EFFECTS OF DIFFERENT MICROBIAL FERMENTATION PROCESSES OF RICE STRAW ON ITS QUALITY AS LIVESTOCK FEED

. *水稻秸秆发酵特性及饲料化利用*

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Keywords: rice straw feed, microbial fermentation, intake rate

ABSTRACT

Rice straw's poor palatability and low nutrition limit its utilization as livestock feed, resulting in low intake rates. To address this, three fermentation treatments (compound bacteria, compound bacteria + enzymes, lactic acid bacteria) were tested over 25, 45, and 65 days, analyzing crude fiber, soluble sugar, and protein to evaluate quality. Results showed compound bacteria + enzymes performed best at 65 days: crude fiber reduced to 34.83%, crude protein increased to 9.45%, and soluble sugar reached 1.89%. Feeding trials with Simmental cattle revealed a 93% intake rate for fermented straw—65% higher than unfermented. This study establishes a microbial degradation technology for rice straw, identifies optimal starters, and forms a feed production process, laying a foundation for enhanced rice straw utilization as livestock feed.

摘要

水稻秸秆适口性差、营养价值低,使其作为畜禽饲料的利用率受限,导致采食率偏低。针对这一问题,本研究 采用三种发酵处理方式(复合菌、复合菌+酶、乳酸菌),分别发酵 25 天、45 天和 65 天,并通过分析粗纤维、可溶性糖和蛋白质含量来评估饲料品质。结果显示,复合菌+酶处理在 65 天时效果最佳:粗纤维含量降至 34.83%,粗蛋白含量提升至 9.45%,可溶性糖含量达 1.89%。对西门塔尔牛的饲喂试验表明,发酵秸秆的采食率为 93%,较未发酵秸秆提高 65%。本研究建立了水稻秸秆的微生物降解技术,筛选出了适宜的发酵剂,形成了秸秆饲料生产工艺,为提升水稻秸秆作为畜禽饲料的利用率奠定了基础。

INTRODUCTION

China is a major rice - producing country, with an annual output of 210 million tons of rice straw (Lu et al., 2022). The main components of rice straw, namely cellulose, hemicellulose, and lignin, are difficult to decompose within a short period. The carbon - nitrogen (C/N) ratio usually ranges from 60:1 to 100:1, leading to poor palatability, low nutritional value, and low intake rates for cattle and sheep (Xu et al., 2020). Microorganisms can secrete cellulase, hemicellulase, and other enzymes. These enzymes break down complex carbohydrates in straw such as crude fiber and lignin, which are difficult for animals to digest directly - into small - molecule substances like glucose and xylose, thereby improving the digestibility of straw (Du et al., 2025). In addition, some microorganisms (e.g., lactic acid bacteria and yeast) utilize carbon and nitrogen sources in straw for metabolism during fermentation, synthesizing nutrients such as proteins, amino acids, and vitamins (e.g., B vitamins). This process increases the content of crude protein, soluble sugars, and other components in straw. For instance, yeast can convert sugars in straw into proteins, significantly enhancing the protein level of straw feed. However, for crop straw with a high C/N ratio, such as rice straw, factors like microbial species, fermentation time, and moisture content during the fermentation process exert a significant impact on fermentation efficiency (Zheng et al., 2019). There remains a lack of clear conclusions regarding the optimization of fermentation for rice straw with a high C/N ratio, such as the compatibility between specific microbial agent combinations and fermentation cycles, which in turn affects livestock intake rates.

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Microbial fermentation technology, when applied with probiotics, can effectively improve the biological and physicochemical properties of rice straw. This process degrades lignin and crude fiber in the straw, thereby increasing the content of nutrients such as protein. Additionally, the softened rice straw can enhance palatability and boost the intake rates of livestock (*Liu et al., 2024*). The strategies of "returning straw to the field through cattle digestion" and "transforming straw into beef and milk via cattle" can contribute to carbon reduction and sequestration, achieving a carbon sequestration rate of 20 - 30% (*Huo et al., 2021, 2022*). Evidently, microbial fermentation of straw is currently the most favorable approach for the comprehensive utilization of rice straw. Exploring the utilization of rice straw as animal feed can not only expand the feed resources for animal husbandry and reduce feed costs but also alleviate the environmental pollution caused by the outdoor stacking and burning of rice straw. This practice is of great significance for increasing farmers' income, and promoting sustainable agricultural development (*Cong et al., 2019; Kyawt et al., 2024*).

