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Analysis of the Nutritional Content, Fiber Fractions, and Methane Emissions of Lemongrass Waste Feed Produced Through Fermentation Using White Rot Fungi

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Abstract. This study was conducted to examine the effectiveness of *Trametes versicolor*, *Phanerochaete chrysosporium, Lentinula edodes*, and a consortium (*Trametes versicolor* + *Phanerochaete chrysosporium*) in enhancing the nutrient content and reducing the fiber fraction of fermented lemongrass waste. This study was conducted at the Faculty of Agriculture, Syiah Kuala University. Using the completely randomized design (CRD) with 5 treatments and 4 replicates, this study examined dry matter, crude protein, crude fiber, crude fat, ash, NFE, ADF, NDF, cellulose, hemicellulose, lignin, and methane emissions. The data were analyzed using ANOVA, followed by Duncan's multiple range test. This study concludes there is a numerical trend toward higher crude protein content in treatment P4 (Consortium; 5.51 ± 1.26) compared with the control (4.12 ± 0.32); however, the difference was not statistically significant ($p = 0.06$). However, in the fiber fraction analysis, the NDF content was lowest in the control group (59.68±0.34) compared to treatments P1, P2, P3, and P4. The control treatment exhibited the highest methane emission (7.1×10^4 ppm), whereas the consortium treatment (P4) produced the lowest methane emission (2.96×10^4 ppm). ANOVA revealed a significant effect of treatment on methane emissions ($p = 0.009$), and Duncan's multiple range test confirmed that the consortium treatment differed significantly from the control. The greatest reduction in the consortium treatment indicates a synergistic effect among the fungi in modifying the substrate, thereby more effectively suppressing methane formation.

Keywords: Fermentation, lemongrass, white rot fungus, fiber fraction, methane emissions

Abstrak. Penelitian ini bertujuan untuk melihat aktivitas penggunaan jamur *Trametes versicolor, Phanerochaete chrysosporium, Lentinula edodes* dan konsorsium (*Trametes versicolor* + *Phanerochaete chrysosporium*) dalam meningkatkan kandungan nutrisi dan menurunkan fraksi serat limbah serai wangi yang difermentasi. Penelitian ini dilakukan pada bulan April 2026 di Fakultas Pertanian Universitas Syiah Kuala. Metode yang digunakan yaitu Rancangan Acak Lengkap (RAL) dengan 5 perlakuan dan 4 ulangan. Parameter yang diperiksa adalah bahan kering, protein kasar, serat kasar, lemak kasar, abu, NFE, ADF, NDF, selulosa, hemiselulosa, lignin, emisi metana. Data dianalisis menggunakan ANOVA dan dilanjutkan dengan Uji Jarak Berganda Duncan. Penelitian ini menyimpulkan adanya kecenderungan numerik peningkatan kandungan protein kasar pada perlakuan P4 (Consortium; 5,51 ± 1,26) dibandingkan dengan kelompok kontrol (4,12 ± 0,32); namun, perbedaan tersebut tidak signifikan secara statistik ($p = 0{,}06$). Namun, dalam analisis fraksi serat, kandungan NDF terendah terdapat pada kelompok kontrol (59,68 ± 0,34) dibandingkan dengan perlakuan P1, P2, P3, dan P4. Perlakuan kontrol menunjukkan emisi metana tertinggi ($7{,}1 \times 10^4$ ppm), sedangkan perlakuan konsorsium (P4) menghasilkan emisi metana terendah ($2{,}96 \times 10^4$ ppm). Analisis ANOVA menunjukkan adanya pengaruh yang signifikan dari perlakuan terhadap emisi metana ($p = 0{,}009$) dan uji rentang berganda Duncan mengonfirmasi bahwa perlakuan konsorsium berbeda secara signifikan dari kelompok kontrol. Penurunan terbesar pada perlakuan konsorsium menunjukkan adanya efek sinergis di antara jamur dalam memodifikasi substrat, sehingga menekan pembentukan metana secara lebih efektif.

Kata kunci: Fermentasi, serai wangi, jamur pelapuk putih, fraksi serat, emisi metan

Introduction

Feed is any type of material that is consumable, safe, digestible, and nutrition-rich to support the livestock's survival, growth, and reproduction (Kamid et al., 2024). High-quality feed contains a sufficient amount of essential nutrients such as energy, protein, fat, minerals, and vitamins to support optimal quality and quantity of livestock production (Anggara et al., 2022). Pratama et al. (2022) state that the assessment of feed ingredient quality must include an analysis of nutrient content and digestibility, as both of these parameters are crucial for the nutritional development of ruminant livestock. Forage is the staple or primary feed for ruminants, comprising nearly 90% of their diet. However, forage availability varies across the year—abundant during the rainy season and scarce in the dry season—resulting in various productivity rates of livestock (Septian et al., 2018).

