Effluent and Aerobic Stability of Cellulase and LAB-Treated Silage of Napier Grass (*Pennisetum purpureum* Schum)

J. Zhang* and S. Kumai

College of Agriculture, Ehime University, Matsuyama 790-0905, Japan

ABSTRACT : The effects of acremonium cellulase (AC) additive and lactic acid bacteria (LAB) inoculant on effluent production and aerobic stability of silage were investigated. Napier grass (*Pennisetum purpureum* Schum) was treated with AC at the rates of 0.05 (AC_1) and 0.1 g/kg (AC_2) and/or with LAB at the rate of 1.0×10^8 cfu/kg fresh grass at ensiling. The treatments of LAB, AC_1, AC_2, LAB+AC_1 and LAB+AC_2 significantly (p<0.01) decreased pH and contents of volatile basic nitrogen and butyric acid, and significantly (p<0.01) increased lactic acid content compared with the control. All treated silages were well preserved with pH of lower than 4.2. There were no significant differences in fermentation quality between the application rates of AC (AC_1 and AC_2) and between the mixtures (AC_1+LAB and AC_2+LAB). AC (AC_1 and AC_2) and AC plus LAB (AC_1+LAB and AC_2+LAB) resulted in more silage effluent than the control and LAB inoculant alone. When the experimental silos were opened, the silages treated with AC and/or LAB were not as stable as the control silage, as shown by pH increase and lactic acid decomposition. (*Asian-Aus. J. Anim. Sci. 2000. Vol. 13, No. 8 : 1063-1067*)

Key Words : Aerobic Stability, Cellulase, Lactic Acid Bacteria, Napier Grass, Silage Effluent

INTRODUCTION

Effluent production and aerobic deterioration are the main causes of nutrient losses during ensiling (Miller and Clifton, 1965) and after opening (Woolford and Cook, 1978; Henderson et al., 1979).

Ensiling high moisture, chopped forage is often accompanied by the production of effluent. The volume of effluent produced in silos depends on several factors: dry matter content of forage (Castle and Watson, 1973; Olsen and Pedersen, 1974; Bastiman, 1976); mechanical treatment of the crop prior to ensiling (Messer and Hawkins, 1977); degree of packing, which affects the quantity of trapped air in the ensiled mass (Greenhill, 1964); the pressure applied to the surface (Alli et al., 1985): type of additives (Pedersen et al., 1973); type of fermentation; and the nature of the forage crop (Perkins and Pratt, 1951).

Well-preserved silages are considered to be more liable to aerobic deterioration than poorly fermented silages which are likely to contain butyric and other volatile fatty acids. These acids, together with ammonia, act as effective preservatives (Ohyama et al., 1975, 1977; Honig and Woolford, 1979). From a study of 84 farm silages, O'Kiely (1989) also found that the well-preserved silages (pH<4.2) were less stable than the poorly preserved silages. Henderson et al. (1979), in a study of the aerobic stability of 18 farm silages, found that only ammonia-N had a significant (negative) correlation with aerobic deterioration.

It has been confirmed that the additions of cellulase and lactic acid bacteria (LAB) have beneficial effects on silage, as they affect the cell wall structure, chemical composition and type of fermentation of silages (Ohyama et al., 1975; McHan, 1985; Zhang et al., 1997a, b). Therefore, cellulase and LAB additives could affect effluent production and aerobic stability of silage. The experiment reported here was designed to investigate the effects of cellulase additive and LAB inoculant on effluent production and aerobic stability of silage, using napier grass which is extensively utilized for ruminants in tropical and sub-tropical areas.

MATERIALS AND METHODS

Grass and additives

Napier grass (*Pennisetum purpureum* Schum), grown on an experimental field at the College of Agriculture, Ehime University (Matsuyama, Japan), was harvested 60 d after third cut on 13 November 1996, and was chopped into approximately 1.5 cm lengths by a forage chopper. After thorough mixing, the grass was sampled for chemical analysis and was used for each treatment.

The additives applied were acremonium cellulase (AC) and LAB inoculant obtained from Meiji Seika Kaisha Ltd. (Tokyo, Japan) and Snow Brand Seed Co., Ltd. (Sapporo, Japan).

