Effects of Cellulase and Compound Probiotics on Whole-Plant Corn Silage Quality (Postprint)

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Abstract

This experiment aimed to investigate the effects of cellulase and compound probiotics on the quality of whole-plant corn silage. Whole-plant corn was used as the experimental material, and the treatments consisted of a control group (without additives), a cellulase (1 g/kg) group, a compound probiotics (2 mL/kg) group, and a combined group of compound probiotics (2 mL/kg) and cellulase (1 g/kg) (enzyme-probiotic combination group), with 3 replicates per group. After 45 days of ensiling, the nutritional components, fermentation quality, and microbial colony counts of whole-plant corn silage were measured to evaluate the effects of cellulase and compound probiotics on silage quality. The results showed that: 1) The crude protein content in whole-plant corn silage of both the cellulase group and the enzyme-probiotic combination group was significantly higher than that of the control group ($P<0.05$); compared with the control group, the enzyme-probiotic combination treatment significantly reduced the contents of neutral detergent fiber (NDF), acid detergent fiber (ADF), and cellulose in whole-plant corn silage ($P<0.05$). 2) Compared with the control group, the sensory quality of whole-plant corn silage in each treatment group was improved, and the soluble carbohydrate content and pH were significantly reduced ($P<0.05$); the enzyme-probiotic combination treatment significantly reduced the ammonia nitrogen/total nitrogen ratio in whole-plant corn silage ($P<0.05$). 3) Compared with the control group, the enzyme-probiotic combination treatment significantly reduced the counts of lactic acid bacteria, yeasts, and aerobic bacteria in whole-plant corn silage ($P<0.05$). It was concluded from this experiment that the combined treatment of cellulase and compound probiotics effectively improved the nutritional value and quality of whole-plant corn silage.

Full Text

Effects of Cellulase and Compound Probiotics on Silage Quality of Whole Corn

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Abstract

This study investigated the effects of cellulase and compound probiotics on the silage quality of whole corn. Whole corn was used as the experimental material and divided into four treatment groups: control (no additive), cellulase (1 g/kg), compound probiotics (2 mL/kg), and a combination of compound probiotics (2 mL/kg) and cellulase (1 g/kg) (enzyme-probiotic combination group), with three replicates per group. After 45 days of ensiling, the nutritional composition, fermentation quality, and microbial colony counts of the whole corn silage were determined to analyze the effects of cellulase and compound probiotics. The results showed that: (1) The crude protein content in both the cellulase group and the enzyme-probiotic combination group was significantly higher than that in the control group ($P < 0.05$). Compared with the control group, the enzyme-probiotic combination treatment significantly reduced the contents of neutral detergent fiber (NDF), acid detergent fiber (ADF), and cellulose ($P < 0.05$). (2) Compared with the control group, the sensory quality of whole corn silage in all treatment groups was improved, with significantly reduced soluble carbohydrate content and pH ($P < 0.05$). The enzyme-probiotic combination treatment significantly decreased the ammonia nitrogen/total nitrogen ratio ($P < 0.05$). (3) Compared with the control group, the enzyme-probiotic combination treatment significantly reduced the counts of lactic acid bacteria, yeasts, and aerobic bacteria in whole corn silage ($P < 0.05$). In conclusion, the combined treatment of cellulase and compound probiotics effectively improved the nutritional value and quality of whole corn silage.

Keywords: whole corn silage; cellulase; compound probiotics; silage quality

Introduction

Silage represents an economical and effective method for forage preservation. During ensiling, fermentation by lactic acid bacteria can improve feed palatability and extend storage duration. A critical factor for enhancing silage quality is promoting the rapid establishment of lactic acid bacteria as the dominant microbial population [1]. Numerous studies have reported that adding lactic acid bacteria can regulate microbial fermentation and improve silage quality [2-4]. Yeasts can enhance the palatability and flavor of silage; under anaerobic conditions, yeasts decompose sugars to produce ethanol, acetic acid, propionic acid, and small amounts of lactic acid [5]. Wang et al. [6] found that adding yeast during ensiling increased the crude protein content of silage. Ren [7] reported that adding alcohol yeast during corn straw ensiling increased crude protein content and produced ethanol and other substances, imparting an alcoholic aroma to the silage.

