Fermentative Quality of Guineagrass Silage by Using Fermented Juice of the Epiphytic Lactic Acid Bacteria (FJLB) as a Silage Additive

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ABSTRACT : This experiment examined the characteristics of fermented juice of epiphytic lactic acid bacteria (FJLB) prepared by the addition of glucose, sucrose and molasses as a fermentation substrate. The effect of FJLB on the fermentative quality and changes in chemical composition during fermentation of guineagrass silage were also investigated. The pH value of the silages treated with FJLB rapidly decreased, and reached to the lowest value within 7 days of start of fermentation, as compared to the control. The number of lactic acid bacteria (LAB) in the treated silages increased for the first 3 days, thereafter the number of LAB declined gradually up to the end of the experiment. Silages treated with FJLB had larger populations of LAB than the control. Ammonia-nitrogen production increased throughout the ensiling period, which in the control and no-sugar added FJLB silages were higher than the other treated silages. Lactic acid levels varied with the time of ensiling and among the silage treatments. For any sugar FJLB treated silages, the lactic acid increased initially, and then slightly reduced to less than 50 g/kg of dry matter until 49 days after ensiling, except the silage treated with glucose added FJLB. Nevertheless, lactic acid content of the control decreased constantly from the beginning of ensiling and was not found after 35 days. Moreover, acetic acid content increased throughout the ensiling period. All the FJLB treated silages had significantly ($p<0.05$) lower pH and ammonia-nitrogen content, while significantly ($p<0.05$) higher lactic acid content and V-score value compared with the control. This study confirmed that the applying of FJLB with any sugar substrate improved fermentative quality of silage. ***(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 6 : 807-811)***

Key Words : Epiphytic Lactic Acid Bacteria, Guineagrass, Silage, Silage Additives

INTRODUCTION

Ensiling has been known as a method to preserve the moist crops by controlling anaerobic fermentation. The success of the ensiling can be achieved when the number of lactic acid bacteria (LAB) is dominant in the fermentation, and the activity of clostridia is restricted. Although it has been well recognized that the epiphytic LAB plays an important role on silage fermentation, the number of epiphytic LAB on the standing crop is tiny and variable (Muck, 1990; Lin et al., 1992), and found to be lower than that of aerobic bacteria, yeast and molds (Hellings et al., 1985; Cai et al., 1998). Therefore, an inoculation of LAB in the ensiling process has been recommended in order to make good quality silage. However, the properties of a bacterial strain vary even within the same species, and some strains are not effective in improving fermentative quality of silage as well (Woolford and Sawczyc, 1984).

Recently, many researchers have reported that manipulating numbers of the epiphytic LAB can be improved by using fermented juice of epiphytic LAB (FJLB) as a silage additive obtained by macerating crop silage with water and anaerobic incubating for 2 days. Oshima et al. (1996) also reported that the application of fermented green juice was more effective on silage fermentation than commercial lactic acid bacteria inoculating in the case of direct cut alfalfa. However, very few researches reported about ensiling of tropical pasture species treated with FJLB. This study is purposed to clarify if the applying FJLB with addition of glucose, sucrose, and molasses as a substrate would enhance the fermentation of the guineagrass silage.

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MATERIALS AND METHODS

FJLB preparation

The FJLB was prepared from guineagrass (*Panicum maximum* Jacq cv. Gatton) before harvesting, and 25 g of fresh grass was macerated with 50 ml of distilled water using a blender. This was filtered through sterilized double cheese cloths and the filtrate was added without any sugar (non-sugar FJLB), with 1% (w/v) glucose and sucrose (FJLB+1% G and FJLB+1% S), and with 5% (w/v) molasses (FJLB+5% M), respectively. The FJLB was kept in an incubator at 30°C for 3 days.

