Comparison of Fermentation Characteristics of Italian Ryegrass (*Lolium multiflorum* Lam.) and Guineagrass (*Panicum maximum* Jacq.) during the Early Stage of Ensiling

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ABSTRACT : The fermentation characteristics and mono- and di-saccharides compositions during the early stage of ensiling were studied with a temperate grass, Italian ryegrass (*Lolium multiflorum* Lam.) and a tropical grass, guineagrass (*Panicum maximum* Jacq.). The laboratory silos were kept in the room set at 25°C, and then were opened on 0.5, 1, 2, 3, 5 and 7 days (14 days in Italian ryegrass) after ensiling, respectively. The Italian ryegrass silage showed a fast and large pH decrease caused by a fast and large production of lactic acid during the first 5 days of ensiling and succeeded to achieve lactic acid type fermentation; high lactic acid/acetic acid and lactic acid content at the end of ensiling (14 days), low values of pH (3.74), acetic acid, ethanol and ammonia-N/total nitrogen, none or only small amounts of Butyric acid, valeric acid and propionic acid. The guineagrass silage showed a slow decrease in pH and a slow increase in lactic acid content during the full ensiling period, causing a high final pH value, low contents of lactic acid, acetic acid, total volatile fatty acids and total organic acids. In Italian ryegrass silage, mono- and di-saccharides compositions decreased largely within the initial 0.5 day (12 h) of ensiling. Sucrose disappeared rapidly within the initial 0.5 day of ensiling, but fructose and glucose contents showed an initial rise by the activity of enzymes in plant tissues, and then decreased gradually. On the other hand, the contents of mono- and di-saccharides in guineagrass showed the largest decreases due mainly to plant respiration within the initial 0.5 day of ensiling, and no initial rises in fructose and glucose contents during the early stage of ensiling because of the absence of fructans which are hydrolyzed into fructose and glucose in temperate grasses. In both silages, the rate of reduction in mono- and di-saccharides compositions within the initial 5 days of ensiling was ranked in the order of glucose>fructose>sucrose, suggesting that glucose and fructose might be more favorably utilized than sucrose by microorganisms and glucose is the first fermentation substrate. It was concluded that the silage made from Italian ryegrass with high moisture content had a good fermentation quality owing to the dominance of lactic acid bacteria and active lactic acid fermentation during the initial stage of ensiling. These results can be explained by rapid plant sap liberation and the high activity of plant enzyme hydrolyzed fructans into fructose and glucose within the initial 2 days of ensiling, which stimulate the homofermentative lactic acid bacteria growth. In ensiling a temperate grass, the physical characteristics may ensure the rapid onset of fermentation phase, which results from the smaller losses of water-soluble carbohydrates during the initial stage of ensiling and providing sufficient water-soluble carbohydrates for lactic acid bacteria. The silage made from guineagrass with intermediate dry matter and high initial mono- and di-saccharides content was stable silage. This could be explained by the higher incorporation of air during the very early stage of ensiling and the restriction of cell breakdown and juice release due to the properties of a tropical grass with coarse porosity and stemmy structures. These physical characteristics delayed the onset of lactic acid bacteria fermentation phase by extending the phases of respiration and aerobic microorganisms activity, causing the higher loss of water-soluble carbohydrates and the shortage of lactic acid bacteria fermentation substrates. ***(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 12 : 1727-1734)***

Key Words : Early Fermentation Characteristics, Mono- and Di-saccharides Compositions, Italian Ryegrass, Guineagrass

INTRODUCTION

It is well known that air is still present in the silage during the early stage of ensiling and this enables plant respiration and aerobic microbial activity to take place: this leads to loss of both nutritive material and fermentation substrates. The rate and efficiency of acid production in the early stage of fermentation are important factors in efficient both silage making and quality (Weinberg et al., 1988), therefore the initial fermentation characteristics are critical to the success or failure of silage making.

Tropical and temperate herbage species are different in their chemical, physical and physiological properties, tropical grasses are generally low in their water-soluble carbohydrates (WSC) but high in polysaccharide contents (Smith, 1973) and have coarse porosity and stemmy structures (Catchpoole and Henzell, 1971). They are usually less dense and presumably more permeable, and relatively

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large quantities of air may be trapped in the forage mass than temperate grasses just after ensiling. These could cause a difference in the ensiling process and the silage quality between these species types (Catchpoole and Henzell, 1971; Kim and Uchida, 1990; Shao et al., 2004). According to McDonald et al. (1991), in temperate origin grass, fructans are the most abundant WSC, however, grasses of tropical and subtropical origins accumulate starches instead of fructans. The WSC can be fermented rapidly by epiphytic lactic acid bacterias (LABs) provided they are readily available, but in contrast, the most naturally occurring LABs do not have the ability to ferment starch directly. Silages made from a number of tropical herbage plants have proved stable against anaerobic decomposition in the silo, as judged by low rate of ammonia-N/total nitrogen (<100 g kg^{-1} TN) (AN/TN) and low butyric acid (BA) content (<2.0 g kg^{-1} DM). However, their chemical compositions do not conform to temperate standards for lactic acid (LA) silage, indicating that their stability was not due to the rapid formation of LA and reduction of pH (Catchpoole and Henzell, 1971).

