

RESEARCH ARTICLE

Effects of Wheat Bran, EM Bacteria, Ammonium Nitrate and Fungal Additives on Fermentation and Forage Quality of Rice Straw Silage

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Abstract: The global ruminant industry is constrained by a persistent shortage of high-quality roughage, thus rendering the bioconversion of agricultural residues, such as rice straw, a critical priority. Nevertheless, the high lignification, silicification, and nitrogen deficiency of rice straw pose significant biological barriers to traditional ensiling. This study investigated the impact of various additives on rice straw silage quality. Six treatments were evaluated: CK (control, no additives), L1 (200 g/kg wheat bran), L2 (L1 + 1 g/kg EM bacteria), L3 (L2 + 20 g/kg ammonium nitrate), L4 (L3 + 1 g/kg *Aspergillus oryzae*), and L5 (L4 + 1 g/kg *Trichoderma longibrachiatum*). All additive concentrations are expressed on a fresh matter basis. After 45 days of ensiling, all treatments significantly improved silage quality compared to the control. Treatments L2-L5 achieved significantly higher sensory scores than CK ($P < 0.05$). All treatments significantly improved silage quality compared to the control, with increased crude protein content, decreased fiber fractions (ADF, NDF), and enhanced Relative Feed Value (RFV) ($P < 0.05$). Fermentation parameters also improved, as evidenced by lower pH values and higher lactic acid concentrations ($P < 0.05$). Results indicate that optimal silage enhancement requires the combined supplementation of fermentable carbohydrates (wheat bran), EM bacteria, and nitrogen source (ammonium nitrate). The addition of *A. oryzae* further improved silage quality, while the effects of *T. longibrachiatum* were less conclusive. Treatment L4 achieved the highest Relative Feed Value (RFV: 79.86) and optimal fermentation profile, while L5 showed comparable pH and lactic acid levels but lower RFV (79.29) and acetic acid content. Based on the current experimental conditions, *T. longibrachiatum* did not provide additional benefits beyond L4 and is not recommended for rice straw silage under these conditions. Overall, treatment L4 produced the most favorable improvements in rice straw silage quality.

Keywords: Rice Straw, Additive, Fermentation Quality, Forage Quality, Multi-Additive Synergy

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Introduction

The scarcity of high-quality, affordable feed resources poses a significant challenge to the ruminant livestock industry in Asia, including China [1]. Rice straw, which consists of the stem and leaves following the harvesting of the grain, constitutes

approximately 50% of the total rice plant biomass [2]. As the world's leading rice producer, China possesses abundant rice straw resources. However, approximately one-third of this resource is discarded annually through environmentally harmful practices such as incineration [3].

Rice straw, which is composed primarily of cellulose, hemicellulose, lignin, protein, and pectin, represents a significant renewable biomass resource. Despite its historical use as roughage in China, the quality, palatability, and efficiency of ruminal fermentation of rice straw are such that they result in reduced feed intake and limited utilisation by ruminants [4, 5]. Consequently, enhancing the quality of rice straw for use as livestock feed has become a research priority. The primary challenge in improving rice straw quality is its resistance to hydrolysis. A range of physical, chemical, and biological treatments have been shown to modify the composition of rice straw cell walls and enhance digestibility. The process of microbial fermentation has been shown to effectively degrade cell wall components, thereby enhancing both palatability and nutritional value [6]. However, the inherent characteristics of rice straw, namely its low water-soluble carbohydrate and nitrogen content, high lignin levels, and insufficient natural lactic acid bacteria, impede efficient fermentation [4].

The findings of research studies indicate that the addition of carbohydrate-rich materials and inorganic nitrogen has a substantial impact on the quality of rice straw silage. The application of cellulose-degrading microorganisms, particularly Effective Microorganism (EM) bacteria, has been extensively implemented across various agricultural sectors [7]. *Aspergillus oryzae*, an aerobic probiotic fungus producing multiple enzymes (cellulase, saccharase, phytase, protease, and amylase), is recognised as safe for direct animal feeding by the Food and Agriculture Organization (FAO) and the World Health Organization (WHO) [8]. In a similar manner, *Trichoderma longibrachiatum* has been shown to be capable of effective degradation of lignin, cellulose, and hemicelluloses [9].

Whilst extant research has generally focused on individual or limited combinations of additives, there has been scant investigation of the combined effects of nitrogen sources, EM bacteria, *A. oryzae* and *T. longibrachiatum*. Despite extensive research on individual additives, critical knowledge gaps remain:

- (1) The quantitative effects of combining multiple microbial inoculants with chemical nitrogen sources
- (2) The mechanistic understanding of fungal enzyme interactions in complex additive systems
- (3) The optimal combination ratio for maximizing both fermentation efficiency and nutritional value. However, the synergistic effects of combining nitrogen sources, EM bacteria, *A. oryzae*, and *T. longibrachiatum* on rice straw silage have not been systematically evaluated.

