Cassava meaf meal inclusion in palm kernel meal diet could improve egg yolk color in post-molted native laying hens[1]

A. Adrizal,* S. Fakhri,* R. Murni,* Y. Yatno,* T. Maranata,* S. Asby,* Y. Yusrizal,* and C. R. Angel†

*Faculty of Animal Husbandry, University of Jambi, Jambi 36361, Indonesia; and †Department of Animal and Avian Sciences, University of Maryland, College Park, MD 20742, USA

ABSTRACT: Our previous study demonstrated that native laying hens receiving 15% or 30% palm kernel meal (PKM) diet had comparable feed conversion ratio (FCR), egg production, and egg quality with those being fed with corn-soybean diet except yolk color score which was significantly lower (paler). The present study was aimed at improving yolk color score of 66-wk old post-molted native laying hens fed PKM diets with cassava leaf meal (CLM). Dietary treatments were a factorial combination of three levels of PKM (0, 15, and 30%) and two levels of CLM (0 and 2.5%), and assigned at random to 90 cages with one hen per cage, so each treatment consisted of 14 to 16 replicates. Experimental diets were isocaloric (2,735 to 2,758 kcal/kg) and contained CP of 17.0 to 18.4%. Diets and water were provided ad libitum for five weeks. Although feed intake was influenced by dietary treatments, hens receiving PKM diets, regardless CLM inclusion, did not show differences in FCR, egg production, and all egg traits except yolk color. Hens receiving the 30% PKM diet laid eggs with lower yolk color score than those received the 15% or 0% PKM diet (6.92 vs. 8.00 vs. 8.00, respectively; $P \leq 0.0001$). Supplementing the PKM diet with CLM clearly improved egg yolk color score from 7.1 (0% CLM) to 8.2 (2.5% CLM) ($P \leq 0.0001$)), and this was further shown by interaction effect of PKM and CLM ($P \leq 0.05$). In conclusion, CLM could be included in the diet containing PKM to improve yolk color for consumers' preference.

Key words: palm kernel meal, native laying hen, cassava leaf meal, feed conversion ratio, egg production, egg yolk color

INTRODUCTION

Indonesia has been the main crude palm oil producing country in the world with a total production of 19 tons recorded in 2009 (United States Department of Agriculture [USDA], 2009). Oil palm tree (*Elaeis gueneensis* Jacq) bears its oil in bunches of fruits, particularly from the mesocarp (major oil storage) and endocarp (kernel). Oil extraction from the kernel of palm fruit leaving a residue called palm kernel cake or meal (**PKM**), a potential poultry feed in palm plantation countries, including Indonesia, where commercial feed price are frequently unaffordable by small-scale poultry farmers (Adrizal et al., 2010). Indonesia produced approximately 2.8 million tons PKM in 2009 (Bromokusumo, 2009) and expected to increase yearly due to plantation expansion.

Optimum dietary levels of feeding broilers and laying hens diets containing PKM and their responses on these diets have been inconsistent (Onwudike, 1988; Perez et al., 2000; and Soltan, 2009; Adrizal et al., 2010). These differences have been attributed to degree of grittiness, fiber content, and amino acid digestibility of PKM (Ravindran and Blair, 1992; Bach-Knudsen, 1997; and Sundu et al., 2006). In case of nutshell separation from the kernel is done prior to oil extraction, which is commonly practiced in Indonesia, grittiness (nutshell contamination) would not be likely a problem in PKM for chicken. In this type of kernel meal, its fiber (nonstarch polysaccharides [**NSP**]) (Düsterhöft et al., 1992; Bach-Knudsen, 1997) content would appear tto be he potential major factor limiting nutrient availability of PKM. Increased digesta viscosity with concomitant poor nutrient