Italian ryegrass and guineagrass are important forage crops. They are now widely distributed throughout temperate and tropical or subtropical regions of the world, they are also major silage crops in China and have been widely used for silage making. However, there is limited information regarding the comparative studies of the initial fermentation characteristics and the changes in mono-and disaccharides between two species grasses during the early stage of ensiling. Based on the consideration of these factors, this study was carried out. The purpose of the present work was to study relations between changes in mono- and di-saccharides contents and the fermentation characteristics during the early ensiling stage of Italian ryegrass and guineagrass silages.

MATERIALS AND METHODS

Silage making

Italian ryegrass was sown on October 15, 1999, in the experimental field of Nanjing Agricultural University and the initial growth of Italian ryegrass was harvested at the internode elongation stage using a hand sickle on April 6, 2000. The harvested Italian ryegrass was chopped into approximately 1 cm length with a forage cutter. Eighty grams of chopped grass were immediately packed into a plastic laboratory silo (100 ml liter capacity) in triplicates, followed by being sealed with a screw top and stored in the room kept at 25°C. The silos were opened on 0.5, 1, 2, 3, 5, 7 and 14 days after ensiling.

Guineagrass was cultivated and the second growth of guineagrass was harvested at the milky ripe stage on October 18, 1999, and used for silage making with the same procedures applied to Italian ryegrass material described above. However, the Eighty-five grams chopped guineagrass was packed into a plastic laboratory silo (100 ml capacity), and the silos were opened on 0.5, 1, 2, 3, 5 and 7 days of ensiling.

Chemical analyses

The chopped grasses were immediately collected for the determination of dry matter (DM), total nitrogen (TN), crude protein (CP) and mono- and di-saccharides (fructose, glucose and sucrose) contents. After the silos were opened and the contents were mixed thoroughly, forty grams of the sample was taken from each silo. This was followed by adding eighty grams of distilled water and macerating at 4°C for 24 h. Then, the extracts were filtered through two layers of cheesecloth and a filter paper (Toyo No. 5A). The filtrate was stored at -20°C prior to chemical analyses. The filtrate was used for determining pH, ammonium nitrogen (AN), LA, ethanol, and volatile fatty acids (VFAs). The pH of silages was measured using a glass electrode pH meter (Horiba Co, Japan). TN was analyzed by the Kjeldahl method (AOAC, 1984) and AN with an ammonia electrode (Model IM-22P, Toa Electronics Ltd, Japan). CP was determined by multiplying TN by 6.25. LA content was determined using the method of Barker and Summerson (1941), and VFAs and ethanol with gas chromatography (Shimadzu GC-17A, Japan, with 12 m capillary column; condition: column temperature at 100°C, injection and detection temperature at 250°C). DM contents of the fresh material and silages were determined by drying in an oven at 60°C for at least 48 h (AOAC, 1984), and DM of silage was recalculated with the contents of volatile compositions. Mono- and di-saccharides compositions of the fresh grass and silages were determined by high performance liquid chromatography (HPLC) as shown in our previous reports (Shao et al., 2002).

Statistical analyses

All data was analyzed statistically by one-way analysis of variance (ANOVA) with storage periods as a factor and statistical significance among storage periods for each item was determined by Fisher's least significant difference test; these were performed by ANOVA using the general linear model (GLM) procedure of the Statistical Analysis System (SAS, 1989).

RESULTS

Characteristics of the Italian ryegrass and guineagrass before ensiling are shown in Table 1. The DM contents of fresh Italian ryegrass and guineagrass were 139.01 g kg^{-1}

Table 1. Characteristics of Italian ryegrass and Guineagrass before ensiled

Forage species	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)
Italian ryegrass	149.53	63.75	23.55	14.22	14.64	52.41
Guineagrass	258.64	79.35	33.54	30.54	80.27	144.34