According to Samadi et al. (2024), agricultural waste is a sustainable alternative to livestock feed that helps reduce environmental pollution. Lemongrass waste is an alternative animal feed to overcome the shortage of forage. Sukamto et al. (2011) reported that lemongrass waste is still nutritious, with 90% dry matter, 7.91% ash, 7.0% crude protein, 3.35% crude fat, 25.73% crude fiber, and 52.10% nitrogen-free extract (NFE). However, lemongrass waste contains lignocellulose (27% lignin and 35% cellulose), resulting in low digestibility for ruminant livestock.

This downside can be optimized through steam treatment followed by fermentation, which reduces the antinutritional compounds and softens the tissue structure of lemongrass waste, thus improving nutrient quality and digestibility and facilitating the fermentation process (Astuti et al., 2024). Similarly, fermentation is a biological processing method in which microorganisms break down complex compounds into simple ones to enhance the digestibility and nutritional value of agricultural waste for use as livestock feed (Samadi et al., 2024). *Lentinula edodes, Trametes versicolor*, and *Phanerochaete chrysosporium*, which are white rot fungi, can be utilized in feed processing. The fermentation process involves microbial activity to produce fiber-degrading enzymes, which contribute to improved substrate utilization and create conditions that are more conducive to rumen microbial activity (Wajizah et al., 2022). Pratama et al. (2025) state that white rot fungi (WRF) are a group of microorganisms capable of degrading lignocellulosic biomass through the production of ligninolytic enzymes, such as lignin peroxidase (LiP), manganese peroxidase (MnP), and laccase. The activity of these enzymes plays a role in breaking down the structure of lignin, making the components of plant cell walls more accessible and usable by microorganisms. Various WRF species, such as *Phanerochaete chrysosporium*, *Trametes versicolor*, and *Lentinula edodes*, exhibit different lignocellulose degradation capacities, resulting in varying fermentation effectiveness in feed materials. Among these species, fermentation using *Phanerochaete chrysosporium* has been shown to improve feed quality through lignocellulose degradation, increase digestibility, and modify fermentation characteristics in the rumen. Therefore, this study aimed to investigate the effects of fermentation using different white rot fungi, including *Phanerochaete chrysosporium*, *Trametes versicolor*, *Lentinula edodes,* and their consortium, on the fiber fractions and methane emissions of lemongrass waste feed.

Materials and Methods

This study was conducted at the Laboratory of Nutrition, Technology, and Forage Crops, Department of Animal Science, Faculty of Agriculture, Syiah Kuala University. We used lemongrass waste, obtained from Blang Kejeran, Gayo Lues, as the primary material. The white rot fungi (*Lentinula edodes*, *Trametes versicolor*, and *Phanerochaete chrysosporium*) used as fermentation agents were sourced from the Indonesian Culture Collection, Cibinong, Indonesia. We rejuvenated the fungal cultures on malt extract agar medium, then propagated them using sterile corn medium as a starter.

The study employed an experimental design with a completely randomized design (CRD), consisting of 5 treatments and 4 replicates (20 experimental units). The treatments were: P0 (control without inoculum), P1 (lemongrass waste fermented with Trametes versicolor), P2 (lemongrass waste fermented with *Phanerochaeta chrysosporium*), P3 (lemongrass waste fermented with *Lentinula edodes*), and P4 (lemongrass waste fermented with a consortium of *Trametes versicolor* and *Phanerochaeta chrysosporium*).

The treatment process began with steam pretreatment of homogenously mixed lemongrass waste, adjusted to a moisture content of 60%. After pretreatment, the substrate was cooled to room temperature and aseptically inoculated with white rot fungi, according to the treatment, at an inoculum level of 5% (w/w) of the substrate. The inoculated substrates were incubated for 21 days following the solid-state fermentation procedure described by Tuyen et al. (2013). After fermentation, the samples were oven-dried at 60°C for 48 hours before chemical analyses. Methane emissions were determined using the in vitro gas production technique.

The fermented feed samples were dried, ground to pass through a 1-mm sieve, and weighed (0.5–1.0 g dry matter) into serum bottles. Buffered rumen fluid, prepared by mixing rumen fluid and buffer solution at a ratio of 1:2, was added to each bottle. We flushed the bottles with CO_2 to maintain anaerobic conditions, sealed them, and incubated them at 39°C for 24–48 hours. At the end of the incubation, the total gas produced was collected, and gas samples (1–2 mL) were analyzed using a gas chromatography (GC) system equipped with a flame ionization detector (FID). The instrument was calibrated using standard methane gas, and methane emissions were expressed as CH_4 concentration (ppm). The data obtained were analyzed using analysis of variance (ANOVA) according to the experimental design. If a significant effect was found, a post-hoc test was conducted to compare the treatments.

