Effect of Ensiling Density on Fermentation Quality of Guineagrass (*Panicum maximum* Jacq.) Silage during the Early Stage of Ensiling

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ABSTRACT : This study is to evaluate the effect of different levels of ensiling density on the fermentation quality of guineagrass silages during the early stage of ensiling. Guineagrass at the milky ripe stage was chopped and ensiled into a small-scale laboratory silo at two ensiling density levels (high density at 95 g/silo and low density at 75 g/silo). Three silos per level were opened after six ensiling periods (0.5, 1, 1.5, 2, 3 and 7 days of ensiling) and the fermentation qualities were analyzed. Within the initial 1.5 days of ensiling there were not significant ($p>0.05$) differences in the fermentation qualities between two density levels silages, and an almost constant pH and no or only small amounts of lactic acid, acetic acid and total volatile fatty acids were detected. However, the high density silage significantly ($p<0.05$) increased the rate and extent of fermentation after 1.5 days of ensiling, which was well reflected in significantly ($p<0.05$) faster and larger pH decline and lactic acid production at each elapsed time as compared with the low density silage. This resulted in significantly ($p<0.05$) lower finial pH and significantly ($p<0.05$) higher lactic acid content at the end of the experiment. Moreover, there was higher AA content relative to LA in both the H-D and L-D silages during the full fermentation course, and resulted in the AA-type silage. There were generally somewhat or significantly ($p<0.05$) higher acetic acid, volatile fatty acids and ammonia-N/total nitrogen in the high density silage than in the low density silage during the initial 3 days of ensiling. However, there were higher ($p>0.05$) ammonia-N/total nitrogen and significantly ($p<0.05$) higher butyric acid content in the low density silage at day 7 of ensiling. The silages of two density levels showed an initial increase in glucose between 0.5 and 1 day for the high density silage and between 1 and 1.5 days for the low density silage, respectively, thereafter showed a large decrease until the end of the experiment. There were not large differences ($p>0.05$) in ethanol content between the low density and high density silages that showed small amounts within initial 3 days of ensiling. However, the low density silage had a significantly ($p<0.05$) higher ethanol content than the high density silage at the end of experiment. From the above results it was suggested that the increase in ensiling density was an effective method to improve the fermentation quality, especially for tropical grasses. ***(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 9 : 1273-1278)***

Key Words : Guineagrass, Silages, Ensiling Density, Fermentation Quality

INTRODUCTION

It is well known that two requirements for efficient preservation of silage are, firstly, exclusion of oxygen and, secondly, prevention of anaerobic decomposition during ensiling (McDonald et al., 1991). The need to exclude oxygen has been described as the basic principle of silage making; when both oxygen and plant sugars remain in the silo, respiration (continuing metabolism of plant cells) and aerobic microorganism activity take place during the very early stages of ensiling. Therefore, the volume of air trapped in a silo affects the duration of respiration and aerobic microorganisms activity. This leads to losses of nutritive material and fermentable substrates being available for satisfactory lactate production. The degree of consolidation, the effectiveness of the final sealing and the texture of the grass determine the amounts of air remaining in silo (Shao et al., 2004). Temperate grasses are recognized to be easy to compress except for wilted silages, but in contrast, most tropical herbages are C_4 plants which usually have high polysaccharide content (Smith, 1962) and coarse, porous and stemmy structures (Catchpoole and Henzell, 1971). The silages made from tropical herbages are usually less dense and presumably more permeable, and relativity large quantities of air may be trapped in the forage mass than its temperate counterparts just after ensiling (Alli et al., 1985). 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). Tropical pasture forages have often failed to settle during ensiling (Catchpoole and Henzell, 1971). The settling process results from death and collapse of plant cells, which occurs within initial periods of ensiling in air-tight laboratory silos (Greenhill, 1964a, b, c). Greenhill (1964a, b, c) reported that cell breakdown and release of intra-cellular plant juice are prerequisites for the initiation of LA

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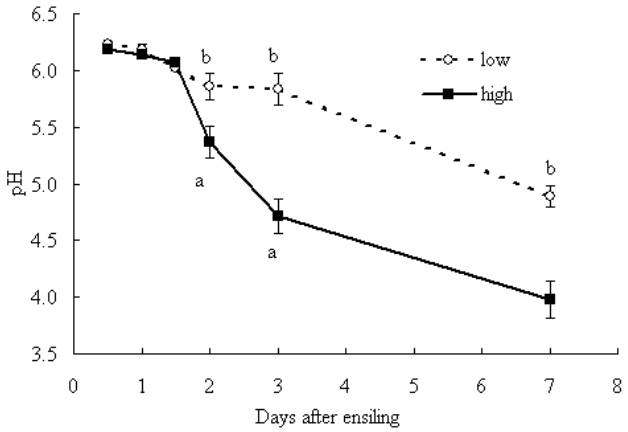

Figure 1. Changes in pH value of guineagrass silages in two ensiling densities (*Values with different letters in the same day show significant differences at $p<0.05$).

