Effects of Adding Glucose, Sorbic Acid and Pre-fermented Juices on the Fermentation Quality of Guineagrass (*Panicum maximum* Jacq.) Silages

Tao Shao[1], N. Ohba, M. Shimojo and Y. Masuda*

Laboratory of Animal Feed Science, Division of Animal Science, Department of Animal and Marine Bioresource Sciences, Faculty of Agriculture, Kyushu University, Fukuoka 812-8581, Japan

[1]College of Animal Science and Technology, Nanjing Agricultural University No.1 Weigang Nanjing 210095, P. R. China

ABSTRACT : This study was conducted to evaluate the effects of adding glucose (G), sorbic acid (S), pre-fermented juice of epiphytic lactic acid bacteria (FJLB) and their combinations on the fermentation qualities and residual mono-and di-saccharides compositions of guineagrass silage. The additives used in this experiment were 1% glucose, 0.1% sorbic acid and FJLB at a theoretical application rate of 9.0×105 CFU g^{-1} on the fresh weight basis of guineagrass, respectively. There was a total of eight treatments in this experiment: (1) C (without additives), (2) FJLB, (3) S, (4) G, (5) FJLB+S, (6) FJLB+G, (7) S+G, (8) FJLB+S+G. After 30 days of storage, the silos were opened for chemical analyses. Based on the results, all additives were efficient in improving the fermentation quality of guineagrass silage. This was well indicated by significantly ($p<0.05$) lower pH and BA content and significantly ($p<0.05$) higher LA content in the treated silages except for the FJLB than in the C. However, there was only a slight increase in LA for the FJLB as compared with the C, which might be due to the low WSC content of the original guineagrass (34.4 g kg^{-1}). When the FJLB+S and FJLB+G were added, there were significant ($p<0.05$) decreases in pH and significant ($p<0.05$) increases in LA as compared with the FJLB alone. This indicated that the G, S and FJLB were of synergestic effects on the silage fermentation quality. The G combination treatments including the G alone showed large improvements in the fermentation quality as compared with the treatments without the G. This suggested that adding fermentable substrates (G) to plant materials such as guineagrass, which contain low WSC, intermediate population of epiphytic LAB, CP and DM content, is more important and efficient for improving the fermentation quality of silages than adding a number of species of domestic LAB (FJLB) and aerobic bacteria inhibitor (S). ***(Asian-Aust. J. Anim. Sci. 2004. Vol 17, No. 6 : 808-813)***

Key Words : Guineagrass Silage, Glucose, Sorbic Acid, Pre-fermented Juices

INTRODUCTION

It is well known that tropical grasses are generally considered to be a particular challenge in silage making and this is attributable to two main factors: first, they tend to have low water-soluble carbohydrate (WSC), secondly, there is essential difference in physical structure between temperate and tropical grasses; tropical grasses often have more porosity, coarseness and permeability than their temperate counterparts (Catchpoole and Henzell, 1971). When tropical grasses are ensiled, relatively large quantities of air may be trapped in the grass mass. This can result in more plant respiration and aerobic microorganisms activity, and consume more WSC, which are required by desirable lactic acid bacteria (LAB), causing fermentable substrates loss (Alli et al., 1985). Aerobic conditions can be improved by proper ensiling, such as chopping, compaction of the forage mass in the silo and rapid sealing of the silo after it has been filled, but tropical grasses are usually more difficult to compress and exclude air completely (Catchpoole and Henzell, 1971). Improving the fermentation quality was achieved by ensiling with some additives such as glucose to increase the supply of available substrates for the growth of LAB, sorbic acid to inhibit the activity of aerobic microorganisms and decrease the loss of WSC in the early stage of ensiling, and the pre-fermented juice of epiphytic LAB (FJLB) to contain more species and numbers of LAB resulting in fast acidification and pH reduction.

