Effect of Additives, Storage Temperature and Regional Difference of Ensiling on the Fermentation Quality of Napier Grass (*Pennisetum purpureum* Schum.) Silage

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ABSTRACT : The effects of addition of cellulases (*Acremonium cellulolyticus* and *Trichoderma viride*, CE), a commercial inoculum containing lactic acid bacteria (*Lactobacillus casei*, LAB), fermented green juice (macerated napier grass with water was incubated anaerobically with 2% glucose for 1 day, FGJ) and glucose (G), and regional difference of ensiling on napier grass (*Pennisetum purpureum* Schum.) silage were studied by using 900 ml laboratory glass bottle silos under 30 and 40℃ storage conditions in 1995 and 1996. Experiment 1 was carried out to compare the addition of CE, LAB, FGJ and the combinations. Silages were stored for 45 days after ensiling. Experiment 2 studied the effects of applications of CE, LAB, FGJ and G. Experiment 3 was carried out using the similar additives as experiment 2 except for LAB. Silages were stored for 60 days in the experiments 2 and 3. Experiments 1 and 2 were done in Nagoya, and experiment 3 in Okinawa. Sugar addition through CE or G improved the fermentation quality in all the experiments, which resulted in a greater decrease in the pH value and an increased level of lactic acid, while butyric acid contents increased under 30℃ storage condition in CE addition. LAB and FGJ additions hardly affected the silage fermentation quality without additional fermentable carbohydrate. But the combination of LAB, FGJ and glucidic addition (CE and G) improved the fermentation quality. The effect of the regional difference of ensiling between temperate (Nagoya; 35° N) and subtropical (Okinawa; 26.5° N) zones on silage fermentation quality was not shown in the present study.
(**Key Words** : Cellulase, Fermented Green Juice, Lactic Acid Bacteria, Napier Grass Silage, Silage Additive, Storage Temperature)

INTRODUCTION

Tropical grasses are generally low in water soluble carbohydrate (WSC), but high in polysaccharide contents (Smith, 1962). Yokota et al. (1991) and Miyagi et al. (1993) reported that a main preservative acid in napier grass (*Pennisetum purpureum* Schum.) silage was lactic acid. Some researchers described that a main preservative acid in silages made from tropical grasses was acetic acid (Kim and Uchida, 1990). It is probably because of WSC content, which is generally lower in tropical grasses than that in temperate grasses (Kim and Uchida, 1990).

Nowadays, there is a general tendency to use biological additives, especially enzymes and inoculants rather than chemical additives (Smith, 1962) to improve fermentation quality of silage. The advantages of biological additives is natural products (Smith, 1962), and can be used safely, non-corrosive and apart from environmental hazards. The application of inoculants containing homofermentative lactic acid bacteria at ensiling has been used since the beginning of this century. McDonald and Durves (1956) and McDonald et al. (1991) cited examples of their use in the early 20th century, and Whittenbury (1961) outlined the properties that a suitable bacterium should possess. Attempts to improve silage fermentation quality by the addition of lactic acid bacteria at ensilage are well documented (McHan, 1986; Hattori et al., 1993; Weinberg et al., 1993). While Ohshima et al. (1996) reported that addition of FGJ, a homemade additive (macerated material grass for silage was incubated anaerobically with glucose for 1 or 2 days) was effective in improving fermentation quality of silage, even when the addition of commercial LAB was ineffective. The addition of cellulase at ensiling resulted in reduction in structural polysaccharide fraction and increased in lactic acid content compared with control silages and chemical treated silages (Van Vuuren et al., 1989; Rooke and Lafilzardeh, 1994), however, reported that cellulase addition did not change fermentation pattern of the silage

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of temperate grasses, Application of cell wall degradable enzymes to guiniagrass at ensilage also improved fermentation quality (Bayorbor et al., 1993).

The objectives of the present experiment were to study the effects of additions of CE, LAB, FGJ and G, and also to study the effects of storage temperature and regional difference of ensiling on silage fermentation quality of napier grass.