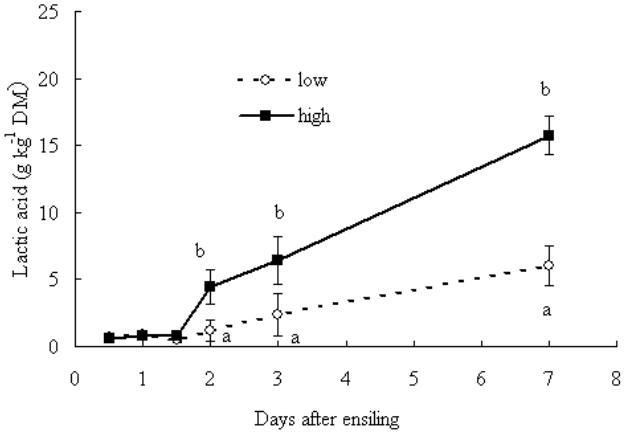

Figure 2. Changes in lactic acid content of guineagrass silages in two ensiling densities (* Values with different letters in the same day show significant differences at $p<0.05$).

fermentation, and the complete exclusion of fresh air from the silage mass can usually be expected to result in cell breakdown and juice release. Thus, the ensiling density is important in the influence on the fermentation course and final fermentation quality. There are experiments of the ensiling density effect on the quality of silages made from temperate grasses (Takahashi, 1968a, b; 1970a, b). However, the information about the ensiling density effects in tropical grasses is limited, especially that in the initial stage of ensiling.

The objective of the present study is to evaluate the effect of different levels of ensiling density on the fermentation quality of guineagrass silages during the early stage of ensiling.

MATERIALS AND METHODS

Silage making

Guineagrass (*Panicum maximum Jacq.*) was grown in the experimental field of Nanjing Agricultural University. The second growth of the guineagrass was hand-harvested with a sickle at the milky ripe stage on 13 November in 1998. The harvested guineagrass, which was collected and chopped into approximately 1 cm length with a forage cutter, and then was immediately packed into a plastic laboratory silo (100 ml liter capacity) in triplicates, followed by being sealed with a screw top and stored in a room kept at 25°C. The silos were opened at 0.5, 1, 1.5, 2, 3 and 7 days after ensiling. The ensiling density levels were as follows: (1) high-density (H-D) silage with 95 g fresh weight (FW)/silo (2) low-density (L-D) silage with 75 g FW/silo.

Chemical analyses

The chopped guineagrass was immediately collected for the determination of dry matter (DM), mono- and di-saccharides compositions, total nitrogen (TN) and crude protein (CP). After the silo was opened, the content was mixed thoroughly and a 35 g sample was taken from each silo. This was followed by adding 70 g 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, ammonia-N (AN), lactic acid (LA), ethanol, glucose and volatile fatty acids (VFAs). The pH of fresh silages was measured with 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 as 6.25 multiplied by TN. The LA content was determined using the method of Barker and Summerson (1941), and VFAs and ethanol were determined with gas chromatography (Shimadzu GC-17A, Japan, with 12 m capillary column, condition: column temperature 100°C, injection temperature 250°C). The DM contents of the fresh material and silages were determined by drying in an oven at 65°C for 48 h (AOAC, 1984), and the DM of silage was recalculated by the contents of volatile components. Glucose was determined using a coupled glucose oxidase-peroxidase system (GLZYME, Eiken Chemical Co.); samples, after the addition of GLZYME, were incubated for 15 minutes at 37°C and the absorbance was read at 500 nm. Mono- and di-saccharide compositions (fructose, glucose and sucrose) in the initial guineagrass were determined using HPLC as shown in our previous report (Shao et al., 2002).