Silage making

Guineagrass was harvested at flowering stage on October 22, 2001 in the experiment field, University of the Ryukyus, Okinawa Japan, and chopped to 1 cm pieces before ensiling. The FJLB were added at 1% (v/w) of fresh material as silage additives. The control silage was added with an equivalent amount of distilled water. Approximately 100 g fresh matter of treated crop then was packed into a plastic pouch in triplicate, and sealed with vacuum sealer (Sharp, Co. Ltd, Vacuum sealer, SQ202). The silos were

Table 1. Chemical composition of guineagrass materials prior to ensiling

Item	
pH	6.12
Moisture (% FM)	74.63
Dry matter (%)	25.37
Buffering capacity M.eq.DM	330.00
WSC (% DM)	3.24
Crude protein (% DM)	7.48
LAB (cfu/g FM)	3.6×10^{4}
Aerobic bacteria (cfu/g FM)	2.9×10^{6}

FM = fresh matter, DM = dry matter.
WSC = water soluble carbohydrate, LAB = lactic acid bacteria.

kept at 25°C and samples were taken 1, 3, 7, 14, 21, 35 and 49 days after ensiling for chemical analysis.

Chemical analysis

The representative 20 g fresh matter of ensiled sample was macerated with 70 ml of distilled water and stored at 4°C for 12 h. Then the extract was filtered using a filter paper, the filtrate was used to determine the pH, ammonia-nitrogen (NH_3-N) and volatile fatty acid content (VFA) of silage. The pH of silage was determined by using a pH meter, the NH_3-N content was measured by using steam distillation technique, and the organic acid content was measured by applying HPLC. Dry matter content of silage was determined by oven drying at 70°C to a constant weight. The content of water soluble carbohydrate (WSC) was estimated colorimetrically using Anthrone method (Murphy, 1958). Buffering capacity of material grass was determined according to the method as described by Playne and McDonald (1966). V-score was evaluated by using the values of organic acid content and NH_3-N (Japan Grassland Farming Forage Seed Association, 1994).

Counts of lactic acid bacteria

One gram of silage was shaken well with 100 ml of sterilized 0.85% NaCl solution, and was serial diluted. The number of lactic acid bacteria (LAB) in FJLB and the extract of crop material and silages were determined by counting the colony forming unit (CFU) after incubation on GYP-$CaCO_3$ agar plate at 35°C for 3 days (Kozaki et al., 1992). Aerobic bacteria were counted on agar plate of nutrient broth after incubation at 37°C for 3 days (Nissui Seiyaku Ltd., Japan). Yeast and molds were counted on a plate of potato dextrose agar (Nissui Seiyaku Ltd., Japan) after incubation at 30°C for 3 days.

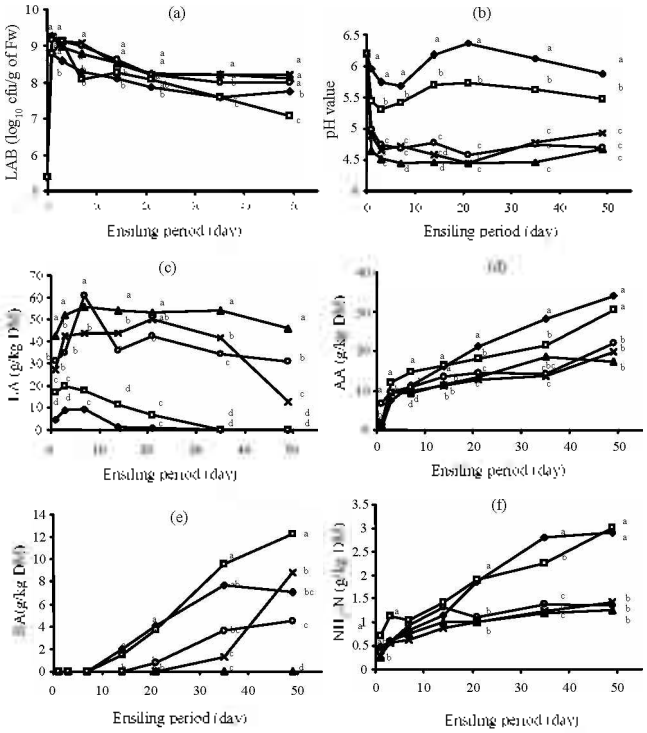

Figure 1a, b, c, d, e, f. Changes in the counts of LAB, pH value, LA , AA, BA and NH3-N contents with (♦) control; (□) non-sugar FJLB; (▲) FJLB+1% glucose; (×) FJLB+1% sucrose; (○) FJLB+5% molasses during the ensiling period. Values are means of three silage samples. Different letters show significant differences between treatments at $p<0.05$.

