

Effect of Glucose and Formic Acid on the Quality of Napiergrass Silage After Treatment With Urea

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ABSTRACT : Urea as a silage additive increases crude protein but reduces fermentation quality of silage by increasing pH and enhancing clostridial bacteria growth, especially in low sugar forages. Glucose and formic acid might be expected to compensate these defects caused by urea addition to grass silage. Thus, in this experiment urea formic acid or urea with glucose was applied to improve N content and the quality of napiergrass (*Pennisetum purpureum* Schumach.) silage. The first growth of napiergrass was harvested at 85 days of age and about 700 g of the grass was ensiled in laboratory silos (1.0 liter polyethylene containers) for 2, 7, 14, and 30 days at room temperature (28°C). The treatments were no additives (control), urea, urea+glucose or urea+formic acid. Urea was added before ensiling at 0.5% of fresh weight of napiergrass and glucose and formic acid were added at 1% of fresh weight, respectively. After opening the silo, pH, dry matter content (DM), contents on DM basis of total N (TN), volatile basic nitrogen (VBN), lactic acid (LA), acetic acid (AA) and butyric acid (BA) were determined. The control at 30 days of fermentation showed 5.89 for pH with 13.8% for VBN/TN and 1.51% for AA. The addition of urea increased TN by about 1.5% units but decreased the fermentation quality by increasing pH from 5.89 to 6.86, increasing VBN/TN from 13.8% to 24.63%, increasing BA from 0.02% to 0.56%, and decreasing LA from 1.03% to 0.02%. Glucose addition with urea significantly decreased VBN/TN from 13.8% to 4.44% by reducing pH from 6.86 to 4.83 because of higher production of LA (2.62%). Adding urea and formic acid resulted in a more pronounced depression of VBN/TN and fermentation than the addition of urea and glucose. This study suggested that the combination of 1% glucose or 1% formic acid with 0.5% urea will improve nutritive value and fermentation quality of napiergrass silage. (*Asian-Aust. J. Anim. Sci.* 2001. Vol. 14, No. 2 : 211-215)

Key Words : Napiergrass, Silage Quality, Urea, Glucose, Formic Acid

INTRODUCTION

Silage quality is first determined by the chemical composition of the forage ensiled (Humphreys, 1991). There are many reports that show silages made from tropical grasses are low in nutritive quality because of low contents of sugar and crude protein, along with a low digestibility for ruminants. They are also low in fermentative quality because of low sugar content (McDonald et al., 1991). Napiergrass (*Pennisetum purpureum* Schumach.), one of the important tropical grasses in tropical and subtropical regions (Skerman and Riveros, 1990), is commonly used for silage making.

Urea is commonly used as a feed additive to improve the quantity of nitrogen content and ruminant digestibility (Hosamani et al., 1998; Jakhmola et al., 1990; Joy et al., 1992; Sindt et al., 1994). Urea, as an additive to silage made from tropical forages, increased crude protein content but decreased fermentation quality by increasing silage pH, and enhancing clostridial bacteria growth (Pancholy et al., 1994). To improve fermentation quality of silage

various additives are used; glucose is a well known additive supplying substrate for fermentation (Archimede et al., 1999), and formic acid has been used extensively for reducing proteolysis and inhibiting clostridial growth in grass silages (Fairbairn et al., 1992; McDonald et al., 1995; Wetherall et al., 1995).

Formic acid reduces ammonia concentration in lucerne silage (Philip et al., 1990). This naturally leads to the combination of urea with formic acid as an additive, but it does not seem that its effect on the nitrogen information of napiergrass silage is known. There are, however, specific antimicrobial effects of formic acid including the activity inhibition of lactic acid bacteria (Chamberlain and Quig, 1987). The lactic acid production was increased by adding molasses to napiergrass silage (Yokota et al., 1991, 1992, 1995), but little seems to be known about the effects of adding sugar and urea on lactic acid and nitrogen contents of napiergrass silage.

Thus, in this experiment urea with formic acid, or urea with glucose, were applied to improve N content and fermentation quality of napiergrass silage.

