



Research Article

Palm Kernel Cake Fermented with *Lentinus edodes* in the Diet of Quail

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Abstract

Background and Objective: Palm kernel cake (PKC) represents an available alternative poultry feed component, fermentation of PKC by *Lentinus edodes* (*L. edodes*), a lignocellulolytic fungus (that produces ligninase and cellulase enzymes), increases the nutrient content and the quality of PKC. The present study was conducted to evaluate the effect of the incorporation of PKC fermented with *L. edodes* (PKCF) in quail diets on the production performance and egg quality. **Materials and Methods:** The present study used a completely randomized design (CRD) with five dietary treatment groups (0, 10, 15, 20 and 25% PKCF) and four replicates per treatment (10 quails per replicate). Two hundred and seven-week-old quails were included in the study. Diets were iso nitrogenous (20% crude protein) and iso caloric (2800 kcal kg⁻¹). The measured parameters included feed consumption, hen -day egg production, egg weight, egg mass production and feed conversion and egg cholesterol content. **Results:** Addition of dietary PKCF up to 25% resulted in good feed consumption, hen-day egg production, egg weight, egg mass and feed conversion and the addition of PKCF decreased ($p < 0.01$) egg cholesterol content. **Conclusion:** It is concluded that up to 25% PKCF in the diet resulted in good egg production performance while reducing the cholesterol content of the quail egg, indicating that this feed supplement should be considered for industrial use.

Key words: Egg cholesterol, fermentation, *Lentinus edodes*, palm kernel cake, production performance of quail,

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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

Indonesia is currently the largest palm oil producer in the world, generating a total of 22.5 million tons per year. Moreover, 70% of the produced palm oil originates from the island of Sumatra¹. During palm oil production, approximately 45-46% of the starting materials are converted to waste products, including palm kernel cake (PKC) and palm oil sludge (POS). Palm kernel cake (PKC) has a potentially beneficial use as a nonconventional animal feed component, based on its production and nutrient content. According to Directorate General of Indonesian Plantations (2014), the area of oil palm plantations in Indonesia was 10,956,231 ha, palm oil production was 29,344,479 t, the production of fresh fruit bunches was approximately 214 t ha⁻¹ year⁻¹ and PKC production was 2.978 million tons¹. The nutrient content of PKC includes 16.30% crude protein, 10.50% fat and 2020.17 kcal kg⁻¹ metabolizable energy^{2,3}. Only up to 10% of PKC can be included in the broiler diets without affecting production and performance^{2,3}. The problem with utilization of PKC is high content of crude fiber (21.75%), which is especially high in cellulose (17.67%) and lignin (16.96%)^{2,3}. Poultry feed containing a high level of crude fiber (e.g., lignin and cellulose) has low digestibility and its utilization in the diet is limited. To improve the quality of PKC as a poultry feed component, the crude fiber content of PKC must be reduced, including the reduction of lignin and cellulose³. Fermentation with a lignocellulolytic-microbe, such as *Lentinus edodes*, is a means to reduce crude fiber content in the substrate.

Lentinus edodes releases exo- β -1,3-glucanase, β -glucosidase, cellobiohydrolase and endoglucanase enzymes to degrade cellulose, hemicellulase to decompose hemicellulose⁴ and xylanase enzymes to degrade xylan⁵. *Lentinus edodes* is able to degrade lignin and cellulose because this fungus can produce lignin-degrading enzymes such as lignin peroxidase (LiP), manganese-dependent peroxidase (MnP) and laccase^{5,6}. *Lentinus edodes* contains protein, fat, carbohydrates, dissolved fiber, eight types of essential amino acids in balanced proportions, vitamins and minerals^{5,6}. *Lentinus edodes* fungus obtains several components of these compounds from cellulose, hemicellulose and lignin which are used by *Lentinus edodes* as a source of nutrition and energy for their growth and development⁶.

