Effects of White-Rot Fungi on Nutritional Value and Antioxidant Properties of Corn Stover (Post-print)

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Date: 2017-11-08T00:00:00+00:00

Abstract

This experiment aimed to investigate the effects of white-rot fungi on the nutritional value and antioxidant capacity of corn straw. Four species of Pleurotus white-rot fungi were selected: Pleurotus diamor, Pleurotus citrinopileatus, Pleurotus eryngii, and Pleurotus sajor-caju. The control group consisted of uninoculated corn straw, while the experimental groups were inoculated with each of the four Pleurotus species, with three replicates per group. Following 20 days of solid-state fermentation, the nutrient composition and in vitro dry matter digestibility (IVDMD) of the fermented products were determined, and their antioxidant capacity was evaluated. The results showed: 1) The total organic matter (TOM) loss rate in the Pleurotus diamor group was significantly higher than that in the Pleurotus citrinopileatus and Pleurotus eryngii groups ($P<0.05$). The acid detergent lignin (ADL) loss rate in the Pleurotus sajor-caju group was significantly higher than that in all other groups ($P<0.05$). The cellulose (CL) loss rate in the Pleurotus citrinopileatus group was significantly higher than that in the Pleurotus eryngii and Pleurotus sajor-caju groups ($P<0.05$). The hemicellulose (HC) loss rate in the Pleurotus citrinopileatus group was significantly higher than that in all other groups ($P<0.05$). 2) The crude protein (CP) content in the Pleurotus citrinopileatus group was significantly higher than that in the control group ($P<0.05$). The ether extract (EE) content in the Pleurotus citrinopileatus, Pleurotus sajor-caju, and Pleurotus diamor groups was significantly higher than that in the control group ($P<0.05$). The total amino acid content in all four white-rot fungi groups was significantly higher than that in the control group ($P<0.05$). The IVDMD of all four white-rot fungi groups was significantly higher than that of the control group ($P<0.05$). 3) The 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging capacity, reducing power, total antioxidant capacity, and total phenolic content in all four white-rot fungi groups were significantly higher than those in the control group ($P<0.05$). These results indicate that corn straw pretreated with white-rot fungi

can improve its IVDMD, CP content, and antioxidant capacity, and can serve as a novel feed resource for ruminants.

Full Text

Effects of White Rot Fungi on Nutritional Value and Antioxidant Properties of Corn Stover

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Abstract: This experiment investigated the effects of white rot fungi on the nutritional value and antioxidant properties of corn stover. Four species of *Pleurotus* white rot fungi were selected: *Pleurotus diamor*, *Pleurotus citrinopileatus*, *Pleurotus eryngii*, and *Pleurotus sajor-caju*. The control group consisted of uninoculated corn stover, while experimental groups were inoculated with each of the four fungal species, with three replicates per group. Following 20 days of solid-state fermentation, the fermented material was analyzed for nutrient composition, in vitro dry matter digestibility (IVDMD), and antioxidant capacity. The results demonstrated: (1) The *P. diamor* group exhibited significantly higher total organic matter (TOM) loss rate compared to *P. citrinopileatus* and *P. eryngii* groups ($P<0.05$). The *P. sajor-caju* group showed significantly higher acid detergent lignin (ADL) loss rate than all other groups ($P<0.05$). The *P. citrinopileatus* group had significantly higher cellulose (CL) loss rate than *P. eryngii* and *P. sajor-caju* groups ($P<0.05$), and its hemicellulose (HC) loss rate was significantly higher than all other groups ($P<0.05$). (2) Crude protein (CP) content in the *P. citrinopileatus* group was significantly higher than the control ($P<0.05$), while ether extract (EE) content in *P. citrinopileatus*, *P. sajor-caju*, and *P. diamor* groups was significantly elevated compared to the control ($P<0.05$). All four fungal groups showed significantly higher total amino acid content and IVDMD than the control group ($P<0.05$). (3) The DPPH free radical scavenging capacity, reducing power, total antioxidant capacity, and total phenolic content were all significantly higher in the four white rot fungi groups compared to the control ($P<0.05$). In conclusion, white rot fungal pretreatment of corn stover can enhance its IVDMD, CP content, and antioxidant properties, making it a promising novel feed resource for ruminant animals.

