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Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorpjn@gmail.com

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Research Article

The Combined Effects of Fungi *Phanerochaete chrysosporium* and *Neurospora crassa* and Fermentation Time to Improve the Quality and Nutrient Content of Palm Oil Sludge

Mirnawati, Gita Ciptaan and Ade Djulardi

Department of Animal Nutrition and Feed Technology, Faculty of Animal Science, Andalas University, Padang 25163, Indonesia

Abstract

Background and Objective: Palm oil sludge, as a byproduct of the palm oil industry, is an agricultural waste product that can be used as an alternative feedstuff for poultry. Palm oil sludge also contains nutrients that can be used as feed ingredients for poultry. Palm oil sludge is limited in use in broiler rations because of its low quality and nutrient content. For this reason, it is necessary to process palm oil sludge by fermentation methods to improve the quality and the nutrient content. This study aimed to determine the combined effect of fungi (*P. chrysosporium* and *N. crassa*) and fermentation time to improve the quality of fermented palm oil sludge. **Materials and Methods:** The materials used in this study were palm oil sludge, the fungi *Phanerochaete chrysosporium* and *Neurospora crassa* and fermentation materials and tools. This experimental study used a completely randomized design (CRD) with a 3×3 factorial pattern with 3 replications. The treatments consisted of factor A (combination of *Phanerochaete chrysosporium* and *Neurospora crassa*), which consisted of A1 (3:1), A2 (3:2) and A3 (4:1) and factor B (fermentation time), which consisted of B1 (7 days), B2 (10 days) and B3 (13 days). **Results:** The results of the analysis of variance showed that there was a significant interaction (p<0.05) between factor A and factor B. Each factor A and B showed a significant effect (p<0.05). **Conclusion:** In this study, it was concluded that the combination of *Phanerochaete chrysosporium* and *Neurospora crassa* (4:1) and 13 days of fermentation time provided optimal results, with 26.20% crude protein, 14.49% crude fiber, 14.54% lignin, 58.20% nitrogen retention and 57.66% crude fiber digestibility.

Key words: Fermentation time, fermentation, *Neurospora crassa*, Nitrogen retention, nutrient content, palm oil sludge, *Phanerochaete chrysosporium*

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Corresponding Author: Mirnawati, Department of Animal Nutrition and Feed Technology, Faculty of Animal Science, Andalas University, Padang 25163, Indonesia

Competing Interest: The authors have declared that no competing interest exists.

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

INTRODUCTION

Fluctuation in the price of feed ingredients is an obstacle that often results in unstable poultry farming in Indonesia. Many feedstuffs are still imported, so the market price is quite high. This condition causes poultry feed costs to reach 60-70% of production costs. To reduce the cost of poultry rations, many efforts have been made, namely, using alternative feed ingredients that are relatively inexpensive, of high nutritional value, are available throughout the year, do not compete with human needs and have a good influence on livestock. For these reasons, alternative feed ingredients are the right choice to overcome these obstacles. Palm oil sludge, as a byproduct of palm oil manufacturing, is an agricultural waste that can be used as an alternative feed ingredient[1].

The Directorate General of Plantations[2] reports that the area of oil palm plantations in Indonesia is 11,312,640 ha, which produces 30,948,931 t of palm oil. Each hectare of oil palm plantation will produce 840-1260 kg of palm oil sludge (POS) and 567 kg of palm kernel cake[3]. The amount of palm oil sludge increases from year to year, which is in line with the increase in palm oil production and is approximately 2% of the total palm oil production[4]. Palm oil sludge also contains nutrients that can be used as feed ingredients for poultry. Mirnawati *et al.*[5] stated that the nutrient content of palm oil sludge was 89.70% dry matter, 14.77% crude protein, 28.34% crude fiber, 8.06% crude lipid, 0.28% Ca, 0.65% P and 1306.42 kcal/metabolizable energy. Although palm oil sludge contains a good amount of nutrients, its use in poultry rations is still limited, at approximately 5%,[6] due to the high crude fiber, lignin and crude fat content[7]. In addition, the lignin content in palm oil sludge is high at 36.40%, which causes limited use in poultry rations due to the inability of poultry to digest lignin[8].

