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The Effect of Nitrogen Fertilization to the Sward on Guineagrass (*Panicum maximum* Jacq cv. Gatton) Silage Fermentation*

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ABSTRACT : To investigate the effect of nitrogen fertilization on the quality of tropical grass silage, guinea grass grown with 3 types of nitrogen fertilizers, namely, urea, ammonium sulfate, and compound fertilizer 804, at 2 fertilization levels, 0.5 and 2.5 kg N a^{-1} (0.5 N and 2.5 N, respectively), was subjected to silage fermentation. Silage fertilized with 0.5 N showed butyrate-dominant fermentation, irrespective of the type of fertilizer used. On the other hand, fermentation of silage fertilized with 2.5 N was significantly affected by the type of fertilizer used; fertilization with ammonium sulfate and compound fertilizer 804 resulted in silage that contained a large amount of butyrate and no lactate; this silage was considered to be of a significantly low quality as compared with silage fertilized with 0.5 N. Among silage fertilized with 2.5 N, the desirable butyrate-free fermentation was found only in urea-fertilized silage, which had the best quality. Grass material fertilized with a high level of urea accumulated a relatively high concentration of nitrate nitrogen (0.22% dry matter). Our results presented here suggest that nitrogen fertilizer management could affect the quality of tropical grass silage and that a relatively high concentration of nitrate in silage may promote butyrate-free fermentation even in tropical grass silage. (**Key Words :** Guinea Grass Silage, Nitrogen Fertilizer, Nitrate Nitrogen)

INTRODUCTION

Ensiling is a moist crop preservation method that has been utilized for centuries to supply feed when pasture production is limited during cool and dry seasons. A variety of techniques, such as additive treatments with lactic acid bacteria, sugars, molasses, and formic acid, have been developed for making well-preserved silage (Jonsson et al., 1990; McDonald et al., 1991; Bolsen et al., 1996; Haigh et al., 1996). Fermented juice of epiphytic lactic acid bacteria has also been developed with the use of temperate pastures to save costs and facilitate convenient self-supply on the farm (Ohshima et al., 1997). Furthermore, with regard to preparation of materials for ensiling, the wilting condition, chopping length, and growth stage of the pasture material have been examined (Uchida et al., 1989; Kim et al., 1990). All these techniques have been developed mainly in temperate regions, although their application has been extended to tropical and subtropical regions (Panditharatne et al., 1986; Tjandraarmadja et al., 1994). The results of previous studies have demonstrated that these techniques improve the fermentation quality of tropical grass silage as well as in temperate grass (Tamada et al., 1998; Shao et al., 2004; Yahaya et al., 2004; Smerjai et al., 2005a, b).

In grassland management, nitrogen fertilization is an important factor for increasing the pasture yield and nutritive value of the plant, including crude protein (CP) content and digestibility, which can improve livestock production (Peyraud and Astigarraga, 1998). Particularly in the management of intensive grazing pastures, nitrogen fertilization is important to maintain the vegetation structure of the sward, allowing high stocking rates. These positive effects of nitrogen fertilization on grasslands in tropical and subtropical regions have been discussed in previous studies

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(Teitzel et al., 1991; Cowan et al., 1995; Hernandez Garay et al., 2004).

In silage fermentation, the quality of silage is known to be influenced by the chemical composition of the grass material (McDonald et al., 1991), which changes depending on the amount of nitrogen fertilizer used. In a previous study increasing the amount of nitrogen fertilizer increased the amount of CP and dry matter (DM) both of herbage and silage, but decreased the amount of herbage water-soluble carbohydrate (WSC) (Wilson, 1969). Another study showed that the extent of pH decline in silage was reduced with increasing nitrogen application, which was accompanied by increased buffering capacity and decreased DM concentration of the herbage at ensiling (Keady and O'Kiely, 1996). Because these studies were conducted on temperate grasses, almost nothing is known about the relationship between the quality of tropical grass silage and nitrogen fertilization of the sward. In the present study, to understand how the amount and type of nitrogen fertilizer affect the chemical composition of tropical grass and the quality of silage, we ensiled guinea grass cultivated under different conditions of nitrogen fertilization, and examined the chemical composition of the sward and silage.

