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Research Article

Improving the Nutritional Quality of Palm Kernel using *Phanerochaete chrysosporium*

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Abstract | *Phanerochaete chrysosporium* is a white-rot fungus widely studied for its strong ability to degrade complex plant materials, particularly lignin. This study aimed to determine the relationship between fermentation duration using *P. chrysosporium* and the nutritional quality of palm kernel. The experiment was conducted using a Completely Randomized Design (CRD) with four treatments consisting of fermentation durations of 8, 10, 12, and 14 days, each with five replications. Data were analyzed using analysis of variance (ANOVA), followed by Duncan's Multiple Range Test (DMRT) at a 5% significance level. The observed variables included crude protein, crude fibre, nitrogen retention, lignin, cellulose, hemicellulose, and crude fibre digestibility. The results showed that fermentation duration had a highly significant effect ($P < 0.01$) on improving the nutritional quality of palm kernel. Based on Duncan's Multiple Range Test (DMRT), 12 days of fermentation (treatment C) resulted in the greatest nutritional improvement, characterized by a 45.00% increase in crude protein, a 46.64% increase in hemicellulose, a 43.86% reduction in crude fibre, a 42.20% reduction in cellulose, a 44.13% reduction in lignin, and a crude fibre digestibility of 55.52%, and nitrogen retention of 67.74%. It can be concluded that fermentation of palm kernel with *P. chrysosporium* for 12 days optimizes its nutritional quality.

Keywords | Palm kernel, Fermentation, *Phanerochaete chrysosporium*, Nutritional quality

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INTRODUCTION

Animal feed is imperative to ensure the success of farming. The availability of the feed ingredients is a problem often faced by farmers. Its fluctuating prices and importance along with basic human needs often cause farmers dilemmas. Therefore, there is a need for alternative feed in order to resolve this issue. Directorate General of Estate Crops (2021) stated that there was an increase in oil palm areas in Indonesia between the years 2019-2021. The areas increased from 14,456,611 Ha to 15,081,021 Ha, while the productions increased from 47,120,247 tons to 49,710,345 tons. The increase of palm oil production also increases waste that can pollute the environment; therefore, the palm oil waste needs to be processed, one of which is to process it to become palm kernel (Sundalin *et al*., 2021). Kernel can be used as an alternative feed ingredient and as a source of vegetable protein for poultry (Azizi *et al*., 2021; Alshelmani *et al*., 2021; Setiana *et al*., 2024). Incorporating 10% of palm kernel into the rations can affect broilers' production and performance as it contains 16.30% crude protein, 10.50% crude fat, and 2020.17 Kcal/Kg energy metabolism (Nuraini *et al*, 2017). However, it also contains a high level of crude fibre at 21.75%, 17.67% cellulose and 17.67% lignin. The level of crude fibre needs to be lowered in order to increase the quality of palm kernel. Fermentation can be used in order to increase the nutritional quality of a feed ingredient (Trisna *et al.*, 2020; Nguru *et al.*, 2024; Ogbuewu and Mbajiorgu, 2024). Fermentation can be done using *Phanerochaete chrysosporium* fungus.

Phanerochaete chrysosporium can produce two extracellular peroxidases which are lignin peroxidase (LiP) and manganese peroxidase (MnP) (Susanti *et al.*, 2022; Kato *et al.*, 2024). *P. chrysosporium* possesses an enzyme that can break down lignin (Zhu *et al.*, 2025) into lignocellulosic components (Rulianah *et al.*, 2017). Additionally, *P. chrysosporium* can increase protein level as it contains protease. Nuraini *et al.* (2015) found that fermenting using *P. chrysosporium* with an inoculum dose of 7% for 10 days yielded the best results; it resulted in 21.86% of crude protein, 14.07% crude fibre with a digestibility value of 49.06% and 62.41% of nitrogen retention. One factor that influences the success of fermentation is the duration of the fermentation (Zhu *et al.*, 2024). The fermentation duration influences the performance of *P. chrysosporium* through its effect on fungal growth, enzyme secretion and lignin/cellulose degradation. During the early days of fermentation the fungus is still establishing and enzyme production is low, while after a certain period enzyme activity and substrate breakdown reach a peak. If fermentation continues beyond optimal duration, nutrient depletion or accumulation of inhibitors may reduce effectiveness. Therefore, selecting an optimum fermentation time is critical to maximise nutrient improvement of lignocellulosic substrate (Zhang *et al.*, 2012; Konan *et al.*, 2024; Praveen and Brogi, 2025). Studies on *P. chrysosporium* have mainly focused on residues like corn stover and rice straw with 7–10 days of fermentation (Zhang *et al.*, 2012). Information on palm kernel is still limited, although its higher lignin content may require different fermentation duration. Compared with other ligninolytic fungi, *P. chrysosporium* produces strong lignin-degrading enzymes (LiP and MnP), enabling efficient lignin breakdown while preserving cellulose (Munir *et al.*, 2015). This study investigates how fermentation duration affects its performance on palm kernel to enhance nutritional quality, so that it can be used as an alternative feed ingredient for poultry.