Cellulase can hydrolyze structural carbohydrates and increase fiber degradation, representing an effective approach for improving silage quality and nutritional value. Cai [8] demonstrated that adding He Shi Bi silage enzyme to corn straw silage significantly improved sensory quality, increased crude protein content, and reduced neutral detergent fiber and acid detergent fiber contents. Chilson et al. [9] showed that adding cellulase and lactic acid bacteria to silage improved silage quality and promoted fiber degradation. Our laboratory previously optimized probiotic combinations and ratios and investigated cellulase addition levels, but the synergistic effects of combined cellulase and compound probiotic treatment require further investigation. Therefore, this study utilized cellulase and compound probiotics to treat whole corn, examining their effects on the nutritional value and fermentation quality of whole corn silage to provide a theoretical basis for the development and application of straw silage additives.

1.1 Experimental Materials

The whole corn used in this experiment was sourced from Henan Sansege Dairy Industry Co., Ltd. The compound probiotics, obtained from the Feed Biotechnology Laboratory of Henan Agricultural University, contained *Lactobacillus plantarum* and *Saccharomyces cerevisiae*, with viable counts adjusted to 1.0×10 CFU/mL. Cellulase was purchased from Shandong Zesheng Biotechnology Co., Ltd., with enzyme activity 20,000 U/g.

1.2 Experimental Design

The experiment consisted of four groups: control (no additive), cellulase group (1 g/kg cellulase), compound probiotics group (2 mL/kg compound probiotics, *Lactobacillus plantarum*:*Saccharomyces cerevisiae* = 1:1), and a cellulase and compound probiotics combination group (enzyme-probiotic combination group, 1 g/kg cellulase + 2 mL/kg compound probiotics), with three replicates per group. After harvesting, 500 g of whole corn was packed into each polyethylene vacuum bag, vacuum-sealed, and ensiled at room temperature for 45 days before opening for determination of sensory scores, nutritional value, and fermentation quality.

1.3.1 Sensory Evaluation

Sensory evaluation was conducted using the German Agricultural Association (DLG) scoring method, which assesses color, odor, and structure to classify silage into four grades: poor (0-4 points), fair (5-9 points), good (10-15 points), and excellent (16-20 points) [10].

1.3.2 Nutritional Composition Analysis

Dry matter content was determined by oven drying to constant weight [11]. Crude protein content was measured using the Kjeldahl method [11]. Crude fat content was determined by Soxhlet extraction [11]. Crude ash content was measured by incineration [11]. Neutral detergent fiber, acid detergent fiber, cellulose, hemicellulose, and acid detergent lignin contents were analyzed according to the Van Soest detergent fiber analysis method [12]. Calcium content was determined by EDTA complexometric titration [13], and phosphorus content by molybdovanadate spectrophotometry [13].

1.3.3 Fermentation Quality Analysis

For pH determination, 10 g of sample was placed in a 250 mL Erlenmeyer flask with 90 mL distilled water, shaken at 150 r/min for 10 min, filtered through qualitative filter paper, and the filtrate was measured using a pHS-3C pH meter. Ammonia nitrogen content was determined by the phenol-hypochlorite colorimetric method [14]. Soluble carbohydrate content was measured by the anthrone-sulfuric acid colorimetric method [15].

1.3.4 Microbial Colony Count Determination

Ten grams of sample was placed in a 250 mL sterilized Erlenmeyer flask with 90 mL of 0.9% sterile saline solution, mixed thoroughly, and serially diluted from 10^{1} to 10 . Lactic acid bacteria were cultured on MRS medium, yeasts on malt extract agar, and aerobic bacteria on LB medium. Lactic acid bacteria and aerobic bacteria were incubated at 37 °C for 48 h, while yeasts were incubated at 30 °C for 48 h. Colony counts were determined by the plate count method and expressed as log CFU/g of feed [16].

1.4 Statistical Analysis

Experimental data were analyzed using SPSS 20.0 software for one-way ANOVA, with Duncan' s multiple comparison test used for inter-group comparisons. Differences were considered significant at $P < 0.05$. Results are expressed as means ± standard deviation.

2.1 Nutritional Composition Analysis of Whole Corn Before Ensiling

Samples were taken before ensiling to determine the nutritional composition of the raw material, as shown in Table 1 . The whole corn contained relatively high crude protein at 8.35% (dry matter basis).

