



## Improvement of Fermentation Quality of Rice Straw Silage by Application of a Bacterial Inoculant and Glucose

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**ABSTRACT :** The improvement of the fermentation quality of rice straw silage by application of lactic acid bacteria (LAB) and glucose was investigated in this study. Sixteen rice varieties were harvested at the maturity stage and the rice straw was ensiled with LAB inoculant ( $1 \times 10^5$  cfu/g of fresh weight) and glucose (2% of fresh weight). Inoculation with LAB improved the fermentation as reflected in reductions in pH, acetic acid (by 3.7 to 78.3%), butyric acid (by -6.0 to 100.0%) and ammonia nitrogen (by 1.0 to 71.7%) concentrations, and increases in lactic acid (by 43.9 to 282.9%) and crude protein concentrations compared with the control. Application of LAB plus glucose was more effective in improving fermentation quality than LAB alone. The variety of rice straw which contained relatively high levels of water soluble carbohydrates (WSC) tended to obtain better fermentation quality. The results suggested that LAB application and selection of rice varieties whose straw contained high levels of WSC were effective in improvement of the fermentation quality of rice straw silage. (**Key Words :** Bacterial Inoculant, Feeding Value, Glucose, Rice Straw, Silage)

### INTRODUCTION

In recent years, the livestock industry, especially herbivorous animal raising, has developed rapidly in China. The shortage of feedstuffs has become a limiting factor for the sustainable development of the livestock industry. In spite of a high production amounting to 192 million tons annually in China (Han et al., 2002), only a small proportion of rice straw is utilized as roughage due to its poor feeding value. The remainder is used as firewood, or directly burnt in the field so as to cause serious air pollution. Therefore, improving availability of rice straw has become a great concern. In the past, some physical, chemical and microbial treatments were tried to improve the feeding value of rice straw in China, but they were proven ineffective. However, in recent years, Japanese and Korean studies showed an improvement in feeding value of rice straw by ensiling (Cai, 2006; Kim et al., 2006).

However, successful ensiling of rice straw is difficult due to its hollow stem, low water soluble carbohydrates (WSC) and less epiphytic lactic acid bacteria (LAB) (Cai,

2006). In order to improve the fermentation quality of rice straw silage, some commercial LAB have been developed as additives for ensiling. For example, Cai (2006) found that application of LAB (Chikuso-1) to round baled, whole-crop rice could improve the silage quality with lower pH value, butyric acid (BA) and ammonia nitrogen ( $\text{NH}_3\text{-N}$ ) concentrations, and higher lactic acid (LA) and crude protein (CP) concentrations. Kim et al. (2006) reported that inoculation with NLRI401 (*Lactobacillus plantarum*) could lower the pH value and BA concentration, and increase LA, acetic acid (AA), and CP concentrations in the rice straw silage. Additionally, inoculation with LAB could also decrease neutral detergent fibre (NDF) and acid detergent fibre (ADF) concentrations, and increase digestibility of dry matter (DM) and NDF of rice straw silage (Xing, 2004).

Sugar or sugar-rich materials are also commonly used as effective additives for ensiling crops that have low WSC. However, adding sugar alone might induce the proliferation of undesirable microorganisms and thus result in fermentation losses (Cai, 2004). Therefore, it is better to use the combination of sugar and LAB for ensiling rice straw (McDonald, 1964; Cai, 2004); however, there is a lack of data for this combination.

Therefore, the objective of this study was to investigate if LAB and glucose application could improve the fermentation quality of straw silage from rice varieties.

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## MATERIALS AND METHODS

### Rice planting and straw ensiling

Ten rice varieties, namely Nekken2, IRAT109, Nipponbare, Dular, Akihikari, Genjah Wangkal, Milyang23, Wuyunjing7, Jinlingxiang and Wuyujing3, were grown in a paddy field in Nanjing, in June 2005. Six rice varieties, namely Zhenxian96, Xudao4, Huaidao262, Hangdao1, Huaidao268 and Liangyoupeijiu, were grown in June 2006.

Each variety was planted in a plot of 15 m<sup>2</sup> with the density of 20×30 cm. The base fertilizer and topdressing were applied at rates of 120 and 200 kg N/ha, respectively. Field management was similar to the standard rice production in Nanjing.

Rice was harvested at the maturity stage. The straw was chopped into 3 to 5 cm lengths and then mixed manually. Three treatments were prepared, including i) control; ii) LAB inoculant (*Lactobacillus plantarum*, Chikuso-1, Snow Brand Ltd., applied at 1×10<sup>5</sup> cfu/g of fresh material); and iii) LAB inoculant (the same as LAB inoculant alone) plus glucose (applied at a rate of 2% of fresh material). The LAB inoculant was dissolved in deionized water and sprayed onto freshly chopped rice straw (5 ml/kg of fresh material) and then mixed thoroughly. Glucose was directly mixed with the chopped straw. Control material was sprayed with deionized water at an equivalent rate. Then, 0.3 kg of the mixture was vacuum-packed in 30×20 cm polyethylene bags with 3 replications for each treatment, and stored at ambient temperature for 60 days.

### Chemical analyses

Rice straw and the associated silage samples were oven-dried at 60°C for 48 h. The dried samples were ground to pass through a 1 mm screen for subsequent analyses of NDF, ADF, WSC, CP and IVOMD. NDF and ADF were determined according to the method of Van Soest et al. (1991). WSC was determined by a colorimetric method (Dubois et al., 1956). CP was determined according to the Association of Official Analytical Chemists (AOAC, 1980). Ash was estimated by combustion of the samples in a muffle furnace at 550°C for 5 h. IVOMD was determined by the method of Goto et al. (1977).