MATERIALS AND METHODS

Palm kernel fermentation

The substrate used consisted of 100% palm kernel, and rice bran was used as the inoculum carrier for *P. chrysosporium*. The fermentation product was made by adding 125 ml of distilled water (60% water content) into 100 g of palm kernel and sterilizing them in an autoclave (at a temperature of 121°C for 15 minutes). Then, the sterilized palm kernel was inoculated with 7% of total substrate's worth of *P. chrysosporium* (Trisna *et al.*, 2020) and leveled with a thickness of 1 cm, then incubated for the predetermined duration (8, 10, 12, and 14 days). Afterwards, it was dried and ground into flour and then, it was observed.

Experimental design

This study used the Completely Randomized Design (CRD) method, which consisted of 4 treatments and 5 replications. The treatments are listed as follows:

- A= Fermentation period of 8 days
- B= Fermentation period of 10 days
- C= Fermentation period of 12 days
- D= Fermentation period of 14 days

Study variables

The variables observed in this study were the increase of crude protein (%) and hemicellulose (%), the decrease of crude fibre (%), cellulose (%) and lignin, as well as measuring the nitrogen retention value (%) and crude fibre digestibility (%). Crude protein and crude fibre contents were determined according to the procedures of AOAC (2016). The contents of cellulose, hemicellulose, and lignin were analyzed following the Van Soest method using neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) analyses (Van Soest *et al.*, 1991). Nitrogen retention and crude fibre digestibility were determined using the Sibbald method (Sibbald, 1982). In this test, 24 broilers aged 6 weeks were used, consisting of 4 chickens in the control group (without fermentation) and 20 chickens divided equally into four treatment groups, with 5 chickens per treatment. The chickens were first fasted for 24 hours before being force fed 30 g of the treatment feed. Excreta were collected for 48 hours, while correction chickens were fasted for 72 hours. The excreta were sprayed with 0.3 N H_2SO_4 every 5 hours to prevent nitrogen loss, after which nitrogen and crude fibre were analyzed to determine nitrogen retention and crude fibre digestibility values.

Data analysis

All data were analysed statistically using Completely Randomized Design (CRD) with 4 treatments and 5 replications. The differences between treatments were tested using Duncan's Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

The effect of treatments on the increase of crude protein (%) and hemicellulose (%)

The effect of the duration of palm kernel fermentation using *P. chrysosporium* on the increase of crude protein and hemicellulose can be seen in Table 1. The analysis of variance results show that the duration of palm kernel fermentation significantly influences ($P<0.01$) the increase of crude protein. The increase of crude protein in treatment C does not have a significant difference ($P>0.05$) with the increase in treatment D, but it has a significant difference ($P<0.01$) with the increase in treatment B and treatment A. The analysis of variance results show that the duration of palm kernel fermentation significantly influences ($P<0.01$) the increase of hemicellulose. The increase of hemicellulose in treatment C does not have a significant difference ($P>0.05$) with the increase in treatment D, but it has a significant difference ($P<0.01$) with the increase in treatment B and treatment A.

Table 1: Effect of fermentation duration of palm kernel using *Phanerochaete chrysosporium* on the percentage increase in crude protein and hemicellulose.

