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Effect of Dietary Supplementation of Palm Oil Waste Fermented with *Phanerochaete chrysosporium* and *Neurospora crassa* on Performance and Some Egg Characterizes of Laying Japanese Quails

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ABSTRACT

The aims of present study was to determine the effect of dietary supplementation of palm oil waste fermented with *Phanerochaete chrysosporium* and *Neurospora crassa* (POWF) with ratio 1:1 on performance and some egg characterizes of laying Japanese quails. This experiment used 200 quails (*Coturnix-coturnix japonica*) aged 20 weeks, with 70% egg production. This study used an experimental method with a completely randomized design (CRD) with four treatments (0%, 8%, 16%, and 24% POWF and five replications). The results of this research showed that the use of POWF in the diet was not affected to feed consumption, daily egg production, egg mass, egg weight, and feed conversion. But, a significant difference in blood total cholesterol, LDL, HDL, egg cholesterol, and index egg yolk color was obsereved, and no significant effect on triglycerides and egg yolk fat. In conclusion, feeding the mixture of palm oil waste fermented with *Phanerochaete chrysosporium*, and *Neurospora crassa* with ratio1:1 can be used up to 24% in laying quail rations, without any adverse effect on egg production. It may decrease the blood cholesterol and egg yolk cholesterol.

Key words: Eqq quality, *Neurospora crassa,* Palm oil waste, Performance, *Phanerochaete chrysosporium,* Japanese quail

INTRODUCTION

The composition of the feed positively influences high egg production, especially in quails (Suwarta and Suryani, 2019; Bejar, 2017). The provision of quality and continuous feeding, especially for poultry, still has problems, because of difficulty in obtaining feed ingredients that do not compete with human needs and high feed prices. About 60-70% of production costs is attributed to the cost of feeding (Thirunalaisamy et al., 2019). The main reason of this expensiveness, especially in developing countries, is due to a great amount of imported ingredients. High feeding costs can be overcome by the use of alternative feeds such as palm oil waste (Sugiharto et al., 2018). According to the TCESI (2017), the area of oil palm planting in Indonesia (2017) is 12.037.677 hectares, producing 7.071.877 tons in 2017. Each hectare of palm oil can produce 4 tons of oil per year, which is obtained around 16 tons of fresh fruit bunches. Based on these data, the palm oil waste can be used as a potential alternative food ingredients because of its abundant availability.

Based on the results of Nuraini et al. (2017), palm oil sludge has nutritional contents; namely crude protein (11.30%), crude fiber (25.80%), lignin (19.19%), cellulose (16.15%), Cuprum (28,169 ppm), and metabolic energy (1550 kcal/kg). According to Nuraini et al. (2019), a palm kernel cake has nutritional contents; crude protein (16.30%), crude fiber (20.42%), lignin (14.19%), cellulose (13.26%), Cuprum (44,62 ppm), and metabolic energy (2017.87 Kcal/kg). According to Nuraini et al. (2017), palm kernel cake can be used in 10% of the ration , and palm oil sludge can only be given as much as 5% in broiler rations (Djulardi et al., 2018). Palm oil sludge and palm kernel cake have a limiting factor in the form of crude fiber content; especially that lignin, and cellulose are high consentrated, so it is difficult for the livestock to digest them (Nuraini et al.,2019).

Fermentation using *Phanerochaete chrysosporium* fungi can produce enzymes of ligninase and cellulase (Wang, 2016). Nuraini et al. (2015) reported that *Neurospora crassa* fungi are orange molds, which provide high β-carotene compared to other carotenogenic fungi that have been isolated from corn cobs. β-carotene can reduce cholesterol by inhibiting the work of the enzyme Hydroxymethyl Glutaryl-COA reductase (HMG-COA reductase), which plays a role in the formation of mevalonate in the process of cholesterol synthesis. *Neurospora crassa* can produce amylase, cellulase, and protease enzymes. Fermentation is influenced by several factors including the composition of the substrate, inoculum dose, and length of fermentation (Nuraini et al., 2017). Fermentation using fungi requires a media/substrate containing carbon, nitrogen, and mineral sources to support the growth and development of mycelium to the fullest.

