Effects of Preheated Additives on the Fermentation Quality of Napiergrass Silage

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ABSTRACT : Hydrolysis of plant protein to non-protein nitrogen (N) or ammonia can reduce quality of silage crops. Heating or non-enzymatic browning is a treatment to inhibit this hydrolysis. This experiment was conducted to examine the effects of pre-heated soybean meal and molasses on the fermentation quality of napiergrass silage. The initial growth of napiergrass was harvested at 85 days of age and immediately chopped into about 1 cm length. About 700 g of the grass was ensiled into a laboratory silo (1.0 liter polyethylene container) and incubated for 30 days at room temperature (28°C). No additives (control), molasses, soybean meal and molasses + soybean meal treatments were prepared. All additives were non-heated or heated in an oven at 150°C for 30 minutes before ensiling. Molasses was added at 3% on the fresh weight basis and soybean meal was added at 0.5% N, respectively. After opening the silo, pH, total nitrogen (TN), volatile basic nitrogen (VBN), lactic acid (LA), acetic acid (AA), butyric acid (BA) and dry matter (DM) contents were determined. The data were analyzed statistically by analysis of variance. Compared with control, molasses addition significantly decreased pH value, VBN/TN, AA and BA and increased LA production. Soybean meal addition significantly increased TN and VBN/TN of silage. Both molasses and soybean meal addition significantly reduced pH value, AA, and BA and increased DM and LA contents of silage. The heating of additives was only effective to reduce VBN/TN production compared with non-heated additives in soybean meal and soybean meal with molasses addition. ***(Asian-Aust. J. Anim. Sci. 2001. Vol 14, No. 11 : 1564-1567)***

Key Words : Fermentation Quality, Napiergrass, Preheated Additives, Silage

INTRODUCTION

Hydrolysis of protein starts after a forage is cut and continues during the storage as silage, where residual proteolytic enzyme activity in the ensiled crop results in the hydrolysis of plant protein and the accumulation of non-protein N including ammonia (McDonald et al., 1991).

Non-enzymatic browning, the Maillard reaction, was discovered by L.C. Maillard, and its mechanism has been widely studied by food chemists (Homma et al., 1982; Yoshimura et al., 1997). The main property of the Maillard reaction is represented by sugar-amino acid groups, the reaction between reducing sugar and amino acid or protein producing strong reducing materials during heat treatment or storage in food (Homma et al., 1970; Yoshimura et al., 1997). Cleale et al. (1987) and Goering et al. (1973) reported that prolonged treatment of proteins under a condition of faster browning can result in formation of indigestible protein and the reaction would be associated with reduced bio-availability of amino acids.

Cleale et al. (1987) reported that factors regulating the rate of non-enzymatic browning include type and concentration of reducing sugar, temperature and duration of heating, water activity and pH of reaction mixture.

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Although urea is cheap material as silage additives to increase N content, it has worse effect on fermentation quality (Yunus et al., 2000). Therefore, alternative material or pretreated material which has low solubility of N should be investigated. The objective of this study was to characterize heated or non-heated soybean meal and molasses as additives to napiergrass silage.

MATERIALS AND METHODS

Characteristics of napiergrass and additives

Napiergrass was cultivated in the experimental field of Kyushu University, Hakozaki, Fukuoka, Japan. The initial growth of napiergrass was harvested at about 15 cm above the ground using a hand sickle in July 1999. The harvested napiergrass was immediately chopped into about 1 cm length using an electric chopper. The chopped grass was immediately collected for the determination of dry matter (DM), total N (TN) and water soluble carbohydrate (WSC) contents. The DM contents of materials were determined by drying in an oven at 80°C for at least 24 h (AOAC, 1984), TN was analyzed by the Kjeldahl method (AOAC, 1984) and WSC by high performance liquid chromatography (HPLC) system from Shimadzu Corp. (Kyoto, Japan) with NH2P-50 column (Shoko. Co., Ltd.). Napiergrass showed 19.4% for DM, 1.5% for TN and 3.06% for WSC on DM basis. Soybean meal with 88.33% for DM and 4.6% for TN (%DM) and molasses with 73.60% for DM and 59% for sugar (%DM) were used as additives.

