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Supplementation of Mangosteen Pericarp Meal and Vitamin E on Egg Quality and Blood Profile of Laying Hens

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ABSTRACT

This research aimed to study the supplementation effects of mangosteen pericarp meal (MPM) and vitamin E (VE) in the diets on the egg quality and blood profile of laying hens. This research used 160 laying hens of Lohman strains 24 weeks of age. The observation was conducted for 11 weeks. A completely randomized design with four treatments and four replications (10 birds each) was used in this experiment. The treatments consisted of R0 (control diet), R1 (R0 + 1 g MPM/kg ration), R2 (R0 + 2 g MPM/kg ration) and R3 (R0 + 200 mg VE/kg ration). Variables measured were egg quality, yolk cholesterol, and blood profiles. The data were analyzed by using analysis of variance (ANOVA) and any significant difference between the treatment means were further tested by Duncan's Multiple Range Test. The results showed that supplementation of 1 g MPM/kg ration in the diet significantly (P<0.05) decreased blood triglycerides compared with the control, laying hens fed with diet suplemented with of 2 g MPM/kg ration, and laying hens with diet suplemented with 200 mg VE/kg ration. Supplementation of MPM and VE did not affect (P>0.05) egg quality (except shell thickness), blood cholesterol, and HDL, respectively. In conclusion, supplementation of 1 g MPM/kg in the diet of laying hens could decrease blood triglycerides.

Key words: blood profile, laying hens, mangosteen pericarp meal, egg quality, vitamin E

ABSTRAK

Penelitian ini bertujuan untuk mengkaji pengaruh suplementasi tepung kulit manggis (TKM) dan vitamin E (VE) di dalam ransum pada kualitas telur dan profil darah ayam petelur. Penelitian ini menggunakan 160 ekor ayam petelur strain Lohman umur 24 minggu yang dipelihara selama 11 minggu. Rancangan yang digunakan adalah rancangan acak lengkap dengan 4 perlakuan dan 4 ulangan (10 ekor setiap ulangan). Perlakuan terdiri atas R0 (pakan kontrol), R1 (R0 + 1 g TKM/kg as fed), R2 (R0 + 2 g TKM/ kg as fed), dan R3 (R0 + 200 mg VE/ kg as fed). Peubah yang diukur adalah kualitas fisik telur, kolesterol telur, dan profil darah. Data yang diperoleh dianalisa secara statistik menggunakan *analysis of variance* (ANOVA) dan jika terdapat perbedaan nyata antar perlakuan maka dilakukan uji lanjut *Duncan's Multiple Range Test*. Hasil penelitian menunjukkan bahwa suplementasi 1 g TKM/kg as fed di dalam ransum menurunkan (P<0,05) trigliserida darah jika dibandingkan dengan kelompok kontrol, ayam petelur yang diberi makan ransum yang disuplementasi 2 g TKM/kg *as fed*, dan 200 mg VE/kg *as fed*. Perlakuan suplementasi TKM dan VE tidak mempengaruhi (P>0,05) kualitas telur (kecuali tebal kerabang), kolesterol darah, dan HDL. Kesimpulannya, suplementasi 1 g TKM/kg as fed dalam ransum ayam petelur dapat menurunkan kadar trigliserida darah.

Kata kunci: profil darah, ayam petelur, tepung kulit manggis, kualitas telur, vitamin E

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INTRODUCTION

Chicken's egg is one of primary poultry products that can be used as animal protein source with rich nutrient content and easily found by consumers. Egg quality is defined by external and internal properties. External properties include shell cleanliness, color, shape and soundness of shell. Meanwhile, internal properties include, relative viscosity of albumen, yolk shape, yolk color and nutrient content (Hussain *et al.*, 2013). Egg quality recently gains attention not only in farmer level but also in industry and consumer levels. Various aspects such as genetic, nutrient, breeding system, environment and post-harvest handling are interesting studies in order to produce and improve the quality of egg.

Ambient temperature is one of important factors that should be considered in breeding chicken in order to produce good quality of eggs. High temperature in Indonesia may cause flocks to experience heat stress leading to the formation of free radical, productivity and metabolism disorder thereby affecting the quality and quantity of eggs (Gu *et al*., 2008; Mujahid *et al*., 2007; Sugito, 2009). Efforts have been done to overcome those problems such as using antioxidant. Recently, mineral and vitamin are commonly used as antioxidants.