Lentinus edodes requires a humid environment, the ideal humidity for growth of *Lentinus edodes* is approximately 80-85% at 10-24°C⁷. *Lentinus edodes* produces an active ingredient, eritadenine, that is also known as a *hypcholesterolemic* agent⁸. The high cholesterol content of quail eggs forces a number of people to avoid the

consumption of quail eggs due to health concerns. Feeds containing eritadenine may reduce the cholesterol content of the livestock products. Eritadenine was shown to play a significant role in lowering the cholesterol levels by binding bile acids, bound bile acids move down to the large intestine and are discharged into the stool^{9,10}. Increased bile acid excretion through the feces will stimulate the body, especially the liver, to synthesize new bile acids derived from cholesterol, so that cholesterol content in the body will be reduced¹⁰. Utilization of 16% palm oil sludge fermented with *Lentinus edodes* produced eritadenine (the hypocholesterolemic agent) and decreased meat cholesterol content by 21.32%^{11,12}. A previous study showed that palm kernel cake fermented (PKCF) by using an 8% inoculum of *Lentinus edodes* for 9 days increased the crude protein content from 12.35-20.16% and increased metabolizable energy from 2017.87-2358.90 kcal kg⁻¹, crude fiber was decreased from 20.42-8.65%, with 64.83% nitrogen retention, 58.30% crude fiber digestion and 123.41 $\mu\text{g g}^{-1}$ eritadenine². Therefore, the present study evaluated the effect of feeding PKCF on quail production performance and egg quality, especially regarding cholesterol content, as well as the ability of PKCF to reduce the necessary amounts of other feed components such as corn and soybean meal.

MATERIALS AND METHODS

Fermented feed preparation: *Lentinus edodes* cultures were reconstituted and subcultured in potato dextrose agar for 7 days. Inoculated subcultures were kept at room temperature. PKCF was prepared according to the procedure described by Nuraini *et al.*² The substrates contained 80% PKC with 20% rice bran and 135 mL added water (water content 70%). The substrates were autoclaved at 121°C for 15 min, cooled, inoculated with an 8% inoculum of *L. edodes* and incubated for 9 days. After incubation, the fermented product was dried, fermented products were ground in a grinding mill and stored until mixed with other feedstuffs.

Experimental design: The present study used a completely randomized design with five dietary treatment groups (0% (control), 10, 15, 20 and 25% PKCF) with four replicates per treatment. The treatments reduced corn and soy bean meal in the quail diets. Rations were formulated to be iso-nitrogenous (20% crude protein) and iso-caloric (2800 kcal kg⁻¹ metabolizable energy). The nutrient and metabolizable energy contents of feedstuffs included in the rations are shown in Table 1 and the ration formulations and the nutrient metabolizable energy contents are shown in Table 2.

Table 1: Nutrient and metabolic energy of the diet formulations used in this study

Ingredients	Formulation (%)				
	A	B	C	D	E
Yellow corn	53.25	45.25	40.75	36.75	32.25
Concentrated CP 126	30.25	30.25	30.25	30.25	30.25
Soybean meal	10.00	7.00	5.75	4.25	3.00
Coconut oil	0.00	1.00	1.75	2.25	3.00
PKCF	0.00	10.00	15.00	20.00	25.00
Bone meal	4.00	4.00	4.00	4.00	4.00
CaCO ₃	1.50	1.50	1.50	1.50	150.00
Top mix	1.00	1.00	1.00	1.00	1.00
Total	100.00	100.00	100.00	100.00	100.00
Crude protein (%)	20.40	20.20	20.17	20.07	20.04
Crude fat	2.88	4.31	5.28	6.00	6.96
Crude fiber	4.71	5.04	5.20	5.36	5.53
Ca	3.54	3.52	3.51	3.50	3.48
P available	0.93	0.93	0.93	0.93	0.93
ME	2861.53	2828.92	2823.24	2806.94	2801.25
Methionine	0.36	0.40	0.43	0.45	0.48
Lysine	0.70	0.77	0.80	0.83	0.87
Eritadenine (mg kg ⁻¹)	0.00	13.45	20.18	26.90	33.63
Total	100.00	100.00	100.00	100.00	100.00

Table 2: The effects of palm kernel cake fermented using *Lentinus edodes* on the production performance of laying quail

	Feed consumption (g bird ⁻¹ day ⁻¹)	Hen-day egg production (%)	Egg weight (g egg ⁻¹)	Egg mass production (g bird ⁻¹ day ⁻¹)	Feed conversion
A (0% PKCF)	21.88	74.46	10.17	7.64	2.86
B (10% PKCF)	21.83	74.73	10.24	7.71	2.83
C (15% PKCF)	21.88	73.21	10.24	7.49	2.92
D (20% PKCF)	21.85	72.41	10.21	7.40	2.95
E (25% PKCF)	21.83	72.59	10.18	7.31	2.96
SE	0.02	1.35	0.14	0.12	0.05

SE: Standard error of the mean

Birds and housing: Two hundred and seven-week-old *Coturnix-coturnix Japonica* quails were included in the present study (10 quails in each box). Quails were randomly assigned to one of the five PKCF treatment group. All quails were provided with a uniform feeder and water space with house boxes reared under the standard management conditions during the 8 weeks of the experiment. Feed and water were provided *ad libitum*.