Parameters (%)	Treatment			
	A (8 days)	B (10 days)	C (12 days)	D (14 days)
Increase in crude protein	23.47 ± 0.62^{c}	29.62 ± 1.67^{b}	45.00 ± 1.72^{a}	43.88 ± 1.10^{a}
Increase in hemicellulose	24.78 ± 1.82^{c}	33.37 ± 0.94^{b}	46.64 ± 0.48^{a}	45.77 ± 0.91^{a}

Values are expressed as mean ± standard deviation. Different superscripts within a row indicate significant differences among treatments ($P < 0.01$).

The increase of crude protein in treatment C and treatment D was higher than the increase in treatment A and treatment B due to the growth of *P. chrysosporium* fungus in the substrate that flourished steadily and turned white. The steadily blooming fungi was marked by the high number of colonies in treatments C and D which were 14.85 log/ml and 14.75 log/ml respectively. *Phanerochaete chrysosporium* in treatment A and B did not develop properly, therefore the protein contribution from the fungi was not maximal and the there were less enzymes excreted which led to lower level of crude protein and marked with fewer numbers of colonies in both treatment A (14.10 log/ml) and B (14.51 log/ml). Crude protein increases due to a change in dry matter and the contribution by microbial cells growth which create single-cell proteins or biomass cells that contain approximately 40-65% protein (Bogale, 2020; Sharif *et al*., 2021).

The increase of hemicellulose in treatments C and D occurred due to the reduction in cellulose and lignin contents. Additionally, the release of lignin bonds with hemicellulose that bind cellulose fibre sheets to form microfibrils that increase cell wall stability and hemicellulose also relates with lignin to form a complex network and provide a strong structure (Li *et al*., 2023; Kong *et al*., 2024). *Phanerochaete chrysosporium* fungus excrete cellulase enzymes and ligninase to degrade cellulose and lignin respectively (Al-Rajhi *et al*., 2024; Mehta and Chelike, 2024). Hemicellulose is obtained from subtracting NDF (cellulose, lignin, hemicellulose) from ADF (cellulose and lignin).

The increase in crude protein (CP) content and hemicellulose obtained in this study (45.00% and 46.64%) was higher than that reported by Nuraini *et al*. (2015), who found that durian fruit waste fermented for 9 days using *P. chrysosporium* and *Neurospora crassa* showed the highest increase in crude protein of 39.44% (from 11.73% to 19.37%), while hemicellulose increased by 47.58% (from 4.99% to 9.52%). The higher improvement observed in the present study may be due to the optimal growth of *P. chrysosporium* on the 12 day of fermentation, during which this fungus actively degraded the palm kernel cake substrate through the production of protease and hemicellulase enzymes that enhance protein synthesis and fibre breakdown.

Table 2: Effect of fermentation duration of palm kernel using *Phanerochaete chrysosporium* on the reduction of crude fibre (%), cellulose (%), and lignin (%).

Parameters (%)	Treatment			
	A (8 days)	B (10 days)	C (12 days)	D (14 days)
Reduction in crude fibre	25.30 ± 1.15^{c}	30.26 ± 1.48^{b}	43.85 ± 1.16^{a}	42.85 ± 1.69^{a}
Reduction in cellulose	24.31 ± 1.64^{c}	33.29 ± 1.27^{b}	42.20 ± 0.59^{a}	41.88 ± 0.86^{a}
Reduction in lignin	23.30 ± 1.77^{c}	33.93 ± 0.96^{b}	44.13 ± 0.80^{a}	43.23 ± 0.83^{a}

Values are expressed as mean ± standard deviation. Different superscripts within a row indicate significant differences among treatments ($P < 0.01$).

