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RESEARCH ARTICLE

# Evaluation of Fermentation Dynamics and Structural Carbohydrate Degradation of Napiergrass Ensiled with Additives of Urea and Molasses

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**ABSTRACT**

This study examined the effects of urea and molasses on fermentation dynamics and structural carbohydrate degradation of Napiergrass (*Pennisetum purpureum* Schumach), which was ensiled in laboratory silos for 3, 7, 14, and 30 days at the ambient temperature. The treatments were additions (fresh weight basis) of: no molasses or urea (control), no molasses and 0.4% urea (U), 4% molasses and 0% urea (M), 4% molasses and 0.4% urea (MU). The results showed that the control group produced an unstable fermentation. U silage always had smallest amount of lactic acid and highest levels of pH, acetic acid, butyric acid and ammonia nitrogen. Compared with control, both M and MU increased water soluble carbohydrate contents which promoted lactic acid fermentation domination, but MU did not restrain clostridial fermentation. After 30 days of ensiling, compared with the control, both M and MU lowered structural carbohydrate contents, and U lowered crude protein content but MU increased this parameter. It was concluded that the combination of 4% molasses with 0.4% urea could improve the fermentation and nutritive qualities of Napiergrass but was not sufficient to inhibit clostridial fermentation.



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## INTRODUCTION

Napiergrass (*Pennisetum purpureum* Schum.), is an important tropical grass with high biomass yield, now widely planted in southern regions of China, where it is commonly used for silage making. However, it has high moisture content and insufficient water soluble carbohydrate (WSC) at vegetative stage, which sometimes resulted in clostridial fermentation (Yahaya *et al*., 2004). Furthermore, its low crude protein content (Yunus *et al*., 2000) and high structural carbohydrate contents (Zhang *et al*., 2011; Bureenok *et al*., 2012) usually lead to low nutritive value of silage.

Molasses, the byproduct of sugar industry, is often used for ensiling of low WSC forages, such as legumes and tropical grasses. Several workers have concluded that the addition of molasses increased the dry matter and lactic acid contents and reduced the pH and ammonia nitrogen contents in treated silages (Tjandraatmadja *et al*., 1994; Bilal, 2009). Dean *et al*. (2008) reported that applying ammonia not only increased crude protein content but also promoted degradation of structural carbohydrate, which will increase organic matter potentially available for utilization by ruminal microorganisms (Yalchi and Hajieghrari, 2010). However, ammonia is a hazardous gas and is also corrosive to zinc, copper and brass and needs special equipments when applying. Urea as a source of ammonia, is relatively safe and convenient in handling method of chemically treating forage and is commonly used as a feed additive to increase crude protein content (Hill and Leaver, 1999; Yunus *et al*., 2001), moreover, urea can improve the digestibility, nitrogen retention and ruminal fermentation (Fang *et al*., 2012). This study was conducted to investigate the effects of adding 0.4% urea or/and 4% molasses on fermentation characteristics, crude protein and structural carbohydrate degradation of Napiergrass silage.

## MATERIALS AND METHODS

**Napiergrass forage:** Napiergrass was cultivated in spring of 2007 in an experimental field at the Nanjing Agricultural University, China. The initial growth of grass was harvested at the vegetative stage on August 24, 2007. The harvested grass was chopped and dried at 65℃ on the ground through a willey mill to 2 mm size. The dry matter (DM) and nitrogen contents of the Napiergrass were determined using methods described by AOAC (1990). The WSC were determined with the anthrone-sulfuric acid reaction assay (Dean *et al*., 2008). The neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), hemi-cellulose and cellulose were determined using methods described by Van-Soest *et al*. (1991). The chopped material contained 19.5% DM, 7.91% crude protein (CP), 8.76% WSC, 58.89% NDF, 35.31% ADF, 5.13% ADL, 23.58% hemi-cellulose and 30.18% cellulose.

**Laboratory scale silage:** After harvesting, the grass was chopped about the size of 2 cm length, a 800 g of the chopped material was mixed thoroughly with additives and filled in a laboratory silo (1 liter capacity) in triplicates and stored for 3, 7, 14 and 30 days at the ambient temperature (29-33℃). The treatments were additions (fresh weight basis) of: no molasses or urea (control), no molasses and 0.4% urea (U), 4% molasses and 0% urea (M), 4% molasses and 0.4% urea (MU). Three silos of each treatment were opened at each fermentation period for determination of pH, lactic acid (LA), ammonia nitrogen ($NH_3$-N) and volatile fatty acids (Shao *et al*., 2005). These samples were also analyzed for DM, nitrogen and WSC by using the methods as mentioned above. The NDF, ADF, ADL, hemi-cellulose and cellulose of 30 days silage for each treatment were also determined by using the methods described by Van-Soest *et al*. (1991).

