.8	0.11	2.54
	N	59.0	64.1	73.4	82.6	84.2	85.9	57.7	28.6	0.13	3.61
NH_3-treated	DM	45.7	48.8	55.2	69.6	75.3	78.6	43.5	36.7	0.08	3.45
	N	68.0	71.3	76.0	86.1	87.8	89.7	66.7	23.5	0.10	2.80

[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 (% hr^{-1}).

it might be ascribed to racemization of amino acids and formation of crosslink amino acids following NaOH treatment of protein (Finot, 1983), the mechanism of protection of protein from ruminal degradation was not fully understood. Ammonia addition provided a soluble nitrogen to the silage and as a consequence, decreased the proportion of slowly degradable fraction (b). When the cell wall digestibility was improved by alkali treatments, potential degradability (a + b) of dry matter could be increased (Ørskov, 1991), but it was not observed in this study. This experiment showed no positive effect of alkali treatment on chemical (fermentation) and nutritional quality of alfalfa silage. However, another experiment made with laboratory silo (unpublished result) indicates that ensiling with NaOH of low moisture alfalfa could decrease the nitrogen solubility and the proteolysis during ensilage without a reduction of lactic acid content. Therefore, denaturation of protein with NaOH treatment could have a potency for modification of ruminal protein degradation when treatment was conducted in a proper condition. Further experiment seems to be necessary to define the mechanisms of the characteristics of protein digestion in NaOH treated forage.

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