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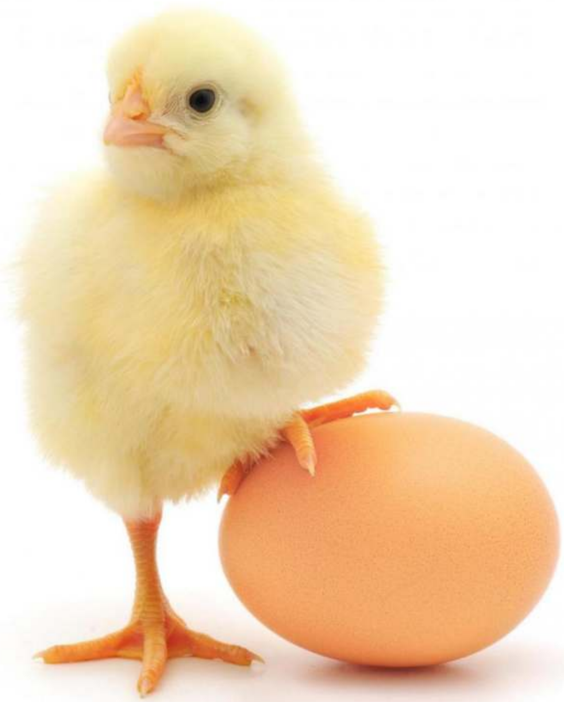

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Research Article
The Effect of Substrate Composition Fermented Using *Pleurotus ostreatus* on the Nutrient Content of Palm Oil Sludge

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Abstract

Background and Objective: Palm oil sludge has been an alternative feed ingredient for poultry but its utilization is limited due to its high content of crude fiber (lignin and cellulose) and its low crude protein content. Fermentation with *Pleurotus ostreatus* (lignocellulolytic fungus producing the enzyme ligninase) was used to degrade the crude fiber to increase the nutritional content of fermented palm oil sludge. This study aimed to determine the best substrate composition of palm oil sludge fermented by *Pleurotus ostratus*. **Materials and Methods:** The completely randomized design (CRD) was used with 4 treatments and 5 replications. The treatments consisted of palm oil sludge (POS) and rice bran (RB): A (100% POS+0% RB), B (90% POS+10% RB), C (80% POS+20% RB) and D (70% POS+30% RB). The parameters measured were crude protein (%), crude fiber (%), cellulase enzymatic activity (U mL^{-1}) and laccase enzymatic activity (U mL^{-1}). **Results:** Fermentation with *Pleurotus ostreatus* showed a highly significant effect ($p<0.01$) on crude protein content, a significant effect ($p<0.05$) on crude fiber content and no significant effects ($p>0.05$) on the enzymatic activities of cellulase and laccase. **Conclusion:** It is concluded that treatment B increased the crude protein, cellulase activity and laccase activity in palm oil sludge but decreased the crude fiber content.

Key words: Cellulase, crude fiber, crude protein, fermentation, laccase, palm oil sludge, *Pleurotus ostreatus*

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Conventional feed is relatively expensive such as yellow corn and soy bean meal. and the use of unconventional feed sources can reduce the dependence on conventional feed use. Feed prices occupy a large portion (60-70%) of total production cost[1]. Therefore, unconventional feed is needed for poultry feed.

Unconventional feed sources, which can be used as animal feed, include agricultural waste or agricultural industrial waste, such as palm oil sludge (waste from the palm oil processing industry). The total area of palm oil plantations in Indonesia reached 10,465,020 ha in 2013. In 2014, the area of palm oil plantations increased to 10,754,801 ha and in 2015, the area of palm oil plantations had again increased to 11,300,370 ha[1]. Palm oil sludge has fairly good nutrient content, with a crude protein content of 11.35% and a metabolic energy of 1550 kcal kg^{-1} [2]. Palm oil sludge contains nutrients that are similar to rice bran; however, it has a greater crude fiber content (25.80%), with the greatest fiber components being lignin (19.19%) and cellulose (16.15%)[3]. In poultry, especially broiler chicken, the utilization of palm oil sludge is just 5%[2,3]. This is due to the high crude fiber content found in palm oil, which can reduce feed consumption, thereby leading to slower growth[4]. Therefore, it is necessary to eliminate, or reduce, the fiber content of palm oil sludge to increase its nutritional value.