For measurements of pH, LA, volatile fatty acid (VFA) and NH<sub>3</sub>-N concentrations, 10 g of silage was blended with 90 ml of deionized water, and stored for 24 h at 4°C. The homogenate was filtered through two layers of cheesecloth and a layer of filter paper. The pH was determined using a glass electrode pH meter (HANNA pH211 Microprocessor pH Meter). LA concentration was determined by a colorimetric method (Madrid et al., 1999). For assessment of VFA concentrations and proportions, the filtrate (5 ml) was combined (5:1) with 25% (v/w) metaphosphoric acid solution. After keeping in ice water for 30 min, the mixture

was centrifuged at 3,400 rpm for 15 min at 4°C. VFA concentrations were determined using a Shimadzu GC-10 gas chromatograph (column: 15 m capillary, 0.53 mm i.d., 1.0 μm film thickness) with flame ionization detector. GC conditions were as follows: injector temperature 180°C, detector 180°C and column 140°C; air pressure 0.5 Mpa, N<sub>2</sub> 0.5 Mpa and H<sub>2</sub> 0.35 Mpa. NH<sub>3</sub>-N concentration was determined by the method of phenol-hypochlorite colorimetry (Weatherburn, 1967). Flieg's scores were calculated on the basis of LA and VFA concentrations in the silage (Woolford, 1984).

### Statistical analyses

The effects of the treatment and rice variety on the quality characteristics of straw silage were evaluated by analyses of variance (ANOVA). If the ANOVA was significant at  $p = 0.05$ , the means of varieties were further compared using the procedure of Duncan's multiple comparison analysis. All these analyses were performed by SAS program (SAS Inst. Inc. Cary, NC, 2001).

## RESULTS AND DISCUSSION

### Chemical composition and IVOMD in rice straw

The chemical composition and IVOMD of the rice straw before ensiling varied greatly among varieties (Table 1). Ranges were 23.9 to 39.3% for DM, 0.5 to 3.1% of fresh weight for WSC, 4.7 to 8.7% of DM for CP, 67.3 to 75.6% of DM for NDF, 43.2 to 53.9% of DM for ADF, 10.9 to 16.2% of DM for ash and 28.1 to 46.4% for IVOMD. Compared with forage crops, rice straw had relatively lower IVOMD for all the varieties in the present study.

### Effect of LAB application on fermentation and nutritional quality of rice straw silage

LAB inoculant at 1×10<sup>5</sup> cfu/g of fresh weight improved fermentation quality of rice straw silage (Tables 2 and 3). Compared with the control, the addition of the LAB inoculant significantly decreased the pH of the silage ( $p < 0.05$ ), except for Nekken2, Xudao4 and Huaidao262. In addition, LAB inoculation increased LA concentration by 43.9 to 283.0%. The AA concentration decreased significantly ( $p < 0.05$ ) except for Nekken2, IRAT109, Dular and Jinlingxiang, while propionic acid (PA) concentration decreased significantly ( $p < 0.05$ ) for all the varieties treated with LAB inoculant. Butyric acid (BA) and NH<sub>3</sub>-N concentrations also tended to be decreased in most varieties by the LAB inoculant treatment. Thus, the Flieg's scores were increased by the LAB application. The results were similar to effects of LAB application on fermentation improvement of the silage of whole crop barley and herbage with low WSC (Rooke et al., 1990; Zahiroddini et al., 2004).

**Table 1.** Chemical composition and IVOMD in rice straw

| Year | Variety        | DM (%) | WSC (% FW) | CP (% DM) | NDF (% DM) | ADF (% DM) | Ash (% DM) | IVOMD (%) |
|------|----------------|--------|------------|-----------|------------|------------|------------|-----------|
| 2005 | Nekken2        | 27.43  | 0.93       | 5.63      | 75.55      | 53.92      | 12.17      | 29.96     |
|      | IRAT109        | 30.48  | 0.51       | 4.80      | 74.92      | 53.62      | 14.05      | 28.07     |
|      | Nipponbare     | 29.19  | 1.93       | 5.03      | 72.03      | 47.88      | 12.20      | 31.25     |
|      | Dular          | 23.87  | 0.87       | 4.70      | 72.24      | 52.11      | 15.38      | 37.44     |
|      | Akihikari      | 29.79  | 1.73       | 6.21      | 70.26      | 48.44      | 12.39      | 31.71     |
|      | Genjah Wangkal | 27.25  | 2.07       | 5.38      | 73.09      | 48.80      | 12.05      | 37.74     |
|      | Milyang23      | 33.31  | 2.24       | 4.71      | 73.50      | 51.77      | 13.69      | 37.08     |
|      | Wuyunjing7     | 29.27  | 2.01       | 6.00      | 72.76      | 51.75      | 16.09      | 39.35     |
|      | Jinlingxiang   | 39.26  | 2.89       | 5.94      | 72.32      | 48.31      | 10.92      | 34.03     |
|      | Wuyujing3      | 34.86  | 3.02       | 7.33      | 70.31      | 48.30      | 10.93      | 38.10     |
| 2006 | Zhenxian96     | 27.68  | 2.29       | 8.63      | 67.81      | 46.23      | 14.89      | 46.40     |
|      | Xudao4         | 31.32  | 1.21       | 5.86      | 73.54      | 53.59      | 13.69      | 37.48     |
|      | Huaidao262     | 29.14  | 1.35       | 7.70      | 72.51      | 52.30      | 13.42      | 35.17     |
|      | Hangdao1       | 33.09  | 3.08       | 8.15      | 67.63      | 43.20      | 11.39      | 42.82     |
|      | Huaidao268     | 33.13  | 2.34       | 6.51      | 72.04      | 49.34      | 13.81      | 42.93     |
|      | Liangyoupeijiu | 34.04  | 1.59       | 5.22      | 72.92      | 52.41      | 16.23      | 43.05     |