**Statistical analyses:** The statistical analysis included two-way analysis of variance with additives and storage periods as factors and Fisher's least significant difference test. These were performed using the GLM procedure of SAS, with the level of statistical significance preset at P<0.05 (SAS 9.0 for Windows).

## RESULTS AND DISCUSSION

**Fermentation characteristics during ensiling:** The good silage should be achieved by a stable fermentation (Zhang *et al*., 2010). In the present experiment, the control group silage produced much LA which caused pH rapidly declining to a low level on day 3, however, it showed significant (P<0.05) decrease in LA and significant (P<0.05) increases in pH and acetic acid (AA) between 14 and 30 days (Table 1), indicating an unstable fermentation. This often had taken place in some silage (Shao *et al*., 2002). Such results might be attributed to some lactic acid bacteria (e.g. *Lactobacillus plantarum*) degrading LA to AA at low WSC content (McDonald *et al*., 1991; Yu *et al*., 2011). Butyric acid (BA) should be less than 2 g/kg DM for good quality silage (Shao *et al*., 2005). However, BA was high in control silage (Table 2), indicating the clostridial fermentation occuring. Zhang *et al*. (2010) reported that there was a critical WSC concentration for inhibiting clostridial fermentation which might enhance when conditions are unfavorable for silage making, such as increase in moisture content and buffering capacity or improper temperature. The Napiergrass had a moderate WSC content (87.6 g/kg DM) and high water content (DM: 195.4 g/kg). Though the WSC content exceeded the 60-70 g/kg DM which is recommended for theoretical requirement to achieve well-preserved fermentation (Wang *et al*., 2009), the high moisture probably elevated the critical WSC level and supplied the advantageous environment for the activity of clostridia (McDonald *et al*., 1991; Bureenok *et al*., 2012).

In this experiment, M treatment significantly (P<0.05) increased the LA contents at all fermentation periods, along with a tendency to increase AA during the first 14 days compared with control group (Table 1). Similar results were also reported by other researchers (Bilal, 2009; Nishino *et al*., 2012). M silage maintained high LA/AA value (Table 1), indicating a stable LA fermentation. This could be attributed to molasses addition to supply sufficient substrate which played an important role in promoting the activity of homofermenters than heterofermenters (Tjandraatmadja *et al*., 1994). M silage always showed a low level of BA contents (<0.8 g/kg DM) at all fermentation periods (Table 2). This might be due to addition of 4% molasses for making the total WSC to approach the critical WSC concentration for inhibiting clostridial fermentation. Both M and MU treatment significantly (P<0.05) increased the DM and WSC contents compared with control (Table 1, Table 2). This might be attributed to high DM and soluble sugar of molasses (Rezaei *et al*., 2009; Denek *et al*., 2011).

In this study, U treated silage showed significantly (P<0.05) lower DM and LA and significantly (P<0.05) higher pH, AA, propionic acid (PA), BA and $NH_3$-N at all fermentation periods compared with control group (Table 1, Table 2). These were similar to the results of Yunus *et al*. (2001). Urea is apt to be hydrolyzed to ammonia due to the activity of urease which is a common enzyme existing in the plants and microbes (McDonald *et al*., 1991). Cesareo and Langton (1992) found that at optimum pH in jack bean (*Canavalia ensiformis*) urease activity was between 7 and 8, and its activity was inhibited at pH 4.6 or below. Urea treated silage always had a pH value of more than 4.6 (Table 1), which could not restrain urease activity, resulting in the production of ammonia. Moreover, the ammonium ion resulting from dissolution of ammonia in solution would increase buffering capacity in the silage, thus decline the level of pH. In such a condition of high pH, the water content and buffering capacity, the clostridium vigorously consumed WSC and LA to produce more BA (Liu *et al*., 2011).