Keywords: white rot fungi; corn stover; antioxidant properties

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China is rich in crop straw resources, accounting for 20%-30% of global straw production. However, most straw is either abandoned or burned, causing environmental pollution and resource waste. Feed utilization represents an effective approach for straw valorization, yet high contents of cellulose (CL), hemicellulose (HC), and lignin severely limit its nutritional value and digestibility, re-

stricting development of straw-based feedstocks. In recent years, various straw processing technologies (physical, chemical, and biological) have matured to improve nutritional value and applicability. Biological methods have emerged as a research focus due to their effectiveness in addressing low digestibility and nutritional value while offering safety, environmental friendliness, and pollution-free operation.

Fungi possess strong capabilities to degrade lignin and other aromatic compounds through extracellular peroxidases that effectively break down lignin, CL, and other macromolecular polymers into monomers, dimers, or low-molecular-weight phenolic compounds. Polysaccharides and polyphenols exhibit biological activities including antioxidant and immunomodulatory effects. Normal physiological metabolism continuously generates free radicals (superoxide anion, peroxyl radicals, nitric oxide, and hydrogen peroxide) that damage essential biomolecules such as proteins, lipids, and DNA. Antioxidants help stabilize these highly reactive radicals, maintaining structural and functional integrity of animal tissues. Animals obtain antioxidants from dietary nutrients to protect against oxidative damage, underscoring the importance of antioxidant components in nutritional feeds for animal health and immune function.

Research on optimizing biological straw treatment has focused on strain screening, enzyme production conditions, and degradation mechanisms. White rot fungi have been identified as the most effective lignin degraders, though studies on their antioxidant capacity remain limited. Therefore, this study selected four *Pleurotus* species—*P. diamor*, *P. citrinopileatus*, *P. eryngii*, and *P. sajor-caju* —to ferment corn stover and investigate their effects on nutritional value and antioxidant properties, aiming to provide theoretical basis for developing more functional feed resources.

1.1 Fungal Strains and Reagents

Four common *Pleurotus* white rot fungal strains were used: *P. diamor*, *P. citrinopileatus*, *P. eryngii*, and *P. sajor-caju*, all purchased from Jiangsu Tianda Edible Fungi Research Institute. Chemical reagents including 1,1-diphenyl-2-picrylhydrazyl (DPPH, 97% purity), potassium ferricyanide (99.5% purity), trichloroacetic acid (99% purity), ferric chloride (99% purity), 2,4,6-tripyridyl-s-triazine (97% purity), iron(III) chloride hexahydrate, iron(II) chloride anhydrous, ferrozine (98% purity), anhydrous methanol, and anhydrous ethanol were obtained from Sinopharm Chemical Reagent Co., Ltd. Gallic acid (99% purity) and amino acid standards (99% purity) were purchased from Sigma-Aldrich (USA).

1.2 Strain Activation

White rot fungi were inoculated onto autoclaved potato dextrose agar medium (200 g potato, 20 g glucose monohydrate, 20 g agar, 1000 mL distilled water, natural pH, sterilized at 121°C for 20 min) using an inoculation loop and incubated

at 25°C for 7 days.

1.3 Liquid Inoculum Preparation

Potato dextrose broth was prepared by boiling 200 g potato in 1000 mL distilled water for 30 min, then adding 20 g glucose monohydrate, 2 g ammonium sulfate, 1 g peptone, 1 g potassium dihydrogen phosphate, and 1 g magnesium sulfate (natural pH). The medium was distributed into 250 mL Erlenmeyer flasks and sterilized at 121°C for 20 min. After cooling to room temperature, eight 8-mm fungal plugs were transferred into each flask and incubated statically at 25°C for 2 days, followed by 4 days in a shaking water bath at 25°C and 150 rpm.

1.4 Experimental Design and Solid-State Fermentation

The substrate composition was: 31.5% corn stover (2-3 cm), 1.0% corn meal, 1.0% urea, 0.5% gypsum, 0.5% vitamins, 0.5% mineral elements, and 65.0% water. After thorough mixing, 200 g portions were packed into heat-resistant polypropylene bags, compacted, and sealed. The substrate was steam-treated for 30 min, left overnight, then autoclaved at 121°C for 30 min. After cooling, the control group remained uninoculated while experimental groups were inoculated with each fungal strain at 10% of substrate dry matter weight. All groups were incubated at 25°C in darkness for 20 days, then dried at 40°C and stored at -20°C until analysis.

