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Palm Oil Sludge Fermentation with *Pleurotus ostreatus* and its Application in Laying Quails' Ration

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Abstract: Palm oil sludge is a byproduct of palm oil processing which is still limited in its use as a constituent of poultry feed. This study aims to improve the quality of palm sludge fermented with *Pleurotus ostreatus* and to determine its effect on the production of laying quail. The study was divided into 2 stages of research. Phase 1 of the laboratory experiment was the determination of the dose of the inoculum and the duration of fermentation of palm sludge. This stage uses a Completely Randomized Design (CRD) 3×3 treatment design with 3 replications. A Factor (inoculum dose) is (A1 = 6%, A2 = 8% and A3 = 10%) of the number of substrates. B Factor (fermentation time) is (B1 = 9 days, B2 = 11 days and B3 = 13 days). The parameters measured were the activity of cellulase enzymes (U/ml), lignin peroxidase (U/ml) and crude fiber content (%), cellulose (%) and lignin (%). Phase 2 biological experiment to laying quails is the application of Fermented Palm Sludge (FPS) products in laying quail rations. This stage uses a Completely Randomized Design (CRD) with 5 treatments and 4 replications. Provision of FPS consisting of: A (0% FPS), B (6% FPS), C (12% FPS), D (18% FPS), E (24% FPS). The parameters measured were feed consumption (g/head/day) and daily egg production (%). The results of the Phase 1 variance analysis showed that there was no interaction ($P>0.05$) between the inoculum dose and the fermentation time for the activity of cellulase enzymes, lignin peroxidase (U/ml) and the content of crude fiber, cellulose and lignin fermented palm sludge, but inoculum doses highly significant effect ($P<0.01$) on the activity of cellulase (U/ml), lignin peroxidase (U/ml), crude fiber content (%), cellulose (%) and lignin (%). The results of the Phase 2 variance analysis showed that the use of FPS with *Pleurotus ostreatus* had no significant effect ($P>0.05$) on ration consumption and egg production. The results of the study concluded that the 8% inoculum dose and 9 days fermentation time was the best treatments to improve the quality of palm sludge. FPS can be used up to 24% in laying quail rations. At this level, it can reduce 27.30% corn and 55.02% soybean meal and can maintain the same ration consumption and egg production as control.

Keywords: Dose of Inoculum, Duration of Fermentation, Pleurotus Ostreatus, Palm Sludge, Laying Quails and Cellulase Enzymes

Introduction

The existence of poultry is very important because it contributes to providing animal protein. One bird whose eggs can be used as animal protein in a large quantity is quail. Quail egg production in one year ranges from 200-300 eggs (Amo *et al*., 2013). According to data from the Director-General of Animal Husbandry and Animal Health, quail population in Indonesia is estimated to 14,570,000 heads. Increasing the quail population requires the availability of sufficient food for the main needs such as basic living, growth, production and reproduction for birds. Conventional feed is relatively expensive and in animal husbandry business, the cost of feed is the largest production cost (60-70%) that must be spent by farmers/ranchers (Amrullah, 2004). But the effort to provide continuous feed is increasingly difficult because the price of feed is expensive and still depends on conventional or commercial feed so that alternative feed (unconventional) is needed which is of good nutritional content, available in large quantities, continuous and inexpensive.

Unconventional feed resources that can be used as animal feed are agricultural waste or agricultural industrial waste. The agricultural industry is constantly increasing especially the palm oil processing industry, which produces waste called palm sludge. Indonesian plantation statistics data released in 2014 by the directorate general of plantations reported that the total area of oil palm plantations in Indonesia in 2013 reached 10,465,020 Ha. In 2014 the area of oil palm plantations continued to increase reaching 10,754,801 Ha and in 2015 the area of oil palm plantations was estimated to reach 11,300,370 Ha DGIP, 2015. For every ton of palm oil yields, around 2-3 tons of palm sludge are produced (Fauzi *et al*., 2012).