Therefore, the present study aimed to explore microbial fermentation technology for the production of rice straw feed and to investigate the effects of different starter cultures and the optimal fermentation duration. By comparing the effects of three treatments—"compound bacteria", "compound bacteria + enzyme", and "lactic acid bacteria"—over fermentation periods of 25 days, 45 days, and 65 days, and integrating nutritional indicators (such as crude fiber and protein) with cattle feeding rate data, this study reveals the efficient degradation effect of rice straw by the synergy of bacteria and enzyme. Key indicators, including the pH value, crude fiber content, crude protein content, soluble sugar levels in the straw after fermentation were evaluated. The results of this study are expected to lay a solid foundation for the further development and utilization of rice straw feed.

MATERIALS AND METHODS

Materials

The rice straw utilized in this study was sourced from the rice field of Shenyang Agricultural University, China. The rice was harvested using a mechanical harvester, resulting in partial mechanical rubbing of the straw during the threshing process. This mechanical treatment affected the straw's physical structure to some extent. The moisture content of the straw was measured to be approximately 35%–45%, which was within a typical range for freshly harvested rice straw and could influence subsequent fermentation processes. Three distinct starter culture treatments were employed:

A compound bacterial mixture consisting of lactic acid bacteria at a concentration of 5×10^5 colony forming units (cfu) per gram and yeast at 1×10^5 cfu per gram;

A compound enzymatic mixture comprising lactic acid bacteria (5 × 10⁵ cfu /g) and cellulase (3 × 10⁵ cfu /g);

Lactic acid bacteria alone, with a concentration of 5 × 10⁵ cfu per gram.

Methods

To systematically investigate the effects of different starter cultures on rice straw feed quality, a four-factor, three-level experiment was designed. Fermentation durations (A1, A2, A3) corresponded to 25, 45, and 65 days, respectively. Starter culture treatments included: B1 (compound bacteria of lactic acid bacteria + yeast), B2 (compound bacteria + cellulase), B3 (lactic acid bacteria alone), and CK (no starter, control). Detailed experimental configurations are shown in Table 1.

Experimental scheme for rice straw fermentation

F4 A	Factor B					
Factor A	B1	B2	В3	CK		
A1	A1B1	A1B2	A1B3	A1CK		
A2	A2B1	A2B2	A2B3	A2CK		
A3	A3B1	A3B2	A3B3	A3CK		

Table 1

Preparation of starter cultures

To prepare starter cultures, brown sugar was dissolved in boiling water, cooled to 40°C, then mixed with starter cultures to homogeneity. The mixture (starter:sugar:water = 1:5:50, mass ratio) was sealed and activated for 3 days before spraying.

1) Starter cultures spraying

Three starter culture types were uniformly sprayed onto each straw piles at a rate of \sim 0.2 g per 100 g straw, adjusting the straw moisture content to 50% - 65%. Then the straw was sealed with plastic strip and incubated in a temperature - controlled climate chamber, with the temperature regime simulating northern China's autumn conditions: 5 °C (0:00–8:00), 10 °C (8:00–12:00), 15 °C (12:00–17:00), and 5 °C (17:00–24:00).

2) Content analysis

Rice straw samples from different fermentation treatments were dried at $105\,^{\circ}$ C, then grounded into powder using a high-speed pulverizer, followed by fine grinding with a planetary ball mill and sieving. Samples for crude protein and soluble sugar analysis were sieved to 80 - 100 mesh, while those for crude fiber analysis were 20 - 30 mesh.

The content of crude fiber

The analysis was carried out in strict compliance with the national standard GB/T 5009.10-2003.

The content of crude protein

The analysis was carried out using the elemental analysis method (Xiao et al., 2018).

The content of soluble sugar

The analysis was conducted in accordance with the national standard NY/T 1278-2007 (Wang et al., 2007).

3) Experimental indices

pH value

To avoid parameter changes from prolonged air exposure, fermented straw samples were collected immediately after opening the packs. After thorough mixing, 10 g of straw was weighed into a wide-mouth bottle, mixed with 100 mL distilled water, and sealed. The bottle was incubated at 10 °C for 12 h, then filtered. The pH of the filtrate was measured using a calibrated pH meter. The pH value is a pivotal metric for evaluating fermentation efficiency. According to established standards (Wang et al., 2011), a pH of \leq 3.8 signifies excellent fermentation quality, 3.9–4.1 indicates good quality, 4.2–4.7 denotes moderate quality, and \geq 4.8 represents poor quality.

Sensory evaluation

On-site sensory evaluation represents the most straightforward, practical, and efficient approach for gauging the quality of straw feed. Fermented straw sensory evaluation followed the Deutsche Landwirtschafts-Gesellschaft scoring method, assessing odor, texture, and color (*Ai et al., 2020*). An evaluation panel comprising ten trained professionals (five males and five females, aged between 25 and 45 years) was assembled to conduct the sensory assessments. Based on total scores, feeds were classified into four quality grades (excellent, good, medium, poor). Detailed scoring criteria are in Table 2.