One effort to solve this problem includes the use of fermentation. Fermentation is one possible method to increase the nutrient content and reduce the crude fiber content of palm oil sludge. Fermentation biotechnology can be used to decrease the crude fiber in palm oil sludge by utilizing microorganisms. Some of these microorganisms are fungi from the Basidiomycetes class that effectively decompose high lignocellulose ingredients[5]. One of the species from the Basidiomycetes class, namely, *Pleurotus ostreatus*, is part of the white rot fungi known as lignocellulolytic fungi[6]. This fungus contains lignin-degrading enzymes, such as the lignin peroxidase (LiP) enzyme, the manganese peroxidase (MnP) enzyme and the laccase enzyme. The species *Phanerochaete chrysosporium* has the following ligninase enzymes: the lignin peroxidase (LiP) enzyme, the manganese peroxidase enzyme (MnP) and the cellulase enzyme.

The success of fermentation is strongly influenced by the composition of the substrate used, which agrees with the opinion of Nuraini *et al.*[7], who stated that the success of solid media fermentation is highly dependent on having optimum conditions, such as substrate composition, substrate thickness, inoculum dose and fermentation time. Rice bran is often used in the fermentation mixture media. The addition of rice bran in fermentation media improves the nutritional value of the fermentation substrate since rice bran contains nitrogen and vitamins (thiamin), which improve the growth of the *Pleurotus ostreatus* mycelium, according to Silverio *et al.*[8], the addition of nitrogen can cause thick and compact mycelium growth.

This study aimed to determine the best substrate composition of palm oil sludge fermented by *Pleurotus ostreatus*, based on crude fiber content, crude protein and cellulase and laccase enzyme activity.

MATERIALS AND METHODS

Palm oil sludge fermentation: Palm oil sludge (POS) was dried under sunlight until the water content was approximately 12-14%. Then, a 100 g substrate, consisting of palm oil sludge (POS) and rice bran (RB), with a composition according to treatment (60% moisture content), was put into a plastic bag and stirred frequently. The substrate was sterilized in an autoclave (121°C for 15 min) and then left to equilibrate to room temperature. The sterile substrate was inoculated with 10% *Pleurotus ostreatus* inoculum. The substrate was then placed in a sterile bottle and incubated for 9 days. After the fermentation process ended, the fermented product was weighed and then dried at 80°C for 2 h, which killed the fungi; the product then continued drying at 60°C for 23-36 h (until dry). After the product was dried, it was stirred frequently, milled and then sampled for analysis and the creation of enzyme extracts.

Enzymatic activity: Enzyme extracts were created by taking 5 g of the sample and soaking it with 50 mL 0.05 M pH 5 acetate buffer. The solution then stood for 2 h, was strained and then the filtrate was taken. The filtrate was centrifuged at 15000 rpm for 15 min and then the supernatant was collected. This supernatant was the crude enzyme extract.

Measurement of cellulase enzyme activity was carried out by taking 1 mL crude enzyme extract and adding 1 mL substrate (0.1% CMC in 0.05 M pH 5 acetate buffer). This was then mixed using a vortex and placed in a shaker water bath for 30 min at 60°C for the reaction to occur. The reaction was stopped by putting it in boiling water for 5 min. Hydrolysis products, namely, Nelson's A and Nelson's B reagents (1 mL), were added and the solution was heated in boiling water for 5 min. A total of 1 mL phosphomolybdate reagent was added, followed by 7 mL of distilled water and then the released glucose was measured using a spectrophotometer at a wavelength of 575 nm.

Measurement of laccase enzyme activity was based on the procedure described by Buswell *et al.*[9]. The sample solution to be analyzed was made with 0.4 mL enzyme filtrate that was mixed with 0.5 mL 0.5 M pH 5 and 0.1 mL ABTS 1 Mm acetate buffers. This mixture was inserted into a cuvette and then shaken. After shaking the solution, the absorbance was measured at a 420 nm wavelength at 0 and 30 min time intervals.

Experimental design: This study used a completely randomized design (CRD) that had 5 treatments performed with 4 replications. The substrate composition consisted of a mixture of palm oil sludge (POS) and rice bran (RB) and each treatment was named as follows; A (100% POS+0% RB), B (90% POS+10% RB), C (80% POS+20% RB) and D (70% POS+30% RB).

Variables: The parameters measured were crude protein (%), crude fiber (%), cellulase enzyme activity (U mL^{-1}) and laccase (U mL^{-1}) enzyme activity.

Data analysis: All data were statistically analyzed using a one-way analysis of variance with completely randomized design (CRD). Significant differences between treatments were determined by using Duncan's Multiple Range Test and a significant level was set at $p<0.05$.

