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Evaluation of White Weathered Mushrooms for Reduction of Lignin and Improvement of Animal Feed Digestion from Coconut Coir Waste

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Abstract: Limited forage availability due to land conversion has become a major constraint in goat farming development. Therefore, the utilization of agricultural waste as alternative feed is increasingly important, including young coconut husk waste which is abundant but underutilized. However, its high lignocellulosic content, particularly lignin, limits digestibility and nutritional value for ruminants. This study aimed to evaluate the ability of several white-rot fungi to reduce lignin content and improve the cellulose characteristics of young coconut husk waste as a potential alternative feed ingredient. The research was conducted in two stages: fungal selection and optimization of fermentation conditions. The first stage used five fungal treatments, namely *Trichoderma* sp. TR3, *Pleurotus sajor-caju*, *Pleurotus ostreatus*, *Trametes versicolor*, and *Lentinus edodes*. Parameters observed included lignin and cellulose contents after 20 days of incubation. The second stage evaluated the effect of incubation period (20, 30, and 40 days) and inoculum volume (0.5, 0.7, and 1.0 mL) using *Pleurotus sajor-caju* as the selected fungus. The results showed that *Pleurotus sajor-caju* produced the lowest lignin content (17.93%) and the highest cellulose content (48.80%) compared with other fungi. In the optimization stage, the lowest lignin content was observed in treatment A3B1 (40 days incubation and 0.5 mL inoculum) at 15.47%, while the highest cellulose content was obtained in treatment A2B1 (30 days incubation and 0.5 mL inoculum) at 61.15%. These findings indicate that *Pleurotus sajor-caju* has strong potential for selective delignification of young coconut husk waste while maintaining cellulose content. Biological fermentation using white-rot fungi can therefore be considered a promising and environmentally friendly approach for improving the quality of lignocellulosic waste as ruminant feed.

Keywords: Alternative feed; Cellulose; Lignin; *Pleurotus sajor-caju*; Young coconut husk waste.

Introduction

Feed is one of the determining factors in the success of the livestock business; goats are among the livestock cultivated by many people in Palu City. Goats are ruminant livestock whose feed is mostly forage, which is currently very limited due to a lack of land to grow forage for livestock feed and to housing for the growing population (Widiastuti et al., 2025). One alternative is to use waste, such as young coconut coir waste. This waste is always available and in sufficient quantities. In addition to being used as animal feed, it also prevents environmental pollution, which can cause a bad smell due to accumulation and give the impression of slums (Denta et al., 2020). One of the inhibiting factors in waste utilization is high lignocellulose content, which reduces

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the digestibility of dry matter and organic matter (Noersidiq et al., 2024). Young coconut coir waste is a waste that is abundant and is only thrown away and generally burned (Jusoh et al., 2020). If it is not addressed now, it will become a problem because waste is building up. Even if only a small part of it is used, this waste still has the potential to be ruminant animal feed because it may contain high levels of cellulose that can replace hjiauan, whose availability is limited.

One area in Central Sulawesi Province with potential for goat farming is Palu City. The potential for livestock development in Palu City is quite high. Goats are the most numerous livestock and are found in almost all sub-districts of Palu City. The average goat population, based on BPS Central Sulawesi data from 2019 to 2022, was 92,108 heads (BPS, 2022). This indicates a high development potential, but it is not offset by the availability of feed. It's just that feed availability is an obstacle to the development of goat livestock. Various types of feed ingredients available can be used as local feed ingredients. These feed ingredients have been used by farmers and have become a local custom or a local wisdom. Farmers also seek good, low-cost feed, such as leaf blades and straw. Leaf blades are also limited in availability, and rice straw is not highly digestible without prior processing (Yohannes et al., 2025). Therefore, this study aims to identify waste that can replace forage as animal feed by fermenting it with white-rot *fungi*.