Statistical analyses

The statistical analysis included two-way analysis of variance with storage periods and ensiling density as factors and Fisher's least significant difference test; these were performed by ANOVA using the GLM procedure of the

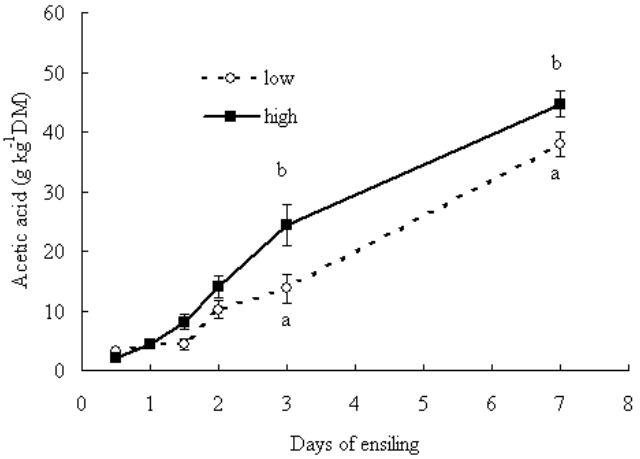

Figure 3. Changes in acetic acid content of guineagrass silages in two ensiling densities (* Values with different letters in the same day show significant differences at $p<0.05$).

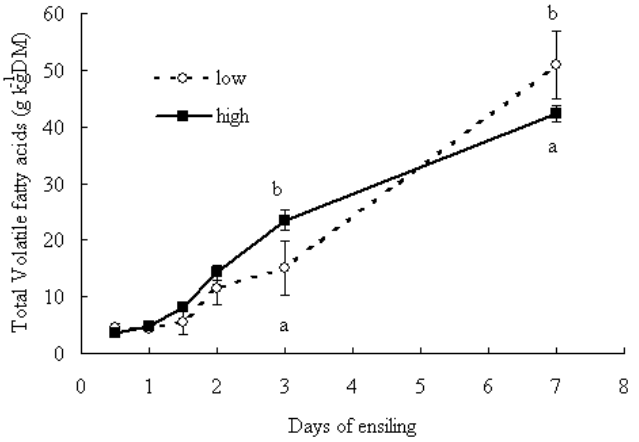

Figure 4. Changes in volatile fatty acids content of guineagrass silages in two ensiling densities (* Values with different letters in the same day show significant differences at $p<0.05$).

Statistical Analysis System, the level of statistical significance was preset at $p<0.05$ (SAS, 1984).

RESULTS AND DISCUSSION

The initial guineagrass had intermediate contents of fructose (23.0 g kg^{-1}DM), glucose (8.0 g kg^{-1} DM), sucrose (19.0 g kg^{-1} DM), total mono- and di-saccharides (50.0 g kg^{-1} DM), CP (76.4 g kg^{-1} DM) and DM (266.2 g kg^{-1}).

Changes in pH value and LA content of silages treated with two levels of ensiling density are shown in Figure 1 and 2. The two density levels silages showed basically similar pH profile with almost constant value, and the LA contents also did not greatly increase within the initial 1.5 days of ensiling, these indicated that there was a time lag before the onset of pH decline and lactic acid production, and the initial lactic acid fermentation was restricted within this period. These results were in agreement with those of our previous study (Shao et al., 2003). It is well known that cell breakdown and the resultant release of plant juices are prerequisites for the production of significant amounts of LA during ensiling, and the complete exclusion of fresh air from the silage mass is usually expected to result in cell breakdown within the initial hours of ensiling (Greenhill, 1964a, b, c). We harvested guineagrass with intermediate DM (266.2 g kg^{-1}) and mono- and di-saccharides (50.0 g kg^{-1} DM) contents, but the guineagrass at the milky ripe stage had very rigid physical properties and would make the cell breakdown and release of plant juices more difficult and slower. Therefore, within the initial 1.5 days of ensiling the fermentation was restricted in two density levels silages. However, the H-D silage showed significantly ($p<0.05$) faster and larger pH decline and LA production at each elapsed time after 1.5 days of ensiling. At day 7 of ensiling the H-D silage decreased the pH to 3.88, but the L-D silage showed a decrease to 4.89, and the highest LA contents of the H-D and L-D silages were 15.75 g kg^{-1} DM and 6.03 g kg^{-1} DM at day 7 of ensiling, respectively, about 2.5 times difference between the two ensiling densities, resulting in significantly ($p<0.05$) lower final pH and significantly ($p<0.05$) higher LA content. This was in agreement with the results of Takahashi et al. (1968a, b., 1970a, b) and Takahashi and Suzuki (1975). This could be explained as follows: the H-D treatment would have a dual action in the silo. Firstly, the H-D silage would provide the larger pressure to crush the plant material, promoting more juice release from grass mass. Secondly, the H-D silage evacuated the more air and decreased the value of O_2 trapped in the silo. The O_2 action was due to the direct and indirect effects, and in the former it acted as an inhibitor to the production of LA, in the latter it was related to the degree of seepage of plant juice resulting from the degree of compaction at ensiling, and higher O_2 content would restrict the rate and extent of plant juice release. In the present study the fermentation quality was improved in the H-D silage. It is suggested that this was mainly from the indirect effect promoting the juice release from grass mass and stimulating epiphytic LAB activity to improve the fermentation quality.