Feed formulation: The poultry feed was formulated with 20% CP to provide 2800 kcal kg⁻¹ metabolic energy (ME). The nutrients and ME of the formulated feed are shown in Table 1. Methods: The experiment was performed using a completely randomized design (CRD) with five treatments (0, 10, 15, 20 and 25% PKCF in the diet) and four replicates per treatment. There were 10 *Coturnix-coturnix Japonica* laying quails per unit of experiment. The formulation, nutrient content and metabolizable energy content of the diets are shown in Table 1. The diet formulation consists of yellow corn, rice bran, soybean meal, bone meal, CP concentrated 126 (PT Charoen Pokphand), PKCF, coconut oil, CaCO₃ flour and topmix (mixed vitamins and minerals).

Variables: The measured variables included feed consumption (g head⁻¹ day⁻¹), quail-day egg production (%), egg weight (g bird⁻¹), egg mass production (g head⁻¹ day⁻¹), feed conversion, egg yolk color and egg cholesterol (mg/100 g).

Data analysis: All data were statistically analyzed using one-way analysis of variance according to the completely randomized design (CRD). Significant differences between the treatments were determined using Duncan's multiple range test and $p < 0.05$ was considered significant.

RESULTS

Feed consumption: The feed consumption of laying quail was not significantly affected ($p > 0.05$) by the levels of PKCF present in the diet. Feed consumption in the control group (21.88 g head⁻¹ day⁻¹) was similar to that in the 25% PKCF group (21.83 g head⁻¹ day⁻¹).

Hen-day egg production: The levels of PKCF in the diet did not influence ($p > 0.05$) the hen-day egg production of laying

Table 3: The effects of palm kernel cake fermented using *Lentinus edodes* on the egg cholesterol of quail

Treatments	Egg cholesterol (mg/100 g)
A (0% PKCF)	616.79 ^a
B (10% PKCF)	586.04 ^a
C (15% PKCF)	565.42 ^{ab}
D (20% PKCF)	541.24 ^{ab}
E (25% PKCF)	490.98 ^b
SE	7.86

^{a-d}Different superscripts within the column correspond to significance ($p < 0.05$), SE: Standard error of the mean

quails. Hen-day egg production in the control group was 74.46% and egg production increased (72.59%) in the 25% PKCF treatment group.

Egg weight: The egg weight of laying quail was not significantly affected ($p > 0.05$) by the levels of PKCF in the diet. The egg weight in the control group (10.17 g egg⁻¹) was similar to that in the 25% PKCF group (10.18 g egg⁻¹).

Egg mass production: The egg mass production of laying quail was not affected ($p > 0.05$) by the levels of PKCF in the diet. The egg mass production in the control group was 7.64 g bird⁻¹ day⁻¹ and increased (7.31 g bird⁻¹ day⁻¹) in the 25% PKCF treatment group.

Feed conversion: The feed conversion ratio of laying quail was not affected ($p > 0.05$) by the levels of PKCF in the diet. The feed conversion in the control was 2.86 and 2.96 in the 25% PKCF treatment group.

The effects of PKCF addition on egg quality of laying quail are illustrated in Table 3.

Egg cholesterol: Inclusion of PKCF in the drinking water of quails significantly decreased ($p < 0.05$) the egg cholesterol content in a concentration-dependent manner. Increasing the amount of PKCF decreased the egg cholesterol content. The egg cholesterol content in the 0% PKCF treatment group was increased (616.79 mg/100 g) compared to that in of the 25% PKCF treatment group (490.98 mg/100 g).