1.5 Sample Extraction

Three grams of sample were accurately weighed into a ground-glass Erlenmeyer flask, mixed with 10 mL anhydrous methanol, and sonicated for 20 min. This extraction was repeated twice, followed by filtration. The filtrate was evaporated to 5 mL using a rotary evaporator at 40°C and stored at 4°C for use within one week.

1.6 Analytical Methods

1.6.1 Nutrient Composition Analysis

Cellulose (CL) and hemicellulose (HC) contents were determined using the Van Soest fiber analysis method. Acid detergent lignin (ADL) was measured according to GB/T 20805-2006. Crude protein (CP) content was determined by the Kjeldahl method, and ether extract (EE) by Soxhlet extraction.

1.6.2 Total Amino Acid Analysis

Samples (0.2-0.5 g) were hydrolyzed in 10 mL of 6 mol/L HCl at 110°C for 24 h. After cooling, the hydrolysate was filtered through 0.22 µm membrane and analyzed using an Agilent 1290 HPLC system. Chromatographic conditions: analytical column (4.6 mm × 150 mm, 3.5 µm) at 40°C, guard column (4.6 mm × 12.5 mm, 5 µm), injection volume 2 µL, flow rate 1.0 mL/min. Mobile phase

A was 40 mmol/L disodium hydrogen phosphate (pH 7.8), and mobile phase B consisted of 45% acetonitrile, 45% methanol, and 10% deionized water.

1.6.3 In Vitro Dry Matter Digestibility (IVDMD) Determination
Artificial rumen fluid was prepared according to Menke et al. Fifty grams (dry weight) of sheep feces were ground and mixed with 1 L artificial rumen fluid under continuous stirring with a magnetic stirrer while maintaining temperature at 39.5°C and anaerobic conditions by CO_2 flushing. Thirty milliliters of rumen fluid were added to anaerobic bottles containing 0.3 g sample using a dispenser. After 48 h incubation at 39.5°C, samples were dried and digested with pepsin solution for 24 h, then rinsed with 90°C distilled water, centrifuged at 10,625×g, and the supernatant discarded. The residue was dried at 105°C and IVDMD was calculated.

1.6.4 DPPH Free Radical Scavenging Assay
Following reference methods, DPPH was dissolved in anhydrous ethanol to prepare 0.1 mmol/L working solution. One milliliter of DPPH solution was mixed with 0.5 mL extract, incubated at room temperature for 30 min, and absorbance measured at 517 nm (A_1). Blank absorbance (A_0) was measured using anhydrous ethanol instead of extract, and control absorbance (A_2) using ethanol instead of DPPH solution with a Shimadzu UV-1800 spectrophotometer. DPPH scavenging capacity (%) = $[1\text{-}(A_1\text{-}A_2)/A_0] \times 100$.

1.6.5 Reducing Power Determination
According to reference methods, 0.5 mL extract was mixed with 0.1 mL of 1% potassium ferricyanide, incubated at 50°C for 30 min, then 50 µL trichloroacetic acid and 50 µL of 1% ferric chloride were added. After 20 min at room temperature, absorbance was measured at 700 nm to calculate reducing power.

1.6.6 Total Antioxidant Capacity Assay
The ferric reducing antioxidant power (FRAP) method was employed. A $FeSO_4$ standard curve was prepared using 0.1, 0.2, 0.4, 0.6, 0.8, and 1.0 mmol/L $FeSO_4$ solutions mixed with 2 mL FRAP reagent and 1 mL distilled water, with absorbance measured at 593 nm. FRAP reagent was prepared by mixing 300 mmol/L acetate buffer (pH 3.6), 10 mmol/L tripyridyltriazine, and 20 mmol/L ferric chloride at a 10:1:1 volume ratio. Two milliliters of FRAP reagent were mixed with 0.5 mL extract and 1 mL distilled water, and absorbance measured at 593 nm to calculate total antioxidant capacity.