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digestibilities, particularly due to the intake of soluble fractions of NSP, including mannans, is well documented in poultry species (Düsterhöft et al., 1992; and Choct, 1997; Lee et al., 2003; and Daskiran et al., 2004). However, dietary mannanase supplementations, in combination with α-galactosidase (Sundu and Dingle, 2003), or with amylase, protease, or other fiber-degrading enzymes (Chong et al., 2008; Soltan, 2009) in PKM diets for broilers or laying hens to overcome NSP effect did not result in consistent responses. Recent study of feeding 48-wk old native laying hens dietary increment PKM levels (0 to 30%) by Adrizal et al. (2010) not only showed a comparable egg production with control, but also demonstrated no beneficial effect of enzyme addition (fiber-degrading enzyme preparations, phytase+protease, or combination of both enzymes). Eventhough, the results clearly showed reduction effect of feeding higher PKM on egg yolk color score (5.61 [control] vs. 7.08 [15%] vs. 8.15 [30% PKM diet]) (Adrizal et al., 2010). Beneficial effect of supplementing poultry diets with leaf meals (e.g. leucaena and cassava) were attributed to their carotene contribution for yolk color pigmentation (D'Mellow et al., 1987; Ravindran, 1993; Onibi et al., 2008). Cassava leaf meal in particular is not only an affordable feedstuff in tropical countries (i.e. Indonesia), good protein source, vitamins, minerals, but also source of carotene that is important for broiler skin and egg yolk, and antioxidant within the cell (D'Mellow et al., 1987; Surai and Speake, 1998; Onibi et al., 2008). Although a recommended level of using CLM in poultry diet varies ranging from 5-10% (Ravindran, 1993), a range of less than 5% of usage is likely sufficient for yolk pigmentation (Ravindran et al., 1983). This study was to reevaluate the use of PKM and the effect of CLM inclusion in PKM diet on egg production and quality, especially yolk color.

MATERIALS AND METHODS

Hens and Dietary Treatments

Animal care and sampling protocol were in compliance with Institute of Laboratory Animal Resources Commission on Life Sciences (National Research Council [NRC], 1996). A total of ninety 66-wk old native laying hens (previously adapted with a commercial layer diet) were used in this study. Newcastle vaccine was given to these birds at hatch, 3-, and 6-month of age. Hens were assigned to 90 cages (one bird per cage with cage dimension of 25 cm wide × 40 cm long × 40 cm high) in a curtain-sided house, where each hen received one of six experimental diets. Daily recordings of morning and afternoon temperatures in the hen's house showed an average of 28.0 ± 1.0°C.

The experimental diets were resulted from a factorial combination of three levels of PKM (0, 15, and 30%) and two levels of CLM (0 and 2.5%) with 14 to 16 replicate hens per treatment. Palm kernel meal, provided by Kresna Duta Agroindo Company (Jambi, Indonesia), was a by-product of expeller extraction of palm kernel. The kernel was separated from the nutshell prior to oil extraction at the processing plant leaving PKM as by-product. The PKM was then ground to pass a 1.5-mm sieve at our farm facility (University of Jambi) before being included in experimental diets. The PKM was sent to laboratory for proximate analysis (Association of Official Analytical Chemists [AOAC], 1980) with the following results: DM, 89.5%; and other nutrients are (per DM basis) CP, 15.4%; ether extract, 8.3%; crude fiber, 16.4%; ash, 4.2%; Ca, 0.46%; and total P, 0.73%. The PKM was also analyzed for gross energy using a bom calorimeter (Model P-202 CA-4PJ, Shimadzu Co., Kyoto, Japan) and gave the value of 4,239 kcal GE/kg. Amino acid assay on PKM using a High Performance Liquid Chromatography (LC-20AD HPLC, Shimadzu Co., Japan) resulted in the following composition: 0.41% Met+Cys, 0.37% Arg, 0.27% Thr, 0.32% Tyr, 0.22% His, 0.17% Ile, 0.33% Lys, 0.49% Leu, 0.47% Val, 0.27% Phe. Based on proximate analysis of CLM (AOAC, 1980), it contained 88.79% DM and other nutrients (DM basis) as follow: CP, 28.96%; ether extract, 1.96%; crude fiber, 11.48%; ash, 6.13%; Ca, 0.52%; and total P, 0.72%.

All diets were formulated to be isocaloric (2,735 to 2,758 kcal/kg) with CP content ranging from 17 to 18%, and contained equivalent amounts of other nutrients (Table 1) that met or exceeded NRC recommendations (1994). Protein content of the 0% PKM diet was set to contain a minimum of 17% whereas in the 15% and 30% PKM diets, the CP contents were allowed to float in order to balance the minimum requirements of all essential amino acids due to the decreased use of soybean meal. The inclusion of CLM was in substitution with soybean meal. Diets and water were provided for ad libitum consumption for five-week feeding trial.

Feed Intake, Feed Conversion Ratio, Egg Collection, and Egg Quality Measurements

Feed intake, FCR (the ratio of feed intake and egg mass [g:g]), and egg production (hen day) were recorded weekly. Eggs were collected daily and weighed. Egg quality traits including egg weight, eggshell surface area, albumen height, haugh unit (**HU**), yolk color, and eggshell thickness were measured from the eggs collected in the last three days on the fourth week of feeding trial.