Silage making

The following treatments were applied to the grass: Untreated (Control);

LAB at 1.0×10^8 colony forming units (cfu)/kg fresh matter (LAB);

    AC applied at 0.05 g/kg fresh matter (AC_1);

    AC applied at 0.1 g/kg fresh matter (AC_2);

    AC_1 plus LAB (AC_1+LAB);

    AC_2 plus LAB (AC_2+LAB).

Batches of the well-mixed chopped grass were put into large basins and the additives diluted with distilled water (20 g/kg fresh grass) were applied using a pipette. The same weight of distilled water was added to the controls. After application of the treatments, the material was thoroughly blended by hand and 450 g of the treated grass was packed into glass bottles (0.85 liter capacity) in six replicates. The bottle silos were sealed with fermentation traps containing water and were stored at ambient temperature. After ensiling for about five months, the silo bottles were opened and three of each treatment were used for the analyses of chemical composition and effluent production; the others were used for an aerobic study.

Analytical methods

The buffering capacity of the grass was determined as described by McDonald and Henderson (1962). Crude protein, crude fat, neutral detergent fibre, acid detergent fibre and water-soluble carbohydrates were determined using the procedures described by Morimoto (1971).

When the bottle silos used for the chemical and effluent analyses were opened, the contents of each replicate were moved into a container pierced with many small holes. Each silage was extracted with a water-jet pump for 60 seconds at the same flow rate of water. All liquid extracted was collected and the volume was measured with a 100 ml measuring cylinder; this was regarded as the total volume of silage effluent. Thereafter, dry matter (DM) content, pH value, organic acid composition and volatile basic nitrogen (VBN) content of each silage were determined. DM contents of the grass and silages were determined by oven drying at 70℃ for 48 hours. VBN was measured by the micro-diffusion method (Morimoto, 1971). The pH of the silage was measured with a pH meter (HM-30S, TOA Electronics Ltd., Tokyo). The organic acid contents were measured by HPLC (column: Shodex Ionpak KC-811×2; detector: Shodex OA VD-1, 430 nm; eluent: 3 mM $HClO_4$, 1.2 ml/min; reagent: Shodex 1/10 ST_3-R, 0.6 ml/min; temperature 40℃). Similarly, the silage effluent was analysed and the constituent losses were calculated. The DM loss during ensiling was calculated, based upon the DM weights in the silo before ensiling and after extracting effluent.

After the bottle silos for the aerobic study were

opened, a sample (about 50 g) of each silage was taken for pH, organic acid and VBN analyses. The silos with the remaining silage were covered with a new cap containing a small hole (diameter 0.5 mm), which allowed air to enter each silo. The 50 g silage sample was handled aseptically and analysed after 2, 4 and 8 days, respectively, as described above.

Statistical analysis

The data were subjected to analysis of variance and treatment means were then compared using Duncan's multiple range method (Duncan, 1955).

RESULTS

The chemical composition of napier grass is shown in table 1. There was a high proportion of cell walls (neutral detergent fibre) and low content of WSC in the grass.

Table 1. Chemical composition of napier grass

Dry matter content (DM, g/kg)	201.2
Crude protein (g/kg DM)	98.9
Crude fat (g/kg DM)	20.5
Netural detergent fibre (g/kg DM)	678.4
Acid detergent fibre (g/kg DM)	408.5
Water-soluble carbohydrates (g/kg DM)	45.7
Buffering capacity (mequiv./kg DM)	261.4

The fermentation quality of silages with LAB and AC treatments is presented in table 2. LAB or AC (AC_1 and AC_2) significantly ($p < 0.01$) decreased pH and butyric acid content, and significantly ($p < 0.01$) increased lactic acid content of silage compared with the control. There were no significant differences between the application rates of AC (AC_1 and AC_2) and between the combinations (AC_1+LAB and AC_2+LAB). Both LAB and AC treatments significantly ($p < 0.01$) decreased the VBN content of silage.

AC (AC_1 and AC_2) or AC plus LAB (AC_1+LAB and AC_2+LAB) resulted in more silage effluent than the control and LAB additive alone (figure 1). The effluent volume tended to increase with the increased AC application rates and the combinations of AC and LAB also promoted effluent production.