According to Nuraini et al. (2019), the optimum condition is 7% inoculum dose and duration of seven days fermentation time with *Phanerochaete chrysospurium* and *Neurospora crassa* with ratio1:1 on a mixture of palm oil sludge and palm kernel cake. This condition may be provided with 13.25% of crude fiber content and 52.87% of crude fiber digestibility, 27.88% of crude protein content, 58.01% of nitrogen retention, 25.42 ppm of Cuprum, and 145.50 mg/kg of β-carotene. Increased crude protein, decreased crude fiber of palm oil waste fermented with *Phanerochaete chrysosporium*, and *Neurospora crassa* were expected to increase the use of palm oil waste in quail rations. The use of a mixture of palm oil waste fermented with *Phanerochaete chrysosporium* and *Neurospora crassa* for laying quails has not been studied yet.

So, the present study aimed to determine the effect of dietary palm oil waste fermentation fermented with *Phanerochaete chrysosporium* and *Neurospora crassa* on performance and some egg characterizes of laying Japanese quails.

MATERIALS AND METHODS

Materials

The material used in this study was a mixture of palm oil waste fermented with *Phanerochaete chrysosporium* and *Neurospora crassa*, yellow corn, bran, 126 concentrates (PT. Charoen Phokphand), bone meal, soybean meal, coconut oil, top mix, and CaCo3 meal. The livestock used in this study were quail (*Coturnix-coturnix japonica*) layer phase of 200 animals, aged 20 weeks with 70% egg production. Food ingredients, food content (%), and metabolic energy (kcal/kg) of components composing rations (as feed) can see in table 1.

Table 1. Feed ingredients, food content and metabolic energy of the diet in laying Japanese quails [a]

Feed ingredients (as feed)	Crude Protein (%)	Fat (%)	Crude Fiber (%)	Calsium (%)	Available Phospor (%)	Energy metabolism[c] (Kcal/kg)	Methionine (%)[c]	Lysine (%)[c]	B-carotene
Concentrate126[b]	38.00	4.00	3.50	5.50	1.00	2910.00[a]	1.00[a]	1.76[a]	-
Milled yellow corn	8.58	2.66	3.90	0.38	0.19	3300.00	-	0.30	33.00
Soybean meal	43.35	2.49	3.50	0.23	0.36	2240.00	0.50	0.60	-
Rice Bran	9.50	5.09	12.84	0.69	0.26	1640.00	0.27	0.67	-
Coconut oil	-	100[b]	-	-	-	8600.00[b]	-	-	-
POWF	20.54	6.97	11.84	0.52	0.26	2200.00[a]	0.42[a]	0.98[a]	95.50
Bone meal	-	-	-	24.00	12.00	-	-	-	-
CaCo3	-	-	-	40.00	-	-	-	-	-
Top mix[d]	-	-	-	0.06	-	-	0.003[c]	0.003[c]	-

Note : [a] Nuraini et al. (2019); [b] PT. Charoen Pokphan (consist of fish meal, soybean meal, dicalcium phosphate, NaCl, niacin, trace mineral and antioxidant) Nutrinet content at this label: crude protein 38-40%, crude fat min 3%, crude fiber max 8%, ash max 20%.). [c]Scott et al. (1982); POWF: Palm Oil Waste fermented with *Phanerochaete chrysosporium* and *Neurospora crassa* . [d]Top mix from PT Medion (Composition /10kg : vitamine A=12.000.000 IU, vitamine D3=2.000.000 IU, vitamine E= 8.000.000 IU, vitamine B1= 2.000mg, vitamine B2= 5.000mg, niacine= 40.000,methionine= 30.000 mg, lysine = 30.000, manganese = 120.000mg, iron= 20.000mg, iodine =200mg, zinc= 100.000mg, cobalt=200mg,copper= 4.000mg)

Fermentation of palm oil waste with *Phanerochaete chrysospurium* and *Neurospora crassa*

Five hundred gram of substrate consisted of 80% palm oil waste (200 g palm oil sludge and 200-gram palm kernel cake), and 20% (100-gram rice bran) with 35 ml of mineral solution added. The mineral composition consisted of $MgSO_47H_2O$ (2.5 g), $FeSO4_7H_2O$ (1 g), KH_2PO_4 (0.01 g), $ZnSO_44H_2O$ (1 g), $MnSO_44H_2O$ (0.01 g), thiamine hydrochloride (0.1225 g) and urea (50 g) and sterile water 1000 ml. The substrate was sterilized in an autoclave (121^0C for 15 minutes). The substrate was inoculated with 8% inoculum of *Phanerochaete chrysosporium* and *Neurospora crassa* with a ratio of 1:1. The substrate was stirred until become homogeneous and flattened to a thickness of 2 cm, and incubated for nine days (Nuraini et al., 2019).