MATERIALS AND METHODS

Napiergrass forage

Napiergrass (*Pennisetum purpureum* Schumach.) was field grown at the Kyushu University, Hakozaki,

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Fukuoka, Japan. The first growth (85 days from planting) was harvested at about 15 cm above the ground level using a hand-sickle. Three nodes were visible on the shoots. The harvested grass was immediately chopped into about 1 cm lengths. The chopped grasses were immediately collected for the determination of DM, TN and water soluble carbohydrate (WSC) contents. The DM content of the material was determined by drying in an oven at 60°C for at least 48 h (AOAC, 1984), TN was analyzed by the Kjeldahl method (AOAC, 1984) and WSC by high performance liquid chromatography (HPLC) system from Shimadzu Corp (Kyoto, Japan) with NH₂P-50 column (Shoko. Co., Ltd.). The chopped material contained 19.4% DM, 1.85% TN and 3.06% WSC.

Silage making

Before ensiling, chopped grasses were mixed with respective additives according to the following treatments: no additive as control (C); urea (U); urea+glucose (UG); urea+formic acid (UFA). Urea was added at the level of 0.5% of napiergrass (fresh weight basis), and glucose and formic acid were added at 1% of napiergrass fresh weight, respectively. Three replicates were prepared for each treatment. About 700 g of the material was ensiled, using a stick to crush the material into a laboratory silo (1.0 liter polyethylene container), followed by incubation for 2, 7, 14, and 30 days at room temperature (28°C).

Chemical analyses of silage

The dry matter (DM) content of the silage was determined by drying a 100 gram sample in an oven at 60°C for at least 48 h (AOAC, 1984). A sample of sixty grams of silage was soaked in 120 ml of water and stored at 2°C for 1 day. The filtrates (silage

extracts) were used for determining pH, volatile basic nitrogen (VBN), lactic acid and volatile fatty acids. The pH was measured with a glass Horiba electrode pH meter. Total nitrogen (TN) was determined by the Kjeldahl method (AOAC, 1984); VBN by steam distillation (AOAC, 1984); lactic acid (LA) by the method of Barker and Summerson (1941); volatile fatty acids were analyzed by gas chromatography (Shimadzu GC-17A with 12 m capillary column, condition: column temperature 100°C, injection and detector temperature 250°C).

The experiment was a factorial design of 4×4 with 3 replicates. The data were analyzed statistically by two-way analysis of variance (ANOVA) using a commercially available package (SAS, 1995).

RESULTS

The pH value and DM content of the silages are shown in figure 1. The results of ANOVA (significant effects) were: DM, main effects for additives ($p<0.01$) and days ($p<0.01$), and interaction for additives×days ($p<0.01$); pH value, main effects for additives ($p<0.01$) and days ($p<0.05$), and interaction for additives×days ($p<0.01$).

Adding urea (U) at the rate of 0.5% significantly increased mean pH value over all fermentation periods compared to C. The silage pH increased rapidly in response to U, being high after only two days of fermentation. Application of 1% glucose or formic acid to urea-treated napiergrass decreased pH value from 8.06 to 4.64 and 3.74, respectively, after 2 days fermentation. Between 7 and 14 days of ensiling, the silage pH significantly increased without any additives and significantly decreased with the U treatment. Days of fermentation had no effect on the pH of UG and