Sensory evaluation criteria of Deutsche Landwirtschafts-Gesellschaft

Indicators	Criteria	Score
Odor	No acid odor, with aromatic fruit or obvious bread aroma A weak acid odor, a strong sour odor or a weak aromatic smell A quite strong acid taste or a pungent sticky or musty odor A strong ammonia odor or almost no acidity	14 10 4 2
Texture	Good stem and leaf structure Poor maintenance of stem and leaf structure The preservation of stem and leaf structures is extremely poor, or there is slight mold contamination Stem and leaf decay or severe pollution	4 2 1 0

Indicators		Score		
Color and luster	Similar to the Slightly disc Severe discoloration, da	2 1 usty odor 0		
Total score	16-20 10-15 5-9			
Level	Excellent (Level 1)	Good (Level 2)	Medium (Level 3)	Bad (Level 4)

RESULTS

Following a designated fermentation period, subtle white flocculent colonies of lactic acid bacteria emerged within the plastic packaging bags, presenting a distinct visual contrast. In stark contrast, the control group (CK) remained unaltered, showing no discernible changes to the naked eye.

The pH value

Figure 1 depicts the dynamic changes in pH values over time for straw samples treated with various starter cultures. Significantly, all three treatment groups with different starter cultures showed notably lower pH values than the control group (CK). The control group maintained pH levels consistently above 5.0 throughout the experiment. Among them, the compound enzyme treatment (B2) stood out with the lowest pH range (3.90–3.93), underscoring its exceptional acidification capability.

These findings strongly suggest that starter cultures promote the fermentation of rice straw by generating substantial amounts of organic acids, thereby effectively reducing pH values. Moreover, organic acids function not only as flavor enhancers to improve feed palatability but also play crucial roles in regulating digestive processes and enhancing animal health (*Wu et al.*, 2022).

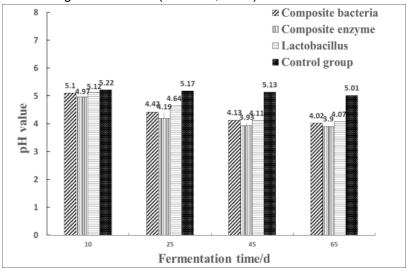


Fig. 1 - Variation of pH values of different groups

Sensory evaluation

As clearly illustrated in Table 3, the three treatment groups outperformed the control group (CK) significantly, registering notably higher sensory scores. Samples rated at Level 1 (Excellent) were characterized by the complete absence of acidic odors, instead exuding a distinct, pleasant aroma reminiscent of fresh bread or natural fragrances, while maintaining an intact texture and structure. Level 2 (Good) samples, though lacking any sour smell, retained a discernible aroma, and the straw underwent a color transformation to light brown. Level 3 (Moderate) samples were marked by a pungent sour odor, a faint aromatic presence, compromised structural stability, and a light yellowish hue. In contrast, Level 4 (Poor) samples emitted strong musty and sour odors, displayed a decomposed texture, and exhibited severe discoloration.

The evaluation outcomes revealed that across all starter cultures tested, a fermentation duration of 45–65 days consistently yielded the highest sensory scores. These comprehensive findings collectively underscore that the application of starter cultures not only elevates the sensory appeal of straw feed but also expedites the degradation of fibers, optimizing its overall quality.

Sensory scores under the criteria of the Deutsche Landwirtschafts-Gesellschaft

Treatment	Ctantan	Sc	Scores of indicators				
group	Starter culture	Odor	Texture	Color and luster	Total score	Quality level	
25d	CK	2	2	0	4	Poor	
	B1	10	2	1	13	Good	
	B2	14	4	2	20	Excellent	
	B3	10	2	1	13	Good	
45d	CK	2	2	0	4	Poor	
	B1	14	4	0	18	Excellent	
	B2	14	4	2	20	Excellent	
	B3	14	4	2	20	Excellent	
65d	CK	2	1	0	3	Poor	
	B1	12	4	0	16	Excellent	
	B2	12	4	2	18	Excellent	
	В3	12	4	2	18	Excellent	

Nutritional indicators

As shown in Table 4, a detailed summary of the nutritional parameters of rice straw from different treatment groups is provided. Statistical analysis was shown in Table 5.