To improve the quality of palm oil sludge so that its utilization in livestock rations can be maximized, it is necessary to perform processing by fermentation. Fermentation can break down cellulose, hemicellulose and two polymers into simple sugars or derivatives and can increase the nutritional content of the original ingredients[9,10]. Fermentation can alter feed ingredients containing protein, fat and carbohydrates that are difficult to digest to be more easily digested while also producing aromas that are preferred by livestock. This fermentation process can also reduce limiting factors such as lignin. For this reason, it is necessary to look for microbes that can produce ligninase enzymes that can reduce the lignin content of palm oil sludge. One of the microbes that produce the ligninase enzyme is *Phanerochaete chrysosporium*. *Phanerochaete chrysosporium* is a weathering fungus that is known for its ability to degrade lignin. According to Zeng *et al.*[11], some species of white rot fungi from the *Basidiomycetes* class are capable of breaking down all lignocellulose components. The results of research conducted by Noferdiman and Yani[12] stated that palm oil sludge fermented with a 6% *P. chrysosporium* inoculum for 8 days was the best combination treatment, with an increase in PK (30.75%) and a decline in SK (40.86%), cellulose (39.78%) and lignin (36.40%) but its utilization is still limited to 15% in broiler rations.

Observation in the field found that palm oil sludge is always overgrown with orange-colored fungi, namely, *Neurospora* sp. Mirnawati *et al.*[13] conducted a study to select three types of fungi that grow in palm oil sludge, namely, *N. crassa*, *Neurospora sitophila* and *Neurospora* sp. From the three types of fungi, *N. crassa* provided the best results in fermented palm oil sludge compared to two other types of *Neurospora*. Palm oil sludge fermented with *N. crassa* can increase crude protein content from 13-20.42% and metabolizable energy from 1105.87-2317.00 kcal kg^{-1}, while decreasing crude fiber from 32.07-20.59% and crude lipid from 12.31-2.08% but its use is still low at 13% in broiler rations[13]. Mirnawati *et al.*[5] carried out fermentation of palm oil sludge with *N. crassa* and the addition of 200 ppm humic acid, which resulted in an increase of crude protein to 23.74%, nitrogen retention to 60.97% and digestibility of crude fiber to 55.63% and reduced crude fiber to 20.14% and crude fat to 2.70%. Furthermore, palm oil sludge fermented with *Neurospora crassa* has been used in broiler rations and it can be used up to 22%[1].

Additionally, fermentation with *Neurospora crassa* aims to produce β-carotene. The fungus *Neurospora crassa* is the highest β-carotene-producing fungus that has been isolated from palm oil sludge[13]. *Neurospora crassa* can also produce amylase, cellulase and protease enzymes. The use of rich β-carotene-feed ingredient in poultry rations can reduce cholesterol in eggs and poultry meat[14].

Based on the descriptions above, it was necessary to conduct a study to combine the fungi *P. chrysosporium* and *N. crassa* to determine which combination was best improved the nutrient content and quality of fermented palm oil sludge. This experiment aimed to determine the combined effect of the fungi *P. chrysosporium* and *N. crassa* and fermentation time to improve the quality of fermented palm oil sludge.

MATERIALS AND METHODS

The materials used in this experiment were as follows: (1) palm oil sludge derived from palm kernel processing and manufacturing by Andalas Agro Industry in Pasaman, West Sumatra, (2) the fungi *Neurospora sitophila* and *Neurospora crassa* obtained from The Research Center of Applied Chemistry, LIPI, Bogor, (3) preparations of potato dextrose agar medium (PDA/Potato Dextrose Agar) produced by Difco-Becton Dickinson, (4) smooth bran and (5) aquades and mineral standards consisting of $(NH_4)2SO_4$ 0.14%, KH_2PO_4 0.2%, $(MgSO_4)7H_2O$ 0.03%, Urea 0.03%, $CaCl_2$ 0.03%, $7H_2O$ 0.0005%, $FeSO_4MnSO_4H_2O$ 0.00016%, $ZnSO_47H_2O$ 0.00014%, $CoCl_2$ 0.0002% and peptone 0.075%[15].

The experiment was conducted using a 3×3 factorial completely randomized design (CRD) with 3 replications[3]. The treatment factors were factor A (combination of *P. chrysosporium* and *N. crassa*): A1 (3:1), A2 (3:2) and A3 (4:1) and factor B (fermentation time): B1 (7 days), B2 (10 days) and B3 (13 days). The variables measured were crude protein, crude fiber, lignin, nitrogen retention and digestibility of crude fiber of fermented palm oil sludge. The data obtained were examined by statistical analysis according to Steel and Torrie[16] and the differences between treatments were tested by Duncan's multiple range test (DMRT).

RESULTS AND DISCUSSION

The average content of crude protein, crude fiber, carotenoids, lignin, nitrogen retention and crude fiber digestion of fermented palm oil sludge in Table 1.

Crude protein content of fermented palm oil sludge: The results of the analysis of variance showed that there was a significant interaction ($p<0.05$) between factor A (combination of *P. chrysosporium* and *N. crassa*) and factor B (fermentation time) for crude protein. Each factor A (combination of *P. chrysosporium* with *N. crassa*) and factor B (fermentation time) also showed a highly significant effect ($p<0.01$) on crude protein content.