1.6.7 Ferrous Ion Chelating Capacity Assay
Following reference methods, 0.5 mL extract was mixed with 1.6 mL deionized water, 0.5 mL ferrous chloride, and 0.1 mL of 5 mmol/L ferrozine, then incubated at 40°C for 10 min. Absorbance was measured at 562 nm (A_1), with blank (A_0) using methanol instead of extract and control (A_2) using water instead of ferrous chloride. Ferrous ion chelating capacity (%) = $[1\text{-}(A_1\text{-}A_2)/A_0] \times 100$.

1.6.8 Total Phenolic Content (TPC) Determination
A standard curve was prepared using gallic acid. Two milliliters of sample solution were mixed with 1 mL Folin-Ciocalteu reagent, shaken for 3-4 min,

then 1 mL of 20% sodium carbonate was added. After 30 min incubation at 30°C in darkness, absorbance was measured at 760 nm to calculate total phenolic content.

1.7 Statistical Analysis

All measurements were performed in triplicate. Data were analyzed using SPSS 16.0 software and expressed as mean ± standard deviation. One-way ANOVA followed by LSD test was used to determine significant differences between groups. Pearson correlation coefficients (R) were calculated to assess relationships between different parameters.

2.1 Effects of White Rot Fungi on Biomass Constituent Loss Rates of Corn Stover

All four white rot fungi effectively degraded corn stover biomass components . The *P. diamor* group showed significantly higher total organic matter (TOM) loss rate compared to *P. citrinopileatus* and *P. eryngii* groups ($P<0.05$). The *P. sajor-caju* group exhibited significantly higher ADL loss rate than all other groups ($P<0.05$), indicating superior ADL degradation capability. The *P. citrinopileatus* group demonstrated significantly higher CL loss rate than *P. eryngii* and *P. sajor-caju* groups ($P<0.05$), and its HC loss rate was significantly higher than all other groups ($P<0.05$), revealing strong CL and HC decomposition abilities. The *P. sajor-caju* group showed the second highest HC degradation capacity after *P. citrinopileatus*.

2.2 Effects of White Rot Fungi on Nutrient Composition and IVDMD of Corn Stover

All fungal treatments increased CP, EE, total amino acid content, and IVDMD compared to the control . The *P. citrinopileatus* group had significantly higher CP content than the control ($P<0.05$), while other fungal groups showed slightly higher but non-significant increases ($P>0.05$). No significant differences in CP content were observed among the four fungal groups ($P>0.05$). The *P. citrinopileatus*, *P. sajor-caju*, and *P. diamor* groups showed significantly higher EE content compared to the control ($P<0.05$), with increases ranging from 29.49% to 79.48%. All four fungal groups exhibited significantly higher total amino acid content than the control ($P<0.05$), though the *P. eryngii* group was significantly lower than the other three fungal groups ($P<0.05$). IVDMD was significantly higher in all fungal groups than the control ($P<0.05$), following the descending order: *P. sajor-caju* > *P. eryngii* > *P. citrinopileatus* > *P. diamor*. The *P. sajor-caju* group increased IVDMD by 6.57% to 90.08% compared to other groups.

2.3 Effects of White Rot Fungi on Antioxidant Properties of Corn Stover

All four fungal treatments enhanced antioxidant properties compared to the control . DPPH radical scavenging capacity, reducing power, total antioxidant capacity, and total phenolic content were significantly higher in all fungal groups ($P<0.05$). The *P. sajor-caju* group showed slightly higher DPPH scavenging capacity than *P. diamor* ($P>0.05$), with both significantly exceeding the other three groups ($P<0.05$). Reducing power was significantly higher in all fungal groups compared to the control ($P<0.05$), though no significant differences existed among fungal groups ($P>0.05$). The *P. sajor-caju* group demonstrated the strongest total antioxidant capacity, followed by *P. diamor*, both significantly higher than other groups ($P<0.05$). Ferrous ion chelating capacity and total phenolic content were highest in the *P. sajor-caju* group ($P<0.05$), followed by *P. diamor*. These results indicate the antioxidant performance of fungal-treated straw decreased in the order: *P. sajor-caju* > *P. diamor* > *P. eryngii* > *P. citrinopileatus*.