The effect of treatments on the reduction of crude fibre (%), cellulose (%), and lignin (%)

The effect of the duration of palm kernel fermentation using *P. chrysosporium* on the reduction of crude fibre, cellulose and lignin could be found in Table 2. The analysis of variance results show that the duration of palm kernel fermentation significantly influences ($P<0.01$) the reduction of crude fibre. The reduction of crude fibre in treatment C does not have a significant difference ($P>0.05$) with the reduction in treatment D, but it has a significant difference ($P<0.01$) with the reduction in treatment B and treatment A. The analysis of variance results show that the duration of palm kernel fermentation significantly influences ($P<0.01$) the reduction of cellulose. The reduction of cellulose in treatment C does not have a significant difference ($P>0.05$) with the reduction in treatment D, but it has a significant difference (P<0.01) with the reduction in treatment B and treatment A. The analysis of variance results show that the duration of palm kernel fermentation significantly influences (P<0.01) the reduction of lignin. The reduction of lignin in treatment C does not have a significant difference (P>0.05) with the reduction in treatment D, but it has a significant difference (P<0.01) with the reduction in treatment and treatment A.

Compared to treatment A and B, in treatments C and D, the *P. chrysosporium* fungus was in their fast growth phase which resulted in more cellulase enzyme that can degrade cellulose and ultimately significantly reducing the level of crude fibre. *Phanerochaete chrysosporium* fungi has the ability to degrade fibre components because besides producing enzymes that can degrade lignin, they are also able to produce enzymes that can degrade cellulose (Kato *et al*., 2024; Kameshwar and Qin, 2017; Xie *et al*., 2021). *Phanerochaete chrysosporium* is a white-rot fungus that can selectively degrade cellulose components by rearranging lignin first, then followed by cellulose (Tuomela *et al*., 2000). In treatment C and D, the *P. chrysosporium* fungus was in their fast growth phase which allowed them to produces more cellulose enzyme to degrade cellulose resulting in a decrease in cellulose due to high production of cellulolytic enzymes. The *P. chrysosporium* fungus in treatment A and B had not grown perfectly yet as they were still in their early growth phase, where the were still not as much energy and nutrients produced resulting in less cellulose rearrangement, so there was not much cellulose reduction.

The reduction of lignin in treatments C and D occurred due to the abundant and uniform growth of *P. chrysosporium* fungus resulting in lignin rearrangement. However, *P. chrysosporium* in treatments A and B had not developed perfectly, therefore, the ligninase had not been able to rearrange the lignin maximally resulting in a nonoptimal reduction in lignin. According to Kato *et al*. (2024), *P. chrysosporium* fungus is able to effectively degrade lignin and its derivatives by producing lignin peroxidase and manganese peroxidase enzymes. Lignin peroxidase is the main catalyst used by *P. chrysosporium* in the ligninolytic process as it is able to breakdown 90% of lignin structures composed by phenolic units (Singh dan Chen, 2008; Vágvölgyi *et al*., 2014). Manganese peroxidase is able to oxidise Mn^{2+} into Mn^{3+} and it helps breakdown phenolic unit of lignin (Kumar and Arora, 2022; Zhang *et al*., 2023; Satwika *et al*., 2024).

The reductions in crude fibre (43.86%), cellulose (42.20%), and lignin (44.13%) observed in this study were greater than those reported in previous studies. Fajrona and Rizal (2024) reported that the fermentation of palm kernel cake (PKC) using *Sclerotium rolfsii* at an 8% inoculum level for 7 days resulted in only a 10.13% decrease in crude fibre (from 21.43% to 19.26%). Similarly, Zhu *et al*. (2024) found that fermentation of PKC with *Bacillus velezensis*, *Saccharomyces cerevisiae*, and *Lactobacillus paracasei* reduced cellulose by 17.6% and lignin by 20.2%. The superior performance of *P. chrysosporium* in the present study is likely due to its strong ability to degrade crude fibre in PKC through the synergistic action of cellulase and ligninase enzymes produced by the fungus. Moreover, the longer fermentation duration (12 days) may have allowed for more optimal fungal growth and enzymatic activity, resulting in a greater reduction in fibre components compared to the shorter fermentation periods reported in previous studies.

The effect of treatments on the value of nitrogen retention (%) and crude fibre digestibility (%)

The effect of the duration of palm kernel fermentation using *P. chrysosporium* on the value of nitrogen retention and crude fibre digestibility is presented in Table 3. The analysis of variance results show that the duration of palm kernel fermentation significantly influences (P<0.01) the value of nitrogen retention and crude fibre digestibility. The value of nitrogen retention and crude fibre digestibility in treatment C does not have a significant difference (P>0.05) with the values in treatment D, but it has a significant difference (P<0.01) with the values in treatment B and treatment A.