Vitamin E (VE) is the major antioxidant component in biological system which plays an important role in metabolism process, protecting cellular structure and maintaining membrane stability from free radical. A number of researches reported that VE is able to fix and increase performance (Ajakaiye *et al*., 2011; Ciftci *et al*., 2005), egg production (Bolukbasi *et al*., 2007; Ciftci *et al*., 2005; Irandoust *et al*., 2012), quality of eggs (Ciftci *et al*., 2005; Jiang *et al*., 2013), immune system (Asli *et al*., 2007; Da Silva *et al*., 2009; Iqbal *et al*., 2015), and decrease malondialdehyde (Brenes *et al*., 2008; Goni *et al*., 2007; Jiang *et al*., 2013; Voljc *et al*., 2011) during heat stress.

Mangosteen pericarp is a part of mangosteen fruit which has long been known as a medicine for diarrhea, skin infections as well as for coloring foodstuffs and textiles. The main chemical compound of mangosteen pericarp is xanthone and its derivative (Jung *et al*., 2006; Kondo *et al*., 2009; Suksamrarn *et al*., 2006), anthocyanin (Palapol *et al*., 2009), saponin and tannin. Some researchers reported that mangosteen compound had an antioxidant (Jung *et al*., 2006; Kondo *et al*., 2009; Suvarnakuta *et al*., 2011), anti-allergic (Chae *et al*., 2012), anti-cancer (Mizushina *et al*., 2013), anti-tumor, anti-bacterial and anti-malaria properties (Akao *et al*., 2008; Gutierrez-orozco & Failla. 2013; Pedraza-Chaveri *et al*., 2008). Accordingly, a study on the supplementation of the laying hens diets with mangosteen pericarp meal (MPM) and VE was carried out to determine their effects on egg physical quality, egg cholesterol, blood cholesterol, triglyceride and high-density lipoprotein.

MATERIALS AND METHODS

Design, Animal, and Diet

This research used 160 laying hens (Lohman strain, Japfacomfeed, Indonesia), aged 24-weeks old. This research used four feeding treatments: R0 (control diet), R1 (R0 + 1 g MPM/kg ration as fed), R2 (R0 + 2 g MPM/kg ration as fed), R3 (R0 + 200 mg VE/kg ration as fed) with four replications. Each replication used 10 chickens (2 hens/cage, the dimensions for each cage were 35 x 36 x 42 cm). Drinking water was provided *ad-libitum* and feeding was given twice a day i.e in the morning (07.00 am) and afternoon (05.00 pm). This research was carried out for 11 weeks. Cage was equipped with lamp for 16L/8D.

Mangosteen pericarp was obtained from smallholders' estate area in Leuwiliang, Bogor, West Java. MPM was processed by using drying oven during 5-6 h (LTE scientific Swallow, UK) at a temperature of 50 °C, then ground using a disk mill machine (Jiayu Electrical Machinery, Taiwan). VE used in this research was vitamin E, 40 mg/g α-tocopherol acetate (Interchemie, Netherlands).

Feed ingredients consisted of maize, soybean meal, fish meal, coconut oil, CaCO3, salt, DL-Methionine, and premix. Feed requirement of laying hens was defined by Leeson & Summers (2005) method with the metabolizable energy content of 2900 kcal/kg and 17% crude protein. The Ingredient and nutrient content used in this research are as shown in Table 1. All research procedures had been approved by the animal ethic committee, Bogor Agricultural University No.12-2014 IPB.

Table 1. Ingredient and nutrient contents of basal diets

Ingredients	Contents
Maize (%)	55.00
Soybean meal (%)	24.00
Fish meal (%)	8.50
Coconut oil (%)	3.00
$CaCO_3$ (%)	8.50
Salt (%)	0.20
Dl-Methionine (%)	0.30
Premix[a] (%)	0.50
Nutrient content [b]:	
Metabolizable energy (kcal/kg)	2900.75
Crude protein (%)	17.19
Crude fiber (%)	2.18
Crude fat (%)	5.32
Ca (%)	3.83
Available P (%)	0.49
Methionine (%)	0.73
Lysine (%)	1.33
Cystine (%)	0.34
Tryptophan (%)	0.27
Threonine (%)	0.95
Arginine (%)	1.42
Na (%)	0.15
Zinc (mg/kg)	38.55

