Fermentation Conditions of Pleurotus eryngii Spent Mushroom Substrate Feed and Its Feeding Effects on Goats (Postprint)

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Abstract

This experiment aimed to investigate the optimal fermentation conditions for a composite fermentation substrate of Pleurotus eryngii spent mushroom substrate with soybean meal and wheat bran, and to study its feeding effects on goats. Experiment 1 used Pleurotus eryngii spent mushroom substrate as the main raw material, with different proportions of wheat bran and soybean meal added (4% and 4%, 4% and 2%, 2% and 4%, 2% and 2%). The fermentation inoculant used was Effective Microorganisms (EM) stock solution, the moisture content of the fermentation mixture was adjusted to approximately 30%, and after 12 days of anaerobic fermentation at room temperature, the fermentation quality and nutritional composition of the fermented Pleurotus eryngii spent substrate feed were evaluated, and the aflatoxin B1 content was measured. The optimal combination was selected for the feeding trial. Experiment 2 selected 60 Matou goats (wethers), which were randomly divided into 5 groups, including 3 treatment groups and 2 control groups. The proportions of fermented Pleurotus eryngii spent substrate feed in the diets of the treatment groups were 30%, 45%, and 60%, respectively, while the proportions of unfermented Pleurotus eryngii spent substrate in the control groups were 30% and 60%, respectively. Each group had 3 replicates, with 4 goats per replicate. The experimental period was 45 days, including a 5-day preliminary period and a 40-day formal experimental period. The results showed: 1) The fermented Pleurotus eryngii spent substrate feed with soybean meal and wheat bran addition ratios of 4% and 2% had the best fermentation quality and lower aflatoxin B1 content. Compared with unfermented Pleurotus eryngii spent substrate, the contents of crude fiber, neutral detergent fiber, and acid detergent fiber decreased by 8.78%, 11.56%, and 18.88%, respectively, while the contents of crude protein and crude ash increased by 19.32% and 8.29%, respectively, with aflatoxin B1 content as low as 3.03 g/kg. 2) There were no significant differences in production performance among the goat groups (P>0.05). With the increase in the proportion

of fermented Pleurotus eryngii spent substrate feed, the body weight gain and average daily gain of the experimental goats showed a decreasing trend, while the feed conversion ratio and feed cost showed an increasing trend. Compared with the 30% unfermented Pleurotus eryngii spent substrate addition, the 30% fermented Pleurotus eryngii spent substrate feed addition improved body weight gain, average daily gain, and dry matter intake, and reduced the feed conversion ratio. The results suggest that using EM stock solution to ferment Pleurotus eryngii spent mushroom substrate, adding 4% soybean meal and 2% wheat bran to the fermentation substrate helps improve fermentation quality, and the optimal inclusion proportion of fermented Pleurotus eryngii spent substrate feed in goat diets is 30%.

Full Text

Fermentation Condition of Pleurotus eryngii Waste Sticks Feed and Its Feed Effects on Goats

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Abstract

This study aimed to explore suitable fermentation conditions for Pleurotus eryngii waste sticks (PEWS) mixed with soybean meal and bran, and to investigate the feeding effects on Matou goats. In Test 1, PEWS served as the main raw material supplemented with different ratios of bran and soybean meal (4% and 4%, 4% and 2%, 2% and 4%, and 2% and 2%). Effective microorganisms (EM) original liquid was used as the fermentation agent, and the moisture content of the fermentation mixture was adjusted to approximately 30%. After 12 days of anaerobic fermentation at room temperature, fermentation quality, nutritional components, and aflatoxin B (AFB) content were evaluated to select the optimal combination for the feeding trial. In Test 2, sixty Matou goats (wethers) with a body weight of (27.98±4.95) kg were randomly divided into five groups, including three experimental groups and two control groups. The experimental groups received diets with fermented PEWS feed at 30%, 45%, and 60%, while the control groups received unfermented PEWS at 30% and 60%. Each group had three replicates with four goats per replicate. The trial lasted 45 days, comprising a 5-day pre-test period and a 40-day formal test period.

The results showed: (1) Fermented PEWS feed with bran and soybean meal supplementation at 4% and 2% achieved the best fermentation quality with lower AFB content. Compared with unfermented PEWS, crude fiber, neutral detergent fiber (NDF), and acid detergent fiber (ADF) contents decreased by 8.78%, 11.56%, and 18.88%, respectively, while crude protein and crude ash contents increased by 19.32% and 8.29%, respectively. The AFB content was as low

as 3.03 g/kg. (2) No significant differences were observed in goat performance among groups (P>0.05). However, with increasing fermented PEWS feed supplementation, body weight gain (BWG) and average daily gain (ADG) tended to decrease, while feed-to-gain ratio (F/G) and feed cost tended to increase. Compared with 30% unfermented PEWS supplementation, the 30% fermented PEWS feed group showed higher BWG, ADG, and dry matter intake (DMI), with lower F/G. In conclusion, fermenting PEWS with EM original liquid and supplementing 4% soybean meal and 2% bran improved fermentation quality, and the optimal supplementation level of fermented PEWS feed in goat diets was 30%.