The highest crude protein content (26.20%) was found in the A3B3 treatment, namely, a combination of *P. chrysosporium* with *N. crassa* (4:1) with a 13-day fermentation time. The high content of crude protein in this treatment was caused by the correct combination of fungi and appropriate fermentation time. Both *P. chrysosporium* and *N. crassa* can produce protease enzymes that break down substrates, especially proteins that are difficult to digest, allowing them to be easily digested.

In addition, high crude protein content is produced by the addition of proteins donated by microbial cells due to their growth, which produces single cell protein products (PST), or cellular biomass that contains approximately 40-65% protein[17,18]. Iyayi[19] stated that the crude protein content of the substrate was a result of the contribution of fungi and enzymes produced during fermentation.

Crude fiber content of fermented palm oil sludge: The results of the analysis of variance showed that there was a significant interaction ($p<0.05$) between factor A (combination

Table 1: Average content of crude protein, crude fiber, lignin, nitrogen retention and crude fiber digestion of fermented palm oil sludge

		Factor B			
Parameters	Factor A	B1 (7 days)	B2 (10 days)	B3 (13 days)	Average
Crude protein (%)	A1 (3:1)	16.34^{cC}	20.53^{bC}	23.32^{aC}	20.06
	A2 (3:2)	18.87^{cB}	22.75^{bB}	24.60^{aB}	22.07
	A3 (4:1)	22.85^{cA}	24.43^{bA}	26.20^{aA}	24.49
	Average	19.35	22.57	24.71	
Crude fiber (%)	A1 (3:1)	20.30^{aA}	18.20^{bA}	16.58^{cA}	18.36
	A2 (3:2)	19.79^{aB}	17.15^{bB}	15.72^{cB}	17.55
	A3 (4:1)	17.48^{aC}	16.75^{bC}	14.49^{cC}	16.24
	Average	19.19	17.37	15.60	
Lignin	A1 (3:1)	28.22^{aA}	24.49^{bA}	22.46^{cA}	25.06
	A2 (3:2)	26.01^{aB}	21.30^{bB}	17.30^{bC}	21.54
	A3 (4:1)	21.37^{aC}	18.57^{bC}	14.54^{cC}	18.16
	Average	25.20	21.45	18.10	
Nitrogen retention (%)	A1 (3:1)	38.61^{cC}	40.95^{bC}	46.64^{aC}	53.73
	A2 (3:2)	40.45^{cB}	45.40^{bB}	50.95^{aB}	53.60
	A3 (4:1)	47.94^{cA}	50.35^{bB}	58.20^{aA}	52.83
	Average	42.33	60.90	56.93	53.39
Digestibility of crude fiber (%)	A1 (3:1)	45.79^{cC}	47.24^{bC}	50.58^{aC}	47.53^{b}
	A2 (3:2)	46.51^{cB}	48.84^{bB}	53.11^{aB}	48.48^{b}
	A3 (4:1)	49.57^{cA}	51.79^{bA}	57.66^{aA}	51.67^{a}
	Average	47.29	49.29	51.11	

The lower case letters and uppercase letters in the same column or line indicate a significant difference ($p<0.05$)

of *P. chrysosporium* and *N. crassa*) and factor B (fermentation time) for crude fiber. Each factor A (combination of *P. chrysosporium* and *N. crassa*) and factor B (fermentation time) also showed a very significant effect ($p<0.01$) on the crude fiber content of fermented palm oil sludge.

Based on the data in Table 1, there was a decrease in the average crude fiber content with an increase in the combination of *P. chrysosporium* and *N. crassa*. A significant reduction (16.57%) in the average crude fiber content was observed in treatment A3, due to the work of cellulase produced from the combination of *P. chrysosporium* and *N. crassa*, as the fungi were able to break down the crude fiber in the substrate. In accordance with the opinion of Ofuya and Nwajiuba[20], the more fungi that grow, the more cellulase is produced to break down cellulose into glucose; at the end of fermentation, the crude fiber content has been decreased. Furthermore, Moore-Landecker[21] and Mirnawati *et al.*[22] stated that cellulase can break cellulose into glucose in the substrate to produce energy so that the crude fiber content decreases. Glucose is used as an energy source for cell growth so that fungi can multiply, as seen from the growth of fertile fungi[23,24].

The crude fiber content decreased with the length of fermentation time. The longer the fermentation time, the more chance the fungi will grow and multiply so that they can break down cellulose, which is useful in breaking down the crude fiber of the substrate and thus decreasing crude fiber. According to Fardiaz[25] and Mirnawati *et al.*[5], short fermentation times result in limited opportunities for fungi to grow and multiply, therefore, the substrate components that are converted into cellular biomass will also be less and fermentation time that exceeds the optimum limit can cause fungi to die due to less food available for growth.