MATERIALS AND METHODS

Silage preparation

The 1st experiment:

Napier grass (Merkeron) was harvested by sickles on October 11 in 1995 at the growing stage at the farm of Nagoya University. Napier grass grew from May to October and the mean daily temperature for the period was from 17 to 29℃ at the farm. After wilting in a glasshouse for 4 hours, the forage was chopped into approximately 1 cm length using a forage chopper and mixed with CE, LAB and the combination of CE and LAB. Silages without additives were also prepared as the control.

Cellulase additive was a mixture of *Acremonium cellulolyticus* and *Trichoderma viride* (1:2) which had 424 U/g of abicerase activity (Snow Brand Seed Co., Ltd. Japan), and 21.2 U of it was added to 1 kg wilted napier grass as 1 ml solution. LAB was a commercial one, which was *Lactobacillus casei* sub sp. *rhamnosus* (Snow Brand Seed Co., Ltd. Japan) and was added as a solution of 1 ml containing 10^8 cfu/kg wilted grass. FGJ was prepared as follows; one day before ensiling, 200 g fresh napier grass was macerated with 600 ml distilled water and filtered through a double cheese cloth. The filtrate was mixed with 20 g glucose in a glass bottle which was attached a gas trap and kept anaerobically for 1 day at 30℃ (Ohshima et al., 1996), and 1 ml of which was added to 1 kg wilted grass.

Six bottle silos of 900 ml capacity with a gas trap on the top were prepared for each additive treatment and they were divided into 2 groups of 3 silos and allocated to the groups of storing temperature at 40 and 30℃ in the incubator. All silos were opened 45 days after ensiling.

The 2nd experiment

Napier grass was harvested by sickles on July 24 in 1996 at the growing stage at the farm of Nagoya University. The grass was treated with CE, LAB, FGJ, CE+LAB, CE+FGJ, G, CE+G, LAB+G, FGJ+G, CE+LAB+G and CE+FGJ+G as same as in experiment 1. Silages without additives were also prepared as the control. The CE additive and the commercial LAB inoculum were the same as experiment 1. FGJ was prepared the same as in experiment 1. Glucose was added at 40 g/kg wilted grass. The other treatments were the same as experiment 1.

The 3rd experiment

Napier grass was harvested by sickles on July 11 in 1996 at the growing stage at Okinawa Prefectural Livestock Experimental Station. Okinawa belongs to sub-tropical zone where the annual highest temperature is 33.9 ℃ and the lowest is 9.1℃. After wilting for 3 hours, the forage was chopped into approximately 1 cm length using a forage chopper and treated with CE, FGJ, CE+FGJ, G, CE+G, FGJ+G and CE+FGJ+G. Silages without additives were also prepared as the control. The CE, FGJ and G additives were the same as experiment 2. The other treatments were the same as experiment 1.

Analytical procedures

The silage and fresh grass were freeze-dried and the weight loss during drying was considered to be the moisture content. A 20 g sample of each silage was macerated with 200 ml distilled water, filtered through a double cheese cloth. The filtrate was used for the analysis. The pH value was measured with an electrode immediately after filtration. Lactic acid was measured by calorimetrically, volatile fatty acids (VFA) by a gaschromatograph and ammonia-N by distillation. The total nitrogen content was determined using a fresh silage sample by Kjeldahl method. WSC in fresh grass and silages were analyzed by the Somogi-Nelson method using freeze-dried samples after extracting with 800 ml/ ethyl alcohol.

Statistics

All data were subjected to analysis of variance. And statistical significance among each treatment and control was determined by Fisher's least significant difference.

RESULTS

Forage

The chemical composition of fresh napier grass used in experiment 1, 2 and 3 is given in table 1. Fresh grass was wilted at a good condition in each experiment, and dry matter (DM) was 22 to 24%. WSC and crude protein contents were 4.22 to 4.89% and 7.83 to 8.51% of DM, respectively. The pH values of FGJ were 5.70 to 5.80 and 3.75 to 4.30, at the time of preparation and at ensiling, respectively (table 2).