We hypothesized that the sequential addition of these additives would provide complementary benefits: wheat bran as a fermentable substrate, EM bacteria for fermentation initiation, ammonium nitrate for nitrogen supplementation, and fungal enzymes for lignocellulose degradation. This study aims to evaluate the effects of these combined additives on fermentation characteristics, nutritional composition, and sensory quality of rice straw silage to identify the optimal treatment combination.

Materials and Methods

Experimental Materials

Rice variety Southern Japonica 9108, harvested in October 2023 from Guohe Town, Lujiang County, Anhui Province, was used as the raw material for this study.

Microbial Inoculants

Effective Microorganism (EM) bacteria composite (containing *Lactobacillus plantarum*, *Saccharomyces cerevisiae*, and *Bacillus subtilis*; viable count $\geq 1 \times 10^8$ CFU/g) was obtained from Shandong Baolai-Leelai Bio-Tech Co., Ltd. (Taian, China). *Aspergillus oryzae* (strain CGMCC 3.5011; viable spore count $\geq 1 \times 10^7$ CFU/g) and *Trichoderma longibrachiatum* (CGMCC 3.1043, viable spore count $\geq 1 \times 10^7$ CFU/g) were purchased from China General Microbiological Culture Collection Center (Beijing, China).

Fungal spore viability was verified before application using the plate count method. Briefly, serial dilutions of each inoculant were plated on Potato Dextrose Agar (PDA) and incubated at 28°C for 48- 72 hours. Colony counts confirmed viable spore concentrations of $\geq 1 \times 10^7$ CFU/g for both *A. oryzae* and *T. longibrachiatum*, meeting the manufacturer's specifications.

For treatment application, microbial inoculants were first dissolved in sterile distilled water (100 mL per kg of rice straw) and then uniformly sprayed onto the chopped rice straw using a hand-operated sprayer while mixing thoroughly to ensure even distribution.

Experimental Design

Freshly harvested rice straw, free from disease and pests, was selected for its cleanliness and appropriate dryness. The straw was cut into uniform sections of 1-2 cm in length.

As shown in Table 1, six treatments were established with varying combinations of bacterial treatments and wheat bran, including CK. Each treatment was prepared in three independent laboratory-scale silos (polyethylene bags, 30×40 cm), with approximately 500 g of fresh rice straw per silo, to ensure statistical validity. Each silo was treated as an independent replicate (n=3 per treatment), with materials sourced from different batches of rice straw to ensure true biological replication rather than technical subsampling. Vacuum sealing was standardized using a DZ-400/2S vacuum packaging machine (Shandong Kangbeite Food Packaging Machinery Co., Ltd., China) with a vacuum pressure of -0.09 MPa maintained for 30 seconds before heat sealing. Silage bags were vacuum-sealed and stored at room temperature (20-25°C) for 45 days before opening to evaluate the quality indices of the rice straw silage.

Additive concentrations were selected based on preliminary experiments and previous literature: wheat bran at 20% (200 g/kg) provides adequate fermentable carbohydrates without excessive cost [19]; EM bacteria at 0.1% (1 g/kg) is the manufacturer's recommended application rate; ammonium nitrate at 2% (20 g/kg) provides supplemental nitrogen while avoiding ammonia toxicity [10]; fungal inoculants at 0.1% (1 g/kg) represent viable spore counts of approximately 1×10^5 CFU/g in the final silage, sufficient for enzymatic activity without overwhelming the system.

Table 1: Experimental design: effects of additive treatments on rice straw silage

Treatment	Bran(g·kg ⁻¹)	EM bacteria (g·kg ⁻¹)	Ammonium nitrate (g·kg ⁻¹)	A.oryzae (g·kg ⁻¹)	T. ongibrachiatum (g·kg ⁻¹)
CK	0	0	0	0	0
L1	200	0	0	0	0
L2	200	1	0	0	0
L3	200	1	20	0	0
L4	200	1	20	1	0
L5	200	1	20	1	1

Note: All additive concentrations are expressed on a Fresh Matter (FM) basis

Measurement Indicators and Methods

Sensory Evaluation

Upon opening the silage bags, sensory evaluation was conducted according to the German Agricultural Society Feed Industry Standards (DLG) [10]. Samples were assessed for odor, texture, and color. Sensory evaluation was conducted by a trained panel of five evaluators with experience in silage quality assessment. Each evaluator independently scored samples in a blinded manner (treatments were coded and randomized). The scoring criteria were: color (0 = black/brown, 1 = greenish-brown, 2 = yellow-green), odor (0-14 scale: 0-2 = strong butyric/putrid, 3-6 = weak butyric/musty, 7-10 = aromatic/weak acid, 11-14 = pleasant lactic acid aroma), and texture (0 = slimy, 2 = moderate moisture, 4 = firm structure). Final scores represent the mean of five panelists' assessments. The scoring scale ranged from 0-2 for color, 0-14 for odor, and 0-4 for texture. For each treatment group, the mean score of three replicates was calculated. The combined scores were classified as follows: 16-20 (excellent), 10-15 (good), 5-9 (moderate), and 0-4 (poor quality).