Glucose addition is to compensate the WSC loss caused by the initial undesirable bacteria activity (yeast, mold and aerobic bacteria) and ensure that a sufficient amount of WSC remains at the vigorous stage of LAB growth and produces lactic acid (LA) enough to decrease pH below 4.2. Advantages in adding glucose are demonstrated by many researches such as Ohyama et al. (1971, 1973, 1975).

Sorbic acid is commonly used as a mold inhibitor in foods (Deuel et al., 1954; Salunkhe, 1956; Lacey, 1989). In addition, potassium sorbate or sorbic acid (Woolford, 1975; Alli and Baker, 1982; Hattori et al., 1996; Cai and Ogawa, 1998) has been shown to inhibit the growth of yeasts and mold, and used as an ingredient of commercial silage additives. These researchers used sorbate or sorbic acid as a silage additive to inhibit the aerobic deterioration, when the silo was opened and exposed to air during the utilization period. In the present study, we used sorbic acid in order to

* Corresponding Author: Yasuhisa Masuda. Tel: +81-92-642-2952, Fax: +81-92-642-2952, E-mail: ymasuda@agr.kyushu-u.ac.jp

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restrict the activity of aerobic microorganisms during the very early stage of ensiling, and decrease the loss of WSC.

It was well documented that adding the FJLB to silages was effective for improving fermentation quality, and often resulted in the increase of LA and in the reduction of ammonia-N (AN) even when the addition of commercial LAB was ineffective (Ohshima et al., 1997a,b,c). In the present study, it is considered that FJLB will promote the onset of LAB fermentation, and accelerate the rate of LA production and pH reduction, and increase the efficiency of WSC utilization for LAB.

There is, however, relatively little published information on the effects of sorbic acid, glucose, FJLB and their combined treatments on the fermentation qualities, especially for tropical grasses. The objectives of the present study are to evaluate the effects of addition of glucose, sorbic acid, FJLB and their combined treatments on the fermentation qualities and residual mono- and di-saccharides compositions of guineagrass silages.

MATERIALS AND METHODS

Additive preparation

The FJLB was prepared from guineagrass according to the following manners: a 100 g sample of freshly cut guineagrass was macerated with 300 ml of distilled water using a blender. The macerated sample was filtered through double layers cheesecloth, and 200 ml of the filtrate was collected into a 500 ml glass bottle to which 4 g of glucose were added. The glass bottle was fitted with a gas trap and maintained at 30°C for 3 days (Ohshima et al., 1997a,b,c). After 3 days of anaerobic incubation, the pH value and the population of LAB of pre-fermented juice were determined just before being added to the silage material.

Silage making

Guineagrass was cultivated in the experimental field of Kyushu University, Hakozaki, Fukuoka, Japan. The second growth of the guineagrass was hand-harvested with a sickle at the middle heading stage on September 4 in 2000. The harvested grass was immediately chopped into about 1cm length prior to treatments. The additives used in the experiment were glucose 1% (G), sorbic acid 0.1% (S) and FJLB at a theoretical application rate of 9.0×10^5 CFU g^{-1} on the fresh weight basis of guineagrass, respectively. There was a total of eight treatments in this experiment: (1) C (without additives), (2) FJLB, (3) S, (4) G, (5) FJLB+S, (6) FJLB+G, (7) S+G, (8) FJLB+S+G. After thorough mixing, a 630-g of guineagrass was ensiled into a laboratory silo (1 liter capacity) in triplicates at each treatment. This was followed by being sealed with a screw top and stored in the room kept at 25°C. All silos were opened after 30 days of storage.

Chemical analyses

The chopped guineagrass was immediately collected for the determination of contents of dry matter (DM), total nitrogen (TN), crude protein (CP) mono-and di-saccharides compositions (fructose, glucose and sucrose), and the population of epiphytic LAB in the initial guineagrass. After opening the silos and mixing the content thoroughly, a 50 g sample was taken from each silo and a 150 g of distilled water was added before being stored in the refrigerator at 4°C for 24 h. Then, the extracts were filtered through double layers cheesecloth and a filter paper (Toyo No. 5A, Japan), and the filtrate was used for the determination of pH, AN, LA, ethanol and volatile fatty acids (VFAs).