MATERIALS AND METHODS

Cultivation of grass material

For the cultivation of silage material under different nitrogen fertilization conditions, guinea grass (*Panicum maximum* Jacq. cv. Gatton) was sown on July 10, 2007, on clay soil (pH 7.0; cation exchange capacity, 17-28 mg 100 g^{-1}) in the Subtropical Field Science Center, University of the Ryukyus. After germination from seed (1 week later), treatment with nitrogen fertilizers was carried out at application rates of 0.5 and 2.5 kg N a^{-1} (0.5 N and 2.5 N, respectively) with the use of 3 types of nitrogen fertilizers, namely, urea (46% N), ammonium sulfate (21% N), and compound fertilizer 804 (18% N, 10% P, and 14% K). The abbreviations used in this study are as follows: UL, 0.5 N urea; ASL, 0.5 N ammonium sulfate; 804L, 0.5 N compound fertilizer 804; UH, 2.5 N urea; ASH, 2.5 N ammonium sulfate; 804H, 2.5 N compound fertilizer 804.

Ensiling

As silage material, guinea grass at the heading stage (height, approximately 1-1.5 m), grown for 45 days after treatment with a nitrogen fertilizer, was harvested (cutting height, 10 cm from the ground) on August 25, 2007. The harvested material was chopped into 1-cm pieces before ensiling, and approximately 150 g of fresh material was immediately packed into a plastic pouch in triplicate and sealed with a vacuum sealer (SQ202; Sharp, Osaka, Japan). The silos were maintained at room temperature, and silage samples were taken for chemical analysis after 40 days of ensiling.

Chemical analysis

The DM content was determined after oven drying the samples at 70°C to a constant weight. The dried sample was ground so that it could be passed through a 1-mm mesh and was then used to analyze the chemical composition of guinea grass material and silage. The amount of CP, neutral detergent fiber (NDF), and acid detergent fiber (ADF) was determined by conventional methods (AOAC, 1990). *In vitro* dry matter digestibility (IVDMD) was determined using the methods described by Goto and Minson (1977). WSC concentration was determined using the method of the Japan Grassland Farming Forage Seed Association (1994). Nitrate nitrogen content was measured in a high-performance liquid chromatograph (HPLC; column, Shim-pack IC-A3; column temperature, 40°C; flow rate, 1.2 ml min^{-1}; Shimadzu, Kyoto, Japan). Total nitrogen content was measured using a Micro Corder JM10 (J Science Lab, Kyoto, Japan).

For evaluation of the quality of the silage, 20 g of fresh silage was macerated with 50 ml distilled water and stored overnight at 4°C. The macerated sample was filtered through filter paper (No. 5A; Advantec, Tokyo, Japan). The water extract was used to determine the pH value, volatile basic nitrogen (VBN), and organic acid content of the silage. VBN content was measured using the steam distillation technique, and organic acid content was determined by HPLC (column, Shim-pack SCR-102H; column temperature, 40°C; flow rate, 0.8 ml min^{-1}; Shimadzu, Kyoto, Japan). Nitrate nitrogen content in the silage and grass material was also determined through HPLC under the conditions described above. To assess the fermentation quality of the silage, the V-score was calculated on the basis of the organic acid and VBN content (JGFFSA, 1994).

Statistical analysis

Silage fermentation data were analyzed using Statview 5.0 (SAS Institute Inc., Cary, NC), and significant differences between the means were identified using the Tukey-Kramer multiple test.

RESULTS AND DISCUSSION

Chemical composition of preensiled materials

Changes in the chemical composition of pre-ensiled materials depending on the amount of nitrogen fertilizer have been demonstrated in temperate grasses; an increase in the amount of fertilizer applied to the sward resulted in decreased DM content (Spraque and Taylor, 1970) and WSC content (Jones et al., 1961), and increased buffering

Table 1. Chemical composition of pre-ensiled guineagrass materials

Item	0.5 N			2.5 N		
	UL	ASL	804L	UH	ASH	804H
Dry matter (% FM)	19.9	19.5	20.0	17.0	16.4	15.6
WSC (% DM)	4.41	3.94	4.00	2.24	3.50	2.42
CP (% DM)	7.70	8.30	8.30	17.3	13.3	11.0
IVDMD (% DM)	61.8	62.5	62.4	60.8	61.4	62.2
NO_3-N (% DM)	0.01	nd	0.01	0.22	0.16	0.15
NDF (% DM)	65.7	66.6	61.4	64.4	66.2	63.9
ADF (% DM)	32.2	31.5	31.6	39.0	35.3	34.6

FM = Fresh matter; WSC = Water soluble carbohydrate; 0.5 N, 0.5 kg Na^{-1}; 2.5 N, 2.5 kg Na^{-1}; UL = Urea low; ASL = Ammonium sulphate low; 804L = Compound fertilizer 804 low; UH = Urea high; ASH = Ammonium sulphate high; 804H = Compound fertilizer 804 high; nd = Not detected.