Table 1. Chemical composition of the fresh napier grass

Experiment No.	Region	Harvest date	Maturity Stage	DM[1]	WSC[2] (%DM)	CP[3] (%DM)
1	Nagoya	11 Oct. '95	growing	22.2	4.22	8.51
2	Nagoya	24 July '96	growing	22.0	4.71	7.83
3	Okinawa	11 July '96	growing	24.0	4.89	8.09

[1] Dry matter. [2] Water soluble carbohydrate. [3] Crude protein.

Table 2. The pH value of the fermented green juice

Experiment No.	pH	
	At preparation	At ensiling
1	5.70	3.75
2	5.78	3.88
3	5.80	4.30

Experiment 1

The fermentation quality and chemical composition of napier grass silages in experiment 1 are shown in table 3. CE added silages (CE, CE+LAB) showed significantly (table 8) lower pH value than any other treatments, and higher contents of lactic acid and WSC at both storage temperature. The single addition of LAB reduced pH value, but not significant (table 8). Ammonia-N was significantly reduced by LAB addition at 40℃ but not at 30℃. FGJ slightly reduced pH value and ammonia-N at 40℃ but not at 30℃ as LAB additives. In all these silages acetic acid was detected, but the other volatile fatty acids were trace in amount.

Experiment 2

Effects of additives with and without glucose addition on the fermentation quality are shown in table 4 and 5, respectively. Moisture contents were not different among all silages. Without glucose addition CE added silages (CE, CE+FGJ and CE+LAB) were significantly low in pH value and ammonia content, and high in lactic acid at both temperatures. But the single addition of LAB or FGJ did not affect fermentation quality, except that the additions decreased ammonia content. With glucose addition, LAB or FGJ added silage decreased pH value at both temperatures, except CE+LAB silages at 30℃. FGJ added silage also decreased ammonia content at both temperatures as well as CE silage at 40℃. Without glucose, CE addition increased butyric acid content at 30℃, but all silages with glucose contained little VFAs without acetic acid.

Table 3. Effect of additives on the quality of napier grass silages in experiment 1

Storage temp.	additives	pH	WSC[1]	Lactic acid	Acetic acid	Propionic acid	Iso-butyric acid	Butyric acid	NH_3-N
			% of DM	······	······	% of fresh silage	······	······	% of TN
40℃	Control	4.37	0.98	1.01	0.24	trace	trace	trace	9.02
	CE[2]	4.22	2.42	1.14	0.25	trace	trace	trace	9.04
	FGJ[3]	4.33	0.61	1.22	0.17	trace	trace	trace	8.88
	LAB[4]	4.29	0.31	1.04	0.18	trace	trace	trace	6.35
	CE+LAB	4.17	2.75	1.15	0.18	trace	trace	trace	6.25
	LSD[5] ($p<0.05$)	0.05	0.59	0.21	0.04				0.48
30℃	Control	4.51	0.09	1.69	0.34	trace	trace	trace	11.29
	CE[2]	4.08	0.30	2.10	0.44	trace	trace	trace	11.46
	FGJ[3]	4.53	0.09	1.34	0.32	trace	trace	trace	13.21
	LAB[4]	4.50	0.10	1.70	0.34	trace	trace	trace	12.45
	CE+LAB	4.07	0.29	2.28	0.41	trace	trace	trace	12.15
	LSD[5] ($p<0.05$)	0.10	0.07	0.88	0.03				1.62

[1] Water-soluble carbohydrate. [2] Cellulase.
[3] Fermented green juice. [4] Lactic acid bacteria inoculant.
[5] Least significant difference.