Feed Quality Evaluation

After 45 days of fermentation, 20-gram silage samples were weighed, dried at 100°C for 16 hours, and cooled to room temperature until reaching constant weight to Determine Dry Matter (DM) content. The dried samples were ground, pulverized, passed through a 40-mesh sieve, and stored in sealed glass bottles for subsequent analyses.

Crude Protein (CP) was determined by the Kjeldahl method [11]. Ether Extract (EE) was measured by Soxhlet extraction [11]. Acid Detergent Fiber (ADF) and Neutral Detergent Fiber (NDF) contents were analyzed using the methods of [12] with an ANKOM²⁰⁰⁰ fiber analyzer. The Relative Feeding Value (RFV) was calculated [13]:

$$RFV = (88.9 - 0.779 \times ADF) \times (120/NDF)/1.29$$

Fermentation Quality Analysis

For each sample, 2.00 g of silage was precisely weighed into 100-ml volumetric flasks, and ultrapure water was added to a volume of 60 ml. Ultrasonic extraction was performed in a 50°C water bath for 20 minutes. After cooling to room temperature, ultrapure water was added to reach the volumetric mark. The mixture was shaken and filtered to obtain the filtrate. Analyses of pH and organic acids, including ammoniacal nitrogen (NH₃-N), Lactic Acid (LA), Acetic Acid (AA), Propionic Acid (PA), and Butyric Acid (BA), were completed within 24 hours. Sample pH was measured using a PHS-25 digital pH meter. Organic acid analysis was conducted using a DUG-20A high-performance liquid chromatography analyzer equipped with a ShimNexUPC18 column [14].

Data Processing Analysis

Data were collected and processed using Excel 2019. Statistical analysis was performed using SPSS 27.0 software (IBM Corp., Armonk, NY, USA). Data were tested for normality (Shapiro-Wilk test) and homogeneity of variance (Levene's test). One-way analysis of variance (ANOVA) was conducted, followed by Duncan's multiple range test for post-hoc comparisons. Each treatment consisted of three independent replicates (n = 3), and each replicate was analyzed separately. Statistical significance was declared at P<0.05. All data are presented as means ± Standard Error of the Mean (SEM, n = 3). Graphical presentation of results was generated using Origin 2021.

Results and Analysis

Effects of Different Treatments on Sensory Evaluation of Rice Straw Silage

Different additive treatments variably improved silage sensory quality (Table 2), indicating their capacity to enhance fermentation. All treatment groups except L1 scored significantly higher than the control group (P<0.05), demonstrating that wheat bran alone provided insufficient fermentable substrate to effectively improve silage quality.

Treatments L3, L4, and L5 achieved significantly higher total sensory scores (16.1-17.3) than CK and L1 (12.0-12.3) (P<0.05) and were graded as "excellent", characterized by no butyric acid odor, preservation of the original material color, and well-maintained structural integrity. Notably, L4 and L5 treatments (which included *A. oryzae*) yielded identical and the highest scores (17.3), representing a 44.2% improvement over the control.

Table 2: Effects of additive treatments on the sensory evaluation of rice straw silage

Treatments	Odor	Texture	Color	Total score	Grade
CK	7.0 ^c	4.0 ^a	1.0 ^b	12.0 ^d	Good
L1	7.6 ^c	4.0 ^a	1.7 ^{ab}	12.3 ^d	Good
L2	8.3 ^{bc}	4.0 ^a	2.0 ^a	14.3 ^c	Good
L3	10.1 ^b	4.0 ^a	2.0 ^a	16.1 ^b	Excellent
L4	11.3 ^a	4.0 ^a	2.0 ^a	17.3 ^a	Excellent
L5	11.3 ^a	4.0 ^a	2.0 ^a	17.3 ^a	Excellent

Note: Values in the same column with different lowercase letters indicate significant differences (P<0.05). Values sharing the same letter or without markers indicate non-significant differences (P>0.05). The texture parameter showed uniform scores (4.0) across all treatments, indicating that all silages maintained firm structure without sliminess. The scoring system effectively differentiated treatments based on odor and color parameters

Effects of Additive Treatments on the Nutritional Quality of Rice Straw Silage

The nutritional profile of rice straw silage was improved by various additive treatments (Figure 1).