Palm sludge has crude protein content (11.35%), crude fiber (25.80%), cellulose (16.15%), lignin (19.19%) and metabolic energy of 1550 kcal/kg (Nuraini and Trisna, 2017). In poultry, especially broiler oil palm sludge ranges from 5% (Sinurat *et al*., 2001), because the increasing content of crude fiber contained in palm sludge in the ration can cause a decrease in chicken performance which can reduce feed consumption and slower growth (Sinurat, 2003). Therefore, to use oil palm sludge, efforts should be made to reduce the limiting factors and improve the quality of oil palm sludge. One way to solve this problem is by palm sludge fermentation to increase nutrient content and reduce the levels of crude fiber sludge. Fermentation biotechnology approach can be used to overcome high crude fiber in palm sludge by utilizing the role of microorganisms. The use of fermented palm sludge with *Lentinus edodes* as a constituent of quail rations can be used up to 20% in rations (Nuraini and Trisna, 2017). Other fungi derived from the Basidiomycete groups, which are also effective in degrading high lignocellulosic materials are white-rot fungi known as *lignocellulolytic fungi,* one of which is *Pleurotus ostreatus* (Hatakka, 2001; Sun and Cheng, 2002).

Nuraini and Mahata (2015) states that the success of a solid media fermentation highly depends on the optimum conditions given such as the composition of the substrate, thickness of the substrate, the dose of the inoculum and the length of fermentation. The use of fermented palm sludge with *Pleurotus ostreatus* against laying quail is unknown. Based on the description above, a study was conducted to determine the best dosage of inoculum and fermentation time with *Pleurotus ostreatus* and test the effect on quail egg production performance.

Materials and Methods

Stage 1. Determination of the Best Inoculum Dosage and Fermentation Time of Palm Sludge Fermented with Pleurotus ostreatus

Palm Oil Sludge Fermentation

The substrate consisting of 90% palm sludge and 10% bran, added to heat-resistant plastic is added with distilled water and Brook solution (60% water content) then homogenized. The substrate is sterilized with an autoclave at 121°C for 15 min. Leave it until the temperature drops to room temperature 25-30°C. After that, the substrate was inoculated with *Pleurotus ostreatus* (6, 8 and 10%) of the total substrate. Then stir evenly and incubated according to the treatment for (9, 11 and 13 days) in a glass bottle and covered with gauze. The fermentation product is dried in an oven at 80°C for 2 h to kill the fungus and continued drying at 60°C for 10 h. The product is stirred evenly and ready to be analyzed.

Enzymatic Activity

5 grams of enzyme and soaked with 50 mL of 0.05 M Ph 5 acetate buffer, then let stand for 2 h, strain and take the filtrate. The filtrate is centrifuged 15000 rpm for 15 min, then a crude enzyme was extracted. Laccase enzyme activity was measured based on the study of (Buswell *et al*., 1995). The sample solution was prepared with a 0.4 mL enzyme filtrate mixed with 0.5 mL acetate buffer 0.5 M Ph 5 and 0.1 mL ABTS 1 Mm. This mixture was put into a cuvette and then shaken. After shaking, the solution was measured at a wavelength of 420 nm with time intervals of 0 and 30 min. Lignin Peroxidase enzyme activity (LiP) was measured based on the method of (Tien and Kirk, 1984). 0.2 mL of filtrated enzyme, 0.5 mL of 5 mM H_2O_2, 0.1 mL of 8 mM vetratril alcohol, 0.2 mL of 0.05 M acetate buffer pH 3 and 0.45 mL of distilled water were added to the cuvette and shaken. The solution was read absorbance at a wavelength of 310 nm with a 0 and 30 min time interval.

Experimental Design

This research was conducted by an experimental method with a Completely Randomized Design (CRD) factorial pattern 3×3 with 3 replications. Factor A (inoculum dose) is (A1 = 6%, A2 = 8% and A3 = 10%) of the number of substrates. Factor B (fermentation time) is (B1 = 9 days, B2 = 11 days and B3 = 13 days).

Variables

The parameters measured were *cellulase enzyme* activity (U/ml), *lignin peroxidase enzyme* activity (U/ml), crude fiber content (%), cellulose content (%) and lignin content (%).

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Data Analysis

Statistical analysis data with the analysis of variance based on a Completely Randomized Design (CRD) factorial pattern 3×3 with 3 replications in Table 2. Differences between treatments were tested by Duncan's Multiple Range Test (DMRT).

Stage 2. Application of Fermented Palm Sludge (FPS) Products with Pleurotus ostreatus in Laying Quail Rations

Palm Sludge Fermentation with Pleurotus ostreatus

The substrate used is 500 g, consisting of 90% palm oil (450 g) and 10% bran (50 g), then adding distilled water and Brook solution (60% water content). The material is sterilized in an autoclave (temperature of 121°C for 15 min), then inoculated with 8% *Pleurotus ostreatus* inoculum, stirred evenly and flattened to a thickness of 1 cm and incubated for 9 days.