Various studies have shown that white-rotting fungi, such as Phanerochaete chrysosporium and Pleurotus spp., are effective at degrading lignocellulose in straw and palm oil waste. The use of fungi as a degrader of lignocellulose is widely used to increase the nutritional value of agro-industrial waste, especially straw and empty oil palm bunches (Donggeng, 2022), using P. crysosporium and CULH (Colombian unidentified ligninolitic hymenomycetes), using P. sajur cayu and P. pulmonaris (Akinfemi et al., 2009; Alvira et al., 2010), using Postreatus and Saccharomyces (Darwish et al., 2012). using P. Crysosporium (Imsya et al., 2013) and then Tricoderma on corn straw (Islamiyati et al., 2013). Pleurotus florida in soybean straw uses Coprinus comatus in straw (Nasehi et al., 2013). Pleurotus florida on wheat straw (Nasehi et al., 2014), Hermiati, et al P.crysosporium and T.versicolor on empty bunches of oil palm (TKKS) (Hermiati et al., 2014) and palm fronds, Coprinus comatus on TKKS (Sabariyah & Hasanuddin, 2021). M. Palmivorus in oil palm and sugarcane waste (Fitriyah et al., 2023). Generally, research focuses only on rice straw and empty oil palm bunches. It has not touched the waste of young coconuts. The researcher hopes that the research results will provide a solution for the provision of a forage substitute feed, especially for farmers in the city of Palu, and to maintain environmental cleanliness. This study uses mushrooms that have been tested for their ability, based on several research results. The digestibility of forage can be determined through In Vitro experiments or through an artificial rumen without directly involving livestock. The in vitro digestibility has two stages: fermentation and enzymatic (Guo et al., 2022). Based on this, it is important to study the types of fungi with strong lignocellulose-degrading abilities in young coconut coir waste so that the waste can be utilized. Therefore, this study aims to evaluate the ability of several types of white-rot fungi to degrade lignocellulose in young coconut fiber waste and improve its in vitro digestibility.

Method

The research method is arranged into three stages: stage 1, mushroom selection; stage 2, ingredient quality test; and stage 3, in vitro test (digestibility test). Mushroom Selection Stage: the research method used is an experiment arranged in 4 stages of activities, namely, first, Inoculum preparation. Second, Substrate preparation. Third, inoculation of the substrate (young coconut fiber). Fourth, Incubation for 20 days. After 20 days, Cellulose and Lignin levels were analyzed.

Stage 1 (Inoculum Preparation): A total of 50 g of Potato Dextrose Agar (PDA) is dissolved in 1000 ml of water, then sterilized by autoclaving at 121°C for 15 minutes. Then it is poured into a 3 ml test tube to make the mushroom growth medium and then cooled. The five pure isolates were then inoculated into the media. The number of tubes made is 5 x 5 = 25, then incubated for 2 x 24 hours.

Stage 2 (Substrate Preparation): a). Mineral manufacturing for substrate enrichment, to make 1 liter of a mixture of KNO3 minerals (2.33 g/liter), 1% bran, from the weight of the substrate, NH4NO3 (0.5%), KCl (0.05%), FeSO4 7H2O (0.001%), Cu SO4 (0.0001%) [17]. b). Minerals are given 10 ml for every 10 grams of young coconut fiber first ground with a size of 40 mesh.c). Young coconut fiber is placed in a petri dish, 1 gram of rice bran is added, then 10 ml of minerals is added, and the mixture is placed in an autoclave and cooled.

Stage 3 (Inoculation): The fungus growing in an inclined tube is then diluted to a suspension with a triple dilution. Then, each mushroom suspension is sprayed onto 10 grams of substrate using an injection device. Then, the cells were incubated for 20 days at room temperature (27 °C). There are many treatments of 5 with 5 repetitions, so there are 25 petri dishes (made in duplicate). An analysis was then carried out to determine lignin content (Datta, 1981). The data obtained were analyzed based on ANOVA.

For stages 2 and 3, the activities are the same, starting from inoculum preparation to incubation, but differing in design because of a factorial with 2 factors, the first factor is the number of inoculum (0.5, 0.75, and 1.0 ml), and the second factor is the incubation period (20, 30, and 40 days).

Results and Discussion

Level 1 Lignin Levels

The implementation of Phase 1 (Mushroom Selection) has 4 stages of activities, namely 1) Preparation of the inoculum, 2) Substrate preparation, 3) Inoculation of the substrate (young coconut coir), 4). Incubation for 20 days. After 20 days, Cellulose and Lignin levels were analyzed. The results of the Lignin Analysis were obtained as follows:

Table 1. Analysis of Cellulose and Lignin levels

Sample Treatment	U1	U2	U3	U4	U5	Quantity	Average
TR3	19.33	19.67	18.67	20.00	20.33	98.00	19.60
PSC	19.33	17.67	17.33	17.33	18.00	89.67	17.93
PO	22.67	24.67	22.33	23.00	20.33	113.00	22.60
TV	22.33	23.00	22.00	22.33	22.67	112.33	22.47
LE	28.67	28.33	27.67	28.33	28.00	141.00	28.20

The data from the research can be seen in Figure 1.