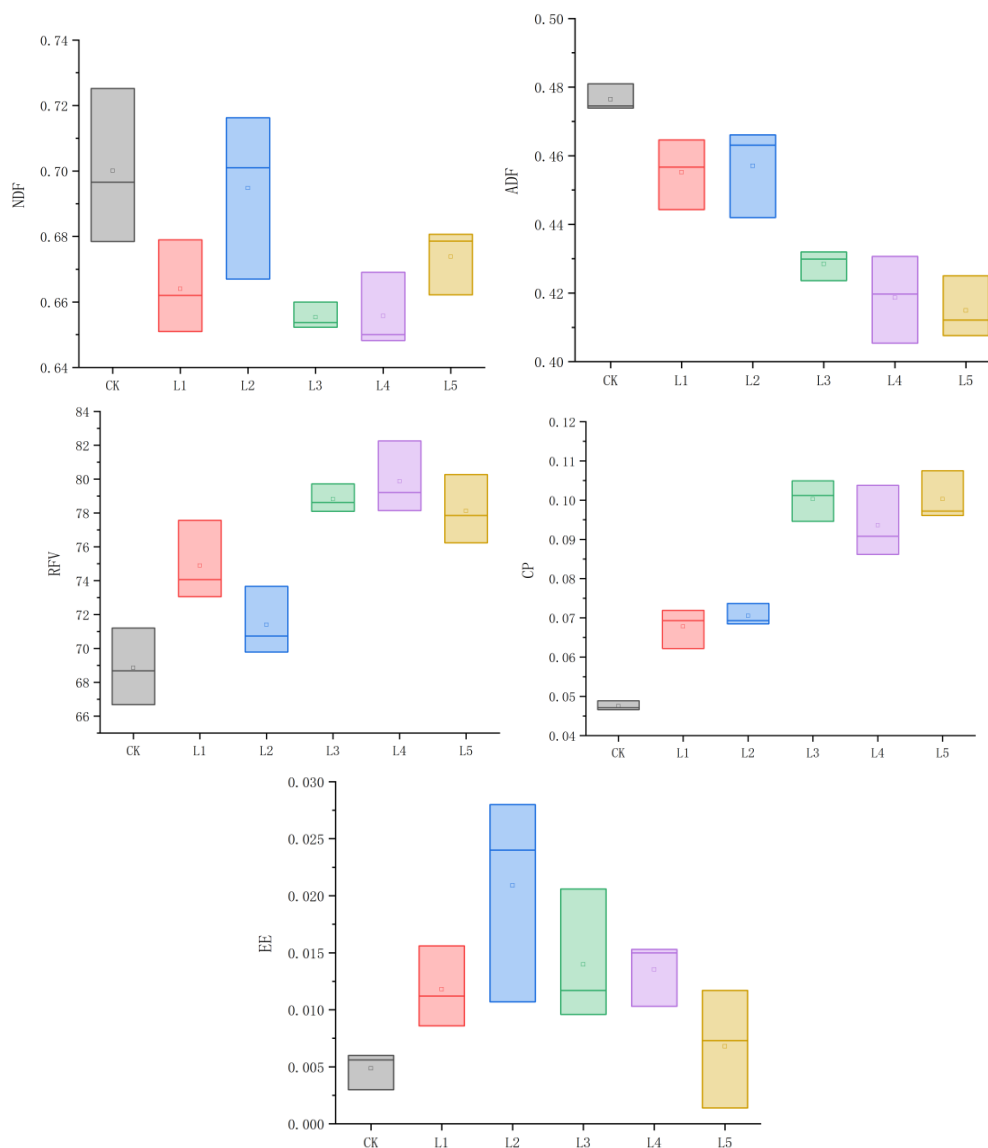


Fig. 1: Effects of different additive treatments on forage quality of rice straw silages

Note: CP = crude protein; EE = ether extract; ADF = acid detergent fiber; NDF = neutral detergent fiber; RFV = relative feed value. Values with different letters indicate significant differences ($P < 0.05$)

All treatments significantly increased Crude Protein (CP) content compared to the control ($P < 0.05$); the addition of wheat bran and EM bacteria promoted rapid lactobacilli fermentation, creating an acidic environment that inhibited undesirable microorganisms and reduced nutrient loss. Treatments L3, L4, and L5 showed the highest CP levels (11.18-11.68% DM), representing a 105-110% increase over CK, which demonstrates that ammonium nitrate (present in these three treatments) effectively enhanced protein content in the silage. It should be noted that CP values in treatments containing ammonium nitrate (L3, L4, L5) include both true protein and Non-Protein Nitrogen (NPN). The total nitrogen content increased due to added ammonium nitrate, which is captured in the CP calculation ($N \times 6.25$). L3 demonstrated the most dramatic increase (110% over CK), while L4 showed slightly lower improvement, possibly because *Aspergillus oryzae* utilized some nitrogen while decomposing fiber and synthesizing microbial biomass, as nitrogen availability substantially affects fungal growth behavior and nutrient assimilation [15].