Data Analysis

All collected data will be statistically analyzed using analysis of variance (ANOVA) with SPSS software. If differences are found among the treatments, Duncan's multiple range test (DMRT) (Steel and Torrie, 1993) will be used for further analysis.

Results and Discussion

Nutritional Content of Fermented Lemongrass Waste Using White Rot Fungus

A nutritional analysis was conducted to evaluate the effect of fermentation using various types of white rot fungi on the nutritional quality of lemongrass waste, which has the potential to be used as feed for ruminant livestock. Table 1 presents the results of the nutritional analysis of fermented lemongrass waste.

Fermentation of lemongrass waste using various white rot fungi over 21 days elicited diverse responses in the substrate's nutrient content, as detailed in Table 1.

Table 1. Nutritional composition of fermented lemongrass waste using various white rot fungi over 21 days

Parameters	Treatment					
(%)	P0	P1	P2	P3	P4	p-Value
Dry matter	93.80±0.18	91.37±5.91	94.10±0.31	90.88±1.58	93.91±0.15	0.32
Crude protein	4.12±0.32	5.11±0.20	4.75±0.56	4.46±0.32	5.51±1.26	0.06
Crude fiber	26.80±0.17	25.09±1.82	26.82±0.49	25.86±0.96	26.22±0.94	0.16
Crude fat	2.91±0.34	2.23±0.38	2.30±0.17	2.31±1.10	2.09±0.27	0.32
Ash	9.02±0.18[a]	9.05±0.43[a]	9.61±0.22[ab]	9.01±0.36[a]	9.85±0.60[b]	0.01
NFE	50.96±0.15	49.89±6.30	50.62±0.90	49.25±0.93	50.24±0.75	0.92

Notes: P0 = Fermentation Control Without Inoculum; P1 = Fermented lemongrass waste + *Trametes versicolor*; P2 = Fermented lemongrass waste + *Phanerochaete chrysosporium*; P3 = Fermented lemongrass waste + *Lentinula edodes*; P4 = Fermented lemongrass waste + Consortium (*Phanerochaete chrysosporium* + *Trametes versicolor*).

Analysis of variance revealed a significant effect (P < 0.05) only on ash content, with dry matter, crude protein, crude fiber, crude fat, and NFE showing no significant differences (P > 0.05). Nevertheless, a numerical trend indicated that fermentation with white rot fungi tended to increase protein content compared to the control.

The dry matter content of fermented lemongrass waste ranged from 90.88% to 94.10%, with no statistically significant differences (P > 0.05) among treatments. However, numerical variations reflected disparities in the metabolic activity of each inoculum. *Phanerochaete chrysosporium* treatment resulted in the highest dry matter content (94.10%), while *Lentinula edodes* exhibited the lowest (90.88%). The reduced dry matter in the *Lentinula edodes* treatment suggests that this fungus preferentially utilizes some of the substrate's organic components for mycelial growth and metabolic activity during fermentation, although direct measurements of material loss due to respiration were not conducted.

Conversely, the higher dry matter content in the *Phanerochaete chrysosporium* treatment indicates that fermentation proceeded without substantial dry matter loss. This disparity is likely attributable to the distinct physiological characteristics of each fungus. *Phanerochaete chrysosporium* is recognized for its highly active ligninolytic enzyme system, particularly lignin peroxidase (LiP) and manganese peroxidase (MnP), whereas *Trametes versicolor predominantly* produces laccase. *Lentinula edodes* generally exhibits a slower substrate colonization rate compared to these two species, leading to differential patterns of substrate utilization during fermentation. These variations in extracellular enzyme composition underscore that substrate utilization efficiency is not uniformly consistent across different fungal species (van Kuijk et al., 2015).

These findings align with the fiber fraction analysis in this study, which demonstrated that NDF, ADF, cellulose, hemicellulose, and lignin contents tended to be elevated in the fermented substrate compared to the control. This implies that during fermentation, the fungi likely consumed the more readily digestible non-structural carbohydrate fractions as an initial energy source, resulting in a relative concentration of cell wall components within the total dry matter. Consequently, despite the distinct physiological characteristics of each inoculum, all treatments successfully maintained the dry matter content of lemongrass waste within a relatively consistent range throughout the 21-day fermentation period. Similar interpretations have been reported in various studies on lignocellulosic biomass fermentation, where changes in chemical composition are influenced by the interplay of organic matter consumption, fungal biomass growth, and shifts in the proportions of cell wall fractions.

Crude protein content exhibited an upward trend following fermentation, particularly in the consortium treatment (5.51%), followed by *Trametes versicolor* (5.11%), *Phanerochaete chrysosporium* (4.75%), and *Lentinula edodes* (4.46%), although these differences were not statistically significant (P=0.06). Numerically, the elevated crude protein value in the consortium treatment suggests that the combination of *Phanerochaete chrysosporium* and *Trametes versicolor* promotes greater mycelial biomass production compared to single inoculants. This is likely due to the complementary nature of their ligninolytic enzyme systems, which can enhance substrate utilization for mycelial growth and microbial protein synthesis (Sanchez, 2020).

