

## Article

# Synergistic Effect of Microorganisms and Enzymes on Nutritional Value of Corn Stover and Wheat Straw

Binglong Chen, Jiancheng Liu, Mengjian Liu, Huiling Zhang, Xuanyue Li, Congcong Tian  and Yong Chen \*

Research Center for Biofeed and Animal Gut Health, College of Animal Science, Xinjiang Agricultural University, Urumqi 830052, China; cblxjau@163.com (B.C.); liujc@xjau.edu.cn (J.L.); liumengjian@xjau.edu.cn (M.L.); hlz080@163.com (H.Z.); xjaulxy@126.com (X.L.); tiancc2023@163.com (C.T.)

\* Correspondence: cy@xjau.edu.cn

**Abstract:** In this study, *Candida utilis*, *Lactobacillus plantarum*, and non-starch polysaccharide enzymes (cellulase, laccase,  $\beta$ -glucanase, xylanase, and mannanase) were employed to examine the effects of various microorganism–enzyme combinations on the nutritional composition, fiber structure, and fermentation quality of corn stover and wheat straw. Furthermore, the synergistic effects of these treatments were assessed through the use of in vitro rumen fermentation. The results showed that the microorganism–enzyme combinations significantly increased the crude protein content ( $p < 0.05$ ), while reducing the acid detergent fiber and neutral detergent fiber levels ( $p < 0.05$ ) in both substrates. The fermentation broth pH decreased ( $p = 0.06$  for corn stover;  $p < 0.05$  for wheat straw) as a result of the treatments, with a significant increase in the lactate concentration ( $p < 0.05$ ). The reducing sugar levels varied across the treatments ( $p < 0.05$ ). Mycotoxin analysis revealed trace amounts of zearalenone, well below the Chinese feed hygiene standard. Scanning electron microscopy showed structural modifications, including fiber breakage and surface wrinkling, in the treated substrates. In vitro rumen fermentation demonstrated significant changes in the  $\text{NH}_3\text{-N}$  production and volatile fatty acid profiles ( $p < 0.05$ ). In conclusion, the addition of different microorganism–enzyme combinations can effectively improve the nutritional composition, fiber structure, and fermentation quality of corn stover and wheat straw. Among the treatments, the T3 group (25% each of *C. utilis*, *L. plantarum*, cellulase, and laccase, with a total addition ratio of 0.3%  $w/w$ ) exhibited the most pronounced improvement in nutritional value for both corn stover and wheat straw. These findings suggest that microorganism–enzyme combinations effectively enhance the nutritional and fermentative quality of agricultural residues.

**Keywords:** microorganism–enzyme synergy; roughages; nutritional composition; fiber structure; fermentation products; mycotoxin; in vitro rumen fermentation



Academic Editor: Qing Zhang

Received: 21 February 2025

Revised: 16 March 2025

Accepted: 8 April 2025

Published: 10 April 2025

**Citation:** Chen, B.; Liu, J.; Liu, M.; Zhang, H.; Li, X.; Tian, C.; Chen, Y. Synergistic Effect of Microorganisms and Enzymes on Nutritional Value of Corn Stover and Wheat Straw.

*Fermentation* **2025**, *11*, 210. <https://doi.org/10.3390/fermentation11040210>

**Copyright:** © 2025 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

## 1. Introduction

Globally, approximately 3 billion tons of agricultural residues are produced annually, with rice straw, corn stover, and wheat straw being the most abundant, accounting for about 90% of the total [1]. Currently, cereal straw is primarily utilized in fertilizers, fuel, and feed [2]. In the livestock industry, it is commonly used as roughage, providing energy and fiber for animals [3]. However, the thick cell walls in straw, primarily composed of cellulose, hemicellulose, and lignin [4], present significant challenges in regard to animal digestion. The complex spatial structure formed by lignin and hemicellulose, tightly encasing cellulose, is a major factor limiting the utilization of straw by animals [5]. Single physical or chemical treatments encounter issues such as high energy consumption, limited

effectiveness, and environmental hazards [6]. While combined physical and chemical pretreatment methods offer better fiber degradation and nutritional improvement outcomes compared to single approaches [7], they are hindered by high costs, complex processes, and biological inhibition [8]. Microbial treatment has been the subject of extensive research and applications due to its cost effectiveness and environmentally friendliness. However, the inherent limitations of microorganisms, such as low efficiency and the need for long incubation periods, restrict their effectiveness [9]. Although enzymatic treatment exhibits high specificity and effectiveness, its low efficiency and high costs limit its development and application [10]. Therefore, the development of more effective treatment methods to enhance straw utilization efficiency, thereby providing higher quality roughage resources for herbivorous livestock, is of considerable significance.

The use of microorganism–enzyme synergistic treatment is an effective approach for the development and utilization of unconventional roughage resources. This method leverages the dual advantages of multi-product fermentation by probiotics and the high specificity and efficiency of enzyme preparations. On one hand, probiotics can degrade macromolecular nutrients into smaller molecules that are more easily digested and absorbed by animals, while simultaneously producing organic acids that lower the pH of the substrate, thereby inhibiting the growth of undesirable microorganisms. On the other hand, the supplementation of specific enzymes addresses the limitations of microbial enzymatic degradation, such as poor efficiency and long processing times [11]. Cellulase is primarily composed of three types of enzymes: endoglucanase, exoglucanase (or cellobiohydrolase), and  $\beta$ -glucosidase. Endoglucanase and exoglucanase act on the amorphous and crystalline regions of cellulose chains, respectively, while  $\beta$ -glucosidase catalyzes the final step of hydrolysis, converting cellobiose into glucose [12]. Xylanase effectively degrades xylan, the major component of hemicellulose [13]. Mannanase achieves degradation by breaking the  $\beta$ -1,4-glycosidic bonds in mannan, a component of hemicellulose [14]. Laccase catalyzes the cleavage of bonds embedded within lignin, facilitating its degradation [15]. During fermentation, the addition of lactic acid bacteria can convert water-soluble carbohydrates into organic acids, lowering the pH and inhibiting the growth of harmful bacteria [16]. Yeast, which secretes cellulase, enhances the nutritional value of feed and improves rumen fermentation [17]. The supplementation of xylanase and *Lactobacillus plantarum* resulted in a higher glucose conversion rate, better fermentation quality, and improved fiber degradation compared to treatments with xylanase or *L. plantarum* alone. Additionally, this combination significantly lowered the pH and increased the lactate content [18]. Similarly, rice straw fermented with a mixture of *Bacillus subtilis*, *Enterococcus faecalis*, cellulase, and xylanase, showed higher volatile fatty acid (VFA) concentrations and a significant increase in the population of carbohydrate-degrading and hydrogen-utilizing bacteria in the rumen compared to the control group [19]. The nutritional value of corn stover fermented with *L. plantarum* and cellulase was significantly enhanced, and its efficient utilization was achieved by improving rumen fermentation in Hu sheep and regulating the rumen microbiota [20]. Liu et al. [21] found that *L. plantarum* and cellulase similarly improved the fermentation quality of the total mixed rations containing rape straw and significantly enhanced its in vitro digestibility. They suggested the application of microbial enzyme-coordinated fermentation technology in feed production. Although there has been considerable research on the microorganism–enzyme synergistic treatment of roughage, the composition and ratio of microorganisms and enzymes when using corn stover and wheat straw as ruminant feed remain inconclusive. The fermentation effects of different microbial and enzyme combinations and their proportions still require experimental verification.

Therefore, considering the structural characteristics of plant fibers and enzymatic hydrolysis sites, this study selected yeast, lactic acid bacteria, and non-starch polysaccharide

enzymes (cellulase, laccase,  $\beta$ -glucanase, xylanase, and mannanase) to investigate the effects of different microorganism–enzyme combinations on the nutritional composition, fiber structure, fermentation quality, mycotoxin levels, and in vitro rumen fermentation parameters of corn stover and wheat straw.

