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The Use of Enzymatic Pre-digestion of Fermented Palm Kernel Cake in the Laying Hens Diet on Production Performance, Nutrient Digestibility, Egg Quality, and Egg Chemical Content

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ABSTRACT

A study was conducted to determine the effect of enzymatic pre-digestion of fermented palm kernel cake in the laying hen's diet on the performance production, nutrient digestibility, egg quality, and egg chemical content. Palm kernel cake (PKC) was added with 3% ammonium sulfate and then fermented by using *Saccharomyces cerevisiae* for 5 days (FPKC). The FPKC was hydrolyzed with mannanase to produce enzymatic pre-digestion of FPKC (EPFPKC). The study used a completely randomized design with 5 treatments and 6 replications. The EPFPKC-containing diets were fed to 240 Lohmann Brown laying hens aged 19 weeks old for 3 months. The diets were D-1 (without EPFPKC), D-2 (5% EPFPKC), D-3 (10% EPFPKC), D-4 (15% EPFPKC), and D-5 (20% EPFPKC). Feed and drinking water were available at all times. Total fecal discharges were collected for three consecutive days to measure feed digestibility. The variables measured were feed digestibility, production performance, egg quality, lauric acid, cholesterol, and beta-carotene content of eggs. Data were analyzed with the variance analysis. The results showed that fermentation could increase the protein content of PKC and decrease the crude fiber content. The addition of EPFPKC in the diets increased dry matter, protein, and crude fiber digestibility and improved the quality of albumen and yolk during 28 days of storage at room temperature. Adding EPFPKC also increased the concentration of lauric acid and beta-carotene in eggs. In conclusion, the addition of 15% of enzymatic pre-digestion of fermented palm kernel cake (D-4) in the diet improves the quality of eggs stored for 28 days, and increases the concentration of lauric acid and beta carotene in eggs.

***Keywords:** beta carotene; enzymatic pre-digestion; fermented palm kernel cake; hens; lauric acid*

INTRODUCTION

Replacing conventional feedstuffs with agricultural by-products has been a big concern in the poultry industry for decades due to the insecurity of, and dependence on, conventional feedstuffs in Indonesia. Currently, soybean meal and corn, two main ingredients in poultry diet, are still imported. The increased use of conventional feedstuffs increases the price of conventional feedstuffs, such as corn, yearly. National corn prices increased from Rp4000 in 2009 to Rp7000 in 2017 (BPS, 2018). The dependency on imported feedstuffs and their increased price puts Indonesia's poultry industry at risk. The way to reduce the dependency on imported feedstuffs should be made by using locally available feedstuffs.

Palm kernel cake (PKC) is widely available in Indonesia, the largest PKC-producing country in the world (FAO, 2018). Palm kernel cake can be used as an alternative to replace imported feed ingredients for poultry due to its moderate content of nutrients and available in large quantities. Palm kernel cake also contains lipids, including a relatively large amount of lauric acid (Purnama *et al.*, 2020) and beta carotene (Sundalian *et al.*, 2022). These two substances (lauric acid and beta carotene) were believed to have antioxidant properties that are beneficial to improve the immune status of poultry (Zeitz *et al.*, 2015). Although this feed ingredient has beneficial nutrients, the use of PKC in poultry diet is limited because PKC contains high cellulose, high mannan (hemicellulose), nutshell contamination (lignin), and low digestibility (Sundu *et al.*, 2006). Efforts to increase the nutritional content, especially protein content, can be made with bioconversion technology (Hafsah *et al.*, 2020). Hafsah *et al.* (2020) found that bioconversion technology using yeast could increase protein content by almost 100% when the fermented substrate was added with ammonium sulfate before the fermentation process. Extrapolating from these findings, the protein content of PKC is expected to improve if the technology used by Hafsah *et al.* (2020) can be successfully applied to PKC.