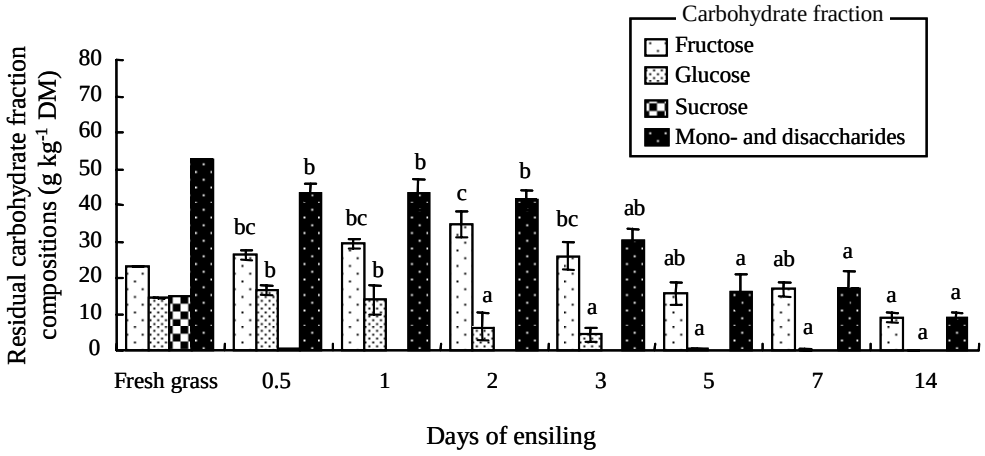

Figure 1. Changes in mono-and di-saccharides during the early stage of ensiling in Italian ryegrass (* Different letters in the same parameter differ significantly at $p<0.05$).

DM and 186.49 g kg^{-1} DM, respectively. Guineagrass had higher DM content than that of Italian ryegrass. Italian ryegrass had lower contents of CP and sucrose, fructose, glucose and total mono- and di-saccharides when compared with guineagrass.

Italian ryegrass fermentation characteristics

Changes in chemical compositions of Italian ryegrass silage are presented in Table 2. The major fermentation products were LA, acetic acid (AA) and ethanol. There was a fast and large reduction ($p<0.05$) in pH from 2 days of ensiling, a sharp decrease ($p<0.05$) to 3.87 on the 5th day, and then pH remained almost constant until day 14 of ensiling. The LA content increased significantly ($p<0.05$) from the second day of ensiling, reaching the highest value (138.83 g kg^{-1} DM) on day 5, and then decreased significantly ($p<0.05$) to 85.83 g kg^{-1} DM at the end of the period.

The AA content showed a significant ($p<0.05$) increase from the second day of ensiling, reaching the peak (6.40 g kg^{-1} DM) on day 3, and then varied between 3.67 and 5.38 g kg^{-1} DM. The ethanol content increased significantly ($p<0.05$) after 2 days of ensiling, and reached the highest concentration (38.22 g kg^{-1} DM) on day 5, and then decreased significantly ($p<0.05$) to 19.20 g kg^{-1} DM at the end of the experiment. The BA, valeric acid (VA) and propionic acid (PA) were absent or detected in only small amounts over the ensiling period. Total organic acids content increased significantly ($p<0.05$) from 3 days of ensiling, reaching the highest value on day 5, followed by an insignificant decrease. The value of LA/AA increased gradually and reached the highest value on day 5 ($p<0.05$), and then tended to decrease. It showed high values in the full fermentation course. The AN/TN increased significantly ($p<0.05$) from 3 days of ensiling and continued to increase to the highest value (75.84 g AN/kg TN) at the end of ensiling. The DM content did not change greatly up to 14 days of ensiling.

Changes in contents of mono- and di-saccharides during the ensiling are shown in Figure 1. Total mono-and di-saccharides decreased largely within initial 0.5 day of ensiling to 43.19 g kg^{-1} DM as compared with the fresh Italian ryegrass (52.41 g kg^{-1} DM), and then showed a gradual decrease until the end of ensiling. Fructose showed an initial rise within 3 days of ensiling from 23.55 g kg^{-1} DM of the fresh Italian ryegrass to 26.0 g kg^{-1} DM, and then gradually decreased to 8.73 g kg^{-1} DM at the end of the experiment. Glucose also tended to increase within 0.5 day of ensiling from 14.22 g kg^{-1} DM of the fresh Italian ryegrass to 16.49 g kg^{-1} DM, and then showed a large decrease ($p<0.05$) between 1 and 2 days (from 13.71 g kg^{-1} DM to 6.28 g kg^{-1} DM), approaching 0 (0.46 g kg^{-1} DM) on day 5. Sucrose disappeared rapidly within only initial 0.5 day of ensiling to 0.45 g kg^{-1} DM as compared with the fresh Italian ryegrass (14.64 g kg^{-1} DM).