Keywords: Pleurotus eryngii waste sticks; fermented feed; Matou goat; performance

Introduction

The scarcity and rising cost of feed ingredients have become critical factors constraining the development of animal husbandry, with the situation worsening in recent years [1]. Therefore, developing novel feed resources is urgently needed. As a major producer of edible mushrooms, China generates approximately 2.5 times the amount of mushroom substrate waste as mushroom output annually. According to statistics, national edible mushroom production reached 2.57×10 tonnes in 2016, yielding about 6.43×10 tonnes of spent mushroom substrate. However, due to limited awareness and technology for resource reuse, most enterprises discard or incinerate mushroom waste, causing resource waste and environmental pollution. Thus, developing technologies for rational utilization of mushroom substrate waste, particularly for feed applications, holds significant ecological importance and market potential.

Mushroom substrate feed is produced from medium-to-high quality spent substrate through appropriate biological treatment and mechanical processing. Numerous studies have demonstrated that adding mushroom substrate feed to ruminant diets yields higher economic benefits than conventional feeds such as corn stalk [2], corn [3], dry rice straw [4], alfalfa hay [5], wheat straw [6], and corn cob [7]. However, mushroom substrate primarily consists of high-fiber materials like wood chips, cottonseed hulls, corn cobs, and agricultural straw, resulting in poor palatability and low digestibility that hinder its utilization [8]. Microbial fermentation effectively addresses these issues by employing enzymatic biodegradation to break down cellulose and hemicellulose in PEWS, improving softness and expansion while producing easily digestible nutrients such as sugars, amino acids, and vitamins that enhance feed value [9-10].

PEWS shows great potential for feed application, with protein content and amino acid composition similar to corn, and crude fiber content comparable to roughage, making it an excellent "neutral" feed. As an inexpensive, easily developed unconventional feed, PEWS is available year-round without seasonal

constraints. This study utilized EM original liquid to ferment PEWS mixed with soybean meal and bran to address palatability issues and high fiber content. Additionally, different inclusion levels of fermented PEWS feed were used in Matou goat diets to investigate effects on performance, providing technical support for developing ruminant feed resources and efficient mushroom waste utilization.

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1. Materials and Methods

1.1 Experimental Materials PEWS were provided by Shaanxi Chinese Mushrooms Tech. Industry Garden Co., Ltd., with corn cob as the main component. Bran, soybean meal, salt, and brown sugar were purchased from a market in Yangling, Shaanxi. Nutrient levels of fermentation raw materials are shown in Table 1 . The fermentation agent was a commercial EM original liquid (containing lactic acid bacteria, yeast, bacillus, actinomycetes, and photosynthetic bacteria, all with viable counts 1×10 CFU/mL).

1.2 Experimental Design

1.2.1 Test 1—PEWS Fermentation Trial Bran and soybean meal were added at dry matter ratios of 4% and 4%, 4% and 2%, 2% and 4%, and 2% and 2%, respectively. EM original liquid was used as the fermentation agent at an inoculation rate of 0.16% (dry matter basis). Fermentation raw materials were mixed with EM solution in a feed mixer, moisture was adjusted to approximately 30%, and the mixture was sealed in bags (1 kg per bag) with three replicates per treatment. After 12 days of fermentation at room temperature (30±2)°C, fermentation quality was evaluated to select the optimal substrate combination for producing fermented PEWS feed for the goat feeding trial.

1.2.2 Test 2—Feeding Trial The feeding trial was conducted at Baoji Yulong Ecological Breeding Cooperative from April 23 to June 6, 2017. The 45-day trial included a 5-day pre-test period and a 40-day formal test period. Sixty Matou goats (wethers) weighing (27.98±4.95) kg were randomly divided into five groups according to the principle of homogeneity, including three experimental groups and two control groups, with three replicates per group and four goats per replicate.

Experimental diets were formulated according to the *Feeding Standard of Meat Sheep* (NY/T 816-2004). While maintaining consistent crude protein and energy levels across groups, experimental groups received fermented PEWS feed at 30%, 45%, and 60%, while control groups received unfermented PEWS at 30% and 60%. Diets were prepared as total mixed pellets. Diet composition and nutrient levels are shown in Table 2 .

Before the trial, goats were numbered, dewormed, vaccinated, and pens were cleaned and disinfected. Goats were housed indoors under identical management conditions and fed by replicate groups. Feed was offered twice daily at 08:00 and 17:00 *ad libitum* with automatic waterers. Residual feed was weighed before the next feeding to determine feed intake.

