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Synergistic effects of enzyme and microbial treatments on corn stover silage quality

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Introduction: This study aimed to investigate the effects of different enzyme preparations and microbial inoculants on the fermentation quality, sensory characteristics, and nutritional composition of corn stover after ensiling, with the objective of identifying the optimal combination for enzyme-microbe co-fermentation of corn stover.

Methods: The experiment was designed using 500 g of corn stover as the fermentation substrate, adjusting the moisture content to 65%, and adding 15 mL of molasses as a supplementary carbon source. A total of 14 treatment groups were established, including nine enzyme preparation treatments (involving different combinations of cellulase, xylanase, lignin peroxidase, manganese peroxidase, and laccase) and five microbial inoculant treatments (comprising varying ratios of lactic acid bacteria, yeast, and *Bacillus subtilis*). The silage samples were subjected to sensory evaluation, and their fermentation quality indicators and nutritional composition were measured. A comprehensive assessment was conducted using the membership function method on all measured indicators.

Results: The results showed that among the enzyme preparation treatments, Group 9 exhibited the best sensory score, the lowest pH value, the most effective lignin degradation, and the highest membership function score. Among the microbial inoculant treatments, the 3:2:1 group (lactic acid bacteria: yeast: *Bacillus subtilis*) demonstrated the best performance in terms of odour, color, highest lactic acid content, and lowest ammonia nitrogen content, achieving a comprehensive score of 0.703. Statistical analysis indicated that appropriate enzyme-microbe combinations significantly reduced the neutral detergent fiber (NDF), acid detergent fiber (ADF), and lignin content of corn stover, increased lactic acid accumulation and sensory quality, and reduced protein degradation. The optimal treatment combinations identified in this experiment were enzyme preparation Group 9 and microbial inoculant group 3:2:1.

Discussion: These combinations not only enhance the nutritional value and digestibility of corn stover silage to meet animal nutritional requirements and reduce feed costs but also promote the resource utilization of agricultural waste and alleviate environmental pressure. This study provides a feasible theoretical basis for advancing the efficient development of the silage industry and agricultural sustainability.

KEYWORDS

bacterial preparation, corn stover, enzyme preparation, fermentation quality, yellow silage

1 Introduction

Corn stover, as the most abundant agricultural by-product in China's agricultural production, has a resource utilization rate of less than 50%. Due to technical limitations in processing, a large amount of stover is either burned or discarded indiscriminately, leading not only to severe atmospheric pollution and resource wastage but also exacerbating pressure on the agro-ecological environment (Fang et al., 2026). Concurrently, China's livestock industry is undergoing large-scale development, where the supply-demand contradiction for high-quality roughage is becoming increasingly acute. As a potential source of high-quality roughage, corn stover is difficult to utilize directly and efficiently due to its compact fibrous structure, high lignin content, and poor palatability. This results in the dual dilemma of "waste surplus" and "feed shortage (Li et al., 2019)."To address this challenge, enzymatic treatment and microbial fermentation technologies have been extensively studied and applied for their respective advantages in degrading lignocellulose and improving feed quality. Among these, microbial fermentation can significantly enhance the nutritional value and utilization efficiency of fermented products through the action of specific microbial consortia.

For instance, Liang et al. found that adding yeast increased the concentration of short-chain fatty acids in the fermentation system by 16.1% and induced favorable changes in the microbial community structure (Liang et al., 2024). Li et al. demonstrated that adding lactic acid bacteria reduced pH, increased lactic acid and the abundance of beneficial bacteria, and improved microbial community structure and fermentation quality, although NH3-N content rose significantly (Li et al., 2026). Liu et al. observed that adding Lactobacillus, cellulase, and xylanase significantly increased the abundance of beneficial bacteria in fermented corn, suppressed the proliferation of harmful bacteria, and elevated lactic acid and water-soluble carbohydrate (WSC) content (Liu et al., 2025). Su et al. further showed that fermentation using enzyme-complex probiotics (including *Bacillus subtilis* and Lactobacillus) increased WSC content and the abundance of Lactobacillus, while reducing ammonia nitrogen and pH, ultimately optimizing bacterial community structure and enhancing the nutritional and fermentation quality of corn stover (Su et al., 2024). However, existing research has predominantly focused on hydrolytic enzymes that degrade cellulose and hemicellulose, with insufficient attention given to oxidative enzymes that break down lignin, such as lignin peroxidase, manganese peroxidase, and laccase. Additionally, studies on microbial agents have largely been confined to the addition of single strains, leaving a gap in systematic research on multifunctional composite microbial fermentation and multi-enzyme complex formulations.

This study, on the one hand, addresses the research gap in "optimizing the synergistic ratio of multi-enzyme and multi-strain complexes" in the enzymatic-microbial co-fermentation technology for corn stover in China, offering a novel solution to the low efficiency of single treatment technologies. On the other hand, by elucidating the mechanisms through which optimal enzyme-microbe combinations enhance fiber degradation and nutrient retention in stover, it aims to increase the digestibility and absorption rate of corn stover to over 50%, providing replicable technical parameters for converting agricultural waste into high-quality roughage. From a practical perspective, the findings can offer guidance on low-cost, high-efficiency stover silage technology for large-scale farming operations. This not only mitigates agricultural non-point source pollution but also alleviates the shortage of high-quality feed, holding significant practical implications and application value for promoting the synergistic development of the "agricultural waste resource utilization–green development of livestock industry" chain.

2 Materials and methods

2.1 Raw materials, enzyme-bacterial inoculants, and laboratory supplies

2.1.1 Prepare experimental materials

Corn stover samples (variety: Beinong Silage 208) were harvested in November 2022 from Chaoyangchuan Town, Yanji City, Jilin Province (approximate geographic coordinates 42°58′N, 129°25′E). The region is characterized by a mid-temperate semi-humid continental monsoon climate, with cold, dry winters, a short frost-free period, and precipitation concentrated primarily in the summer. The samples were collected from local corn fields during the plant's senescence period. After preliminary laboratory processing, the stover was chopped to a length of 2–2.5 cm for subsequent use. Molasses samples were sourced from a feed material market in Yitong Manchu Autonomous County, Siping City, Jilin Province.

For the enzyme preparation treatments on corn stover, nine treatment groups were established, each with three replicates. For the microbial agent treatments on corn stover, five treatment groups were established, each with three replicates.

2.1.2 Enzyme preparations

The enzymes used in this experiment are listed in Table 1.

2.1.3 Culture preparation

The three bacterial strains were selected, isolated, and identified by our laboratory. Refer to Table 2, liquid culture media were prepared, and each type of strain was cultured separately in pure culture. The concentrations of the fermentation broths were measured using bacterial counting chamber method. The results showed that the concentration of *Lactobacilli* was 6×10^8 CFU/mL, the concentration of yeast was 3×10^8 CFU/mL, and the concentration of *Bacillus subtilis* was 2×10^8 CFU/mL. All microbial strains were obtained from the China General Microbiological Culture Collection Center (CGMCC).

2.2 Preparation of corn stover silage

2.2.1 Enzyme treatment of corn stover

The experiment on enzyme treatment of corn stover included nine treatment groups, with three replicates each. Each group used 500 g of corn stover as the fermentation substrate. The content was adjusted to 65%, and 15 mL of molasses was added as a supplementary carbon source. According to the enzyme dosage specified in Table 3, the corresponding enzymes were fully activated dissolved in 325 mL of distilled water to prepare a uniform enzyme solution. This solution was then evenly sprayed onto the surface of the corn stover. The treated stover was transferred into a one-way container. Degassing fermentation bags (40 × 50 cm; material: food-grade seven-layer co-extruded nylon film PA/PE, thickness 0.18 mm). The bags were then vacuum-sealed using a vacuum packaging machine. The mixture was thoroughly homogenized, packed into silage bags, and fermented at 28–32°C for 14 days. It was stored under controlled conditions.