Ether Extract (EE) content increased significantly across all treatments ($P < 0.05$), with L2 showing the highest level (2.09%) over four times that of the control. While EE is not a major component of rice straw, its measurement provides insight into lipid preservation during fermentation [16]. The significant increase in EE across treatments, particularly in L2, suggests that rapid pH reduction by EM bacteria effectively inhibited lipolytic enzyme activity, thereby preserving lipid content that would otherwise be degraded during poor-quality fermentation. This suggests that EM bacteria alone (L2) promoted lipid preservation by rapidly reducing pH and inhibiting lipolytic enzyme activity, without requiring additional nitrogen or fungal additives.

All treatments significantly reduced both Acid Detergent Fiber (ADF) and Neutral Detergent Fiber (NDF) contents compared to the control ($P < 0.05$). Treatments L3, L4, and L5 achieved the lowest fiber contents. L5 showed the lowest ADF value (41.49% DM), representing a 12.6% reduction from CK. NDF was reduced by 5.13-6.40% across treatments L3-L5, with L3 and L4 achieving the greatest reductions (6.40% and 6.34%, respectively), while L5 showed a smaller decrease (5.13%). The smaller NDF reduction in L5 compared to L4 possibly suggests complex interactions between *T. longibrachiatum* and *A. oryzae* that warrant further investigation. This fiber reduction corresponds with significantly higher Relative Feeding Value (RFV) in these three treatments ($P < 0.05$), indicating enhanced digestibility and nutritional quality. The combined supply of fermentable substrate (wheat bran) and nitrogen (ammonium nitrate) may have enhanced fungal metabolic activity and biomass-associated fiber transformation, as observed in fungal fermentation systems under varying carbon and nitrogen regimes [17,18].

Effects of Different Additive Treatments on Fermentation Characteristics of Rice Straw Silage

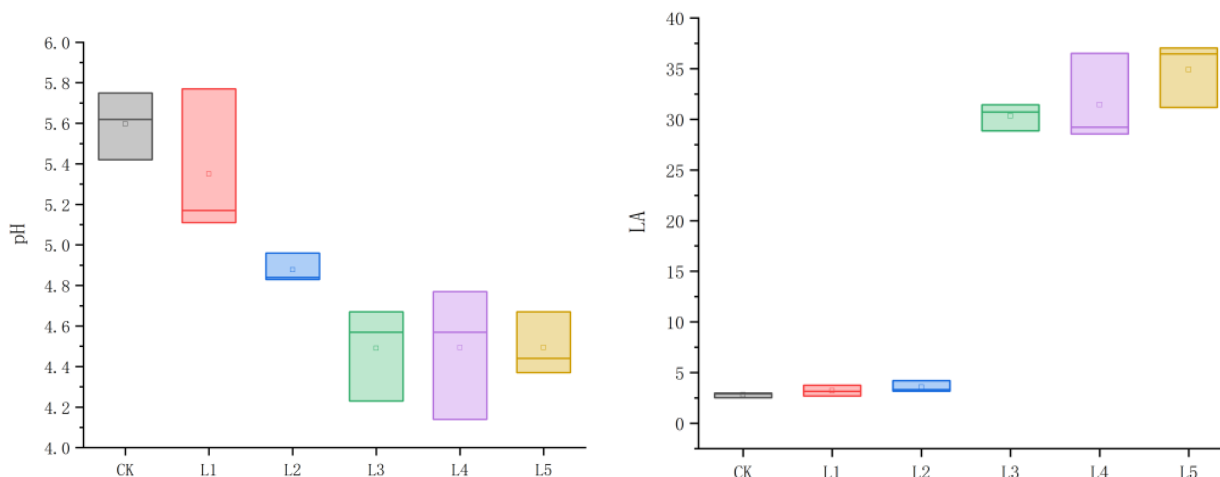
The fermentation quality was variably improved by different additive treatments (Figure 2).

pH values decreased in all treatments compared to the control, with treatments L2-L5 showing significant reductions ($P < 0.05$). L5 achieved the lowest pH (4.09), indicating the most efficient acid production. This pH range (4.09-4.31) for treatments L3-L5 is optimal for preventing undesirable microbial activity while maintaining nutrient preservation.

Lactic acid (LA) content increased across all treatments, with treatments L3, L4, and L5 showing significantly higher levels (29.83-34.88 g·kg⁻¹ DM) than both CK (12.82 g·kg⁻¹ DM) and the L1-L2 treatments ($P < 0.05$), representing dramatic increases of 133-172%. L5 achieved the highest LA content (34.88 g·kg⁻¹), suggesting that the combination of both fungal species with EM bacteria and ammonium nitrate created optimal conditions for lactic acid production.