Most parameters showed significant correlations . ADL loss rate was significantly positively correlated with IVDMD and total phenolic content ($P<0.01$). Total phenolic content exhibited significant positive correlations with DPPH scavenging capacity, reducing power, total antioxidant capacity, and ferrous ion chelating capacity ($P<0.05$ or $P<0.001$).

3.1 Effects of White Rot Fungi on Biomass Loss Rates of Corn Stover

Biological degradation effectively addresses the challenges of lignocellulose recalcitrance and poor digestibility in herbivores. White rot fungi are the most efficient lignin degraders in nature, having evolved a unique enzymatic system that secretes multiple oxidative enzymes for lignin decomposition. This study demonstrated that fermentation with four white rot fungi significantly altered corn stover biomass composition, with ADL showing the greatest degradation, followed by HC and CL. This selective degradation reflects the preferential targeting of lignin by white rot fungi. Dietary CL content affects feed digestibility in ruminants, and lignin content negatively correlates with digestibility, representing a key limiting factor. Reduced lignin content improves both digestibility and palatability. While many studies confirm white rot fungi degrade lignocellulose, significant interspecific variation exists in degradation capacity. The superior ADL degradation by *P. sajor-caju* aligns with Jafari et al., whereas *P. citrinopileatus* showed stronger CL and HC degradation abilities.

3.2 Effects of White Rot Fungi on Nutrient Composition and IVDMD of Corn Stover

China' s livestock industry faces chronic protein feed shortages, with substantial grain crops diverted to animal feed, intensifying competition between human and animal nutrition. White rot fungi can convert various nitrogen sources

into fungal protein, increasing protein content in fermented corn stover. The Kjeldahl method used for CP determination cannot fully recover nitrate and nitrite nitrogen, while white rot fungi convert non-protein nitrogen into fungal protein (protein nitrogen), potentially contributing to increased CP values. Additionally, lignocellulose oxidation to CO_2 during fermentation reduces dry matter content, increasing protein concentration per unit mass. This study showed that four *Pleurotus* species utilized nitrogen sources in the substrate to synthesize protein and amino acids while improving digestibility, consistent with Arora et al. Enhanced digestibility may result from reduced lignocellulose content and increased protein levels. Research indicates dietary protein and fat levels regulate fiber digestibility in ruminants, as elevated ruminal ammonia nitrogen (NH_3-N) provides essential nitrogen for microbial growth, increasing bacterial and protozoal populations and promoting cellulolytic activity.

3.3 Effects of White Rot Fungi on Antioxidant Properties of Corn Stover

Under solid-state fermentation, white rot fungi can transform low-quality agricultural waste into high-nutrition feed supplements. Antioxidant capacity serves as a key indicator of feed quality, with DPPH scavenging, reducing power, total antioxidant capacity, ferrous chelation, and total phenolic content being critical evaluation parameters. This study found all four fungal treatments significantly increased these antioxidant parameters compared to the control, indicating production of antioxidant compounds during fermentation that block free radical chain reactions and form stable complexes with ferrous ions, thereby preventing oxidative damage to proteins and carbohydrates. The positive correlations between total phenolic content and all antioxidant parameters, and between ADL loss rate and phenolic content, suggest lignin degradation releases phenolic compounds that enhance antioxidant activity, consistent with Chandra et al. and Assi et al. Antioxidants maintain cellular structure and function while improving immune capacity; limited antioxidant capacity shortens immune cell lifespan and increases disease susceptibility. Dietary antioxidant supplementation enhances immune function and growth performance in animals, highlighting the important regulatory role of fungal-fermented straw as an antioxidant feed additive.

Conclusions: 1. All four white rot fungi effectively degraded corn stover biomass (CL, HC, and ADL), with *P. citrinopileatus* showing strongest CL and HC degradation and *P. sajor-caju* exhibiting superior ADL degradation. 2. Fermentation with all four fungi improved nutritional content and IVDMD of corn stover, with *P. citrinopileatus* producing the highest total amino acid content and *P. sajor-caju* achieving significantly highest IVDMD. 3. White rot fungal fermentation enhanced the antioxidant capacity of corn stover.

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