The *Trametes versicolor* treatment also demonstrated a comparatively higher protein content than *Phanerochaete chrysosporium* and *Lentinula edodes*, indicating proficient substrate colonization. In contrast, the comparatively modest increase in protein content for *Lentinula edodes* is thought to be linked to differences in adaptability and mycelial growth rates on the lemongrass waste substrate. These results are consistent with the fiber fraction analysis, which indicated that fermentation altered cell wall composition without inducing significant changes in crude protein content. Thus, while inoculum type tends to influence fungal biomass formation during fermentation, the effect had not yet reached statistical significance.

Crude fiber content ranged from 25.09% to 26.82% and did not differ significantly (P > 0.05) among treatments. However, numerically, the *Trametes versicolor* treatment yielded the lowest crude fiber content (25.09%), while the *Phanerochaete chrysosporium* treatment resulted in the highest (26.82%). This discrepancy is likely associated with the characteristics of the lignocellulolytic enzymes possessed by each fungus. *Trametes versicolor* is known for its high laccase activity, which enables it to modify lignocellulose structure and facilitate the utilization of certain fiber components during fermentation (Sanchez, 2020).

Conversely, *Phanerochaete chrysosporium* possesses a ligninolytic enzyme system dominated by lignin peroxidase (LiP) and manganese peroxidase (MnP), leading to a substrate utilization pattern distinct from that of *Trametes versicolor* (van Kuijk et al., 2015). The *Lentinula edodes* treatment yielded a crude fiber content intermediate to the other two treatments, suggesting a moderately effective ability to degrade lignocellulose in lemongrass waste. The consortium treatment exhibited a lower crude fiber value than the control, but not lower than the single *Trametes versicolor* treatment. This indicates that the combination of the two fungi did not produce a sufficiently significant synergistic effect on crude fiber content changes during the 21-day fermentation.

Furthermore, the efficacy of white rot fungi in cellulose degradation is considerably influenced by substrate type, degree of lignification, fermentation duration, and the fungi's selectivity in breaking down lignin relative to other carbohydrate components (Qi et al., 2023).

Crude fat content showed a declining trend after fermentation, with the lowest value in the consortium treatment (2.09%) and the highest in the control (2.91%), although this difference was not statistically significant (P > 0.05). Numerically, the consortium treatment demonstrated a more pronounced decrease in fat content compared to the single inoculum. This is likely due to the more diverse metabolic activity of the two fungi, leading to a more intensive utilization of organic compounds, including lipids, during mycelial growth. The Trametes versicolor, *Phanerochaete chrysosporium*, and *Lentinula edodes* treatments also exhibited lower fat levels compared to the control, indicating that each fungus possesses a distinct capacity to utilize lipids as an energy source during fermentation (Elisashvili et al., 2008). However, this effect was not substantial enough to result in a statistically significant difference. According to Sanchez (2020), changes in fat content during fermentation are generally a secondary effect of microbial metabolic activity rather than the primary target of white rot fungi's degradation.

Fermentation significantly affected (P < 0.05) ash content. The highest ash content was observed in the consortium treatment (9.85%), followed by *Phanerochaete chrysosporium* (9.61%), while *Lentinula edodes* (9.01%) and the control (9.02%) exhibited the lowest values. The increase in ash content in the consortium treatment suggests that the combination of *Phanerochaete chrysosporium* and *Trametes versicolor* is more effective at utilizing the organic components of the substrate during fermentation, resulting in a higher proportion of residual minerals. The *Phanerochaete chrysosporium* treatment also showed a relatively high ash content, which is thought to be linked to the activity of its extracellular enzymes in utilizing lignocellulosic components as a nutrient source. In contrast, *Lentinula edodes* produced ash content relatively similar to the control, indicating that changes in substrate composition during fermentation were less pronounced compared to the other treatments. These differences suggest that each inoculum possesses a distinct capacity for substrate utilization during fermentation, leading to varying changes in ash content.

NFE levels, ranging from 49.25% to 50.96%, did not demonstrate significant differences across treatments (P > 0.05). However, numerically, the *Lentinula edodes* treatment yielded the lowest NFE value (49.25%), while the control exhibited the highest (50.96%). The relatively stable NFE values suggest that fermentation with various white rot fungi did not induce significant alterations in the non-structural carbohydrate fraction. This is probably because each fungus possesses a distinct substrate utilization pattern; however, most metabolic activity is directed towards supporting mycelial growth and utilizing available organic components rather than substantially modifying NFE content.

