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Natural lactic acid bacteria population of tropical grasses and their fermentation factor analysis of silage prepared with cellulase and inoculant

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ABSTRACT

Natural lactic acid bacteria (LAB) populations in tropical grasses and their fermentation characteristics on silage prepared with cellulase enzyme and LAB inoculants were studied. A commercial inoculant *Lactobacillus plantarum* Chikuso 1 (CH), a local selected strain *Lactobacillus casei* TH14 (TH14), and 2 cellulases, *Acremonium* cellulase (AC) and Maicelase (MC; Meiji Seika Pharma Co. Ltd., Tokyo, Japan), were used as additives to silage preparation with fresh and wilted (6 h) Guinea grass and Napier grass. Silage was prepared using a laboratory-scale fermentation system. Treatments were CH, TH14, AC at 0.01% fresh matter, AC 0.1%, MC 0.01%, MC 0.1%, CH+AC 0.01%, CH+AC 0.1%, CH+MC 0.01%, CH+MC 0.1%, TH14+AC 0.1%, TH14+AC 0.01%, TH14+MC 0.1%, and TH14+MC 0.01%. Microorganism counts of Guinea grass and Napier grass before ensiling were 10^2 LAB and 10^6 aerobic bacteria; these increased during wilting. Based on morphological and biochemical characteristics, and 16S rRNA gene sequence analysis, natural strains from both grasses were identified as *L. plantarum*, *L. casei*, *Lactobacillus acidipiscis*, *Leuconostoc pseudomesenteroides*, *Leuconostoc garlicum*, *Weissella confusa*, and *Lactococcus lactis*. *Lactobacillus plantarum* and *L. casei* are the dominant species and could grow at lower pH and produce more lactic acid than the other isolates. Crude protein and neutral detergent fiber were 5.8 and 83.7% of dry matter (DM) for Guinea grass, and 7.5 and 77.1% of DM for Napier grass. Guinea grass had a low level of water-soluble carbohydrates (0.39% of DM). Guinea grass silage treated with cellulase had a lower pH and higher lactic acid content than control and LAB treatments. The 0.1% AC and MC treatments had the best result for fermentation quality. All high water-soluble carbohydrate (2.38% DM) Napier grass silages showed good fermentation quality. Compared with control and LAB-inoculated silage, the cellulase-treated silages had significantly higher crude protein content and lower neutral detergent fiber and acid detergent fiber contents. The results confirmed that cellulase could improve tropical silage quality, inhibiting protein degradation and promoting fiber degradation.
Key words: cellulase, fermentation factor, lactic acid bacteria, tropical silage

INTRODUCTION

The major constraint for dairying in the tropics is shortage of feed in terms of quality and quantity, especially in the dry season. The main feed sources for dairy cows are native grasses and byproducts from agriculture. Dairy cows fed low-quality roughage have low milk production. To establish a forage production system to cover the shortage of animal feed in the dry season, technologies using many grass varieties have been developed. These include the testing and cultivation of forages, studying their adaptability to various conditions and their nutritive value and productivity (Phaikaew et al., 2001). Purple Guinea grass (*Panicum maximum* 'TD 58') and Napier grass (*Pennisetum purpureum* 'Pak Chong 1') are widely used for ruminant feed in the tropics, including Thailand. They can both grow well in the rainy season, are high in DM yield, and are drought tolerant (Tudsri et al., 2002; Hare et al., 2009). They need to be conserved to supply feed for ruminants during the dry season.

Silage preparation and storage is one of the most effective techniques for animal feed supply in the dry season in the tropics. High quality tropical silages are difficult to create because of low lactic acid bacteria (**LAB**) and water-soluble carbohydrate (**WSC**) contents in the forage (Pholsen et al., 2016). In this experiment, LAB inoculants and cellulase enzyme were selected as microbial additives to improve silage quality.

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Cellulase improves fiber degradation, increasing WSC as a substrate for LAB to produce lactic acid (Cai et al., 1999).

The moisture content of the grass also directly affects bacterial activity during the fermentation phase. The activity of silage microorganisms slows as grass DM content increases and as silage pH decreases. Microorganism activity stops at a higher pH as grass DM content increases (McDonald et al., 1991). Usually, tropical grass has a high moisture content (>80%), which causes butyric acid fermentation leading to unsuccessful ensiling (Pholsen et al., 2016). Grass wilting could inhibit undesirable microorganisms and reduce nutrient loss. However, the characteristics of LAB and cellulase, and their true function in silage making under different moisture conditions need further study.

