Effects of Lactic Acid Bacteria Inoculant on Silage Quality and Nutritional Composition of Whole-Plant Corn: Postprint

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Abstract

This study investigated the effects of lactic acid bacteria preparation on the quality and nutritional composition of whole-plant corn silage. Fresh whole-plant corn (720 kg) was selected and evenly divided into 4 groups, with 12 replicates per group and 15 kg per replicate. The control group received no lactic acid bacteria preparation, while the experimental groups were supplemented with 2, 10, and 20 mg/kg of lactic acid bacteria preparation, respectively, and stored at room temperature for a trial period of 60 days. Sampling and analysis were conducted at 45 and 60 days. The results showed that, compared with the control group: 1) Sensory evaluation indices showed no significant changes in any of the experimental groups ($P>0.05$). 2) The number of lactic acid bacteria in the experimental groups increased significantly ($P<0.05$), and the mold count in the 10 mg/kg group at 45 days and in all experimental groups at 60 days decreased significantly ($P<0.05$). 3) At 45 days, the contents of lactic acid and acetic acid in the experimental groups increased significantly ($P<0.05$), while the butyric acid content decreased significantly ($P<0.05$); at 60 days, the acetic acid content in the 2 and 10 mg/kg groups increased significantly ($P<0.05$). 4) At 45 days, the crude protein content in the 2 and 10 mg/kg groups increased significantly ($P<0.05$), the neutral detergent fiber content in the 2 mg/kg group increased significantly ($P<0.05$), and the ammonia nitrogen content in all experimental groups decreased significantly ($P<0.05$); at 60 days, the water-soluble carbohydrate content in the 20 mg/kg group decreased significantly ($P<0.05$). Therefore, the addition of lactic acid bacteria preparation can improve the quality of whole-plant corn silage and enhance its nutritional value; based on the lowest mold count during fermentation, the recommended addition level of lactic acid bacteria preparation is 10 mg/kg.

Full Text

Effects of Lactic Acid Bacteria Preparation on Quality and Nutrient Composition of Whole Corn Silage

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Abstract: This study investigated the effects of lactic acid bacteria preparation on the quality and nutrient composition of whole corn silage. A total of 720 kg of fresh whole corn was divided into four groups with twelve replicates per group (15 kg per replicate). The control group received no lactic acid bacteria preparation, while the experimental groups were supplemented with 2, 10, and 20 mg/kg of lactic acid bacteria preparation, respectively. All silages were stored at room temperature for 60 days, with samples collected for analysis on days 45 and 60. Compared with the control group, the results showed that: 1) sensory evaluation indices did not differ significantly among experimental groups ($P > 0.05$); 2) lactic acid bacteria counts increased significantly in all experimental groups ($P < 0.05$), while mold counts decreased significantly in the 10 mg/kg group at day 45 and in all experimental groups at day 60 ($P < 0.05$); 3) lactic acid and acetic acid contents increased significantly while butyric acid content decreased significantly in experimental groups at day 45 ($P < 0.05$), and acetic acid content increased significantly in the 2 and 10 mg/kg groups at day 60 ($P < 0.05$); 4) at day 45, crude protein content increased significantly in the 2 and 10 mg/kg groups ($P < 0.05$), neutral detergent fiber content increased significantly in the 2 mg/kg group ($P < 0.05$), and ammonia nitrogen content decreased significantly in all experimental groups ($P < 0.05$); at day 60, water-soluble carbohydrate content decreased significantly in the 20 mg/kg group ($P < 0.05$). These findings indicate that lactic acid bacteria preparation can improve both the quality and nutritional value of whole corn silage, with a recommended supplementation rate of 10 mg/kg based on minimal mold counts during fermentation.