Note: [a]Premix provided (in mg/kg premix)= vit A 500,000 IU; vit D 100,000 IU; vit E 150 mg; vit K 50 mg; vit B1 50 mg; vit B2 250 mg; vit B12 250 mcg; niacinamide 375 mg; Ca-d-panthotenate 125 mg; folic acid 25 mg; choline cloride 5,000 mg; Glysine 3,750 mg; Dl-methionine 5,000 mg; Mg sulphate 1,700 mg; Fe sulphate 1,250 mg; Mn sulphate 2,500 mg; Cu sulphate 25 mg; Zn sulphate 500 mg; K iodine 5 mg. [b] Calculated value.

Variables Observed

Physical quality of egg. Physical quality off egg was observed on day 21 after diet treatments. The observation was carried out for five consecutive weeks with eggs were taken randomly. Samples were taken once a week (3 eggs from each replication). Parameters consisted of egg weight (g) measured by using digital scale (Osuka-HWH®, Japan), percentage of egg shell (%), yolk (%), and the albumen (%). Shell thickness (mm) was measured by using digital caliper (150 Digital Caliper, Nankai®, Japan). Haugh unit (HU) were calculated from the records of egg weight and albumen height by using the formula: HU= 100 log10 (H - 1.7 W0.37+7.56), where H= height of the albumen (mm), and W= egg weight (g). Yolk color was determined by comparing the yolk color standard to Egg Roche Yolk Colour Fan (Ovo Color, Aktiengesellscharft BASF, Germany). Shell color was determined by comparing the shell color according to Brown Color Indicator (Max care, Trouw Nutrition International, Netherlands).

Yolk cholesterol. Yolk cholesterol was determined at the end of the study by using one egg for each replication which was taken randomly. Egg cholesterol was analyzed by using Liebermann Burchard method (Burke *et al.*, 1974), the absorbance was read by using spectrophotometer (Hitachi U-2001, Japan) at a wavelength of (λ) 420 nm.

Blood high-density lipoprotein, cholesterol and triglycerides. Blood sampling was carried out at the end of the study. Chickens were randomly selected from each treatment (one chicken per replication). Blood was taken as much as 3 ml at the vena jugularis which located in the neck by using a sterile syringe. Blood high-density lipoproteins (HDL) and cholesterol were analyzed by using CHOD-PAD (cholesterol oxidation-phenol-4-aminoantipyrine-peroxidase) method. Triglycerides (TG) level was analyzed by using glycerol-3-phosphate oxidase (GPO) colorimetic method which read at a wavelength (λ) of 500 nm using spectrophotometer (Hitachi U-2001, Japan).

Data Analysis

Data were then statistically analyzed by using Analysis of Variance (ANOVA) by means of Statistical Package for the Social Sciences (IBM®SPSS® version 21.0). Duncan Multiple Range Test (DMRT) was applied to determine the differences among treatments (Steel & Torrie, 1995). Differences were considered significant at $P<0.05$.

RESULTS AND DISCUSSION

Physical Quality of Egg

The physical quality of egg as resulted of MPM and VE supplementation on hen's diet is shown in Table 2. The results showed that supplementation of MPM 2 g/kg ration as fed and VE 200 g/kg ration as fed significantly reduced shell weight compared to the control and MPM supplementation of 1 g/kg ration as fed ($P<0.05$). However, supplementation of MPM and VE in the diet did not affect ($P>0.05$) the egg weight, percentage of egg shell, yolk, albumen, yolk color, shell color and haugh unit.

Shell thickness decreased in R3 treatment compared with R0 and R1 treatments. This is possibly due to the interaction of vitamin E with vitamin D. Excess of VE in the diet can reduce Ca and P in the blood and bone (Murphy *et al.*, 1981), absorption and utilization of vitamin A and D_3 (Leeson & Summers, 2001). In this case the vitamin D is dissolved in the unhydrolyzed and possibly nonsolubilized tocopheryl acetate and passes through the gut unabsorbed, consequently impaired bone and shell calcification. The reduced shell thickness did not affect the shell weight in this study.