Guineagrass fermentation characteristics

The early fermentation qualities of the guineagrass silage are presented in Table 3. The pH value decreased slowly from 5.68 to 5.35, a slight but significant ($p<0.05$)

Table 2. Changes in fermentation qualities in Italian ryegrass silage during the early stage of ensiling

Item	Storage period						
	0.5 day	1 day	2 days	3 days	5 days	7 days	14 days
pH (SD)	5.98 (0.13)$^{d, 1}$	5.99 (0.03)^{d}	4.60 (0.15)^{c}	4.06 (0.05)^{b}	3.87 (0.04)^{a}	3.77 (0.04)^{a}	3.74 (0.02)^{a}
Dry matter (SD) (g kg^{-1})	144.39 (0.90)^{a}	140.37 (3.64)^{a}	140.01 (2.39)^{a}	145.29 (3.12)^{a}	138.90 (0.38)^{a}	143.65 (0.33)^{a}	143.35 (5.25)^{a}
Ethanol (SD) (g kg^{-1} DM)	20.34 (3.89)^{a}	21.80 (1.41)^{ab}	23.54 (0.24)^{ab}	27.91(1.30)^{b}	38.22 (9.76)^{c}	22.74 (3.83)^{ab}	19.20 (0.85)^{a}
Lactic acid (SD) (g kg^{-1} DM)	2.29 (0.74)^{a}	4.98 (0.47)^{a}	58.23 (9.87)^{b}	86.67 (4.04)^{b}	138.83 (16.16)^{c}	87.73 (15.21)^{b}	85.83 (14.86)^{b}
Acetic acid (SD) (g kg^{-1} DM)	0.71 (0.18)^{a}	0.53 (0.20)^{a}	3.00 (0.72)^{b}	6.40 (1.21)^{d}	4.21 (1.66)^{bc}	3.67 (1.03)^{b}	5.38 (1.21)^{cd}
Butyric acid (SD) (g kg^{-1} DM)	0.02 (0.04)^{a}	0.00 (0.00)^{a}	0.01 (0.02)^{a}	0.01 (0.02)^{a}	0.01 (0.02)^{a}	0.02 (0.01)^{a}	0.01 (0.01)^{a}
Valeric acid (SD) (g kg^{-1} DM)	0.07 (0.12)^{a}	0.00 (0.00)^{a}	0.00 (0.00)^{a}	0.00 (0.00)^{a}	0.00 (0.00)^{a}	0.00 (0.00)^{a}	0.00 (0.00)^{a}
Propionic acid (SD) (g kg^{-1} DM)	0.16 (0.27)^{a}	0.00 (0.00)^{a}	0.07 (0.12)^{a}	0.06 (0.11)^{a}	0.11 (0.09)^{a}	0.11 (0.08)^{a}	0.07 (0.06)^{a}
Volatile fatty acids (SD) (g kg^{-1} DM)	0.96 (0.36)^{a}	0.53 (0.35)^{a}	3.08 (0.80)^{b}	6.47 (0.94)^{d}	4.33 (1.09)^{bc}	3.80 (1.32)^{b}	5.46 (1.23)^{cd}
Organic acids (g kg^{-1} DM)	3.25 (1.28)^{a}	5.51 (0.57)^{a}	61.31 (10.45)^{ab}	93.14 (5.14)^{bc}	143.16 (20.11)^{c}	91.53 (25.42)^{bc}	91.29 (25.36)^{bc}
Lactic acid/acetic acid (SD)	3.23 (0.96)^{a}	9.40 (3.15)^{ab}	19.41 (1.96)^{bc}	13.54 (1.91)^{abc}	32.98 (16.17)^{d}	23.91(8.82)^{cd}	15.95 (4.48)^{bc}
AN/TN (SD) (g AN kg^{-1} TN)^{2}	21.67 (3.85)^{a}	21.55 (2.31)^{a}	35.16 (4.88)^{a}	64.13 (10.14)^{bc}	55.53 (18.82)^{b}	57.56 (8.95)^{b}	75.84 (15.12)^{c}

[1] Values followed by different letters in the same row show significant differences at $p<0.05$.

[2] AN: ammonia-N, TN: total nitrogen.