Experimental Design

Rations were prepared with a content of 20% crude protein and 2800 kcal/kg metabolic energy. This research was conducted using an experimental method designed with a Completely Randomized Design (CRD) method with 5 treatments and 4 replications, each consisting of 10 quails as a unit of the experiment. Provision of FPS consisting of: A (0% FPS), B (6% FPS), C (12% FPS), D (18% FPS), E (24% FPS). Feed ingredients, the content of food substances and metabolic energy of the ingredients of ration (as feed) can be seen from Table 1. While the composition of rations, food content and energy of research rations can be seen in Table 2. The ration was prepared with isoprotein (20%) and isoenergy (2800 kcal/kg) according to (Djulardi, 1995).

Birds

The birds used in this study are 200 quails (*Coturnix-coturnix japonica*) of the 20-week laying phase.

Table 1: The content of food substances (%), metabolic energy (kcal/kg) and ration ingredients (as feed)

Feed ingredients	Crude pretein	Fat	Rough fiber	Ca	P	ME (kkal)	Metionin	Lysin
Corn	8,20	2,66	2,90	0,38	0,19	3300,00	0,18	0,26
Consentrate 126	38,00	4,00	8,00	5,50	1,00	2910,00	1,00	1,76
Soybean	45,35	2,49	7,50	0,63	0,36	2240,00	0,50	0,60
Coconut Oil	0,00	100,00	0,00	0,00	0,00	8600,00	0,0	0,00
FPM	21,87	3,12	14,21	0,24	0,29	2107,47	0,55[a]	1,14[a]
Flour	0,00	0,00	0,00	24,00	12,00	0,00	0,00	0,00
Lysin								99,00
CaCO3				40,00				0,00
Top mix				5,38	1,44		0,30	0,30
Dedak	9,28	4,09	16,02	0,69	0,26	1640	0,27	0,67

Table 2: Ration composition (%), food content (%) and metabolic energy (kcal/kg) of research ration

	Composition of treatment ration				
Feed ingredients	A	B	C	D	E
Corn	50,55	47,75	44,00	40,45	36,75
Concentrate 126	23,75	23,75	23,75	23,75	23,75
Soybean meal	14,45	12,65	10,55	8,45	6,50
Coconut oil	0,50	1,00	1,85	2,50	3,00
Fermented palm sludge	0,00	6,00	12,00	18,00	24,00
Flour	4,00	4,15	4,15	4,20	4,40
Lisin sintesis	0,25	0,20	0,20	0,15	0,10
$CaCO_3$	1,00	1,00	1,00	1,00	1,00
Top Mix	0,50	0,50	0,50	0,50	0,50
Bran	5,00	3,00	2,00	1,00	0,00
Total	100,00	100,00	100,00	100,00	100,00
Protein	20,19	20,27	20,23	20,20	20,24
Fat	3,36	3,84	4,69	5,34	5,84
SK	5,25	5,57	5,99	6,42	6,86
Ca	3,01	3,03	3,01	3,00	3,03
Available P	0,89	0,90	0,90	0,91	0,94
ME	2807,96	2811,88	2824,24	2826,00	2813,27
Metionin	0,42	0,43	0,44	0,46	0,47
Lisin	0,92	0,91	0,95	0,94	0,93

Variables

The parameters observed were feed consumption (g/head/day) and daily egg production (%).

Data Analysis

The data obtained are processed statistically by analysis of diversity according to (Steel and Torrie, 1995). Differences between treatments were tested with Duncan's Multiple Range Test (DMRT).

Results

Stage 1. Determination of the Best Inoculum Dosage and Fermentation Time of Palm Sludge Fermented with Pleurotus ostreatus

a. Effect of Treatment on Cellulase Enzymes and Lignin Peroxidase Activity

The effect of inoculum dose treatment and fermentation time on the average activity of *cellulase* (U/ml) enzyme and *lignin peroxidase* (U/ml) is presented in Table 3. The results of the diversity analysis showed that there was no interaction (P>0.05) between A factor (dose of inoculum) with B factor (fermentation time) on cellulase enzyme activity and lignin peroxidase enzyme activity from palm sludge fermented with the fungus *Pleurotus ostreatus*. The dose of inoculum has different effects (P<0.01) on the activity of *cellulase enzymes* and *lignin peroxidase enzymes*.

b. Effect of Treatment on Crude Fiber Content, Cellulose and Lignin

The effect of inoculum dosage treatment and fermentation time on crude fiber content, cellulose and lignin are presented in Table 4. The results of the diversity analysis showed that there was no interaction (P>0.05) between factor A (inoculum dose) and factor B (fermentation time) to crude fiber, cellulose and lignin content from palm sludge fermented with the fungus *Pleurotus ostreatus*. The dose of inoculum had a very significant effect (P<0.01) on the content of crude fiber, cellulose and lignin.