1.3 Measurement Indicators and Methods

1.3.1 Fermentation Quality Evaluation of Fermented PEWS Feed

1.3.1.1 Sensory Evaluation

Following silage quality evaluation methods, sensory assessment of fermented PEWS feed was conducted based on color, odor, and softness [11].

1.3.1.2 Fermentation Characteristics and Nutritional Analysis

Three grams of fresh fermented feed sample were mixed with 30 mL deionized water, extracted for 40 minutes, and pH was measured with a pH meter. Lactic acid (LA) content was determined by the hydroxydiphenyl colorimetric method [12-13]. Dry matter recovery (DMR) and gas loss rate (GL) were calculated as follows:

DMR (%) = (Dry matter content of sample / Dry matter content of sample) × 100
GL (%) = (Mass of sample after fermentation / Dry matter content of sample) × 100

After fermentation, samples were dried in a forced-air oven at 65°C to constant weight, equilibrated for 24 hours, ground through a 40-mesh sieve, and stored. Dry matter content was determined using a moisture analyzer. Crude fiber was measured according to GB/T 6434-1994, ADF according to NY/T 1459-2007, NDF according to GB/T 20806-2006, hemicellulose calculated as the difference between NDF and ADF, crude ash according to GB/T 6438-2007, crude protein according to GB/T 6432-1994, water-soluble carbohydrates by sulfuric acid-anthrone colorimetry [14], and organic matter calculated as the difference between dry matter and crude ash.

1.3.1.3 Aflatoxin B (AFB) Determination

AFB content was detected using an ELISA rapid test kit from Beacon Company (USA) following the manufacturer' s instructions, with a Multiskan-FC microplate reader from Thermo Fisher Scientific (USA).

1.3.2 Goat Performance Measurement Body weight gain (BWG) was calculated as the difference between initial and final weights measured on fasting goats on the first and last days of the formal test period. Average daily gain (ADG) was BWG divided by trial days. Feed-to-gain ratio (F/G) was dry matter intake (DMI) divided by BWG. Feed cost per kg gain was F/G multiplied by feed price per kg.

1.4 Data Analysis Data were analyzed using one-way ANOVA with SPSS 20.0 software. Duncan' s multiple comparison test was used to detect significant differences, with $P<0.05$ as the significance threshold.

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2. Results

2.1 Sensory Evaluation of Fermented PEWS Feed Fermented PEWS feed exhibited a dark yellow color, while unfermented substrate appeared whitish. No mold was observed in fermented PEWS feed, which had a loose texture, non-sticky feel, and rich aroma of alcohol and lactic acid.

2.2 Fermentation Characteristics and Nutritional Analysis of Fermented PEWS Feed As shown in Table 3 , bran and soybean meal ratios had no significant effect on pH ($P>0.05$). DMR was significantly higher when bran and soybean meal ratios were 4% and 4% or 2% and 2% compared with other groups ($P<0.05$). GL was significantly higher at the 4% and 2% ratio than other groups ($P<0.05$). Lactic acid content was significantly higher at 4% and 4% or 2% and 4% ratios compared with the 2% and 2% ratio ($P<0.05$).

Crude fiber, NDF, and ADF contents were significantly lower at 4% and 4% or 4% and 2% ratios than other groups ($P<0.05$). Hemicellulose content was significantly lower at the 2% and 2% ratio than other groups ($P<0.05$). Crude ash content was significantly higher at 4% and 2% or 2% and 2% ratios than other groups ($P<0.05$). Crude protein content was significantly higher at 4% and 4% or 2% and 4% ratios than the other two groups ($P<0.05$). Organic matter content was significantly higher at the 2% and 4% ratio than other groups ($P<0.05$). Water-soluble carbohydrate content was significantly higher at 2% and 4% or 2% and 2% ratios than the other two groups ($P<0.05$).

2.3 Feed Hygiene Test Results of Fermented PEWS Feed As shown in Table 4 , AFB contents in all fermented PEWS feed samples were within the range required by the national *Feed Hygiene Standard* GB 13078-2017 (10-20 g/kg). Bran and soybean meal ratios had no significant effect on AFB content ($P>0.05$).

Considering all factors, fermented PEWS feed with 4% soybean meal and 2% bran showed the best fermentation quality with lower AFB content. Therefore, this formulation and fermentation process were selected for producing feed for the goat feeding study.

2.4 Effects of Fermented PEWS Feed on Goat Performance As shown in Table 5 , no significant differences were observed in goat performance among groups ($P>0.05$). However, with increasing fermented PEWS feed supplementation, BWG and ADG tended to decrease while F/G tended to increase. The 30% fermented PEWS feed group showed higher BWG, ADG, and DMI, and

lower F/G compared with the 30% unfermented PEWS group, though differences were not significant (P>0.05). Feed costs also did not differ significantly (P>0.05). The 60% fermented PEWS feed group showed lower BWG, ADG, and DMI, and higher F/G compared with the 60% unfermented PEWS group, with no significant differences (P>0.05) and no significant difference in feed cost (P>0.05).