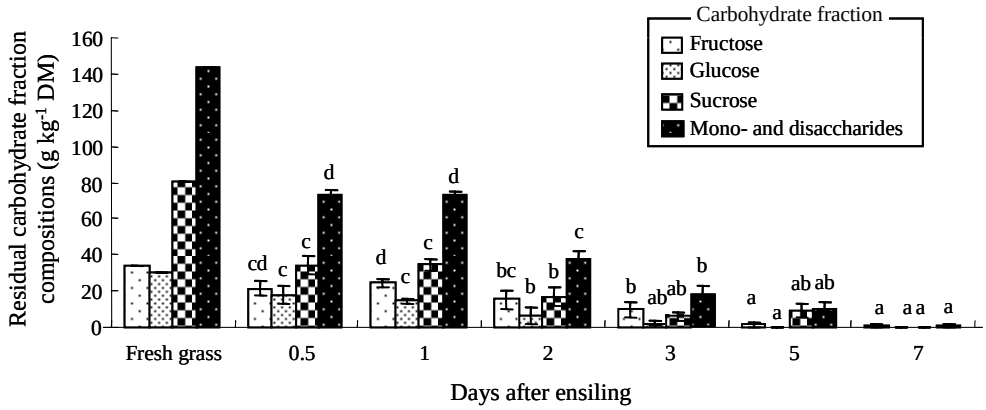

Figure 2. Changes in mono- and di-saccharides during the early stage of ensiling in guineagrass (* Different letters in the same parameter differ significantly at $p<0.05$).

decrease from 2 days of ensiling. The production rate of LA was also very slow from 1.53 to 6.15 g kg^{-1} DM, a small but significant ($p<0.05$) increase from 2 days of ensiling. The AA content did not greatly change within the initial 3 days of ensiling, and then increased significantly ($p<0.05$) to 4.42 g kg^{-1} DM. The LA/AA gradually increased and reached the peak (5.86) on day 3 of ensiling ($p<0.05$), and then decreased significantly ($p<0.05$) to 1.42. The total VFAs content showed a similar profile with AA, almost constant within the initial 3 days, and then increased significantly ($p<0.05$) to 8.12 g kg^{-1} DM at the end of the experiment. Total organic acids increased significantly ($p<0.05$) from 2 days of ensiling, and reached to 14.2 g kg^{-1} DM at the end of ensilage. The ethanol content gradually increased within the initial 2 days of ensiling and then kept on significantly ($p<0.05$) increasing to 20.76 g kg^{-1} DM on the 7th day. The BA, VA and PA were hardly detected during the initial 5 days of ensiling, and there was a slight but significant increase in BA and PA contents on day 7 of ensiling. The AN/TN significantly ($p<0.05$) increased from 2 days of ensiling and reached 45.54 g AN/kg TN at the end of ensiling. The DM content did not change greatly up to 7 days of ensiling.

Changes in the contents of mono-and di-saccharides compositions during the ensiling period are shown in Figure 2. There was the largest decrease in total mono- and di-saccharides within the initial 0.5 day of ensiling to 73.26 g kg^{-1} DM from 144.34 g kg^{-1} DM of the fresh grass. However, between 0.5 day and 1 day of ensiling they were almost constant and then decreased significantly ($p<0.05$) from 2 days of ensiling to 0.87 g kg^{-1} DM at the end of the storage.

Similarly, fructose, glucose and sucrose also decreased largely within initial 0.5 day of ensiling from the original values of fresh grass. Fructose showed a slight increase between 0.5 day and 1 day of ensiling, and then decreased significantly ($p<0.05$) from 3 days of ensiling to 0.87 g kg^{-1} DM at the end of ensilage. Glucose decreased slowly between 0.5 day and 1 day of ensiling, and then decreased significantly ($p<0.05$) to zero until 5 days of ensilage. Sucrose showed no changes between 0.5 day and 1 day of ensiling, and then decreased significantly ($p<0.05$) to zero on the 7th day of ensiling. The rate of disappearance in mono-and di-saccharides compositions was ranked in the following order: glucose>fructose>sucrose within the initial 5 days of ensiling.

Table 3. Changes in fermentation qualities in guineagrass silage during the early stage of ensiling