The pH of silage was measured using a glass electrode pH meter (Horiba Co. Japan). The TN contents of fresh guineagrass and silages were analyzed by the Kjeldahl method (AOAC, 1984) and the CP of fresh grass was determined with TN multiplied by 6.25. The LA content was determined using the method of Barker and Summerson (1941), and the contents of VFAs and ethanol with gas chromatography (Shimadzu, Japan, GC-17A with 12 m capillary column, condition: column temperature 100°C, injection and detection temperature 250°C). The AN content with an ammonia electrode meter (Model IM-22P, Toa Electronics Ltd. Japan). The DM contents of the fresh guineagrass and silages were determined by drying in an oven at 60°C for at least 48 h (AOAC, 1984), and the DM of silages was recalculated with the contents of volatile compositions. Mono-and di-saccharides compositions of the fresh guineagrass and silages were determined by high performance liquid chromatography (HPLC) as shown in a previous report (Shao et al., 2002). The population of epiphytic LABs in the fresh guineagrass and the FJLB was determined by counting the CFU with GYP-$CaCO_3$ agar plate (Masuko et al., 1992).

Statistical analyses

Statistical analyses included one-way analysis of variance with treatments as a factor and Fisher's least significant difference test; these were performed by ANOVA using the GLM procedure of the Statistical Analysis System (SAS, 1984).

RESULTS

From the characteristics of the initial guineagrass and FJLB in this experiment (Table 1), it is apparent that the guineagrass had low contents of fructose (16.2 g kg^{-1}), glucose (11.7 g kg^{-1}), sucrose (6.4 g kg^{-1}) and total mono- and di-saccharides (34.4 g kg^{-1}), and intermediate contents of CP (79.4 g kg^{-1}) and DM (290.1 g kg^{-1}). The population of epiphytic LAB was 4.74×10^5 CFU g^{-1} for the fresh

Table 1. Characteristics of guineagrass and FJLB[1] before ensiled

Silage material							FJLB	
Dry matter (g kg^{-1})	Crude protein (g kg^{-1} DM)	Fructose (g kg^{-1} DM)	Glucose (g kg^{-1} DM)	Sucrose (g kg^{-1} DM)	Mono- and di-saccharides (g kg^{-1} DM)	LAB [2] (CFU g^{-1} FW)	LAB [3] (CFU ml^{-1})	pH
290.1	79.4	16.2	11.7	6.4	34.4	4.74×10^{5}	5.64×10^{8}	3.87

[1] Pre-fermentated juice of epiphytic lactic acid bacteria.
[2] The population of epiphytic lactic acid bacteria in initial fresh grass expressed as colony forming unit (CFU) per g fresh weight (FW).
[3] The population of lactic acid bacteria in FJLB prior to being added expressed as colony forming unit (CFU) per ml FJLB.

Table 2. Fermentation quality of guineagrass silages treated with glucose, sorbic acid, FJLB and their combinations