The H-D silage generally showed somewhat ($p>0.05$) or significantly ($p<0.05$) higher contents of AA and VFAs as compared with the L-D silage during ensiling (Figures 3 and 4). This indicated that the H-D silage had more extensive fermentation than the L-D silage, which was attributed to the faster and larger juice release from the silage mass during ensiling. However, at day 7 of ensiling there were significantly ($p<0.05$) higher VFAs in the L-D silage than in the H-D silage (Figure 7), which stemmed from the achievement of significantly ($p<0.05$) higher BA content (Figure 5). The high pH value (4.89) was the main factor to result in the clostridial activity occurring in the L-

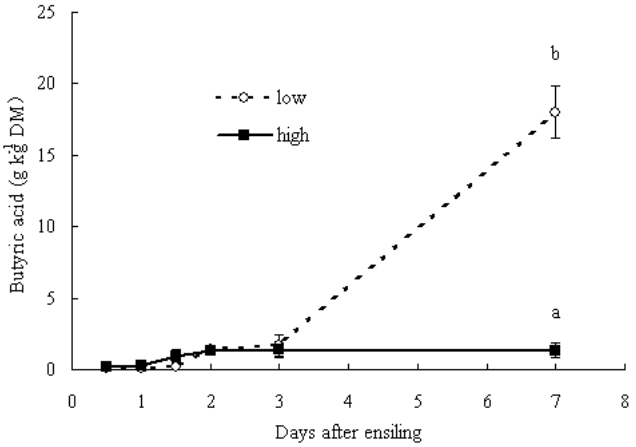

Figure 5. Changes in butyric acid content of guineagrass silages in two ensiling densities (* Values with different letters in the same day show significant differences at $p<0.05$).

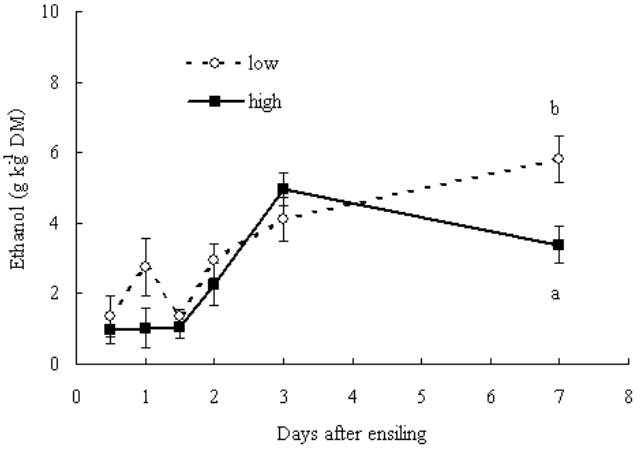

Figure 6. Changes in ethanol content of guineagrass silages in two ensiling densities (* Values with different letters in the same day show significant differences at $p<0.05$).

D silage after 3 days of ensiling. Moreover, there was higher AA content relative to LA in both the H-D and L-D silages during the full fermentation course (Figures 2 and 3), indicating the AA-type silage in the present case. This result is in agreement with the earlier finding by some researchers (Catchpoole, 1970; Catchpoole and Henzell, 1971; Panditharatne et al., 1986), where it was shown that AA was the major fermentation end-product associated with the ensiling of tropical grasses. However, it is still not clear. Yokota et al. (1991) and Miyagi et al. (1993) reported that the ensiling nature of tropical species was LA fermentation. They suggested that the WSC content of the original grass might determine the LA or AA-type fermentation. Moreover, Niimi and Kawamura (1998) suggested that hemicellulose fermentation plays an important role in ensiling of tropical grass. When the WSC was scarce in the tropical grass silage during ensiling, hemicellulose may eventually become available as a result of hydrolysis brought about by the