RESULTS

The effects of each treatment on the crude protein, crude fiber and cellulase and laccase enzyme activities are shown in Table 1.

Effect of treatment on crude protein content: The results of the analysis of variance showed that there was a significant treatment effect ($p<0.01$) on crude protein content. The highest crude protein percentage (25.1622%) was found in treatment B and the lowest percentage (21.3248%) was found in treatment A.

Effect of treatment on crude fiber content: The results of the analysis of variance showed that there was a significant treatment effect ($p<0.05$) on crude fiber content. The lowest crude fiber percentage (15.50%) was found in treatment B and the highest percentage (18.08%) was found in treatment A.

Effect of treatment on cellulase enzyme activity (U mL^{-1}): The results of the analysis of variance showed that there was no significant effect of treatment ($p>0.05$) on cellulase enzyme activity. The greatest cellulase enzyme activity (3.95 U mL^{-1}) was found in treatment B and the lowest activity (3.90 U mL^{-1}) was found in treatment A.

Effect of treatment on laccase enzyme activity (U mL^{-1}): The results of the analysis of variance showed that there was no significant effect of treatment ($p>0.05$) on laccase enzyme activity. The greatest laccase enzyme activity (14.42 U mL^{-1}) was found in treatment B and the lowest activity (12.38 U mL^{-1}) was found in treatment A.

DISCUSSION

The high crude protein content in treatment B caused the *Pleurotus ostreatus* fungus to grow well because it had enough nutrients from the carbon and nitrogen present in palm oil sludge and rice bran. The high crude protein content in treatment B created a C:N balance of 14.93: 1, which is suitable for the growth of *Pleurotus ostreatus*. According to Nuraini *et al.*[10], the C:N balance that is suitable for the growth of molds and fungi ranges from 10:1 to 15:1. The mycelium cell wall of the fungus contains protein, which contributes to the total protein content. The fungal cell walls contain 6.3% protein, while fungal cell membranes that contain hyphae are 24-45% protein[11]. During the fermentation process, the fungus secretes an enzyme, which is also protein; thus, the microbe itself is also a source of single cell protein[12]. The low content of crude protein in treatment A led to little growth of the microorganism because the substrate had a C:N of 20:1 since it only contained palm oil sludge without the addition of rice bran, which is a source of nitrogen.

Table 1: Average crude protein (%), crude fiber (%), cellulase and laccase enzyme activity (U mL^{-1})

Treatments	CP**	CF*	Cellulase^{ns}	Laccase^{ns}
A (100% POS+0 RB)	21.32^{c}	18.08^{a}	3.90	12.38
B (90% POS+10% RB)	25.16^{a}	15.50^{b}	3.95	14.42
C (80% POS+20% RB)	24.05^{ab}	16.06^{b}	3.93	13.08
D (70% POS+30% RB)	23.18^{b}	16.71^{ab}	3.92	12.96
SE	0.47	0.62	0.03	0.65

**Highly significant ($p<0.01$) *significant ($p<0.05$), ^{ns}Non significant ($p>0.05$)

The low crude fiber content in treatment B increased the activity of the cellulase enzyme to 3.95 U mL^{-1} and the activity of the laccase enzyme to 14.42 U mL^{-1}. Rice bran increased the nutritional value of palm oil sludge fermentation (POSF) because rice bran contains natural nitrogen, which is needed for the growth of *Pleurotus ostreatus*. Rice bran has been widely used to induce growth of the mycelium in fungi cultivation because of its nitrogen, carbohydrate, carbon and vitamin B1 (thiamine) content[13]. Rice bran can be used as a natural substrate to increase the thickness and density of the mycelium[8].

The high crude fiber content in treatment A correlated with the lowest cellulase enzyme activity. The decreased cellulase enzyme activity in treatment A was due to the incomplete growth of the *Pleurotus ostreatus* mycelium. The growth of the mycelium was limited because no rice bran was added to the substrate, whereas in the other treatments, rice bran was added. The low cellulase enzyme activity in treatment A was as low as the cellulase enzyme activity in treatment D (which had the addition of 30% rice bran). The addition of a larger quantity of rice bran disrupted the growth of *Pleurotus ostreatus*, thereby decreasing its ability to degrade POS. This fungus can only grow in the presence of small quantities of nitrogen. Excessive amounts of nitrogen from rice bran can form ammonia, which negatively impacts fungal growth[14].