The DM losses during ensiling and losses in effluent are given in table 3. The total DM losses, caused by respiration, fermentation and in effluent, were higher in silages of AC_1+LAB and AC_2+LAB and lower in silages of LAB and AC_1; AC_2 had similar DM losses to the control at about 70 g/kg. Due to effluent production, cellulase-treatment increased organic acid losses by 5 to 9 times and total nitrogen losses by 2 to 3 times over the control and LAB inoculant alone.

Table 2. Effects of cellulase additive and LAB inoculant on the fermentation quality of napier grass silage

	Silage treatments						Significance		
	Control	LAB	AC_1	AC_2	AC_1+ LAB	AC_2+ LAB	Cellu-lase	LAB	Inter-action
Dry matter (g/kg)	193.0^B	197.5^B	210.0^A	214.5^A	208.0^{AB}	205.0^{AB}	**	NS	NS
pH	4.68^A	4.17^B	4.05^{BC}	3.90^C	3.95^C	3.93^C	**	**	NS
Acetic acid (g/kg DM)	17.9	13.1	13.3	12.6	11.5	10.2	NS	NS	NS
Butyric acid (g/kg DM)	30.6^A	7.1^B	3.8^B	6.5^B	1.9^B	2.9^B	**	**	**
Lactic acid (g/kg DM)	5.1^C	45.5^B	59.0^{AB}	72.0^A	68.8^{AB}	67.3^{AB}	**	**	NS
Propionic acid (g/kg DM)	3.6^A	1.0^B	0.7^B	0.7^B	1.0^B	0.7^B	**	**	NS
Valeric acid (g/kg DM)	2.8	3.3	1.7	1.4	2.9	2.4	NS	NS	NS
Total acids (g/kg DM)	60.6^C	70.2^{BC}	78.6^{ABC}	93.2^A	86.4^{AB}	83.4^{ABC}	**	NS	NS
VBN (g/kg DM)	1.48^A	1.11^B	1.00^B	0.93^B	1.06^B	0.88^B	**	**	NS

LAB: Lactic acid bacteria; AC_1 and AC_2: Acremonium cellulase at 0.05 and at 0.1 g/kg, respectively.
VBN: Volatile basic nitrogen; NS: Not significant; * and **: Significant at $p<0.05$ and 0.01, respectively.
Values within the same row with different superscripts differ significantly from each other at $p<0.01$.
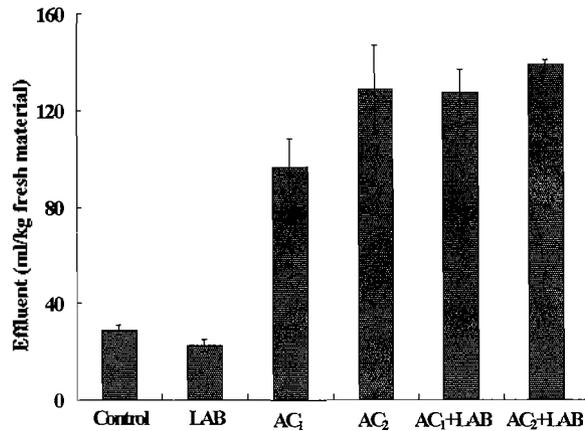

Figure 1. Effects of cellulase and LAB on the effluent volume of silage. Values are means and standard deviations are represented by vertical bars (n=3). LAB: Lactic acid bacteria, AC_1 and AC_2: Acremonium cellulase at 0.05 and 0.1 g/kg, respectively.

Figure 2. pH changes of silages in the aerobic phase. Each point on the curve is the mean for three silages. LAB: Lactic acid bacteria, AC_1 and AC_2: Acremonium cellulase at 0.05 and 0.1 g/kg, respectively.

Figure 2 showed that pH was static for the control silage and increased slightly for the silages treated with AC and/or LAB, after the silages exposed to air.