2.2.2 Microbial inoculant treatment of corn stover

The experiment on microbial inoculant treatment of corn stover included five treatment groups with three replicates each. For all groups,500 g of corn stover as the fermentation substrate. The moisture content was adjusted to 65%, and 15 mL of molasses was added as a supplementary carbon source. The overall inoculation amount was 1×10^8 CFU/g, ensuring consistent microbial inoculation across different treatments. According to the inoculant amounts specified in Table 4, fermentation liquids were prepared and mixed thoroughly. The liquid was then evenly sprayed onto the corn stover. The treated stover was transferred into one-way degassing fermentation bags (40 × 50 cm; material: food-grade seven-layer co-extruded nylon film PA/PE, thickness 0.18 mm). The bags were finally vacuum-sealed using a vacuum packaging machine.

TABLE 1 Sources of enzyme preparations and reaction sites in this experiment.

Name in this study	Enzyme TyPe	Strain number	Supplier	Enzyme activity	Catalytic reaction
C	Cellulase	C805042	Macklin	1×10^4 U/g	Hydrolysis of glycosidic bonds in cellulose
X	Xylanase	X832362	Macklin	1×10^5 U/g	Hydrolysis of glycosidic bonds in the xylan backbone
LiP	Lignin Peroxidase	L824022	Macklin	100U/g	Oxidation of phenolic and non-phenolic aromatic compounds
MnP	Manganese Peroxidase	M923136	Macklin	10 U/g	Oxidation of phenolic compounds or aromatic amines
Lac	Laccase	L871938	Macklin	500U/g	Oxidation of diphenol structural analogs

All enzyme preparations were stored in a laboratory refrigerator at 4°C.

TABLE 2 Composition of bacterial preparations and their culture media.

Name	Strain number	Source	Medium composition/L
Yeast	CICC 138631	The strains were stored in the laboratory's -80°C freezer.	Potato starch: 5 g; Glucose: 20 g; Chloramphenicol: 0.1 g; Distilled water: 1.0 L
Bacillus subtilis	CICC 10089	The strains were stored in the laboratory's -80°C freezer.	Peptone: 10.0 g; Beef extract: 10.0 g; NaCl: 5.0 g; Distilled water: 1.0 L
Lactobacilli	CICC137997	The strains were stored in the laboratory's -80°C freezer.	Peptone: 10 g; Beef extract: 10 g; *Yeast* extract: 5 g; Dipotassium hydrogen phosphate: 2 g; Diammonium citrate: 2 g; Sodium acetate: 5 g; Glucose: 20 g; Tween 80: 1.0 mL; Magnesium sulfate: 0.5 g; Manganese sulfate: 0.25 g; Distilled water: 1.0 L

The above medium must be sterilized by autoclaving at 121°C for 20 minutes before use.

TABLE 3 Additions of different enzyme preparations.

Test group	Cellulase	Xylanase	Lignin peroxidase	Manganese peroxidase	Laccase
1	–	–	–	–	–
2	0.50g	0.50g	–	–	–
3	0.33g	0.33g	0.33g	–	–
4	0.33g	0.33g	–	0.33g	–
5	0.33g	0.33g	–	–	0.33g
6	0.25g	0.25g	0.25g	0.25g	–
7	0.25g	0.25g	0.25g	–	0.25g
8	0.25g	0.25g	–	0.25g	0.25g
9	0.20g	0.20g	0.20g	0.20g	0.20g

- Not added.

2.2.3 Opening of corn stover silage

The enzyme-treated and inoculant-treated corn stover silage bags were opened on day 14. Immediately after opening, quantitative sampling was performed. The samples were divided into two parts for subsequent analysis. The first sample portion (200 g) was dried in a 65°C oven to constant weight, then ground and passed through a 40-mesh sieve. These prepared samples were sealed and stored for nutritional composition analysis. The second sample portion (50 g) was used to prepare an extract for analyzing fermentation-related indicators.

2.3 Parameters and methods

2.3.1 Sensory evaluation

The quality of the yellow stover silage was evaluated using the silage sensory assessment standard established by the German Agricultural Society (DLG). The evaluation was based on three main aspects: odor, structure, and color. To reduce the influence of subjective factors, each sensory evaluation was performed independently by 4–5 assessors (Yosefe et al., 2023). The specific evaluation criteria are shown in Table 5.

2.3.2 Determination of fermentation quality

Analysis of corn stover silage fermentation quality was conducted using extracts. The specific procedure was as follows: Exactly 20 g of the sample was weighed, and 180 mL of distilled water was added. The mixture was homogenized for 30 seconds using a blender at medium-high speed. The homogenate was stored at 4°C for 24 hours to facilitate extraction. It was then filtered through four layers of medical gauze, and the residue was squeezed thoroughly to collect the extract. The extract was further filtered through quantitative filter paper into a conical flask. A 10 mL portion of the filtrate was aliquoted into 15 mL centrifuge tubes and stored at -20°C for later use. This extract was used to measure pH, lactic acid (LA), ammonia nitrogen (NH_3-N), and volatile fatty acids (VFA), which included acetic acid (AA), propionic acid (PA), butyric acid (BA), and valeric acid (VA) (Du et al., 2025). The pH value was measured directly with a pH meter, and this value was recorded as the sample's pH. The contents of lactic acid and VFA were determined using a Shimadzu LC-2010A high-performance liquid chromatograph and a GC-2014 gas chromatograph, respectively (Aramrueang et al., 2022). The ammonia nitrogen content was then analyzed by the phenol-sodium hypochlorite colorimetric method (Broderick and Kang, 1980).

TABLE 4 Addition of different bacterial formulations.

Treatments	Lactobacillus concentration	*Yeast* inoculation rate	*Bacillus subtilis* inoculation rate	Distilled water
Control group (0:0:0)	–	–	–	325 ml
1:1:0	41.7 ml	83.3 ml	–	200 ml
1:0:1	41.7 ml	–	125 ml	158.3 ml
1:1:1	28.3 ml	56.7 ml	85 ml	155 ml
3:2:1	41.7 ml	56.7 ml	41.5 ml	185.1 ml

- Not added

TABLE 5 Sensory assessment criteria.

Parameters	Scoring criteria			Score
Smell	It is free from a butyric odor and shows either a fruity fragrance or a distinct bread-like scent.			14
	A slight butyric odor is present, along with a strong sour taste and a weak aromatic note.			10
	A strong butyric odor is present. Alternatively, there may be a pungent burnt or musty smell.			4
	There is a strong butyric or ammonia odor. Alternatively, there is almost no sour taste.			2
Texture	The veins are clearly visible.			4
	Some veins are clear.			2
	The leaf veins are faint.			1
	It is clumped together.			0
Color	It looks like the raw straw material and is yellow-brown.			2
	It is slightly discolored and brown.			1
	The color has changed greatly and has become dark brown or black.			0
Total Score	16-20	10-15	5-9	0-4
Grade	Grade 1 (Excellent)	Grade 2 (Fair)	Grade 3 (Average)	Grade 4 (Poor)

2.3.3 Determination of nutritional quality in corn stover

A 200 g sample of corn silage was placed in a 65°C oven and dried to a constant weight. It was then ground and passed through a 40-mesh sieve before being sealed and stored for later use. All nutritional indicators were determined in accordance with national standards or widely recognized industry methods. The dry matter (DM) content was measured according to GOST 31640-2012. The sample was dried in a 105°C oven, cooled in a desiccator for 30 minutes, and then weighed. Crude protein (CP) and total nitrogen (TN) content were determined using the Kjeldahl method (Chen et al., 2025). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) content were analyzed by the Van Soest method. Crude ash (Ash) content was measured by high-temperature ashing. Water-soluble carbohydrates (WSC) were quantified using the anthrone-sulfuric acid colorimetric method, and acid detergent lignin (ADL) content was determined according to the GB/T 20805–2006 method. Based on these results, hemicellulose content was calculated as NDF minus ADF, and cellulose content was calculated as ADF minus ADL.