Effect of FPS Fermented with Pleurotus ostreatus in the Ration on the Performance of Quail Egg Production

Effect of treatment on feed consumption and quail egg production using fermented palm sludge with *Pleurotus ostreatus* in the ration can be seen in Table 5. The results of the diversity analysis show that the use of fermented palm sludge with *Pleurotus ostreatus* has no significant effect (P>0.05) on ration consumption and egg production.

Table 3: Cellulase (U/ml) and lignin peroxidase (U/ml) enzyme activities

Variable	A factor (Inoculum dose)	B Factor (Duration of fermentation) B1 (9 days)	B2 (11 days)	B3 (13 days)	Average
Cellulase (U/ml)	A1 (6%)	3,79	3,81	3,82	3,81[a]
	A2 (8%)	3,94	3,96	3,97	3,96[b]
	A3 (10%)	3,96	3,98	3,98	3,98[b]
	Average	3,90	3,92	3,93	
Lignin Peroxidase (U/ml)	A1 (6%)	38,03	38,23	38,53	38,26[a]
	A2 (8%)	41,92	42,11	42,31	42,11[b]
	A3 (10%)	42,02	42,21	42,51	42,25[b]
	Average	40,65	40,85	41,12	

Note: Different superscripts in the same column show very significant different effects (P<0.01)

Table 4: Content of crude fiber (%), Cellulose (%) and Lignin (%)

Variable	A Factor (inoculum dose)	B Factor (Duration of fermentation) B1(9 days)	B2(11days)	B3(13days)	Average
Rough Fiber	A1 (6%)	19,85	20,32	20,66	20,27[a]
	A2 (8%)	15,95	16,44	16,72	16,37[b]
	A3 (10%)	14,87	15,75	15,81	15,48[b]
	Average	16,89	17,50	17,73	
Cellulose	A1 (6%)	14,03	14,19	14,37	14,20[a]
	A2 (8%)	10,98	11,69	11,97	11,55[b]
	A3 (10%)	10,13	11,55	11,91	11,20[b]
	Average	12,72	12,48	12,75	
Lignin	A1 (6%)	13,04	13,23	13,49	13,25[a]
	A2 (8%)	10,55	10,61	10,88	10,68[b]
	A3 (10%)	9,99	10,45	10,72	10,39[b]
	Average	11,20	11,43	11,70	

Note: Different superscripts in the same column show very significant different effects (P <0.01).

Table 5: Consumption of ration (g/head/day) and egg production (%)

Treatment	Ration consumption (g/head/day)	Egg production (%)
A (0% FPM)	22.76	80.00
B (6% FPM)	22.71	79.91
C (12% FPM)	22.74	79.73
D (18% FPM)	22.76	79.55
E (24% FPM)	22.77	79.64
SE	0.02	0.67

Note: FPM = Fermented Palm Sludge

Discussion

Fermented palm sludge with the fungus *Pleurotus ostreatus* has an average *cellulase enzyme* activity between 3.79 to 3.98 U/ml and *lignin peroxidase enzyme* activity ranging from 38.03 to 42.51 U/ml. The highest cellulase enzyme activity and lignin peroxidase enzyme activity were in the treatment of A2 and A3 by showing the same effect between the two. The length of fermentation has no significant effect (P>0.05) on *cellulase enzyme* and *lignin peroxidase enzyme* activities.

The influence of factor B (fermentation time) on the activity of *cellulase* and *lignin peroxidase enzymes* due to the fungus *Pleurotus ostreatus* on days 9 to 13 is still in the exponential phase so that the growth of the fungus *Pleurotus ostreatus* is fertile and evenly distributed. While factor A (inoculum dose) gives a significant effect between treatments because the more the dose increases the more fertile the fungus *Pleurotus ostreatus* and causes increased enzyme activity. The higher the doses of inoculum, the faster the fermentation process because with a high dose of inoculum causes the growth of microbes on the substrate more and more and the enzyme activity also increases. Inoculum dose and fermentation time work together to increase the activity of enzyme activity (Noferdiman and Yani, 2013).