capacity (Keady and O'Kiely, 1996). As shown in Table 1, similar changes in chemical composition were observed in the present study in which guinea grass was used. The 2.5 N-fertilized silage had lower DM and WSC contents and considerably higher CP content, which was about 1.3-2.2 times greater than that of the 0.5 N-fertilized silage, irrespective of the type of nitrogen fertilizer used. Moreover, a relatively high amount of nitrate nitrogen (0.15-0.22%) was accumulated in the 2.5 N-fertilized silage, whereas nitrate nitrogen was almost absent in the 0.5N-fertilized silage. No significant difference was observed between the 0.5 N-fertilized silage and 2.5 N-fertilized silage with respect to NDF, ADF, and IVDMD.

The WSC content of guinea grass material (range, 2.2-4.4%) used in the present study seemed insufficient for the promotion of lactate fermentation because a WSC content higher than 6% or 7% has been reported to be favorable for lactate fermentation (Oshima et al., 1997). Moreover, the fundamental characteristics of tropical grasses used as silage material are that they have a high buffering capacity and low number of epiphytic lactic acid bacteria compared with temperate grasses (Catchpoole and Henzell, 1971). Therefore, our guinea grass material, particularly that fertilized with 2.5 N, seemed to be unsuitable for preparing well-preserved silage through lactate fermentation.

Fermentation quality of guinea grass silage

The silos containing guinea grass silage that had been ensiled for 40 days were opened, and the fermentation quality index was examined (Table 2). The fermentation quality of guinea grass silage differed among the different amounts of nitrogen fertilizer (0.5 N and 2.5 N) used, whereas the type of nitrogen fertilizer was found to influence the fermentation quality of only 2.5 N-fertilized silage.

In 0.5 N-fertilized silage, although only a small amount of lactate was produced (1.70-3.03 g kg^{-1}), all silage showed butyrate-dominant fermentation, in which butyrate

Table 2. Fermentation quality of guineagrass silages affected by different nitrogen fertilization

	0.5 N			2.5 N			LSD
	UL	ASL	804L	UH	ASH	804H	
pH	4.53^{b}	4.83^{ab}	4.54^{b}	5.02^{ab}	4.93^{ab}	5.19^{a}	0.62
VBN (g kg^{-1} DM)	1.33^{c}	1.81^{c}	1.13^{c}	3.87^{b}	5.78^{a}	6.43^{a}	1.89
Lactate (g kg^{-1} DM)	2.72^{b}	1.70^{b}	3.03^{b}	17.78^{a}	nd^{b}	nd^{b}	4.39
Formate (g kg^{-1} DM)	0.03^{b}	0.06^{b}	0.16^{b}	0.89^{a}	0.11^{b}	0.07^{b}	0.24
Acetate (g kg^{-1} DM)	29.61^{bc}	20.81^{c}	21.62^{c}	33.83^{bc}	58.93^{a}	45.32^{ab}	22.88
Propionate (g kg^{-1} DM)	2.34^{bc}	5.25^{abc}	1.33^{c}	1.65^{c}	6.60^{ab}	7.21^{a}	4.33
Butyrate (g kg^{-1} DM)	46.39^{a}	42.93^{a}	50.21^{a}	nd^{b}	58.02^{a}	44.69^{a}	20.69
Valerate (g kg^{-1} DM)	nd	nd	nd	nd	nd	2.34	2.82
Total acid (g kg^{-1} DM)	81.10^{bc}	70.76^{bc}	76.37^{bc}	54.17^{c}	123.65^{a}	99.64^{ab}	36.64
LA/TA	0.03^{b}	0.03^{b}	0.04^{b}	0.33^{a}	0.00^{b}	0.00^{b}	0.09
AA/TA	0.37^{b}	0.26^{b}	0.28^{b}	0.63^{a}	0.48^{ab}	0.45^{ab}	0.26
BA/TA	0.57^{bc}	0.63^{ab}	0.66^{ab}	0.00^{d}	0.47^{c}	0.44^{c}	0.18
VBN/TN	0.09^{c}	0.12^{c}	0.08^{c}	0.15^{bc}	0.24^{ab}	0.28^{a}	0.10

VBN = Volatile basic nitrogen; LA/TA = The ratio of lactate to total acid; AA/TA = The ratio of acetate to total acid; BA/TA = The ratio of lactate to total acid 0.5 N, 0.5 kg Na^{-1}; 2.5 N, 2.5 kg Na^{-1}; UL = Urea low; ASL = Ammonium sulphate low; 804L = Compound fertilizer 804 low; UH = Urea high; ASH = Ammonium sulphate high; 804H = Compound fertilizer 804 high; nd = Not detected.