Item	C	FJLB	S	G	FJLB+S	FJLB+G	S+G	FJLB+S+G
Dry matter (SD) (g kg^{-1})	266.0 (4.77)^{a}	266.1 (1.34)^{a}	263.4 (4.26)^{a}	278.0 (6.85)^{b}	264.0 (6.01)^{a}	265.0 (0.91)^{a}	279.1 (4.06)^{b}	262.0 (3.62)^{a}
pH (SD)	4.58 (0.17)^{d}	4.17 (0.04)^{c}	4.19 (0.10)^{c}	3.86 (0.02)^{ab}	4.00 (0.04)^{b}	3.85 (0.04)^{ab}	3.72 (0.02)^{a}	3.79 (0.03)^{a}
Lactic acid (SD) (g kg^{-1} DM)	18.7 (4.62)^{a}	21.7 (4.96)^{ab}	32.7 (5.54)^{bc}	61.8 (4.60)^{e}	38.6 (5.31)^{c}	44.4 (4.24)^{cd}	55.4 (18.08)^{de}	55.2 (12.13)^{de}
Acetic acid (SD) (g kg^{-1} DM)	13.6 (6.31)^{bc}	14.2 (3.63)^{c}	8.5 (2.74)^{ab}	9.1 (4.06)^{abc}	9.5 (0.79)^{abc}	11.5 (4.79)^{abc}	7.4 (1.88)^{a}	7.4 (0.45)^{a}
Volatile fatty acids (SD) (g kg^{-1} DM)	27.0 (2.72)^{d}	18.1 (4.84)^{cd}	14.3 (5.21)^{bc}	10.5 (4.26)^{ab}	10.8 (1.70)^{ab}	12.2 (4.64)^{abc}	8.8 (2.42)^{a}	7.9 (0.17)^{a}
Butyric acid (SD) (g kg^{-1} DM)	12.5 (3.65)^{c}	3.7 (1.43)^{ab}	5.1 (4.60)^{b}	1.2 (0.47)^{a}	1.1 (1.62)^{a}	0.3 (0.22)^{a}	1.2 (1.59)^{a}	0.1 (0.20)^{a}
Valeric acid (SD) (g kg^{-1} DM)	0.0 (0.04)^{a}	0.0 (0.00)^{a}	0.0 (0.00)^{a}	0.0 (0.00)^{a}	0.0 (0.00)^{a}	0.0 (0.07)^{a}	0.0 (0.00)^{a}	0.0 (0.00)^{a}
Propionic acid (SD) (g kg^{-1} DM)	0.8 (0.04)^{b}	0.2 (0.26)^{a}	0.7 (0.21)^{b}	0.2 (0.43)^{a}	0.2 (0.41)^{a}	0.4 (0.48)^{a}	0.1 (0.24)^{a}	0.4 (0.41)^{a}
Ethanol (SD) (g kg^{-1} DM)	11.7 (2.89)^{abc}	9.8 (3.75)^{ab}	10.8 (3.68)^{ab}	17.0 (9.67)^{c}	7.8 (1.24)^{ab}	14.4 (1.12)^{bc}	7.3 (0.94)^{a}	10.8 (0.66)^{abc}
AN/TN (SD) (g AN kg^{-1} TN)	88.4 (24.42)^{bc}	92.6 (19.22)^{c}	70.6 (6.17)^{ab}	53.0 (7.14)^{a}	86.9 (14.26)^{bc}	70.5 (12.87)^{ab}	65.5 (12.24)^{a}	62.5 (6.08)^{a}
Lactic acid/acetic acid (SD)	1.4 (1.48)^{a}	1.5 (1.48)^{a}	3.9 (1.11)^{ab}	6.8 (3.61)^{c}	4.1 (0.58)^{ab}	3.9 (2.40)^{ab}	7.5 (1.68)^{c}	7.4 (1.34)^{c}

$^{a, b, c}$ Values followed by different letters in the same row show significant differences at $p<0.05$.
C: control. FJLB: pre-fermented juice of epiphytic lactic acid bacteria, S: sorbic acid, G: glucose, AN: NH_3-N, TN: total N.

guineagrass and 5.64×10^{8} CFU ml^{-1} for the FJLB having pH value of 3.87.

The fermentation qualities of guineagrass silages treated with glucose, sorbic acid, FJLB and their combinations are presented in Table 2. The G and S+G silages significantly ($p<0.05$) increased DM content, and the other treated silages did not show significant ($p>0.05$) differences as compared with the C. All treated silages showed significantly ($p<0.05$) lower pH values than the C. When the FJLB+S and FJLB+G were added, there were significant ($p<0.05$) decreases in pH as compared with the FJLB alone. Similarly the FJLB+S and S+G silages also significantly ($p<0.05$) decreased pH as compared with the S alone. The G addition showed significantly ($p<0.05$) lower pH than the FJLB and S additions. There were no significant ($p>0.05$) differences between the G alone and G combination silages, but they had the largest pH reduction as compared with the other treated silages.