**Table 2.** Fermentation characteristics of the rice straw silage (2005)<sup>1</sup>

|                             | Treat-Ment <sup>3</sup> | Nekken2             | IRAT109              | Nipponbare           | Dular                | Akihikari            | Genjah Wangkal       | Milyang23            | Wuyunjing7           | Jinlingxiang       | Wuyujing3           | SEM  |
|-----------------------------|-------------------------|---------------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|--------------------|---------------------|------|
| pH                          | C                       | 4.89 <sup>Aa</sup>  | 4.91 <sup>Aa</sup>   | 4.70 <sup>BCDa</sup> | 4.79 <sup>ABCa</sup> | 4.86 <sup>Aa</sup>   | 4.80 <sup>ABa</sup>  | 4.66 <sup>CDa</sup>  | 4.66 <sup>CDa</sup>  | 4.58 <sup>Da</sup> | 4.56 <sup>Da</sup>  | 0.04 |
|                             | L                       | 4.79 <sup>Aa</sup>  | 4.80 <sup>Ab</sup>   | 4.51 <sup>Cb</sup>   | 4.69 <sup>ABb</sup>  | 4.67 <sup>Bb</sup>   | 4.39 <sup>CDb</sup>  | 4.25 <sup>Eb</sup>   | 4.40 <sup>CDb</sup>  | 4.26 <sup>Eb</sup> | 4.35 <sup>DEb</sup> |      |
|                             | LG                      | 3.86 <sup>BCb</sup> | 3.94 <sup>ABc</sup>  | 3.79 <sup>CDEc</sup> | 3.72 <sup>Ec</sup>   | 3.81 <sup>CDc</sup>  | 3.77 <sup>DEc</sup>  | 3.79 <sup>CDEc</sup> | 3.81 <sup>CDc</sup>  | 3.97 <sup>Ac</sup> | 3.90 <sup>ABc</sup> |      |
| Lactic acid (% DM)          | C                       | 0.46 <sup>CDb</sup> | 0.34 <sup>Db</sup>   | 0.65 <sup>CDc</sup>  | 0.45 <sup>Dc</sup>   | 0.79 <sup>Cc</sup>   | 1.20 <sup>Bc</sup>   | 1.12 <sup>Bc</sup>   | 1.18 <sup>Bc</sup>   | 1.82 <sup>Ab</sup> | 1.89 <sup>Ac</sup>  | 2.03 |
|                             | L                       | 0.74 <sup>Db</sup>  | 0.40 <sup>Db</sup>   | 1.34 <sup>Cb</sup>   | 0.81 <sup>Db</sup>   | 1.46 <sup>Cb</sup>   | 2.77 <sup>Ab</sup>   | 2.71 <sup>Ab</sup>   | 2.10 <sup>Bb</sup>   | 3.05 <sup>Aa</sup> | 3.06 <sup>Ab</sup>  |      |
|                             | LG                      | 5.47 <sup>Ba</sup>  | 4.25 <sup>DEFa</sup> | 4.00 <sup>Efa</sup>  | 6.24 <sup>Aa</sup>   | 5.03 <sup>BCa</sup>  | 6.28 <sup>Aa</sup>   | 4.48 <sup>CDEa</sup> | 4.86 <sup>BCDa</sup> | 3.72 <sup>Fa</sup> | 5.04 <sup>BCa</sup> |      |
| Acetic acid (% DM)          | C                       | 2.26 <sup>Ba</sup>  | 1.68 <sup>Ca</sup>   | 2.60 <sup>Aa</sup>   | 1.88 <sup>Ca</sup>   | 2.12 <sup>Ba</sup>   | 2.14 <sup>Ba</sup>   | 1.07 <sup>Da</sup>   | 0.67 <sup>Ea</sup>   | 0.35 <sup>Fa</sup> | 0.34 <sup>Fa</sup>  | 0.88 |
|                             | L                       | 1.94 <sup>Aa</sup>  | 1.53 <sup>Bca</sup>  | 1.67 <sup>ABCb</sup> | 1.79 <sup>ABa</sup>  | 1.63 <sup>ABCb</sup> | 1.38 <sup>Cb</sup>   | 0.87 <sup>Db</sup>   | 0.35 <sup>Fb</sup>   | 0.27 <sup>Ea</sup> | 0.18 <sup>Eb</sup>  |      |
|                             | LG                      | 0.12 <sup>CDb</sup> | 0.43 <sup>Ab</sup>   | 0.34 <sup>ABc</sup>  | 0.23 <sup>BCb</sup>  | 0.33 <sup>ABc</sup>  | 0.12 <sup>CDc</sup>  | 0.09 <sup>Dc</sup>   | 0.03 <sup>Dc</sup>   | 0.03 <sup>Db</sup> | 0.02 <sup>Dc</sup>  |      |
| Propionic acid (% DM)       | C                       | 0.55 <sup>Aa</sup>  | 0.58 <sup>Aa</sup>   | 0.18 <sup>Bca</sup>  | 0.22 <sup>Ba</sup>   | 0.66 <sup>Aa</sup>   | 0.19 <sup>Ba</sup>   | 0.04 <sup>BC</sup>   | 0.10 <sup>BC</sup>   | ND                 | 0.04 <sup>BC</sup>  | 0.21 |
|                             | L                       | 0.23 <sup>Bb</sup>  | 0.27 <sup>Bb</sup>   | 0.07 <sup>Cb</sup>   | 0.88 <sup>Cb</sup>   | 0.41 <sup>Ab</sup>   | ND                   | ND                   | ND                   | ND                 | ND                  |      |
|                             | LG                      | ND <sup>4</sup>     | ND                   | ND                   | ND                   | ND                   | 0.01 <sup>a</sup>    | ND                   | ND                   | ND                 | ND                  |      |
| Butyric acid (% DM)         | C                       | 0.81 <sup>Ba</sup>  | 0.64 <sup>Da</sup>   | 0.69 <sup>CDa</sup>  | 1.12 <sup>Aa</sup>   | 0.64 <sup>Da</sup>   | 0.77 <sup>BCa</sup>  | 0.03 <sup>F</sup>    | 0.35 <sup>Ea</sup>   | 0.04 <sup>F</sup>  | 0.07 <sup>F</sup>   | 0.40 |
|                             | L                       | 0.72 <sup>Bb</sup>  | 0.59 <sup>Bca</sup>  | 0.58 <sup>Ca</sup>   | 1.19 <sup>Aa</sup>   | 0.64 <sup>BCa</sup>  | 0.18 <sup>Db</sup>   | ND                   | 0.14 <sup>Db</sup>   | ND                 | ND                  |      |
|                             | LG                      | ND                  | 0.03 <sup>Ab</sup>   | ND                   | ND                   | 0.01 <sup>Bb</sup>   | ND                   | ND                   | ND                   | ND                 | ND                  |      |
| NH <sub>3</sub> -N (% TN)   | C                       | 23.39 <sup>Aa</sup> | 22.61 <sup>Aa</sup>  | 21.47 <sup>ABa</sup> | 17.49 <sup>Ca</sup>  | 22.83 <sup>Aa</sup>  | 22.73 <sup>Aa</sup>  | 7.19 <sup>Da</sup>   | 19.48 <sup>BCa</sup> | 5.66 <sup>Da</sup> | 7.39 <sup>Da</sup>  | 0.87 |
|                             | L                       | 16.43 <sup>Ab</sup> | 15.11 <sup>Ab</sup>  | 15.88 <sup>Ab</sup>  | 16.98 <sup>Aa</sup>  | 17.43 <sup>Ab</sup>  | 11.38 <sup>Bb</sup>  | 4.51 <sup>Cb</sup>   | 5.39 <sup>Cb</sup>   | 3.18 <sup>Cb</sup> | 3.90 <sup>Cb</sup>  |      |
|                             | LG                      | 2.68 <sup>Bc</sup>  | 3.38 <sup>Ac</sup>   | 2.23 <sup>CDc</sup>  | 2.35 <sup>BCb</sup>  | 3.07 <sup>Ac</sup>   | 1.96 <sup>CDEc</sup> | 1.85 <sup>DEc</sup>  | 0.53 <sup>Fc</sup>   | 1.60 <sup>Ec</sup> | 2.28 <sup>Cc</sup>  |      |
| Flieg's scores <sup>2</sup> | C                       | 26                  | 28                   | 25                   | 18                   | 33                   | 35                   | 69                   | 60                   | 91                 | 88                  |      |
|                             | L                       | 28                  | 25                   | 44                   | 22                   | 46                   | 76                   | 97                   | 88                   | 100                | 100                 |      |
|                             | LG                      | 100                 | 96                   | 100                  | 100                  | 99                   | 92                   | 100                  | 100                  | 100                | 100                 |      |