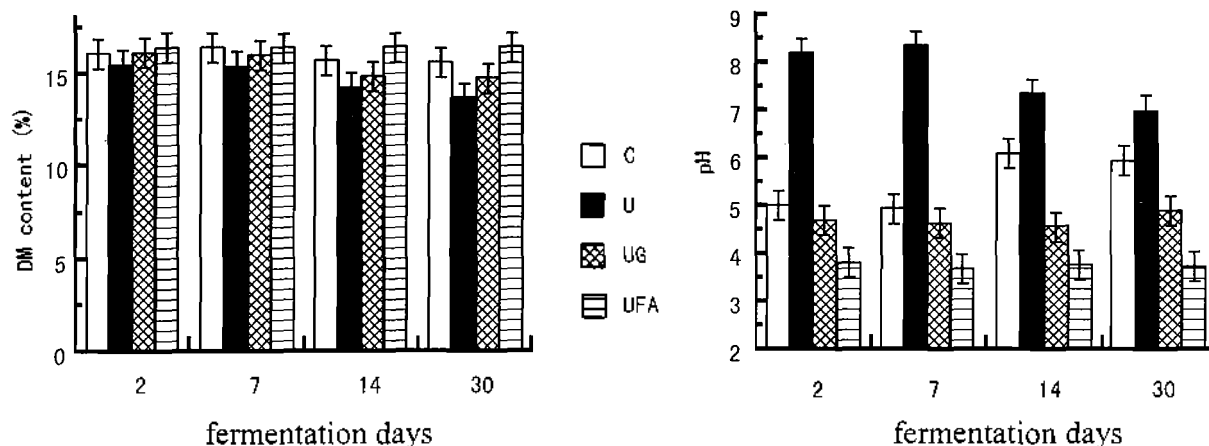


Figure 1. The effects of urea, urea and glucose or urea and formic acid additives and fermentation time on the dry matter content (DM%) and pH value of napiergrass silage. C=control (no additive), U=urea, UG=U+glucose, and UFA=U+formic acid. Mean±SD (three replicates)

UFA silages. The final pH values after 30 days of storage were 5.89 for C, 6.86 for U, 4.83 for UG and 3.69 for UFA, respectively.

U and UG treatments significantly lowered the DM content of the silages compared with the control, but UFA showed significantly higher DM compared with C (only after 30 days fermentation) and U treatments. C, U and UG tended to decrease DM content as fermentation days increased, but in UFA treatments fermentation days did not affect this parameter. The final DM content of silage was 15.22% for C, 13.29% for U, 14.39% for UG and 16.09% for UFA, respectively.

TN and VBN/TN in silages are shown in figure 2. Why VBN/TN was calculated is that this ratio is associated with the quality of silage (McDonald et al., 1991). The results of ANOVA (significant effects) were: TN, main effects for additives ($p < 0.01$) and days ($p < 0.01$), and interaction for additives \times days ($p < 0.05$); VBN/TN, main effects for additives ($p < 0.01$) and days ($p < 0.01$), and interaction for additives \times days ($p < 0.01$).

Urea addition significantly increased TN compared with the control, but there were no significant differences between U, UG and UFA silages for TN content. After 30 days, compared with 13.80% of VBN/TN for C, U treatment increased it to 24.63%, but UG and UFA decreased this parameter to 4.44% and 1.43%, respectively. VBN/TN increased with the days of storage and became stable after 14 days in C, and after 7 days of storage in U. UG and UFA treatments tended to increase VBN/TN as fermentation time increased.

Figure 3 shows the effects of treatments on lactic acid (LA), acetic acid (AA) and butyric acid (BA) production in the silage. The results of ANOVA

(significant effects) were; LA: main effects for additives ($p < 0.01$) and days ($p < 0.05$), and interaction for additives \times days ($p < 0.01$); AA: main effects for additives ($p < 0.01$) and days ($p < 0.01$), and interaction for additives \times days ($p < 0.01$); BA: main effects for additives ($p < 0.05$).

The lactic acid content of silages showed large variation; LA was present in small amounts or was not detected in the U silage compared with the other silages. When compared with C (1.03), LA content in UG treatment showed a significant increase to 2.62% in the final silage, whereas UFA treatment did not show a significant increase (1.31%).

When compared with C, U and UG treatments significantly increased AA production from 2 days until 30 days, whereas UFA treatment significantly decreased this parameter after 30 days. In C and all treatments AA production tended to increase with fermentation time and reached the highest values after 30 days.

U treatment showed significantly higher BA production than the other treatments and it tended to increase with fermentation time reaching the highest value after 30 days. BA was present in small amounts or was not detected in C, UG and UFA silages.