Methods

Experimental design

This research was conducted using the Completely Randomized Design (CRD) method with four treatments and five replications, in the following procedures: group A (control) contained 0% Palm Oil Waste Fermented with *Phanerochaete chrysospurium* and *Neurospora crassa* (POWF), group B contained 8% POWF, group C contained 16% POWF, and group D contained 24% POWF in diets. The composition of the treatment diet was indicated in table 2, and the content of the treatment diet in table 3.

Measurement of variables

The variables in laying quail have included: feed consumption (g/head/day), quail egg production per day (%), egg weight (g/head/day), quail egg mass (g/head/day), feed conversion, blood cholesterol (mg/dl), HDL (mg/dl), Triglyceride (mg/dl), LDL (mg/dl), egg yolk cholesterol (mg/100g), egg yolk fat (%), and egg yolk color index. The egg yolk color index was evaluated visually by the usual La Roche scale (Bovšková et al., 2014).

Data analysis

The data was statistically analyzed by a one-way analysis of variance in CRD. Significant differences among treatments were determined using Duncan's Multiple Range Test (DMRT), P values<0.05 was considered to be substantial.

Table 2. Composition of experimental rations

Feed ingredients	Treatment (%)			
	A	B	C	D
Yellow corn	46.25	43.25	41.25	39.00
Concentrate126	28.50	28.50	28.50	28.50
Soybean meal	10.50	6.75	3.25	0.00
Rice Bran	8.50	5.25	2.75	0.25
Coconut oil	1.25	1.25	1.25	1.25
POWF	0.00	8.00	16.00	24.00
Bone meal	3.00	3.00	3.00	3.00
Top Mix	0.50	0.50	0.50	0.50
CaCO3	1.50	1.50	1.50	1.50
Total	100.00	100.00	100.00	100.00

Note: POWF: Palm Oil Waste fermented with *Phanerochaete chrysospurium* and *Neurospora crassa*

Table 3. Food content and metabolic energy of the experimental ration

Substance content Food and Energy Metabolism	Ration			
	A	B	C	D
Crude protein	20.16	20.14	20.20	20.35
Crude Fiber	3.80	4.22	4.61	5.06
Fat	4.31	4.60	4.88	5.17
Ca	3.19	3.18	3.17	3.17
P available	0.73	0.73	0.74	0.74
ME (kkal/kg)	2837.70	2835.10	2834.00	2821.95
Methionine	0.36	0.37	0.38	0.39
Lysine	0.76	0.79	0.83	0.86
B-carotene	15.26	22.41	29.55	36.45

*Note: The data of this table is calculated based on tables 1 and 2

RESULTS

The effects of inclusion POWF on the production performance of laying quails was indicated in table 4.

Food consumption

The food consumption of laying quail was not affected by the levels of POWF presenting in the diet. Utilization of POWF until 24% POWF (24.22 g/head/day) was similar to food consumption in control (24.09 g/head/day).

Hen-day egg production

The levels of POWF in the diet were not significantly affected the hen-day egg production of laying quails (p > 0.05). Increasing POWF levels to 24% (79.93 g/head/day) was similar to 0% POWF (79.80 g/head/day).

Egg mass production

The egg mass production of laying quail was not affected by the levels of CPF in the diet (P>0.05). Increasing fermented product levels to 24% POWF (7.97 g/head/day) in the diet was similar to that in the 0% POWF (7.84 g/head/day) control group on egg mass production.

Egg weight

The egg weight of laying quail was not significantly affected (*ns*) by utilization of POWF in the diet. The egg weight in the control treatment (9.81 g/egg) was similar to that in the 24% POWF (9.96 g/egg) group.