Nutritional indicators of rice straw feed (%)

Table 4

Table 3

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Treatment _ group		Nutritional indicators					
		Crude fiber	Crude protein	Soluble sugar			
25d	B1	39.41±3.05Ac	8.10±0.00Bc	1.55±0.00Aa			
45d	B1	37.97±2.22ABc	9.10±0.06Aa	1.57±0.43Ab			
65d	B1	36.75±1.30Bc	9.38±0.02Aa	1.56±0.17Ab			
25d	B2	37.67±3.40Ad	8.34±0.03Ba	1.52±0.12Ba			
45d	B2	36.96±2.13Ad	8.94±0.01Bab	1.78±0.01Aa			
65d	B2	34.83±0.58Bd	9.45±0.35Aa	1.89±0.01Aa			
25d	В3	41.71±0.86Ab	8.25±0.45Bb	1.22±0.00Bb			
45d	В3	40.41±1.92Bb	8.59±0.26Ab	1.49±0.00Ab			
65d	В3	38.41±2.22Cb	8.79±0.00Ab	1.44±0.10Ab			
25d	CK	43.25±1.82Aa	5.87±0.02Ac	1.10±0.00Ac			
45d	CK	43.73±2.36Aa	5.39±0.03Ac	1.14±0.02Ac			
65d	CK	42.39±3.18Aa	5.36±0.15Ac	1.25±0.16Ac			

Note: Capital letters are employed to denote significant variations in the impact of fermentation time on nutritional indicators when the same starter culture treatments are applied. Conversely, lowercase letters signify significant disparities in the effects of different starter culture types on nutritional indicators under identical fermentation time conditions.

Analysis of variance for nutritional indicators

Table 5

Source of variation	Dependent variable	Sum of squares	degree of freedom	Mean square	F-value	P-value
Treatment group	Crude fiber	36.723	4	18.362	3.630	0.042
	Crude protein	2.227	4	1.114	31.714	0.000
	Soluble sugar	0.234	4	0.117	5.311	0.012
Starter cultures	Crude fiber	223.031	6	74.344	14.697	0.000
	Crude protein	71.182	6	23.727	675.673	0.000
	Soluble sugar	1.591	6	0.530	24.071	0.000
Treatment group * Starter cultures	Crude fiber	6.367	12	1.061	0.210	0.970
	Crude protein	3.280	12	0.547	15.568	0.000
	Soluble sugar	0.143	12	0.024	1.083	0.400
Error	Crude fiber	121.406	48	5.059		
	Crude protein	0.843	48	0.035		
	Soluble sugar	0.529	48	0.022		
Total	Crude fiber	56435.722	72			
	Crude protein	2360.461	72			
	Soluble sugar	79.147	72			

The changes in the content of crude fiber

During the entire fermentation process, the crude fiber content in treatment groups B1 (compound bacteria), B2 (compound enzyme), and B3 (lactic acid bacteria) was significantly lower than that in the control group (CK) (P<0.05). Notably, treatment B2 consistently showed the lowest crude fiber content across all fermentation stages. After 65 days of fermentation, its crude fiber content reached a minimum of 34.83%, representing a 13.56% reduction compared to the CK group. Statistical analysis revealed that, with the extension of fermentation time, the crude fiber content exhibited a significant decreasing trend (P < 0.05), while the fermentation duration exerted no significant effect on the CK group. Collectively, these findings strongly suggest that the application of starter cultures effectively promotes straw degradation, consequently leading to a notable reduction in the crude fiber content of the final feed product.

Crude protein

The choice of starter cultures exerted a pronounced influence on the crude protein content of the straw feed. Across all treatments involving starter cultures, the levels of crude protein increased steadily as fermentation progressed, culminating in a peak at the 65 day mark. Notably, the B1 treatment group achieved a crude protein content of 9.38%, the B2 group reached 9.45%, and the B3 group recorded 8.79%. In stark contrast, the control group (CK) experienced a marginal decrease, with its crude protein content dropping to 8.38% by the 65th day of fermentation. These findings unequivocally indicate that the application of starter cultures facilitates the accumulation of crude protein in rice straw, thereby substantially enhancing the nutritional profile of the feed. The main reasons for the increase in crude protein include:

Soluble sugar

During the fermentation process, a significant upward trend in soluble sugar content was observed across all treatment groups, whereas the control group (CK) remained unchanged. Statistical analysis confirmed that starter cultures exerted a significant influence on soluble sugar levels (P < 0.05) compared to the CK. In treatment group B1, the soluble sugar content increased initially and plateaued between 45 and 65 days of fermentation. In contrast, treatment group B2 exhibited a continuous increase, with significantly higher soluble sugar values than groups B1 and B3 at both 45 and 65 days. These results suggest that starter cultures enhance straw fermentation by increasing the soluble sugar content in the feed. Comparative analysis indicated that the B2 treatment achieved the optimal fermentation results during the 45 - 65-day period.