Acetic Acid (AA) content varied among treatments, with L4 showing a significant increase (70% higher than CK) and L5 showing a significant decrease (16% lower than CK) ($P < 0.05$). The dramatic variation in AA content among treatments reflects different metabolic pathways: heterofermentative lactic acid bacteria and fungal metabolism in L4 produced more AA, while *T. longibrachiatum* in L5 may have metabolized AA as a carbon source. However, this interpretation remains speculative in the absence of direct measurements of fungal growth or enzyme activity.

Propionic Acid (PA) content increased significantly in all treatments ($P < 0.05$), with L5, L2, and L1 showing the highest levels. The elevated PA in these treatments may contribute to improved aerobic stability of the silage upon exposure to air.



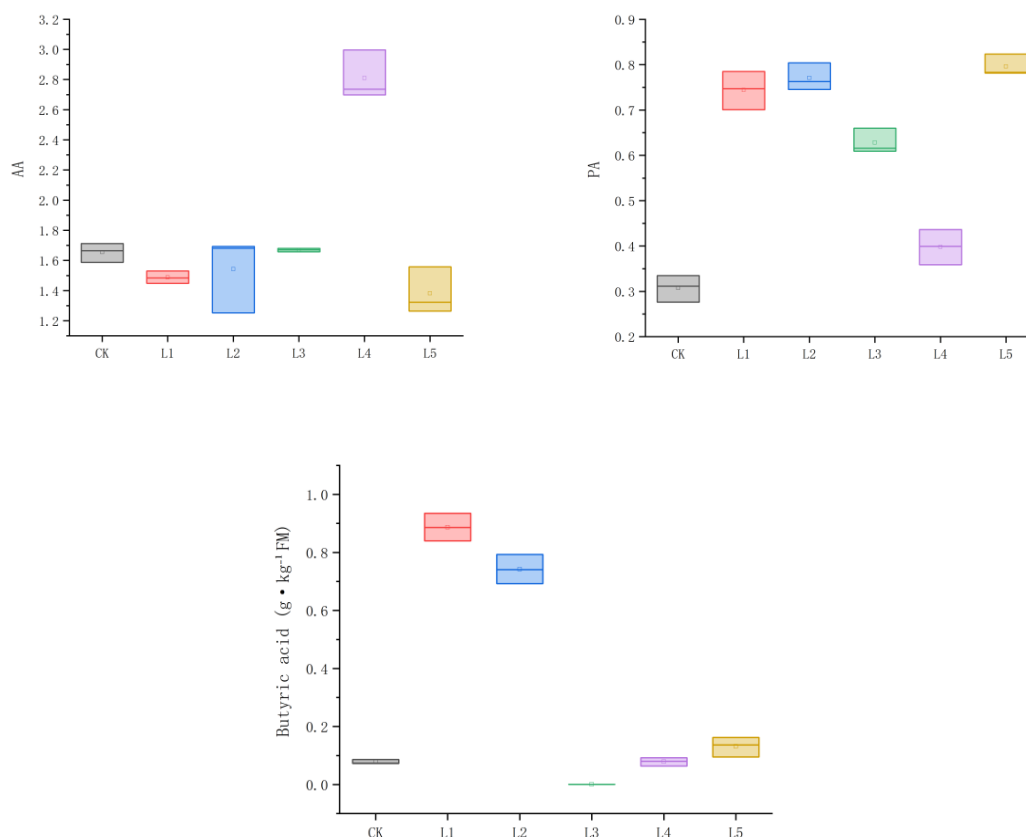


Fig. 2: Effects of different additive treatments on fermentation quality of rice straw silages

Note: LA = lactic acid; AA = acetic acid; PA = propionic acid; BA = butyric acid; $\text{NH}_3\text{-N}$ = ammoniacal nitrogen. Values with different letters indicate significant differences ($P < 0.05$)

Most notably, Butyric Acid (BA) an indicator of undesirable clostridial fermentation was completely eliminated in treatments L3 and L4, and reduced by 85% in L5 compared to the control. This absence of butyric acid corresponds with the excellent sensory scores and indicates high-quality fermentation in these treatments.

Overall, treatments L3, L4, and L5 consistently demonstrated superior fermentation characteristics, with L4 (containing ammonium nitrate and *A. oryzae*) showing the optimal balance of desirable acids, complete elimination of butyric acid, and the lowest ammonium nitrogen content, suggesting the most efficient fermentation process.