Item	Storage period					
	0.5 day	1 day	2 days	3 days	5 days	7 days
pH (SD)	5.68 (0.05)^{c} [1]	5.68 (0.02)^{c}	5.56 (0.02)^{b}	5.55 (0.05)^{b}	5.56 (0.10)^{b}	5.35 (0.10)^{a}
Dry matter (SD) (g kg^{-1})	256.10 (0.74)^{a}	254.50 (4.22)^{a}	253.89 (1.65)^{a}	256.38 (3.25)^{a}	254.64 (6.20)^{a}	256.60 (7.05)^{a}
Ethanol (SD) (g kg^{-1} DM)	2.49 (0.55)^{a}	3.52 (0.57)^{a}	5.36 (1.08)^{ab}	7.83 (1.75)^{b}	15.30 (4.84)^{c}	20.76 (0.88)^{d}
Lactic acid (SD) (g kg^{-1}DM)	1.53 (0.21)^{a}	2.23 (0.68)^{ab}	3.85 (0.20)^{bc}	5.48 (0.82)^{cd}	5.01 (2.13)^{cd}	6.15 (0.69)^{d}
Acetic acid (SD) (g kg^{-1} DM)	0.55 (0.10)^{a}	1.03 (0.11)^{a}	1.04 (0.14)^{a}	0.93 (0.04)^{a}	3.80 (0.53)^{b}	4.42 (0.61)^{c}
Butyric acid (SD) (g kg^{-1}DM)	0.14 (0.17)^{a}	0.02 (0.04)^{a}	0.00 (0.00)^{a}	0.07 (0.00)^{a}	0.39 (0.27)^{a}	2.71 (1.30)^{b}
Valeric acid (SD) (g kg^{-1} DM)	0.13 (0.13)^{b}	0.00 (0.00)^{a}	0.00 (0.00)^{a}	0.00 (0.00)^{a}	0.02 (0.04)^{a}	0.02 (0.04) ^{a}
Propionic acid (SD) (g kg^{-1} DM)	0.32 (0.21)^{a}	0.09 (0.15)^{a}	0.00 (0.00)^{a}	0.00 (0.00)^{a}	0.06 (0.05)^{a}	0.91 (0.48)^{b}
Volatile fatty acids(SD) (g kg^{-1} DM)	1.14 (0.55)^{a}	1.14 (0.29)^{a}	1.04 (0.14)^{a}	1.01 (0.03)^{a}	4.28 (0.45)^{b}	8.06 (2.12)^{c}
Organic acids (SD) (g kg^{-1} DM)	2.67 (0.49)^{a}	3.37 (0.96)^{ab}	4.89 (0.20)^{bc}	6.48 (0.83)^{c}	9.28 (1.72)^{d}	14.22 (1.82)^{e}
Lactic acid/acetic acid (SD)	2.83 (0.71)^{bc}	2.14 (0.43)^{ab}	3.76 (0.56)^{c}	5.86 (0.93)^{d}	1.39 (0.79)^{a}	1.42 (0.33)^{a}
AN/TN (SD) (g AN kg^{-1} TN)[2]	2.64 (0.04)^{a}	9.01 (2.26)^{ab}	15.81 (1.29)^{bc}	21.19 (1.23)^{c}	35.55 (3.66)^{d}	45.54 (9.22)^{e}

[1] Values followed by different letters in the same row show significant differences at $p<0.05$.

[2] AN: ammonia-N, TN: total nitrogen.

DISCUSSION

Italian ryegrass fermentation characteristics

Italian ryegrass silage in the present study showed a good fermentation quality as judged from the report by Catchpoole and Henzell (1971); a low pH value (3.74), high LA content (85.83 g kg^{-1} DM), low contents of AA (5.38 g kg^{-1} DM), ethanol (19.20 g kg^{-1} DM) and AN/TN (75.84 g AN/kg TN), with none or only small amounts of BA, VA and PA at the end of ensiling (Figure 1 and Table 2). It was shown in 14 day-fermentation dynamics study of Italian ryegrass silage that the rate and extent of reduction in pH was large within initial 5 days of ensiling. This was mainly caused by a rapid and intensive production of LA between 1 and 5 days. During the 14 days of ensiling LA continued to be the major fermentation product with a small production of AA, resulting in the high value of LA/AA over the storage periods (from 3.28 to 19.45). These indicate that acidification was initiated by homofermentative LAB, and this was dominant during the fermentation course. The dominance of homofermentative LAB in the early stage of ensiling in non-additive-treated Italian ryegrass silages was in agreement with the reports by McDonald et al. (1991) and Rooke and Kafilzadeh (1994). However, after 5 days of ensiling LA and LA/AA showed significant decreases, indicating that there was a significant shift from homofermentative to heterofermentative activity of LAB. This finding was also in agreement with other studies as reviewed by Beck (1972). Beck (1972) also found that, in well-preserved silages, acidification was initiated by homofermentative strains, but after only 4 days 85% of the strains were heterofermentative, suggesting the eventual dominance of heterofermentative strains due to their greater tolerance to AA and low pH (Beck, 1978). Total organic acids content showed a significant ($p<0.05$) increase from 3 days of ensiling, and reached the peak on day 5. This indicates that the greatest fermentation activity occurred within the initial 5 days of ensiling. It has been well known that the cell breakdown and the resultant release of plant juices are prerequisite for the production of significant amounts of LA during ensiling. Gibson et al. (1961) reported that the modern precision-chop forage harvester could chop herbage into a short length and ensure a rapid plant sap liberation, which stimulates the LAB growth, especially homofermentative LAB in the early fermentation stage. The harvested Italian ryegrass at the immature stage (internode elongation stage) with a high moisture content and low cell wall constituents, was cut at once into 1 cm and crushed, thus it was considered that the cell breakdown and the release of plant juices were faster and easier. Therefore, the ensiled grass produced LA rapidly just after ensiling. The increases in AA and ethanol contents during the fermentation period suggested the activity of some heterofermentative LAB or yeast. Ethanol content was reported to be higher than AA during the storage period due probably to the survival of some yeasts throughout the ensilage period (Weinberg et al., 1988; Driehuis et al., 1997; Henderson and McDonald, 1971). It may be considered in accordance with Ashbell and Kashanchi (1987) that although the laboratory silo was sealed completely, there might be still a little amount of O_2 in the silo and sufficient fermentable sugars were available for yeasts to maintain their metabolism.