Table 4. Effect of additives without glucose supplementation on the quality of napier grass silages in experiment 2 (at Nagoya)

Storage temp.	additives	pH	Moisture	Lactic acid	Acetic acid	Propionic acid	Iso-butyric acid	Butyric acid	NH_3-N
			% of fresh silage						% of TN
40℃	Control	4.51	83.65	1.27	0.10	trace	trace	0.02	10.37
	CE [1]	4.11	81.53	2.08	0.18	trace	trace	0.01	7.45
	FGJ [2]	4.48	82.48	1.66	0.12	trace	trace	0.02	9.74
	CE+FGJ	4.05	82.97	2.10	0.14	trace	trace	0.01	4.82
	LAB [3]	4.41	82.91	1.37	0.14	trace	trace	0.00	6.24
	CE+LAB	4.22	83.14	1.71	0.20	trace	trace	0.00	7.25
	LSD [4] ($p<0.05$)	0.12		0.42	0.03			0.03	1.65
30℃	Control	4.79	85.14	1.29	0.24	trace	trace	0.00	12.57
	CE [1]	4.06	84.22	2.21	0.23	trace	trace	0.30	8.43
	FGJ [2]	4.79	82.46	1.36	0.23	trace	trace	0.02	11.79
	CE+FGJ	4.08	82.28	2.26	0.24	trace	trace	0.00	8.05
	LAB [3]	4.66	85.42	1.56	0.24	trace	trace	0.00	14.27
	CE+LAB	4.06	83.21	2.27	0.24	trace	trace	0.00	8.13
	LSD [4] ($p<0.05$)	0.09		0.65	0.05			0.37	1.17

[1] Cellulase. [2] Fermented green juice.
[3] Lactic acid bacteria inoculant. [4] Least significant difference.

Table 5. Effect of additives with glucose supplementation on the quality of napier grass silages in experiment 2 (at Nagoya)

Storage temp.	additives	pH	Moisture	Lactic acid	Acetic acid	Propionic acid	Iso-butyric acid	Butyric acid	NH_3-N
			% of fresh silage						% of TN
40℃	Control	4.51	81.10	1.64	0.12	trace	trace	trace	7.63
	CE [1]	4.54	81.75	1.74	0.13	trace	trace	trace	6.64
	FGJ [2]	3.80	81.92	2.40	0.11	trace	trace	trace	5.87
	CE+FGJ	3.94	81.73	2.34	0.13	trace	trace	trace	5.48
	LAB [3]	3.99	81.80	2.20	0.11	trace	trace	trace	8.83
	CE+LAB	3.61	80.99	2.49	0.13	trace	trace	trace	7.95
	LSD [4] ($p<0.05$)	0.03		2.28	0.02				0.76
30℃	Control	3.87	82.53	2.39	0.16	trace	trace	trace	8.57
	CE [1]	3.85	81.34	2.33	0.13	trace	trace	trace	8.00
	FGJ [2]	3.75	82.75	2.38	0.12	trace	trace	trace	5.50
	CE+FGJ	3.67	82.61	2.39	0.14	trace	trace	trace	4.68
	LAB [3]	3.78	81.89	2.11	0.13	trace	trace	trace	8.11
	CE+LAB	4.09	80.58	2.06	0.19	trace	trace	trace	8.56
	LSD [4] ($p<0.05$)	0.03		0.53	0.07				1.08

[1] Cellulase. [2] Fermented green juice.
[3] Lactic acid bacteria inoculant. [4] Least significant difference.

Table 6. Effect of additives without glucose supplementation on the quality of napier grass silages in experiment 3 (at Okinawa)

Storage temp.	additives	pH	Moisture	Lactic acid	Acetic acid	Propionic acid	Iso-butyric acid	Butyric acid	NH_3-N
			% of fresh silage						% of TN
40℃	Control	4.51	81.80	1.56	0.16	trace	trace	trace	15.15
	CE[1]	4.20	82.39	1.78	0.19	trace	trace	trace	12.80
	FGJ[2]	4.34	82.21	1.69	0.13	trace	trace	trace	14.31
	CE+FGJ	3.73	80.73	2.03	0.09	trace	trace	trace	11.87
	LSD[3] ($p<0.05$)	0.05		0.48	0.63				0.97
30℃	Control	4.62	82.68	1.50	0.53	trace	trace	0.05	12.52
	CE[1]	4.03	82.30	1.89	0.17	trace	trace	2.77	10.17
	FGJ[2]	4.79	80.58	1.32	0.16	trace	trace	0.10	14.71
	CE+FGJ	4.01	82.21	1.87	0.19	trace	trace	0.01	8.43
	LSD[3] ($p<0.05$)	0.27		0.56	0.15			2.72	1.80