Discussion

Effect of Additive Treatments on Sensory Evaluation

Bran alone (L1) produced no significant improvement, suggesting rice straw lacks sufficient native fermenting bacteria. While L1 and L2 achieved "good" quality, L3-L5 reached "excellent" standards. This indicates that optimal EM bacterial fermentation requires both nitrogen and carbohydrate sources [10, 19]. Among nitrogen-containing treatments, L4 and L5 significantly outperformed L3, confirming that fungal additives (*Aspergillus oryzae* and *Trichoderma longibrachiatum*) enhance fermentation by suppressing harmful bacteria and improving enzymatic degradation of complex carbohydrates.

Effects of Additive Treatments on Nutritional Quality of Rice Straw Silage

All treatments significantly increased Relative Feeding Value (RFV) compared to the control ($P < 0.05$). L4 achieved the highest RFV (79.86), closely followed by L5 (79.29) and L3 (78.81), indicating that these combinations of additives most

effectively improved feed quality. The slightly lower RFV in L5 compared to L4 suggests some complex interactions between *T. longibrachiatum* and *A. oryzae*.

The significantly lower ammonium nitrogen ($\text{NH}_3\text{-N}$) content in L4 compared to L3 and L5 suggests effective incorporation of inorganic nitrogen into microbial biomass. This indicates that the added ammonium nitrate served as a substrate for microbial protein synthesis rather than remaining as free ammonia. Therefore, the elevated CP in L4 represents not merely NPN supplementation but actual improvement in true protein content through enhanced microbial growth and activity. The nitrogen availability in L4 likely promoted *A. oryzae* biomass formation and metabolic activity, as C/N ratio and nitrogen sources substantially influence fungal growth dynamics and nutrient assimilation patterns [15].

Our results align with [7], who reported that *Aspergillus* inoculation reduced NDF by 8-12% in rice straw silage, comparable to our 6.34% reduction in L4. However, unlike [6], who found *Trichoderma viride* beneficial when combined with wheat bran and LAB, our L5 treatment showed diminished benefits compared to L4, suggesting species-specific effects between *T. longibrachiatum* and *A. oryzae*. Additionally, the absence of a treatment containing *A. oryzae* without ammonium nitrate (e.g., L2 + *A. oryzae*) limits our ability to definitively separate the direct enzymatic effects of *A. oryzae* from potential synergistic interactions with supplemental nitrogen. This represents a limitation in the experimental design that should be addressed in future studies to fully understand the mechanisms underlying the superior performance of L4.

The less conclusive effects of *T. longibrachiatum* in L5 may be related to its ligninolytic activity. While *T. longibrachiatum* is known for lignin degradation capacity, we did not measure lignin content or specific enzyme activities (cellulase, xylanase, ligninase) in this study. Future work should include lignin quantification and enzyme assays to elucidate the mechanistic basis for the observed differences between *A. oryzae* and *T. longibrachiatum* treatments. From a practical perspective, the multi-additive approach in L4 demonstrated higher economic potential than L3 by significantly improving the Relative Feeding Value (RFV) by over 15%, which may reduce the need for expensive concentrate supplements in beef cattle diets.

Effects of Additive Treatments on Fermentation Quality

pH is a crucial indicator of silage fermentation quality, with lower values inhibiting harmful microorganisms and preserving nutrients. All treatment groups showed significantly lower pH than the control, consistent with previous findings on lactic acid bacteria additions to agricultural residues [20, 21].

Lactic Acid (LA) content increased in all treatments, with L3, L4, and L5 showing significantly higher levels than both CK and the L1-L2 treatments ($P < 0.05$). This demonstrates that optimal fermentation requires adequate nitrogen and carbon sources along with effective microbial inoculants. The limited improvement in L1 and L2 suggests that carbon source and EM bacteria alone are insufficient without adequate nitrogen.

Acetic Acid (AA) inhibits fungal growth and enhances aerobic stability in silage [22, 23]. AA content varied among treatments: L3 and L4 increased by 1.20% and 70.30%, respectively, compared to CK, while L5 decreased by 16% with the addition of *T. longibrachiatum*. L4 showed significantly higher AA content than L5, which may theoretically enhance aerobic stability, as acetic acid is known to inhibit yeast and mold growth. However, this hypothesis requires validation through aerobic stability testing. The suggestion that *T. longibrachiatum* metabolized acetic acid in L5 remains speculative without direct measurements of fungal growth, enzyme activity, or substrate utilization patterns. Future research should include microbial enumeration and enzyme assays to substantiate metabolic interpretations.