The BA, VA and PA were absent or detected in only small amounts over the ensiling period. This was attributed to a rapid reduction in pH because of the rapid production of LA, restricting the growth of clostridia and other bacteria (Catchpoole and Henzell, 1971; McDonald et al., 1991; Henderson, 1993). The AN/TN increased significantly ($p<0.05$) from 3 days of ensiling and continued to increase to the highest value (75.84 g AN/kg TN) at the end of ensiling, but the value was less than 80 g kg^{-1} DM at the end of ensiling. This was associated with the production of some ammonia from other sources such as the breakdown of nitrates and nitrites, by the action of plant enzymes and Enterobacteriaceae (Seale, 1986).

Fructose content tended to increase within 3 days of ensiling and glucose content also showed an initial rise,

whereas sucrose rapidly disappeared in a short time within 0.5 day. These findings agreed with those of Masaki and Ohyama (1979). It can be considered that the increase in fructose or in glucose was due to the hydrolysis of sucrose and fructans, suggesting that residual plant enzymes were active in the early period of ensiling (Clark, 1974; Bousset et al., 1972; Gouet et al., 1970). Glucose decreased more rapidly to 0.5 g kg^{-1} DM on the 5th day than fructose, which indicated the role of glucose as the first fermentation substrates (Hattori et al., 1993).

Total mono- and di-saccharides showed a large decrease within initial 0.5 day of ensiling (from 52.41 to 43.19 g kg^{-1} DM), and then decreased gradually until the end of the ensiling. This was partly due to the respiration loss during initial 0.5 day of ensiling, which was similar to that found by other workers (Wylam, 1953; Carpintero et al., 1969; Seale, 1986). The sum amount of fermented carbohydrates calculated from the amount of LA produced and remaining mono- and di-saccharides proved to be larger than the quantity of mono- and di-saccharides in the initial grass. From this fact, it is suggested that considerable amounts of LA were produced from some other substrates than initial mono- and di-saccharides as mentioned by Masaki and Ohyama (1979). This was not surprising in Italian ryegrass as a temperate grass in which fructans are the most abundant source of WSC, and in the initial stage of ensilage most of the fructans were hydrolyzed into fructose and glucose.

Guineagrass silage fermentation characteristics

Evidence has shown that the rate and extent of guineagrass silage fermentation was restricted throughout the ensiling period in this experiment. This was well indicated by a slow decrease in pH and a slow increase in LA content, resulting in high pH value, low contents of LA, AA, VFAs and total organic acids, and low AN/TN value at the end of ensiling. These results were different from the previous study on Italian ryegrass silage, but they seem to be similar to the fermentation quality of wilted Italian ryegrass silages (McDonald et al., 1968; Anderson and Jackson, 1970; Marsh, 1979; Castle and Watson, 1982; Haigh and Parker, 1985; Driehuis et al., 1997; Gordon et al., 1999). It is well known that the rate and extent of fermentation during ensiling were influenced by DM content of the grass and decreased with increasing DM content, which was explained from the decreasing growth rate of LAB with decreasing water activity. We harvested guineagrass at the milky ripe stage with intermediate DM content (258.64 g kg^{-1}) and high mono- and di-saccharides content (144.34 g kg^{-1} DM), but the material plant was very rigid. The restricted fermentation process could probably be attributed to the rigid physical properties of guineagrass at the milky ripe stage. It had coarse porosity and stemmy structures, and thus there might be a relatively larger quantity of air trapped in the forage mass just after ensiling, causing the delay in time for the air disappearance. These factors would make the cell breakdown and release of plant juice more difficult and slow, this delayed the onset time of juice liberation and restricted the rate and extent of fermentation of guineagrass silage by epiphytic LAB. McDonald et al. (1991) suggested that rates of diffusion of WSC from intact and ruptured cells into the aqueous phase might be more important than absolute amounts in the crop for silage making. Greenhill (1964a, b, c) also reported that cell breakdown and release of intra-cellular plant juices are prerequisite for the initiation of LAB fermentation, and the complete exclusion of fresh air from the silage mass can usually be expected to result in cell breakdown and juice release. The ethanol content continuously increased as a major fermentation product, whereas mono- and di-saccharides compositions (fructose, glucose and sucrose) continuously decreased throughout the fermentation process, a larger amount of ethanol relative to LA and AA. The occurrence of ethanol fermentation rather than LA fermentation (Table 3) was probably the result of the activity of yeasts (aerobic bacteria) (McDonald et al., 1991). The reasons for the occurrence of the predominance of ethanol rather than LA fermentation when guineagrass with high mono- and di-saccharides was ensiled are not clear. McDonald et al. (1968) found more yeasts in silages of high DM content. In the present study this is probably due to the essentially different physical structure of guineagrass at the milky ripe stage as compared with temperate grasses, resulting in the slow release of juice and inhibition of the LA fermentation. The high pH value and low productions of LA and AA might stimulate yeasts activity and result in continuous ethanol increase during ensiling (Alli et al., 1985; Driehuis et al., 1997; Guan et al., 2002). In addition there were low total fermentation products (organic acids and ethanol) and almost no residual mono- and di-saccharides was detected at the end of ensiling, although the microflora and gas production analyses were not carried out during ensiling in this study, it was assumed that some amounts of mono- and di-saccharides were consumed by some other bacterial activity than yeasts and LAB during the early stage of ensiling. There was a small but significantly higher BA and PA contents on the 7th day of ensiling, which indicated that some clostridial activity occurred. This was attributed to the high pH and some residual sucrose on the 5th day of ensiling. Low AN/TN value (45.54 g AN/kg TN) and none or only small amounts of BA, VA and PA indicated that guineagrass produced a stable silage in spite of high pH value and low LA content at the end of ensiling, as was shown by Catchpoole and Henzell (1971). Yokota et al. (1991, 1995) and Miyagi et al. (1993) reported that the ensiling nature of tropical species