The enzyme activity of laccase in treatment B was 14.42 U mL^{-1}, which was higher than that in treatments C, D and A, since the growth of *Pleurotus ostreatus* in treatment B was better than that in the other treatments. This result was due to a sufficient addition of rice bran in treatment B for the growth of *Pleurotus ostreatus,* which increased its ability to degrade lignocellulose in palm oil sludge. Laccase enzyme activity, in this study, ranged from 12.38-14.42 U mL^{-1}. This result was higher than what Subowo[15] found in liquid fermentation, where the activity was 8.29 U mL^{-1}. The increased laccase enzyme activity also caused the humidity of the fermentation to be in the range of 60%. Optimal laccase enzyme activity occurs at a humidity level between 40-60% and when the humidity level increases above 60%, the laccase enzyme activity begins to decrease[16].

CONCLUSION

When the composition of the substrate is 90% POS+10% RB and it is fermented with *Pleurotus ostreatus*, then the crude protein content and cellulase and laccase enzyme activity increase and the crude fiber content decreases.

SIGNIFICANCE STATEMENT

The results of the present study will aid in the development of fermentation with *Pleurotus ostreatus*, which will not only increase the growth of *Pleurotus ostreatus* but it will also increase protein content and ligninase and cellulose enzyme activity and reduce crude fiber content.

REFERENCES

1. DGoIP., 2015. Indonesian plantation statistics 2013-2015. Directorate General of Plantation, Ministry of Agriculture, Jakarta, Republic of Indonesia.
2. Nuraini, A. Djulardi and A. Trisna, 2017. Palm oil sludge fermented by using lignocellulolytic fungi as poultry diet. Int. J. Poult. Sci., 16: 6-10.
3. Djulardi, A., N. Nuraini and A. Trisna, 2018. Palm oil sludge fermented with *Lentinus edodes* in the diet of broilers. Int. J. Poult. Sci., 17: 306-310.
4. Aritonang, D., 1984. Effect of the use of palm kernel cake in growing pig rations. Master's Thesis, Faculty of Animal Husbandry, Bogor Agricultural Institute, Bogor.
5. Sun, Y. and J. Cheng, 2002. Hydrolysis of lignocellulosic materials for ethanol production: A review. Bioresour. Technol., 83: 1-11.
6. Hatakka, A., 2001. Biodegredaion of Lignin. In: Biopolymers, Lignin, Humic Substances and Coal, Steinbuchel, A. (Ed.)., Vol. 1, Wiley VCH., Germany, pp: 129-180.
7. Nuraini, Sabrina and S.A. Latif, 2009. Improving the quality of tapioca by product through fermentation by *Neurospora crassa* to produce β carotene rich feed. Pak. J. Nutr., 8: 487-490.
8. Silverio, C.M., I.C. Viela., F.L. Guilatco and N.B. Hernandes, 1981. Mushroom culture on enriched composed Sawdust. NSDB, Technol. J., 6: 22-40.
9. Buswell, J.A., Y. Cai and S.T. Chang, 1995. Effect of nutrient nitrogen and manganese on manganese peroxidase and laccase production by *Lentinula* (*Lentinus*) edodes. FEMS Microbiol. Lett., 128: 81-87.
10. Nuraini, Y.S. Nur and A. Djulardi, 2018. Improving the quality of cocoa pods fermented with *Pleurotus ostreatus* to produce low cholesterol of poultry eggs. Research report., Dikti Competency Grant, LPPM Unand.
11. Garraway, M.O. and R.C. Evans, 1984. Fungal Nutrition and Physiology. John Wiley and Sons, New York, ISBN: 0471058440.

12. Fardiaz, S., 1987. Practice Guide to Food Microbiology. Information Resource Institution, Bogor, Indonesia.
13. Okwujiako, I.A., 1990. The effect of vitamin on the vegetative growth and fruitbodi of *Pleurotus sajorcaju*. J. Mush. Trop., 10: 35-39.
14. Parlindungan, A.K., 2003. Characteristics of growth and production of white oyster mushrooms (*Pleurotus ostreatus*) and gray oyster mushrooms (*Pleurotus sajor* Caju) in the log of reeds. J. Natur Indonesia, 5: 152-156.
15. Subowo, Y.B., 2015. Fungal Selection of Ligninase Enzymes and their Ability to Decompose Palm Oil Liquid Waste. Vol. 1, Indonesian Institute of Sciences (LIPI), Bogor, Indonesia, pp: 1766-1770.
16. Patel, H., A. Gupte and S. Gupte, 2009. Effect of different culture conditions and inducers on production of laccase by a basidiomycete fungal isolate *Pleurotus ostreatus* HP-1 under solid state fermentation. BioResources, 4: 268-284.