Statistical analysis

Data obtained on chemical composition of silage ensiled for 49 days were analyzed using the GLM procedure of SAS and the significance of the difference among treatment means was determined by the Duncan's multiple-range test (SAS, 1998).

RESULTS

The chemical composition of material prior to ensiling was shown in Table 1. The epiphytic lactic acid bacteria (LAB) counts of the extract of fresh material were 10^4 cfu/ml and increased to the range of 10^8-10^9 cfu/ml after incubation (Table 2).

The changes in the number of LAB count, pH value,

Table 2. The pH value, microorganism, organic acid and WSC contained in each FJLB before use as silage additives

	pH	LAB	Aerobic bacteria	Yeast	Lactic acid	Acetic acid	Residual WSC
		cfu/ml			mg/ml		
Non-sugar FJLB	5.83	2.29×10^{8}	1.97×10^{8}	ND	0.00	0.11	0.00
FJLB+1% glucose	4.24	2.00×10^{8}	1.50×10^{6}	ND	3.51	0.06	0.00
FJLB+1% sucrose	4.03	1.96×10^{8}	8.00×10^{6}	ND	4.80	0.10	0.00
FJLB+5% molasses	4.12	1.85×10^{9}	1.20×10^{6}	ND	15.42	0.36	0.10

lactic acid and acetic acid contents during silage fermentation are shown in Figure 1. The numbers of LAB counts increased rapidly and reached a maximum population number within one day after ensiling for all sugar FJLB treated silages and then constantly decreased from 10^9 to 10^7-10^8 cfu/g until 49 d of ensiling period (Figure 1a). The LAB counts in the control and non-sugar FJLB silages were lower ($p<0.05$) than the other treated silage in any ensiling time.

The pH value of the control silage was higher ($p<0.05$) than the other treated silage (Figure 1b). The pH of non-sugar FJLB treated silage slightly reduced and reached to 5.5, and no further declined until the end of experiment. In contrast, the pH of sugar-FJLB treated silages declined rapidly on the first day of fermentation and maintained almost the same value until 49 d of ensiling period, and the values were significantly ($p<0.05$) lower than that of the control and the non-sugar FJLB.

In the control and sugar free FJLB treated silages, the lactic acid content slowly increased up to 3 d, and constantly decreased and was not observed at 35 d eventually. Conversely, the other FJLB treated silages had significantly higher value than the former two silages ($p<0.05$), though the lactic acid content after 7 d slightly decreased to less than 50 g/kg of dry matter (Figure 1c), except the glucose-FJLB treated silage. The concentration of lactic acid in the molasses-FJLB treated silage was dramatically higher ($p<0.05$) than the other silages at 7 d. The glucose-FJLB treated silage contained the largest amount of lactic acid at 49 d of ensiling period (Table 3).

Generally, acetic acid content was low at the beginning (Figure 1d) and gradually increased until the end of ensiling period. Nevertheless, after third day of ensiling the acetic acid content in the control and non-sugar FJLB were higher ($p<0.05$) than all the sugar-FJLB treated silages at any ensiling time. In case of butyric acid, it was not found until 14 d in the control and 21 d in the treated silages of the ensiling period (Figure 1e). Glucose-FJLB treated silage was not detected this acid in any ensiling time.

NH_3-N production steadily increased throughout the ensiling time for all silages (Figure 1f), though the values were less than 3 g/ kg of DM until 49 d after ensiling. There were no differences among the control and the non-sugar FJLB treated silages until 14 days after ensiling, while all sugar FJLB treated silages showed the lower ($p<0.05$) concentration of NH_3-N on 49 d after ensiling than the control and non-sugar FJLB treated silage (Table 3). Moreover, NH_3-N content in all sugar treated silages was not significantly different.

In summary, the control and non-sugar FJLB treated silages showed poor fermentation with a high pH and NH_3-N content, low V-score and lack of lactic acid (Table 3). Precisely, all the silages treated with FJLB added with any sugar substrate revealed better quality, compared to respective opposite experiment result. In addition, glucose containing FJLB treated silage presented the highest quality, considering the content of lactic acid ($p<0.05$).