Means with different superscript within each column are significantly different at $p<0.05$ tested by Tukey-Kramer method.

formation exceeds lactate or acetate production. In 0.5 N-fertilized silage fermentation, there was no apparent influence of the type of nitrogen fertilizer on the fermentation quality. On the other hand, organic acid production in 2.5 N-fertilized silage was influenced by the type of nitrogen fertilizer. Although both silages fertilized with ASH and 804H mainly produced acetate and butyrate but no lactate, the highest amount of lactate was found and no butyrate production was detected in UH-fertilized silages.

To evaluate the fermentation quality of the silage, the V-score of each silage was calculated (Figure 1). The V-score was calculated from both VBN/total nitrogen (TN) and volatile fatty acid content by using multiple regression, which has been developed for the assessment of silage quality of temperate grasses (Japan Grassland Farming Forage Seed Association, 1994). Values greater than 80, from 60 to 80, and less than 60 are defined as very good, good, and bad, respectively, in terms of fermentation quality. In the guinea grass silage prepared in the present study, the V-score of silage fertilized with UL, ASL, 804L, UH, ASH, and 804H was found to be 47, 50, 54, 68, 6, and 12, respectively. Most of the silage prepared in this study was considered to be of bad fermentation quality, except for that fertilized with UH.

The 0.5 N-fertilized silage seemed to undergo butyrate-dominant fermentation (low quality), which is characteristic of natural fermentation in tropical grass silage that is not subjected to any additive treatment (Kim and Uchida, 1991; Imura et al., 2001; Nimi et al., 2006). On the other hand, the fermentation of 2.5 N-fertilized silage was affected by the type of nitrogen fertilizer; silages fertilized with ASH and 804H were of poorer quality than those fertilized with UH, which had no lactate, high butyrate, and a remarkably low V-score (Figure 1). Vigorous protein decomposition probably occurred during ensiling in silage fertilized with ASH and 804H, which was mainly because of a considerably high CP content and low DM and WSC contents in pre-ensiled materials. Because forage containing high CP tends to result in silage containing a high concentration of VBN, silage fertilized with ASH and 804H also produced significantly high VBN/TN (0.24 and 0.28 in silage fertilized with ASH and 804H, respectively) as compared with 0.5 N-fertilized silage (in the range of 0.08-0.12). On the other hand, UH-fertilized silage prepared from the grass material accumulating a high concentration of nitrate produced no butyrate and the highest amount of lactate, and had the best V-score in the silage we prepared, which contrasted with ASH- and 804H-fertilized silage.

Nitrate nitrogen in guinea grass silage

The nitrate nitrogen content of each silage is shown in Figure 2. The accumulated nitrate nitrogen found in pre-ensiled materials fertilized with ASH and 804H disappeared completely during 40 days of ensiling. Although a small amount of nitrate nitrogen (0.06% DM) was detected in UH-fertilized silage, 72% of the original nitrate in the pre-ensiled material disappeared during the ensiling period. The nitrate nitrogen detected in UH-fertilized silage was not

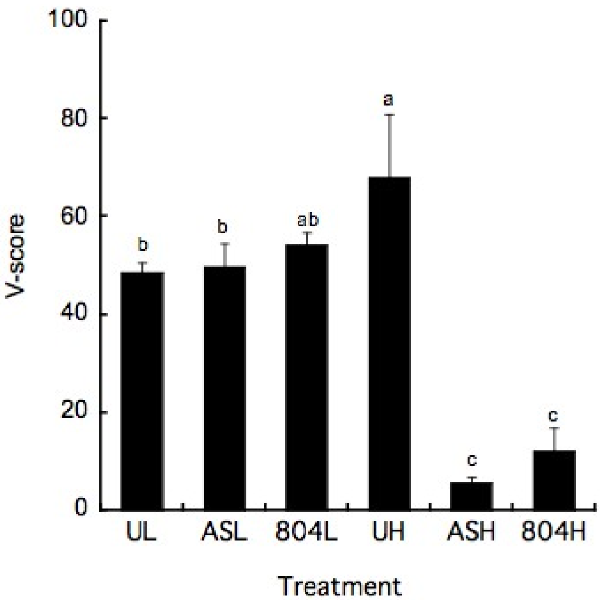

Figure 1. V-score of guineagrass silages. Vertical bar shows SD. Different letters indicate significant differences at p<0.05 by Tukey-Kramer test. UL, urea 0.5 N: ASL, ammonium sulphate 0.5 N: 804L, compound fertilizer 804 0.5 N: UH, urea 2.5 N: ASH, ammonium sulphate 2.5 N: 804H, compound fertilizer 804 2.5 N.

