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E-mail: editorijps@gmail.com

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Research Article
Palm Oil Sludge Fermented by Using Lignocellulolytic Fungi as Poultry Diet

Nuraini, Ade Djulardi and Ade Trisna

Faculty of Animal Science, Kampus Limau Manis, University of Andalas, Padang, Indonesia

Abstract

Background: Palm Oil Sludge (POS) represents an alternative to poultry feed but its utilization is limited due to the high content of crude fiber (lignin and cellulose) and low content of crude protein. Fermentation with lignocellulolytic fungi, which include *Lentinus edodes*, *Pleurotus ostreatus* and *Phanerochaete chrysosporium*, produce ligninase and cellulase enzyme have been used as an approach to increase POS nutrient levels and quality. This study evaluated the nutrient content and quality of POS after fermentation using different ligninocellulolytic fungi. **Methodology:** Two experiments were conducted in this study. Experiment 1 was performed as a 3×3 factorial arrangement in a completely randomized design with 3 replicates. The first factor was lignocellulolytic fungi (*Lentinus edodes*, *Pleurotus ostreatus* and *Phanerochaete chrysosporium*) and the second factor was incubation time (7, 11 and 15 days). Subsequently, Crude Fiber (CF), Crude Protein (CP), cellulose, lignin and cellulose enzyme activity were measured. Experiment 2 compared the nutrient content of fermented (the best treatment identified in experiment 1) and unfermented POS. In this experiment, the nitrogen retention, crude fiber digestion, Metabolizable Energy (ME) and amino acid profile of POS was measured. **Results:** In experiment 1, it was found that *Pleurotus ostreatus* significantly reduced CF, cellulose and lignin levels and increased CP content as well as cellulase enzyme activity after 11 days of incubation ($p<0.01$) Moreover, experiment 2 showed that nitrogen retention, crude fiber digestion and ME content levels in fermented POS were higher than in unfermented POS. Fermentation also improved the amino acid profile of POS. **Conclusion:** An 11 day fermentation with *Pleurotus ostreatus* was identified as the best condition for improving the nutrient content and quality of POS.

Key words: Palm oil sludge, fermentation, lignocellulolytic fungi, nutrient content and quality, poultry feed

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Corresponding Author: Nuraini, Faculty of Animal Science, Kampus Limau Manis, University of Andalas, Padang, Indonesia
Tel: +62 (751) 71464 Fax: +62 (751) 71464

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

INTRODUCTION

Palm Oil Sludge (POS) can be used as a non-conventional animal feed. Indonesia is currently the largest palm oil producer in the world, producing a total of 22.5 million tons per year. Moreover, 70% of the palm oil production originates from the island of Sumatra. During palm oil production, approximately 45-46% of the material results in waste, including POS and Palm Kernel Cake (PKC). The POS consists of approximately 11.30% crude protein, 10.43% fat, 25.80% crude fiber, 19.19% lignin, 16.15% cellulose and 1550 kcal kg^{-1} energy metabolism[1].

The utilization of POS as poultry feed is currently low with only 5% being used in broiler ration[1]. Poultry feed containing high content of crude fiber (lignin and cellulose) has low digestibility and therefore, utilization in the diet is also limited. Lignocellulose consists of three major components: Cellulose, hemicellulose and lignin[2]. Improving the nutrient quality of POS requires reducing the crude fiber levels through microorganism fermentation, which has been shown to increase the nutrient content, digestibility value and palatability of feed[3-7].

It has been shown that fermentation using cellulolytic fungi can reduce the cellulose content of POS but lignin content remains high. Therefore, fermentation using lignocellulolytic fungi (*Lentinula edodes*, *Pleorotus ostreatus* and *Phanerochaete chrysosporium*) may reduce lignin levels due to the production of ligninase and cellulase enzymes. The ligninase enzyme produced by these fungi can degrade lignin as a substrate[4-6,8-12]. Previous studies have also shown these fungi produce cellulase enzymes[4-13].

The POS fermented with an 8% inoculum of *Phanerochaete chrysosporium* for 7 days decreased crude fiber levels (lignin and cellulose) and increased application in a broiler diet by 15%. Therefore, fermentation of POS with other types of lignocellulolytic fungi, including *Lentinula edodes*, *Pleorotus ostreatus* and *Phanerochaete chrysosporium*, would likely improve the nutrient content and quality of POS as well as reduce crude fiber, especially lignin and cellulose. In this study we assessed the effects of different types of lignocellulolytic fungi and incubation times on the nutrient quality of POS.

MATERIALS AND METHODS

The main objective of this study was to assess improvements in nutrient quality of POS through fermentation using several lignocellulolytic fungi and incubation times.