DISCUSSION

McDonald et al. (1991) has classified silage additives into five main categories. Those are fermentation stimulants, fermentation inhibitors, aerobic deterioration inhibitors, nutrients and absorbents. In this experiment urea was used as a nutrient, glucose as a fermentation stimulant and formic acid as a fermentation inhibitor. Application of urea at the rate of 0.5% on a fresh basis increased total nitrogen about 1.5%

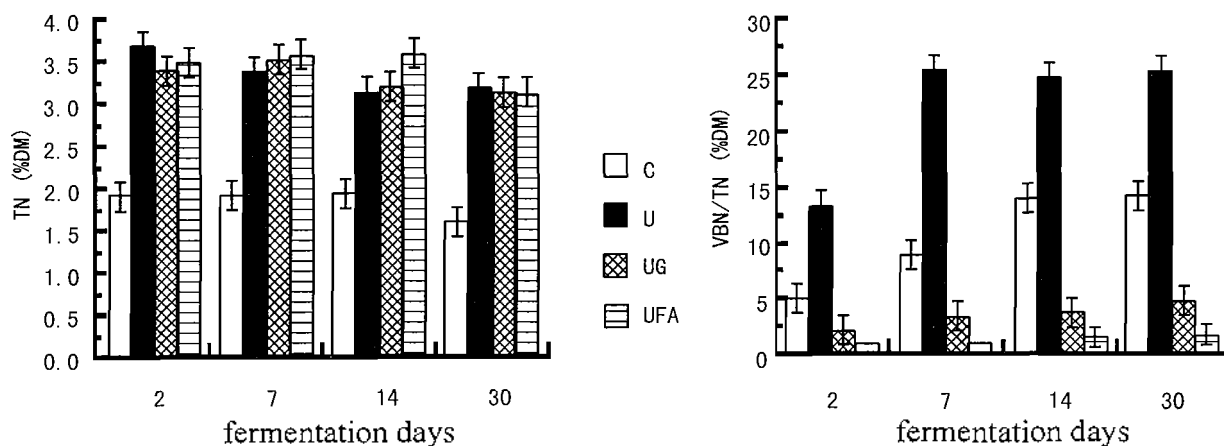


Figure 2. The effects of urea, urea and glucose or urea and formic acid additives and fermentation time on total nitrogen (TN) and volatile basic nitrogen (VBN) of napiergrass silage. C=control (no additive), U=urea, UG=U+glucose and UFA=U+formic acid. Mean \pm SD (three replicates)

unit, but decreased the silage fermentation quality by ammonia release from urea which resulted in additional buffering capacity in the final silage.