<sup>1</sup> Means followed by different uppercase in the same row differ ( $p < 0.05$ ); Means followed by different lowercase letters in the same column differ ( $p < 0.05$ ).

<sup>2</sup> Flieg's scores: <20, very bad; 21-40, bad; 41-60, medium; 61-80, good; 81-100, very good.

<sup>3</sup> C = Control; L = LAB inoculation treatment; LG = LAB inoculation plus glucose treatment.

<sup>4</sup> ND = Not detected.

**Table 3.** Fermentation characteristics of the rice straw silage (2006)<sup>1</sup>

|                                | Treat-<br>Ment <sup>3</sup> | Zhenxian96          | Xudao4              | Huaidao262           | Hangdao1            | Huaidao268           | Liangyoupeijiu       | SEM  |
|--------------------------------|-----------------------------|---------------------|---------------------|----------------------|---------------------|----------------------|----------------------|------|
| pH                             | C                           | 4.69 <sup>ABa</sup> | 4.77 <sup>ABa</sup> | 4.70 <sup>ABa</sup>  | 4.26 <sup>Ca</sup>  | 4.64 <sup>Ba</sup>   | 4.81 <sup>Aa</sup>   | 0.04 |
|                                | L                           | 4.24 <sup>Cb</sup>  | 4.64 <sup>Aa</sup>  | 4.58 <sup>Aa</sup>   | 4.06 <sup>Db</sup>  | 4.23 <sup>Cb</sup>   | 4.40 <sup>Bb</sup>   |      |
| Lactic acid<br>(% DM)          | C                           | 9.04 <sup>Bb</sup>  | 6.05 <sup>Bb</sup>  | 5.76 <sup>Bb</sup>   | 26.57 <sup>Ab</sup> | 9.99 <sup>Bb</sup>   | 5.27 <sup>Bb</sup>   | 2.12 |
|                                | L                           | 32.38 <sup>Ba</sup> | 17.61 <sup>Ca</sup> | 18.46 <sup>Ca</sup>  | 45.38 <sup>Aa</sup> | 26.90 <sup>BCa</sup> | 19.06 <sup>Ca</sup>  |      |
| Acetic acid<br>(% DM)          | C                           | 17.31 <sup>Ba</sup> | 19.70 <sup>Aa</sup> | 19.29 <sup>ABa</sup> | 8.37 <sup>Ca</sup>  | 17.25 <sup>Ba</sup>  | 18.44 <sup>ABa</sup> | 1.08 |
|                                | L                           | 4.38 <sup>Bb</sup>  | 10.32 <sup>Ab</sup> | 4.25 <sup>Bb</sup>   | 2.64 <sup>Bb</sup>  | 5.25 <sup>Bb</sup>   | 11.28 <sup>Ab</sup>  |      |
| Propionic acid<br>(% DM)       | C                           | 5.47 <sup>ABa</sup> | 3.39 <sup>Ba</sup>  | 3.74 <sup>Ba</sup>   | 1.79 <sup>B</sup>   | 8.20 <sup>ABa</sup>  | 6.38 <sup>Aa</sup>   | 1.00 |
|                                | L                           | 2.33 <sup>Ab</sup>  | 0.81 <sup>ABb</sup> | 1.68 <sup>ABb</sup>  | ND <sup>4</sup>     | 2.58 <sup>Ab</sup>   | 1.92 <sup>ABb</sup>  |      |
| Butyric acid<br>(% DM)         | C                           | 6.05 <sup>Ba</sup>  | 7.67 <sup>Aa</sup>  | 5.90 <sup>Ba</sup>   | 2.22 <sup>D</sup>   | 3.45 <sup>CD</sup>   | 6.58 <sup>ABa</sup>  | 0.44 |
|                                | L                           | 3.82 <sup>Bb</sup>  | 6.43 <sup>Ab</sup>  | 2.65 <sup>Bb</sup>   | ND                  | ND                   | 2.36 <sup>Bb</sup>   |      |
| NH <sub>3</sub> -N<br>(% TN)   | C                           | 15.37 <sup>Ba</sup> | 14.28 <sup>Ba</sup> | 24.08 <sup>Aa</sup>  | 7.14 <sup>Ca</sup>  | 7.93 <sup>Ca</sup>   | 8.13 <sup>Ca</sup>   | 1.01 |
|                                | L                           | 5.64 <sup>Cb</sup>  | 6.63 <sup>Bb</sup>  | 9.10 <sup>Ab</sup>   | 3.07 <sup>Db</sup>  | 2.90 <sup>Db</sup>   | 4.88 <sup>Cb</sup>   |      |
| Flieg's<br>scores <sup>2</sup> | C                           | 37                  | 26                  | 29                   | 81                  | 46                   | 37                   |      |
|                                | L                           | 81                  | 56                  | 82                   | 100                 | 99                   | 70                   |      |

<sup>1</sup> Means followed by different uppercase in the same row differ (p<0.05); Means followed by different lowercase letters in the same column differ (p<0.05).

<sup>2</sup> Flieg's scores: <20, very bad; 21-40, bad; 41-60, medium; 61-80, good; 81-100, very good.

<sup>3</sup> C = Control; L = LAB inoculation treatment. <sup>4</sup> ND = Not detected.

LAB inoculation also led to a slight increase in CP concentration of the silage (Tables 4 and 5). This could be attributed to the inhibition of undesirable microorganisms and plant enzymes by a more rapid fall in pH (Muck, 1993). WSC was not affected by LAB inoculation, except in Wuyujing3, Zhenxian96 and Huaidao268 silages (Table 4 and 5), which is consistent with the results of Taylor et al. (2002). The rice straw with higher WSC tended to have higher WSC residue in the silage. NDF and ADF tended to decline, while IVOMD increased slightly (Table 4 and 5)

which might be attributed to the slight hydrolysis of the cell wall by the LAB, and is in agreement with the results of Wu et al. (2005).

#### Effect of application of LAB plus glucose on fermentation and nutritional quality of rice straw silage

The application of LAB plus glucose resulted in satisfactory fermentation for all the varieties in the present study (Table 2). For all varieties, rice straw had a low concentration of WSC (0.5 to 3.1% of fresh weight, Table