Key words: lactic acid bacteria; whole corn; silage quality; nutrient composition

Silage production utilizes naturally occurring lactic acid bacteria on the surface of fresh forage materials. Under anaerobic conditions, these bacteria ferment soluble carbohydrates into organic acids such as lactic and acetic acid, which lower pH and inhibit the growth of spoilage microorganisms like molds, thereby preserving the nutritional characteristics of the forage [1]. Whole corn silage plays a crucial role in ruminant nutrition, offering high nutritional value, good palatability, and easy storage, which promotes the development of "grain-saving" and "straw-based" livestock production systems [2]. However, inadequate silage techniques and insufficient native lactic acid bacteria on forage surfaces often fail to shorten the aerobic phase effectively, leading to spoilage microbial proliferation, severe nutrient loss, and compromised silage quality. Consequently, producing high-quality, safe corn silage has become a major industry concern. Recent research on silage additives has focused on microbial preparations, enzymes, organic acids, and sugars. Among these, lactic acid bacteria-based microbial preparations have attracted particular attention due to their ability to effectively inhibit spoilage microorganisms, reduce nutrient loss, and improve silage quality with stable results.

Lactic acid bacteria are classified into two main types based on their fermentation patterns: homofermentative and heterofermentative. Homofermentative bacteria produce primarily lactic acid from glucose, while heterofermentative bacteria generate lactic acid along with acetic acid, ethanol, and carbon dioxide [2-3]. Previous studies indicate that homofermentative lactic acid bacteria effectively improve silage quality during fermentation, whereas heterofermentative strains enhance aerobic stability [4-6]. Most existing research has focused on single-strain applications, with both types demonstrating distinct advantages. However, studies on the synergistic effects of combined homofermentative and heterofermentative lactic acid bacteria remain limited. Furthermore, results vary due to environmental conditions [7] and application rates [8], necessitating further investigation. Therefore, this study evaluated the effects of different doses of a combined homofermentative and heterofermentative lactic acid bacteria preparation on whole corn silage quality and nutrient composition, using corn harvested in Shandong Province in September 2016, to provide a reference for practical application.

1.1 Experimental Materials

Fresh whole corn harvested in September 2016 from Shandong Province served as the experimental material, with nutrient contents (on a dry matter basis) of: organic matter (OM) 86.84%, crude protein (CP) 8.27%, neutral detergent fiber (NDF) 55.95%, and acid detergent fiber (ADF) 26.03%. Silage bags (75 cm × 40 cm × 8 cm) featured a double-layer design with polypropylene outer layers and polyethylene inner layers. The lactic acid bacteria preparation (11GFT, Pioneer Hi-Bred, Canada) contained 1.1×10^{11} CFU/g of lactic acid bacteria, including *Lactobacillus buchneri* and *Lactobacillus casei*.

1.2 Experimental Design

A total of 720 kg of fresh whole corn (cv. Denghai 605) at the dough stage, with a moisture content of 73.67% and chopped to 1–4 cm lengths, was divided into four groups (twelve replicates per group, 15 kg per replicate). The control group received 45 mL of deionized water sprayed per replicate, while experimental groups received 2, 10, and 20 mg/kg of lactic acid bacteria preparation (prediluted in 45 mL deionized water). All replicates were mixed thoroughly, packed rapidly into silage bags, compacted, sealed, and stored at room temperature for 60 days.

1.3 Sample Collection and Analysis

Samples were collected from six replicates per group on days 45 and 60 for various analyses.

1.3.1 Sensory Evaluation Sensory evaluation followed the German Agricultural Society (DLG) silage quality scoring system [9], assessing odor (14 points), structure (4 points), and color (2 points). Total scores of 16–20 were rated as excellent, 10–15 as acceptable.

1.3.2 Microbial Counts Thirty grams of fresh sample was mixed with 270 mL of physiological saline and diluted serially from 10^{-1} to 10^{-7}. Plate count methods were employed for microbial enumeration. Lactic acid bacteria were cultured on MRS agar (64.25 g MRS medium per liter distilled water, autoclaved at 121°C for 15 minutes) under anaerobic conditions at 37°C for 2 days. Molds were enumerated on potato dextrose agar (40.1 g per liter distilled water, autoclaved at 121°C for 20 minutes) at 37°C for 2–4 days. Valid plate counts ranged from 30–300 colonies per plate.

1.3.3 pH Determination Ten grams of fresh sample was extracted with 90 mL deionized water at 4°C for 24 hours, and the filtrate pH was measured using a pH meter (HI9025, Hanna Instruments, Italy).