In the present study, the natural lactic acid bacteria populations and fermentation quality of tropical grasses were examined. To analyze the fermentation factors, the fresh and wilted silages were also prepared with additives, with particular reference to cellulase enzyme and LAB inoculants; these are considered most important in silage fermentation quality improvement.

MATERIALS AND METHODS

Ensiling Materials and Silage Preparation

Purple guinea (*Panicum maximum* 'TD 58') and Napier (*Pennisetum purpureum* × *Pennisetum americanum* 'Pak Chong 1') grasses were grown in May 2013, at the experimental farm, Department of Animal Science, Faculty of Agriculture, Khon Kaen University, Khon Kaen, Thailand, in areas of 800 and 1,600 m^2 on Korat soil series (Oxic Paleustults), respectively. The plot of purple Guinea grass was ploughed twice and harrowed once, and 17,778 populations of root stock of purple Guinea grass were planted into rows by hand at distances between and within rows of 75 × 75 cm, respectively. Cattle manure was applied at a rate of 24,000 kg/ha (4 equal portions of 6,000 kg/ha were split applied for 4 cuts) for high DM yield of organic grass (Yoottasanong et al., 2015). The plot of Napier grass was ploughed and harrowed once, and 11,111 populations of stem cuttings of the Napier grass were planted into rows by hand at distances between and within rows of 120 × 75 cm, respectively. For high DM yield, basal dressing fertilizers of NPK (15–15–15) and cattle manure were applied at 300 and 12,500 kg/ha, and nitrogen fertilizer (urea) at a rate of 60 kg/ha was split applied (Kiyothong, 2014). On April 10, 2014, both grasses were cut to adjust the height to 10 cm above ground level. The recommended rate of cattle manure was applied to purple Guinea and urea was applied to Napier grass. Both grasses were harvested at 60 d of regrowth on June 10, 2014. To study the relationship between moisture adjustment and silage fermentation, 50% of each grass was cut and chopped to 1 cm length (Supachai chopper, Kanchanaburi, Thailand) in the early morning and then wilted for 6 h in the shade. Another 50% was cut and chopped for fresh silage preparation.

A local selected LAB *Lactobacillus casei* strain TH14 (Pholsen et al., 2016), a commercial inoculant strain Chikuso 1 (CH, *L. plantarum*, Snow Brand Seed Co., Ltd., Sapporo, Japan), and 2 commercial cellulase enzymes (**AC**, *Acremonium* cellulase; **MC**, Maicelase, Meiji Seika Pharma Co. Ltd., Tokyo, Japan) were used as silage additives. The production strain, main composition, and carboxymethyl-cellulase activity of cellulase used in this study are shown in Table 1. Strain TH14 was isolated from sweet corn (*Zea mays* L.) stover silage. This strain grows well in a low pH environment and produces high lactic acid content (Pholsen et al., 2016). Lactobacilli de Man, Rogosa, Sharpe (**MRS**) broth (Difco Laboratories, Detroit, MI) was inoculated with strains TH14 and CH and incubated overnight. After incubation, the optical density at 620 nm of the suspension was adjusted with sterile 0.85% NaCl solution to 0.42 nm. The LAB inoculum was 1 mL of suspension/kg of fresh matter (**FM**). The LAB was inoculated at 1.0×10^5 cfu/g of FM. Both AC and MC cellulase were added at 0.01 and 0.1% FM. Four types of ensiled material (fresh and wilted Guinea grass and fresh and wilted Napier grass) were treated with 15 combinations of additives: control (untreated), CH, TH14, AC 0.01% FM, AC 0.1%, MC 0.01%, MC 0.1%, CH+AC 0.01%, CH+AC 0.1%, CH+MC 0.01%, CH+MC 0.1%, TH14+AC 0.1%, TH14+AC 0.01%, TH14+MC 0.1%, and TH14+MC 0.01%. The experimental design was a 2 × 15 factorial arrangement in a completely randomized design (grasses × additives) with 3 replications. One thousand-gram portions of grass, chopped to 20-mm length, were mixed well with additives, packed into a bag silo with laminated nylon and polyethylene (Hiryu KN, Asahikasei, Tokyo, Japan), and sealed using a vacuum sealer (SQ–303, Asahi Kasei Pax Corp., Tokyo, Japan). All silos were stored at room temperature (25 to 37°C). At d 30 after ensiling, 3 bags per treatment were opened for evaluation of fermentation end products, chemical, and microorganism compositions.

Microorganism Analysis of Pre-Ensiled Grass and Silage

Pre-ensiled grasses and silage samples at 30 d (3 replications) after fermentation were used for microorganism analysis. The microorganism counts were done