2. Materials and Methods

2.1. Substrates, Microorganisms, and Enzymes

Corn stover and wheat straw were collected from Sanping experimental farm, Xinjiang Agricultural University (China); the specific characteristics and nutritional value of the crops are presented in Table 1. *Candida utilis* (Henneberg) Lodder et Kreger-van Rij ( $3.0 \times 10^9$  colony-forming units (CFU)/g) was obtained from Cangzhou Huayu Biotechnology Co., Ltd. (Cangzhou, China), and *L. plantarum* ( $3.0 \times 10^9$  CFU/g) was purchased from Yiwukang Biotechnology (Linyi, China). The enzymatic preparations, including cellulase (enzyme activity  $\approx 10,000$  U/g, EC 3.2.1.4), laccase (enzyme activity  $\approx 10,000$  U/L, EC 1.10.3.2),  $\beta$ -glucanase (enzyme activity  $\approx 50,000$  U/g, EC 3.2.1.58), xylanase (enzyme activity  $\approx 30,000$  U/g, EC 3.2.1.8), and mannanase (enzyme activity  $\approx 25,000$  U/g, EC 3.2.1.25), were supplied by Sunson Industry Group (Beijing, China). All the chemical reagents used in this study were of analytical grade.

Table 1. The specific characteristics and nutritional value of the crops.

| Crop Stalks | Variety | Harvesting Date | Maturity Stage         | DM (%) | ADF (%) | NDF (%) | CP (%) | Reducing Sugars (mg/g) |
|-------------|---------|-----------------|------------------------|--------|---------|---------|--------|------------------------|
| Corn stover | XF806   | 2023.09         | Late collection period | 96.02  | 63.92   | 31.44   | 5.40   | 8.04                   |
| Wheat straw | XM1807  | 2023.08         | Late collection period | 96.27  | 61.15   | 34.91   | 5.45   | 4.34                   |

2.2. Treatment with Microorganisms and Enzymes

The experiment comprised of seven groups, with the control group (C) receiving no microbial or enzymatic additives, while the treatment groups (T1–T6) were supplemented with different microorganism–enzyme combinations, as detailed in Table 2. Prior to fermentation, the corn stover and wheat straw were chopped into 2–3 cm lengths. For each fermentation unit, 100 g of chopped sample was mixed with the microorganism–enzyme combinations equivalent to 0.3% (*w/w*, air-dried basis) of its weight, supplemented with 3.0 g of molasses and 1.0 g of urea. Distilled water was sprayed on the sample to adjust the moisture content to approximately 65%. The mixtures were thoroughly mixed, packed into polyethylene bags (30  $\times$  40 cm), and the air was removed using a portable vacuum pump (14891, Deli, Ningbo, China). The bags were then sealed and subjected to fermentation at room temperature ( $25 \pm 2$  °C) for 30 days. Each treatment was replicated three times to ensure statistical reliability.

Table 2. Microorganism–enzyme combinations used in this study.

| Microorganisms and Enzymes | Groups |      |      |      |      |      |      |
|----------------------------|--------|------|------|------|------|------|------|
|                            | C      | T1   | T2   | T3   | T4   | T5   | T6   |
| <i>C. utilis</i> (%)       | -      | 5.0  | 15.0 | 25.0 | 5.0  | 15.0 | 25.0 |
| <i>L. plantarum</i> (%)    | -      | 5.0  | 15.0 | 25.0 | 5.0  | 15.0 | 25.0 |
| Cellulase (%)              | -      | 45.0 | 35.0 | 25.0 | -    | -    | -    |
| Laccase (%)                | -      | 45.0 | 35.0 | 25.0 | 22.5 | 17.5 | 12.5 |

Table 2. Cont.

| Microorganisms and Enzymes | Groups |    |    |    |      |      |      |
|----------------------------|--------|----|----|----|------|------|------|
|                            | C      | T1 | T2 | T3 | T4   | T5   | T6   |
| $\beta$ -glucanase (%)     | -      | -  | -  | -  | 22.5 | 17.5 | 12.5 |
| Xylanase (%)               | -      | -  | -  | -  | 22.5 | 17.5 | 12.5 |
| Mannanase (%)              | -      | -  | -  | -  | 22.5 | 17.5 | 12.5 |

### 2.3. Determination of Nutritional Components and Fermentation Products

After 30 days of fermentation, the bags were opened, and the samples were dried at 65 °C for 48 h to determine the dry matter (DM) content. The samples were then ground through an 80-mesh sieve for subsequent analysis. The crude ash content was determined according to the AOAC [22], and the organic matter (OM) was calculated. The crude protein (CP) was measured using an automatic Kjeldahl analyzer (K1100, Hanon, Jinan, China). The neutral detergent fiber (NDF) and acid detergent fiber (ADF) were analyzed using an automatic fiber analyzer (F2000, Hanon, Jinan, China) and the filter bag method.

The pH value was determined according to the method described in a previous report [23]. Briefly, 30 g of the fermented sample was mixed with 270 mL of distilled water and homogenized in a blender for 1 min. The homogenate was filtered through four layers of medical gauze, and the pH of the filtrate was measured using a pH meter (FE20-FiveEasy, Mettler Toledo, Shanghai, China). The reducing sugar content was quantified using the 3,5-dinitrosalicylic acid (DNS) method, as outlined in a previous report [24]. The filtrate was appropriately diluted, incubated in a water bath at 50 °C for 20 min, and centrifuged at 4000 × g for 15 min. A 0.5 mL aliquot of the supernatant was mixed with 0.5 mL of DNS solution, heated in a boiling water bath for 5 min, and immediately cooled using tap water. After adding 4 mL of distilled water, the absorbance was measured at 540 nm, and the reducing sugar content was calculated using a standard curve.

The ammoniacal nitrogen (NH<sub>3</sub>-N) concentration in the filtrate was determined using the phenol–sodium hypochlorite colorimetric method with a plate reader (Infinite M200, Tecan, Männedorf, Switzerland), as described in reference [25]. Lactate was measured using a lactate analyzer (LM5, Anolox Instruments, Stourbridge, UK). The concentrations of VFA, including acetate and propionate, were analyzed using a gas chromatograph (GC-2010, Shimadzu, Kyoto, Japan) [26].

For the mycotoxin analysis, the concentrations of aflatoxin B<sub>1</sub> (AFB<sub>1</sub>), zearalenone (ZON), and deoxynivalenol (DON) in the fermented and ground straw samples were quantified using a quantitative rapid mycotoxin fluorescence detector (FD-600, Femdetection Bio-Tech, Shanghai, China), according to the manufacturer's instructions.

### 2.4. In Vitro Rumen Fermentation and Determination of Fermentation Parameters

The culture medium was prepared according to the formulation described by Zhou et al. [27], with the addition of 0.7 g/L sodium isoascorbate for oxygen removal [28]. A total of 0.400 g of the fermented sample was weighed, ground, and placed into fiber analysis filter bags (FB2000, Hanon, Jinan, China), which were then sealed and labeled. Each treatment group included six replicates and three blank fiber bags without samples. Four 7-month-old male small-tailed Han sheep, fitted with permanent ruminal fistulas and maintained with a daily feed intake of 1.5 kg, were selected as donors of ruminal microorganisms. Fresh rumen fluid was collected, filtered through eight layers of gauze, and immediately transferred into a pre-warmed thermos flask. The filtrate was mixed with a pre-warmed culture medium at a ratio of 1:2 to prepare the mixed artificial rumen culture. Subsequently, 60 mL of the mixture was dispensed into 100 mL glass syringes, with 1 mL graduations.

The syringes were sealed and incubated horizontally in a constant-temperature shaker at 39 °C for 72 h, with the medium mixed every 2 h.

After fermentation, the pH value and NH<sub>3</sub>-N concentration were immediately measured. The VFAs, including acetate, propionate, butyrate, isobutyrate, isovalerate, and valerate, were quantified, and the total VFA (TVFAs) concentration and acetate/propionate (A/P) ratio were calculated. The fiber analysis filter bags were rinsed with tap water until they were colorless, dried at 65 °C to a constant weight, and used to calculate the DM degradation rate (DMD). For the protozoa counting, 0.5 mL of filtered rumen fluid was mixed with 1 mL of methyl green formaldehyde solution (MFS), gently shaken, and allowed to stain for 10 min. The stained sample was then observed, and the protozoa number was counted under a microscope at 400× magnification, using a counting chamber (1.0 × 1.0 × 0.5 mm) [29].

### 2.5. Fiber Structure Analysis

Dried samples were coated with a conductive layer using a carbon evaporation coater and observed under a scanning electron microscope (Quanta FEG250, FEI, La Vergne, TN, USA) at 500× magnification to capture images of the fiber structure.