1.3.4 Organic Acids and Ammonia Nitrogen Thirty-five grams of fresh sample was extracted with 70 mL deionized water at 4°C for 24 hours, then filtered through two layers of gauze and filter paper. The filtrate was stored at -20°C for subsequent analysis of lactic acid (LA), volatile fatty acids (VFA), and ammonia nitrogen (NH_3-N). Lactic acid content was determined by the p-hydroxydiphenyl method [10] using calcium lactate standards, expressed as g/kg DM. VFAs (acetic, propionic, and butyric acids) were analyzed by gas chromatography (GC-2010, Shimadzu, Japan) [11] and expressed as g/kg DM. Ammonia nitrogen was measured by the phenol-hypochlorite method [12] using ammonium chloride standards, expressed as g/kg total nitrogen (TN).

1.3.5 Chemical Composition Air-dried samples were analyzed for OM, CP, NDF, and ADF according to AOAC (2015) methods [13]. Water-soluble carbohydrate (WSC) content was determined by the anthrone-sulfuric acid method [14] using glucose standards, expressed as g/kg DM.

1.4 Statistical Analysis

Data were initially processed using Excel 2007, then subjected to one-way ANOVA using SPSS 16.0. Duncan' s multiple range test was used for post-hoc comparisons. Results are presented as means with standard error of the mean (SEM), with $P < 0.05$ considered statistically significant.

2.1 Effects of Lactic Acid Bacteria Preparation on Sensory Evaluation

As shown in Table 1 , no significant differences were observed in odor, structure, color, or total sensory scores among all groups ($P > 0.05$). Values in the same row with no letter or the same letter superscripts indicate no significant difference ($P > 0.05$), while different lowercase letters indicate significant difference ($P < 0.05$). The same notation applies below.

2.2 Effects of Lactic Acid Bacteria Preparation on Microbial Counts

Table 2 shows that compared with the control group, lactic acid bacteria counts increased significantly by 24.54%, 25.96%, and 25.11% in the 2, 10, and 20 mg/kg groups at day 45 ($P < 0.05$), while mold counts decreased by 10.09% ($P > 0.05$), 20.00% ($P < 0.05$), and 14.13% ($P > 0.05$), respectively. At day 60, lactic acid bacteria counts increased significantly by 17.44%, 20.93%, and 20.54% in the 2, 10, and 20 mg/kg groups ($P < 0.05$), and mold counts decreased significantly by 31.01%, 29.65%, and 28.29% ($P < 0.05$).

2.3 Effects of Lactic Acid Bacteria Preparation on pH and Organic Acid Contents

As presented in Table 3 , pH values did not differ significantly among groups at either 45 or 60 days ($P > 0.05$). At day 45, lactic acid content increased significantly by 10.63%, 7.90%, and 8.05% in the 2, 10, and 20 mg/kg groups ($P < 0.05$), while acetic acid content increased significantly by 364.10%, 288.76%, and 285.40% ($P < 0.05$). Butyric acid content decreased significantly by 17.72%, 15.19%, and 11.39% ($P < 0.05$). At day 60, acetic acid content increased significantly by 767.17% and 171.73% in the 2 and 10 mg/kg groups ($P < 0.05$), with no other significant differences observed ($P > 0.05$).

2.4 Effects of Lactic Acid Bacteria Preparation on Chemical Composition

Table 4 shows that at day 45, CP content increased significantly by 7.70% and 11.85% in the 2 and 20 mg/kg groups ($P < 0.05$), NDF content increased significantly by 15.90% in the 2 mg/kg group ($P < 0.05$), and ammonia nitrogen content decreased significantly by 15.44%, 21.87%, and 18.60% in the 2, 10, and 20 mg/kg groups ($P < 0.05$). At day 60, WSC content decreased significantly by 33.14% in the 20 mg/kg group ($P < 0.05$), with no other significant differences among groups ($P > 0.05$).

Discussion

3.1 Effects of Lactic Acid Bacteria Preparation on Silage Quality

The fundamental principle of silage production involves creating anaerobic conditions through the respiration of aerobic microorganisms and plant cells, followed by lactic acid fermentation that produces organic acids to inhibit spoilage microorganisms, thereby preserving feed for long-term storage [15]. This process typically includes aerobic bacterial activity, lactic acid fermentation, and stabilization phases [16]. In this study, all groups achieved total sensory scores of 16–20, indicating excellent silage quality. The significant increase in lactic acid bacteria counts and reduction in mold counts in treated groups align with previous findings [17-18], demonstrating that lactic acid bacteria preparation promotes beneficial microbes as the dominant population, shortens the aerobic phase, and suppresses harmful microorganisms like molds [17].