Although fermentation technology can increase the content of certain nutrients, such as protein content, its effectiveness in increasing digestibility produces inconsistent results. Accordingly, attempts to improve the digestibility of fermented substrate need to be made. The addition of enzymes has become a solution to increasing feed digestibility, especially in poultry feed (Sundu *et al.,* 2006). Conventionally, enzymes are added directly to animal feed. This technique can cause enzymes to become inactive due to storage, transportation, and further feed processing. Therefore, enzymatic pre-digestion of a feed ingredient in the incubator can be a way to increase digestibility and minimize the possibility of enzyme inactivation. The novelty of this study is related to the use of combined fermentation and pre-digestion technologies to improve low-quality feedstuffs. A study was conducted to determine the effect of enzymatic pre-digestion of fermented PKC on the nutritional value of PKC, feed digestibility, production performance of laying hens, the quality of egg stored for 28 days at room temperature, the contents of lauric acid, beta carotene, and cholesterol of egg.

MATERIALS AND METHODS

Fermentation and Hydrolyzation Process

The fermentation process was based on the procedure by Hafsah *et al*. (2020). The ground PKC was used as a solid substrate for fermentation. The PKC was steamed using an autoclave for 30 minutes and then cooled. The substrate of PKC was added with 3% ammonium sulfate (fertilizer) and mixed thoroughly. The substrate was soaked with sterile distilled water until the estimated water content was 70%-80%. *Saccharomyces cerevisiae* yeast was added at 0.2% of the weight of the substrate (equivalent to 346 cfu/g) for the fermentation process. The mixture was incubated aerobically in plastic bags for 5 days. The fermented PKC (FPKC) was then harvested and oven-dried at 50 ^{o}C for 48 hours.

The dried substrate of FPKC was ground and added with 0.25% mannanase enzyme (Hemicell®HT). This enzyme product contains 16 x 10^{6} units/kg beta mannanase activity. The mixtures between ground FPKC and mannanase were sprayed with sterile water to produce PKC with 80% moisture content. The substrate was then incubated for 48 hours at 40-50 ^{o}C. The hydrolyzation of FPKC (EPFPKC) product was dried and used for the study.

Experimental Animal and Cages

The protocol of this study was verified by the animal ethics committee at the Universitas Tadulako, Faculty of Animal Science and Fisheries, with an approval number 72/AEC-Untad/6/2022. A total of 240 Lohmann Brown laying hens aged 19 weeks were used in this study. The open-sided house with battery cages was used. Two hens were placed in each battery cage for 3 months. Each cage was facilitated with a feed trough and a drinker. The cage and house were routinely cleaned. The hens were vaccinated against New Castle Diseases 4 days after arrival. During the first week, the laying hens were habituated to the experimental diets and cages. Monitoring and selection of hens were done during the first week of arrival. Any hen showing a sign of the disease was discarded.

Experimental Diets

Feed ingredients used in this study were purchased locally. The diets (Table 1) were formulated using the UFFF software version 1.11 (Pesti *et al.,* 1986). The treatment diets were levels of enzymatic pre-digestion of fermented palm kernel cake (EPFPKC). The EPFPKC contains 36.38% protein, 27.63% crude fiber, and 16.90% total amino acids, while PKC has 18.81% protein, 42.32% crude fiber and 12.87% total amino acids. The diets were D-1 (without EPFPKC), D-2 (5% EPFPKC), D-3 (10% EPFPKC), D-4 (15% EPFPKC), and D-5 (20% EPFPKC). The diet and drinking water were provided *ad libitum*.

Experimental Design

This study used a completely randomized design with 5 treatments and 6 replicate cages. Each cage consists of 8 chickens.

Measurement of Variables

The EPFPKC product was analyzed for proximate analysis (AOAC, 1990) to get the data on protein, lipid, crude fiber, and lipid contents. A procedure used by Sundu *et al*. (2008) was adopted in this study to analyze amino acid concentration. Digestibility's of dry matter, crude protein, lipid, crude fiber, and metabolizable energy were measured by using a total collection of fecal discharges method. In week 4, total excreta collection was carried out every day for 3 consecutive days. The excreta were weighed after discarding any contaminant. The fresh excreta were immediately oven-dried at 50 ^{o}C for 48 hours. Feed intake and excreta discharges during 3 days were recorded to measure digestibility.