2.4 Comprehensive evaluation using membership functions

To systematically evaluate the combined effects of different enzyme and microbial agents on the fermentation quality and nutritional value of corn stover silage, this study used membership function analysis to provide an overall assessment of key indicators and to identify the best treatment. Specifically, the average membership function values were calculated separately based on fermentation characteristics and nutritional quality indicators, and the treatments were ranked accordingly. Among these indicators, dry matter (DM), crude protein (CP), neutral detergent fiber (NDF), water-soluble carbohydrates (WSC), pH, lactic acid (LA), and acetic acid (AA) were considered positive indicators, meaning higher values suggest better fermentation quality or nutritional value. In contrast, acid detergent fiber (ADF), crude ash (Ash), propionic acid (PA), and ammonia nitrogen (NH_3–N) were treated as negative indicators, with lower values reflecting better overall quality. A higher value obtained from the membership function analysis indicates a better overall nutritional value of the silage under that treatment combination.

2.5 Equations

Formula for Positive Correlation $Y(+) = (X_{avg} - X_{min})/(X_{max} - X_{min})$Formula for Negative Correlation: $Y(-) = 1 - Y(+)$

Where: X is the measured value of an indicator for a sample.

Y(+) is the membership function value for a positive correlation indicator.

Y(-) is the membership function value for a negative correlation indicator.

Xavg is the measured value of an indicator for a sample.

Xmax is the maximum measured value of a certain indicator among all samples.

Xmin is the minimum measured value of a certain indicator among all samples.

2.5.1 Data processing and statistical analysis

In this study, Microsoft Excel 2016 software was employed for the preliminary organization and calculation of data. Relevant graphs were generated using Origin Pro 2018.

All quantitative data are expressed as “Mean ± Standard Deviation (Mean ± SD)”. Prior to comparing differences among treatment groups, the data were subjected to normality testing (Shapiro–Wilk test) and homogeneity of variance testing (Levene test) to verify the underlying assumptions for parametric statistical methods.

For data meeting the assumptions of normality and homogeneity of variance, one-way analysis of variance (One-way ANOVA) was used to test for significant differences. Where ANOVA indicated significant differences, Tukey's HSD (Honestly Significant Difference) test was subsequently applied for multiple comparisons between groups. All statistical analyses were performed using SPSS software, with a threshold of $P < 0.05$ considered indicative of statistical significance.

3 Results

3.1 Evaluation of sensory quality in corn stover silage treated with different enzyme and microbial agents

Sensory evaluation results showed that all nine treatment groups achieved an overall score of Grade 2 or higher, indicating that all treatments resulted in good fermentation. Among all treatments, the highest total score was 14.33 points (Group 9), and the lowest was 11.33 points (Group 5). In terms of odor, Group 9 received the highest score, but showed no significant difference compared to Group 1. In terms of texture, Group 9 performed significantly better than all other treatment groups ($P < 0.01$). Group 9 also received the highest score in color, but showed no significant difference compared to Groups 8, 7, and 4. Based on the overall sensory evaluation ranking of the nine treatments, the order was as follows: Group 9 > Group 1 = Group 8 > Group 2 > Group 6 = Group 7 > Group 3 > Group 4 > Group 5 (Table 6).

Based on the sensory evaluation results, all treatment groups except the control group achieved a sensory grade of 2 or higher, indicating generally good fermentation quality of corn stover silage under the four treatment conditions. Among them, the 3:2:1 group and the 1:1:1 group reached Grade 1, demonstrating excellent fermentation outcomes. The total sensory scores of all treatment groups ranged from 9.00 to 16.33, with the highest score observed in the 3:2:1 group and the lowest in the control group. Further analysis showed that the 3:2:1 group scored significantly higher in both odor and color than the other treatment groups ($P < 0.01$), while no significant differences in texture were found among the groups ($P > 0.05$). Overall, the fermentation quality of the five treatment groups, ranked from best to worst, was as follows: 3:2:1 group > 1:1:1 group > 1:0:1 group > 1:1:0 group > control group (Table 7).

3.2 Effects of different enzyme and microbial agents on the fermentation quality of corn stover silage

The pH values across all treatment groups ranged from 4.05 to 4.56. Only Group 9 had a pH below 4.2 (pH = 4.05) (Figure 1), which was significantly lower than all other groups ($P < 0.01$). In terms of organic acid composition, there were no significant differences in lactic acid content among the treatments ($P > 0.05$) (Figure 2). For acetic acid content, Group 2 had the highest level, which was significantly greater than the other treatments ($P < 0.05$). Groups 3 and 6 showed the lowest acetic acid content, but the difference was not significant when compared to Groups 1, 4, 5, 7, 8, and 9 ($P > 0.05$). Propionic acid was detected in all treatments. Group 4 contained the highest propionic acid level, significantly exceeding all other groups ($P < 0.01$). Groups 2 and 5 had the lowest propionic acid content, and this did not differ significantly from Groups 1, 6, 8, and 9 ($P > 0.05$). Butyric acid was only detected in Group 9 and not found in any other groups. Ammonia nitrogen (NH_3-N) content is a key indicator for evaluating fermentation quality, and higher values are negatively correlated with the production of high-quality silage. In this study, Groups 1 and 3 had significantly higher NH_3-N content than the other treatments ($P < 0.01$). Groups 7 and 8 showed the lowest NH_3-N levels, and

TABLE 6 Effect of different enzyme preparations on sensory evaluation of fermentation quality of corn straws.

Test group	Smell	Texture	Color	Total score	Grade	Comprehensive ranking
1	8.40^{AB}	3.50^{B}	1.40^{DF}	13.30^{B}	Grade 2	2
2	8.20^{BC}	3.53^{B}	1.47^{CDF}	13.20^{B}	Grade 2	3
3	7.80^{CD}	3.57^{B}	1.30^{F}	12.67^{BC}	Grade 2	5
4	7.30^{E}	3.20^{C}	1.63^{ABC}	12.13^{C}	Grade 2	6
5	6.23^{F}	3.60^{B}	1.50^{CD}	11.33^{D}	Grade 2	7
6	7.67^{DE}	3.50^{B}	1.60^{BC}	12.77^{BC}	Grade 2	4
7	7.70^{DE}	3.40^{BC}	1.67^{AB}	12.77^{BC}	Grade 2	4
8	7.97^{C}	3.50^{B}	1.73^{AB}	13.20^{B}	Grade 2	3
9	8.70^{A}	3.83^{A}	1.80^{A}	14.33^{A}	Grade 2	1
SEM	0.14	0.04	0.03	0.16	–	–
P-value	<0.01	<0.01	<0.01	<0.01	–	–

[1] Differences between shoulder-marked letters (A, B, C, D, E, and F) in the same column indicate significant differences ($P < 0.05$); [2] Standard error of the SEM mean.

TABLE 7 Effect of different ratios of *Lactobacillus*, *Saccharomyces* and *Bacillus subtilis* on the sensory evaluation of the fermentation quality of corn stover.

Treatments	Smell	Texture	Color	Total score	Grade	Comprehensive ranking
Control group (0:0:0)	6.43^{E}	1.23	1.33^{C}	9.00^{C}	Grade 3	5
1:1:0	9.7^{D}	2.67	1.67^{AB}	14.03^{B}	Grade 2	4
1:0:1	10.33^{C}	2.77	1.57^{BC}	14.67^{B}	Grade 2	3
1:1:1	11.33^{B}	2.07	1.77^{A}	16.00^{A}	Grade 1	2
3:2:1	12.33^{A}	2.60	1.70^{A}	16.63^{A}	Grade 1	1
SEM	0.36	0.22	0.03	0.50	–	–
P-value	<0.01	NS	<0.01	<0.01	–	–

[1]Differences between shoulder-marked letters (A, B, C, D, E, and F) in the same column indicate significant differences ($P < 0.05$); [2]Standard error of the SEM mean.

these did not differ significantly from Groups 5 and 6 ($P > 0.05$). For the AN/TN (ammonia nitrogen/total nitrogen) ratio, Group 9 had the lowest value, but no significant differences were observed between any of the groups ($P > 0.05$) (Table 8).