DISCUSSION

Many researchers have reported that the guineagrass silages without any additive reveal poor lactic acid fermentation with a high level of acetic acid and scarce lactic acid content at the end of ensiling time (Niimi and Kawamura, 1998; Imura et al., 2001; Shao et al., 2004). In this study, the applying of any sugar treated FJLB as a silage additive showed improved the fermentative quality of silage with a high concentration of lactic acid and a low concentration of NH_3-N.

In the control and non-sugar FJLB treated silages, the pH value was higher from beginning to end of ensiling period compared to treated sample. Moreover, the LAB number of these silages was lower than the treated silages in any ensiling time. The study clearly revealed that all sugar FJLB silages at the earlier ensiling period were able to decrease pH more rapidly and increase the LAB number dramatically in the same time.

Considering the acid content, it was clearly shown that the sugar treated FJLB caused higher lactic acid formation. However, it was found that the lactic acid content of all silages decreased with the extension of storage period. Meanwhile, the increasing acetic acid content was found during ensiling period. There are two evidents for these results. Firstly, the population of lactic acid bacteria had

Table 3. Chemical composition of 49 days silage

	Control	Non-sugar FJLB	FJLB+1% glucose	FJLB+1% sucrose	FJLB+5% molasses	SEM[3]
pH	5.88[a, 1]	5.46[b]	4.67[c]	4.92[c]	4.69[c]	0.03
LA[2] (g/kg DM)	0.00[d]	0.00[d]	45.88[a]	12.54[b]	31.18[c]	0.13
AA (g/kg DM)	33.89[a]	30.44[a]	17.42[b]	19.71[b]	21.97[b]	0.91
BA (g/kg DM)	7.12[ab]	16.00[a]	3.33[b]	7.23[ab]	3.60[b]	0.28
NH_3-N (g/kg TN)	283.97[a]	343.67[a]	107.02[b]	126.90[b]	107.85[b]	7.31
V-score	32.80[b]	18.78[b]	79.21[a]	63.22[a]	77.94[a]	1.15

[1] Mean values within rows with different superscript letters were significantly different ($p<0.05$).

[2] LA = lactic acid, AA = acetic acid, BA = butyric acid, NH_3-N = ammonia-nitrogen.

[3] SEM = Standard error of mean.

changed from homolactic to heterolactic during the fermentation (Shockey et al., 1988). Secondly, when the fresh material had low level of WSC content or even no more available carbohydrate, LAB were able to utilize lactic acid and produce more acetic acid (Lindgren et al., 1990).

Butyric acid content was slightly high in non-sugar FJLB silages. This result was probably due to the lack of a substrate for the FJLB predominantly grown in the incubation period. Generally, plant juice contains many kinds of microorganism such as LAB, coliform bacteria and fungi. LAB number is usually lower than the other consuming sugar competitor in plant juice (Hellings et al., 1985; Cai et al., 1998; Yahaya et al., 2004). In this experiment, when juice was incubated without sugar, these bacteria could only use sugar that present in the plant juice for their growing. Therefore, LAB could not grow predominantly with the other bacteria. Finally, non-sugar FJLB may contain the same number of LAB or less, compared to the other bacteria (Ohmomo et al., 2002). In addition, after application of FJLB to material crop, these undesirable bacteria could compete to utilize sugar with the LAB, with decreasing in the amount of WSC to LAB. This might be explained by high pH and low lactic acid content during the ensiling period. Moreover, the growth of butyric acid bacteria could be stimulated by these conditions as indicated a high content of butyric acid at the end of ensiling was present.

This experiment also showed significantly different results with the variety of sugar added in FJLB. It was found that the glucose-added FJLB was able to decrease the pH value more rapidly than the others, and fortunately, lactic acid content constantly remained during the ensiling period. Although, these FJLB were made from the same crop and the LAB counts were similar before the used of silage additives, the difference of the fermentative quality was may be due to the change of LAB species and their percentages in each substrate during incubation and after ensiling. This study suggests that the FJLB treated with glucose probably consisted of effective LAB species with the highest percentage than that in other sugar substrate.

CONCLUSION

The results obviously confirmed that using of any sugar treated FJLB additive somehow would be one of the ways to improve the fermentative quality of silage in the tropical area. Though, the FJLB also contain many kinds of microorganism and a variety of strains as mentioned, further researches must be needed to identify the actual useful species of bacteria, and to clarify the mechanism of the bacteria for improving quality of silage.

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