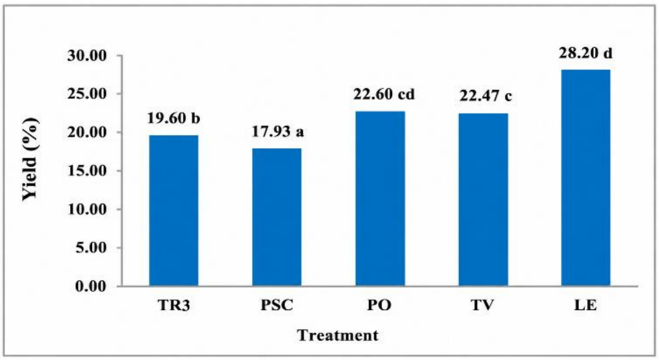

Figure 1. Results of Crude Lignin Content Test of Coconut Coir Media with Different Mushroom Inoculation

Based on the BNJ test at the 5% level, there is a very noticeable difference between the treatment type and the measured parameters. The best results were shown by Pleurotus sajor-caju (17.93%), followed by Trichoderma sp. TR3 (19.60%), Trametes versicolor (22.47%), Pleurotus ostreatus (22.60%), and highest in Lentinus edodes (28.20%).

The *lowest value of Pleurotus sajor-caju* indicates optimal efficiency in the process studied. *P. sajor-caju* has the characteristic of more efficient metabolism in the utilization of substrates, resulting in lower but more desirable parameters. This indicates that this species has a superior bioconversion mechanism.

Trichoderma sp. The TR3 shows good performance with a second low value. *Trichoderma* has an efficient, controlled enzymatic system that supports optimal metabolic processes (Chen et al., 2025; Verma et al., 2007). These characteristics make *Trichoderma* a good alternative for applications that require low parameters.

Trametes versicolor and *Pleurotus ostreatus* show relatively equivalent but higher values. These two white weathering fungi exhibit high metabolic activity, which can cause measured parameters to be higher than those of other species (Panngoen & Leksawasdi, 2023).

Lentinus edodes showed the highest value (28.20%), indicating the lowest efficiency in this study. These results suggest that *L. edodes* exhibits high enzymatic activity and excessive metabolic intensity, which can result in suboptimal performance in certain applications.

The results show that Pleurotus sajor-caju has the greatest potential for application to young coconut fiber, as its average lignin content is the lowest among the mushrooms.

Cellulose Level 1 Levels

In the cellulose parameters, the highest value was shown by *Pleurotus sajor-caju* (48.80%), followed by *Trichoderma* sp. TR3 (44.20%), *Trametes versicolor* (33.00%), *Pleurotus ostreatus* (32.60%), and lowest in *Lentinus edodes* (27.20%). Research by Liu et al., (2021) shows that *Pleurotus* spp. is able to maintain high cellulose content during the degradation of lignocellulose on agricultural substrates.

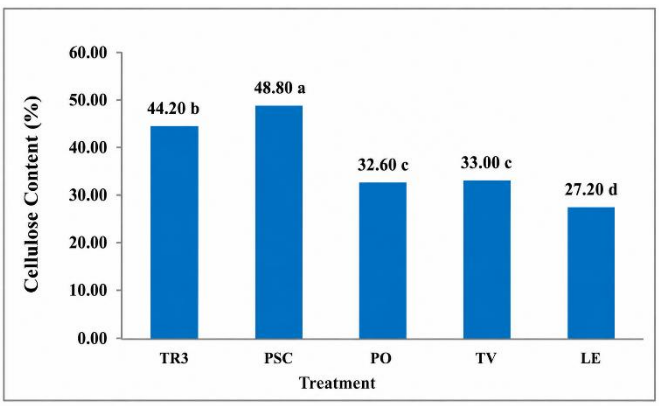

Figure 2. Results of Cellulose Content Test of Coconut Coir Media with Different Mushroom Inoculation

When associated with lignin data, *Pleurotus sajor-caju,* which has the lowest lignin levels and the highest cellulose levels, shows the best bioconversion performance. This condition suggests that the fungus effectively degrades lignin without excessively degrading cellulose, resulting in a material that is easier for livestock to digest, as reported in studies on cotton stalks (Sabariyah & Hasanuddin, 2021; Yang et al., 2019).