Table 1 Nutrition composition of whole corn (DM basis)

2.2 Effects of Different Additive Treatments on Nutritional Composition of Whole Corn Silage

As shown in Table 2 , the crude protein content in both the cellulase group and enzyme-probiotic combination group was significantly higher than in the control group ($P < 0.05$), with the enzyme-probiotic combination group achieving the highest crude protein content at 9.95%. The enzyme-probiotic combination group exhibited significantly lower neutral detergent fiber and acid detergent fiber contents compared with the control, cellulase, and compound probiotics groups ($P < 0.05$). The cellulose content in the enzyme-probiotic combination group was significantly lower than in the control group ($P < 0.05$) but did not differ significantly from the cellulase group ($P > 0.05$). The acid detergent lignin content in the enzyme-probiotic combination group was significantly lower than in the cellulase group ($P < 0.05$). Both the enzyme-probiotic combination group and cellulase group showed significantly higher crude ash content compared with the other two groups ($P < 0.05$). No significant differences were observed among groups in crude fat, hemicellulose, calcium, or phosphorus contents ($P > 0.05$).

Table 2 Nutrition composition of whole corn silage in each group (DM basis)

Items	Control group	Cellulase group	Compound probiotics group	Enzyme-probiotic combination group
Crude protein	9.05 ± 0.24	9.89 ± 0.16	9.46 ± 0.22	9.95 ± 0.44
Crude fat	6.19 ± 1.28	6.45 ± 0.77	6.58 ± 1.56	6.21 ± 1.52
Neutral detergent fiber	70.81 ± 1.58	68.75 ± 1.13	70.29 ± 1.77	66.16 ± 0.63
Acid detergent fiber	39.61 ± 1.51	38.59 ± 1.00	39.18 ± 0.53	35.12 ± 1.46
Cellulose	27.59 ± 1.27	25.61 ± 1.51	28.78 ± 1.56	24.56 ± 0.54

Items	Control group	Cellulase group	Compound probiotics group	Enzyme-probiotic combination group
Hemicellulose	31.20 ± 1.63	30.16 ± 2.11	31.11 ± 1.31	31.04 ± 1.36
Acid detergent lignin	8.79 ± 0.72	9.72 ± 0.94	9.16 ± 1.26	7.36 ± 1.24
Crude ash	7.97 ± 0.20	8.93 ± 0.13	8.25 ± 0.12	8.86 ± 0.35
Calcium	0.39 ± 0.03	0.45 ± 0.02	0.41 ± 0.04	0.41 ± 0.04
Phosphorus	0.25 ± 0.01	0.26 ± 0.01	0.28 ± 0.02	0.27 ± 0.02

In the same row, values with different small letter superscripts indicate significant difference ($P < 0.05$), while values with no letter or the same letter superscripts indicate no significant difference ($P > 0.05$). The same applies below.

2.3 Effects of Different Additive Treatments on Fermentation Quality of Whole Corn Silage

As shown in Table 3 , the sensory scores of whole corn silage in all treatment groups were higher than that of the control group, with the enzyme-probiotic combination group achieving the highest score of 19 points. All treatment groups scored between 16-19 points, indicating excellent quality with strong aroma. Compared with the control group, pH values in all treatment groups were significantly reduced ($P < 0.05$), with the compound probiotics group showing the lowest pH. The ammonia nitrogen/total nitrogen ratio in the enzyme-probiotic combination group was significantly lower than in the control and cellulase groups ($P < 0.05$). Soluble carbohydrate content in all treatment groups was significantly lower than in the control group ($P < 0.05$).

Table 3 Fermentation quality of whole corn silage in each group

Items	Control group	Cellulase group	Compound probiotics group	Enzyme-probiotic combination group
Sensory score	16	17	18	19
pH	3.61 ± 0.02	3.45 ± 0.02	3.40 ± 0.03	3.43 ± 0.02

Items	Control group	Cellulase group	Compound probiotics group	Enzyme-probiotic combination group
NH -N/TN (%)	4.03 ± 0.20	4.05 ± 0.35	3.86 ± 0.19	3.54 ± 0.11
Soluble carbohydrates (% DM)	4.10 ± 0.22	3.57 ± 0.23	3.37 ± 0.31	3.31 ± 0.30

2.4 Effects of Different Additive Treatments on Microbial Colonies in Whole Corn Silage

As shown in Table 4 , the counts of lactic acid bacteria in the control and compound probiotics groups were significantly higher than those in the cellulase and enzyme-probiotic combination groups ($P < 0.05$). The yeast count in the enzyme-probiotic combination group was significantly lower than in all other groups ($P < 0.05$). The aerobic bacteria count in the enzyme-probiotic combination group was significantly lower than in the compound probiotics group ($P < 0.05$), with no significant differences from other groups ($P > 0.05$).