The consortium treatment yielded an NFE value nearly identical to that of *Phanerochaete chrysosporium*, indicating that the combination of the two fungi maintained balanced substrate utilization during fermentation. As per McDonald et al. (2022), changes in NFE generally mirror changes in its constituent components; thus, if most proximate components remain largely unchanged, the NFE value also tends to remain constant. Consequently, inoculum type did not significantly affect the NFE content of lemongrass waste after 21 days of fermentation (Sanchez, 2020).

Overall, the study's findings indicate that fermenting lemongrass waste with white rot fungi for 21 days can preserve dry matter content, augment crude protein content, reduce crude fat, and elevate ash content; however, it has not yet led to significant changes in most nutritional components. This suggests that the fermentation process primarily enhances the biological quality and lignocellulosic structure of the substrate rather than drastically altering its nutrient composition.

Fiber Fraction of Fermented Lemongrass Waste Using White Rot Fungi

Fiber fraction analysis was conducted to evaluate the impact of white rot fungi fermentation on changes in the cell wall components of lemongrass waste, encompassing NDF, ADF, cellulose, hemicellulose, and lignin. The nutritional analysis results for lemongrass waste fermented with white rot fungi for 21 days are presented in Figure 1.

Based on the research results, the NDF value in the control treatment was 59.68 ± 0.34%, while the *Trametes versicolor* treatment (66.86 ± 3.34%), *Phanerochaete chrysosporium* (67.42 ± 3.19%), *Lentinula edodes* (63.33 ± 2.02%), and consortium (66.07 ± 2.25%) treatments showed higher values than the control. Neutral Detergent Fiber (NDF) is a fiber fraction consisting of hemicellulose, cellulose, and lignin, which make up plant cell walls. NDF values are often used as an indicator of forage quality because they are closely related to feed intake and digestibility in ruminants. Generally, the higher the NDF content, the lower the dry matter intake, because the rumen fills more quickly and the rate of feed degradation slows down (Carrillo-Díaz et al., 2022).

The increase in NDF values in the *Trametes versicolor*, *Phanerochaete chrysosporium, Lentinula edodes*, and consortium treatments compared to the control is thought to have occurred because, during the fermentation process, microorganisms utilized more of the water-soluble components, such as simple sugars, starch, and part of the plant cell contents. As a result, the proportion of cell wall fractions became relatively higher, causing the NDF percentage to increase.