As mentioned in the text, compared with the control group (CK), the addition of the starter culture significantly affects the level of soluble sugars (P < 0.05). The microorganisms in different types of starter cultures (such as treatment groups B1 and B2) have different species and metabolic characteristics, which can promote the decomposition of polysaccharides in the substrate or the synthesis of soluble sugars. Certain microorganisms in the starter culture (such as yeasts and lactic acid bacteria) can secrete relevant enzymes (such as amylase and cellulase). These enzymes can hydrolyze polysaccharides (such as starch, cellulose, hemicellulose, etc.) in raw materials like straw into soluble sugars (such as glucose and xylose), thereby increasing the content of soluble sugars in the fermentation system (*Jabbour et al., 2013; Guo. et al., 2022*).

Feeding Experiment of Cattle

The production process of rice straw feed was conducted as follows: the preparation of the starter culture adhered to the same protocol described in Part 2. Following the spraying of the starter culture, the moisture content of the rice straw was adjusted to approximately 50%–65%. The feed production apparatus included a baling machine and a sealing machine. A spraying device was installed at the inlet of the baling machine, enabling a sequential operation workflow that included starter culture spraying, baling, and plastic sealing. The resultant straw bales were standardized with dimensions of 80 cm in diameter and 80 cm in length, achieving a consistent density of 480 kg/m³. Each bale was packaged with three layers of plastic to ensure integrity during storage and handling. For experimental purposes, rice straw fermented for 65 days with the combined treatment of compound bacteria and enzymes was designated as the experimental feed. In contrast, untreated rice straw was employed as the control group (CK). All samples were derived from the Shennong 9903 rice cultivar, ensuring uniformity in the raw material source for accurate experimental comparison.

The study subjects consisted of 20 Simmental cattle originating from Longpao Village, Qingyuan County, Liaoning Province, China. These cattle were approximately 4 months old, demonstrated normal growth patterns, had moderate fat deposition, were in good health condition, and their body weights ranged from 140 to 185 kg.

The feeding protocol was divided into two distinct phases: a preparation phase lasting for 3 days and an experimental phase spanning 3 weeks. The twenty cattle were randomly assigned into two groups, with 10 cattle in each group. The control group was fed unfermented rice straw, while the experimental group was provided with fermented rice straw.

During the experimental period, the daily diet of the cattle consisted of both coarse feed and concentrated feed. Specifically, the coarse feed was corn silage, with each group being supplied 75 kg of it daily. The concentrated feed was commercial fattening-cattle feed, with 8 kg provided to each group per day. These two types of feed were mixed and administered to the cattle twice a day, at 6:00 a.m. and 6:00 p.m. For the control group (CK), the coarse feed was substituted with unfermented rice straw, whereas the experimental group received fermented rice straw. Throughout the entire trial, the concentrated feed remained unchanged for both groups.

The daily amounts of coarse and concentrated feed remained consistent across all groups, and the feeding schedules were strictly adhered to. Throughout the experiment, the cattle were ensured unrestricted access to drinking water and feed. During the preparation phase, the coarse feed was gradually replaced with either unfermented rice straw (in the control group, CK) or fermented rice straw (in the experimental group). One-third of the coarse feed was replaced daily until a complete substitution was achieved. By the first day of the experimental phase, the coarse feed was fully replaced with rice-straw feed. Daily, the coarse and concentrated feeds were homogeneously blended according to the prescribed proportions. The remaining coarse feed from the previous day was weighed to calculate the intake rates (refer to Equation 1).

$$Intake\ rate = \frac{Total\ amount - Remaining\ amount}{Total amount} \times 100\% \tag{1}$$

Table 6

Results of feeding experiment

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	Week 1		Week 2		Week 3			Average
Groups	Feeding amount / kg	Residual amount / kg	Feeding amount / kg	Residual amount / kg	Feeding amount / kg	Residual amount / kg	Average intake / kg	intake rate / %
Control group	75.0	57.6	75.0	52.3	75.0	51.1	21.33	28
Experime ntal group	75.0	1.30	75.0	1.83	75.0	2.12	69.75	93

Table 6 illustrates that fermented rice straw proved to be more advantageous for Simmental cattle. In contrast, unfermented rice straw displayed suboptimal palatability, leading to lower acceptance by the cattle. Notably, the intake rate of fermented rice straw reached an impressive 93%, marking a substantial 65% increase when compared to the mere 28% intake rate of unfermented rice straw.