### 2.6. Data Analysis

The experimental data were analyzed using IBM SPSS software (v. 23.0). A one-way analysis of variance (one-way ANOVA) was performed, followed by Duncan's multiple range test to determine any significant differences. The model is as follows:

$y_i = \mu + \alpha_j + \varepsilon_i$ , where  $y_i$  is an observation;  $\mu$  is the overall mean;  $\alpha$  is the effect of the additives ( $j = 1, 7$ ); and  $\varepsilon_i$  is the residual error.

The normality of the data was assessed using the Shapiro–Wilk test, the independence of the data was verified using the Durbin–Watson test, and the homogeneity of the variances across the groups was confirmed using Levene's test. Statistical significance was set at  $p < 0.05$ ; the experimental results are presented as mean values and the standard error of the mean (SEM).

## 3. Results

### 3.1. Nutritional Composition

As shown in Table 3, for corn stover, compared to the control group, the OM content in groups T3–T6 was significantly reduced ( $p < 0.05$ ), while the CP content was significantly increased ( $p < 0.05$ ). The NDF content in all six microorganism–enzyme-treated groups was significantly decreased ( $p < 0.05$ ). Except for groups T2 and T6, the ADF content in the other microorganism–enzyme-treated groups was also significantly reduced ( $p < 0.05$ ).

For wheat straw, all six treatment groups demonstrated significantly higher CP content ( $p < 0.05$ ) compared to the control group. Conversely, the NDF and ADF contents were significantly reduced ( $p < 0.05$ ) in all the treatment groups.

**Table 3.** Effects of synergistic microorganism–enzyme treatment on the nutritional composition of corn stover and wheat straw (dry matter basis).

| Roughage    | Items   | C                  | T1                  | T2                  | T3                  | T4                 | T5                 | T6                 | SEM   | <i>p</i> -Value |
|-------------|---------|--------------------|---------------------|---------------------|---------------------|--------------------|--------------------|--------------------|-------|-----------------|
| Corn stover | OM (%)  | 90.26 <sup>a</sup> | 89.92 <sup>ab</sup> | 89.93 <sup>ab</sup> | 89.04 <sup>bc</sup> | 88.22 <sup>c</sup> | 88.79 <sup>c</sup> | 88.69 <sup>c</sup> | 0.183 | <0.001          |
|             | CP (%)  | 7.37 <sup>c</sup>  | 7.75 <sup>bc</sup>  | 7.85 <sup>bc</sup>  | 8.24 <sup>ab</sup>  | 8.09 <sup>ab</sup> | 8.27 <sup>ab</sup> | 8.48 <sup>a</sup>  | 0.087 | <0.001          |
|             | NDF (%) | 64.39 <sup>a</sup> | 55.00 <sup>bc</sup> | 57.44 <sup>b</sup>  | 51.9 <sup>c</sup>   | 53.73 <sup>c</sup> | 54.02 <sup>c</sup> | 52.56 <sup>c</sup> | 0.917 | <0.001          |
|             | ADF (%) | 33.94 <sup>a</sup> | 30.89 <sup>b</sup>  | 32.67 <sup>ab</sup> | 30.76 <sup>b</sup>  | 29.99 <sup>b</sup> | 31.01 <sup>b</sup> | 34.17 <sup>a</sup> | 0.442 | 0.021           |

Table 3. Cont.

| Roughage    | Items   | C                  | T1                  | T2                  | T3                 | T4                 | T5                  | T6                 | SEM   | p-Value |
|-------------|---------|--------------------|---------------------|---------------------|--------------------|--------------------|---------------------|--------------------|-------|---------|
| Wheat straw | OM (%)  | 85.65              | 85.60               | 84.65               | 85.39              | 85.20              | 85.09               | 84.29              | 0.216 | 0.658   |
|             | CP (%)  | 3.61 <sup>b</sup>  | 6.62 <sup>a</sup>   | 6.67 <sup>a</sup>   | 6.74 <sup>a</sup>  | 6.52 <sup>a</sup>  | 6.61 <sup>a</sup>   | 6.51 <sup>a</sup>  | 0.242 | <0.001  |
|             | NDF (%) | 70.00 <sup>a</sup> | 66.75 <sup>bc</sup> | 65.92 <sup>c</sup>  | 66.05 <sup>c</sup> | 67.00 <sup>b</sup> | 66.46 <sup>bc</sup> | 62.16 <sup>d</sup> | 0.483 | <0.001  |
|             | ADF (%) | 41.43 <sup>a</sup> | 37.78 <sup>c</sup>  | 37.14 <sup>cd</sup> | 36.26 <sup>d</sup> | 37.54 <sup>c</sup> | 38.93 <sup>b</sup>  | 32.48 <sup>e</sup> | 0.573 | <0.001  |

Within the same row, values without superscript letters or with the same superscript letters are not significantly different ( $p > 0.05$ ), whereas values with different superscript letters are significantly different ( $p < 0.05$ ). OM, organic matter; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; SEM, standard error of the mean.

### 3.2. Fermentation Products

As shown in Table 4, the pH values of corn stover in all six treatment groups exhibited a decreasing trend ( $p = 0.060$ ) compared to the control group. Significant increases ( $p < 0.05$ ) were observed in regard to the lactate and  $\text{NH}_3\text{-N}$  contents across all the treatment groups. Notably, the reducing sugar content in the T1 group was significantly higher ( $p < 0.05$ ) than that of the other treatment groups.

For wheat straw, the pH values in all six treatment groups were significantly lower ( $p < 0.05$ ) than that of the control group. The reducing sugar content in the T1 group showed a significantly higher value ( $p < 0.05$ ) compared to the other treatment groups. Furthermore, the lactate contents in the T1-T3 groups were significantly elevated ( $p < 0.05$ ) relative to both the T5-T6 groups and the control group.

**Table 4.** Effects of synergistic microorganism–enzyme treatment on fermentation products of corn stover and wheat straw.

| Roughage    | Items                        | C                  | T1                 | T2                  | T3                  | T4                  | T5                 | T6                | SEM   | p-Value |
|-------------|------------------------------|--------------------|--------------------|---------------------|---------------------|---------------------|--------------------|-------------------|-------|---------|
| Corn stover | pH                           | 4.89               | 4.50               | 4.42                | 4.58                | 4.73                | 4.68               | 4.29              | 0.057 | 0.060   |
|             | Reducing sugars (mg/g)       | 17.50 <sup>b</sup> | 27.03 <sup>a</sup> | 13.35 <sup>bc</sup> | 12.71 <sup>bc</sup> | 12.42 <sup>bc</sup> | 9.38 <sup>c</sup>  | 9.50 <sup>c</sup> | 1.408 | <0.001  |
|             | Lactate (mM)                 | 4.14 <sup>b</sup>  | 7.46 <sup>a</sup>  | 6.18 <sup>ab</sup>  | 8.59 <sup>a</sup>   | 7.39 <sup>a</sup>   | 7.18 <sup>a</sup>  | 7.33 <sup>a</sup> | 0.386 | 0.041   |
|             | Acetate (mM)                 | 8.64               | 8.42               | 7.70                | 8.55                | 7.63                | 7.64               | 8.10              | 0.342 | 0.977   |
|             | Propionate (mM)              | ND                 | ND                 | ND                  | ND                  | ND                  | ND                 | ND                | -     | -       |
|             | $\text{NH}_3\text{-N}$ (mM)  | 2.61 <sup>c</sup>  | 7.02 <sup>ab</sup> | 5.22 <sup>b</sup>   | 9.28 <sup>a</sup>   | 5.93 <sup>b</sup>   | 6.36 <sup>b</sup>  | 5.85 <sup>b</sup> | 0.484 | 0.002   |
| Wheat straw | pH                           | 4.86 <sup>a</sup>  | 4.27 <sup>cd</sup> | 4.35 <sup>c</sup>   | 4.36 <sup>c</sup>   | 4.61 <sup>b</sup>   | 4.07 <sup>e</sup>  | 4.22 <sup>d</sup> | 0.056 | <0.001  |
|             | Reducing sugars (mg/g)       | 5.87 <sup>cd</sup> | 11.33 <sup>a</sup> | 8.50 <sup>b</sup>   | 7.75 <sup>bc</sup>  | 5.63 <sup>cd</sup>  | 6.26 <sup>bc</sup> | 5.22 <sup>d</sup> | 0.502 | <0.001  |
|             | Lactate (mM)                 | 5.28 <sup>cd</sup> | 8.23 <sup>ab</sup> | 7.80 <sup>ab</sup>  | 9.32 <sup>a</sup>   | 7.02 <sup>bc</sup>  | 5.25 <sup>cd</sup> | 4.85 <sup>d</sup> | 0.401 | <0.001  |
|             | Acetate (mM)                 | 6.87               | 9.82               | 6.41                | 6.94                | 6.17                | 4.71               | 6.65              | 0.467 | 0.136   |
|             | Propionate ( $\mu\text{M}$ ) | 91.70              | 21.20              | 11.30               | 12.00               | 4.80                | 20.30              | 41.50             | 0.012 | 0.604   |
|             | $\text{NH}_3\text{-N}$ (mM)  | 1.68               | 2.82               | 2.36                | 2.65                | 2.48                | 3.07               | 1.17              | 0.257 | 0.488   |

Within the same row, values without superscript letters or with the same superscript letters are not significantly different ( $p > 0.05$ ), whereas values with different superscript letters are significantly different ( $p < 0.05$ ). ND, not detected; SEM, standard error of the mean.