With the exception of the FJLB, all treated silages significantly ($p<0.05$) increased LA content as compared with the C. The FJLB+S and FJLB+G silages had significantly ($p<0.05$) higher LA content than the FJLB alone. The S+G silage showed significantly ($p<0.05$) higher LA content and the FJLB+S tended to increase ($p>0.05$) it as compared with the S alone. The G and G combination treatments (FJLB+G, S+G, FJLB+S+G) greatly improved the fermentation quality of silage with larger LA production than the silages without the G. There were not significant ($p>0.05$) differences in LA content among the G, S+G and FJLB+S+G treated silages, but the FJLB+G had a significantly ($p<0.05$) lower LA content as compared with the G alone.

The S+G and FJLB+S+G silages significantly ($p<0.05$) decreased AA content as compared with the C and FJLB. The other treated silages did not show significant ($p>0.05$) differences in AA content from the C. With the exception of the FJLB all treated silages significantly ($p<0.05$) decreased the content of VFAs as compared with the C. Silages treated with the S+G and FJLB+S+G showed the lowest VFAs that were significantly ($p<0.05$) lower than the C, FJLB and S treated silages. There were no significant ($p>0.05$) differences among the other treated silages in VFAs content.

When additives were given, there were significant ($p<0.05$) decreases in BA content as compared with the C. However, the S or FJLB had a significant ($p<0.05$) or a slight ($p>0.05$) increase in BA content than the other treated silages. The valeric acid (VA) was hardly found in all silages. There were very low levels of propionic acid (PA) in all silages, but the C and S showed slightly but significantly ($p<0.05$) higher PA than the other treated silages. The ethanol content did not show significant ($p>0.05$) differences among all silages except for the G and FJLB+G that had a higher tendency.

When compared with 88.4 g kg^{-1} for AN/TN of the C, there were significant ($p<0.05$) decreases in the G, S+G and S+G+FJLB, respectively. The FJLB+G addition significantly ($p<0.05$) decreased AN/TN but the FJLB+S showed an insignificant decrease ($p>0.05$) as compared with the FJLB alone. There were no significant ($p>0.05$) differences in AN/TN between the FJLB+S and S, and between the S+G and S.

The FJLB and C had a similar LA/AA. The S addition tended to increase it as compared with the C, but there were significant ($p<0.05$) increases in the G, S+G and FJLB+S+G.