**Table 4.** Nutritional value of the rice straw silage (2005)<sup>1</sup>

|               | Treat-<br>Ment <sup>2</sup> | Nekken2               | IRAT109              | Nipponbare             | Dular                 | Akihikari             | Genjah<br>Wangkal     | Milyang23             | Wuyunjing7            | Jinlingxiang          | Wuyujing3             | SEM  |
|---------------|-----------------------------|-----------------------|----------------------|------------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|------|
| DM<br>(%)     | C                           | 24.93 <sup>Eb</sup>   | 28.32 <sup>Cb</sup>  | 28.74 <sup>Ca</sup>    | 22.34 <sup>Fa</sup>   | 27.14 <sup>Db</sup>   | 23.11 <sup>Fb</sup>   | 30.46 <sup>Bb</sup>   | 27.17 <sup>Da</sup>   | 35.61 <sup>Ab</sup>   | 31.34 <sup>Bb</sup>   | 0.43 |
|               | L                           | 25.66 <sup>Fb</sup>   | 28.00 <sup>Db</sup>  | 28.09 <sup>Da</sup>    | 22.79 <sup>Ha</sup>   | 27.08 <sup>Eb</sup>   | 23.96 <sup>Gab</sup>  | 31.05 <sup>Cb</sup>   | 26.85 <sup>Ea</sup>   | 37.19 <sup>Aa</sup>   | 31.96 <sup>Bb</sup>   |      |
|               | LG                          | 28.50 <sup>Ea</sup>   | 29.26 <sup>Da</sup>  | 28.84 <sup>DEa</sup>   | 23.67 <sup>Hb</sup>   | 29.02 <sup>DEa</sup>  | 24.51 <sup>Ga</sup>   | 32.89 <sup>Ca</sup>   | 27.45 <sup>Fa</sup>   | 37.87 <sup>Aa</sup>   | 34.22 <sup>Ba</sup>   |      |
| WSC<br>(% DM) | C                           | 0.80 <sup>CDEab</sup> | 0.73 <sup>DEb</sup>  | 0.72 <sup>DEb</sup>    | 0.62 <sup>Eb</sup>    | 1.02 <sup>Cb</sup>    | 0.67 <sup>Eb</sup>    | 0.97 <sup>CDb</sup>   | 0.76 <sup>CDEb</sup>  | 1.37 <sup>Bb</sup>    | 1.67 <sup>Ac</sup>    | 0.08 |
|               | L                           | 0.65 <sup>Db</sup>    | 0.66 <sup>Db</sup>   | 0.74 <sup>Db</sup>     | 0.56 <sup>Db</sup>    | 1.28 <sup>Bab</sup>   | 0.76 <sup>Db</sup>    | 1.04 <sup>Cb</sup>    | 0.74 <sup>Db</sup>    | 1.98 <sup>Ab</sup>    | 2.10 <sup>Ab</sup>    |      |
|               | LG                          | 1.12 <sup>DEa</sup>   | 0.94 <sup>Ea</sup>   | 1.58 <sup>CDa</sup>    | 0.79 <sup>Ea</sup>    | 1.53 <sup>CDa</sup>   | 1.71 <sup>Ca</sup>    | 2.25 <sup>Ba</sup>    | 2.00 <sup>BCa</sup>   | 3.54 <sup>Aa</sup>    | 3.21 <sup>Aa</sup>    |      |
| CP<br>(% DM)  | C                           | 5.38 <sup>CDc</sup>   | 4.63 <sup>Eb</sup>   | 5.00 <sup>CDEb</sup>   | 4.84 <sup>DEb</sup>   | 6.09 <sup>Bc</sup>    | 4.84 <sup>DEc</sup>   | 4.63 <sup>Ea</sup>    | 5.31 <sup>CDc</sup>   | 5.57 <sup>BCa</sup>   | 7.62 <sup>Aa</sup>    | 0.10 |
|               | L                           | 5.86 <sup>Cb</sup>    | 5.15 <sup>Dab</sup>  | 5.20 <sup>Db</sup>     | 4.73 <sup>Eb</sup>    | 6.42 <sup>Bb</sup>    | 5.20 <sup>Db</sup>    | 4.77 <sup>Ea</sup>    | 5.93 <sup>Cb</sup>    | 6.14 <sup>BCa</sup>   | 7.80 <sup>Aa</sup>    |      |