Food conversion

The food conversion ratio of laying quail was not affected (*ns*) by the levels of POWF in the diet. Increasing POWF levels to 24% (3.04) was similar to the control (3.08).

Plasma lipid profile

The total blood cholesterol significantly decreased (p<0.01) in POWF groups. The plasma lipid in the treated group with 24% POWF was decreased (152.26 mg/dl) compared to the group that had no POWF in the diet (298.86 mg/dl). The serum LDL content of laying quail was highly (P<0.01) decreased affected by POWF. The serum LDL content ranged in the treated group with 24% POWF was decreased (59.60 mg/dl) compared to the goup that received any POWF treatment (102.18 mg/dl). The serum HDL content of laying quail was significantly (P<0.01) increased affected by POWF. The serum HDL in treated group with 24% POWF was increased (113.18 mg/dl) compared the group with no POWF (82.04 mg/dl). Triglycerides in the group received any POWF (442.64 mg/dl) was not significantly different from the group with 24% POWF (422.86 mg/dl).

Egg Yolk Cholesterol

The inclusion of POWF in the diet of laying quails highly affected (P<0.01) on the egg yolk cholesterol. Increasing the amount of POWF has decreased the egg yolk cholesterol content. The egg yolk cholesterol in the 24% POWF treated group was reduced (681.51 mg/100g) compared to that in the group consumed no POWF treatment (854.94 mg/100g).

Egg yolk fat

The egg yolk fat of laying quail was not affected by the levels of POWF in the diet. The egg yolk fat in the control group was 28.40%, and 28.42% in the 24% POWF treatedgroup.

Egg yolk color index

The inclusion of POWF in the diet of quails was highly increased (P<0.01) on the egg yolk color index. The index of egg yolk color in the group treated with 24% POWF was increased (6.63) compared to that one with 24% POWF (7.91).

Table 4. The effects of inclusion Palm Oil Waste fermented with *Phanerochaete chrysospurium* on the production performance of Japanese laying quails aged 20-25 weeks

Treatment	Feed consumption (g/head/day)	Egg production (%)	Egg weight (g/egg)	Egg mass (g/head/day)	Feed conversion
A (0% POWF)	24.09	79.80	9.81	7.84	3.08
B (8% POWF)	24.07	79.20	9.89	7.88	3.05
C (16% POWF)	24.18	79.27	9.93	7.91	3.06
D (24% POWF)	24.22	79.93	9.96	7.97	3.04
SE	0.09	0.69	0.08	0.12	0.05

*Note: ns = not significantly different effect (P> 0.05); SE = Default error; POWF: Palm Oil Waste fermented with *Phanerochaete chrysospurium* and *Neurospora crassa*

Table 5. Plasma lipid profile for each treatment at the end of the experiment in laying Japanese quails aged 20-25 weeks influenced by palm oil waste fermented in the diet

Treatment	Total cholesterol (mg/dl)	Triglycerides (mg/dl) ^{ns}	HDL (mg/ dl)	LDL (mg/ dl)
A (0%POWF)	298.86^{a}	442.64	82.04^{c}	102.18^{a}
B (8%POWF)	274.68^{a}	426.94	96.26^{b}	99.07^{a}
C (16%POWF)	197.24^{c}	426.50	99.48^{b}	72.80^{b}
D (24%POWF)	152.26^{d}	422.86	113.18^{a}	59.60^{c}
SE	7.73	6.80	3.65	3.14

Note: $^{a,b\,=}$ Superscript difference in the same column affected highly significant (P<0.05). POWF: Palm Oil Waste fermented with *Phanerochaete chrysospurium* and *Neurospora crassa*

Tabel 6. Effect of utilization of palm oil waste fermented in the diet of Japanese quails aged 20-25 weeks on the quality of egg

Treatment	Egg Cholesterol (mg/100g) **	Egg Yolk Fat (%) ns	Egg Yolk Color **
A (0%POWF)	854.94a	28.40	6.63b
B (8%POWF)	742.70b	28.21	6.56b
C (16%POWF)	732.56b	28.67	7.48a
D (24%POWF)	681.51b	28.42	7.91a
SE	9.25	0.68	0.13

Note: a,b = Superscript difference in the same column affected highly significant ($p < 0.05$). POWF: Palm Oil Waste Fermented with *Phanerochaete chrysospurium* and *Neurospora crassa*