Discussion

This study assessed three starter cultures (compound bacteria, compound bacteria + enzymes, lactic acid bacteria) on rice straw fermentation over 25, 45, 65 days, focusing on nutrition and intake rates. Findings show microbial fermentation enhances feed quality, with 65-day compound bacteria + enzyme (B2) most effective.

Treated groups (especially B2) had significantly reduced crude fiber, with B2 reaching 34.83% at 65 days (13.56% lower than CK), due to cellulase hydrolyzing cellulose β -1,4-glycosidic bonds and disrupting hemicellulose-lignin interactions (*Su et al., 2012; Tem et al., 2019*). B2's continuous crude fiber decline indicates sustained enzymatic activity, unlike CK's stability.

B2's crude protein peaked at 9.45% at 65 days, via microbial-enzyme synergy: lactic acid bacteria/yeast use straw nitrogen for microbial proteins (*Zhu et al., 2021*), while cellulase aids carbon access and nitrogen conversion (*Hall et al., 2001*), outperforming B1 (9.38%) and B3 (8.79%).

B2's soluble sugar (1.89% at 65 days) rose continuously, unlike B1's plateau, due to microbial cellulase/amylase hydrolyzing polysaccharides into glucose/xylose (*Jabbour et al., 2013; Guo et al., 2022*), boosting nutrition and palatability.

pH and sensory attributes: Treated groups had a lower pH than CK (pH > 5.0); Specifically, the B2 group had a pH of 3.90–3.93 (excellent quality) (*Wang et al., 2011*).

This quality advantage is attributed to the spoilage-inhibiting effect of lactic acid (*Sun, 2023; Zhang et al., 2021*). B2 scored highest in sensory evaluation (bread-like aroma, intact texture, raw material-like color), contributing to 93% intake rate (*Liu et al., 2024*).

Feeding trial implications. Fermented straw's 93% intake rate (vs. 28% unfermented) stems from reduced crude fiber, increased soluble sugars, and improved texture, addressing low straw utilization in ruminants (*Xu et al., 2020*). Standardized production (50%–65% moisture, 480 kg/m³ density, 65-day B2) supports scalability, aligning with carbon sequestration via "straw-to-feed" (*Huo et al., 2021, 2022*).

Findings support microbial fermentation's role (*Du et al., 2025; Feng et al., 2021*), but B2's superiority highlights microbe-enzyme synergy, contrasting with microbial-only studies (*Zheng et al., 2019*) and emphasizing exogenous enzymes in high C/N substrates.

Limitations: unanalyzed microbial dynamics, intake rate-focused feeding trial, single rice cultivar (Shennong 9903). Future work: metagenomic analysis of microbe-enzyme interactions, long-term large-cohort trials, parameter optimization for scaling. In summary, 65-day B2 optimizes rice straw feed quality (reduced crude fiber, increased nutrients, enhanced palatability) with 93% intake rate, supporting sustainable straw use in livestock.

CONCLUSIONS

This study systematically explored the impacts of starter cultures, specifically compound bacteria and enzymes, along with fermentation duration, on the nutritional characteristics of rice straw. The key findings are comprehensively summarized as follows:

- (1) The microbial degradation experiment provided compelling evidence of significant enhancements in the sensory indicators of rice straw. Compared with the control group (CK), the treated samples exhibited marked improvements. Notably, the pH values of the groups treated with starter cultures decreased substantially relative to the CK, with the compound bacteria enzyme treatment group achieving the lowest pH value of 3.90. Moreover, pronounced differences were detected in the contents of crude fiber and soluble sugar after the application of starter cultures. At the 65 day fermentation mark, the compound bacteria enzyme treatment yielded the most outstanding results: it achieved the highest levels of crude protein (9.45%) and soluble sugar (1.89%), while simultaneously presenting the lowest crude fiber content (34.83%). The addition of the three types of starter cultures effectively enhanced the aromatic profile, softened the texture, and significantly elevated the overall quality of the straw feed. Among these, the compound bacteria enzyme treatment emerged as the most effective approach, attaining optimal outcomes.
- (2) The feeding experiment conducted on Simmental steers yielded remarkable results. Fermented straw feed demonstrated an impressively high intake rate of 93%, in stark contrast to the mere 28% intake rate of unfermented rice straw. Collectively, these findings clearly demonstrate that rice straw feed treated with starter cultures undergoes notable improvements in sensory quality, palatability, and nutritional parameters. More importantly, it significantly boosts the intake rate in ruminant livestock, highlighting the practical significance and potential of this treatment method in animal husbandry applications.