### 3.3. Mycotoxins

As shown in Table 5, for corn stover, the ZON content in the T6 group was significantly elevated compared to that of the other groups ( $p < 0.05$ ). For wheat straw, the ZON content in the T1, T4, and T6 groups was significantly lower than that of the control group ( $p < 0.05$ ). Furthermore, the levels of  $\text{AFB}_1$ , ZON, and DON in both corn stover and wheat straw were within the safety limits specified by the Chinese feed hygiene standard (GB 13078-2017 [30],  $\text{AFB}_1 \leq 30 \mu\text{g/kg}$ ,  $\text{ZON} \leq 1000 \mu\text{g/kg}$ , and  $\text{DON} \leq 5000 \mu\text{g/kg}$ ).

**Table 5.** Effects of synergistic microorganism–enzyme treatment on mycotoxin levels in corn stover and wheat straw.

| Roughage    | Mycotoxins               | C                  | T1                  | T2                    | T3                  | T4                 | T5                   | T6                   | SEM   | <i>p</i> -Value |
|-------------|--------------------------|--------------------|---------------------|-----------------------|---------------------|--------------------|----------------------|----------------------|-------|-----------------|
| Corn stover | AFB <sub>1</sub> (ug/kg) | <1                 | <1                  | <1                    | <1                  | <1                 | <1                   | 3.45                 | -     | -               |
|             | ZON (ug/kg)              | 47.37 <sup>b</sup> | 36.53 <sup>b</sup>  | 32.62 <sup>b</sup>    | 43.29 <sup>b</sup>  | 39.63 <sup>b</sup> | 35.07 <sup>b</sup>   | 151.84 <sup>a</sup>  | 9.159 | <0.001          |
|             | DON (ug/kg)              | <100               | <100                | <100                  | <100                | <100               | <100                 | <100                 | -     | -               |
| Wheat straw | AFB <sub>1</sub> (ug/kg) | <1                 | <1                  | <1                    | <1                  | <1                 | <1                   | <1                   | -     | -               |
|             | ZON (ug/kg)              | 39.48 <sup>a</sup> | 20.10 <sup>cd</sup> | 28.80 <sup>abcd</sup> | 33.66 <sup>ab</sup> | 17.53 <sup>d</sup> | 30.81 <sup>abc</sup> | 21.94 <sup>bcd</sup> | 2.043 | 0.011           |
|             | DON (ug/kg)              | <100               | <100                | <100                  | <100                | <100               | <100                 | <100                 | -     | -               |

In the same row, values with no superscript letters or the same superscript letters mean no significant difference ( $p > 0.05$ ), while values with different superscript letters mean that there is a significant difference ( $p < 0.05$ ). AFB<sub>1</sub>, aflatoxin B<sub>1</sub>; ZON, zearalenone; DON, deoxynivalenol; SEM, standard error of the mean.

### 3.4. In Vitro Rumen Fermentation Parameters

As presented in Table 6, for corn stover, the NH<sub>3</sub>-N levels in the T3 and T5 groups were significantly higher than that of the T2, T4, and T6 groups ( $p < 0.05$ ). Moreover, the concentrations of individual VFAs and TVFAs in the T3 and T5 groups were significantly higher than that of the control and the T6 group ( $p < 0.05$ ).

**Table 6.** Effects of synergistic microorganism–enzyme treatment on in vitro rumen fermentation.

| Roughage    | Parameters              | C                   | T1                   | T2                  | T3                   | T4                  | T5                   | T6                 | SEM     | <i>p</i> -Value |
|-------------|-------------------------|---------------------|----------------------|---------------------|----------------------|---------------------|----------------------|--------------------|---------|-----------------|
| Corn stover | pH                      | 6.43                | 6.62                 | 6.51                | 6.48                 | 6.46                | 6.45                 | 6.51               | 0.022   | 0.265           |
|             | DMD (%)                 | 76.76               | 73.07                | 72.95               | 71.72                | 72.61               | 72.01                | 74.15              | 0.557   | 0.227           |
|             | NH <sub>3</sub> -N (mM) | 22.08 <sup>ab</sup> | 21.79 <sup>ab</sup>  | 19.22 <sup>bc</sup> | 24.46 <sup>a</sup>   | 20.45 <sup>b</sup>  | 25.24 <sup>a</sup>   | 16.43 <sup>c</sup> | 0.586   | <0.001          |
|             | Protozoa (lg counts/mL) | 5.96                | 5.74                 | 5.89                | 6.02                 | 5.99                | 5.98                 | 5.76               | 0.042   | 0.447           |
|             | Acetate (mM)            | 35.93 <sup>cd</sup> | 42.17 <sup>abc</sup> | 39.72 <sup>bc</sup> | 48.69 <sup>ab</sup>  | 36.70 <sup>cd</sup> | 50.08 <sup>a</sup>   | 29.09 <sup>d</sup> | 1.471   | <0.001          |
|             | Propionate (mM)         | 9.81 <sup>cd</sup>  | 11.34 <sup>bc</sup>  | 10.75 <sup>c</sup>  | 13.44 <sup>ab</sup>  | 10.10 <sup>cd</sup> | 13.79 <sup>a</sup>   | 8.05 <sup>d</sup>  | 0.375   | <0.001          |
|             | Isobutyrate (mM)        | 0.80 <sup>bc</sup>  | 0.93 <sup>ab</sup>   | 0.83 <sup>bc</sup>  | 1.10 <sup>a</sup>    | 0.81 <sup>bc</sup>  | 1.09 <sup>a</sup>    | 0.65 <sup>c</sup>  | 0.031   | <0.001          |
|             | Butyrate (mM)           | 5.47 <sup>cd</sup>  | 6.49 <sup>bc</sup>   | 5.94 <sup>cd</sup>  | 7.53 <sup>ab</sup>   | 5.72 <sup>cd</sup>  | 7.89 <sup>a</sup>    | 4.76 <sup>d</sup>  | 0.207   | <0.001          |
|             | Isovalerate (mM)        | 1.61 <sup>bc</sup>  | 1.89 <sup>ab</sup>   | 1.71 <sup>bc</sup>  | 2.27 <sup>a</sup>    | 1.67 <sup>bc</sup>  | 2.30 <sup>a</sup>    | 1.34 <sup>c</sup>  | 0.067   | <0.001          |
|             | Valerate (mM)           | 0.69 <sup>bc</sup>  | 0.80 <sup>b</sup>    | 0.74 <sup>bc</sup>  | 0.99 <sup>a</sup>    | 0.72 <sup>bc</sup>  | 1.01 <sup>a</sup>    | 0.58 <sup>c</sup>  | 0.030   | <0.001          |
|             | TVFAs (mM)              | 54.30 <sup>bc</sup> | 63.63 <sup>ab</sup>  | 59.69 <sup>b</sup>  | 74.01 <sup>a</sup>   | 55.71 <sup>bc</sup> | 76.16 <sup>a</sup>   | 44.47 <sup>c</sup> | 2.174   | <0.001          |
|             | A/P                     | 3.65                | 3.70                 | 3.70                | 3.62                 | 3.57                | 3.62                 | 3.60               | 0.020   | 0.540           |
| Wheat straw | pH                      | 6.57 <sup>bc</sup>  | 6.58 <sup>abc</sup>  | 6.61 <sup>ab</sup>  | 6.56 <sup>c</sup>    | 6.58 <sup>bc</sup>  | 6.62 <sup>a</sup>    | 6.62 <sup>a</sup>  | 0.005   | 0.002           |
|             | DMD (%)                 | 76.91               | 75.13                | 76.48               | 75.85                | 75.35               | 75.55                | 77.52              | 0.375   | 0.602           |
|             | NH <sub>3</sub> -N (mM) | 9.47 <sup>cd</sup>  | 11.76 <sup>a</sup>   | 10.23 <sup>bc</sup> | 10.88 <sup>abc</sup> | 11.58 <sup>ab</sup> | 10.95 <sup>abc</sup> | 8.79 <sup>d</sup>  | 0.20126 | <0.001          |
|             | Protozoa (lg counts/mL) | 5.70                | 5.82                 | 5.86                | 5.86                 | 5.70                | 5.83                 | 5.76               | 0.025   | 0.332           |
|             | Acetate (mM)            | 24.37 <sup>ab</sup> | 24.89 <sup>a</sup>   | 20.54 <sup>bc</sup> | 25.27 <sup>a</sup>   | 26.18 <sup>a</sup>  | 23.86 <sup>ab</sup>  | 17.37 <sup>c</sup> | 0.561   | <0.001          |
|             | Propionate (mM)         | 6.54 <sup>ab</sup>  | 6.99 <sup>a</sup>    | 5.65 <sup>bc</sup>  | 7.17 <sup>a</sup>    | 7.27 <sup>a</sup>   | 6.64 <sup>ab</sup>   | 4.84 <sup>c</sup>  | 0.162   | <0.001          |
|             | Isobutyrate (mM)        | 0.40 <sup>ab</sup>  | 0.41 <sup>ab</sup>   | 0.35 <sup>b</sup>   | 0.41 <sup>ab</sup>   | 0.43 <sup>a</sup>   | 0.39 <sup>ab</sup>   | 0.28 <sup>c</sup>  | 0.009   | <0.001          |
|             | Butyrate (mM)           | 2.46 <sup>ab</sup>  | 0.73 <sup>a</sup>    | 2.21 <sup>bc</sup>  | 2.59 <sup>ab</sup>   | 2.60 <sup>ab</sup>  | 2.46 <sup>ab</sup>   | 1.97 <sup>c</sup>  | 0.053   | <0.001          |
|             | Isovalerate (mM)        | 0.55 <sup>a</sup>   | 0.58 <sup>a</sup>    | 0.49 <sup>a</sup>   | 0.56 <sup>a</sup>    | 0.59 <sup>a</sup>   | 0.53 <sup>a</sup>    | 0.39 <sup>b</sup>  | 0.014   | <0.001          |
|             | Valerate (mM)           | 0.17 <sup>a</sup>   | 0.20 <sup>a</sup>    | 0.15 <sup>ab</sup>  | 0.19 <sup>a</sup>    | 0.19 <sup>a</sup>   | 0.18 <sup>a</sup>    | 0.11 <sup>b</sup>  | 0.006   | <0.001          |
|             | TVFAs (mM)              | 34.49 <sup>ab</sup> | 35.81 <sup>a</sup>   | 29.39 <sup>bc</sup> | 36.19 <sup>a</sup>   | 37.26 <sup>a</sup>  | 34.05 <sup>ab</sup>  | 24.95 <sup>c</sup> | 0.800   | <0.001          |
|             | A/P                     | 3.74                | 3.57                 | 3.63                | 3.56                 | 3.60                | 3.61                 | 3.62               | 0.018   | 0.118           |