DISCUSSION

Using up to 25% PKCF in the diet of the quails had no significant effect on any of the measured production performance parameters, except the cholesterol content in the egg. The feed consumption of quails in the present study ranged from 21.83- 21.88 g hen⁻¹ day⁻¹. In fact, increasing utilization of PKCF up to 25% resulted in a similar feed consumption. Utilization of up to 25% PKCF (with a 41.90%

reduction in corn and a 45% reduction in soy bean meal) yielded feed that was just as palatable as the 0% PKCF diet (higher utilization of corn and soy bean meal content) for quails. According to Djulardi *et al.*¹², palatability is determined by taste, smell and color. The color of the control group (0% PKCF in the diet) was just as bright as that in the group with 25% PKCF in the diet. The bright color of the ration in the control group was due to utilization of a large amount of corn and soybean meal, while the 25% PKCF diet was bright because of certain fermented products (PKCF). Fermented products are preferred by the poultry compared to the nonfermented material^{2,11,12} due to the good flavor. More-over, fermented product has increased content of amino acids and introduces additional vitamins (e.g., vitamins B1, B2 and B12). Feed consumption was similar between the treatments from A (0% PKCF) to E (25% PKCF), indicating that the quality of the rations was also similar. The quality of treatment A was good due to the presence of corn and soybean meal with good nutrients, treatments B, C, D and E also had good quality due to the supplementation with the fermented product. The quality is related to the inclusion of *Lentinus edodes* that produced the cellulase enzymes, which break down the materials that are difficult to digest to easily digested materials, for example cellulose can be broken down into simple sugars to increase the nutritional value and metabolic energy². According to Lee *et al.*⁴ *Lentinus edodes* releases the exo- β -1,3-glucanase enzyme, β -glucosidase, cellobiohydrolase and endoglucanase to degrade cellulose and xylanase to decrease xylan.

The feed consumption of laying quail fed with 25% PKCF in the diet (21.83 g head⁻¹ day⁻¹) was similar to that described in the study of Costa *et al.*¹³ who reported that the feed consumption of laying quail (6-13 weeks of age) supplemented with prebiotic and organic minerals ranged from 21.23 to 22.03 g head⁻¹ day⁻¹ however feed consumption was lower than that reported by Nuraini *et al.*¹⁴, who observed that the feed consumption of laying quail aged 8-12 weeks was 22.36 g head⁻¹ day⁻¹.

The similar hen day egg production of the 25 and 0% (control) PKCF groups was most likely caused by the similar feed consumption. Similar feed consumption indicates that the amount of nutrients consumed and digested are also similar, especially protein and energy, resulting in similar daily egg production. Egg production is affected by the nutrient content in the diet and feed intake¹³.

The similarity of hen day egg production indicated that utilization of up to 25% PKCF was still favored by quail despite a 41.90% reduction in utilization of corn and a 45% reduction in soybean meal content in the ration, however the quail

tolerated the changes and produced the same number of eggs as detected in the case of the rations that use considerable amounts of corn and soybean meal. The lack of corn and soybean meal in the E treatment group can be compensated by the PKCF products that were obtained by the inclusion of *Lentinus edodes*, which produces higher level of essential amino acids, notably lysine and methionine. PKC fermented by *Lentinus edodes* was found to have higher levels of lysine (1.00%) and methionine (0.62%)². Feed that is fermented with microorganisms has higher amino acid content than the original ingredients and this amino acids are produced by microorganisms. The average hen day egg production observed in the present study (72.59%) was higher than that observed by Nuraini *et al.*² (70.56%) in quails fed 20% palm oil sludge fermented with *Lentinus edodes* and also by Aldi¹⁵ (71.69%) in the case of the hen day egg production at the age of 11-15 weeks.

The same protein consumption used in each treatment means that the amount of food substances, especially proteins that are eaten and used for egg formation are also the same, thus giving-yielding the same egg weight. According to Amrullah¹⁶, the egg weight is influenced by dietary consumption and especially protein. Ivy and Glaves¹⁷ stated that the egg weight is influenced by the balance of food substances, by amino acids from the ration in particular. Al-Daraji *et al.*¹⁸ stated that some of the nutrient content of the feed can determine the egg weight, including the energy content of the feed, feed protein content, methionine acid, unsaturated fatty acids, especially linoleic acid, minerals, especially phosphorus and antinutrients.