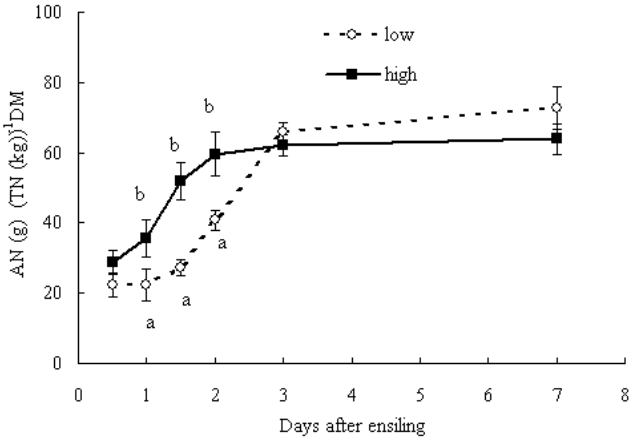

Figure 7. Changes in AN/TN rate of guineagrass silages in two ensiling densities AN/TN: ammonia-N / total nitrogen (* Values with different letters in the same day show significant differences at $p<0.05$).

action of enzymes present in the plant itself, resulting in heterofermentation.

There was not a large difference in ethanol content between the L-D and H-D silages (Figure 6), except that the L-D density silage had a slightly and significantly ($p<0.05$) higher ethanol content than the H-D silage at the end of ensiling. This suggested that there was not a remarkable occurrence of yeast activity in the two density levels silages, and only indicated some heterofermentive LAB activity (Alli et al., 1985; Driehuis et al., 1997; Guan et al., 2002). Just after ensiling AN/TN kept on increasing during 3 days of ensiling, and the H-D silage showed significantly ($p<0.05$) higher AN/TN than the L-D silage between 1 and 2 days of ensiling (Figure 7). This was probably due to the activity of some plant enzymes or the H-D silage had more extensive fermentation than the L-D silage in the early stage of ensiling (Driehuis et al., 1997). There were not so high values of AN/TN in all silages (<80 g/kg DM), implying stable silage (Catchpoole and Henzell, 1971). However, at day 7 of ensiling the L-D silage showed a higher AN/TN than the H-D silage (Figure 7), which was probably due to some clostridial activity occurring, because there was a significantly ($p<0.05$) higher BA (18.01 g kg^{-1} DM) content at day 7 (Figure 5).

There was an initial increase in glucose content with two density levels silages between 0.5 and 1 day for the H-D silage and between 1 and 1.5 days for the L-D silage, respectively (Figure 8). This was probably from the activity of plant enzymes, which hydrolyzed some substrates such as sucrose, cellulose and hemicellulose to glucose (McDonald et al., 1991; Yahaya et al., 2000, 2001).

In conclusion, the present study showed that the silage made from guineagrass with two density levels was restricted in the fermentation in the very early stage, which was attributed to rigid physical properties of guineagrass at

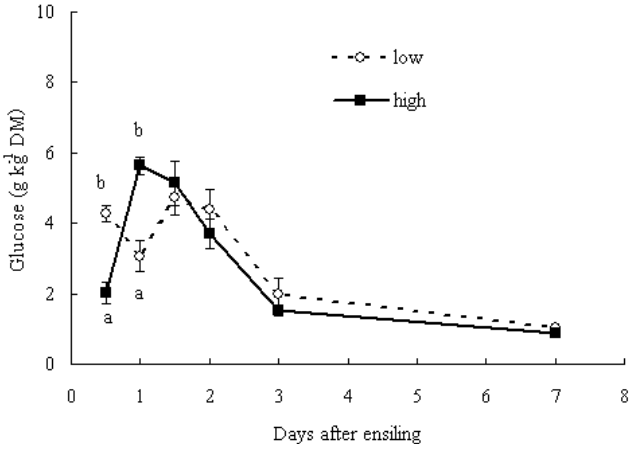

Figure 8. Changes in glucose content of guineagrass silage in two ensiling densities (* Values with different letters in the same day show significant differences at $p<0.05$).

the milky ripe stage. However, the H-D silage substantially increased the rate and extent of fermentation and showed significantly ($p<0.05$) faster and larger pH decline and LA production after 1.5 days of ensiling. This resulted in significantly ($p<0.05$) lower final pH and significantly ($p<0.05$) higher LA content as compared with the L-D silage. It was suggested that H-D silage could shorten the duration of juice release from grass mass and increased the utilization efficiency of WSC during the early stage of ensiling and decreased the loss of WSC, leaving more amounts of fermentation substrates for LA fermentation. It is recommended that increasing ensiling density is an effective method to improve the fermentation quality, especially for tropical grasses.

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