|               | LG                          | 6.40 <sup>Ca</sup>    | 5.53 <sup>Da</sup>   | 6.39 <sup>Ca</sup>     | 5.81 <sup>Da</sup>    | 7.32 <sup>Ba</sup>    | 5.81 <sup>Da</sup>    | 4.92 <sup>Ea</sup>    | 6.43 <sup>Ca</sup>    | 6.35 <sup>Ca</sup>    | 8.00 <sup>Aa</sup>    |      |
| NDF<br>(% DM) | C                           | 77.66 <sup>Aa</sup>   | 77.31 <sup>ABa</sup> | 74.78 <sup>BCDa</sup>  | 74.75 <sup>BCDa</sup> | 72.87 <sup>CDa</sup>  | 74.19 <sup>CDa</sup>  | 75.17 <sup>ABCa</sup> | 74.72 <sup>BCDa</sup> | 75.58 <sup>ABCa</sup> | 72.34 <sup>Da</sup>   | 0.30 |
|               | L                           | 76.41 <sup>Aa</sup>   | 75.36 <sup>ABb</sup> | 73.58 <sup>ABCab</sup> | 73.24 <sup>BCab</sup> | 75.31 <sup>Ca</sup>   | 74.05 <sup>ABCa</sup> | 73.90 <sup>ABCa</sup> | 72.66 <sup>BCb</sup>  | 74.90 <sup>ABa</sup>  | 71.88 <sup>Ca</sup>   |      |
|               | LG                          | 70.77 <sup>ABb</sup>  | 72.08 <sup>Ac</sup>  | 69.85 <sup>ABb</sup>   | 70.36 <sup>ABb</sup>  | 69.42 <sup>ABb</sup>  | 68.80 <sup>Bb</sup>   | 70.32 <sup>ABb</sup>  | 69.89 <sup>ABc</sup>  | 70.51 <sup>ABb</sup>  | 69.92 <sup>ABa</sup>  |      |
| ADF<br>(% DM) | C                           | 55.12 <sup>Aa</sup>   | 54.86 <sup>Aa</sup>  | 50.03 <sup>Da</sup>    | 55.61 <sup>Aa</sup>   | 51.39 <sup>CDa</sup>  | 52.54 <sup>BCa</sup>  | 52.32 <sup>BCa</sup>  | 53.04 <sup>Ba</sup>   | 50.24 <sup>Da</sup>   | 51.91 <sup>BCa</sup>  | 0.31 |
|               | L                           | 54.75 <sup>Aa</sup>   | 53.15 <sup>ABb</sup> | 50.42 <sup>CDa</sup>   | 54.79 <sup>Aa</sup>   | 49.11 <sup>Db</sup>   | 51.20 <sup>BCDa</sup> | 50.89 <sup>BCDa</sup> | 52.11 <sup>BCa</sup>  | 49.56 <sup>Da</sup>   | 50.08 <sup>CDab</sup> |      |
|               | LG                          | 51.71 <sup>Ab</sup>   | 50.25 <sup>Bc</sup>  | 48.07 <sup>Cb</sup>    | 50.04 <sup>Bb</sup>   | 45.49 <sup>Ec</sup>   | 48.12 <sup>Cb</sup>   | 47.15 <sup>CDb</sup>  | 47.00 <sup>CDEb</sup> | 46.33 <sup>DEb</sup>  | 47.27 <sup>CDb</sup>  |      |
| IVOMD<br>(%)  | C                           | 27.82 <sup>Da</sup>   | 26.48 <sup>Db</sup>  | 31.54 <sup>Cb</sup>    | 37.28 <sup>ABb</sup>  | 31.37 <sup>Cb</sup>   | 34.83 <sup>Bb</sup>   | 35.62 <sup>ABa</sup>  | 37.72 <sup>ABb</sup>  | 38.39 <sup>Ab</sup>   | 38.36 <sup>Aa</sup>   | 0.54 |
|               | L                           | 28.29 <sup>Da</sup>   | 27.86 <sup>Db</sup>  | 32.79 <sup>Cb</sup>    | 38.11 <sup>ABab</sup> | 32.71 <sup>Cb</sup>   | 35.00 <sup>BCb</sup>  | 37.44 <sup>ABa</sup>  | 38.13 <sup>ABb</sup>  | 39.23 <sup>Ab</sup>   | 39.79 <sup>Aa</sup>   |      |
|               | LG                          | 33.13 <sup>Da</sup>   | 33.54 <sup>Da</sup>  | 36.45 <sup>CDa</sup>   | 42.49 <sup>ABa</sup>  | 39.86 <sup>ABCa</sup> | 38.67 <sup>BCa</sup>  | 40.37 <sup>ABCa</sup> | 43.43 <sup>Aa</sup>   | 42.34 <sup>ABa</sup>  | 40.40 <sup>ABCa</sup> |      |