Trichoderma sp. TR3 also shows good results. A high cellulose value is consistent with a previously obtained low lignin level Zhu et al., (2025) found that *Trichoderma asperellum* effectively reduced lignin in wheat hay through solid fermentation, while retaining cellulose for animal feed.

Trametes versicolor and *Pleurotus ostreatus* had lower cellulose levels and higher lignin levels than in the previous two treatments. This is related to the metabolic activity of white-rot fungi, which tend to degrade lignin and cellulose simultaneously, so that cellulose is degraded and the fermentation process is suboptimal for improving feed quality (Hartono et al., 2015).

Lentinus edodes showed the highest lignin levels and the lowest cellulose levels, indicating excessive cellulose degradation, while lignin does not break down effectively. Such non-selective enzymatic activity makes these fungi less suitable for applications that require increased availability of easily digestible fiber by livestock (Nicholas et al., 2020). Overall, the relationship between lignin and cellulose indicates that high lignin levels accompanied by low cellulose levels indicate an underoptimal bioconversion process. *Pleurotus sajor-caju* is the best treatment because it can efficiently lower lignin while maintaining high cellulose levels.

Lignin level 2 (Pleuretus Sajor Caju Inoculation)

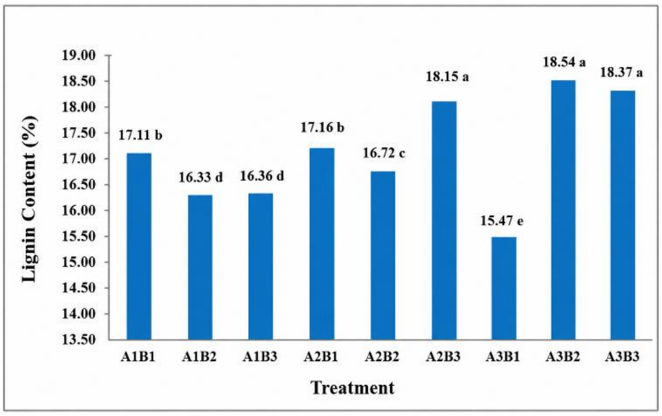

Figure 3. Results of Lignin Level Test of Coconut Coir Media with Mushroom Inoculation *Pleurotus sajor-caju*

Based *on* the results of lignin level testing in the second stage, it was observed that treatment with the fungus Pleurotus sajor-caju resulted in a significant difference in lignin reduction, depending on the length of inoculation and the volume of inoculum used. The lowest lignin levels were obtained in the A3B1 treatment (40 days with 0.5 ml inoculum) at 15.47%, while the highest lignin levels were found in the A3B2 and A3B3 treatments (40 days with 0.7 ml and 1 ml inoculum) at 18.54% and 18.37%, respectively. This suggests that a longer inoculation period is not always associated with a greater decrease in lignin when excessive inoculum volumes are used.

Treatments with inoculation durations of 20 and 30 days (A1 and A2) tended to produce higher lignin levels than the 40-day duration at low inoculum volumes (B1). However, increasing the inoculum volume from 0.5 ml to 0.7 ml or 1 ml at the same inoculation duration did not necessarily yield a greater decrease in lignin; even after 40 days, lignin levels increased at higher inoculum volumes. This suggests a possible inhibitory effect of high inoculum volumes on fungal enzymatic activity during delignification.

This data aligns with the literature, which indicates that the delignification process by the Pleurotus sajor-caju fungus is strongly influenced by fermentation duration and optimal inoculum concentration. According to research by Sabariyah & Hasanuddin, (2021), a sufficiently long fermentation duration allows fungi to selectively delignify, resulting in a significant decrease in lignin, but too many inocula can lead to nutrient and oxygen competition that limits fungal activity, thereby reducing the efficiency of lignin degradation.

The results also show that excessive inoculum doses in solid fermentation can decrease the activity of lignolytic enzymes, such as lignin peroxidase and manganese peroxidase, which play an important role in lignin degradation. This condition supports the results from the B2 and B3 treatments with a duration of 40 days, which showed lignin levels not lower than those in the B1 treatment.