The silages, except for the control with low lactic acid content, had approximately 32-64% of lactic acid decomposed during 8-d aeration (figure 3: LAB 63.6%, AC_1 43.1%, AC_2 41.0, AC_1+LAB 31.5%, AC_2+LAB 38.6%). VBN contents showed no significant changes for all silages.

DISCUSSION

Cellulase addition greatly improved the fermentation quality of napier grass silage but resulted in higher volume of effluent. This could be because cellulase
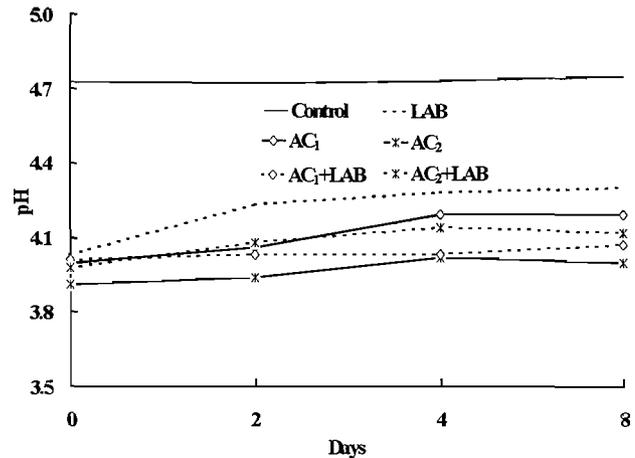
addition altered the structural integrity of the plant material so that more juice escaped from the cells. Jacobs and McAllan (1991) also found highest effluent volumes with enzyme-treated perennial ryegrass silages compared with both untreated and formic acid-treated. Furthermore, effluent tended to increase with increasing AC application rates, implying that the higher the application rate of cellulase the lower the moisture-holding capacity of silage. LAB inoculant alone produced lower effluent but when applied with AC effluent production was stimulated. There is no clear explanation for their synergistic effect on effluent production.

Ensiling, like any fermentation process, involves considerable changes in the composition of the material being preserved. Because of these changes, some losses of DM and herbage constituents will

Table 3. Losses of dry matter and constituents of napier grass silage with cellulase and LAB treatments

	Silage treatments						Significance		
	Control	LAB	AC_1	AC_2	AC_1+ LAB	AC_2+ LAB	Cellulase	LAB	Interaction
Dry matter (g/kg)[1]	70.1[ab]	39.6[b]	56.7[ab]	71.4[a]	97.5[a]	115.6[a]	*	NS	*
Organic acid losses[2] (g/kg DM)									
Acetic acid	0.48[AB]	0.29[B]	1.60[AB]	2.00[A]	1.59[AB]	1.39[AB]	**	NS	NS
Butyric acid	0.81[A]	0.06[B]	0.58[AB]	0.52[AB]	0.19[AB]	0.21[AB]	NS	**	NS
Lactic acid	0.09[C]	1.52[C]	6.84[B]	11.42[A]	9.59[AB]	9.80[AB]	**	*	NS
Propionic acid	0.09	0.03	0.05	0.13	0.19	0.07	NS	NS	NS
Valeric acid	0.08	0.03	0.15	1.45	0.32	0.28	NS	NS	NS
Total acids	1.55[B]	1.93[B]	9.22[A]	14.52[A]	11.88[A]	11.75[A]	**	NS	NS
Total N losses[2] (mg/kg DM)	35.2[b]	42.8[b]	104.3[ab]	145.9[a]	95.7[ab]	159.5[ab]	*	NS	NS

LAB: Lactic acid bacteria; AC_1 and AC_2: Acremonium cellulase at 0.05 and at 0.1g kg^{-1}, respectively;
[1] The total losses during ensiling; [2] The losses resulted from effluent.
NS: Not significant; * and **: Significant at $p<0.05$ and 0.01, respectively.
Values within the same row with different superscripts differ significantly from each other at $p<0.01$(A, B) or 0.05 (a, b).
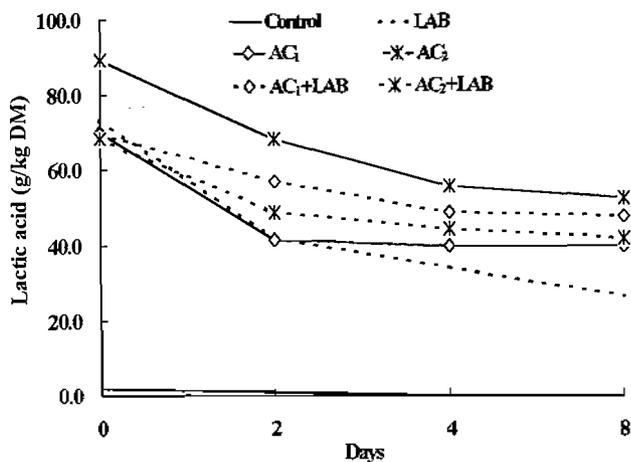