<sup>1</sup> Means followed by different uppercase in the same row differ (p<0.05); Means followed by different lowercase letters in the same column differ (p<0.05).

<sup>2</sup> C = Control; L = LAB inoculation treatment; LG = LAB inoculation plus glucose treatment.

**Table 5.** Nutritional value of the rice straw silage (2006)<sup>1</sup>

|        | Treat-<br>Ment <sup>2</sup> | Zhenxian96           | Xudao4              | Huaidao262           | Hangdao1             | Huaidao268           | Liangyoupeijiu       | SEM  |
|--------|-----------------------------|----------------------|---------------------|----------------------|----------------------|----------------------|----------------------|------|
| DM     | C                           | 25.12 <sup>Ea</sup>  | 29.99 <sup>Bb</sup> | 26.86 <sup>Db</sup>  | 28.12 <sup>Cb</sup>  | 29.77 <sup>Ba</sup>  | 31.13 <sup>Ab</sup>  | 0.40 |
| (%)    | L                           | 25.10 <sup>Ea</sup>  | 31.35 <sup>Ba</sup> | 27.68 <sup>Da</sup>  | 29.69 <sup>Ca</sup>  | 30.61 <sup>Ba</sup>  | 32.57 <sup>Aa</sup>  |      |
| WSC    | C                           | 0.68 <sup>Cb</sup>   | 0.64 <sup>Ca</sup>  | 0.70 <sup>Ca</sup>   | 1.63 <sup>Aa</sup>   | 1.02 <sup>Bb</sup>   | 0.76 <sup>Ca</sup>   | 0.07 |
| (% DM) | L                           | 0.86 <sup>Ca</sup>   | 0.68 <sup>Da</sup>  | 0.64 <sup>Da</sup>   | 1.90 <sup>Aa</sup>   | 1.31 <sup>Ba</sup>   | 0.73 <sup>Da</sup>   |      |
| CP     | C                           | 6.59 <sup>Bb</sup>   | 4.95 <sup>Da</sup>  | 5.87 <sup>Cb</sup>   | 7.89 <sup>Aa</sup>   | 5.86 <sup>Ca</sup>   | 4.58 <sup>Da</sup>   | 0.24 |
| (% DM) | L                           | 8.62 <sup>Aa</sup>   | 5.92 <sup>Ba</sup>  | 7.64 <sup>Aa</sup>   | 8.37 <sup>Aa</sup>   | 6.11 <sup>Ba</sup>   | 5.16 <sup>Ba</sup>   |      |
| NDF    | C                           | 71.73 <sup>Ca</sup>  | 75.46 <sup>Aa</sup> | 73.96 <sup>Ba</sup>  | 71.28 <sup>Ca</sup>  | 75.69 <sup>Aa</sup>  | 73.19 <sup>Ba</sup>  | 3.18 |
| (% DM) | L                           | 69.89 <sup>Db</sup>  | 74.25 <sup>Aa</sup> | 72.24 <sup>Bb</sup>  | 70.49 <sup>CDa</sup> | 72.12 <sup>Bb</sup>  | 71.36 <sup>BCb</sup> |      |
| ADF    | C                           | 52.54 <sup>DEa</sup> | 55.86 <sup>Aa</sup> | 55.10 <sup>ABa</sup> | 47.99 <sup>Fa</sup>  | 53.51 <sup>CDa</sup> | 54.41 <sup>BCa</sup> | 0.52 |
| (% DM) | L                           | 51.69 <sup>Ca</sup>  | 55.00 <sup>Aa</sup> | 53.95 <sup>ABa</sup> | 45.01 <sup>Db</sup>  | 51.85 <sup>Cb</sup>  | 52.97 <sup>BCa</sup> |      |
| IVOMD  | C                           | 38.08 <sup>Aa</sup>  | 29.94 <sup>Ba</sup> | 27.74 <sup>Ba</sup>  | 38.77 <sup>Aa</sup>  | 36.23 <sup>Ba</sup>  | 31.51 <sup>Aa</sup>  | 0.80 |
| (%)    | L                           | 40.36 <sup>Aa</sup>  | 30.04 <sup>Ca</sup> | 29.55 <sup>Ca</sup>  | 38.59 <sup>ABa</sup> | 36.33 <sup>ABa</sup> | 33.77 <sup>BCa</sup> |      |