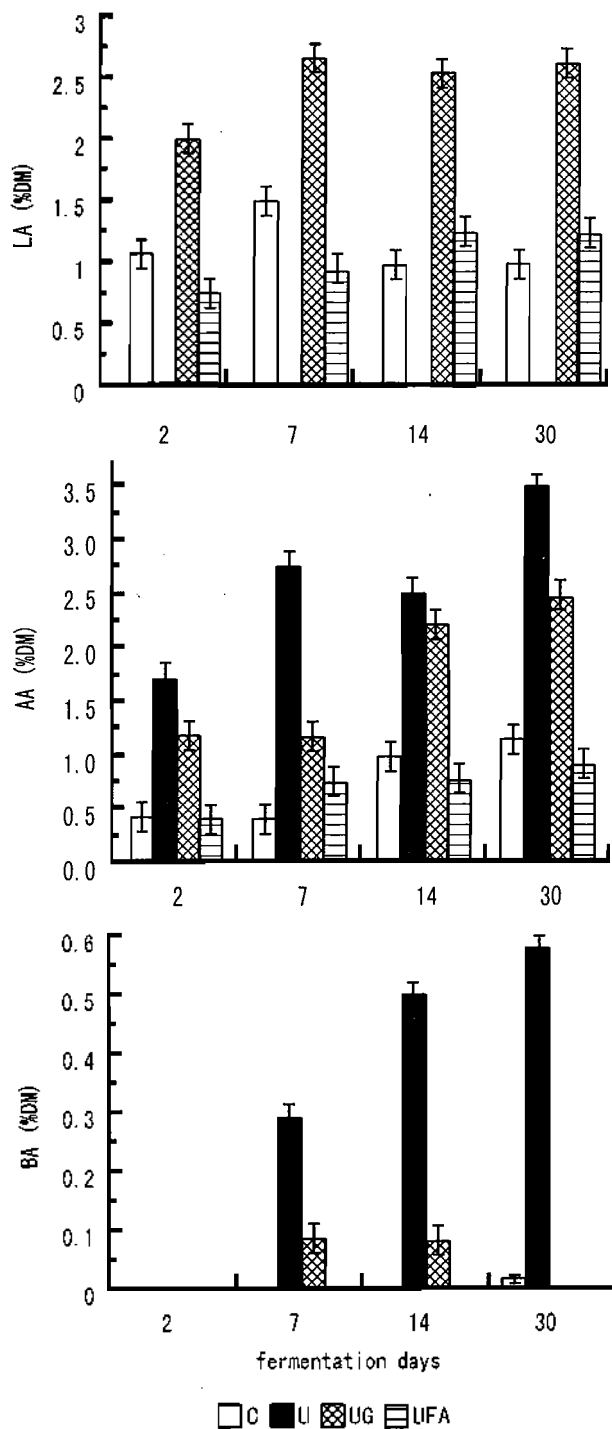


Figure 3. The effects of urea, urea and glucose or urea and formic acid additives and fermentation time on lactic acid (LA), acetic acid (AA) and butyric acid (BA) content of napiergrass silage. C=control (no additive), U=urea, UG=U+glucose and UFA=U+formic acid. Mean \pm SD (three replicates)

(Lessard et al., 1978).

Glucose addition to urea treatment reduced pH value from 6.86 to 4.83 and VBN/TN from 24.63 to 4.44% in the final silage. Many researchers conclude that the addition of sugars in silage making would increase silage quality by increasing dry matter and lactic acid contents and reduce the pH value and ammonia levels, because the sugar would supply available energy for lactic acid bacteria growth (Catchpoole and Henzell, 1971; Henderson, 1993; McDonald et al., 1991; Yokota et al., 1992). In the present experiment, application of 1% glucose to urea treatment improved fermentation quality by increasing lactic acid and dry matter contents and by reducing pH value, resulting in the depression of volatile basic nitrogen and butyric acid production in the final silage.

Combination of 1% formic acid with 0.5% urea resulted in a lower pH value and volatile basic nitrogen content of napiergrass silage, but this silage did not show large lactic acid content. Formic acid treatment is reported to bring an immediate reduction in pH value (Phillip et al., 1990) and to make the fermentation less sensitive to sugar content (Pettersson and Lindgren, 1990). Formic acid as a silage additive may also reduce proteolysis and amino acid degradation and increase nutritive value of silage (Wetherall et al., 1995). In the present experiment the application of formic acid to napiergrass treated with urea reduced pH value and this low pH inhibited urease activity (Stephanie and Simon, 1992) from 2 days and kept it low until 30 days.

In the final silage, butyric acid production was detected in C and U treatment, but it was not detected in UG and UFA treatments. The silage with formic acid addition showed a lower pH value than the silage with glucose addition, but lactic acid production was higher in the silage with glucose addition. This result suggested that formic acid inhibited fermentation and the activity of both urease and lactic acid bacteria, but the addition of glucose was associated with promoting fermentation and suppressing secondary fermentation (McDonald et al., 1991). Combination of 0.6% urea with 5% molasses resulted in a good fermentation quality in our previous study (Yunus et al., 2000). From the present study we suggest that the combination of 1% glucose or 1% formic acid with 0.5% urea will improve nutritive value and fermentation quality of napiergrass silage.

REFERENCES

- AOAC. 1984. Official Methods of Analysis. Association of Official and Analytical Chemists 14th Ed. Arlington, Virginia-22201.
- Archimede, H., G. Aumont, G. Saminadin, E. Depres, P. Despois and A. Xande. 1999. Effects of urea and