After treatment with composite bacterial agents at different ratios, the pH of the fermentation system in all treatment groups decreased significantly ($P < 0.01$) and was significantly lower than that of the control group (Figure 3). Among them, the 3:2:1 ratio group had the lowest pH value of 4.12, but the difference was not significant compared to the 1:1:0 group and the 1:1:1 group. In terms of lactic acid accumulation, the 3:2:1 group produced significantly more lactic acid than all other groups ($P < 0.01$) (Figure 4). There was no significant difference in lactic acid content among the 1:1:0, 1:0:1, and 1:1:1 groups, but all were significantly higher than the control group ($P < 0.01$). Significant differences were also observed in acetic acid content among the treatment groups, with the 3:2:1 group showing the highest level, significantly exceeding the other groups ($P < 0.05$). The groups ranked in descending order of acetic acid content were: 3:2:1 group > 1:0:1 group > 1:1:0 group > 1:1:1 group > control group. Small amounts of propionic acid and butyric acid were detected in all treatment groups. The propionic acid content in the 3:2:1, 1:0:1, and 1:1:1 groups was significantly higher than in the 1:1:0 and control groups ($P < 0.01$). The butyric acid content in the 3:2:1 and 1:1:1 groups was significantly higher than in the other groups ($P < 0.01$). For ammonia nitrogen (AN) content, the 3:2:1 group had the lowest value, but showed no significant difference compared to the 1:0:1, 1:1:0, and 1:1:1 groups ($P > 0.05$). Additionally, the 3:2:1 group had the lowest ammonia nitrogen/total nitrogen (AN/TN) ratio among all treatments, but no significant differences in AN/TN values were observed among the groups ($P > 0.05$) (Table 9).

3.3 Effects of different enzyme and microbial agents on the nutritional composition of corn stover silage

The results showed significant differences ($P < 0.01$) in key nutritional indicators among the treatment groups, including dry

TABLE 8 Changes in the chemical composition of corn stover after enzymatic digestion by different treatments.

Test group	pH	LA (g/kg)	AA (g/kg)	PA (g/kg)	BA (g/kg)	NH_3-N (g/kg)	AN/TN (g/kg)
1	4.53^{AB}	30.00	3.20^{B}	1.27^{C}	0^{B}	3.33^{A}	4.79
2	4.37^{B}	29.57	4.20^{A}	1.23^{C}	0^{B}	3.16^{B}	4.68
3	4.40^{B}	30.93	2.80^{B}	1.37^{AB}	0^{B}	3.29^{A}	4.83
4	4.33^{BC}	30.63	3.03^{B}	1.53^{A}	0^{B}	3.163^{B}	4.62
5	4.56^{A}	30.00	3.07^{B}	1.23^{C}	0^{B}	3.09^{BC}	4.54
6	4.33^{BC}	29.43	2.90^{B}	1.27^{C}	0^{B}	3.07^{BC}	4.48
7	4.25^{C}	31.27	3.07^{B}	1.50^{AB}	0^{B}	3.01^{C}	4.23
8	4.31^{BC}	29.90	3.13^{B}	1.33^{C}	0^{B}	3.01^{C}	4.39
9	4.05^{D}	30.73	3.13^{B}	1.37^{ABC}	0.22^{A}	3.12^{B}	4.42
SEM	0.03	0.21	0.09	0.03	0.01	0.02	0.05
P-value	<0.01	NS	<0.01	<0.01	<0.01	<0.01	NS

[1]LA, lactic acid; AA, acetic acid; PA, propionic acid; BA, butyric acid; NH_3-N, ammoniacal nitrogen; AN/TN, Ammonia Nitrogen/Total Nitrogen; WSC, water-soluble carbohydrates; [2]Differences between shoulder-scripted letters in the same column (A, B, C, D, and E) denote significant differences ($p < 0.05$); [3]Standard error of the mean for SEM; and [4]NS denotes non-significant differences ($p > 0.05$).

TABLE 9 Effect of different ratios of *Lactobacillus, Saccharomyces* and *Bacillus subtilis* on the fermentation quality of corn stover.

Treatments	pH	LA (g/kg)	AA (g/kg)	PA (g/kg)	BA (g/kg)	NH_3-N (g/kg)	AN/TN (g/kg)
Control group(0:0:0)	5.25^{A}	30.74^{C}	4.29^{E}	1.65^{C}	0.22^{BC}	3.97^{A}	5.60
1:1:0	4.21^{BC}	37.30^{B}	4.88^{C}	2.42^{B}	0.21^{C}	3.58^{AB}	5.00
1:0:1	4.25^{B}	37.33^{B}	5.07^{B}	2.65^{A}	0.25^{B}	3.59^{AB}	5.32
1:1:1	4.21^{BC}	37.37^{B}	4.70^{D}	2.65^{A}	0.38^{A}	3.59^{AB}	5.00
3:2:1	4.12^{C}	39.87^{A}	6.07^{A}	2.64^{A}	0.41^{A}	3.13^{B}	4.24
SEM	0.22	0.84	0.19	0.11	0.02	0.03	0.14
P-value	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

[1]LA, lactic acid; AA, acetic acid; PA, propionic acid; BA, butyric acid; NH_3-N, ammoniacal nitrogen; AN/TN, Ammonia Nitrogen/Tota Nitrogen; WSC, water-soluble carbohydrates; [2]Differences between shoulder-scripted letters in the same column (A, B, C, D, and E) denote significant differences ($p < 0.05$); [3]Standard error of the mean for SEM; and [4]NS denotes non-significant differences ($p > 0.05$).

matter (DM), crude protein (CP), cellulose (ADF), hemicellulose (NDF), and lignin. Group 8 had the highest DM content, but the difference was not significant compared to Groups 1, 2, 5, and 9. The contents of CP, ADF, and Ash also showed significant differences among the treatment groups. In terms of fiber components, Group 9 had the lowest NDF content among all treatments, but showed no significant difference compared to Groups 1, 4, 5, 6, 7, and 8. Further analysis showed that Group 9 had relatively low cellulose and hemicellulose content, but showed no significant difference compared to Groups 6, 7, and 8. Lignin is a major anti-nutritional factor that limits the feeding value of straw, and a decrease in its content helps improve the overall nutritional quality. Group 9 had the lowest lignin content, but the difference was not significant compared to Groups 5, 6, 7, and 8. There were no significant differences in WSC content among all treatment groups (P > 0.05) (Table 10).

Analysis of the effects of different microbial agent treatments on the nutritional composition of corn stover showed significant differences in multiple nutritional indicators among the treatment groups. Compared to the control group, the dry matter (DM) content of the 3:2:1, 1:1:1, 1:0:1, and 1:1:0 groups was significantly reduced (P < 0.01), with no significant differences among these four groups. Among them, the 1:0:1 group had the lowest DM content. CP content can reflect fermentation quality. Among the different treatments, the 3:2:1 group had the highest CP content, showing no significant difference compared to the 1:1:1, 1:1:0, and control groups, while the CP content of the 1:0:1 group was significantly lower than all other groups (P < 0.05). In terms of fiber components, no significant differences were observed in acid detergent fiber (ADF) content among the treatment groups (P > 0.05), but there were significant differences in neutral detergent fiber (NDF) content among the groups (P < 0.05). Among them, the 3:2:1 group had the lowest NDF content, showing no significant difference compared to the 1:1:1 and 1:0:1 groups, but was significantly lower than the control group (P < 0.05). In terms of structural carbohydrates, no significant changes were observed in hemicellulose content among all treatment groups (P > 0.05). The 1:0:1 group had the lowest cellulose content, showing no significant difference compared to the

TABLE 10 Changes in nutrient composition of corn stover treated with different enzyme preparations (%).