was LA fermentation. However, others (Catchpoole and Henzell, 1971; Kim and Uchida, 1990) demonstrated that a main preservation in silages made from tropical grasses was AA fermentation, which is probably because WSC content is generally lower in tropical grasses than in temperate grasses. These were different from the present results, in which guineagrass had higher mono- and di-saccharides content but showed neither LA-type nor AA-type fermentation. It contained ethanol as a major fermentation product. The mechanism determining the final type of fermentation of tropical grasses is still not clear but it may be related to the physical structure and WSC content and/or the interaction among these factors.

Although the contents of mono- and di-saccharides compositions (fructose, glucose and sucrose) showed the largest decreases within the initial 0.5 day of ensiling (Figure 2), very small amounts of organic acids and ethanol products were detected in this period (Table 3), suggesting that the loss was caused mainly by plant respiration. This was similar to that found by previous study with Italian ryegrass, but the loss amounts of total mono- and di-saccharides compositions were higher compared with Italian ryegrass silage. It is suggested that the respiration loss of WSC in guineagrass silage is larger than that in Italian ryegrass silage within the initial 0.5 day of ensiling. The rate of reduction in mono- and di-saccharides compositions within the initial 5 days of ensiling was ranked in the order of glucose>fructose>sucrose, suggesting that glucose and fructose might be more favorably utilized by microorganisms than sucrose. The rate of disappearance in sucrose was slower than that in Italian ryegrass, which suggested that the activity of plant enzymes of guineagrass in the very early stage of ensiling was lower compared with Italian ryegrass. The initial rises in fructose and glucose contents, it was found in Italian ryegrass silage, however, there was a slight increase between 0.5 day and 1 day of ensiling in guineagrass. This may be due to the hydrolysis of only sucrose and the absence of fructans in guineagrass, because tropical grasses accumulate starches instead of fructans.

In conclusion, this study showed that the silage from Italian ryegrass with high moisture content had a good fermentation quality, where active LA fermentation took place in the initial stage of ensiling due to the activity of homofermentative LAB that dominated in the earlier stage rather than the later stage of ensilage. These results can be explained by a rapid plant sap liberation, which stimulates the homofermentative LAB growth (Greenhill, 1964a, b, c). Changes in the contents of mono- and di-saccharides compositions also suggested the high activity of plant enzymes within initial 2 days of ensiling. In ensiling a temperate grass, the physical characteristics may ensure the rapid onset of fermentation phase, resulting in the smaller losses of WSC during the very early stage of ensiling and providing sufficient substrates for LAB. The guineagrass with intermediate DM and high initial mono-and di-saccharides content was stable silage. This could be explained by the higher incorporation of air at ensiling and the restriction of cell breakdown and juice release due to the properties of a tropical grass with coarse porosity and stemmy structures. These physical characteristics delayed the onset of LAB fermentation phase during the very early stage of ensiling by extending respiration and aerobic microbial activity, causing the higher loss of WSC and the shortage of LAB fermentation substrates.

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