Egg surface area was calculated by using a formula described by Paganelli et al. (1974) as follow: egg surface = $4.835 \times W^{0.662}$, where W is egg weight. Having measured egg surface, egg was broken up equatorially on the shell and the content was placed onto a flat-surface glass. Height of thick albumen was measured with albumen height apparatus (Teclock Corporation, Japan) approximately at 1 cm away from yolk. The formula of Williams (1997) was used to calculate haugh unit (HU) as follow: HU value = $100 \text{ Log } (H + 7.7 - 1.7W^{0.37})$; where H is albumen height (mm) and W is egg weight (g). Egg yolk color was scored using a 15-point scale of yolk color fan of DSM (DSM Nutritional Products Ltd., Basel, CH-4002, Switzerland). Eggshell thickness was an average measure taken at two different locations of eggshell using a stainless steel digital caliper (Mitutoyo, Model 500-196-20, Japan).

Statistical Analysis

Data were subjected to two way ANOVA based on a 3 × 2 factorial arrangement, and Tukey test was performed on data showing significance among treatment means ($P \leq 0.05$) (SAS Institute, 2008). Significance among treatment means was accepted at a $P \leq 0.05$.

RESULTS AND DISCUSSION

Mortality was not found during the five-wk feeding trial. Although hens receiving the 15% PKM diet consumed less feed (3,110 g/hen) than those receiving the control diet (3,266 g/hen) or the 30% PKM diet (3,263 g/hen) ($P \leq 0.0001$), there were no differences in FCR between the control (4.48) and all the PKM groups (4.49 and 4.89 for the 15% and 30% PKM, respectively) (Table 2). Previous study with native laying hens showed that, a linear relationship between dietary levels of PKM (0 to 30 %) with feed consumption was observed, but this did not affect FCR or egg production (Adrizal et al., 2010). Chong et al. (2008) in a study of feeding laying hens a diet containing up to 25% PKM also reported a positive correlation between dietary PKM levels and feed intake, however, those receiving the PKM diets had poorer FCR than those received the control diet. Therefore, the present feed intake data confirms not only our previous finding of tolerable this current particular PKM diet up to 30% by native laying hens (Adrizal et al., 2010), but also demonstrates comparable nutrients of PKM to corn-soybean diet when PKM diet was formulated isocaloric and with equivalent of all other nutrients, including amino acids. Data on egg production, egg mass, egg weight, egg number (Table 2) and egg quality (Table 3), except yolk color, support this statement.

The inclusion of CLM in the present experimental diets reduced protein concentration slightly compared with their corresponding control or PKM diets (Table 1), but this protein reduction does not alter other essential nutrients. Therefore, it would expectedly to see no adverse effect of CLM on feed intake, FCR, egg production, and egg quality (except yolk color). Fasuyi and Aletor (2005) did a study replacing fishmeal in broiler's diet (fishmeal was used at 5% of the diet) with cassava leaf protein concentrate, and found no deleterious effect of substituting 60% of fishmeal in the diet. In another study with broiler chickens, Onibi et al. (2008) fed broiler a 10.5% CLM diet (in replacing 30% of soybean meal protein [soybean meal was used at 14% in the diet]), and found that the replacement could optimize not only broiler growth but also more pigmented shanks of the birds. In his review article, Ravindran et al. (1983) suggested not to use more than 15% CLM in the diet, unless with methionine supplementation and energy balanced. Ravindran et al. (1983) further recommended to limiting CLM usage to 5%, perhaps, for the satisfactory effect on growth and pigmentation.

Because, higher level of CLM inclusion in the diet will increase crude fiber and other antinutritive factors (e.g. cyanoglucoside and tannins) that may cause gastrointestinal disorder and impair protein digestibility (Ravindran, 1993). A significant interaction effect between PKM and CLM on feed intake discernable at the level of 0% PKM at least demonstrated CLM contribution of some nutrients, although this effect was not evident at higher level of PKM.