Percentage of yolk ranged between 23.08%-23.88%, while the albumen ranged between 63.88%-65.01%. Percentage of yolk and albumen was influenced by the content of protein in the diet. Based on this research, protein content (17%) in the diet and feed intake (unpublished data) were not significantly different among treatments. This condition produced similar weight of yolk and albumen, relatively.

Table 2. Physical quality of eggs produced by laying hens suplemented with mangosteen pericarp meal (MPM) and vitamin E (VE)

Variables	Treatments			
	R0	R1	R2	R3
Egg weight (g)	53.91±1.68	53.79±0.77	52.33±1.27	53.69±1.11
Egg shell (%)	12.19±0.58	12.24±0.57	12.27±0.29	11.91±0.23
Yolk (%)	23.19±1.01	23.88±0.90	23.69±0.43	23.08±1.06
Albumin (%)	64.63±1.19	63.88±1.24	64.05±0.23	65.01±1.08
Shell thickness (mm)	0.33±0.01^{a}	0.33±0.00^{a}	0.32±0.01^{ab}	0.31±0.01^{b}
Yolk color	8.05±0.37	8.13±0.36	8.27±0.20	8.55±0.45
Haugh unit	98.01±1.41	98.93±2.44	98.75±1.47	98.27±2.79
Shell color	8.52±0.04	8.19±0.31	8.23±0.47	8.16±0.72

Note: Means in the same row with different superscripts differ significantly ($P<0.05$). R0= control feed, R1= R0 + 1 g MPM/kg ration as fed, R2= R0 + 2 g MPM/kg ration as fed, R3= R0 + 200 mg VE/kg ration as fed.

Yolk color is influenced by the type of pigment contained in the diet. The application of MPM and VE in the diet (Table 2) did not increase yolk color score, althought MPM contained pigment which derived from secondary metabolites such as: anthocyanins (Palapol *et al*., 2009).

Shell color in this study was not affected by the supplementation of MPM and VE. The main factor that affect shell color is biliverdin pigment (Zhao *et al*., 2006; Wang *et al*., 2009), phorpyrin (Wang *et al*., 2009), genetic (Zhang *et al*., 2005), stress level, age and disease (Aygun, 2014). Brown shell color is influenced by phorpyrin pigment which composed of protophorpyrin, coproporphyrin, pentacarboxylic porphyrin, urophorpyrin and some types of unidentified phorpyrin (Wang *et al*., 2007). Protophorpyrin pigment is produced during the hemoglobin (Hb) process (Kennedy & Vevers, 1973). This pigment is transported from the liver to the uterus through the blood. Based on Hb values (unpublished data), there was a correlation between Hb and shell color. The increasing of Hb increased shell color and vice versa.

Haugh units (HU) of egg found in this study was not influenced by the supplementation of MPM and VE. This finding is in accordance with the finding of Irandoust *et al.* (2012) that the addition of VE 348 IU/ kg does not affect the HU value. HU values in this study ranged between 98.01-98.93 and these values were classified into AA quality i.e. > 72 (Unites State Department of Agriculture, 2000).

Yolk Cholesterol

Yolk cholesterol as resulted of the supplementation of MPM and VE in the diet is shown in Table 3. The result showed that the supplementation of 1 g MPM/ kg ration as fed significantly increased egg cholesterol ($P<0.05$). The average value of yolk cholesterol was R0 (4.91 mg/g), R1 (6.15 mg/g), R2 (5.41 mg/g) and R3 (5.24 mg/g). Supplementation of 1 g MPM/kg ration as fed increased the egg cholesterol as compared to control, supplementation of 2 g MPM/kg ration as fed and 200 mg VE/kg ration as fed. This is due to the ability of MPM as an antioxidant that can protect lipid (TG, phospholipid and cholesterol). It also can protect protein which role as a precursor for yolk formation that it used for the development of embryo by inhibiting free radical resulted from high temperature that can damage tissue.

Yolk precursor (lipid and lipoprotein) is synthesized in the liver supported by estrogen hormone and then circulated to ovaries by blood for follicle maturation and formation. Cholesterol is classified into lipid which is required by a hen as a source of feed material for small cell (blastoderm) and then used by embryo during hatching and to support growth after hatching (McGraw, 2006).