Figure 3. Changes in lactic acid contents of silages in the aerobic phase. Each point on the curve is the mean for three silages. LAB: Lactic acid bacteria, AC_1 and AC_2: Acremonium cellulase at 0.05 and 0.1 g/kg, respectively.

occur. The degree to which they occur is affected by many variables, of which the use of additives is but one. Jacobs and McAllan (1991) reported that losses of DM with cellulase-treated silages were higher than with untreated silage, contrasting with the results of Kennedy (1988). In the present experiment, DM losses were not increased by AC alone (≤ 0.1 g/kg^{-1}), but DM losses increased as the application rates of AC increased, and the combination of AC and LAB resulted in higher DM losses than AC or LAB additive alone.

Microbiological activity normally increases in silages upon exposure to air (Lindgren et al., 1985). This activity is associated with increased CO_2

production and temperature. Thereafter, pH increases and lactic acid content decreases. Thus, the analyses of pH and chemical composition give a good indication of silage susceptibility to aerobic deterioration. Some silages show signs of deterioration in less than 24 h, whereas others remain unchanged and stable during weeks of aerobic exposure (Jonsson and Pahlow, 1984; Lindgren et al., 1985). The present experiment showed that lactic acid decreased and pH increased slightly in all silages except for the control over 8 days of aerobic exposure. The control silage was more stable than the treated silages, probably resulting from higher contents of volatile fatty acids and VBN, which inhibited the development of yeasts and moulds (Ohyama et al., 1975; O'Kiely, 1989). This was also confirmed by the fact that there were many viable colonies of micro-organisms with white color (mainly yeasts) in LAB, AC and AC+LAB silages, while they were not detected in the control silage, even after exposing to air for 6 days.

Although about a half of the lactic acid in treated silages was broken down, their pH values did not greatly increase, possibly because the VBN contents changed little during aeration (data not shown). Billington et al. (1987) found that the VBN content increased during aerobic deterioration but Selmer-Olsen et al. (1993) reported that it decreased. However, Woolford (1986) found that the VBN content of grass silage increased, whereas in maize silage it decreased. This demonstrates that aerobic deterioration is not always associated with increased VBN.

We conclude that although cellulase-treatment improved the fermentation quality, it also increased effluent production during ensiling and lowered silage stability under aerobic conditions compared with the control. Thus, material with low DM content should

be properly wilted before ensiling and careful management of the silage will be required after opening, when cellulase is applied.

REFERENCES

Alli, I., C. Thiffault and B. Baker. 1985. A device for monitoring silage effluent produced in laboratory silos. J. Dairy Sci. 68:3355-3359.

Bastiman, B. 1976. Factors affecting silage effluent production. Exp. Husb. 31:40-46.

Billington, R. S., R. P. Hoxey and J. F. Lowe. 1987. The effect of consolidation on the aerobic deterioration of grass silage. In Proc. 8th Silage Conf., Hurley. pp. 39-40.

Castle, M. E. and N. J. Watson. 1973. The relationship between the DM content of herbage for silage making and effluent production. J. Br. Grassl. Soc. 28:135-138.

Duncan, D. B. 1955. Multiple range and multiple F-test. Biometrics. 11:1-42.

Greenhill, W. L. 1964. Plant juices in relation to silage fermentation. 1. The role of the juice. J. Br. Grassl. Soc. 19:30-37.

Henderson, A. R., M. G. Ewart and G. M. Robertson. 1979. Studies on the aerobic stability of commercial silages. J. Sci. Food Agric. 30:223-228.