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REFERENCES

- [1] Ai, Q., Jiang, H., Guo, R., Jiang, T., Su, H., (2020). Effects of adding defective apple fermentation in rice straw on quality and microbial numbers of mixed silage (添加残次苹果发酵物对稻草青贮品质及其微生物数量的影响). *Transactions of the Chinese Society of Agricultural Engineering*, Vol. 36, 316-323, Beijing/China. DOI: 10.11975/j.issn.1002-6819.2020.20.035
- [2] Cong, H., Yao, Z., Zhao, L., Meng, H., Wang, J., Huo, L., Yuan, Y., Jia, J., Xie, T., Wu, Y., (2019). Analysis of long-term mechanism for development of straw industry in northeast China under guidance of "Green-concept" (中国农作物秸秆资源分布及其产业体系与利用路径). *Transactions of the Chinese Society of Agricultural Engineering*, Vol. 36, pp. 132-140, Beijing/China. DOI: CNKI:SUN:NYGU.0.2019-22-015:
- [3] Du, S., Xu, L., Jiang. C., Xiao, Y., (2025). Novel strategy to understand the bacteria-enzyme synergy action regulates the ensiling performance of wheat straw silage by multi-omics analysis, *International*

- Journal of Biological Macromolecules, Vol. 289, pp. 138864, Netherlands, DOI: org/10.1016/j.ijbiomac.2024.138864
- [4] Feng, P., Wu, H., Meng, F., Zheng, H., Yang, Z., Wang, J., Shen, Z., (2021). Effects of different compatible biological agents on quality and feeding value of rice straw silage (不同配伍生物制剂对水稻秸秆 青 贮 品 质 及 饲 喂 价 值 的 影 响). *China Feed*, Vol. 17, pp. 122-128., Beijing/China. DOI: 10.15906/j.cnki.cn11-2975/s.20211725
- [5] Guo, Z., Wang, L., Yu, C., (2022). Increasing the glucose metabolism enhances the bioelectricity generation in microbial fuel cells. *Acta Biochimica et Biophysica Sinica*, Vol 54. pp. 1566-1567. DOI: 10.3724/abbs.2022129
- [6] Hall M.B., Herejk C., Differences in Yields of Microbial Crude Protein from In Vitro Fermentation of Carbohydrates, (2001). *Journal of Dairy Science*, Vol 84, pp. 2486-2493. DOI: 10.3168/jds.S0022-0302(01)74699-1
- [7] Huo, L., Yao, Z., Zhao, L., Luo, J., Zhang, P., (2022). Contribution and Potential of Comprehensive Utilization of Straw in GHG Emission Reduction and Carbon Sequestration (秸秆综合利用减排固碳贡献与潜力研究). *Transactions of the Chinese Society for Agricultural Machinery*, Vol. 53, pp. 349-359, Beijing/China.
- [8] Huo, L., Zhao, L., Yao, Z., Luo, J., Zhang, P., Xie, T., Jia, J., Deng Y., Wei, X., (2021). Potentiality of agricultural biomass energy for greenhouse gas emission reduction (农业生物质能温室气体减排潜力). *Transactions of the Chinese Society of Agricultural Engineering*, Vol. 37, pp. 179-187, Beijing/China.
- [9] Jinpeng, Z., Jie, Z., Junfeng, L., Zhihao, D., Lei, C., Yunfeng, B., Yushan, J., Tao, S., n.d. (2019). Effect of different additives on fermentation quality and structural carbohydrates compositions of rice straw silage (不同添加剂对水稻秸秆青贮发酵品质和结构性碳水化合物组分的影响). *Journal of Nanjing Agricultural University*, Vol. 42(1), pp. 152-159, Nanjng/China. DOI: 10.7685/jnau.201803011
- [10] Jabbour, D., Borrusch, M. S., Goutami B., Jonathan D. W. (2013). Enhancement of fermentable sugar yields by α-xylosidase supplementation of commercial cellulases. *Biotechnology for Biofuels*, Vol. 6:58. DOI:10.1186/1754-6834-6-58
- [11] Kyawt, Y., Aung, M., Xu, Y., Sun, Z., Zhou, Y., Zhu, W., Padmakumar, V., Tan, Z., Cheng, Y., (2024). Dynamic changes of rumen microbiota and serum metabolome revealed increases in meat quality and growth performances of sheep fed bio-fermented rice straw. *Journal of Animal Science and Biotechnology*, Vol. 15, Beijing/China. DOI: 10.1186/s40104-023-00983-5
- [12] Liu, E.Y., Wang, Shuiping, Wang, Shibo, Khan, N.A., Zhou, X., Tang, S., Zhou, C., Tan, Z., Liu, Y., (2024). Bacterial inoculants and enzymes based silage cocktails boost the ensiling quality of biomasses from reed, corn and rice straw. *Chem. Biol. Technol. Agric*, Vol. 11, England. DOI: 10.1186/s40538-024-00549-1
- [13] Lu, H., Chen, Y., Zhang, P., Huan, H., Xie, H., Hu, H., (2022). Impacts of farmland size and benefit expectations on the utilization of straw resources: Evidence from crop straw incorporation in China. *Soil Use and Management*, Vol. 38, pp. 929–939, England. DOI: 10.1111/sum.12739
- [14] Su Y., Zhao G., Wei Z., Yan C., Liu S. (2012). Mutation of Cellulose Synthase Gene Improves the Nutritive Value of Rice Straw, Asian - Australasian Journal of Animal Science, Vol. 25, pp. 800-805. DOI: 10.5713/ajas.2011.11409
- [15] Sun, Y., Sun, Q.L., Tang, Y., Li, Q., Tian, C., Sun, H., (2023). Integrated microbiology and metabolomic analysis reveal the improvement of rice straw silage quality by inoculation of Lactobacillus brevis, *Biotechnology for Biofuels and Bioproducts*, Vol. 16, England. DOI: 10.1186/s13068-023-02431-y
- [16] Tem T. D., Thava V., (2019). Modification of rice bran dietary fiber concentrates using enzyme and extrusion cooking. *Food Hydrocolloids*, Vol 89, pp. 773-782. DOI: 10.1016/j.foodhyd.2018.11.024
- [17] Wang, D., Buckmaster, D.R., Jiang, Y., Hua, J., n.d., (2011). Experimental study on baling rice straw silage. *International Journal of Agricultural and Biological Engineering*, Vol.4, Beijing/China. DOI: 10.3965/ijabe.v4i1.262
- [18] Wang, X., Liu, S., Liu, Z., Yang, M., Chen, N., (2007). Determination of Soluble Sugars in Vegetables and Their Products by Copper-Reduction Iodometric Method (NY/T 1278-2007)(蔬菜及其制品中可溶性糖的测定铜还原碘量法), Ministry of Agriculture of the People's Republic of China, Beijing. 2007: 1 4
- [19] Wu, Y., Ding, H., Shao, T., Zhao, J., Dong, D., Dai, T., Yin, X., Zong, C., Li, J., (2022). Effects of enzyme preparation on fermentation quality and in vitro digestion characteristics of rice straw silage (酶制剂对水