DISCUSSION

Effects of dietary supplementation POWF on the Japanese laying quail performance

The effects of the utilization of a mixture of POWF with *Phanerochaete chrysosporium* and *Neurospora crassa* with ratio 1:1 on the food consumption, quail day egg production, egg weight, egg mass, and food conversion are shown in table 4. There was no signficant differences in feed consumption in treatment groups A, B, C, and D which showed that the use of POWF in the diet preferred by quail. Althoughtreatments B, C, and D contain POWF, and provide a small amount of corn, concentrate, and bran, they have similar palatability to treatment A (without a mixture of POWF). however they contain more corn, soybean meal, and bran. According to Djulardi et al. (2018), the factors influenced food consumption in poultry are palatability, food quality, crude fiber in the food, and taste of food.

Palatability was similar among treatments A and treatments B, C, and D showing that the quality of the rations were as same as reach other. The fermentation process can break down the complex food substances into simple ones to improve foods' quality and digestibility. Nuraini et al. (2017), fermented products can produce a desired flavor, having several preferred vitamins to the livestock compared to the original products. food consumption of laying quail at the age of 20 weeks in treatment D (24% POWF) was 24.22 g/head/day. Castro (2018) reported food consumption of laying quail from 23.5 up to 25.5 g/head/day.

According to Indreswari (2016), food consumption, especially protein intake, affects egg production. Daily egg production was not different in treatment A, in comparison with treatments B, C, and D, due to the food consumption; especially protein intake was also similar. similar protein intake may result in equal daily egg production.

Reducing the use of corn, bean, and soybean meal in treatments B, C, and D has resulted in a decrease in amino acid content of lysine and methionine. However, a mixture of POWF and fermented bran with *Phanerochaete chrysospurium* and *Neurospora crassa* can cover the deficiency of amino acid lysine and methionine. The amino acid content of lysine and methionine in POWF with *Phanerochaete chrysospurium* and *Neurospora crassa* includes 0.98% lysin and 0.42% methionine. Daily egg production of laying quail at the age of 20 weeks in treatment D (24% POWF) is 79.93 g/head/day.

Because of same food intake, food conversion in treatment A, it was similar to treatment B, C, and D , hence egg mass was also identical. Food conversion can indicate the production coefficiency; a smaller value indicates more efficient use of food to produce eggs. Khairani (2016) suggested that the food's conversion values will remain balanced even if there is an increase in food conversion affected by food intake and egg mass

The food conversion of laying quail at the age of 20 weeks in treatment D (24% POWF) was determined to be in the ratio of 3.04. Indreswari (2016) reported that food conversion of laying quail was 3.06-3.80.

Effects of POWF utilization on the blood cholesterol

The blood cholesterol of quail (mg/dl) treated with POWF was indicated in table 5. The use of a mixture of POWF with *Phanerochaete chrysospurium* and *Neurospora crassa* with ratio 1:1 was found to reduce cholesterol levels in the serum of laying quail. Utilization of POWF with *Phanerochaete chrysospurium* and *Neurospora crassa* with rate 1:1 up to the level of 24% can reduce blood cholesterol by as much as 50.5%. The blood cholesterol reduction in treatments C and D compared to treatments A and B is related to the use of POWF with *Phanerochaete chrysospurium* and *Neurospora crassa* containing β-carotene. The higher the use of POWF with *Phanerochaete chrysospurium* and *Neurospora crassa* in the ration, the higher the content of β-carotene in the diet; so that it can reduce cholesterol levels in the serum of laying quail. According to Nuraini et al. (2015), β-carotene can lower the cholesterol by inhibiting Hydroxymethyl Glutaryl reductase enzyme (HMG-KOA reductase). This enzyme is needed in the formation of mevalonate in the process of cholesterol synthesis.