Table 4 Number of microbial colonies of whole corn silage in each group (log CFU/g)

Items	Control group	Cellulase group	Compound probiotics group	Enzyme-probiotic combination group
Lactic acid bacteria	7.84 ± 0.08	7.49 ± 0.05	7.90 ± 0.11	7.57 ± 0.06
Yeasts	6.96 ± 0.04	6.94 ± 0.14	7.12 ± 0.25	6.60 ± 0.16
Aerobic bacteria	7.58 ± 0.03	7.56 ± 0.12	7.66 ± 0.17	7.41 ± 0.11

3.1 Nutritional Composition of Whole Corn

The nutritional value of silage depends primarily on the nutritional composition of the raw material and the quality of fermentation. Developed animal husbandry countries in Europe and America predominantly use whole-plant silage for straw utilization. In this experiment, the selected whole corn contained relatively high crude protein and neutral detergent fiber, which may be related to the corn variety. Xu et al. [17] demonstrated significant variations in crude protein, neutral detergent fiber, and acid detergent fiber contents among different corn varieties. The variety selected for this study was Denghai 518, a widely promoted whole-plant silage corn variety in the Nanyang region of Henan Province.

3.2 Effects of Different Additive Treatments on Nutritional Composition of Whole Corn Silage

Cellulase degrades structural polysaccharides in plant cell walls into monosaccharides, providing sufficient substrates for microbial fermentation and improving silage quality [18-19]. In this study, the significantly higher crude protein content in the cellulase and enzyme-probiotic combination groups may be attributed to enhanced microbial growth resulting from fiber degradation. Yeasts contain high levels of protein and are widely used in feed fermentation; adding yeast to silage can increase crude protein content and improve aroma [20-21]. Zhang et al. [22] found that combined *Lactobacillus* and *Saccharomyces cerevisiae* inoculants increased crude protein content in silage. Zhang et al. [23] reported that cellulase and lactic acid bacteria preparations increased crude protein content in corn silage.

The enzyme-probiotic combination group exhibited significantly lower acid detergent fiber and neutral detergent fiber contents compared with all other groups, with cellulose content significantly lower than the control and compound probiotics groups, and acid detergent lignin content significantly lower than the cellulase group. These results demonstrate that the enzyme-probiotic combination treatment was most effective for degrading fiber components in whole corn. The neutral detergent fiber and acid detergent fiber contents in the cellulase and compound probiotics groups did not differ significantly from the control group, indicating that single additions of either enzyme or probiotics alone could not promote fiber degradation in whole corn. Ni et al. [24] reported that combined cellulase and lactic acid bacteria significantly reduced neutral detergent fiber and acid detergent fiber contents in silage. Tan et al. [25] similarly demonstrated that compound enzyme and lactic acid bacteria preparations significantly reduced neutral detergent fiber and acid detergent fiber contents in corn straw silage. Wang [26] found that lactic acid bacteria plus enzyme preparation significantly increased crude protein content and dramatically decreased neutral detergent fiber and acid detergent fiber contents in rice straw silage, with the combined treatment showing superior improvement compared to single treatments. In this study, the cellulase group showed significantly increased crude ash content, the specific reasons for which require further investigation. Overall results indicate that the enzyme-probiotic combination group significantly improved silage nutritional value compared with single treatments.

The degradation of straw fiber components by enzyme preparations has been demonstrated in numerous studies [27-30]. In this experiment, the cellulase group showed significantly lower cellulose content compared with the compound probiotics group, with neutral detergent fiber and acid detergent fiber contents showing a decreasing trend, though not significantly. This suggests that cellulase treatment was more effective than compound probiotics for degrading fiber components in whole corn, likely because the lactic acid bacteria and yeasts in the compound probiotics cannot secrete cellulase and thus have no direct effect on fiber degradation.

3.3 Effects of Different Additive Treatments on Fermentation Quality of Whole Corn Silage

Both cellulase and compound probiotics treatments improved silage sensory scores, with the enzyme-probiotic combination treatment showing particularly pronounced effects, producing silage with strong acidic aroma and soft texture. These results align with Zhang et al. [22] regarding the quality improvement effects of combined *Lactobacillus* and *Saccharomyces cerevisiae* inoculants, and are consistent with the conclusion that enzyme-probiotic combination treatment most effectively degrades fiber components.