Experiment 1: Palm Oil Sludge (POS) was dried under sunlight until the water content reach approximately 12-14%. This experiment was designed as a 3×3 factorial arrangement in a completely randomized design with 3 replicates per treatment. The first factor was the type of lignocellulolytic fungi (*Lentinus edodes*, *Pleorotus ostreatus* and *Phanerochaete chrysosporium*) assessed and the second factor was the incubation time (7, 11 and 15 days). In the first experiment, 100 g of substrate containing 80% POS and 20% rice bran mixture per experimental unit was added together with 100 mL of aquadest (moisture content 70%). The mixture was then sterilized in an autoclave (121°C for 15 min). After cooling to 27-30°C, the sample was inoculated with 8% *Lentinus edodes*, *Pleorotus ostreatus* and *Phanerochaete chrysosporium* together with 100 ppm calcium and 40 ppm manganese. These mixtures were then incubated for 7, 11 and 15 days.

All of the data obtained were statistically analyzed by analysis of variance. The differences among treatments were determined by using Duncan Multiple Range Test (DMRT)[14].

Experiment 2: The second experiment was designed to compare nutrient content between fermented (the best treatment obtained in the first experiment) and unfermented POS. Measured variables included the following: Nitrogen retention, crude fiber digestion, Metabolizable Energy (ME) and amino acid profile. The fermentation sample used in this experiment was based on the best conditions obtained in the first experiment. Amino acid analysis was performed by HPLC. All data obtained from the second experiment were compared numerically.

RESULTS

Experiment 1

Effect of lignocellulolytic fungi and incubation time on crude fiber, lignin, cellulose and cellulase enzyme activity: The effects of lignocellulolytic fungi and incubation time on crude fiber, lignin, cellulose content and cellulase enzyme activity of POS are shown in Table 1-4, respectively. We found that the combination of lignocellulolytic fungi and incubation time had a significant effect on crude fiber, lignin and cellulose content as well as cellulase enzyme activity of POS ($p<0.05$).

Effect of lignocellulolytic fungi and incubation time on crude protein: The effect of lignocellulolytic fungi and incubation time on crude protein content of POS is shown in Table 5. It was found that the combination of the type of

Table 1: Effect of lignocellulolytic fungi and incubation time on crude fiber (%)

Treatments	B1 (7 days)	B2 (11 days)	B3 (15 days)	Averages
A1 (Le)	18.36^{Aab}	14.97^{Ba}	14.59^{Ba}	15.97
A2 (Po)	17.96^{Ab}	14.04^{Bb}	14.12^{Bb}	15.37
A3 (Pc)	18.74^{Aa}	14.91^{Ba}	14.95^{Ba}	16.20
Averages	18.35	14.64	14.55	

Table 2: Effect of lignocellulolytic fungi and incubation time on lignin (%)

Treatments	B1 (7 days)	B2 (11 days)	B3 (15 days)	Averages
A1 (Le)	16.17^{Aa}	14.70^{Bb}	14.78^{Ba}	15.22
A2 (Po)	15.14^{Ab}	13.22^{Bb}	13.39^{Bb}	13.91
A3 (Pc)	16.00^{Aa}	14.49^{Ba}	14.58^{Aa}	15.02
Averages	15.77	14.14	14.25	

Table 3: Effect of lignocellulolytic fungi and incubation time on cellulose (%)

Treatments	B1 (7 days)	B2 (11 days)	B3 (15 days)	Averages
A1 (Le)	9.33^{Aab}	7.81^{Bb}	7.84^{Bb}	8.33
A2 (Po)	8.90^{Ab}	7.68^{Bb}	7.99^{Bb}	8.19
A3 (Pc)	9.98^{Aa}	8.82^{Ba}	8.50^{Aa}	9.10
Averages	9.40	9.10	8.11	

Table 4: Effect of lignocellulolytic fungi and incubation time on cellulase enzyme activity (U mL^{-1})

Treatments	B1 (7 days)	B2 (11 days)	B3 (15 days)	Averages
A1 (Le)	4.83^{Bb}	5.09^{Aa}	4.87^{Bb}	4.93
A2 (Po)	5.03^{Aa}	5.22^{Aa}	5.02^{Aa}	5.09
A3 (Pc)	4.84^{Ab}	5.01^{Aa}	4.80^{Ab}	4.88
Averages	4.90	5.11	4.90	

Table 5: Effect of lignocellulolytic fungi and incubation time on crude protein (%)

Treatments	B1 (7 days)	B2 (11 days)	B3 (15 days)	Averages
A1 (Le)	14.04^{Cc}	20.11^{Ac}	19.05^{Bc}	17.73
A2 (Po)	15.05^{Ca}	21.86^{Aa}	20.55^{Ba}	19.15
A3 (Pc)	14.51^{Bb}	20.46^{Ab}	20.40^{Ab}	18.46
Averages	14.53	20.81	20.00	

lignocellulolytic fungi and incubation time had a strong significant effect on crude protein content of POS ($p<0.01$).

Experiment 2

Comparison of nitrogen retention, crude fiber digestibility, metabolizable energy and amino acid profile between unfermented vs., fermented POS: Nitrogen retention, crude fiber digestion and metabolizable energy of POS under fermented and unfermented conditions are shown in Table 6. The amino acid profiles under both conditions are shown in Table 7.