Test group	DM	CP	NDF	ADF	Ash	WSC	Cellulose	Hemicellulose	Lignin
1	63.70^{A}	4.34	82.76^{AB}	37.46	7.11	1.39	37.00^{A}	28.99^{A}	7.86^{A}
2	63.63^{A}	4.23	83.44^{A}	38.56	7.19	1.30	34.69^{B}	26.80^{B}	7.78^{B}
3	61.83^{B}	4.27	84.23^{A}	37.36	7.17	1.38	34.19^{D}	25.01^{C}	7.38^{C}
4	62.63^{AB}	4.22	79.82^{AB}	36.67	7.34	1.42	36.00^{C}	24.85^{C}	7.78^{C}
5	63.43^{A}	4.29	80.66^{AB}	37.82	7.40	1.38	33.56^{D}	24.01^{D}	7.65^{DF}
6	61.67^{B}	4.28	77.62^{B}	35.55	7.17	1.38	32.89 ^{DF}	23.18^{E}	7.76^{F}
7	62.93^{AB}	4.45	79.9^{AB}	36.54	7.19	1.38	32.46^{F}	26.20^{F}	7.52^{F}
8	63.97^{A}	4.30	80.40^{AB}	34.80	7.32	1.39	32.46^{F}	26.18^{F}	7.28^{F}
9	63.3^{A}	4.42	77.51^{B}	35.52	7.03	1.43	31.89^{F}	25.18^{F}	7.17^{E}
SEM	0.19	0.04	0.63	0.32	0.04	0.01	0.25	0.40	0.30
P-value	<0.01	NS	0.48	NS	NS	NS	<0.01	<0.01	<0.01

[1]DM, dry matter; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; Ash, crude ash; WSC, water-soluble carbohydrates; Cellulose; Hemicellulose; Lignin.
[2]Values in the same column with different letters (A, B, C, D, E, F) indicate significant differences ($p < 0.05$). [3]SEM, standard error of the mean. [4]NS indicates no significant difference ($p > 0.05$).

3:2:1 and 1:0:1 groups, but differing significantly from the 1:1:1 group and control group ($P < 0.05$). The 3:2:1 group had the lowest lignin content, with no significant difference compared to the 1:1:1 and 1:1:0 groups, but differing significantly from the 1:0:1 group and control group ($P < 0.05$). No significant differences were found in ash content among all treatment groups. Additionally, the water-soluble carbohydrate (WSC) content was highest in the 3:2:1 and 1:1:0 groups, and significantly higher than in all other groups ($P < 0.05$) (Table 11).

3.4 Comprehensive evaluation of different enzyme and microbial agents on corn stover silage effectiveness

Based on the results of the membership function analysis, the comprehensive evaluation of fermentation quality of corn stover silage treated with different enzyme and microbial agents showed that Group 9 had the highest comprehensive score (membership function value 0.705), indicating the best fermentation effect. The comprehensive evaluation scores of the remaining treatment groups, from high to low, were as follows: Group 8, Group 7, Group 1, Group 3, Group 2, Group 4, Group 5, and Group 6. Among the 15 indicators, the ones that contributed the most were acid detergent fiber (ADF), Ash, hemicellulose, butyric acid, WSC, and pH value (Table 12).

Based on the membership function analysis, the comprehensive quality evaluation of the straw silage fermentation system under different microbial agent ratios showed significant differences. The 3:2:1 treatment group had the highest comprehensive evaluation score (0.703), indicating the best fermentation quality. The comprehensive scores of the remaining treatment groups, from high to low, were as follows: 1:1:1 group, 1:1:0 group, control group, and 1:0:1 group. Among the 15 indicators evaluated, crude protein (CP) content contributed the most to the comprehensive evaluation and was the key factor affecting the differences in fermentation quality among the treatment groups (Table 13).

4 Discussion

4.1 Effects of different enzyme and microbial agents on the sensory quality of corn stover

The sensory properties of silage (such as color, odor, and texture) are important criteria for evaluating its fermentation quality and can directly reflect the effectiveness of the microbial fermentation process. From a research perspective, existing studies have predominantly focused on the improvement of silage quality using single enzyme preparations (such as cellulase) or single microbial strains. For instance, Song et al. demonstrated that cellulase effectively reduces the fiber content of silage; however, there is limited systematic reporting on its comprehensive enhancement of sensory characteristics (Payne et al. 2015; Song et al., 2025). In contrast, this study innovatively and systematically compares the effects of various complex enzyme preparations, with particular emphasis on the quantitative evaluation of sensory quality—a dimension directly linked to animal feeding behavior. This approach provides more direct evidence for the development of yellow silage additives tailored to practical production needs. The results of this study show that treatments with different enzyme and microbial agents significantly affected the sensory properties of corn stover silage. The experimental results indicate that the odor scores of Group 9 and Group 1 were significantly higher than those of the other groups. This shows that these enzyme agents can enhance the aroma of corn stover. The enzyme agents also helped break down pigments in the stover and improved its color. The texture and color scores of Group 9 were also significantly higher than those of the other groups, indicating that the enzyme formulation used in this group performed well in improving the taste and appearance of corn stover. By breaking down structural polysaccharides such as cellulose, hemicellulose, and lignin, the enzyme preparations not only improved the physical structure of the straw, increasing its softness and palatability, but also promoted pigment decomposition and the formation of volatile aromatic compounds, thereby enhancing the overall sensory quality

TABLE 11 Changes in nutrient composition of corn stover treated with different microbial preparations (%).

Treatments	DM	CP	NDF	ADF	Ash	WSC	Cellulose	Hemicellulose	Lignin
Control group (0:0:0)	62.50^{A}	4.43^{AB}	84.11^{A}	40.82	7.22	0.77^{B}	34.49^{A}	28.66	7.57^{A}
1:1:0	60.10^{B}	4.48^{A}	83.95^{A}	41.92	7.52	0.80^{AB}	33.33^{AB}	27.65	7.33^{AB}
1:0:1	58.56^{B}	4.22^{B}	79.60^{AB}	39.50	7.65	0.79^{B}	31.32^{B}	28.17	7.49^{A}
1:1:1	60.08^{B}	4.47^{A}	80.54^{AB}	38.72	7.35	0.78^{B}	35.10^{A}	26.67	7.29^{AB}
3:2:1	58.85^{B}	4.62^{A}	76.71^{B}	38.35	7.20	0.83^{A}	31.45^{B}	26.81	6.96^{B}
SEM	0.44	0.04	0.92	0.59	0.06	0.01	0.48	0.30	0.08
P-value	<0.01	0.04	0.02	NS	NS	0.03	<0.01	NS	0.04

[1] DM, dry matter; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; Ash, crude ash; WSC, water-soluble carbohydrates; Cellulose; Hemicellulose; Lignin.

[2] Values in the same column with different letters (A, B, C, D, E, F) indicate significant differences ($p < 0.05$). [3] SEM, standard error of the mean. [4] NS indicates no significant difference ($p > 0.05$).

TABLE 12 Comprehensive evaluation of the effect of different enzyme preparations on the yellow storage of corn stover.

Group Item	1	2	3	4	5	6	7	8	9
DM	1.00	0.97	0.08	0.48	0.87	0.00	0.62	1.13	0.80
CP	0.51	0.07	0.21	0.00	0.30	0.29	1.00	0.37	0.86
NDF	0.78	0.88	1.00	0.34	0.47	0.02	0.36	0.43	0.00
ADF	0.36	0.00	0.39	0.62	0.24	0.99	0.66	1.24	1.00
Ash	0.79	0.58	0.63	0.16	0.00	0.63	0.58	0.21	1.00
Cellulose	1.00	0.55	0.45	0.80	0.33	0.20	0.11	0.11	0.00
Hemicellulose	0.00	0.38	0.69	0.71	0.86	1.00	0.48	0.48	0.66
Lignin	0.00	0.12	0.70	0.12	0.30	0.14	0.49	0.84	1.00
LA	0.38	0.09	1.00	0.80	0.38	0.00	1.22	0.31	0.87
AA	0.29	1.00	0.00	0.17	0.19	0.07	0.19	0.24	0.24
PA	0.87	1.00	0.50	0.12	1.00	0.87	0.00	0.63	0.50
BA	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
WSC	0.69	0.00	0.62	0.92	0.62	0.62	0.62	0.69	1.00
pH	0.06	0.37	0.31	0.45	0.00	0.45	0.61	0.49	1.00
NH_3-N	0.00	0.53	0.13	0.52	0.75	0.81	1.00	1.00	0.66
Comprehensive Score	0.449	0.435	0.447	0.414	0.420	0.406	0.530	0.545	0.705
Sorting	4	6	5	7	8	9	3	2	1

TABLE 13 Comprehensive evaluation of the effect of different microbial preparations on yellow storage of corn stover.