Silage making

The harvested grass was immediately chopped into about 1 cm length and the 700 grams were ensiled into a laboratory silo (1.0 liter polyethylene container) and incubated for 30 days at room temperature (28°C). The treatments were as follows: napiergrass only as control (C), napiergrass with: molasses (M), molasses heated (MH), soybean meal (S), soybean meal heated (SH), soybean meal + molasses (SM) and soy bean meal + molasses which were both heated (SHMH). Soybean meal was added at a level of 0.5% N and molasses was added at 3% on the fresh weight basis of napiergrass, respectively. Before ensiling soybean meal and molasses were heated in an oven at 150°C for 30 minutes for SH, MH and SHMH treatments. Before heating, appropriate quantities of the reducing sugars were mixed into additives, which previously had been treated with NaOH to achieve pH 8.5 (Cleale et al., 1987).

Three replicates were prepared for each treatment.

Chemical analyses of silage

After opening the silo, DM content was determined by drying in an oven at 80°C for at least 24 h (AOAC, 1984). Sixty grams of silage sample were soaked in 120 ml of water and stored at 4°C for one day. The silage extracts were used for determining pH, volatile basic nitrogen (VBN), lactic acid (LA) and volatile fatty acids. The pH of silage was measured from silage juice using a glass electrode pH meter (Horiba Co.). TN was analyzed by the Kjeldahl method (AOAC, 1984) and VBN by the steam distillation method (AOAC, 1984), LA using the method of Barker and Summerson (1941), and acetic acid (AA) and butyric acid (BA) with gas chromatography (Shimadzu GC-17A with 12 m capillary column, condition: column temperature 100°C, injection temperature 250°C).

Statistical analysis

The data were analyzed statistically by one-way analysis of variance (ANOVA) using a commercially available package (SAS, 1985).

RESULTS

The DM content, pH and chemical composition of silages are shown in table 1. Molasses addition at the rate of 3% slightly increased DM of napiergrass silage from 19.57% (C) to 20.86% (M), and there was a significant ($p<0.01$) increase when molasses heated (MH) was added (22.00%). When soybean meal was added, DM was found to be 20.21% for non-heated treatment (S) and 20.75% for heated treatment (SH), respectively. There were not significant differences between C, S and SH. The highest DM value (23.48%) was found when both soybean meal and molasses were added (SM), and there was no significant increase (22.72%) when both additives were heated (SHMH).

Compared with 5.13 for the pH value of control (C), M treatment significantly ($p<0.01$) decreased pH to 3.97, but there was not a significant difference between M and MH treatments. The highest pH value was found in S treatment (5.36), there was not a significant difference between SH (5.02) and C (5.13). SM and SHMH treatments showed a pH value of 4.05 and 4.07, respectively. These two values were significantly ($p<0.01$) lower than control (C), S and SH, but did not show significant differences when compared with M and MH.

The addition of molasses at the rate of 3% tended to decrease TN from 1.80% to 1.56% and 1.55%, respectively. The highest TN was shown at S (2.20%), a value significantly ($p<0.01$) different from SH (1.88%). SM and SHMH treatments showed 1.74% and 1.79% for TN, respectively. These two values were significantly ($p<0.01$) different from S treatment.

When compared with 11.53% for VBN/TN of C, there were significant ($p<0.05$) decreases in M (8.23%), MH (8.37%) and SHMH (8.85%), respectively. M and MH did not show significant differences, but there was a significant ($p<0.01$) decrease from SM (10.96%) to SHMH (8.85%). There was an extremely high VBN/TN (24.08%) in S and there was a significant ($p<0.01$) decrease in SH (12.92%).

Molasses addition gave higher LA production, 14.30% and 14.45% for M and MH treatments, respectively. There were slightly lower values when soybean meal coexisted (12.56% for SM and 13.23% for SHMH). There were no significant differences between C (1.24%), S (1.22%) and SH (1.70%). The heating of additives did not show significant differences in LA content compared with non-heated corresponding additives.

The presence of molasses in silages showed significant decreases in AA production compared with the C and S addition. AA production in S (1.49%) and SH (1.27%) was not different from C (1.31%), whereas in M, MH, SM and SHMH there was lower production of 0.79, 0.81, 0.69 and 0.63%, respectively were recorded.

The control silage showed significantly higher BA production (0.25%) than M (0.01%), MH (0.07%), SM (0.01%) and SHMH (0.00%), but did not show significant differences from S (0.11%) and SH (0.13%). The heating of additives did not show significant differences in BA production compared with non-heated corresponding additives.