[1] Cellulase. [2] Fermented green juice. [3] Least significant difference.

Table 7. Effect of additives with glucose supplementation on the quality of napier grass silages in experiment 3 (at Okinawa)

Storage temp.	additives	pH	Moisture	Lactic acid	Acetic acid	Propionic acid	Iso-butyric acid	Butyric acid	NH_3-N acid
			% of fresh silage						% of TN
40℃	Control	3.89	82.34	2.14	0.15	trace	trace	trace	14.43
	CE[1]	3.79	80.59	2.27	0.15	trace	trace	trace	9.93
	FGJ[2]	3.79	79.82	2.15	0.10	trace	trace	trace	9.45
	CE+FGJ	4.00	79.84	1.74	0.10	trace	trace	trace	9.44
	LSD[3] ($p<0.05$)	0.69		0.69	0.01				2.00
30℃	Control	4.01	80.86	1.51	0.11	trace	trace	trace	10.39
	CE[1]	3.93	80.55	1.79	0.13	trace	trace	trace	9.11
	FGJ[2]	3.78	79.96	1.61	0.16	trace	trace	trace	6.68
	CE+FGJ	3.60	81.27	2.00	0.14	trace	trace	trace	6.82
	LSD[3] ($p<0.05$)	0.44		0.52	0.08				0.83

[1] Cellulase. [2] Fermented green juice. [3] Least significant difference.

Experiment 3

This experiment was done in Okinawa to compare the results with those obtained in Nagoya (Experiment 2). Without glucose, CE added silages (CE and CE+FGJ) decreased pH value and ammonia content at both temperatures. FGJ silage also decreased pH value at 40℃ and combined addition of CE and FGJ was more effective than the single addition of them. As in experiment 2, CE silage contained high butyric acid at 30℃. With glucose addition all the additives decreased ammonia content at both temperatures, especially in the case of FGJ additive at 40℃.

DISCUSSION

On participation of lactic acid bacteria for silage fermentation, their number increased rapidly at the first stage of fermentation, and the pH value of the silage decreased (Rooke et al., 1990: Bayorbor et al., 1993). The low pH value of the silage restrained butyric acid bacteria and a lot of other microorganisms (Henderson et al., 1982; Bayorbor et al., 1993), and consequently, decreased DM loss in the silage (Henderson et al., 1982). Activity of lactic acid bacteria on silage fermentation was affected by chemical ingredients in the grass, especially WSC, and

Table 8. Significance of F values derived from analysis of treatments for the silage