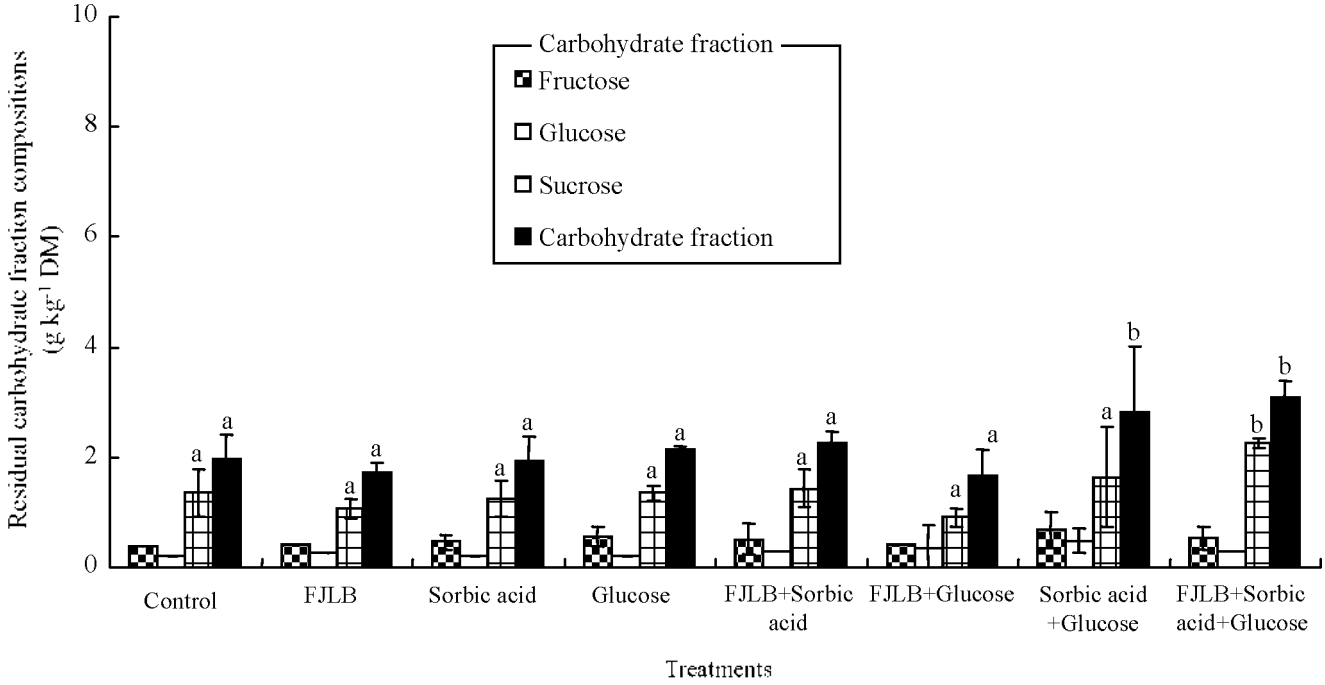

Figure 1. Effect of additives on the residual carbohydrate fraction of guineagrass silages. FJLB: pre-fermentated juice of epiphytic lactic acid bacteria. Different letters in the same fraction show significant differences at $p<0.05$.

The additions of the FJLB+S and FJLB+G showed a tendency of increase in LA/AA as compared with the FJLB alone. There was not a significant ($p>0.05$) difference between the S alone and FJLB+S, but the S+G showed significantly ($p<0.05$) higher LA/AA than the S alone.

The contents of residual mono-and di-saccharides compositions are shown in Figure 1. The S+G and FJLB+S+G additions showed slightly but significantly ($p<0.05$) higher residual total mono- and di-saccharides contents. There were no significant ($p>0.05$) differences in residual glucose, fructose and sucrose among all silages that showed very low contents except for the FJLB+S+G having a slight but significantly ($p<0.05$) higher sucrose content.

DISCUSSION

All treated silages significantly ($p<0.05$) decreased pH and BA whereas significantly ($p<0.05$) increased LA with the exception of LA in the FJLB as compared with the C. These indicated that the additives improved the fermentation quality. However, the FJLB addition showed a small LA increase, which was probably caused by low WSC content of the original guineagrass (34.4 g kg^{-1}), rather than the FJLB inoculant itself. This is in agreement with the report of Uchida and Kitamura (1987), which showed that LAB additives did not have a large effect on fermentation quality of tropical grass with low WSC content. Seale (1986) also reported that LAB inoculant was less effective if the fermentable substrate was low or insufficient.

When the FJLB+S and FJLB+G were added, there was an evidence of further increase in LA and decrease in pH as well as a tendency of increase in LA/AA as compared with the FJLB alone. This indicated that the S, G and FJLB were of synergistic effects on the silage fermentation quality. Moreover, it also indicated that the efficiency of fermentation in silages with the additive of FJLB alone was limited by low WSC content of the original grass. This could probably be explained as follows. First, the FJLB combined with G (FJLB+G) supplied the fermentable substrate for LAB to produce more amounts of LA, resulting in lower pH (Ohyama et al., 1971, 1973, 1975). Second, FJLB combined with the S (FJLB+S) restricted the activity of aerobic bacteria during very early stages of ensiling, and decreased the loss of WSC for LAB (Woolford, 1975, Alli et al., 1985). The S+G had significantly ($p<0.05$) higher LA than the S+FJLB and S, but there was not a large difference ($p>0.05$) between the S+FJLB and S. This indicated that the S+G addition was more effective than the S+FJLB and S alone, because the low WSC content in the original material was the major limiting factor in the effect on the fermentation quality. Although the S alone addition decreased the loss of WSC from the undesirable bacteria activity, there was not enough WSC for LAB as compared with the S+G addition silage.