Group Item	Control group(0:0:0)	1:1:0	1:0:1	1:1:1	3:2:1
DM	1.00	0.39	0.00	0.39	0.07
CP	0.80	1.00	0.00	0.97	1.51
NDF	1.00	0.98	0.39	0.52	0.00
ADF	0.31	0.00	0.68	0.90	1.00
Ash	0.94	0.28	0.00	0.66	1.00
Cellulose	0.84	0.53	0.00	1.00	0.03
Hemicellulose	0.00	0.51	0.25	1.00	0.93
Lignin	0.00	0.38	0.13	0.45	1.00
LA	0.00	0.72	0.72	0.73	1.00
AA	0.00	0.33	0.43	0.23	1.00
PA	1.00	0.23	0.00	0.00	0.01
BA	0.05	0.00	0.20	0.84	1.00
WSC	0.00	0.50	0.33	0.17	1.00
PH	0.00	1.00	0.00	1.00	0.00
NH_3-N	0.00	0.46	0.45	0.45	1.00
Comprehensive Score	0.396	0.488	0.239	0.620	0.703
Sorting	4	3	5	2	1

(Song et al., 2025). The superior sensory characteristics observed in the enzyme preparation of Group 9 are likely attributable to the synergistic interactions among its composite enzyme components, particularly the lignin-degrading enzymes. As noted by Reddy et al., the physical encapsulation of cellulose and hemicellulose by lignin constitutes a key barrier limiting the degradation and conversion of straw (Reddy et al., 2025). Meanwhile, hemicellulase breaks down hemicellulose into low-molecular-weight polysaccharides, further improving the flexibility and plasticity of the straw. Ligninase in the enzyme preparations breaks down lignin in the straw into small molecular compounds, reducing its encapsulating effect on other components and further improving the air permeability and heat transfer of the straw (Chauhan, 2020). We hypothesize that the enzyme preparation in Group 9 likely contains lignin-degrading enzymes such as laccase and manganese peroxidase, which, together with cellulase and hemicellulase, form an efficient synergistic degradation system. Cai et al. indicated that ligninases initially attack and depolymerize the rigid lignin network, thereby exposing the internal cellulose and hemicellulose, which allows subsequent carbohydrate hydrolases to act more fully and efficiently (Cai et al., 2023). This "synergistic wall-breaking" effect not only significantly improves the physical texture of straw and promotes the release and degradation of pigments (enhancing color), but also, as noted by Wang et al., provides substrates such as phenolic precursors from lignin degradation and soluble sugars from fiber degradation for reactions like the Maillard reaction, potentially generating more pleasant volatile aromatic compounds (improving odor) (Wang et al., 2025). This explains why Group 9 achieved the highest scores in all three sensory dimensions: color, texture, and odor. Overall, Group 9 had a significantly higher total score than the other groups. This indicates that the enzyme formulation in this group significantly improved the sensory evaluation of corn stover fermentation quality. The overall sensory ranking of the treatment groups was: Group 9 > Group 1 > Group 2 > Group 6 = Group 7 > Group 3 > Group 4 > Group 5. This study shows that compared to other enzyme formulations, the enzyme preparation in Group 9 can improve the fermentation quality of corn stover and enhance its sensory evaluation in terms of taste, appearance, and aroma.

In the sensory evaluation of corn stover fermentation quality, different microbial agent ratios significantly affected the results. First, we observed that the 3:2:1 group scored significantly higher in odor than the other groups ($P < 0.01$). This shows that this microbial formulation best improved the odor of corn stover silage. For texture evaluation, no significant differences were found among the treatment groups ($P > 0.05$). This indicates that the different microbial agent ratios had similar effects on improving straw texture. In color, both the 3:2:1 and 1:1:1 groups scored significantly higher than the other groups ($P < 0.01$). This demonstrates that these two ratios were clearly better at enhancing the color of the fermented product. For overall scores, the 3:2:1 and 1:1:1 groups had significantly higher comprehensive scores than the other treatments ($P < 0.01$). This further confirms that these two ratios were effective in improving the overall fermentation quality of corn stover. According to the overall sensory ranking, the 3:2:1 group had the highest score and was clearly better than the other groups. A study (Wang Yuxia et al., China, on β-glucosidase from Trichosporon asahii) showed that *yeast* primarily improves the color of corn stover by secreting enzymes such as β-glucosidase to break down pigments. In contrast, lactic *Lactobacilli* mainly produce lactic acid through fermentation, which lowers the stover's pH, inhibits the growth of spoilage bacteria, and also generates organic acids and esters that improve its odor. Meanwhile, *Bacillus subtilis* can secrete various enzymes and has a strong ability to degrade cellulose, thereby improving the stover's texture (Su et al., 2024). By optimizing the ratios of *yeast*, *Lactobacilli*, and *Bacillus subtilis* microbial agents, we can effectively improve the color, odor, and texture of corn stover (Guoqiang et al., 2023; Brandão et al., 2025). In conclusion, among the four microbial agent ratios studied, the 3:2:1 ratio performed best in enhancing the sensory evaluation of corn stover fermentation quality and shows good application potential.

4.2 Effects of different enzyme and microbial agents on the fermentation quality of corn stover silage

During the ensiling of corn stover, adding different enzyme and microbial agents significantly affects the composition and quality of the fermentation products. Key indicators for evaluating fermentation quality include pH, lactic acid, ammonia nitrogen, volatile fatty acids (such as acetic acid, propionic acid, and butyric acid), and water-soluble carbohydrate content. These parameters interact in complex ways and collectively determine the final quality of the silage (Obinwanne C et al., 2022). Among them, pH is one of the most important indicators for measuring fermentation success. A suitable pH environment supports the growth and metabolism of *Lactobacilli*. If the pH is too low or too high, it can inhibit beneficial microbial activity, reduce lactic acid accumulation, and lower fermentation quality (Luo et al., 2021). It is generally accepted that high-quality silage should have a pH below 4.2. If the pH is too high, it often indicates that spoilage microbial activity has not been effectively controlled, and the fermentation process may be abnormal. Also, the total amount and composition of organic acids are key for evaluating silage fermentation. Ideal silage should have lactic acid as the main product, a low amount of acetic acid, and no butyric acid. A higher proportion of lactic acid usually indicates better fermentation. In contrast, if acetic acid is high and lactic acid is low, it reflects poor fermentation quality. Another important indicator is ammonia nitrogen, which is a product of protein breakdown and shows how stable the protein is in silage. A suitable amount of ammonia nitrogen can provide usable nitrogen for ruminant animals. However, if the content is too high, it may reduce the feed's palatability and could even negatively affect animal health (Peng et al., 2025). The ratio of ammonia nitrogen to total nitrogen is often used to show the degree of protein breakdown. A higher ratio means more intense protein decomposition.

Among the different enzyme treatments, Group 9 had a much lower pH than the other groups, showing a significant difference ($P < 0.01$). Using composite enzyme preparations in feed has several benefits. One important role is to lower the feed's pH. This happens because some enzymes in these preparations have acid-base catalytic activity and can help adjust the feed's acid-base balance. They also break down certain substances in the feed, releasing acidic compounds that further reduce pH (Rahikainen et al., 2013). In addition, composite enzymes improve the breakdown and digestion of nutritional components in the feed, which also helps lower pH. For example, hemicellulase and cellulase act on their specific substrates, breaking them down and producing more acidic components, which further promotes pH reduction (Romsaiyud et al., 2009). Ammonia nitrogen (AN) content is another key indicator for evaluating the quality of fermented feed. In this study, the ammonia nitrogen content of Groups 1 and 3 was significantly higher than that of the other treatment groups ($P < 0.01$). Group 9 had the lowest AN/TN (ammonia nitrogen/total nitrogen) ratio among all groups, but statistical results showed no significant differences in AN/TN among the treatment groups ($P > 0.05$). Regarding fermentation products, the butyric acid content of Group 9 was significantly higher than that of the other groups ($P < 0.01$). This suggests that the fermentation process in this group may be more prone to anaerobic metabolism and butyric acid accumulation. Butyric acid is a characteristic product of anaerobic fermentation (Jiang et al., 2018), and its high content may indicate stronger anaerobic fermentation capacity in Group 9. No significant differences were observed in lactic acid content among the treatment groups ($P > 0.05$), indicating that enzyme treatments had limited effects on lactic acid production. In contrast, the acetic acid content of Group 2 was significantly higher than that of the other groups (P < 0.01), suggesting that its fermentation pathway may favor acetic acid production. Acetic acid is a common product in fermentation processes, and its high content may positively influence fermentation quality. Group 4 showed the highest propionic acid content (P < 0.01). The increase in propionic acid content may indicate stronger microbial metabolic activity under these conditions, contributing to improved fermentation efficiency and quality. Overall, different enzyme combinations had significant effects on the fermentation quality of corn stover, with Group 9 showing clearly better fermentation performance than the other groups.