Table 1. Ingredient and nutrient composition (as is basis) of diets containing three levels of palm kernel meal (PKM; 0, 15, and 30%) and two levels of cassava leaf meal (CLM; 0 and 2.5%)

	0PKM	15PKM	30PKM	0PKM + CLM	15PKM + CLM	30PKM + CLM
Ingredients				(%)		
Yellow corn	49.17	33.11	17.40	49.17	33.11	17.40
Soybean meal[1]	28.00	27.00	25.64	25.50	24.50	23.14
PKM (expeller extraction)	0.00	15.00	30.00	0.00	15.00	30.00
CLM	0.00	0.00	0.00	2.50	2.50	2.50
Rice bran	10.00	10.00	10.00	10.00	10.00	10.00
Palm oil	2.50	4.75	7.00	2.50	4.75	7.00
DL-Methionine	0.08	0.09	0.10	0.08	0.09	0.10
Dicalcium phosphate	1.55	1.55	1.55	1.55	1.55	1.55
Calcium carbonate	8.00	7.80	7.60	8.00	7.80	7.60
Sodium chloride	0.20	0.20	0.21	0.20	0.20	0.21
Sodium bicarbonate	0.30	0.30	0.30	0.30	0.30	0.30
Vitamin-mineral premix[2]	0.20	0.20	0.20	0.20	0.20	0.20
Calculated nutrients:						
MEn, kcal/kg	2,758	2,754	2,753	2,740	2735	2735
Crude protein, %	17.51	18.02	18.39	17.02	17.52	17.89
Lysine, %	0.92	0.92	0.92	0.88	0.88	0.88
Methionine, %	0.36	0.37	0.39	0.35	0.37	0.38
Methionine + Cystine, %	0.65	0.65	0.65	0.63	0.63	0.63
Ether extract, %	5.54	8.40	11.28	5.64	8.51	11.38
Crude fiber, %	4.46	6.51	8.53	4.77	6.81	8.84
Calcium, %	3.64	3.65	3.65	3.64	3.65	3.65
Total phosphorus, %	0.60	0.62	0.64	0.60	0.62	0.63
Non phytate phosphorus, %	0.29	0.29	0.30	0.28	0.29	0.29
Na, %	0.18	0.18	0.18	0.18	0.18	0.18
Cl, %	0.16	0.15	0.15	0.16	0.15	0.15
Total carotene[3]	9.74	6.63	3.59	9.90	6.79	3.75
Analyzed nutrients[4]:						
DM. %	89.44	90.00	91.35	-	-	-
GE, kcal/kg (air-dry basis)	4,491	4,782	5,003	-	-	-
Crude protein, %	18.42	18.00	19.32	18.02	17.60	18.92
Ether extract, %	7.78	8,61	12,47	7.84	8.67	12.53
Crude fiber, %	7.95	12.57	15.43	8.32	12.94	15.80
Calcium, %	3.34	3.18	3.59	3.34	3.18	3.59
Total phosphorus, %	0.51	0.59	0.65	0.51	0.59	0.65

[1]Soybean meal contained 88% DM, 45.6% CP, 2.75% EE, 5.16% CF, 0.39% Ca, and 0.91% total P.

[2]Provided (per kg diet): vitamin A: 2,500 IU; vitamin D_3: 500 IU; Vitamin E: 1.5 IU; vitamin K_3: 0.4 mg; thiamine: 0.3 mg; riboflavin: 1 mg; pyridoxine: 1 mg: cyanocobalamin: 2.4 µg; vitamin C: 6 mg; niacin: 7 mg; Ca-d-pantothenate: 1 mg; manganese: 20 mg; iron: 5 mg; iodium: 0.04 mg; zinc: 20 mg; cobalt: 0.04 mg; copper: 0.6 mg; antioxidant: 2 mg; methionine: 7 mg; and lysine: 7 mg.

[3]Total carotenes contained in the diets were calculated based on data of NRC (1994), Ravindran and Blair (1991), Zin (2006), Calderon et al. (2007), and Verschoyle et al. (2007).

[4]Analyzed nutrients of CLM diets were obtained after substituting the amount of nutrients contributed by 2.5% of soybean meal in the diets with the amount of nutrients provided by 2.5% of CLM.