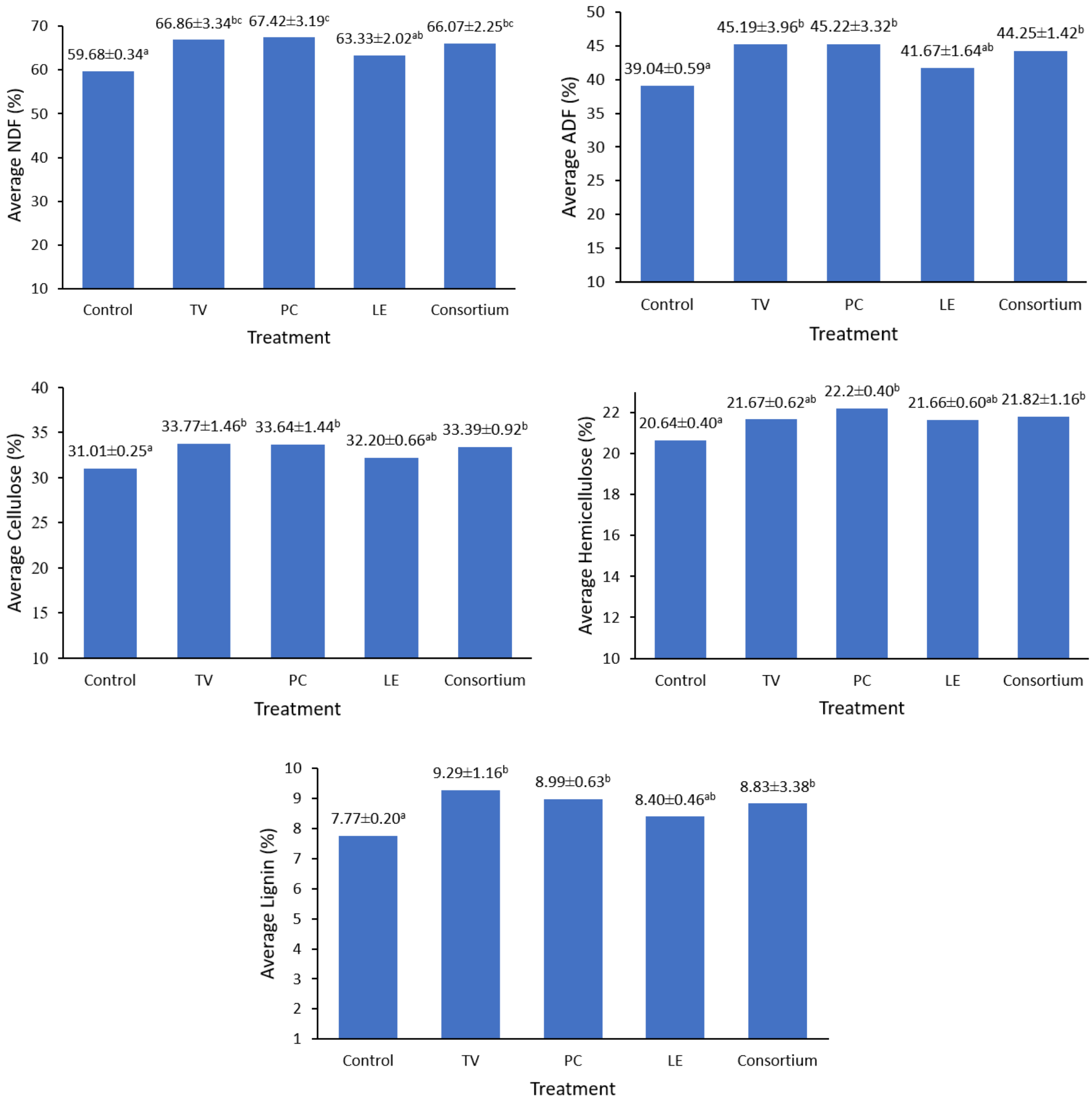

Figure 1. Fiber fraction of lemongrass waste after fermentation using various types of white rot fungi for 21 days.

The increase in NDF content in the fermented lemongrass waste indicates that the degradation of cell wall components has not yet occurred optimally. The increase in NDF content in the fermented lemongrass waste indicates that the degradation of cell wall components has not yet occurred optimally.

This finding differs from several previous studies, which reported that the effect of fungal inoculum on lignocellulosic components depends on fungal species, substrate characteristics, and incubation period. Some white-rot fungi are capable of selectively degrading lignin and reducing NDF, ADF, cellulose, and hemicellulose, whereas other fungal species primarily consume readily available nutrients during the early stages of fermentation, resulting in little or no reduction in cell wall fractions (Tuyen et al., 2013). The NDF value in the control treatment was 59.68 ± 0.34%, whereas the *Trametes versicolor* treatment (66.86 ± 3.34%), *Phanerochaete chrysosporium* (67.42 ± 3.19%), *Lentinula edodes* (63.33 ± 2.02%), and consortium (66.07 ± 2.25%) treatments exhibited higher values than the control.

Neutral Detergent Fiber (NDF) is a fiber fraction composed of hemicellulose, cellulose, and lignin, which constitute plant cell walls. NDF values commonly serve as an indicator of forage quality due to their close relationship with feed intake and digestibility in ruminants. Generally, a higher NDF content correlates with lower dry matter intake, as the rumen fills more quickly and the rate of feed degradation decreases (Carrillo-Díaz et al., 2022). The observed increase in NDF values in the Trametes versicolor, *Phanerochaete chrysosporium*, *Lentinula edodes*, and consortium treatments compared to the control is thought to be a result of microorganisms utilizing more water-soluble components, such as simple sugars, starch, and a portion of plant cell contents, during the fermentation process. Consequently, the proportion of cell wall fractions became relatively higher, leading to an increase in the NDF percentage.

This rise in NDF content in the fermented lemongrass waste suggests that the degradation of cell wall components has not yet reached optimal levels. This finding diverges from several prior studies, which reported that the effect of fungal inoculum on lignocellulosic components depends on fungal species, substrate characteristics, and incubation period. Some white-rot fungi are capable of selectively degrading lignin and reducing NDF, ADF, cellulose, and hemicellulose, whereas other fungal species primarily consume readily available nutrients during the initial stages of fermentation, resulting in little to no reduction in cell wall fractions (Tuyen et al., 2013).

Analysis results indicated that the fermentation treatment had a highly significant effect ($P < 0.01$) on ADF content. The ADF values in the fermentation treatment tended to be higher than those in the control group. ADF is a fiber fraction comprising cellulose and lignin and is used as an indicator of feed digestibility. An increase in ADF signifies a relative accumulation of indigestible fiber components during fermentation. White rot fungi are known to produce lignin-degrading enzymes such as laccase, manganese peroxidase (MnP), and lignin peroxidase (LiP); however, the efficacy of these enzymes is significantly influenced by the type of substrate, pH, moisture content, and fermentation duration.