In the same row, the values with no superscript letters or the same superscript letters mean there was no significant difference ( $p > 0.05$ ), while values with different superscript letters mean that there was a significant difference ( $p < 0.05$ ). DMD, dry matter degradation rate; TVFAs, total volatile fatty acids; A/P, ratio of acetate to propionate; SEM, standard error of the mean.

For wheat straw, compared to the control group, the T3 group exhibited a significant reduction in pH ( $p < 0.05$ ), while the concentrations of acetate, propionate, and TVFAs in the T3 group were significantly higher than that of the T2 group ( $p < 0.05$ ). Furthermore, concentrations of individual VFAs and TVFAs were significantly decreased in the T6 group ( $p < 0.05$ ).

### 3.5. Scanning Electron Microscopy

As shown in Figure 1a,c, the surfaces of untreated corn stover and wheat straw were smooth and intact, with well-organized fiber structures and clearly defined cell arrangements, without any signs of damage. In contrast, as depicted in Figure 1b,d, the surfaces of corn stover and wheat straw treated with the microorganism–enzyme combinations exhibited significant structural alterations. The fiber structures were disrupted, showing signs of breakage and delamination, while the cell structures were partially damaged and dissolved.

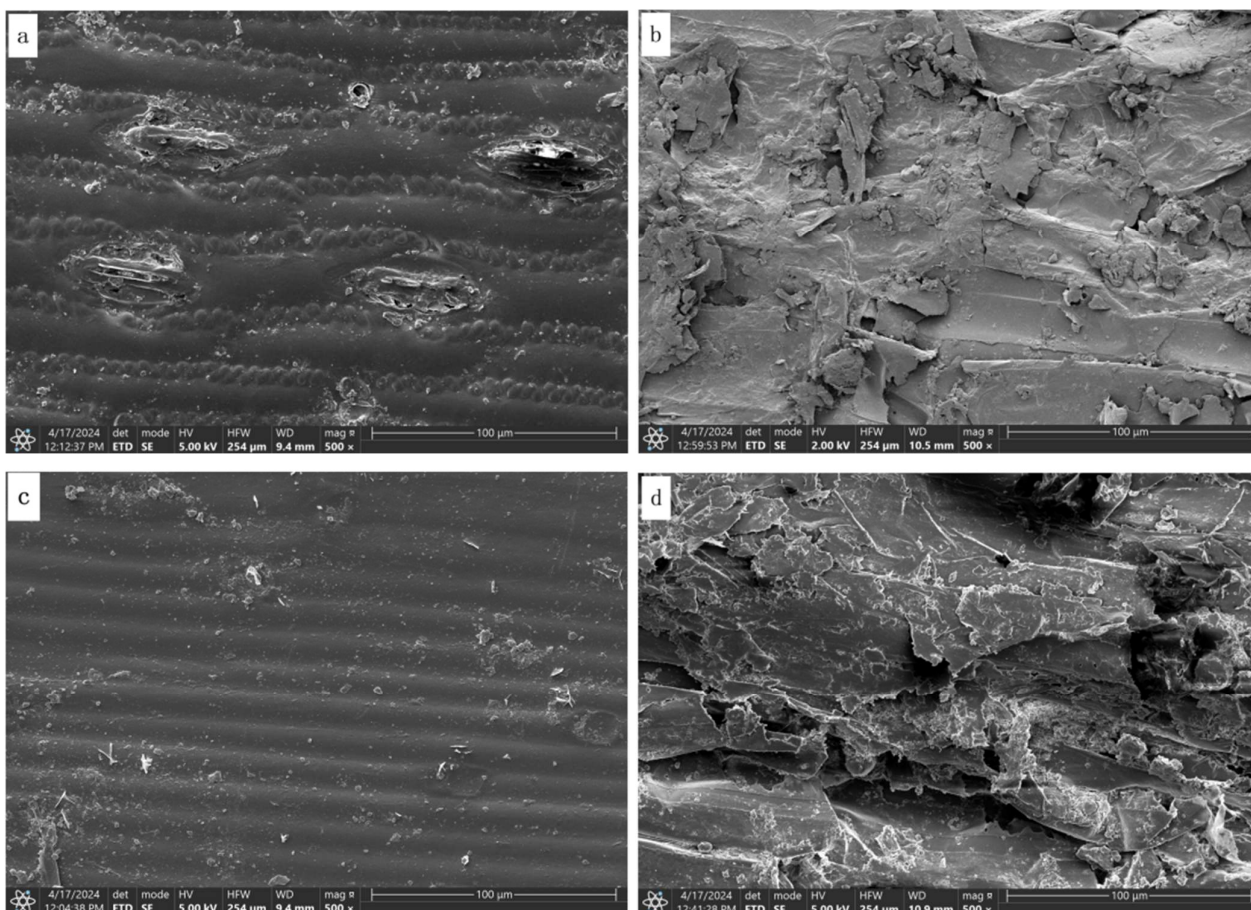


**Figure 1.** Scanning electron microscopy images of corn stover and wheat straw treated with microorganisms and enzymes: (a) corn stover (control); (b) corn stover (T3); (c) wheat straw (control); and (d) wheat straw (T6). Images were obtained at an accelerating voltage of 5.00 kV and a magnification of 500 $\times$ .