Eggs were collected on day 30 to measure the contents of protein, lipids, cholesterol, beta-carotene, and lauric acid. One day after collection, the egg was broken. Yolk and albumen were mixed and then oven-dried at 50 ^{o}C for 48 hours. Total lipid content and protein were measured using the AOAC method (AOAC, 1990). The lauric acid, cholesterol, and β-carotene contents were respectively analyzed using the methods of Beynen & Katan (1985), Bragagnolo and Rodriguez-Amaya (2003), and Islam *et al*. (2017). All the egg chemical content analyses were done at PT Saraswanti Indo Genetech, Bogor, Indonesia.

On day 40, the eggs were collected and stored for 28 days before measuring egg qualities: egg shape index, egg mass loss, yolk index, and the Haugh unit. Roche's yolk color fan was used to determine the yolk color of the egg. The formulas for the parameters measured were:

Shape index of egg = Length (mm)/ Width (mm)

Egg mass loss (%) = [(Egg mass on day 1 – Egg mass on the day of measurement) / Egg mass on day 1] x 100

Yolk index = Yolk height (mm) / Yolk diameter (mm)

The Haugh unit = 100 log (H + 7.57 - 1.7$W^{0.37}$)

Table 1. Composition and nutrient contents (calculating) of laying hens ration with different level of enzymatic pre-digestion of fermented palm kernel cake in the diets

Items	D-1	D-2	D-3	D-4	D-5
Ingredients					
EPFPKC (%)	0.00	5.00	10.00	15.00	20.00
Soybean meal (%)	15.70	14.20	10.40	7.00	5.00
Corn (%)	54.50	53.00	52.00	50.90	48.90
Fish meal (%)	8.00	8.00	10.00	11.80	12.50
Rice bran (%)	13.50	10.70	9.00	6.50	4.00
Palm oil (%)	0.00	1.00	1.50	2.00	3.00
$CaCO_3$ (%)	6.50	6.00	6.00	5.80	5.80
Dicalcium phosphate (%)	1.00	0.30	0.30	0.20	0.10
Table salt (%)	0.30	0.30	0.30	0.30	0.20
Methionine (%)	0.10	0.10	0.10	0.10	0.10
Lysine (%)	0.10	0.10	0.10	0.10	0.10
Vitamin and mineral mix (%)	0.30	0.30	0.30	0.30	0.30
Calculated nutrients					
Crude protein (%)	17.80	17.80	17.70	17.70	17.70
Total amino acids	13.64	13.63	14.03	14.34	14.49
Crude fiber (%)	3.56	4.53	5.10	5.89	6.57
Dry matter (%)	90.60	90.20	89.50	89.70	89.40
Ether extract (%)	5.55	7.85	7.88	9.08	10.95
Metabolizable energy (kcal/kg)	2785	2787	2783	2762	2758
Calcium (%)	3.38	3.25	3.15	3.16	3.19
Phosphorous (%)	0.55	0.61	0.54	0.61	0.71
Lysine (%)	1.07	1.08	1.07	1.08	1.08
Methionine (%)	0.46	0.47	0.48	0.5	0.51
Cysteine (%)	0.29	0.29	0.28	0.27	0.26

Note: Calculated nutrients were based on NRC (1994) and current analysis for EPFPKC. EPFPKC= Enzymatic Pre-digestion of fermented palm kernel cake, Vitamin and mineral Mix contains Vitamin mixture (mg or IU if mentioned kg diet): 2400 IU retinyl acetate, 400 IU Cholecalciferol, 16 IU DL-Tocopherol acetate, 5.0 menadione, 2.4 Pyridoxine, 10 riboflavin, 12 Ca Pantothenate, 0.4 Biotin, 4.0 Thiamine, 45 Niacin, 0.04 Cobalamin. Mineral mixture (mg kg diet-1): 14 Iron, 24 Manganese, 50 Zinc, 8 Copper, 0.4 Iodine. D-1= without EPFPKC, D-2= 5% EPFPKC, D-3= 10% EPFPKC, D-4= 15% EPFPKC, D-5= 20% EPFPKC.

where H was albumen height (mm) and W was egg mass (g).