Treatments	pH	WSC	Lact.	Acet.	Prop.	iso-But.	But.	NH_3-N
Experiment 1								
CE	*	*	*	ns	ns	ns	ns	ns
LAB	ns	ns	ns	ns	ns	ns	ns	*
FGJ	ns	ns	ns	ns	ns	ns	ns	ns
× Temp.								
CE	*	*	*	*	ns	ns	ns	*
LAB	ns	ns	ns	*	ns	ns	ns	*
FGJ	ns	ns	ns	*	ns	ns	ns	*
Experiment 2								
CE	*	–	*	ns	ns	ns	ns	*
LAB	ns	–	ns	ns	ns	ns	ns	*
FGJ	ns	–	ns	ns	ns	ns	ns	*
G	*	–	*	ns	ns	ns	ns	*
× Glucose								
CE	ns	–	ns	ns	ns	ns	ns	*
LAB	*	–	*	ns	ns	ns	ns	*
FGJ	*	–	*	ns	ns	ns	ns	*
× Temp.								
CE	*	–	*	*	ns	ns	*	*
LAB	ns	–	ns	*	ns	ns	ns	*
FGJ	ns	–	ns	*	ns	ns	ns	*
G	ns	–	ns	ns	ns	ns	ns	ns
Experiment 3								
CE	*	–	*	ns	ns	ns	ns	*
FGJ	ns	–	ns	ns	ns	ns	ns	ns
G	*	–	*	ns	ns	ns	ns	ns
× Glucose								
CE	ns	–	ns	ns	ns	ns	ns	*
FGJ	*	–	*	ns	ns	ns	ns	*
× Temp.								
CE	*	–	*	*	ns	ns	*	*
FGJ	ns	–	ns	ns	ns	ns	ns	ns
G	ns	–	ns	ns	ns	ns	ns	ns
Experiment 2 and 3								
Region	ns	–	ns	ns	ns	ns	ns	ns

ns = not significant; * = significant at 5% levels.

silage fermentation was controlled by species and levels of lactic acid bacteria, which adhered on material grass (Henderson, 1982).

CE added silages (CE and CE+LAB) contained higher content of WSC which was produced from polysaccharides of the material grass than the other silages in experiment 1 (table 3). Such degrading action of cellulolytic enzymes have been observed by other researchers (Kim and Uchida, 1990). WSC in CE added silages was less at 30℃ than at 40℃, and higher lactic acid content was observed at 30℃. It would be probable that activity of lactic acid bacteria was higher at 30℃ and WSC generated during ensilage had already fermented to lactic acid at opening the silos at 30℃. There are a lot of reports that LAB additives were effective for temperate grasses in decreasing pH value and increasing lactic acid contents (McHan, 1986; Uchida and Kitamura, 1987; McDonald et al., 1991; Miyagi et al., 1993). LAB additive, however, was not always effective in this experiment for naiper grass. Yokota et al. (1991) and Miyagi et al. (1993) reported that napier grass silage mainly produced lactic acid rather than acetic acid. In this experiment, it is probable that number of lactic acid bacteria were enough to ferment the material napier grass (table 8), because single addition of LAB or FGJ did not decrease pH value or increase lactic acid.

Some reports described that WSC content for making high quality silage was more than 6 to 7% of DM (Chang et al., 1988-89; Ohshima et al., 1996), but in this experiment WSC was 4.22 to 4.89% DM. Sugar additives such as molasses (McDonald and Purves, 1956; Whittenbury, 1961; Uchida and Kitamura, 1987; Rooke et al., 1990; Yokota et al., 1992; Masuko et al., 1996) and glucose (McDonald and Purves, 1956; Chang et al., 1988-89) increased silage quality. Application of CE tended to be similar efficiency with glucose addition. But effect of CE was different between storage temperatures; a suitable temperature for CE activity in this experiment was 30℃. In this condition, however, butyric acid was increased. The reason was unclear.

At ensilage the epiphytic inoculant microorganisms interact with DM, WSC, and buffering capacity in material forages, and these factors affect silage quality (Smith, 1962; Kumai et al., 1995). Ohshima et al. (1996, 1997a,b) reported that FGJ addition was more effective on silage fermentation than commercial lactic acid bacteria inoculant in the case of direct cut alfalfa. FGJ had more species of lactic acid bacteria than commercial lactic acid bacteria inoculant and worked efficiently for the silage fermentation. In the present study, FGJ had similar efficacy to LAB in improving silage quality and their addition decreased pH value and increased lactic acid content in all the experiments when enough fermentable carbohydrates were secured by adding CE or glucose, and deficient in epiphytic LAB.

In the present experiment pH value of napier grass silage without additives decreased at 40℃ compared to 30℃. This fact suggests that napier grass silage could be conserved at good quality in the case of anaerobic condition even if the ambient temperature is so high. Moreover, addition of cellulase improves silage fermentation quality.

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