Lignin content of fermented palm oil sludge: The results of the analysis of variance showed that there was a significant interaction ($p<0.05$) between factor A (combination of *P. chrysosporium* and *N. crassa*) and factor B (fermentation time) for lignin. Each factor A (combination of *P. chrysosporium* and *N. crassa*) and factor B (fermentation time) also showed a very significant effect ($p<0.01$) on lignin in palm oil sludge. The content of lignin decreased with an increasing combination of fungi and a longer fermentation time. The lowest lignin content was found in the A3B3 treatment, namely, a combination of *P. chrysosporium* and *N. crassa* (4:1) with a 13-day fermentation duration. This was because *P. chrysosporium* was higher in this treatment than the others, so the chance for the fungus to grow was higher, increasing the ability to produce ligninase enzymes. This was indicated by the large number of *P. chrysosporium* fungal mycelium that covered the surface of the substrate. The fungi that grew substantially caused an increase in ligninase so that the lignin content decreased. There are 3 types of enzymes produced by *P. chrysosporium*, namely, LiP (lignin peroxidase), MnP (manganese peroxidase) and laccase, which play important roles in degrading lignin by 7-30%, depending on the type of lignin and the incubation time[26].

The content of lignin decreased with the length of fermentation time; the longer the fermentation time, the more the fungus has the opportunity to grow and multiply so that it can produce ligninase enzymes.

Nitrogen retention of fermented palm oil sludge: Results of the analysis of variance showed that there were highly significant interactions ($p<0.05$) between factor A (combination of *P. chrysosporium* and *N. crassa*) and factor B (fermentation time) for nitrogen retention. Factor A and factor B also significantly affected ($p<0.01$) the nitrogen retention of fermented palm oil sludge.

From Table 1, it can be seen that the nitrogen retention was highest in the A3B3 treatment at 58.20%. The result of the A3B3 treatment was due to more protein being consumed than being excreted through feces and urine. Nitrogen retention will be positive if the nitrogen consumed is more than that excreted. The high nitrogen retention in treatment A1B2 was also caused by fungi that can alter the protein structure of the substrate, such that when given to poultry, it will facilitate the work of protease enzymes in the digestive tract of poultry to break down the protein components contained in the feed[5]. This occurs because fermentation process activated microbial metabolism that produced vitamin and essential amino acid ,increases protein and reduces crude fiber composition[9].

Crude fiber digestibility of fermented palm oil sludge: The results of the analysis of variance showed that there was a significant interaction ($p<0.05$) between factor A (combination of *P. chrysosporium* and *N. crassa*) and factor B (fermentation time) for the crude fiber digestibility of fermented palm oil sludge. Each factor A (combination of *P. chrysosporium* and *N. crassa*) and factor B (fermentation time) also showed a very significant effect ($p<0.01$) on crude fiber digestibility in fermented palm oil sludge.

The crude fiber digestibility of fermented palm oil sludge increased with the combination of fungi and fermentation time. The highest crude fiber digestibility was found in the A3B3 treatment, which was 57.66%. This was due to the low amount of crude fiber consumed. The less crude fiber consumed, the greater the digestibility of crude fiber. In accordance with the opinion of Mirnawati *et al*.[16], the digestibility of crude fiber depends on the content of crude fiber in the feed ingredients; the higher the crude fiber content, the lower the digestibility of crude fiber because of the limitations of poultry to digest crude fiber. High crude fiber levels interfere with the digestion of other substances. Digestion is also influenced by several factors, including the content of crude fiber in feed, composition of crude fiber and activity of microorganisms[27].

The longer the fermentation time, the greater the opportunity to grow and multiply, allowing fungi to break down cellulose and alter the raw fibers in the substrate. Mirnawati *et al*.[18] stated that there is a positive relationship between cellulose growth and production with the growth of fungi, as cellulase is produced to break down cellulose into glucose so that it can increase the digestibility of crude fiber at the end of fermentation.

CONCLUSION

From the results of this study, it can be concluded that the combination of *Phanerochaete chrysosporium* and *Neurospora crassa* (4:1) fermented for 13 days gave optimal results, with 26.20% crude protein, 14.49% crude fiber, 14.54% lignin, 58.20% nitrogen retention and 57.66% crude fiber digestibility of fermented palm oil sludge.

SIGNIFICANCE STATEMENT

This study determined the optimal conditions to improve the quality and nutrient content of palm oil sludge by combining the fungi *Phanerochaete chrysosporium* and *Neurospora crassa* with fermentation time. From the results of this study, it is expected that fermented palm oil sludge can be used as a feed ingredient for poultry.

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