Lemongrass waste likely still contains essential oils and a complex lignocellulosic structure, which may impede optimal lignin degradation. The rise in ADF suggests that the proportion of cellulose and lignin in the material remains relatively high. According to Adesogan et al. (2019), high ADF content is typically negatively correlated with dry matter digestibility and available energy for livestock. Similar findings were reported by Olagunju et al. (2024) in the fermentation of sorghum using white rot fungi, where the ADF content increased from 5.87% in the control to 7.32–8.21% after fermentation. This increase in ADF is believed to be due to the fungi more readily utilizing easily degradable non-structural components as an energy source for growth, leading to a relatively higher concentration of the cell wall fraction composed of cellulose and lignin. Consequently, the ADF value measured after fermentation was higher than that of the control material.

Based on the research findings, the average cellulose content across the various treatments ranged from 31.01% to 33.77%. The highest value was observed in the *Trametes versicolor* treatment (33.77 ± 1.46%), followed by *Phanerochaete chrysosporium* (33.64 ± 1.44%), Consortium (33.39 ± 0.92%), *Lentinula edodes* (32.20 ± 0.66%), and the lowest in the Control (31.01 ± 0.25%). In general, all biological treatments tended to increase the proportion of cellulose compared to the control. The increase in cellulose content in the fermentation treatment, accompanied by high lignin content, suggests that lignin degradation has not yet proceeded optimally.

This condition is thought to occur because fungi utilize non-fibrous components or, more specifically, hemicellulose as their primary energy source, causing the proportion of cellulose in the material to increase relatively, even though the lignin content remains high. Additionally, partial degradation of lignin may occur, leading to greater exposure of the cellulose fraction and an increase in its proportion in the dry matter. According to Chen et al. (2024), the biological delignification process can enhance cellulose accessibility by breaking the lignin-carbohydrate bonds that coat plant fibers.

The hemicellulose content ranged from 20.64% to 22.20% and showed no significant differences among treatments ($P > 0.05$). Nevertheless, all fermentation treatments exhibited higher hemicellulose values than the control. The numerical increase in hemicellulose indicates that hemicellulose degradation has not yet progressed intensively. Hemicellulose is a polysaccharide that degrades more easily than cellulose and lignin; however, in complex lignocellulosic substrates, hemicellulose is often protected by the lignin matrix, thereby limiting access by hemicellulase enzymes. According to Wu et al. (2022), changes in hemicellulose content during fermentation are influenced not only by the degradation of hemicellulose itself but also by the loss of other components, which leads to changes in their relative proportions in the dry matter. Therefore, the observed increase in hemicellulose in this study likely reflects a change in relative composition rather than an actual increase in the amount of hemicellulose.

The study results demonstrated that the fermentation treatment had a highly significant effect ($P < 0.01$) on lignin content. Lignin content increased from 7.77% in the control group to 8.40–9.29% in the fermentation treatment group. The highest value was observed in the *Trametes versicolor* treatment (9.29%). Theoretically, white rot fungi possess the ability to degrade lignin through the production of enzymes such as laccase, manganese peroxidase, and lignin peroxidase. However, the increased lignin measured in this study does not imply new lignin synthesis during fermentation. This increase is more likely due to a reduction in non-fiber components, resulting in a higher proportion of lignin in the dry matter. A similar phenomenon is often observed in the fermentation of lignocellulosic biomass with a relatively short incubation time. According to Khan et al. (2024), the success of delignification is significantly influenced by fermentation duration, substrate type, and the activity of the ligninolytic enzymes produced by the fungi. Therefore, the 21-day fermentation period in this study is believed to have been insufficient to produce greater lignin degradation compared to the loss of non-structural components.

Overall, the 21-day fermentation of lemongrass waste using Trametes versicolor, *Phanerochaete chrysosporium*, *Lentinula edodes*, and a consortium resulted in an increase in NDF, ADF, cellulose, hemicellulose, and lignin. This increase is believed to stem from the fungi's utilization of non-structural components, causing the cell wall fraction to become more concentrated in the dry matter. These results indicate that the delignification process has not yet been optimized, meaning fermentation has had a greater effect on altering the proportions of nutrient components than on significantly reducing the structural fiber content. These findings suggest that the incubation time, inoculum dose, or fermentation conditions need to be optimized to improve the effectiveness of lignocellulose degradation in lemongrass waste.

Methane Production Potential of Fermented Lemongrass Waste Using White Rot Fungi

A methane emission analysis was conducted to evaluate the effect of fermentation using white rot fungi on the potential for methane gas production from lemongrass waste during the rumen fermentation process. Methane emissions are an indicator of the efficiency of feed utilization by rumen microorganisms, which is closely related to changes in nutrient composition and fiber fractions, particularly the content of NDF, ADF, cellulose, hemicellulose, and lignin.