Compared with U, MU silage produced more LA, which rapidly declined the pH to a level of below 4.2 on day 7 and maintained this low pH level until the end of ensiling (Table 1). After 7 days, the LA/AA value of MU silage was also high and stable (Table 1). This indicates that MU silage obtained a stable fermentation. However, the MU silage showed high amount of BA and $NH_3$-N

**Table 1:** Effects of adding urea and molasses on dry matter, pH, lactic acid, acetic acid and lactic acid/acetic acid of Napiergrass silage

| Parameters | Treatments | Fermentation days | | | |
|---|---|---|---|---|---|
| | | 3 | 7 | 14 | 30 |
| Dry matter (g/kg FW) | Control | 190.6±4.2aC | 186.5±9.1abB | 179.0±3.7bC | 176.9±6.1bB |
| | U | 173.8±3.7aD | 160.7±5.4bC | 159.8±6.6bD | 153.6±6.8bC |
| | M | 211.9±8.7A | 210.7±5.3A | 211.6±5.2A | 210.2±5.0A |
| | MU | 201.2±0.9B | 202.6±2.5A | 202.0±3.8B | 201.4±2.0A |
| pH | Control | 3.8±0.0bC | 3.8±0.1bC | 3.8±0.1bC | 4.1±0.1aB |
| | U | 5.3±0.1aA | 5.1±0.0bA | 4.9±0.1cA | 4.7± 0.0dA |
| | M | 3.7±0.0aC | 3.7±0.0aC | 3.7±0.1aD | 3.5±0.1bC |
| | MU | 4.6±0.0aB | 4.1±0.1bB | 4.0±0.1bB | 4.0±0.0bB |
| Lactic acid (g/kg DM) | Control | 49.2±3.3bB | 63.2±5.3aB | 64.0±4.5aB | 37.6±8.1cB |
| | U | 1.4±0.1cC | 1.6±0.5bcC | 2.9±1.2bC | 14.5±0.7aC |
| | M | 73.1±4.0A | 79.8±10.0A | 78.5±4.6A | 86.5±14.7A |
| | MU | 52.5±0.1bB | 76.3±5.9aA | 80.7±2.4aA | 77.3±2.0aA |
| Acetic acid (g/kg DM) | Control | 6.7±2.0bC | 6.7±0.7bB | 7.8±1.1bB | 12.7±3.7aB |
| | U | 34.2±1.6cA | 45.5±4.8bA | 52.5±4.3aA | 56.2±2.4aA |
| | M | 8.8±1.1BC | 9.6±1.8B | 8.8±2.4B | 8.7±1.1B |
| | MU | 10.6±1.1B | 10.5±1.0B | 9.7±1.3B | 10.1±2.8B |
| Lactic acid/acetic acid | Control | 8.0±3.2aAB | 9.4±0.5aA | 8.4±1.7aA | 3.0±0.3bB |
| | U | 0.0±0.0bC | 0.0±0.0bB | 0.1±0.0bB | 0.3±0.0aC |
| | M | 8.4±1.4A | 8.7±2.9A | 9.4±2.8A | 9.9±1.4A |
| | MU | 5.0±0.6bB | 7.3±1.3abA | 8.4±1.2aA | 8.0±2.1aA |

Control: no additives, U: urea, M: molasses, MU: molasses + urea; Values (Mean±SD) with different small letters among fermentation days and capital letters among a specific parameter within the same fermentation day differ significantly (P<0.05).

**Table 2:** Effects of adding urea and molasses on propionic acid, butyric acid, $NH_3$-N and water soluble carbohydrate of Napiergrass silage

| Parameters | Treatments | Fermentation days | | | |
|---|---|---|---|---|---|
| | | 3 | 7 | 14 | 30 |
| Propionic acid (g/kg DM) | Control | 0.2±0.1B | 0.1±0.1B | 0.3±0.2B | 0.1±0.0B |
| | U | 0.6±0.1A | 1.1±0.7A | 0.8±0.3A | 0.9±0.3A |
| | M | 0.1±0.0abB | 0.0±0.0bB | 0.1±0.0aB | 0.1±0.0aB |
| | MU | 0.1±0.0bcB | 0.0±0.0cB | 0.3±0.1aB | 0.2±0.1abB |
| Butyric acid (g/kg DM) | Control | 0.6±0.1bC | 1.2±0.8bC | 2.6±2.2bC | 12.1±1.2aB |
| | U | 28.4±2.6cA | 42.1±6.1bA | 52.8±3.3aA | 54.1±4.4aA |
| | M | 0.6±0.2C | 0.7±0.2C | 0.6±0.1C | 0.5±0.1C |
| | MU | 10.1±0.5abB | 8.8±1.2bB | 10.5±1.0abB | 12.1±1.9aB |
| $NH_3$-N (g/kg TN) | Control | 58.3±3.2bD | 68.9±6.7bC | 73.5±11.0bC | 100.1±10.6aC |
| | U | 488.6±14.1bA | 581.9±20.2aA | 585.4±14.0aA | 592.7±8.8aA |
| | M | 86.8±6.9C | 91.8±10.5C | 87.0±5.7C | 87.6±7.6C |
| | MU | 337.6±8.4bB | 338.0±16.9bB | 362.4±13.7aB | 370.4±11.3aB |
| Water soluble carbohydrate (g/kg DM) | Control | 18.5±2.5aC | 15.5±1.8abC | 13.5±1.6bC | 12.2±1.8bC |
| | U | 10.7±0.5aD | 7.5±0.1bD | 7.1±0.1bD | 5.5±0.3cD |
| | M | 42.9±3.8aA | 28.6±1.8bA | 24.6±1.3bcA | 22.4±0.9cA |
| | MU | 30.3±2.0aB | 21.0±1.2bB | 18.2±1.1bcB | 17.4±1.5cB |