Honig, H. and M. K. Woolford. 1979. Changes in silage on exposure to air. Occasional Symposium, British Grassland Society, pp. 76-87.

Jacobs, J. L. and A. B. McAllan. 1991. Enzymes as silage additives. 1. Silage quality, digestion, digestibility and performance in growing cattle. Grass Forage Sci. 46:63-73.

Jonsson, A. and G. Pahlow. 1984. Systematic classification and biochemical characterization of yeasts growing in grass silage inoculated with Lactobacillus cultures. Anim. Res. Devel. 20:7-22.

Kennedy, S. J. 1988. Enzymes as silage additives. In Proc. 12th General Meeting of the European Grassland Federation, Dublin. pp. 233-238.

Lindgren, S., K. Pettersson, A. Kaxpersson, A. Johsson and P. Lingvall. 1985. Microbial dynamics during aerobic deterioration of silages. J. Sci. Food Agric. 36:765-774.

McDonald, P. and A. R. Henderson. 1962. Buffering capacity of herbage samples as a factor in ensilage. J. Sci. Food Agric. 13:395-400.

McHan, F. 1985. Cellulase-treated coastal bermudagrass silage and production of soluble carbohydrates, silage acids, and digestibility. J. Dairy Sci. 69:431-438.

Messer, H. J. M. and J. C. Hawkins. 1977. The influence of moisture content and chop length of forage maize on silage bulk density and the pressure on bunker silo walls. J. Agric. Engin. Res. 22:175-182.

Miller, W. J. and C. M. Clifton. 1965. Relation of dry matter content in ensiled material and other factors to nutrient losses by seepage. J. Dairy Sci. 48:917-923.

Morimoto, H. 1971. The Experimental Methods for Animal Nutrition. Yokendo, Tokyo.

Ohyama, Y., S. Hara and S. Masaki. 1977. The use of caproic acid to prevent aerobic deterioration of silages after opening, with special reference to the amounts and time of application. J. Food Agric. Sci. 28:369-374.

Ohyama, Y., T. Morichi and S. Masaki. 1975. The effect of inoculation with Lactobacillus plantarum and the addition of glucose at ensiling on the quality of aerated silages. J. Food Agric. Sci. 26:1001-1008.

O'Kiely, P. 1989. Aerobic stability of farm silage. Irish J. Agric. Res. 28:102-103.

Olsen, R. A. and T. A. Pedersen. 1974. Growth of Torulopsis glabrate in effluents of grass silage. Acta Agric. Scand. 24:160-168.

Pedersen, T. A., R. A. Olsen and D. M. Guttormsen. 1973. Numbers and types of micro-organisms in silage and effluent from grass ensiled with different additives. Acta Agric. Scand. 23:109-120.

Perkins, A. E. and A. D. Pratt. 1951. A laboratory silo and its uses. J. Dairy Sci. 34:606-614.

Selmer-Olsen, I., A. R. Henderson, S. Robertson and R. McGinn. 1993. Cell wall degrading enzymes for silage. 2. Aerobic stability of enzyme-treated laboratory silages. Grass Forage Sci. 48:55-63.

Woolford, M. K. 1986. Aerobic deterioration of silage. In: Development in Silage (Ed. B. Stark and M. Wilkinson). Chalcombe Publication. pp. 45-65.

Woolford, M. K. and J. E. Cook. 1978. A note on the effect on the aerobic deterioration of maize silage of the manipulation of the microflora by means of antibiotics. Anim. Feed Sci. Technol. 3:89-94.

Zhang, J. G., S. Kumai and R. Fukumi. 1997a. Effects of microwave radiation and cellulase addition on silage quality of fresh straw of rice (Oryza sativa L.). Anim. Sci. Technol. (Jpn.). 68(2):131-137.

Zhang, J. G., S. Kumai, R. Fukumi, I. Hattori and T. Kono. 1997b. Effects of additives of lactic acid bacteria and cellulases on the fermentation quality of naked barley (Hordeum vulgare L. emand Lam) straw silage. Grassl. Sci. 43(2):88-94.