Cholesterol content of yolk significantly affected the lipid composition of embryonic tissue. Avian embryonic tissue contains unsaturated fatty acids in the fat fraction, thus it requires an effective antioxidant protection. Antioxidant defense tissue of newly hatched birds are composed of fat-soluble antioxidants (vitamin E, carotenoids), water soluble (ascorbic acid and glutathione), and antioxidant enzyme (SOD, GSH-Px and catalase) (Surai, 2003).

Blood Profiles

The effect of ration supplemented with MPM and VE on cholesterol, TG, and HDL is presented in Table 3. The result showed that MPM supplementation in the diet significantly reduced TG level ($P<0.05$). However, MPM and VE supplementation did not affect ($P>0.05$) cholesterol and HDL concentrations in the blood.

TG ranged between 522.63-753.9 mg/dl. MPM supplementation in the treatment of R1 and R2 showed lower TG levels compared with R0 and R3 treatment. Low level of TG was due to the influence of antioxidant which contained in MPM diet that inhibited free radical that could interfere TG hydrolysis. Antioxidant contained in MPM is not only α-mangosteen (40 ppm), but also there are saponin (8.24 g/100g) and tannin (32.49 g/100g). Saponin (0.08 g) and tannin (0.32 g) can reduce TG. In this case tannin can increase the activity of lipoprotein lipase (LPL) enzyme (Kothari *et al*., 2011). The increasing activity of LPL enzyme will break down TG into glycerol and fatty acids and will be released into the blood vessels to be transferred to the destined cells (Piliang & Djojosoebagio, 2006). The tolerance limit of tannins in chicken feed is 2.6 g/kg (Kumar *et al*., 2005) and saponins at 4.5 g/kg ration (Abbas, 2013).

In R3 treatment, supplementation of vitamin E in the diet was not able to lower TG level in the blood. R3 resulted higher TG level compared to R0 treatment. The increased TG was relatively followed by the increased VLDL and LDL. On the contrary, the increased of HDL was relatively followed by the decreased of VLDL and LDL, vice versa.

Table 3. Yolk cholesterol, blood cholesterol, triglyceride, and blood high-density lipoproteins (HDL) in laying hens suplemented with mangosteen pericarp meal (MPM) and vitamin E (VE)

Variables	Treatments			
	R0	R1	R2	R3
Yolk cholesterol (mg/g)	4.91± 0.15^{a}	6.15± 0.31^{b}	5.41± 0.44^{a}	5.24± 0.43^{a}
Blood cholesterol (mg/dl)	138.91±11.24	106.00±17.89	115.82± 27.87	118.73±20.25
Triglyceride (mg/dl)	730.45±66.81^{a}	522.63±67.71^{b}	612.65±118.60^{ab}	753.09±84.14^{a}
HDL (mg/dl)	14.89± 1.48	15.75± 1.95	14.00± 1.39	17.58±3.02

Note: Means in the same row with different superscripts differ significantly ($P<0.05$). R0= control feed, R1= R0 + 1 g MPM/kg ration as fed, R2= R0 + 2 g MPM/kg ration as fed, R3= R0 + 200 mg VE/kg ration as fed.

Table 3 showed that there was a decrease trend in blood cholesterol of hen suplemented with MPM and VE. It is due to MPM and VE act as an antioxidant which can protect lipids from free radicals. Therefore, cholesterol that has been synthesized in the liver during production phase, then carried by the blood in the form of lipoproteins and stored in follicle growing and forwarded to the ovary. MPM absorption in the body is associated with fat digestion in the small intestine (Guiterez-orozco & Failla, 2013) as well as VE (Surai, 2003).

MPM and VE supplementation in the ration did not affect on HDL (Table 3). In this case, MPM and VE can not increase the synthesis and secretion of HDL in the liver and intestine. The functions of HDL, i.e.: (1) transport LDL cholesterol from peripheral tissues to the liver (reverse cholesterol transport) to be converted in the form of bile salts (Hardinia *et al.*, 2007), (2) storage area for apolipoprotein C and E which are needed in the chylomicrons and VLDL catabolism. Apolipoprotein C is a cofactor of lipase lipoprotein and apoliprotein E is a ligand for the LDL receptor (Adiputro *et al.*, 2013).

CONCLUSION

Supplementation of 1 g MPM/kg as fed in laying hens diet could reduce TG concentration in the blood. The supplementation of MPM and VE did not affect the egg quality (except shell thickness), blood cholesterol, and HDL.

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