Table 6: Nitrogen retention, crude fiber digestion and metabolizable energy of unfermented and fermented POS (A3B2)

Compound and energy	Unfermented	Fermented
Nitrogen retention (%)	44.05	69.02
Crude fiber digestion (%)	40.48	60.93
Metabolizable energy (kcal kg^{-1})	1540.09	2050.87

Table 7: Amino acid profile of fermented and unfermented POS (A3B2)

	Unfermented	Fermented
Amino acids	----------(DM%)----------	
Aspartate	1.18	1.28
Glutamate	2.52	2.63
Serine	0.54	0.61
Histidine	0.49	0.57
Glycine	0.38	0.43
Threonine	0.51	0.58
Arginine	0.62	0.69
Alanine	0.98	1.14
Tyrosine	0.19	0.28
Methionine	0.50	0.58
Valine	0.43	0.52
Phenylalanine	0.52	0.59
Iso-leucine	0.39	0.47
Leucine	1.12	1.24
Lysine	1.06	1.21
Cysteine	0.51	0.61
Proline	0.27	0.35

DISCUSSION

The crude fiber, lignin and cellulose levels of POS were decreased after fermentation with *Lentinus edodes*, *Pleorotus ostreatus* and *Phanerochaete chrysosporium*, which was due to degradation by ligninase and cellulose enzyme produced in the fungi. The lowest crude fiber content of POS was observed in the fermentation reaction with *Pleorotus ostreatus* after 11 and 15 days of incubation (reduction of 31.04 and 30.64%, respectively). Bentil[7] also found that fermentation of cocoa bean shells with *Pleurotus ostreatus* decreased the crude fiber content by 30.60 and 49.93% at the 2nd and 6th weeks of incubation, respectively. In addition, Akinfemi *et al.*[4] also found that the crude fiber content of peanut husk fermented with *Pleorotus ostreatus* decreased from 26.20-16.9% (35.49% decrease) after 3 weeks of incubation.

The lowest cellulose and lignin levels in POS were also observed after fermentation with *Pleurotus ostreatus* for 11 and 15 days (7.68 and 7.99% for cellulose and 13.22 and 13.39% for lignin, respectively). Cellulose degradation was likely facilitated by the hydrolytic enzymes (cellulases) secreted by the fungus during fermentation[2,5,7]. The cellulase enzyme activity in POS fermented with *Pleorotus ostreatus* also increased by the 11th and 15th days of incubation (5.22 and 5.02 U mL^{-1}, respectively). Fungi are capable of producing a range of enzymes extracellularly and *P. ostreatus* is known to produce cellulases, hemicellulases and laccases that help degrade complex carbohydrates into soluble sugars[9,4].

Fermentation of POS with *Lentinus edodes* also exhibited reductions in crude fiber content at the 11th and 15th days of incubation by 26.76 and 26.57%, respectively. Elisashvili *et al.*[8]

previously reported that *L. edodes* IBB 123 reached maximal laccase activity on day 7 of cultivation but *Pleurotus* spp., achieved maximum acitivity after 10 days of cultivation. It was also observed a 26.76 and 26.57% reduction in crude fiber content in POS after 11 and 15 days of incubation, respectively, with *Phanerochaete chrysosporium*. Moreover, Nuraini and Nirwansyah[13] reported that fermentation of cocoa pod with *Phanerochaete chrysosporium* and *Monascus purpureus* reduced the crude fiber, lignin and cellulose content.

The highest crude protein levels in POS were observed after fermentation with *Pleurotus ostreatus* for 11 days. Fungi growth likely contributed to the increase in crude protein content in the POS, thus augmenting the crude protein content in the fermented product. The observed increase in protein levels could be due to bioconversion of some of the soluble carbohydrates in the colonized substrate into mycelia protein or Single Cell Protein (SCP) by the growing fungus[5,6,13].

The increase in crude protein content of POS after fermentation was associated with the process of protein enrichment, which was similar to the formation of a single cell protein and therefore could not be separated from proteins produced by the fungi. The increase in crude protein content of fermented POS was also related to the enzymes produced by the fungi (cellulase and ligninase).

Nitrogen retention and metabolizable energy were enhanced when POS was fermented using *Pleorotus ostreatus.* In addition, all of the amino acids assessed were increased in fermented POS compared to unfermented POS. Importantly, the first limiting amino acids in a poultry diet (i.e., methionine and lysine) were slightly increased from 0.50, 1.06-0.58 and 1.21%, respectively, in the fermented POS compared to the unfermented POS.

CONCLUSION

Fermentation with *Pleurotus ostreatus* for 11 days was identified as the best condition for improving nutrient content (reducing crude fiber, lignin and cellulose levels as well as increasing crude protein and cellulose enzyme activity) and nutrient quality of POS.

SIGNIFICANCE STATEMENT

This study assessed the utilization of Palm Oil Sludge (POS) through fermentation using different lignocellulolytic fungi. The use of POS waste as an alternative poultry feed is still limited due to the crude fiber content and low protein content. This study found that fermenting POS using different lignocellulolytic fungi significantly reduced the crude fiber content.

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