Thus, optimizing the duration of inoculation and the inoculum volume is very important in the lignocellulose bioconversion process using Pleurotus sajor-caju, ensuring maximum delignification without inhibiting fungal growth or enzymatic activity. The 40-day inoculation period with an inoculum volume of 0.5 ml (A3B1) can be considered the optimal condition in this study for achieving the lowest lignin levels, which, of course, has the potential to improve the digestibility of feed ingredients for livestock.

Cellulose level 2 (Pleuretus Sajor Caju Inoculation)

Analysis of cellulose content data showed a significant effect of inoculation duration and inoculum volume on cellulose content in the substrate after fermentation. The A2B1 treatment (30 days with 0.5 ml inoculum) produced the highest cellulose level (61.15%), suggesting that, under these conditions, the fungus was able to maintain or even increase cellulose levels relative to other treatments.

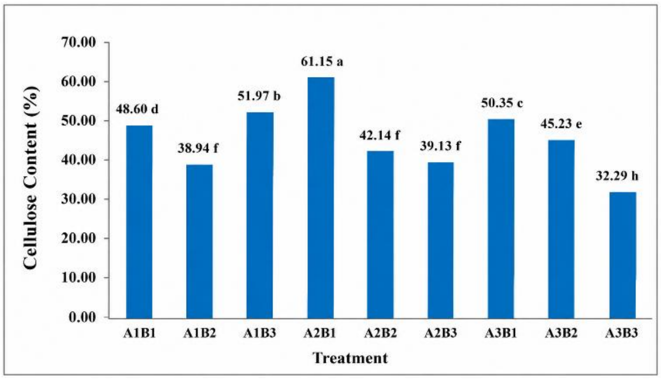

Figure 4. Results of Cellulose Content Test of Coconut Coir Media with Mushroom Inoculation *Pleurotus sajor-caju*

In contrast, the treatment with the longest inoculation duration (A3 = 40 days) and the highest inoculum volume (B3 = 1 ml) resulted in the lowest cellulose content of 32.29%. This indicates that using excessive volumes of inoculum over a long fermentation period can lead to excessive cellulose degradation, thereby significantly lowering cellulose levels. This condition is less than ideal because cellulose is the main energy source for rumen microbes in animal feed.

In addition, treatment with an inoculation duration of 20 days (A1) and an inoculum volume of 0.7 ml (B2) also showed a fairly low level of cellulose (38.94%), indicating that the too short fermentation time did not allow the fungus to work optimally in balancing delignification and maintenance of cellulose structure.

These results are consistent with the literature, which states that the fermentation process using white weathering fungi, such as Pleurotus sajor-caju, must be optimized for the duration and dose of the inoculum so that delignification occurs selectively without damaging too much cellulose. According to a study by Helmiati et al., (2020), an increase in fermentation duration has the potential to significantly lower cellulose levels if the volume of inoculum is not properly controlled, as excessive activity of the cellulase enzyme will break down the cellulose into simpler products.

Based on these data, the A2B1 treatment (30 days, 0.5 ml inoculum) can be considered the optimal fermentation condition for maintaining high cellulose levels while allowing good differentiation. This is very important for maintaining the nutritional value of feed ingredients, as sufficiently high cellulose levels help ensure the availability of energy for rumen microbes and improve the digestion efficiency of livestock.

Conclusion

Based on the results of the above research, it can be concluded that among the five types of mushrooms used, the one most effective at reducing lignin while maintaining high cellulose levels is *the Pleurotus sajor-caju mushroom.* In addition, optimal fermentation conditions were identified to maintain high cellulose levels while allowing good delignification, namely in the A2B1 treatment (30 days, 0.5 ml inoculum).

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Conflict of Interest

The author states that there is no conflict of interest regarding the publication of this manuscript.

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Author Contributions

Siti Sabariyah conceptualized the study, designed the methodology, supervised the research activities, conducted data analysis, and prepared the original manuscript draft. Muhammad Jufri contributed to data interpretation, statistical analysis, and manuscript revision. Sayani assisted in laboratory experiments, sample preparation, and data collection. Rahman Dani Lasamadi contributed to literature review, validation of results, and final manuscript editing. All authors have read and approved the final version of the manuscript.

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