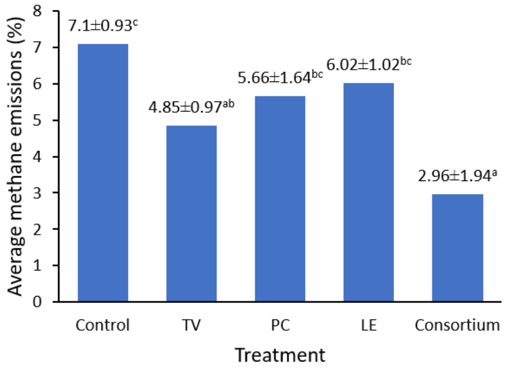

Figure 2. Methane emissions from lemongrass waste after fermentation using various types of white rot fungi

The results of the methane emissions analysis of fermented lemongrass waste using white rot fungi over 21 days are presented in Figure 2.

The results of the study show that the fermentation treatment using fungi was able to reduce methane emissions compared to the control. The highest methane emissions were observed in the control treatment, at 7.1, while the lowest methane emissions were observed in the consortium treatment, at 2.96. The greatest reduction observed in the consortium treatment indicates a synergistic effect among the fungi in modifying the substrate, enabling them to suppress methane formation more effectively. Methane is produced by methanogenic archaea in the rumen through the utilization of hydrogen (H_2) and carbon dioxide (CO_2) formed during the fermentation of structural carbohydrates. Methane production generally increases in fermentation that produces high levels of acetate because that pathway generates more free hydrogen, which is then utilized by methanogens. Conversely, if hydrogen is diverted to other fermentation pathways, such as propionate formation, or is utilized by non-methanogenic microorganisms, methane production will decrease (Kumar et al., 2013). This study shows that a reduction in methane emissions is not always accompanied by a decrease in fiber content. The NDF, ADF, cellulose, hemicellulose, and lignin values in the fermentation treatments actually tended to be higher than those in the control group. This finding suggests that the decrease in methane is most likely due to changes in rumen fermentation characteristics resulting from the biological activity of fungi, rather than solely due to changes in fiber content. White rot fungi are known to produce lignolytic enzymes such as laccase, manganese peroxidase, and lignin peroxidase, which are capable of altering the structure of lignocellulose and producing secondary metabolites that can influence rumen microbial activity (Daniso and Spanghero, 2026).

The results of this study are consistent with the findings of Kholif et al. (2022), who reported that the addition of *Pleurotus ostreatus* to agricultural waste significantly reduced CH_4 and CO_2 production during in vitro rumen fermentation. The researchers stated that fungal treatment not only alters the fiber composition but also affects the fermentation pattern, thereby reducing the availability of hydrogen to methanogens. The significant reduction in methane emissions in the consortium treatment is thought to be related to the ability of several fungal species to work synergistically in degrading lignocellulosic components. Interactions among fungi allow for the formation of a broader spectrum of enzymes than in single-strain cultures, resulting in more intensive substrate modification. These changes can alter the rumen fermentation profile and reduce methanogen activity. This was evident from a 58.28% reduction in methane emissions in the consortium treatment compared to the control. A study by Astudillo-Neira et al. (2023) also reported that the use of hay biodegraded by *Pleurotus ostreatus* can increase dry matter degradation while reducing methane production during in vitro incubation. The researchers concluded that fungal treatment improves substrate quality and alters fermentation patterns, thereby reducing methane production.

Overall, the study results indicate that fungal fermentation treatments, particularly those using a consortium, have high potential as a strategy for mitigating methane emissions. Although the NDF, ADF, cellulose, hemicellulose, and lignin contents were relatively higher than those of the control, methane emissions decreased significantly. This finding suggests that factors other than fiber fraction composition, potentially including changes in rumen fermentation characteristics and fungal biological activity, may have contributed to methane mitigation. However, these mechanisms were not directly evaluated in the present study, as rumen fermentation parameters such as volatile fatty acids (VFAs), pH, and methanogen populations were not measured. Therefore, further studies are needed to investigate these parameters to better elucidate the mechanisms underlying methane reduction following fermentation with white rot fungi.

Conclusions

Fermentation of lemongrass waste using white rot fungi for 21 days did not significantly affect most proximate nutrient components, although it increased ash content and showed a numerical trend toward higher crude protein content. Fermentation also increased the NDF, ADF, cellulose, hemicellulose, and lignin contents, likely reflecting an increased proportion of structural carbohydrates following the degradation of non-structural components. Despite the increase in fiber fractions, methane emissions were significantly reduced across all fermentation treatments, with the consortium of *Phanerochaete chrysosporium* and *Trametes versicolor* producing the lowest methane emissions. These findings suggest that consortium fermentation has potential as a biological treatment to improve the utilization of lemongrass waste as ruminant feed while mitigating methane emissions.

Future studies should optimize fermentation conditions, including inoculum dosage and fermentation duration, to maximize feed quality and methane mitigation. In addition, further investigations should evaluate rumen fermentation characteristics, such as volatile fatty acid profiles, pH, methanogen populations, and animal performance under in vivo conditions, to clarify the mechanisms underlying methane reduction and support the practical application of this technology in ruminant production systems.

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