The table 5, shows that the blood cholesterol of quail given POWF with *Phanerochaete chrysospurium* and *Neurospora crassa* range 152.26 - 298.86 mg/dl. This research has shown differnt results in comparison with findings of Aetin et al. (2017) suggested that blood cholesterol content of quails ranged 222-322.2 mg/dl. The average serum LDL of quails fed a mixture of POWF with *Phanerochaete chrysospurium* and *Neurospora crassa* ranged 59.60-102.18 mg/dl. The results of this research on POWF supplementation with *Phanerochaete chrysospurium* and *Neurospora crassa* showed a decrease in serum LDL levels in quails' blood. The LDL content in the study was higher than that of Aetin et al. (2017), ranged 37.48 - 51.60 mg/dl. The decrease in LDL levels is related to the β-carotene content, which can prevent lipid oxidation, and be able to inhibit the activity of the HMG CoA reductase enzyme resulting in mevalonates formation, which is needed for cholesterol synthesis. The inhibition of HMG-CoA reductase will reduce the cholesterol synthesis in liver, thereby reducing the combination of APO B, and increasing LDL receptors on the surface of the liver (Rahastuti et al., 2011).

The average serum HDL of quails' blood with the use of a mixture of POWF and fermented bran with *Phanerochaete chrysospurium* and *Neurospora crassa* ranged 82.04 - 113.18 mg/dl. The HDL content in this study showed a high value compared to the research of Khabib Arrosichin et al. (2016), where the HDL content of quails' blood at ranges of 86.6 - 99.8 mg/dl. The average of serum triglycerides of quails (80 days) with the use of a mixture of POWF with *Phanerochaete chrysospurium* and *Neurospora crassa* ranged 422.86-442.64 mg/dl. The triglycerides content in this study was higher than Parizadian (2011) reported that the triglycerides blood of quail (70 days) ranged 149.80 - 154.62 mg/dl

Effect of POWF on egg quality of the Japanese quails

The use of POWF in the diet is found to reduce egg yolk cholesterol in quails, due to an increase in β-carotene content in the diet. The β-carotene content in treatment A (no POWF) was 15.26 mg/kg, while in treatment D (24% POWF) was 36.45 mg/kg. Ramakrishnan et al. (2017), also recommended that β-carotene is a compound that can reduce cholesterol by inhibiting the action of the HMG-CoA reductase enzyme. According to USDA (2017), the egg yolk cholesterol content of quails was 844 mg/100 g. Table 5 indicate that the egg yolk fat content in treatment A (no POWF) is 28.40%, and in treatment D (24% POWF) is 28.42%. Because crude fat content in both treatment diets were not much different, egg yolk fat content in both treatments A and D was similar (Tatiana, 2018).

Increasing utilization of POWF also caused an increase in the β-carotene content, so that the intensity color of egg yolk was also higher. The treatment diet with 24% POWF containing high β carotene content increased the score of egg yolk (orange-yellow) compared to the control treatment. β carotene is an unstable carotenoid group compound because it quickly oxidizes to xanthophyll. Xanthophyll serves to stain the yolk. The quails' body cannot synthesize xanthophyll; therefore, xanthophyll is obtained from diets consisting of xanthophylls in the ingredients. The egg yolk color index obtained by using 24% POWF in the diet of 7.91. El-Tarabany (2016) indicated the range of egg yolk color index between 7 - 9 with the treatment of cage stocking density on egg quality traits in Japanese quail.

CONCLUSION

Based on the results of the present study, it is concluded that the use of a mixture of palm oil waste fermented with *Phanerochaete chrysospurium* and *Neurospora crassa* with ratio 1:1 up to 24% level in diet of laying quail can be adequate. In other words, with this kind of dietary supplementation, food consumption was 24.22 g/head/day, egg production was 79.93%, egg weight was 9.96 g/head/day, egg mass was 7.97 g/head/day, food conversion was 3.04, total cholesterol was 152.26 (mg/dl), triglycerides was 422.86 (mg/dl), LDL was 59.60 (mg/dl), HDL was 113.18 (mg/dl), egg cholesterol was 681.51 (mg/100g), egg yolk fat was 28.42%, and egg color index was 7.91.

DECLARATIONS

Author's contribution

Nuraini contributed to created the idea, designed the experiment (fermentation and utilization POWF to quail), analyzed data, and wrote this article. Ade Djulardi committed to using POWF to quail. Dwi Yuzaria contributed to analyzed data and checked the written report. All authors confirmed the final revised form of article for publishing in this journal.

Competing interests

The authors declared that they have no competing interests.

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