## 4. Discussion

### 4.1. Effects of Microorganisms and Enzymes on the Nutritional Composition of Corn Stover and Wheat Straw

Corn stover and wheat straw, abundant agricultural by-products globally, are limited in terms of feed utilization due to their high fiber content, low protein levels, and poor digestibility [31]. Numerous studies have demonstrated that microorganism–enzyme treatments effectively enhance the CP content, while reducing NDF and ADF levels in forages [32,33]. This phenomenon may be attributed to the hydrolysis of structural carbohydrates into soluble carbohydrates by microorganisms and enzymes during fermentation, thereby improving nutrient bioavailability for animals [34]. Concurrently, lignin–cellulose degradation releases additional plant proteins and facilitates the synthesis of microbial protein through the probiotic utilization of fermentation substrates [35]. Consistent with

previous studies [32,33], our findings demonstrated that all treatment groups exhibited increased CP content and reduced NDF and ADF levels compared to the control group. Among all the experimental groups, the T3 group in terms of corn stover demonstrated the highest reduction in NDF and ADF content, whereas the T6 group in terms of wheat stover showed the most pronounced decrease in NDF and ADF levels. These results collectively indicate that the synergistic microorganism–enzyme treatment improves the nutritional value of different types of roughage.

#### 4.2. Effects of Microorganisms and Enzymes on Fermentation Products of Corn Stover and Wheat Straw

The pH and organic acid content are critical indicators of feed fermentation quality. *Lactobacillus* sp. can utilize reducing sugars to produce substantial amounts of organic acids, such as lactate, acetate, and propionate, which promote a decrease in pH and inhibit the activity of spoilage microorganisms and proteolysis [36]. Additionally, high concentrations of lactate can suppress the growth of harmful bacteria and reduce the production of butyrate, thereby improving fermentation quality. Acetate is produced by heterofermentative *Lactobacillus* sp. during fermentation and can also be formed through the hydrolysis of acetyl groups in hemicellulose [31]. The addition of *Lactobacillus* sp. and cellulase significantly reduced the pH, propionate content, and mold count in mixed silage made of amaranth and corn stover, while increasing the levels of lactate and acetate [37]. The results of this experiment indicated that the pH of the wheat straw treatment group was significantly lower than that of the control group, whereas no significant change was observed in the pH of the corn stover treatment group, which may be attributed to the buffering capacity of corn stover [38].

The negative correlation between cellulose content and reducing sugar levels [39] stems from the conversion of degraded cellulose and other non-starch polysaccharides into soluble sugars. Reducing sugars serve as carbon sources and energy substrates for microbial proliferation, thereby enhancing fermentation efficiency [40]. Notably, no significant correlation was observed between the reducing sugar content and NDF/ADF levels in this study, which may be attributed to the dual roles of reducing sugar generation and consumption during microbial fermentation. For instance, while the T1 group initially improved the reducing sugar content in wheat straw, incremental additions of *C. utilis* and *L. plantarum* progressively decreased the reducing sugar levels, indicating that net consumption exceeded production.

Ammonia formation reflects partial protein hydrolysis during fermentation. The presence of undesirable microorganisms, such as *Clostridium perfringens* can inhibit *Lactobacillus* sp. growth, promote butyrate production, and catalyze protein degradation into NH<sub>3</sub>-N, leading to feed nutrient loss [41]. In this experiment, the NH<sub>3</sub>-N concentrations in all the treatment groups involving corn stover were significantly higher than that of the control group, which contrasts with the findings by Ma et al. [37]. This discrepancy may be attributed to differences in microbial metabolism and the degradation of enzymes.

#### 4.3. Effects of Microorganisms and Enzymes on Mycotoxin Production in Corn Stover and Wheat Straw

Mycotoxins are toxic secondary metabolites produced by fungi, primarily including aflatoxin B<sub>1</sub>, fumonisin B<sub>1</sub>, zearalenone, and deoxynivalenol (vomitoxin), which pose significant threats to animals and even human health [42]. The effect of *L. buchneri*, *L. plantarum*, and a combination of *L. buchneri* and *L. plantarum* could effectively reduce the levels of aflatoxin B<sub>1</sub>, fumonisin B<sub>1</sub>, zearalenone, and deoxynivalenol, thereby mitigating the adverse effects of fungal infection on the quality of corn silage [43]. These findings align with the results in the present study, suggesting that the addition of *L. plantarum* can reduce

mycotoxin production during fermentation. This effect may be attributed to the antifungal properties of bioactive substances produced by *Lactobacillus* sp. [44].

#### 4.4. In Vitro Rumen Fermentation of Straw Treated with Microorganism–Enzyme Combinations

The production of VFAs in the rumen is closely associated with the digestion rate of NDF. Straw subjected to microorganism–enzyme synergistic fermentation exhibits enhanced fiber digestibility, which accelerates the fermentation rate, while reducing ammonia production and increasing the concentration of VFAs [45]. In this experiment, variations in the concentrations of  $\text{NH}_3\text{-N}$  and TVFAs were observed. The type of feed significantly influences the TVFA concentration and its proportion in the rumen [46]. In this study, corn stover subjected to microorganism–enzyme treatment demonstrated significantly higher VFA production during in vitro rumen fermentation compared to the untreated controls. In contrast, wheat straw showed no significant change in VFA production following a similar treatment. These findings suggest that microorganism–enzyme treatment enhances the ruminal degradability of corn stover.

#### 4.5. Effects of Microorganisms and Enzymes on the Fiber Structure of Corn Stover and Wheat Straw

The fiber-degrading activity of rumen microorganisms is dependent on their attachment to the fiber surface [47]. Consequently, the pretreatment of roughage to disrupt the cell wall structure and expose the cell contents can effectively enhance lignocellulose degradation. Previous research has demonstrated that both lactic acid bacteria alone and in combination with molasses induce structural damage to the surface of rice straw fibers [48]. In this study, scanning electron microscopy was employed to analyze the fiber structure degradation in roughage before and after microorganism–enzyme synergistic treatment. The results revealed that microorganism–enzyme combinations altered the surface morphology of roughage fibers, resulting in structural modifications, including fracturing and delamination. These findings indicate that the treated samples provided more colonization sites for rumen microorganisms, thereby facilitating cellulose degradation.

## 5. Conclusions

The synergistic application of *C. utilis*, *L. plantarum*, and non-starch polysaccharide enzymes modifies the fiber architecture of roughage, thereby enhancing fermentation efficiency and nutritional quality. In this study, the T3 treatment, comprising 25% each of *C. utilis*, *L. plantarum*, cellulase, and laccase, with a total addition ratio of 0.3% (*w/w*), demonstrated optimal efficacy in regard to both corn stover and wheat straw fermentation. The findings in this study provide important technical insights and practical support for improving the development and utilization of unconventional roughage resources. Specifically, the results highlight the potential of bacterial–enzymatic synergistic treatment to enhance the nutritional value and fermentability of straw-based roughage. To further advance this field, future research should focus on optimizing key fermentation parameters, including time, temperature, and the composition of the microbes and enzymes. Additionally, it is essential to elucidate the mechanistic basis of microbial–enzymatic synergism and its subsequent impact on animal physiology and performance. These investigations will not only refine the fermentation process, but also facilitate the broader application of this technology in animal production systems, ultimately contributing to the sustainable intensification of livestock production.

**Author Contributions:** Conceptualization, Y.C. and J.L.; methodology, M.L.; software, B.C.; validation, X.L. and C.T.; formal analysis, B.C.; investigation, B.C.; resources, Y.C.; data curation, Y.C.; writing—original draft preparation, B.C.; writing—review and editing, Y.C. and J.L.; visualization, H.Z.;

supervision, Y.C. and H.Z.; project administration, Y.C.; funding acquisition, Y.C. All authors have read and agreed to the published version of the manuscript.