Organic acid analysis revealed that lactic acid bacteria preparation increased lactic acid content at day 45, indicating enhanced early fermentation. However, no significant differences were observed at day 60, with lactic acid content decreasing compared to day 45. This likely reflects the mixed culture of *L. buchneri* (heterofermentative) and *L. casei* (homofermentative) used in this study. *L. buchneri* can metabolize lactic acid into volatile fatty acids such as acetic acid. Kung et al. [19] reported that inoculating alfalfa with three doses of *L. buchneri* (1$\times10^{5}$, 5×10^{5}, 1×10^{6} CFU/g) for 56 days reduced lactic acid and increased acetic acid content. Similarly, Lü et al. [20] found that low-dose *L. buchneri* initially increased lactic acid production, but higher doses and extended storage significantly reduced lactic acid while increasing acetic acid.

Lactobacillus casei is a facultative heterofermentative species. Nishino et al. [4] reported that inoculating whole corn (2$\times10^{6}$ CFU/g) and total mixed ration (3$\times10^{6}$ CFU/g) with *L. casei* increased lactic acid and decreased acetic acid after 60 days. Subsequent studies by Nishino et al. [5] on fescue, whole corn, and total mixed ration silage produced similar results, confirming that *L. casei* enhances lactic acid production while reducing acetic acid. The increased lactic acid content observed at day 45 in this study likely reflects early colonization and fermentation by *L. casei*. However, as fermentation progressed, *L. buchneri* may have proliferated [21] and utilized lactic acid produced by *L. casei*, resulting in lower lactic acid content at day 60. The concurrent increase in acetic acid content, particularly with prolonged fermentation, reflects the conversion of lactic acid to acetic acid and propanediol by *L. buchneri*. Volatile fatty acids like acetic acid are more effective antifungal agents than lactic acid, thereby improving aerobic stability. Thus, the observed changes in lactic and acetic acid contents likely represent the combined effects of both bacterial species. Butyric acid, produced by spoilage and clostridial bacteria through protein, glucose, and lactic acid degradation, serves as an indicator of silage quality—higher concentrations indicate poorer quality [7,22]. The reduced butyric acid content at day 45 in treated groups corresponds with decreased mold counts.

3.2 Effects of Lactic Acid Bacteria Preparation on Nutrient Composition

Nutrient composition is another critical indicator of silage quality. This study demonstrated that lactic acid bacteria preparation increased CP content and reduced ammonia nitrogen proportion. Ammonia nitrogen in silage primarily results from plant enzyme-mediated protein degradation and microbial proteolysis, reflecting the extent of protein breakdown [23-24]. Nishino et al. [5] reported significant reductions in ammonia nitrogen when *L. buchneri* and *L. casei* were inoculated into fescue and total mixed ration silage. Li et al. [25] also found that complex lactic acid bacteria inoculant significantly reduced ammonia nitrogen and increased CP content in corn straw silage after 150 days. These consistent results suggest that lactic acid bacteria may reduce protein degradation during storage or inhibit proteolytic spoilage microorganisms, thereby decreasing ammonia nitrogen and indirectly increasing CP content.

Additionally, treated groups showed increased NDF content and decreased WSC content, similar to findings by Addah et al. [22]. Corn stalks contain abundant WSC that provides nutrients for lactic acid bacteria colonization. Bacterial utilization of WSC may have increased the relative proportion of less digestible carbohydrate fractions such as NDF [2]. Although CP content increased in treated groups, OM content did not differ significantly, suggesting a reduction in non-nitrogenous compounds. This may be attributed to lactic acid bacteria utilizing soluble carbohydrates for growth while simultaneously inhibiting protein degradation by spoilage microorganisms, thereby maintaining high silage quality. This interpretation aligns with the observed reductions in OM and WSC content from day 45 to day 60, while CP content remained relatively stable.

Conclusion

Under the conditions of this experiment, lactic acid bacteria preparation improved whole corn silage quality and nutritional value. Based on minimal mold counts during fermentation, a supplementation rate of 10 mg/kg is recommended.

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