The egg weight of quail at the age of 12-16 weeks fed with the ration containing 25% PKCF was 10.18 g egg⁻¹. Nuraini¹⁴ reported that the egg weight of laying quail aged 8-12 weeks was 9.70 g egg⁻¹. The values of the egg weight are influenced by the age of quail. The egg weight is higher in older quail reaches a stable weight of 10 g at week 9-13²¹.

The egg mass depends on egg weight and egg production and not significantly influenced ($p>0.05$) because egg mass is the result of egg production and egg weight. The egg mass is closely related to the weight of the eggs and egg production. The egg mass depends on the percentage of daily egg production and egg weight. The egg mass corresponds to the ratio of egg production ability between the groups or poultry strains due to feeding and better management programs^{18,19}.

Moreover, the feed conversion ratios for all PKCF treatment groups were similar because there was no significant change in feed consumption or average daily egg production. The feed conversion ratio is the ratio between

feed intake and the amount of egg produced^{16,18,19}. Feed conversion ratio can be used as an indicator of the production coefficient, where a lower value indicates more efficient use of the feed to produce the eggs. In the present study, feed conversion ratio was defined as the ratio between the feed intake and egg mass production. Feed conversion ratio was similar between the 0% PKCF and 25% PKCF treatment groups (2.96) of the present study, demonstrating that substitution of up to 25% PKCF for corn and soy bean meal in the diet of the broilers does not significantly affect feed intake or average daily egg production. The quality of the ration determined the values of the conversion, good quality rations with a balanced nutritional content and high palatability produce better conversion of the rations, whereas low quality rations with low palatability produce low conversion^{16,17}.

Feed conversion ratio is influenced by genetics, body size, environmental temperature, health, adequate nutrients and the number and weight of produced eggs. Feed conversion ratio is affected by the ability of livestock to digest feed ingredients, the adequacy of feed substances for basic life and, growth and the type of feed consumed. The feed conversion ratio of 12-16week old laying quail fed with 25% PKCF ratio was 2.96. The feed conversion ratio of 8-12weeks old laying quail fed this ration was 2.87².

Interestingly, the egg cholesterol content decreased by 20.40% (from 616.79 mg/100 g in the 0% PKCF treatment group to 490.98 mg/100 g in the 25% PKCF treatment group), apparently due to the increased amount of the fermentation product, eritadenine, in the quail diet. Eritadenine is a product of *L. edodes* fermentation with known hypocholesterolemic effects⁷⁻⁹. The adenosine derivative eritadenine [2^o, 3(R)-dihydroxy-4-(9-adenyl)-butyric acid, lentinacin and lentysine) has been isolated and identified as an active hypocholesterolemic component of the shiitake mushroom. Eritadenine reduces serum cholesterol levels in mice by accelerating the excretion of ingested cholesterol and its metabolic decomposition¹⁰. Furthermore, eritadenine affects the metabolism of cholesterol and the metabolism of phospholipids and fatty acids in rats⁹. Increased levels of eritadenine in the ration resulted in an increase the amount of eritadenine consumed, thus lowering the cholesterol content of quail egg yolk. *Lentinus edodes* is a fungus that produces eritadenine compounds that can reduce cholesterol. Eritadenine can reduce cholesterol by binding to bile acids, the bound bile acids migrate to the large intestine and are then discharged with the feces. Increased bile acid excretion through feces will stimulate the body, especially the liver, to synthesize new bile acids derived from cholesterol, thus reducing, the cholesterol levels in the body²⁰.

The use of PKCF in laying quail rations in treatment E (25% PKCF) with eritadenine content of 33.63 mg kg can reduce cholesterol in the quail egg yolk by 20.39%. The reduction of cholesterol levels in the quail egg yolk is higher than that observed by Nuraini¹⁴ who used palm oil sludge fermented with *Lentinus edodes* in the laying quail rations.

CONCLUSION

Inclusion up to 25% PKCF in quail diets did not significantly affect feed consumption (21.83 g hen⁻¹ day⁻¹), average daily egg production (72.59%) and feed conversion ratio (2.96) but it reduced the egg yolk cholesterol content by 20.39%.

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SIGNIFICANCE STATEMENT

The present study discovered an alternative feed component (PKCF) which resulted in good egg production performance and egg quality, especially regarding cholesterol content. This result will aid in the development of PKCF formulations for quail rations, that will provide good egg production performance (no negative effects), increase egg quality and reduce costs for farmers.

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