Egg production was done by directly weighing the total eggs based on each experimental unit. Feed intake was measured by subtracting the total feed offered from the leftover feed throughout the study, while the feed conversion ratio was calculated by dividing the amount of diet ingested with egg production.

Statistical Analysis

The data obtained were analyzed by analysis of variance and differences among treatments, and they were tested using Duncan's Multiple Region Test (Steel & Torrie, 1980). The statistical analysis was done by using Minitab software.

RESULTS

Data on the nutrient profiles of either PKC or enzymatic pre-digestion of fermented palm kernel cake (EPFPKC) were mentioned in the Experimental Diets sub-section. Data on egg production, feed intake, digestible dry matter intake, and FCR are shown in Table 2. Egg productions in terms of egg mass or total egg production of hens fed 5%-15% EPFPKC were the same as the control hens (diet without EPFPKC). However, egg production dropped significantly ($p<0.05$) when the 20% EPFPKC was added to the diet. The FCR of the 20% EPFPKC diet also decreased significantly.

Data on feed digestibility and AME of the diets with or without EPFPKC can be seen in Table 3. There was no significant difference in the AME of the diets, lipid digestibility, or dry matter excreta due to the addition of EPFPKC. The present study indicates that treating PKC with fermentation and enzymatic pre-digestion could maintain dry matter digestibility when the maximal inclusion rate is 15%. The addition of 20% EPFPKC in the diets decreased the digestibility's of dry matter, protein, and crude fiber ($p<0.05$), compared to the diet without EPFPKC (D-1).

The chemical content of eggs and the qualities of eggs stored for 28 days at room temperature are presented in Tables 4 and 5. There was no significant difference in the concentrations of lipids, cholesterol, and protein in eggs due to the addition of EPFPKC in the diet. The addition of 15% EPFPKC in the diet improves significantly ($p<0.05$) beta carotene of eggs. Lauric acid concentration of eggs increased significantly ($p<0.05$) over the increased levels of EPFPKC in the diets (Table 4).

The effect of the additional EPFPKC in the diets on the qualities of eggs stored for 28 days at room temperature was significant ($p<0.05$). The positive effect

Table 2. Egg production, feed intake, and feed conversion ratio of laying hens fed diets containing enzymatic pre-digestion of fermented palm kernel cake

Variables	Treatment diets					p-value	SEM
	D-1	D-2	D-3	D-4	D-5		
Egg mass (g/egg)	52.92[a]	52.55[ab]	52.87[ab]	52.75[ab]	52.33[b]	0.003	0.061
Egg production (%)	90.0[a]	89.9[a]	89.9[a]	89.8[a]	81.9[b]	<0.001	0.181
Total egg production (g/bird)	21,333[a]	21,160[a]	21,288[a]	21,214[a]	19,198[b]	<0.001	49.2
Feed intake (g/bird)	53,529	53,667	53,904	53,842	53,637	0.12	48.2
DDMI (g/bird)	42,633	42,5426	42,699	42,328	41,540	<0.001	63.3
FCR	2.53[b]	2.54[b]	2.53[b]	2.54[b]	2.79[a]	< 0.001	0.006

Note: D-1= without EPFPKC, D-2= 5% EPFPKC, D-3= 10% EPFPKC, D-4= 15% EPFPKC, D-5= 20% EPFPKC. DDMI= Digestible dry matter intake; FCR= feed conversion ratio; SEM= Standard error of means. Means in the same row with different superscripts differ significantly (p<0.05).