Both cellulase and compound probiotics additions significantly reduced silage pH, with compound probiotics showing a more pronounced effect. Lactic acid bacteria are crucial for successful ensiling, requiring populations exceeding 10 CFU/g of material for rapid proliferation [7]. Yeasts can improve silage palatability and nutritional value [31-32]; therefore, adding compound probiotics (*Lactobacillus plantarum* and *Saccharomyces cerevisiae*) increases lactic acid bacteria populations, rapidly reduces pH, inhibits harmful microorganisms, reduces nutrient loss, and improves silage quality. The significant pH reduction observed in this study demonstrates the effectiveness of these additives.

Ammonia nitrogen in silage is primarily produced through protein degradation by plant enzymes and microorganisms, with the ammonia nitrogen/total nitrogen ratio reflecting the extent of protein degradation [23]. The significantly lower ammonia nitrogen/total nitrogen ratio in the enzyme-probiotic combination group indicates superior fermentation quality, likely due to rapid pH decline inhibiting aerobic microorganisms and plant enzyme activity, thereby reducing crude protein decomposition.

The combined addition of cellulase and compound probiotics showed superior effects on silage quality. Chen et al. [33] found that combined enzyme and lactic acid bacteria preparations improved silage fermentation quality more than single additives. Nadeau et al. [34] reported that simultaneous addition of compound enzyme and lactic acid bacteria preparations increased lactic acid bacteria populations during early fermentation while providing additional substrates for lactic acid fermentation, producing synergistic effects. Xing et al. [35] demonstrated that combined lactic acid bacteria and cellulase significantly reduced pH and ammonia nitrogen/total nitrogen in corn silage. In this study, soluble carbohydrate content was significantly lower in all treatment groups compared with the control. Whole corn naturally contains abundant soluble carbohydrates that provide nutrients for lactic acid bacteria colonization; therefore, probiotic growth may have utilized these carbohydrates, though more complex interactions between microorganisms and enzymes may also be involved, requiring further investigation.

3.4 Effects of Different Additive Treatments on Microbial Colonies in Whole Corn Silage

In this study, the enzyme-probiotic combination group showed significantly lower lactic acid bacteria and yeast counts compared with the control, likely related to the lower pH in this group. Xing et al. [28] and Cai et al. [36] demonstrated that low pH inhibits microbial growth. Although the compound probiotics group had low pH, its lactic acid bacteria count was significantly higher than in the cellulase and enzyme-probiotic combination groups, possibly because the added *Lactobacillus* became the dominant silage microorganism and proliferated extensively.

Lactic acid bacteria are essential for successful ensiling, rapidly producing large amounts of lactic acid during early fermentation to reduce pH below 4.2 and inhibit harmful microorganisms. Yeasts improve silage palatability and flavor, fermenting sugars to produce ethanol, acetic acid, propionic acid, and small amounts of lactic acid under anaerobic conditions, while oxidizing sugars to water and carbon dioxide under aerobic conditions [37]. Silage contains numerous microorganisms, with lactic acid bacteria being the most abundant and functionally important, while yeasts and aerobic bacteria are present in smaller numbers during storage but become active and cause spoilage upon exposure to air [38]. In this study, aerobic bacteria counts after 45 days of ensiling were higher than those reported by Liu et al. [19] and Xing et al. [28], possibly due to the polyethylene bag ensiling method. Miao et al. [39] used the same method with different bacterial inoculants and found significantly reduced aerobic bacteria counts after 60 days compared with initial counts. Xing et al. [35] reported yeast counts exceeding 10 CFU/g in lactic acid bacteria-inoculated whole corn silage. Since microbial populations in whole corn before fermentation were not determined in this study, the reasons for these relatively high microbial counts require further investigation. The significantly reduced yeast and aerobic bacteria counts in the enzyme-probiotic combination group indicate relatively better silage quality after fermentation.

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4 Conclusion

The combined treatment of cellulase and compound probiotics significantly increased crude protein content, reduced neutral detergent fiber, acid detergent fiber, and cellulose contents, decreased pH and ammonia nitrogen/total nitrogen ratio, and reduced lactic acid bacteria, yeast, and aerobic bacteria counts in whole corn silage, effectively improving the nutritional value and quality of whole corn silage.

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