Table 3: Effect of fermentation duration of palm kernel using *Phanerochaete chrysosporium* on the value of nitrogen retention (%) and crude fibre digestibility (%).

Parameters (%)	Treatment			
	A (8 days)	B (10 days)	C (12 days)	D (14 days)
Nitrogen retention	62.00 ± 1.50^{c}	64.25 ± 1.40^{b}	67.74 ± 2.13^{a}	67.48 ± 1.32^{a}
Fibre digestibility	41.77 ± 2.03^{c}	46.95 ± 0.69^{b}	55.52 ± 0.56^{a}	54.84 ± 0.77^{a}

Values are expressed as mean ± standard deviation. Different superscripts within a row indicate significant differences among treatments (P < 0.01).

The high nitrogen retention level in treatments C and D is related to the high amount of crude protein consumed, which is linked to the higher increase of crude protein in treatment C and D which were 25.74% and 25.54%, respectively. The high level of nitrogen retention in treatments C and D is also due to the high amount of nitrogen consumed which were 0.75 (g) and 0.74 (g), respectively. The high nitrogen retention levels in treatments C (67.74%) and D (67.48%) show that the protein quality in treatments C and D is better than in other treatments. According to Prawitasari *et al*. (2012), the level of protein digestibility depends on the protein content of the feed and the amount of protein that enters the digestive system.

The crude fibre digestibility value in treatments C and D is related to the low crude fibre contents which were 12.03% and 12.24% respectively. On the other hand, there is a higher level of crude fibre content in treatments A and B at 16.00% and 14.94%, respectively. The low crude fibre content is occurred because the lignin and cellulose contents in the BISF had been broken down by ligninase and cellulose enzymes produced by *P. chrysosporium* which increases the digestibility of the feed. Fermented food tends to have a better nutritional value as microorganisms are catabolic and able to breakdown complex substances into simpler ones for that they can be digested easily (Sharma *et al*., 2020; Shah *et al*., 2023; Knez *et al*., 2023). Furthermore, there is a high digestibility of crude fibre in the two treatments because there is also a high level of hemicellulose. Hemicellulose is a soluble fibre that can be digested easily.

The nitrogen retention (67.74%) and crude fibre digestibility (55.52%) values obtained in this study were higher than those reported in previous studies. Maulana *et al*. (2021) reported nitrogen retention of 55.09% and crude fibre digestibility of 54.02% in palm oil waste fermented with *Lentinus edodes* for 9 days. The higher values observed in the present study indicate that *P. chrysosporium* was more effective in improving nitrogen retention and crude fibre digestibility. This improvement is likely associated with the longer fermentation duration (12 days), which enhanced the enzymatic degradation of complex fibre structures in palm kernel cake, making the fermented product more digestible for poultry.

CONCLUSION

It can be concluded that fermenting palm kernel with *Phanerochaete chrysosporium* for 12 days yields the best improvement in its nutritional quality. The 12 days treatment exhibited that crude protein increased by 45.00%, hemicellulose increased by 46.64%, crude fibre decreased by 43.86%, cellulose decreased by 42.20%, lignin decreased by 44.13%, crude fibre digestibility reached 55.52%, and nitrogen retention reached 67.74%.

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NOVELTY STATEMENT

This study shows that the nutritional value of palm kernel can be improved through careful adjustment of fermentation time using *Phanerochaete chrysosporium*. By comparing several fermentation periods under controlled conditions, the study highlights how biological treatment influences protein levels, structural fiber reduction, digestibility, and nitrogen retention. Together, these results support the use of white-rot fungal fermentation as a practical approach for enhancing palm kernel as a feed ingredient.

AUTHORS' CONTRIBUTION

AT and MA was responsible for formulating the study and assessing the manuscript. AT, NN, and MA. They conducted field and laboratory investigations and gathered information. MA and FM conducted the data analysis and wrote and polished the manuscript. All authors have reviewed, assessed, and approved the final manuscript.

Generative AI and AI-assisted technology statement

The authors declare that no Generative AI or AI-assisted technologies were used in the creation of this manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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