**Funding:** This work was funded by the Program for Science and Technology Innovation Talents (2022TSYCLJ0014), the Xinjiang Key Research and Development Program of China (2023B02015); and the Special Project of the Central Government Guidance on Local Science and Technology Development (ZYYD2023B09).

**Institutional Review Board Statement:** The experimental procedures that involved animals were approved by the Experimental Animal Welfare Ethics Committee of Xinjiang Agricultural University (protocol number: 20230712).

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** The data are contained within the article.

**Conflicts of Interest:** The authors declare that there are no conflicts of interest.

## Abbreviations

The following abbreviations are used in this manuscript:

|                    |                             |
|--------------------|-----------------------------|
| CFU                | Colony-forming units        |
| DM                 | Dry matter                  |
| OM                 | Organic matter              |
| CP                 | Crude protein               |
| NDF                | Neutral detergent fiber     |
| ADF                | Acid detergent fiber        |
| NH <sub>3</sub> -N | Ammonia nitrogen            |
| VFAs               | Volatile fatty acids        |
| TVFAs              | Total volatile fatty acids  |
| A/P                | Acetate/Propionate          |
| DMD                | Dry matter degradation rate |
| AFB <sub>1</sub>   | Aflatoxin B <sub>1</sub>    |
| ZON                | Zearalenone                 |
| DON                | Deoxynivalenol              |
| SEM                | Standard error of the mean  |

## References

1. Ma, Y.; Shen, Y.; Liu, Y. State of the art of straw treatment technology: Challenges and solutions forward. *Bioresour. Technol.* **2020**, *313*, 123656. [\[CrossRef\]](#) [\[PubMed\]](#)
2. Maślowski, M.; Miedzianowska, J.; Strzelec, K. The potential application of cereal straw as a bio-filler for elastomer composites. *Polym. Bull.* **2020**, *77*, 2021–2038. [\[CrossRef\]](#)
3. Tao, Q.; Li, B.; Chen, Y.; Zhao, J.; Li, Q.; Chen, Y.; Peng, Q.; Yuan, S.; Li, H.; Huang, R.; et al. An integrated method to produce fermented liquid feed and biologically modified biochar as cadmium adsorbents using corn stalks. *Waste Manag.* **2021**, *127*, 112–120. [\[CrossRef\]](#)
4. Kim, J.S.; Lee, Y.Y.; Kim, T.H. A review on alkaline pretreatment technology for bioconversion of lignocellulosic biomass. *Bioresour. Technol.* **2016**, *199*, 42–48. [\[CrossRef\]](#) [\[PubMed\]](#)
5. Yat, S.C.; Berger, A.; Shonnard, D.R. Kinetic characterization for dilute sulfuric acid hydrolysis of timber varieties and switchgrass. *Bioresour. Technol.* **2008**, *99*, 3855–3863. [\[CrossRef\]](#) [\[PubMed\]](#)
6. Yuan, W.; Gong, Z.; Wang, G.; Zhou, W.; Liu, Y.; Wang, X.; Zhao, M. Alkaline organosolv pretreatment of corn stover for enhancing the enzymatic digestibility. *Bioresour. Technol.* **2018**, *265*, 464–470. [\[CrossRef\]](#)
7. Wang, P.; Liu, C.; Chang, J.; Yin, Q.; Huang, W.; Liu, Y.; Dang, X.; Gao, T.; Lu, F. Effect of physicochemical pretreatments plus enzymatic hydrolysis on the composition and morphologic structure of corn straw. *Renew. Energy* **2019**, *138*, 502–508. [\[CrossRef\]](#)
8. Rusănescu, C.; Ciobanu, M.; Rusănescu, M.; Dinculoiu, R. Pretreatments applied to wheat straw to obtain bioethanol. *Appl. Sci.* **2024**, *14*, 1612. [\[CrossRef\]](#)
9. Saha, B.; Qureshi, N.; Kennedy, G.; Cotta, M. Biological pretreatment of corn stover with white-rot fungus for improved enzymatic hydrolysis. *Int. Biodeterior. Biodegrad.* **2016**, *109*, 29–35. [\[CrossRef\]](#)

10. Agrawal, R.; Satlewal, A.; Gaur, R.; Mathur, A.; Kumar, R.; Gupta, R.; Tuli, D. Pilot scale pretreatment of wheat straw and comparative evaluation of commercial enzyme preparations for biomass saccharification and fermentation. *Biochem. Eng. J.* **2015**, *102*, 54–61. [\[CrossRef\]](#)
11. Wang, Z.; Tang, H.; Liu, G.; Gong, H.; Li, Y.; Chen, Y.; Yang, Y. Compound probiotics producing cellulase could replace cellulase preparations during solid-state fermentation of millet bran. *Bioresour. Technol.* **2023**, *385*, 129457. [\[CrossRef\]](#) [\[PubMed\]](#)
12. Ilić, N.; Milić, M.; Beluhan, S.; Dimitrijević-Branković, S. Cellulases: From lignocellulosic biomass to improved production. *Energies* **2023**, *16*, 3598. [\[CrossRef\]](#)
13. Walia, A.; Guleria, S.; Mehta, P.; Chauhan, A.; Parkash, J. Microbial xylanases and their industrial application in pulp and paper biobleaching: A review. *3 Biotech* **2017**, *7*, 11. [\[CrossRef\]](#) [\[PubMed\]](#)
14. Wang, P.; Pei, X.; Zhou, W.; Zhao, Y.; Gu, P.; Li, Y.; Gao, J. Research and application progress of microbial  $\beta$ -mannanases: A mini-review. *World J. Microbiol. Biotechnol.* **2024**, *40*, 169. [\[CrossRef\]](#) [\[PubMed\]](#)
15. Zhang, W.; Wang, W.; Wang, J.; Shen, G.; Yuan, Y.; Yan, L.; Tang, H.; Wang, W. Isolation and characterization of a novel laccase for lignin degradation, LacZ1. *Appl. Environ. Microbiol.* **2021**, *87*, e0135521. [\[CrossRef\]](#)
16. Yan, J.; Sun, Y.; Kang, Y.; Meng, X.; Zhang, H.; Cai, Y.; Zhu, W.; Yuan, X.; Cui, Z. An innovative strategy to enhance the ensiling quality and methane production of excessively wilted wheat straw: Using acetic acid or hetero-fermentative lactic acid bacterial community as additives. *Waste Manag.* **2022**, *149*, 11–20. [\[CrossRef\]](#)
17. Suntara, C.; Cherdthong, A.; Uriyapongson, S.; Wanapat, M.; Chanjula, P. Comparison effects of ruminal crabtree-negative yeasts and crabtree-positive yeasts for improving ensiled rice straw quality and ruminal digestion using *in vitro* gas production. *J. Fungi* **2020**, *6*, 109. [\[CrossRef\]](#)
18. Zhao, J.; Dong, Z.; Li, J.; Chen, L.; Bai, Y.; Jia, Y.; Shao, T. Ensiling as pretreatment of rice straw: The effect of hemicellulase and *Lactobacillus plantarum* on hemicellulose degradation and cellulose conversion. *Bioresour. Technol.* **2018**, *266*, 158–165. [\[CrossRef\]](#)
19. Hu, Y.; He, Y.; Gao, S.; Liao, Z.; Lai, T.; Zhou, H.; Chen, Q.; Li, L.; Gao, H.; Lu, W. The effect of a diet based on rice straw co-fermented with probiotics and enzymes versus a fresh corn Stover-based diet on the rumen bacterial community and metabolites of beef cattle. *Sci. Rep.* **2020**, *10*, 10721. [\[CrossRef\]](#)
20. Wang, L.; Wang, J.; Wang, P.; Liu, C.; Li, X.; Chang, J.; Jin, S.; Yin, Q.; Zhu, Q. Effect of corn straw treated with *Lactobacillus plantarum* and cellulase on ruminal fermentation and microbiota of Hu sheep. *Fermentation* **2024**, *10*, 402. [\[CrossRef\]](#)
21. Liu, Q.; Li, X.; Desta, S.; Zhang, J.; Shao, T. Effects of *Lactobacillus plantarum* and fibrolytic enzyme on the fermentation quality and *in vitro* digestibility of total mixed rations silage including rape straw. *J. Integr. Agric.* **2016**, *15*, 2087–2096. [\[CrossRef\]](#)
22. AOAC. *Official Methods of Analysis*; Association of Official Analytical Chemists: Arlington, TX, USA, 1995.
23. Yan, Y.; Li, X.; Guan, H.; Huang, L.; Ma, X.; Peng, Y.; Li, Z.; Nie, G.; Zhou, J.; Yang, W.; et al. Microbial community and fermentation characteristic of Italian ryegrass silage prepared with corn stover and lactic acid bacteria. *Bioresour. Technol.* **2019**, *279*, 166–173. [\[CrossRef\]](#) [\[PubMed\]](#)
24. Song, H.; Gao, Y.; Yang, Y.; Xiao, W.; Liu, S.; Xia, W.; Liu, Z.; Yi, L.; Jiang, Z. Synergistic effect of cellulase and xylanase during hydrolysis of natural lignocellulosic substrates. *Bioresour. Technol.* **2016**, *219*, 710–715. [\[CrossRef\]](#) [\[PubMed\]](#)
25. Broderick, G.; Kang, J. Automated simultaneous determination of ammonia and total amino acids in ruminal fluid and *in vitro* media. *J. Dairy Sci.* **1980**, *63*, 64–75. [\[CrossRef\]](#)
26. Wei, H.; Liu, J.; Liu, M.; Zhang, H.; Chen, Y. Rumen fermentation and microbial diversity of sheep fed a high-concentrate diet supplemented with hydroethanolic extract of walnut green husks. *Anim. Biosci.* **2024**, *37*, 655–667. [\[CrossRef\]](#)
27. Zhou, Y.; Cheng, Y.; Zhu, W. The Process and matters needing attention of *in vitro* fermentation of rumen microorganisms. In *Microbiome Protocols Ebook*; Bio-protocol LLC: Sunnyvale, CA, USA, 2021; Volume Bio-101, p. e2003663. (In Chinese) [\[CrossRef\]](#)
28. Li, J.; Yan, H.; Chen, J.; Duan, C.; Guo, Y.; Liu, Y.; Zhang, Y.; Ji, S. Correlation of ruminal fermentation parameters and rumen bacterial community by comparing those of the goat, sheep, and cow *in vitro*. *Fermentation* **2022**, *8*, 427. [\[CrossRef\]](#)
29. Wang, J. *Research Methods of Ruminant Nutrition*; Modern Education Press: Beijing, China, 2011.
30. GB 13078-2017; Hygienical Standard for Feeds. National Standard of the People's Republic of China: Beijing, China, 2017.
31. Liu, X.; Wang, A.; Zhu, L.; Guo, W.; Guo, X.; Zhu, B.; Yang, M. Effect of additive cellulase on fermentation quality of whole-plant corn silage ensiling by a *Bacillus* inoculant and dynamic microbial community analysis. *Front. Microbiol.* **2024**, *14*, 1330538. [\[CrossRef\]](#)
32. Gao, J.; Wang, P.; Zhou, C.; Li, P.; Tang, H.; Zhang, J.; Cai, Y. Chemical composition and *in vitro* digestibility of corn stover during field exposure and their fermentation characteristics of silage prepared with microbial additives. *Asian-Australas. J. Anim. Sci.* **2019**, *32*, 1854–1863. [\[CrossRef\]](#)
33. Babaeinasab, Y.; Rouzbehan, Y.; Fazaeli, H.; Rezaei, J. Chemical composition, silage fermentation characteristics, and *in vitro* ruminal fermentation parameters of potato-wheat straw silage treated with molasses and lactic acid bacteria and corn silage. *J. Anim. Sci.* **2015**, *93*, 4377–4386. [\[CrossRef\]](#)
34. He, L.; Zhou, W.; Wang, C.; Yang, F.; Chen, X.; Zhang, Q. Effect of cellulase and *Lactobacillus casei* on ensiling characteristics, chemical composition, antioxidant activity, and digestibility of mulberry leaf silage. *J. Dairy Sci.* **2019**, *102*, 9919–9931. [\[CrossRef\]](#)