This study investigated the effects of co-inoculation with *Lactobacilli* (LAB), *yeast* (YS), and *Bacillus subtilis* (BS) at different ratios on the fermentation quality of corn stover. The results showed that the pH values of all treatment groups were significantly lower than that of the control group. This indicates that the three microbial species, working together, can effectively reduce the pH of the fermentation system. Regarding ammonia nitrogen and AN/TN content, the 3:2:1 group had the lowest levels, with no significant difference compared to the 1:0:1, 1:1:0, and 1:1:1 groups. This suggests that this ratio helps suppress excessive protein breakdown and reduces ammonia nitrogen accumulation. LAB, as the primary acid-producing microorganisms, lower the system pH by producing lactic acid, thereby creating a suitable environment for the growth of *yeast* and *Bacillus subtilis*. In terms of lactic acid content, the 3:2:1 group was significantly higher than all other groups, indicating that this ratio favors lactic acid accumulation. Increased lactic acid content signifies improved fermentation quality, as lactic acid inhibits the growth of harmful bacteria and enhances the feed's nutritional value. Furthermore, significant differences in acetic acid content were observed among the treatment groups, with the 3:2:1 group having the highest level. The ranking of groups by acetic acid content, from high to low, was: 3:2:1 group > 1:0:1 group > 1:1:0 group > 1:1:1 group > control group. Regarding propionic and butyric acid content, low levels of both were detected in all treatment groups. The propionic acid content in the 3:2:1, 1:0:1, and 1:1:1 groups was significantly higher than in the 1:1:0 and control groups. Meanwhile, the butyric acid content in the 3:2:1 and 1:1:1 groups was also significantly higher than in the other groups. In summary, during fermented feed production, adjusting the ratios of LAB, *yeast*, and *Bacillus subtilis* can effectively improve fermentation quality and enhance feed utilization. Under the experimental conditions here, the LAB: YS : BS = 3:2:1 treatment demonstrated the best fermentation characteristics, including higher lactic and acetic acid content, lower ammonia nitrogen accumulation, and moderate propionic and butyric acid production. This indicates that this ratio has good potential for improving fermentation quality and enhancing feed utilization efficiency.

4.3 Effects of different enzyme and microbial agents on the nutritional quality of corn stover silage

The nutritional quality of silage largely depends on its chemical composition, which directly affects its practical feeding value. Dry matter (DM) content is a fundamental indicator for evaluating roughage quality, and its level directly influences both storage stability and nutritional value. This is because the DM content is directly related to the moisture content of the roughage. Excessively high moisture can make the roughage prone to mold and spoilage, thereby reducing its feeding value. Crude protein (CP) content, which includes both true protein and non-protein nitrogen, is a key measure of the nutritional status of the feed (Zhang et al., 2022). A higher CP content in silage generally reflects better fermentation outcomes. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) are critical parameters for evaluating fiber components and significantly influence the feed's palatability to animals and its digestibility. Specifically, NDF content is negatively correlated with an animal's dry matter intake, while ADF content directly affects the feed's digestibility. Generally, lower NDF content helps increase feed intake, and lower ADF content often indicates less indigestible fiber in the feed and a higher proportion of digestible dry matter, suggesting successful ensiling. Furthermore, lignin, hemicellulose, and cellulose are the main components of the plant cell wall. Their levels directly affect the feed's digestibility and utilization in the animal's body. A reduction in the content of these components usually helps improve the overall nutritional quality of the feed (Li et al., 2020) (Sharma and Arora, 2013).

The results of this experiment on the nutritional composition of corn stover treated with different enzyme preparations showed that the DM content of Groups 1, 2, 5, 8, and 9 was significantly higher than that of the other groups. This result demonstrates that enzyme treatments can effectively increase the dry matter content of corn stover (Zhao et al., 2022). Crude protein (CP) is a key indicator for evaluating the nutritional quality of roughage, and its content directly affects the feeding value of the feed. Generally, higher crude protein content helps meet the nitrogen requirements for animal growth and development, thereby improving the overall nutritional value of the feed (Chornolata et al., 2021) (Peñuela-Sierra et al., 2024). However, in this study, no significant differences were observed in crude protein (CP), acid detergent fiber (ADF), or ash content among the treatment groups, indicating that different enzyme treatments had limited effects on these components. Notably, the NDF content of Group 9 was significantly lower than that of the other groups, which is likely related to the type and concentration of enzymes used in this group. Furthermore, the cellulose, hemicellulose, and lignin contents of Groups 7, 8, and 9 were all significantly reduced, indicating that these enzyme preparations effectively broke down the structural carbohydrates and lignin in corn stover, thereby improving its digestibility and nutritional value. In summary, enzyme treatments had clear regulatory effects on the nutritional composition of corn stover, with the treatments in Groups 7, 8, and 9 showing particularly notable results.

Different ratios of *Lactobacilli*, *yeast*, and *Bacillus subtilis* had significant effects on the nutritional composition of corn stover. Among the various combinations, the dry matter (DM) content of the 3:2:1, 1:1:1, 1:0:1, and 1:1:0 groups was significantly lower than that of the control group, and the differences were highly significant (P < 0.01). For other nutritional components, we found no significant

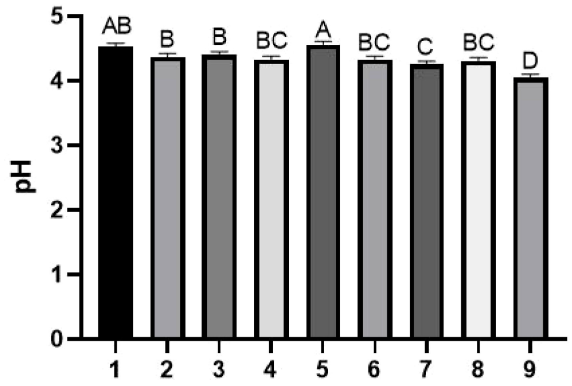

FIGURE 1
Effect of different enzyme preparations on pH.

differences in ADF and hemicellulose content among all treatment groups, and there were also no statistical differences compared to the control group (P > 0.05). However, the crude protein (CP) content of the 1:0:1 group was significantly lower than that of the other treatment groups, suggesting potential issues with protein degradation in this group (P < 0.05). Furthermore, the neutral detergent fiber (NDF), lignin, cellulose, and ash content of the 3:2:1 treatment group were significantly lower than those of the other treatment groups (P < 0.05). These results indicate that this microbial ratio may have a strong degrading effect on these components in corn stover (P < 0.05).

4.4 Comprehensive evaluation of the effects of different enzyme and microbial agents on corn stover silage fermentation

In the comprehensive evaluation of corn stover silage fermentation systems treated with different enzyme and microbial agents, Group 9 achieved the highest comprehensive score of 0.705. This study employs a multi-index comprehensive evaluation method to screen the optimal treatment, aligning with international research trends, such as the approach adopted by Tharangani et al., who

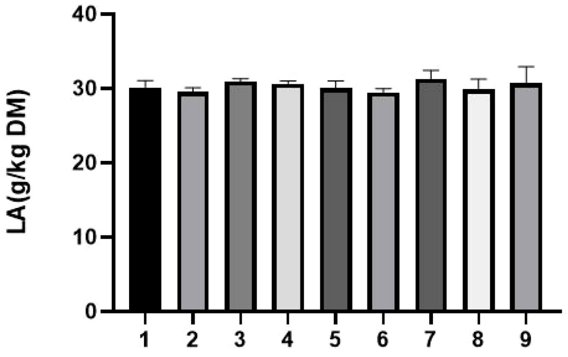

FIGURE 2
Effect of different enzyme preparations on lactic acid production. No significant differences were observed among the groups.