- 稻秸秆青贮发酵品质及体外消化特性的影响). *Acta Prataculturae Sinica*, Vol. 31, pp. 167-177, Gansu/China. DOI: 10.11686/cyxb2021312
- [20] Xiao, Z., Fan, X., Ma, D., Li, L., Wang, S., Jia, Z., Liu, X., Wang, Z., Liu, J., (2018). GB/T6432-2018, Determination of crude protein in feeds—Kjeldahl method (饲料中粗蛋白的测定-凯氏定氮法), National Feed Quality Supervision and Testing Center (Beijing)
- [21] Xu, C., Yuan, Q., Zhao, S., He, T., Song, N., (2020). Effects of pretreatments on physical and chemical characteristics of wheat straw used as a maintenance-free compressed green roof substrate material. *Journal of Cleaner Production*, Vol. 277, United States. DOI: 10.1016/j.jclepro.2020.123381
- [22] Zhang, H., Cheng, X., Elsabagh, M., Lin, B., Wang, H., (2021). Effects of formic acid and corn flour supplementation of banana pseudostem silages on nutritional quality of silage, growth, digestion, rumen fermentation and cellulolytic bacterial community of Nubian black goats. *Journal of Integrative Agriculture*, Vol. 20, pp. 2214-2226, Beijing/China. DOI: 10.1016/s2095-3119(20)63470-0
- [23] Zheng, G., Zhou, C., Yin, T., Lu, Z., & Ai, S., (2019). Optimization of fermentation factors to enhance rice straw degradation ability using a microbial consortium lzf-12. *International Journal of Agricultural and Biological Engineering*. Beijing/China, DOI: 10.25165/ijabe.v12i3.4638
- [24] Zhu X., Wang L., Zhang Z., Ding L., Hang S. (2021). Combination of fiber-degrading enzymatic hydrolysis and lactobacilli fermentation enhances utilization of fiber and protein in rapeseed meal as revealed in simulated pig digestion and fermentation in vitro. *Animal Feed Science and Technology*, Vol. 278, pp. 115001-. DOI: 10.1016/j.anifeedsci.2021.115001