DISCUSSION

According to McDonald et al. (1991) protein degradation occurs during silage fermentation through the action of *clostridia* on amino acids, degrading them to ammonia, amines and volatile fatty acids. The enterobacteria can

Table 1. Effect of preheated additives on the quality of napiergrass silage

Treatments	DM (SD)	pH (SD)	Chemical composition (%DM)				
			TN (SD)	VBN/TN (SD)	LA (SD)	AA (SD)	BA (SD)
C	19.6 (0.3)[a]	5.13 (0.03)[b]	1.80 (0.07)[bc]	11.53 (0.41)[b]	1.24 (0.12)[a]	1.31 (0.12)[b]	0.25 (0.12)[b]
M	20.9 (0.6)[ab]	3.97 (0.21)[a]	1.56 (0.03)[ab]	8.23 (0.86)[a]	14.30 (2.09)[b]	0.79 (0.13)[a]	0.01 (0.01)[a]
MH	22.0 (0.2)[b]	4.17 (0.14)[a]	1.55 (0.03)[a]	8.37 (0.70)[a]	14.45 (0.97)[b]	0.81 (0.08)[a]	0.07 (0.07)[a]
S	20.2 (0.2)[a]	5.36 (0.15)[b]	2.20 (0.03)[d]	24.08 (1.24)[c]	1.22 (0.06)[a]	1.49 (0.17)[b]	0.11 (0.03)[ab]
SH	20.8 (0.5)[ab]	5.02 (0.28)[b]	1.88 (0.17)[c]	12.92 (0.27)[b]	1.70 (0.65)[a]	1.27 (0.15)[b]	0.13 (0.05)[ab]
SM	23.5 (0.3)[c]	4.05 (0.08)[a]	1.74 (0.07)[ab]	10.96 (0.40)[b]	12.56 (1.55)[b]	0.69 (0.05)[a]	0.01 (0.01)[a]
SHMH	22.7 (0.7)[bc]	4.07 (0.09)[a]	1.79 (0.05)[abc]	8.85 (0.40)[a]	13.23 (0.85)[b]	0.63 (0.07)[a]	0.00 (0.00)[a]
Level of significance	$p<0.01$	$p<0.01$	$p<0.01$	$p<0.01$	$p<0.01$	$p<0.01$	$p<0.05$

C: Control, M: Molasses, MH: Molasses heated, S: Soybean meal, SH: Soybean meal heated, SM: Soybean meal and molasses, SHMH: Soybean meal heated and molasses heated, DM: Dry matter, TN: Total nitrogen, VBN/TN: Volatile basic nitrogen/total nitrogen, LA: Lactic acid, AA: Acetic acid, and BA: Butyric acid.

[a,b,c] Different letters indicate significant difference at $p<0.01$ or $p<0.05$.

SD: Standard deviation.

also degrade amino acids. Higher ammonia content in silage is a good indicator of clostridial activity, destroying lactic acid and leading to pH rise, which results in inferior preservation and a reduction in the nutritive value of silage by degrading amino acids (Ohshima and McDonald, 1978).

Data from this experiment indicated that the presence of molasses resulted in a good quality of silage by increasing LA production and decreasing pH value as shown in other works (Yokota et al., 1991, 1992, 1995; Yunus et al., 2000). Higher water soluble carbohydrates addition in silage making enhanced LA production and caused lower pH value, this consequence would inhibit clostridial bacteria growth (Davies et al., 1997; McDonald et al., 1991).

In the present study soybean meal addition increased TN but lowered fermentation quality by increasing the value of VBN/TN and pH value and by decreasing LA production of silage. The addition of both molasses and soybean meal led to good fermentation quality of silage and this result agrees with the report of Catchpoole and Henzell (1971) who standardized a good quality of silage prepared from tropical forages.

The heating effectively reduced VBN production in soybean meal or soybean meal with molasses treatment, but did not affect the treatment with molasses only. Cleale et al. (1987) also found that heating would cause reduced ammonia release from soybean meal in ruminal fermentation. Furthermore, Cleale et al. (1987) also reported that *in vitro* ammonia release from soybean meal was suppressed by non-enzymatic browning and was also influenced by many factors, including source and quantity of added reducing sugar, moisture and pH of sample, and temperature and duration of heating. Based from those results of the present experiment, we can conclude that heating of soybean meal additive before ensiling with napiergrass is effective to increase fermentation quality by reduced ammonia production from the silage.

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