Control: no additives, U: urea, M: molasses, MU: molasses + urea; Values (Mean±SD) with different small letters among fermentation days and capital letters among a specific parameter within the same fermentation day differ significantly (P<0.05).

**Table 3:** Effects of adding urea and molasses on crude protein and structural carbohydrates of 30 days silage

| Parameters | Treatments | | | |
|---|---|---|---|---|
| | Control | U | M | MU |
| Crude protein (g/kg DM) | 60.2±1.7B | 55.1±0.7C | 60.1±1.3B | 85.4±1.7A |
| Neutral detergent fiber (g/kg DM) | 594.7±4.6B | 648.8±7.1A | 528.3±7.3C | 538.6±3.6C |
| Acid detergent fiber (g/kg DM) | 376.0±3.8B | 416.3±4.7A | 332.8±5.2D | 346.7±2.6C |
| Acid detergent lignin (g/kg DM) | 53.7±2.3B | 63.4±1.9A | 46.5±1.6C | 54.7±2.3B |
| Hemicellulose (g/kg DM) | 218.7±8.1B | 232.5±2.5A | 195.5±3.4C | 191.9±0.9C |
| Cellulose (g/kg DM) | 322.3±2.6B | 352.9±4.2A | 286.3±6.0C | 292.0±5.0C |

Control: no additives, U: urea, M: molasses, MU: molasses + urea; Values (Mean±SD) with different letters among treatments differ significantly (P<0.05).

contents (Table 2). This might be due to the ammonia which is released from urea increasing the buffering capacity which increased the critical WSC concentration for inhibiting clostridial fermentation.

**CP and structural carbohydrate of 30 days silage:** Increase of CP due to urea addition was reported by some researchers (McDonald *et al*., 1991; Hill and Leaver, 1999; Yunus *et al*., 2000). However, U treatment significantly (P<0.05) decreased CP contents but MU treatment significantly (P<0.05) increased this parameter (Table 3). This might result from the CP data which was determined based on oven dried samples, which led to loss of volatile nitrogen compounds, principally the ammonia. U treated silage had high ammonia contents which was lost during drying, while MU improved fermentation quality and reduced the loss of ammonia (less loss during drying).

Dean *et al*. (2008) reported that ammonia could act as an alkali-upgrading chemical to promote structural carbohydrate degradation of hays due to the hydrolytic action of ammonia on linkages between lignin and structural polysaccharides. Yahaya *et al*. (2002) reported that clostridial fermentation could increase hemi-cellulose degradation. But clostridial fermentation often led to large loss of nutrients such as WSC and protein (Shao *et al*., 2005; Bureenok *et al*., 2012). In our experiment, although there was high levels of ammonia and BA, U treatment significantly (P<0.05) increased structural carbohydrates contents compared with control (Table 3). This can be explained as follows: Firstly, in U treated silage the clostridial fermentation caused more loss in easy degradable nutrients than in structural carbohydrates, which indirectly increased the proportion of structural carbohydrates. Secondly, in high moisture content forages, any ammonia released from urea hydrolysis would dissolve and form the ammonium ion, reducing the presence and diffusion of ammonia gas across the silage, thus urea could not act as an alkali-upgrading chemical (Hill and Leaver, 1999). In this case, the degradation of lignocellulose linkages was not properly enhanced by the ammoniation process. Both M and MU significantly ($P<0.05$) decreased structural carbohydrate contents compared with control (Table 3). This was in agreement with the reports of Rezaei *et al*. (2009) and Baytok *et al*. (2005). These decreases might be attributed to the lower fiber contents of molasses and enhancement of cell wall hydrolysis by increased organic acids production due to the sugars in molasses.

**Conclusion:** High water contents and high buffering capacity would increase critical WSC concentration for inhibiting clostridial fermentation. Adding 0.4% urea alone is not advisable due to poor fermentation quality and large loss of easy degradable nutrients, whereas 4% molasses promoted the LA fermentation domination and structural carbohydrate degradation. Thus more than 4% molasses to 0.4% urea treated Napiergrass might be necessary for restraining clostridial fermentation.

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