35. Yu, P.; Christensen, D.A.; McKinnon, J.J. In situ rumen degradation kinetics of timothy and alfalfa as affected by cultivar and stage of maturity. *Can. J. Anim. Sci.* **2004**, *84*, 255–263. [[CrossRef](#)]
36. Heinritz, S.N.; Martens, S.D.; Avila, P.; Hoedtke, S. The effect of inoculant and sucrose addition on the silage quality of tropical forage legumes with varying ensilability. *Anim. Feed. Sci. Technol.* **2012**, *174*, 201–210. [[CrossRef](#)]
37. Ma, J.; Fan, X.; Ma, Z.; Huang, X.; Tang, M.; Yin, F.; Zhao, Z.; Gan, S. Silage additives improve fermentation quality, aerobic stability and rumen degradation in mixed silage composed of amaranth and corn straw. *Front. Plant Sci.* **2023**, *14*, 1189747. [[CrossRef](#)]
38. Hu, W.; Schmidt, R.; McDonell, E.; Klingerman, C.; Kung, L. The effect of *Lactobacillus buchneri* 40788 or *Lactobacillus plantarum* MTD-1 on the fermentation and aerobic stability of corn silages ensiled at two dry matter contents. *J. Dairy Sci.* **2009**, *92*, 3907–3914. [[CrossRef](#)] [[PubMed](#)]
39. Li, Y.; Fan, X.; Zhang, H.; Ai, F.; Jiao, Y.; Zhang, Q.; Zhang, Z. Pretreatment of corn stover by torrefaction for improving reducing sugar and biohydrogen production. *Bioresour. Technol.* **2022**, *351*, 126905. [[CrossRef](#)]
40. Zhang, T.; Jiang, D.; Li, Y.; Zhang, H.; Zhang, Z.; Jing, Y.; Lu, C.; Zhang, Y.; Xia, C.; Zhang, Q. Lignin removal, reducing sugar yield and photo-fermentative biohydrogen production capability of corn stover: Effects of different pretreatments. *Bioresour. Technol.* **2022**, *346*, 126437. [[CrossRef](#)]
41. Muck, R.; Nadeau, E.; McAllister, T.; Contreras-Govea, F.; Santos, M.; Kung, L. Silage review: Recent advances and future uses of silage additives. *J. Dairy Sci.* **2018**, *101*, 3980–4000. [[CrossRef](#)]
42. Tolosa, J.; Rodríguez-Carrasco, Y.; Ruiz, M.; Vila-Donat, P. Multi-mycotoxin occurrence in feed, metabolism and carry-over to animal-derived food products: A review. *Food Chem. Toxicol.* **2021**, *158*, 112661. [[CrossRef](#)]
43. Li, J.; Wang, W.; Chen, S.; Shao, T.; Tao, X.; Yuan, X. Effect of lactic acid bacteria on the fermentation quality and mycotoxins concentrations of corn silage infested with mycotoxigenic fungi. *Toxins* **2021**, *13*, 699. [[CrossRef](#)]
44. Ahlberg, S.; Joutsjoki, V.; Korhonen, H. Potential of lactic acid bacteria in aflatoxin risk mitigation. *Int. J. Food Microbiol.* **2015**, *207*, 87–102. [[CrossRef](#)]
45. Froetschel, M.; Amos, H. Effects of dietary fiber and feeding frequency on ruminal fermentation, digesta water-holding capacity, and fractional turnover of contents. *J. Anim. Sci.* **1991**, *69*, 1312–1321. [[CrossRef](#)] [[PubMed](#)]
46. Liu, C.; Wu, H.; Liu, S.; Chai, S.; Meng, Q.; Zhou, Z. Dynamic alterations in yak rumen bacteria community and metabolome characteristics in response to feed type. *Front. Microbiol.* **2019**, *10*, 1116. [[CrossRef](#)] [[PubMed](#)]
47. Hua, D.; Hendriks, W.; Xiong, B.; Pellikaan, W. Starch and cellulose degradation in the rumen and applications of metagenomics on ruminal microorganisms. *Animals* **2022**, *12*, 3020. [[CrossRef](#)] [[PubMed](#)]
48. Chen, X.; Ma, Y.; Khan, M.; Xiao, J.; Alugongo, G.; Li, S.; Wang, Y.; Cao, Z. A combination of lactic acid bacteria and molasses improves fermentation quality, chemical composition, physicochemical structure, in vitro degradability and rumen microbiota colonization of rice straw. *Front. Vet. Sci.* **2022**, *9*, 900764. [[CrossRef](#)]

**Disclaimer/Publisher’s Note:** The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.