<sup>1</sup> Means followed by different uppercase in the same row differ ( $p < 0.05$ ); Means followed by different lowercase letters in the same column differ ( $p < 0.05$ ).

<sup>2</sup> C = Control; L = LAB inoculation treatment.

1), which was a limiting factor in LA fermentation as mentioned above. Glucose supplied LAB with enough fermentation substrate, which accelerated ( $p < 0.05$ ) LA accumulation and thus pH decline. The addition of glucose with LAB also significantly decreased AA and  $\text{NH}_3\text{-N}$  concentrations ( $p < 0.05$ , Table 2). PA and BA were not detected in most varieties (Table 2). This was also the case in lucerne silage (Seale et al., 1986; Jones et al., 1992). It indicated that application of LAB plus glucose was more efficient in improvement of fermentation than LAB alone.

Furthermore, the application of LAB plus glucose increased DM, WSC, CP concentrations and IVOMD, but decreased NDF and ADF concentrations of the silage ( $p < 0.05$ , Table 4). This was consistent with results obtained with lucerne and corn silage (Nishino et al., 1999; Aksu et al., 2005).

#### Comparison of the fermentation and nutritional quality of rice straw silage among the rice varieties

The fermentation characteristics of rice straw silage differed among the rice varieties. According to the fermentation characteristics of the silage, the rice varieties were sorted into three groups: i) Good, including Jinlingxiang, Wuyujing3 and Hangdao1. These silages had high Flieg's scores with high LA concentration, and low pH, AA, PA, BA and  $\text{NH}_3\text{-N}$  concentrations, even if no LAB and/or sugar were added. ii) Medium, including Genjah Wangkal, Milyang23, Wuyunjing7, Zhenxian96, Huaidao262, Huaidao268 and Liangyoupeijiu. The silage quality was poor if no additives were added, but achieved "Good" quality if LAB was added. iii) Unsatisfactory, including Nekken2, IRAT109, Nipponbare, Dular, Akihikari and Xudao4. The silage quality was still unsatisfactory even

after LAB inoculation. These silages had high pH, AA, PA, BA and  $\text{NH}_3\text{-N}$  concentrations, and low LA concentration and Flieg's scores. They could achieve a satisfactory quality only if both LAB and sugar were added (Table 2; the rice straw of Xudao4 was not treated with LAB plus glucose).

The variation in fermentation characteristics between rice varieties could be mainly attributed to WSC concentration in the rice straw. The LAB in an inoculant would be unable to produce enough LA to lower pH to an acceptable level if there was insufficient WSC in the original crop (Seale et al., 1986). For example, Nekken2 and IRAT109 straw in the present study had low WSC, and inoculation with LAB alone did not obtain a satisfactory fermentation, whereas Jinlingxiang and Wuyujing3 straw had higher WSC, and thus had a satisfactory fermentation quality. The correlation analyses indicated that pH value and LA concentration of the silage significantly correlated to WSC concentration of rice straw ( $r = -0.789, 0.908$ , respectively). Therefore, the addition of LAB alone might be enough for rice varieties that contained sufficient WSC in the straw (Nishino et al., 1999), but sugar or sugar-rich materials should be added to ensure good fermentation quality for rice varieties whose straw has low WSC.

Nutritional characteristics of the silage also differed greatly among the rice varieties. In general, Nekken2, IRAT109, Nipponbare, Xudao4 and Huaidao262 silages had relatively low nutritional value, while Wuyunjing7, Jinlingxiang, Wuyujing3, Zhenxian96, Hangdao1 and Huaidao268 silages had better nutritional value (Tables 4 and 5). The differences in nutritional value among the varieties could be attributed to the chemical composition of the rice straw material.

## IMPLICATIONS

Rice straw is difficult to ensile due to its low WSC concentration and less epiphytic LAB. Inoculation with LAB could improve the silage fermentation of rice straw that contains sufficient fermentable WSC, and application of LAB plus glucose was proven more effective in improving fermentation quality than LAB alone. Difference in the fermentation quality of silage among rice varieties was mainly because of the WSC concentration in the straw; LAB application and selection of rice varieties whose straw contains high levels of WSC are a good strategy for ensiling rice straw.

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