Table 3. Nutrient digestibilities and apparent metabolizable energy of the diets containing enzymatic pre-digestion of fermented palm kernel cake with the addition of 3% ammonium sulfate

Variables	Treatments					p-value	SEM
	D-1	D-2	D-3	D-4	D-5		
Dig. dry matter (%)	79.6[a]	79.3[a]	79.2[a]	78.6[a]	77.4[b]	<0.001	0.118
Dry matter excreta (%)	28.5	32	29.9	31.4	32.3	0.071	0.449
AME (kcal/kg)	2,800	2,779	2,777	2,775	2,768	0.480	5.810
Dig. Crude protein (%)	80.6[a]	79.3[ab]	78.9[ab]	78.9[ab]	77.5[b]	0.007	0.235
Dig. Crude fiber (%)	22.5[a]	21.5[a]	21.1[a]	21.1[a]	19.2[b]	<0.001	0.184
Dig. Lipid (%)	90.1	88.9	88.2	88.1	88.1	0.053	0.217

Note: D-1= without EPFPKC, D-2= 5% EPFPKC, D-3= 10% EPFPKC, D-4= 15% EPFPKC, D-5= 20% EPFPKC. EPFPKC= enzymatic pre-digestion of fermented palm kernel cake; SEM= Standard error of means; Dig= Digestibility, AME= Apparent metabolizable energy. Means in the same row with different superscripts differ significantly (p<0.05).

Table 4. Lauric acid, beta-carotene, cholesterol, lipid, and protein of the egg produced by laying hens fed diets containing enzymatic pre-digestion of fermented palm kernel cake

Treatment diets	Variables				
	Lauric acid (%)	β-carotene (mg/kg)	Lipid (%)	Cholesterol (mg/100 g)	Protein (%)
D-1	0.0074[c]	0.1400[b]	35.4	1511	52.7
D-2	0.0098[b]	0.1200[b]	34.7	1577	53.9
D-3	0.0107[b]	0.1350[b]	36.4	1483	51.8
D-4	0.0114[b]	0.4733[a]	35.9	1466	52.3
D-5	0.0213[a]	0.1633[b]	36.2	1499	52.1
SEM	0.000185	0.0218	0.321	30.8	0.230
P value	0000	0000	0.516	0.819	0.074

Note: D-1= without EPFPKC, D-2= 5% EPFPKC, D-3= 10% EPFPKC, D-4= 15% EPFPKC, D-5= 20% EPFPKC. EPFPKC= enzymatic pre-digestion of fermented palm kernel cake; SEM= Standard error of means. Means in the same column with different superscripts differ significantly (p<0.05).

of the addition of EPFPKC becomes more evident as the deterioration of the qualities of albumen (the height of albumen and the Haugh unit), yolk (the height of yolk and yolk index), and egg mass loss can be slowed down (Table 5). Albumen has been used as an indicator to determine the decreased quality of eggs concerning storage time. The albumen height of eggs stored for 28 days showed significant differences from the control eggs. Eggshell thickness, yolk color, and egg shape index are not affected by the addition of EPFPKC in the diets.

DISCUSSION

Nutritional Profiles of Enzymatic Pre-digestion of Fermented Palm Kernel Cake

Data on the nutritional profiles of PKC and enzymatic pre-digestion of fermented PKC (EPFPKC) with the addition of 3% ammonium sulfate showed changes in protein and crude fiber contents. The PKC used in this study had 42.3% crude fiber and fermentation and enzymatic treatment of PKC led to a decrease in crude fiber content to 27.63%. The decrease in crude fiber content is understandable, as microorganisms can degrade complex fiber into simpler fractions due to their production of enzymes during the fermentation process (Bahri *et al*., 2019; Hatta *et al*., 2014).

The addition of 3% ammonium sulfate as a source of nitrogen led to an increase in protein content to 36.4%. Total amino acid content also improves from 12.9 in PKC to 16.9% in EPFPKC with the addition of 3% ammonium sulfate. This fact indicates that nitrogen from ammonium sulfate was converted into amino acids. The ability of microorganisms, particularly yeast *Saccharomyces cerevisiae*, to bio-convert inorganic minerals into organic substances has been reported by Hafsah *et al*. (2020) and Demirci *et al*. (1999). Hafsah *et*