The additions of the G, S+G and FJLB+S+G greatly increased LA and LA/AA and decreased pH as compared with the other treated silages. This indicated that these additives further promoted homofermentative LAB

fermentation as compared with the other treated silages. It was confirmed that adding the fermentable substrate (G) to the guineagrass was more important and effective to make high quality silages than adding the FJLB and S. The other treated silages showed insignificant ($p>0.05$) increases in LA/AA compared with the C, suggesting that these treatments without the G had not enough amounts of fermentable substrates to further increase the activity of homofermentative LAB in the production of more LA.

There was an interesting result that the G+FJLB silage showed lower LA and LA/AA as well as a tendency of higher AA than the G alone. The increase of AA in the FJLB additive silages was also found in other experiments (Ohshima et al., 1997 a,b,c), suggesting some herterofermentatative LAB activity occurring. This may be explained as follows. FJLB contain more species and numbers of LABs from the original grass, which was different from the commercial LAB containing only homofermentative LAB. When the FJLB was added to grass mass, the numbers of both homo- and hetero-fermentative LAB were enhanced, thus resulting in higher activity of not only homofermentative LAB but also heterofermentative LAB.

There were significantly ($p<0.05$) lower values of AN/TN in the G, S+G and S+G+FJLB silages than in the C. This indicated that the rate of LA production and that of pH reduction were faster in these silages, thus inhibiting the activity of proteolysis by plant enzymes and other undesired bacteria activity during the early stage of ensiling. The FJLB or S alone had a slightly higher BA or PA content than the other treated silages, which indicated some clostridial bacteria activity occurring. This was due to low WSC of the initial guineagrass, and there were not sufficient LA and enough low pH to inhibit the activity of clostridial bacteria during ensiling.

Some researchers (Yokota et al., 1991; Miyagi et al., 1993) reported that the ensiling nature of tropical species was LA-type fermentation, but others (Catchpoole, 1970; Catchpoole and Henzell, 1971; Panditharatne et al., 1986; Kim and Uchida, 1990) demonstrated that a main preservation in silages made from tropical grasses was AA-type. They suggested that the WSC content of the original grass might determine the LA or AA-type fermentation. In the present study the guineagrass contained low amounts of WSC (34.4 g kg^{-1}), but the LA content was higher relative to AA and ethanol in all silages, not the AA-type silage. This was still not clear, but there was probably a high activity of lactate producing bacteria adherent to the original guineagrass (4.74×10^5 CFU g^{-1} FW).

There were very low contents of residual fructose, glucose, sucrose and total mono- and di-saccharide in all silages. This indicated that the WSC content of original guineagrass was low and not adequate, therefore, almost all the fermentation substrates were consumed during 30 days of ensiling. The S+G and FJLB+S+G additions showed a slightly but significantly ($p<0.05$) higher sucrose and total mono-and di-saccharides contents as compared with the other treated silages. This suggests that the combination of the G with S additions improved the utilization efficiency of WSC compared with the G or S alone.

Based on this study, all additives had a beneficial effect on the improvement of fermentation quality, but the FJLB alone had less benefit as compared with the other additives. The FJLB in the combination with S or G further increased LA content and decreased pH value, and which were the synergestic effects on the silage fermentation quality. Especially the G and G combination treatments greatly improved the fermentation quality. These suggest that adding fermentable substrates (G) to plant materials such as guineagrass, which contain low WSC, intermediate population of epiphytic LAB, CP and DM content, is more important and efficient for improving the fermentation quality of silages than adding a number of species of domestic LABs (FJLB) and aerobic inhibitor (sorbic acid).

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