- saccharose on intake and digestion of a *Digitaria decumbens* hay by black belly sheep. *Anim. Sci.* 69:403-410.
- Barker, S. B. and W. H. Summerson. 1941. The colorimetric determination of lactic acid in biological material. *J. Biol. Chem.* 138:535-554.
- Catchpole, V. R. and E. F. Henzell. 1971. Silage and silage making from tropical herbage species. *Herb. Abstr.* 41:213-219.
- Chamberlain, D. G. and J. Quig. 1987. The effects of the rate of addition of formic acid and sulfuric acid on the ensilage of perennial ryegrass in laboratory silo. *J. Sci. Food Agric.* 38:217-228.
- Fairbairn, R. L., I. Alli and L. E. Phillip. 1992. Proteolysis and amino acid degradation during ensilage of untreated or formic acid-treated lucerne and maize. *Grass Forage Sci.* 47:382-390.
- Henderson, N. 1993. Silage additives. *Anim. Feed Sci. Technol.* 45:35-56.
- Hosamani, S. V., U. R. Mehra and R. S. Dass. 1998. Effect of different planes of nutrition on urea molasses mineral block intake, nutrient utilization, rumen fermentation pattern and blood profile in murrah buffaloes (*Bubalus bubalis*). *Anim. Feed Sci. Technol.* 76:117-128.
- Humphreys, L. R. 1991. *Tropical Pasture Utilisation*. Cambridge University Press, New York. pp. 143-148.
- Jakhmola, R. C., J. R. Weddell and J. F. D. Greenhalgh. 1990. Effect of straw, cellulase enzyme and urea on chemical composition of grass and grass/legume silage. *Anim. Feed Sci. Technol.* 28:39-50.
- Joy, M., X. Alibes and F. Munoz. 1992. Chemical treatment of lignocellulosic residues with urea. *Anim. Feed Sci. Technol.* 38:319-333.
- Lessard, J. R., J. D. Erfle, F. D. Sauer and S. Mahadevan. 1978. Protein and free amino acid patterns in maize ensiled with or without urea. *J. Sci. Food Agric.* 29:506-512.
- McDonald, P., A. R. Henderson and S. J. E. Heron. 1991. *The Biochemistry of Silage*. 2nd edition. Cambrian Printers Ltd. Aberystwyth. pp. 184-236.
- McDonald, P., R. A. Edwards, J. F. D. Greenhalgh and C. A. Morgan. 1995. *Animal Nutrition*. 5th edition. Longman Scientific and Technical, New York. pp. 451-464.
- Pancholy, R., P. C. Mali and D. Mathur. 1994. Effect of urea-molasses and lactic culture on silage fermentation of *Cenchrus ciliaris*. *Ann. Arid Zone.* 33:147-150.
- Petterson, K. L. and S. Lindgren. 1990. The influence of the carbohydrate fraction and additives on silage quality. *Grass Forage Sci.* 45:223-233.
- Phillip, L. E., L. Underhill and H. Garino. 1990. Effects of treating lucerne with an inoculum of lactic acid bacteria or formic acid upon chemical changes during fermentation, and upon the nutritive value of the silage for lambs. *Grass Forage Sci.* 45:337-344.
- SAS. 1985. *SAS Users Guide: Statistics* (SAS Institute, Cary).
- Sindt, M. H., R. A. Stock and T. J. Klopfenstein. 1994. Urea vs. urea and escape protein for finishing calves and yearlings. *Anim. Feed Sci. Technol.* 49:103-117.
- Skerman, P. J. and F. Riveros. 1990. *Tropical Grasses*. FAO. pp. 621-627.
- Stephanie, D. C. and R. L. Simon. 1992. Kinetic properties of *Helicobacter pylori* urease compared with jack bean urease. *FEMS Microbiol. Lett.* 99:5-21.
- Wetherall, J. A., D. G. Armstrong, H. J. Finlayson and J. A. Rooke. 1995. Reduction of proteolysis during ensilage of perennial ryegrass by protease inhibitors. *J. Sci. Food Agric.* 68:497-505.
- Yokota, H., T. Okajima and M. Ohshima. 1991. Effect of environmental temperature and addition of molasses on the quality of napiergrass (*Pennisetum purpureum* Schum.) silage. *Asian-Aus. J. Anim. Sci.* 4:377-382.
- Yokota, H., J. H. Kim, T. Okajima and M. Ohshima. 1992. Nutritional quality of wilted napiergrass (*Pennisetum purpureum* Schum.) ensiled with or without molasses. *Asian-Aus. J. Anim. Sci.* 5:673-679.
- Yokota, H., M. Ohshima, K. J. Huang and T. Okajima. 1995. Lactic acid production in napiergrass (*Pennisetum purpureum* Schum.). *Jpn. Grassl. Sci.* 41:207-211.
- Yunus, M., N. Ohba, M. Shimojo, M. Furuse and Y. Masuda. 2000. Effects of adding urea and molasses on napiergrass silage quality. *Asian-Aus. J. Anim. Sci.* 13:1542-1547.