Table 5. The qualities of eggs were stored for 28 days at room temperature

Variables	Treatments					p-value	SEM
	D-1	D-2	D-3	D-4	D-5		
Yolk (%)	29.4	27.6	27.9	27.5	28.3	0.710	0.473
Albumen (%)	56.9	58.6	58.3	59.0	58.1	0.763	0.537
Eggshell (%)	13.7	13.8	13.8	13.4	13.6	0.969	0.182
Yolk color	7.75	7.50	7.75	7.75	7.71	0.803	0.077
Egg shape index	0.762	0.763	0.748	0.748	0.751	0.499	0.0035
Index of yolk	0.194[c]	0.198[bc]	0.220[a]	0.215[ab]	0.217[ab]	0.002	0.0023
The Haugh unit	44.5[c]	45.9[bc]	50.3[a]	49.8[ab]	49.3[ab]	<0.001	0.428
Egg mass loss (%)	6.18[a]	5.11[b]	5.11[b]	5.12[b]	5.11[b]	0.004	0.097
Eggshell thickness (mm)	0.325	0.326	0.340	0.321	0.325	0.442	0.0033
Yolk height (mm)	9.23[c]	9.38[bc]	10.01[a]	9.90[ab]	9.92[ab]	0.001	0.066
Albumen height (mm)	2.44[b]	2.72[a]	2.85[a]	2.87[a]	2.94[a]	<0.001	0.0255

Note: D-1= without EPFPKC, D-2= 5% EPFPKC, D-3= 10% EPFPKC, D-4= 15% EPFPKC, D-5= 20% EPFPKC. EPFPKC= enzymatic pre-digestion of fermented palm kernel cake; SEM= Standard error of means. Means in the same row with different superscripts differ significantly ($p<0.05$).

al. (2020) found that adding ammonium sulfate in the coconut dregs substrate before fermentation increased the amino acid content of the substrate. A detailed study by Demirci *et al.* (1999) indicated that *Saccharomyces cerevisiae* could bio-convert 0.3% of organic selenium into amino acid (seleno-methionine).

The influence of the EPFPKC on feed digestibility is not available in the database. The decreased digestibility's of dry matter, protein, and crude fiber was found in the diet with 20% EPFPKC, compared to the diet without EPFPKC (D-1). This may indicate that the 20% EPFPKC diet was beyond the capacity of laying hens to optimally digest the diet as there was a linear decrease in dry matter digestibility over the increased inclusion of EPFPKC with the regression equation of $Y= 1.1020 X + 79.84$ and $R^2 = 0.8533$.

Egg Production and Nutritional Profiles of the Egg

Although PKC is believed to be of low quality (Sundu *et al.*, 2006), the fermentation followed by enzymatic pre-digestion technology could optimize egg production to the same level as the production of eggs of laying hen fed without the addition of PKC (D-1). These data indicate that the technology of fermentation plus enzymatic pre-digestion is compatible when the high-fiber ingredient of PKC is used in the diet formula for laying hens. Using 15% EPFPKC is tolerable for laying hens without reducing egg production. The addition of 20% of EPFPKC (D-5) significantly reduced egg production. These findings may explain that laying hens fed a 20% EPFPKC cannot optimally utilize the nutrients present in the diet or spend more energy to digest the high-fiber diet. The egg mass produced by hens fed the D-5 diet was also smaller than that of control eggs (52.3 vs 52.9 g).

It has been well-accepted that egg production is associated with feed consumption. Although feed intake in this current finding was the same, egg production was affected by the treatment diets. The logic behind this finding is the differences in nutrient digestibility, either dry matter digestibility or protein digestibility. Sitindaon *et al*. (2021) found that fermentation can increase the digestibility of PKC protein from 51% to 55%. The drop in digestibility of diets containing a high percentage of PKC in D-5 led to a decrease in the digestible dry matter intake in this study (Table 3). The lower egg production of laying hens fed with the D-5 diet led to the laying hens having a higher FCR than other diets.

Lauric acid has been reported to be highly digestible in the digestive tract of poultry and has anti-oxidant and bactericidal properties (Sandhya *et al.*, 2016; Zimboran *et al.*, 2022). These properties are beneficial to promote growth and production. Many researchers have studied the use of lauric acid on poultry performance (Zeitz *et al.*, 2015; Jola *et al.*, 2017). The addition of lauric acid-rich fats in the diet increases lauric acid content in the thigh and breast muscles of broilers (Zeitz *et al.*, 2015). Palm oil or palm kernel oil has been identified to have a large amount of lauric acid, 48% in palm kernel oil (Silalahi *et al.*, 2018). Since lauric acid can be easily absorbed in the digestive tract of poultry (Hankel *et al.*, 2018), the addition of 5%-20% EPFPKC in the diets enhanced the lauric acid content of eggs by 32% at D-2 to 188% at D-5.