Overall, supplementation of 30% fermented PEWS feed in the diet achieved better feeding effects.

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3. Discussion

3.1 Effects of Compound Fermentation on PEWS Feed Quality Spent mushroom substrate is the solid culture medium discarded after mushroom production, composed of mycelium and agricultural wastes such as cottonseed hulls, wood chips, and straw, with high organic matter content and complete nutrients [15]. Through enzymatic biodegradation, the flavor and nutritional value of mushroom substrate improve after fermentation [16]. Previous studies have investigated mushroom substrate fermentation processes. Ban et al. [17] fermented *Ganoderma lucidum* substrate with *Bacillus subtilis* and *Candida utilis*, achieving 47.20% lignocellulose degradation and increasing crude protein from 6.12% to 13.84%. Li et al. [18] optimized fermentation conditions for *Pleurotus nebrodensis* substrate: yeast to lactic acid bacteria ratio of 1:3, 3% inoculum, and 15 days at 35°C. Xu et al. [19] used 88% PEWS, 2% urea, and 10% bran, fermented sequentially with *Ganoderma lucidum* and *Saccharomyces cerevisiae* for 11 days at 28°C, pH natural, and water-to-material ratio of 2:1, increasing true protein and crude polysaccharide contents by 68.54% and 132.12%, respectively. Li et al. [20] reported that substrate to cornmeal ratio of 90:10 with lactic acid bacteria to yeast ratio of 1:3 at 28°C yielded 18.24% crude protein.

Our results showed that fermented PEWS feed with 4% soybean meal and 2% bran achieved the best fermentation quality with lower AFB content. Compared with pre-fermentation, crude fiber decreased by 8.78%, NDF by 11.56%, ADF by 18.88%, crude protein increased by 19.32%, crude ash increased by 8.29%, and AFB content was as low as 3.03 g/kg. The mushroom production process consumes some carbon and nitrogen sources from the substrate; supplementing appropriate ratios of soybean meal and bran replenishes these nutrients, promoting microbial growth and accelerating fermentation [21]. The fermentation process involves multiple live microorganisms and their metabolites, creating a rich, mellow flavor that improves palatability [22]. Lactic acid produced during fermentation inhibits harmful bacteria, reducing *Aspergillus flavus* growth and effectively suppressing AFB production.

3.2 Effects of Fermented PEWS Feed on Goat Performance Mushroom substrate contains high levels of cellulose and lignin from wood chips and

corn cobs, resulting in poor palatability, reduced feed intake, and impaired performance. Fermentation with EM original liquid improves PEWS flavor, reduces fiber hardness, enhances goat appetite, and increases productivity. Previous studies have reported varying optimal inclusion levels of mushroom substrate feed for fattening sheep. Cheng et al. [23] found that adding 20% PEWS to Boer crossbred goat diets increased profit by 3.3 yuan per goat. Fazaeli et al. [24] reported that feeding wheat straw substrate at less than 20% to meat sheep did not significantly affect ADG, DMI, or digestibility. Li et al. [3] replaced corn in finishing sheep concentrate with 5%-15% oyster mushroom substrate, achieving optimal weight gain and economic benefits. Li et al. [25] improved meat quality in Boer goats with 20% PEWS supplementation. Guo et al. [26-27] reported ADG of 122-137 g/d in Boer goat kids fed 12%-20% fermented PEWS feed, with 10% enoki mushroom substrate showing optimal weight gain and economic returns. Lu et al. [28] found that 50% fermented mushroom substrate was suitable for fattening Boer crossbred goats.

Our study showed that although weight gain differences were not significant among groups, goat performance decreased with increasing fermented PEWS feed supplementation, indicating that inclusion levels should not be too high. At the optimal inclusion level (30%), fermented PEWS feed produced higher ADG and BWG and lower F/G than unfermented PEWS, consistent with findings by Liu et al. [6] and Guo et al. [29]. This may be attributed to: (1) improved conversion of mineral elements through probiotic fermentation, which combines with proteins, amino acids, and polysaccharides in organic forms that are easily absorbed with high bioavailability [30-31]; and (2) beneficial bacteria in fermented feed multiplying in the animal digestive tract, producing various digestive enzymes that enhance degradation of fiber and other macromolecules while inducing endogenous enzyme secretion, thereby improving feed conversion efficiency [32]. Effects of fermented PEWS feed on apparent digestibility in goats will be reported separately.

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4. Conclusion

1. Fermenting PEWS with EM original liquid and supplementing 4% soybean meal and 2% bran improved fermentation quality.
2. Supplementing 30% fermented PEWS feed in goat diets achieved satisfactory feeding effects.

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