Important limitations of this study must be acknowledged:

- (1) The absence of a treatment containing *A. oryzae* without ammonium nitrate (e.g., L2 + *A. oryzae*) prevents definitive conclusions about whether the improvements in L4 over L3 are solely attributable to fungal enzymes or require nitrogen-enzyme synergy
- (2) Lignin content and specific enzyme activities (cellulase, xylanase, ligninase) were not measured, limiting mechanistic understanding of fungal effects
- (3) Aerobic stability was not assessed, despite theoretical implications from acetic acid differences
- (4) Dry matter recovery and microbial enumeration (lactic acid bacteria, yeasts, molds) were not conducted, which would provide a more complete profile of silage quality and storage characteristics

A limitation of our experimental design is the absence of a treatment containing *A. oryzae* without ammonium nitrate (e.g., L2 + *A. oryzae*). This control would definitively separate the direct effects of *A. oryzae* from potential interactions with supplemental nitrogen. Future studies should include this treatment to clarify whether the improvements in L4 over L3 are solely attributable to fungal enzymes or require nitrogen-enzyme synergy.

This study focused on fermentation and nutritional outcomes after 45 days of ensiling. Future research should include measurements of dry matter recovery, aerobic stability (measured as temperature rise upon air exposure), and microbial enumeration (lactic acid bacteria, yeasts, molds) to provide a complete profile of silage quality and storage characteristics.

For practical farm-scale application, several factors must be considered:

- (1) Storage and handling: EM bacteria and fungal inoculants require refrigeration and have limited shelf life
- (2) Cost-effectiveness: the multi-additive approach (L4) must demonstrate economic returns through improved animal performance to justify additional input costs
- (3) Scalability: the mixing and application procedures described here (small-scale bags) need validation in large-scale bunker silos or bale wrapping systems
- (4) Seasonal variation: Rice straw quality varies with harvest conditions, potentially affecting optimal additive ratios

While L4 demonstrated superior fermentation and nutritional quality, the economic viability of this multi-additive approach compared to simpler formulations (e.g., L3) requires formal cost-benefit analysis. The additional cost of *A. oryzae* inoculant must be weighed against the incremental improvements in RFV, fiber reduction, and fermentation quality [24]. Such economic analysis should consider not only additive costs but also potential improvements in animal performance (feed intake, digestibility, production) that would justify the investment. Future research should include on-farm trials and economic analysis to facilitate technology transfer.

Conclusion

Based on the comparative analysis between treatment groups and the control, the following conclusions can be drawn:

The addition of wheat bran, EM bacteria, ammonium nitrate, *Aspergillus oryzae*, and *Trichoderma longibrachiatum* significantly enhanced both nutritional and fermentation qualities of rice straw silage, effectively transforming low-value agricultural residue into high-quality feed.

Optimal fermentation required the synergistic action of multiple additives. The combination of soluble carbohydrates (wheat bran), EM bacteria, and nitrogen source (ammonium nitrate) significantly improved silage quality, with *A. oryzae* providing additional enhancement. Under the experimental conditions of this study, *T. longibrachiatum* did not provide additional benefits beyond the L4 treatment and is not recommended for rice straw silage under these conditions. The lack of improvement with *T. longibrachiatum* may be due to competitive interactions with *A. oryzae*, suboptimal environmental conditions for its ligninolytic activity, or insufficient ensiling duration for lignin degradation. Further investigation with modified conditions (longer ensiling periods, different inoculation rates, or sequential inoculation strategies) is warranted before definitive conclusions can be drawn about its utility in rice straw silage systems. The effects of *T. longibrachiatum* were mixed and warrant further investigation.

Among all treatments tested, the most effective formulation was the L4 treatment containing 200 g/kg wheat bran, 1 g/kg EM bacteria, 20 g/kg ammonium nitrate, and 1 g/kg *A. oryzae* (all on a fresh matter basis), which yielded the highest overall quality improvements in rice straw silage.

From a practical perspective, while L4 demonstrated the best overall performance, economic considerations are important for on-farm adoption. The cost of L4 (multiple additives including fungal species) should be evaluated against simpler formulations like L3. Future studies should conduct cost-benefit analysis considering additive costs, labor, and the economic value of improved feed quality.

For practical on-farm application, we recommend:

- (1) Chop rice straw to 1-2 cm length

- (2) Add 200 g/kg wheat bran on fresh matter basis
- (3) Dissolve all microbial and chemical additives in water and spray uniformly
- (4) Compact tightly and seal to ensure anaerobic conditions
- (5) Store for a minimum 45 days before feeding

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Author's Contributions

Bin Yang: Designed the experiment, collated the data, and prepared the paper.

Jiahuan Liu and Dongmei Ma: Performed the experiment, analyzed the data, and wrote the paper.

Lingyu Li, Rundong Qin, and Junhao Li: Participated in the experiment, collated the data, and revised the manuscript.

Lie Yang: Supervised the research, provided funding, and revised the manuscript.

Ethics

The authors assume full responsibility for any ethical dilemmas that may emerge following the manuscript's publication.

Conflict of Interest

The authors declare that they have no competing interests.

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