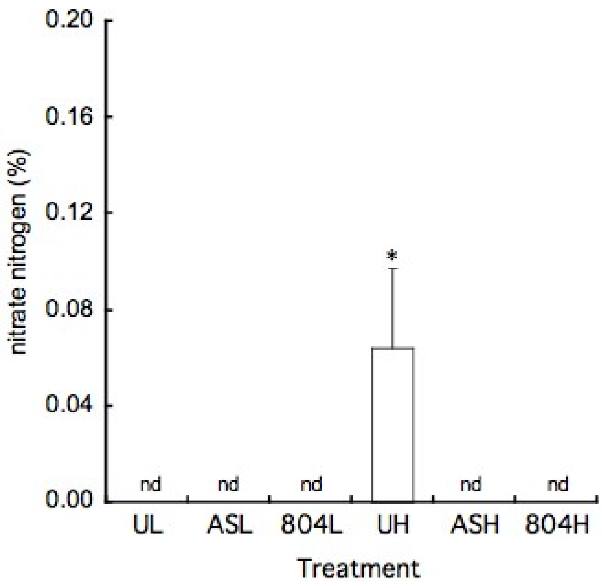

Figure 2. Nitrate nitrogen content in guineagrass silage at the end of ensiling. Vertical bar shows SD. nd denotes not detected. Asterisk indicates significant difference at p<0.05 by Tukey-Kramer test. UL, urea 0.5 N: ASL, ammonium sulphate 0.5 N: 804L, compound fertilizer 804 0.5 N: UH, urea 2.5 N: ASH, ammonium sulphate 2.5 N: 804H, compound fertilizer 804 2.5 N.

considered likely to cause nitrate poisoning of bovines because it was lower than the critical concentration for animals (0.2% DM; Miyazaki, 1977).

In general, nitrate nitrogen in grass material is reduced to nitrite nitrogen or nitric oxide in the early stage of ensiling (Ataku et al., 1981; Spoelstra, 1985), and these nitrogenous compounds inhibit clostridial activity in the silo (Spoelstra, 1983; Wilkinson, 1999). Ataku et al. (1981) examined the relationship between nitrate nitrogen content in pre-ensiled silage and silage fermentation in orchard grass, and showed that grass material containing nitrate nitrogen higher than 0.19% DM resulted in butyrate-free silage without exception. In the present study on guinea grass, butyrate-free fermentation occurred only in UH-fertilized silage, and the nitrate nitrogen content in its pre-ensiled material reached 0.22% DM. Therefore, although low DM and WSC content and high CP content were found in UH-fertilized silage, it is probable that protein decomposition by proteolytic clostridia during ensiling was prevented by toxic compounds derived from nitrate nitrogen. Thus, fermentation in UH-fertilized silage appeared to be inactive compared with any other silage, judging from the lowest organic acid production (54.2 g kg^{-1}) and the small amount of nitrate nitrogen remaining in the silage (0.06% DM). Hence, lactate-dominant fermentation found in UH-fertilized silage was likely to be the result of the repression of undesirable butyrate fermentation but not the promotion of lactate fermentation. Application of high amounts of nitrogen fertilizer to the sward normally causes accumulation of nitrate nitrogen in the forage (Look and Mackenzie, 1970; Hojjati et al., 1972; Prins, 1983). Moreover, Crawford et al. (1961) showed that the type of nitrogen fertilizer could influence the accumulation of nitrate nitrogen in oat plants harvested at the vegetative stage, and urea rather than ammonium sulfate tended to cause accumulation of nitrate nitrogen in the forage; these results were also found in our study. However, it is unclear why such a large amount of nitrate nitrogen accumulated only in UH-fertilized forage despite the similar experimental conditions used, such as the amount of nitrogen fertilizer used and the growing period after fertilization.

In conclusion, the present study suggests that application of nitrogen fertilizers to tropical pastures for ensiling is important to the quality of silage fermentation, and that high levels of nitrogen fertilization do not necessarily lead to undesirable butyrate fermentation of tropical silage. However, a more detailed study is needed to examine the effect of nitrogen fertilization on the fermentation of tropical forage silage, taking into account other factors such as regrowth conditions, wilting, and ensiling period.

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