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Original research article

Effects of applying lactic acid bacteria to the fermentation on a mixture of corn steep liquor and air-dried rice straw

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ABSTRACT

This study was to determine the fermentation quality of a mixture of corn steep liquor (CSL) (178 g/kg wet basis) and air-dried rice straw (356 g/kg wet basis) after being treated with inoculants of different types of lactic acid bacteria (LAB). The treatments included the addition of no LAB additive (control), which was deionized water; homo-fermentative LAB alone (^{ho}LAB), which was *Lactobacillus plantarum* alone), and a mixture of homo-fermentative and hetero-fermentative LAB ($^{he + ho}$LAB), which were *L. plantarum*, *Lactobacillus casei*, and *Lactobacillus buchneri*. The results showed that the inoculation of the mixture of CSL and air-dried rice straw with $^{he + ho}$LAB significantly increased the concentration of acetic acid and lactic acid compared with the control ($P < 0.05$). The addition of $^{he + ho}$LAB effectively inhibited the growth of yeast in the silage. The concentration of total lactic acid bacteria in the $^{he + ho}$LAB-treated silage was significant higher than those obtained in other groups ($P < 0.05$). The duration of the aerobic stability of the silages increased from 56 h to >372 h. The control group was the first to spoil, whereas the silage treated with $^{he + ho}$LAB remained stable throughout the 372 h period of monitoring. The results demonstrated that the $^{he + ho}$LAB could effectively improve the fermentation quality and aerobic stability of the silage.

1. Introduction

China is an agricultural country with abundant straw resources (Bi et al., 2008). However, only 15% of the rice straw is fed to ruminants; the rest is burnt or buried in the field (Wang et al., 2011). Under natural conditions, fresh rice straws rapidly become air-dried. Thus, it is not a suitable feed for animals because of its low digestibility, poor palatability, high crude fibre, and low protein content. Corn steep liquor (CSL) is a major by-product obtained from the wet-milling industry. It contains a rich complement of organic nitrogen and vitamins, which is capable of replacing yeast extract in a variety of fermentation process (Nascimento et al., 2009). Corn steep liquor has been successfully used in the production of enzymes (Silveira et al., 2001), lactic acid (Agarwal et al., 2006; Liu et al., 2010), and ethanol (Silveira et al., 2001; Saxena and Tanner, 2012). Consequently, air-dried rice straw, as an extensive source of absorbers, can be mixed and ensilaged with CSL effectively. However, the stems of air-dried rice straw have weak natural adhesion to lactic acid bacteria (LAB), thus it is essential to add silage bacterial additives to the mixture of CSL and rice straw to improve the concentration of LAB (Wilkinson, 2005).

Commercial homo-fermentative LAB (^{ho}LAB) inoculants have been developed to ensure rapid and efficient fermentation of water-soluble carbohydrate (WSC) into lactic acid, a rapid decrease in pH, and improved silage conservation with minimal fermentation losses (Huisden et al., 2009; Weinberg et al., 1993). However, such inoculants have also been responsible for decreasing the aerobic stability of silages observed in many studies (Weinberg et al., 1993; Kleinschmit et al., 2005) because antifungal volatile

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fatty acid (VFA) are lowered and lactic acid is easily oxidized by aerobic microorganisms (MacDonald et al., 1991; Kleinschmit et al., 2005). The aerobic stability of forage with *Lactobacillus buchneri* inoculation can be considerably enhanced by the metabolic activity of converting lactic acid to acetic acid under anaerobic conditions, and the silage could remain cool; thus, it does not spoil as long as 30 d when it is exposed to air (Driehuis et al., 1999; Elferink et al., 2001). Recently, dual-purpose inoculants containing homo-fermentative and hetero-fermentative bacteria have been developed to overcome the limitations of inoculants containing either type of bacteria alone, and the combination of both types of organisms has the potential to improve the speed of fermentation and enhance the aerobic stability (Nishino et al., 2007; Reich and Kung, 2010; Schmidt and Kung Jr, 2010; Conaghan et al., 2010; Kung et al., 2003), but the fermentation of a mixture of CSL and air-dried rice straw with LAB inoculants (*Lactobacillus plantarum*, *L. buchneri* and *Lactobacillus casei*) has not yet been studied.

The present work was to study the effects of ensiling a mixture of CSL and air-dried rice straw with ^{ho}LAB alone or in combination with hetero-fermentative LAB ($^{he\ +\ ho}$LAB) on the fermentation quality and aerobic stability. This experiment was performed by the application of the microorganisms to laboratory silages. The ability to successfully convert a mixture of CSL and air-dried rice straw into a new type of ruminant fermentation feed will promote the improvement in the ecological environment and the cyclic development of the agricultural economy.

2. Materials and methods

2.1. Forage and ensiling

2.1.1. Experimental materials

Rice straws were collected from the Xiangfang Experimental Farm of Northeast Agricultural University (Harbin, China), air-dried to 10% dry matter (DM), and chopped to a theoretical length of 2 to 3 cm. Corn steep liquor obtained from the Cargill Biochemistry Co., Ltd. (Songyuan, China) was used in the study.