Although the carotenoid content in palm kernel oil (2.24-3.46 ppm) is lower than that of palm oil, being 28.5 ppm (Izuddin *et al.*, 2022), the use of PKC is often associated with the addition of palm oil to meet energy requirements for poultry. In this study, 5%–20% PKC resulted in additional use of 1 to 3% palm oil in the diet. This brings about an increased concentration of β-carotene in the diet. The hens fed the diets added with EPFPKC and palm oil produced more β-carotene in the eggs, especially in the 15% EPFPKC (D-4). The addition of more or less than a 15% EPFPKC in the diet could not produce a significant improvement of β-carotene in eggs. It is hard to explain why adding 20% EPFPKC could not improve the β-carotene of eggs to a significant concentration. It is possible that beta-carotene is not fully available as feed digestibility dropped significantly in the D-5 diet. The contents of cholesterol, lipids, and egg protein were not affected by the fermentation and pre-digestion treatment of PKC.

Qualities of Eggs Stored for 28 Days at Room Temperature

The percentage of eggshell, albumen, and yolk in all eggs was not significantly different. This is probably due to the uniformity of some of the nutritional contents of the diets from all treatments, especially calcium, phosphorus, and protein. Calcium and phosphorus are the main components of eggshell formation (Leeson, 2008), and protein is the main component in the yolk and albumen. Since the diets were made of iso-calcium and iso-phosphorus, the percentage and the thickness of eggshell in this study were the same, ranging from 12.76%–13.76% and 0.318–0.325 mm, respectively. The percentages of albumen and yolk of all eggs were also similar.

The egg is a biological product that is easily damaged during storage, especially if the storage temperature does not match the ideal temperature (Gravena *et al.,* 2011). The largest egg mass loss was obtained in the control eggs. Eggs produced by laying hens fed the diet-containing EPFPKC experienced a small mass loss when stored for 28 days at room temperature and were statistically different from control eggs (D-1). The high antioxidant present in palm oil and PKC could be the reason behind this finding. Antioxidants such as β-carotene (Stahl & Sies, 2003) and lauric acid (Zimboran *et al.,* 2022), which are abundant in PKC, may slow down the process of decomposition of albumen, which in turn potentially inhibits the process of water evaporation contained in albumen.

The very first part of the egg undergoing the process of damage during storage is the albumen (Hatta *et al.,* 2020). The difference in albumen damage in terms of the Haugh unit was detected when the diets were added with more than 10% EPFPKC. The increase in the quality of albumen was in line with the increase in the level of EPFPKC in the diets. During 28 days of storage at room temperature, the higher the use of EPFPKC, the better the prevention of decreased albumen height, with values of $R^2 = 0.8449$ and $Y= 0.023x + 2.534$.

CONCLUSION

Bioconversion technology through fermentation with the addition of ammonium sulfate followed by enzymatic pre-digestion of palm kernel cake increased the protein content and reduced the crude fiber content. The concentration of lauric acid and beta-carotene in eggs improved when the enzymatic pre-digestion of fermented palm kernel cake was added to the diet. The technology of enzymatic pre-digestion of fermented palm kernel cake slowed down egg mass loss and deterioration of albumen and yolk during 28 days of storage at room temperature. The use of enzymatic pre-digestion of fermented palm kernel cake at a level of 15% in the laying hen's diet was highly recommended. This technology has the potential to be used in poultry diets as conventional feedstuffs are getting more expensive in the future.

CONFLICT OF INTEREST

B. Sundu serves as an editor of the Tropical Animal Science Journal but has no role in the decision to publish this article. The authors ensure no conflict of interest with companies, institutions, and the funding body included in this article.

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