The cellulase enzyme activity of *Pleurotus ostreatus* obtained from the results of this study was slightly lower (3.79 to 3.98 U/ml) than the enzyme activity rate reported by (Daba *et al*., 2011) which was 4 U/ml on the 8th day of fermentation. While the activity of the *Lignin Peroxidase* enzymes (LiP) obtained from this study is also still below (38.03 to 42.51 U/ml) of the enzyme activity reported by (Bilal and Asgher, 2016) that is equal to 45, 2 U/ml on the 10th day of fermentation on rice straw substrate.

The best average of cellulase enzyme activity and lignin peroxidase enzyme activity based on the efficiency of the inoculum dose and the fermentation time is in the same treatment, namely the A2B1 treatment (8% inoculum dose and 9 days fermentation time) of 3.94 U/ml (*cellulase enzyme* activity) and 41.92 U/ml (lignin peroxidase enzyme activity).

Fermented palm sludge with the fungus *Pleurotus ostreatus* has an average crude fiber content of 3.79 to 3.98 U/ml and the activity of the lignin peroxidase enzyme ranges from 38.03 to 42.51 U/ml. The highest cellulase enzyme activity and lignin peroxidase enzyme activity were in the treatment of A2 and A3 by showing the same effect between the two. The length of fermentation has no significant effect (P>0.05) on crude fiber content, cellulose content and lignin content.

The low content of crude fiber, cellulose and lignin as a whole in A2 and A3 treatments is due to the activity of cellulase enzymes which are also high in A2 and A3 treatments so that they can break down the contents of crude fiber, cellulose and lignin. The low lignin content in A2 and A3 treatments was also proven by the high lignin peroxidase enzyme activity in A2 and A3 doses so that it was able to degrade lignin and to lower the lignin content. The research report (Noferdiman and Yani, 2013) explained that the addition of higher doses caused the fungus to grow more so that it increased the activity of cellulase and *lignin peroxidase* enzymes in degrading palm sludge. Extracellular enzymes produced by fungi degrade crude fiber cell wall components (Chesson, 1993). The fungus *Pleurotus ostreatus* contains *lignin peroxidase* and *endocellulase* enzymes (Chang and Chui, 1992). *Pleurotus Ostreatus* produces *lignin peroxidase* enzymes, manganese peroxidase and laccase (Howard *et al*., 2003; Kirk and Farrell, 1987) which can hydrolyze lignin, cellulose and hemicellulose into simpler components.

The best reduction of crude fiber, cellulose and lignin content based on the efficiency of the inoculum dose and the length of fermentation is in the treatment of A2B1 (8% inoculum dose and 9 days fermentation time). The treatment obtained crude fiber content (15.95%), cellulose content (10.98%) and lignin content (10.55%).

Consumption of quail ration gives a significantly different result by giving FPS up to the level of 24% in the ration. This shows that FPS is favored by quail, although at that level the use of corn is reduced by 27.30% and soybean meal is reduced by 55.02%. According to (Haddadin *et al*., 1996), the amount of feed consumed by birds is affected by palatability. Fermented products can produce flavors that are preferred by birds and have several vitamins (B1, B2, B12) so that birds are preferred compared to the original ingredients (Murugesan *et al*., 2005).

The effect of the treatment on daily egg production from treatment A to treatment E gave no significantly different results. This is caused by the consumption of rations that are also the same in each treatment. The same consumption shows that the amount of nutrients contained in the ration for egg formation is also the same, so egg production is also the same. The difference in egg production is also caused by protein consumption is also the same in every treatment. Protein consumption in treatment A to treatment E ranged from 4.59 to 4.61%. Egg production is influenced by ration consumption, especially protein consumption (Rasyaf, 1992).

Conclusion

Inoculum dose A2 (8%) and B1 fermentation time (9 days) from *Pleurotus ostreatus* in palm sludge are the best treatment to improve the quality of palm sludge. In this condition *cellulase enzyme* activity (3.94 U/ml), *lignin peroxidase* enzyme activity (41.92 U/ml), crude fiber content (15.95%), cellulose content (10.98%) and lignin content (10.55%) as showed in Table 3. Fermentation of palm sludge with *Pleurotus ostreatus* can be used as much as 24% in laying quail rations. In this condition, the consumption of 22.56 g/head/day was obtained and 80% of egg production was obtained daily.

Authors' Contributions

All the authors equally contributed to this study.

Ethics

This research has been approved by the Committee of Research Ethics of the Faculty of Animal Science of Andalas University Padang, Indonesia and therefore, no ethical issues may arise after its publication.

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