2.1.2. Treatments

The CSL (178 g/kg, wet basis) were mixed with 356 g/kg air-dried rice straw (wet basis) thoroughly before the application of the different fermentation inoculants. The mixture was then assigned to one of the following treatments: 1) deionized water, untreated (control); 2) *L. plantarum*, alone (^{ho}LAB); 3) *L. plantarum*, *L. casei*, and *L. buchneri*, a mixture of homo- and hetero-fermentative LAB ($^{he\ +\ ho}$LAB). The application rate of each inoculant to the fresh forage was 1×10^6 cfu/g of fresh matter (FM). The L. *plantarum* strain isolated from a commercial inoculant (Silage. help) was used. The $^{he\ +\ ho}$LAB obtained from Northeast Agricultural University consisted of 2 strains of ^{ho}LAB (*L. plantarum* and *L. casei*) in combination with a ^{he}LAB (*L. buchneri*). In this experiment, the bacterial additive of each group was inoculated in De Man, Rogosa, or Sharpe (MRS) broth for 48 h, and the bacteria were then plated on MRS agar overnight to confirm their viability. Appropriate amounts of the inoculants were then used to achieve the desired application rate. The inoculants were applied at a rate of 50 mL/kg (wet basis) forage with a sprayer. Approximately 416 mL/kg (wet basis) deionized water was sprayed onto the mixture to achieve a final moisture content of 60%. To ensure that the amount of moisture was equal to what was found in the microbial-treated group, the control silage was sprayed with 466 mL/kg (wet basis) deionized water. Approximately 300 g (wet basis) of forage from each treatment were packed into a plastic bag (polyethylene; 400 mm × 500 mm), and all of the bags were sealed with a vacuum sealer and stored indoors for 60 days at ambient temperature (18 ± 2 °C). Duplicate silos for each treatment were opened after 0, 3, 5, 7, 10, 30, 45 and 60 days. The silages were randomly subsampled from several different positions, and then mixed to generate a composite sample for microbiological and chemical analysis. The rest of the bag was subjected to an aerobic stability test after 60 days.

2.2. Chemical and microbial analysis

The silage samples were dried at 65 °C and analysed for DM according to AOAC (1990) procedures. The nitrogen (N) content was measured using the Kjeldahl method (AOAC, 1990). The CP was calculated as N × 6.25. The acid detergent fibre (ADF) and neutral detergent fibre (NDF) values were analysed according to the procedures described by Van Soest et al. (1991) using the Ankom system (Ankom 220 fibre analyser; Ankom) with heat-stable α-amylase. The WSC concentration was measured through the colorimetric method (Dubois et al., 1956). Both fresh and silage juice were extracted by blending 10 g forage (wet basis) in 90 mL of distilled water and storing the mixture for 24 h at 4 °C in a refrigerator (Nishino and Uchida, 1999). The slurry mixture was then filtered through 4 layers of cheesecloth (Xing et al., 2009), and the filtrate was used for pH, ammonia-N, lactic acid and VFA determination. The pH was directly measured using a pH meter (Sartorius Basic pH Meter, Germany). The ammonia-N (NH_3–N) concentration was determined using an ammonia-sensing electrode (Expandable Ion Analyser EA 940, Orion, USA). Samples for VFA analysis were prepared as described by Li and Meng (2006). The concentrations of VFA were analysed using a gas–liquid chromatography (GC, 2010, Tokyo, Japan) equipped with a flame-ionization detector and a free fatty acid phase (FFAP) capillary column (HP-INNOWAX, 30 m × 0.250 mm × 0.25 μm). The lactic acid content was determined using a high-performance liquid chromatography (Waters 600, Tokyo, Japan) following the procedure described by Muck and Dickerson (1987).

Another portion of the fresh silage juices was extracted by blending 10 g forages (wet basis) in 90 mL distilled water for 30 min at ambient temperature, and then filtered through a double layer of cheesecloth. The filtrate was divided into 2 sets of LAB by pour-plating on MRS agar, and the yeasts and moulds were enumerated by pouring the filtrate onto malt extract agar (Oxoid CM0059). The plates were incubated at 37 °C for 48 h, and then numbers of colony-forming units were counted.

All of the chemical analyses were conducted in triplicates, and the results were expressed on a DM basis except that the microbiological data were transformed to log units (% fresh matter), DM content (% fresh matter) and NH_3–N (% total nitrogen).

2.3. Statistical analysis

The data were analysed as a completely randomized design using the ANOVA procedure of SAS 9.2 (SAS Institute, 1999). The results were presented as the mean values and standard error of the means. Differences between treatment means were determined by Duncan's multiple range test method.

3. Results

The chemical compositions of CSL, air-dried rice straw, and their mixture before ensiling are showed in Table 1. The dry matter content of each treatment was adjusted to 40%. The content of CSL (characterized by high protein content) was up to 37% DM, which was favourable for the growth of bacteria. As shown in Table 1, the application of CSL effectively increased the protein content of the dry rice straw to 9.95%. The content of NDF and ADF of dry rice