Table 2. Feed intake, FCR, and egg production of post-molted native hens fed diets containing palm kernel meal (PKM) with or without cassava leaf meal (CLM) supplementation for five-week feeding

	Feed intake	FCR	Egg production (Hen day)	Egg Mass	Egg Weight	Egg Number
Factor	g	g/g	%	g/hen	g/egg	N
PKM, %:						
0	3266[a]	4.48	48.5	770	45.5	17.0
15	3110[b]	4.49	46.6	737	45.3	16.3
30	3263[a]	4.89	43.8	701	45.7	15.3
CLM (%):						
0	3225	4.65	46.0	734	45.7	16.1
2.5	3201	4.61	46.6	738	45.4	16.3
PKM × CLM:						
0 × 0	3322[a]	4.61	47.1	764	46.3	16.5
0 × 2.5	3209[bc]	4.36	49.8	777	44.7	17.4
15 × 0	3109[c]	4.19	48.8	772	45.2	17.1
15 × 2.5	3111[c]	4.78	44.5	703	45.4	15.6
30 × 0	3244[ab]	5.16	42.1	667	45.5	14.8
30 × 2.5	3283[ab]	4.70	45.5	734	46.0	15.9
SEM[1]	25	0.32	2.8	44	0.7	1.0
Source of variance:	——— Probabilities ($P \leq$ F) ———					
PKM	0.0001	0.2637	0.2599	0.2963	0.8228	0.2599
CLM	0.2425	0.8804	0.7989	0.9184	0.6051	0.7898
PKM × CLM	0.0107	0.2053	0.3102	0.2933	0.3386	0.3102

[a-c]Means within a column with no common superscripts differ significantly ($P \leq 0.05$).
[1]Standard error of means, means of 14 to 16 replicates per treatment.

Table 3. Egg quality of native hens fed diets containing palm kernel meal (PKM) with or without cassava leaf meal (CLM) supplementation during five-wk feeding trial

	Albumen height	Haugh unit	Yolk color score	Eggshell thickness
Factor	mm			mm
PKM, % :				
0	4.52	69.1	8.00[a]	0.405
15	4.32	66.4	8.00[a]	0.382
30	4.58	68.9	6.93[b]	0.382
CLM, %:				
0	4.41	67.4	7.07[b]	0.388
2.5	4.53	68.8	8.21[a]	0.391
PKM × CLM:				
0 × 0	4.28	66.2	7.79[ab]	0.391
0 × 2.5	4.77	71.9	8.21[ab]	0.418
15 × 0	4.41	66.9	7.38[b]	0.386
15 × 2.5	4.23	66.0	8.63[a]	0.377
30 × 0	4.55	69.0	6.06[c]	0.386
30 × 2.5	4.60	68.8	7.80[ab]	0.378
SEM[1]	0.25	2.2	0.25	0.011
Source of variance:	——— Probabilities ($P \leq$ F) ———			
PKM	0.5459	0.4182	0.0001	0.0769
CLM	0.5593	0.4165	0.0001	0.7623
PKM × CLM	0.4116	0.2854	0.0375	0.2110

[a-c]Means within a column with no common superscripts differ significantly ($P \leq 0.05$).
[1]Standard error of means, means of 14 to 16 replicate hens per treatment.

Significant effect of PKM and CLM was observed on yolk color score where dietary PKM reduced the score (8.00 vs. 8.00 vs. 6.93 for the 0%, 15%, and 30% PKM, respectively; $P \leq 0.0001$), and in contrast, dietary CLM improved the score (7.07 vs. 8.21 for the 0 % and 2.5% CLM, respectively; $P \leq 0.0001$) (Table 3). Because oil extraction of PKM at processing plants could have removed up to 80% of the oil (May, 1994), most of fat-soluble vitamins including carotenoids could have been also removed as well. This could be the main cause of reducing effect of PKM on yolk color. Although increasing dietary level of PKM also resulted in increased oil supplementation, this (palm) oil is a cooking oil which has been refined, colorless, and contains less of carotenoids. Lower yolk color score has also been reported in laying hens fed 25% PKM diet and palm oil (Chong et al., 2008). On the other hand, CLM is the source of carotene, which could reach the level of >800 mg/kg (Wobeto et al., 2006), much greater than corn (17 mg/kg, NRC, 1994), rice bran (< 1.5 mg/kg, Verschoyle et al., 2007) or soybean meal (< 3 mg/kg, Calderon et al., 2007). An interaction effect between PKM and CLM on yolk color ($P \leq 0.04$) further strengthens the benefit of CLM supplementation to PKM diet as carotene source. A study with quail indicated a satisfactory effect of CLM at 5% for yolk yellowness than control (Nguyen et al., 2003).

CONCLUSIONS

Palm kernel meal can be included in the diet up to 30% without negative effect on egg production. All egg quality traits (eggshell surface and thickness, albumen height and haugh unit) except yolk color were comparable among hens given control and PKM diets The reducing effect of PKM on yolk color score was alleviated by CLM inclusion at 2.5%.

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