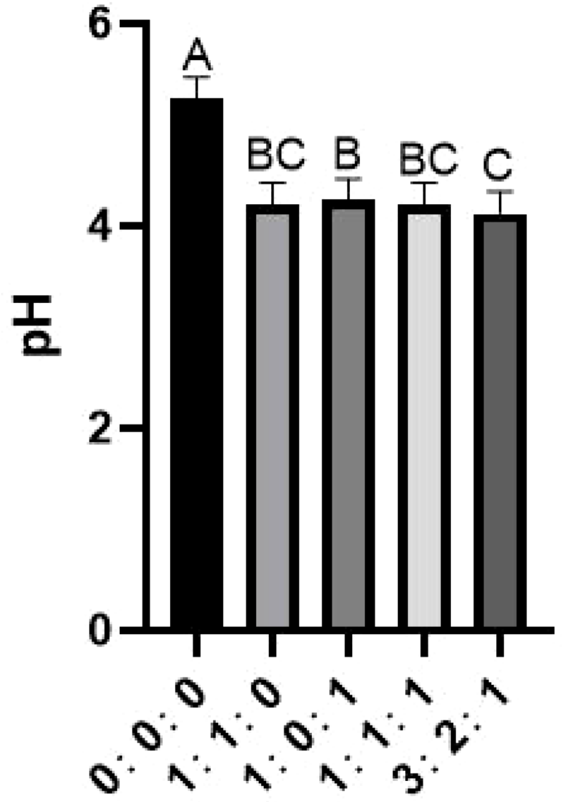

FIGURE 3
Effect of bacterial inoculants on pH dynamics.

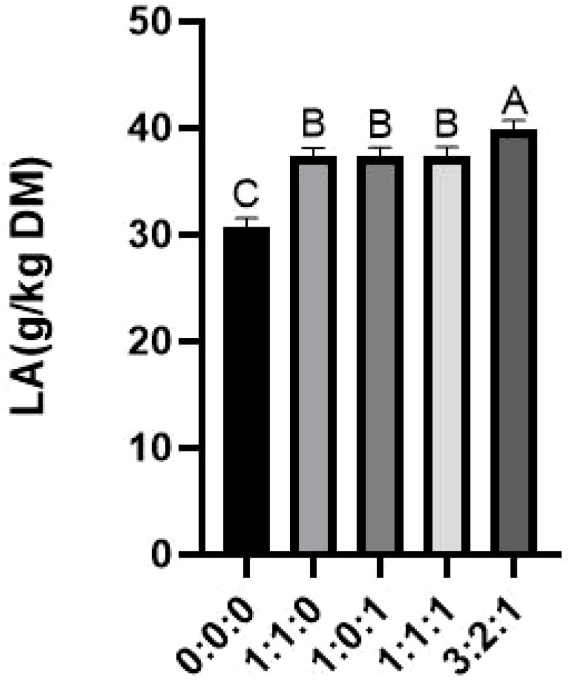

FIGURE 4
Effect of microbial additives on lactic acid production.

evaluated silage quality through key fermentation parameters (Tharangani et al., 2021). However, compared to most studies focusing on single or a few core indicators (e.g., pH, lactic acid), the evaluation system in this study integrates multidimensional data, including fiber composition, organic acid profiles, and dry matter basis, to more comprehensively reflect the impact of additives on the overall fermentation conversion efficiency of straw. Its objective lies in identifying comprehensive treatment schemes that achieve dual optimization of "structural degradation" and "fermentation regulation." This high score was thanks to its excellent performance across multiple indicators. The most important contributing indicators were acid detergent fiber (ADF), ash, hemicellulose, butyric acid, WSC, and pH. The performance of these key indicators collectively points to a core mechanism: the composite enzyme preparation in Group 9 likely drives profound optimization of the fermentation system through efficient synergistic action, particularly via effective depolymerization of lignin. This aligns with the findings of Mi et al., who demonstrated that effective pretreatment of lignin can significantly enhance fiber accessibility and saccharification efficiency in subsequent fermentation processes (Mi et al., 2016). The strong performance in these indicators fully demonstrates the superiority of Group 9 in the stover silage fermentation process. The comprehensive scores of the remaining treatment groups were as follows: Group 8 had the next highest score, ranking second. Group 7 and Group 1 ranked third and fourth, respectively. Groups 3, 2, 4, 5, and 6 had relatively lower scores, ranking fifth to ninth, respectively. These results further confirm the leading position of Group 9 in the stover silage fermentation process.

In the corn stover silage fermentation process, significant differences were observed in the fermentation effectiveness among treatment groups with different microbial agent ratios. The 3:2:1 group showed the best performance, achieving a comprehensive evaluation score of 0.703. Among all indicators, the CP value contributed most significantly to this group's high score. The comprehensive evaluation rankings of the other groups were as follows: 1:1:1 group > 1:1:0 group > control group > 1:0:1 group. These results indicate that the ratio of microbial agents significantly affects stover silage fermentation effectiveness. In the 3:2:1 group, the synergistic effects between the microbial strains were likely optimal, leading to the best performance in indicators such as CP value.

This study focuses on the quality at the 14-day fermentation stage as the core analysis target, aiming to provide direct reference for current production practices. By optimizing the fermentation initiation conditions and evaluating its short-term effects, we found that the Group 9 enzyme preparation combined with the 3:2:1 microbial agent ratio exhibited excellent performance at the laboratory scale, laying an important foundation for subsequent scale-up experiments. Follow-up research should prioritize pilot-scale trials at the ton level to verify the stability of this process with different batches of raw materials, and further optimize key parameters such as inoculation dosage, moisture control, and compaction density, thereby advancing its transition toward practical application.

While studies on aerobic stability, nutrient degradation, and impacts on animal feeding behavior during long-term storage (e.g., over 30 days) hold significant scientific value, these aspects are more appropriately addressed in subsequent stability evaluations. For large-scale farm implementation, a comprehensive assessment is required, including the cost of enzyme-microbial preparations, equipment investment, operational convenience, and the enhancement of feed quality as reflected in improved animal production performance (such as feed intake, daily weight gain, and milk yield). Feeding trials should also be conducted to clarify the economic benefits.

Furthermore, future research could extend the application of this enzyme-microbe synergistic technology to the resource utilization of other agricultural wastes (such as rice straw and wheat straw), and conduct in-depth analysis of microbial community succession and metabolic functions during the fermentation process, to achieve precise regulation of the fermentation system.

5 Conclusion

The results of this chapter show that Enzyme Preparation Group 9 has a clear advantage over other enzyme preparations in improving the fermentation quality of corn stover, with the highest membership function score of 0.705. Through comparative analysis of the four microbial preparation ratios, the 3:2:1 ratio was found to be most effective in improving the sensory evaluation of corn stover fermentation quality, with the highest membership function score of 0.703. This study has identified suitable microbial and enzyme preparations for compound enzyme-microbe fermentation, thus providing an experimental basis for subsequent compound fermentation research (Figures 1–4).

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Ethics statement

All experimental procedures involving animals were approved by the Institutional Animal Care and Use Committee of Yanbian University (approval code: YBU201908027).

Author contributions

MZ: Writing – original draft, Investigation, Writing – review & editing. WM: Formal Analysis, Investigation, Writing – review & editing. MS: Formal Analysis, Investigation, Writing – review & editing. BW: Formal Analysis, Investigation, Writing – review & editing. JW: Formal Analysis, Investigation, Writing – review & editing. XL: Writing – review & editing. CW: Conceptualization, Methodology, Supervision, Writing – review & editing. YW: Conceptualization, Methodology, Supervision, Writing – review & editing.

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Conflict of interest

The author(s) declared that this work was conducted in theabsence of any commercial or financial relationships that could beconstrued as a potential conflict of interest.

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