The Nutritional Content of Egg and Blood’s Lipid Profile of Layer Fed by Kepok Banana Meal (Musa paradisiaca L.) as Feed Supplement

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The Nutritional Content of Egg and Blood's Lipid Profile of Layer Fed by Kepok Banana Meal (*Musa paradisiaca L.*) as Feed Supplement

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Abstract. This study was aimed to evaluate the nutritional of content of egg and the lipid profile of layers by feeding of *kepok* Banana flour (*Musa paradisiaca formantypica*) as feed supplement. A total of 200 laying 58-week-old hens were used in the study, with 5 treatments and 5 replications, each of which was filled with 8 chickens. The treatments were applied in a completely randomized design, continued with Duncan's test. The treatments given by *kepok* banana flour were 0%, 5%, 10%, 15% and 20% substitution of corn flour. This research was conducted for 8 weeks. The parameters measure included egg crude protein (%), egg crude fat (%), egg white crude protein (%), egg yellow fat (%), egg cholesterol (mg/100 g), blood cholesterol (mg/dl), blood LDL cholesterol (mg/dl), and blood HDL cholesterol (mg/dl) of the laying hens. The results showed that the utilization of *kepok* banana flour up to 20% substitution of yellow corn had a highly significant effect on egg protein content, egg white protein, egg fat content, egg cholesterol content, chicken blood cholesterol, chicken blood HDL, chicken blood LDL and triglycerides of chicken blood. It was concluded that *kepok* banana flour substitution of up to 20% as a feed source can increase the nutritional content of chicken eggs and the fat profile of laying hens.

Keywords: *kepok* banana flour, nutritional content of eggs, lipid profile, layer

Abstrak. Penelitian ini bertujuan untuk mengetahui kandungan nutrisi telur dan profil lemak ayam petelur dengan pemberian pisang kepok *(Musa Paradisiaca formantypica)* sebagai supplement pakan. Sebanyak 200 ekor ayam petelur yang digunakan dalam penelitian, dengan 5 perlakuan dan 5 ulangan setiap ulangan diisi oleh 8 ekor ayam. Penelitian ini menggunakan rancangan acak lengkap, dan uji lanjutkan uji Duncan's test. Perlakuan yang diberikan tepung pisang kepok 0 %, 5%,10 %,15 % dan 20% menggantikan tepung jagung. Penelitian ini dilaksanakan selama 8 minggu. Parameter yang dicatat meliputi protein kasar telur (%), lemak kasar telur (%), protein kasar putih telur (%), lemak kuning telur (%), kolesterol telur (mg/100g), kolesterol darah (mg/dl), darah kolesterol LDL (mg/dl), dan kolesterol HDL darah (mg/dl) ayam petelur. Hasil penelitian menunjukkan bahwa penggunaan tepung pisang kepok sampai 20% menggantikan tepung jagung dapat memberikan pengaruh yang sangat nyata terhadap kandungan protein telur, protein putih telur, kandungan lemak telur, kandungan kolesterol telur, kolesterol darah ayam, HDL darah ayam,LDL darah ayam dan trigliserida darah ayam. Simpulan tepung pisang kepok sampai 20 % sebagai sumber dapat meningkatkan kandungan nutrisi telur ayam dan profil lemak ayam petelur.

Kata Kunci: tepung pisang kepok, kandungan nutrisi telur, profil lemak, ayam petelur

Introduction

Food security is important as source of protein, energy and essential macro nutrients for the human population. Eggs are a source of animal protein consumed globally in increasing food security (Sharma et al., 2020). Public awareness regarding healthy living has prompted people to choose well-nourished foods. Eggs are an unprocessed food with high nutritional value and one of the highest nutrient concentration indexes. However, eggs vary widely in quality, depending on various internal and external factors that affect the acceptance or rejection of these foods by consumers. Eggs are animal food products that consist of the required nutritional needs for humans. The nutritional content of egg yolks is 17.07% protein, 32.21% fat and 2.03% minerals (Faitarone et al., 2013). Cholesterol content in poultry products is one of the most essential qualitative characteristics for consumers, and its concentration can be easily modified through feed formulation (Dhama et al., 2015). This situation calls for a concerted effort on finding other alternatives with lower costs for feed ingredients. Using cheaper and easily accessible feed ingredients in poultry feed production can reduce production costs (Ghayas et al., 2017). In North Sulawesi, *kepok* banana plants are easier to find than corn. In Indonesia, bananas are ranked first among other fruits with regards to distribution, planting area, and production. Bananas are rich in dietary fiber, protein, essential amino acids, cellulose, hemicellulose, lignin, starch, resistant starch, polyunsaturated fatty acids and potassium (Rodríguez-Ambriz et al., 2008; Sarawong et al., 2014). Currently, industrial flour production from green bananas is in great demand because of its nutritional value, especially high resistant starch between 40.9-58.5% and food fibre 6.0-15.5% (Tribess et al., 2009). Research on bananas produced as banana flour is used up to 54.08% level in laying hens feed, through cutting bananas into pieces, dried in an oven for two days, then ground and converted into flour (Koni et al., 2020).

Research on the effect of providing *kepok* bananas in the ration has been conducted in response to immunoglobulins, vitamins (A, B1, B6, D2 dan D3) and egg cholesterol. The level of banana *kepok* used is 5 %, 10 %, 15 % dan 20 % replacing corn flour. The results of the study showed the increased of IgY, vitamins (A, B1, D2 and D3) and egg cholesterol but gave the same results to vitamin B6 (Leke et al., 2022). Based on above description, the purpose of this research was to analyze the nutritional content and lipid profile of layer egg fed by *kepok* banana *(Musa Paradisiaca* L.*)* as feed supplement.

Materials and Methods

Samples Preparation

Kepok bananas were obtained from Tongkaina village, Bunaken district, Manado city. The *kepok* bananas parts that were used as treatment materials are the skin and inner flesh of the *kepok* bananas, with dark green outer skin and black spots. These were thinly sliced and dried in the sun for 3-4 days. The *kepok* bananas were then dried in the sun and milled until they become flour. Banana *kepok* flour was analyzed proximate at the Center for Food and Nutrition Studies, PAU of Gadjah Mada University. The results of the analysis were 89.51 % dry matter content, 23.80 % ash, 4.15 % crude protein, 3.77 % crude fat, 5.11 % crude fiber and gross energy of 4259.43 Kcal/kg.

The materials that were used in this study included 200 laying 58-week-old hens. The animals were housed in a battery cage, and each cage accommodated 8 chickens. The feed ingredients used in this study were 41 % corn, 15 % rice bran, 2 % $CaCO_3$, 42% concentrate (CLK) and banana *kepok* flour. The research method used was a completely randomized design with 5 treatments and 5 replications, and each replication contained 8 laying hens. Feeding was given with an interval of 2 weeks of adaptation and 8 weeks of treatment feeding. Feed was given twice in the morning and in the afternoon. The water was provided ad libitum. Results of the calculated composition and nutrient are, then presented in Table 1.

The variables determined in this study were egg cholesterol levels measured by the Lieberman-Burchard method. Liebermann Burchard's method, known as the acetic anhydride test, was applied to detect cholesterol. Sampling was done in 80 weeks old chickens, where 25 chickens from the sample in each unit were individually weighed with a digital scale to an accuracy of 1 gram. After 6 hours of fasting, blood samples were collected by puncturing the ulnar vein using a sterile syringe and needle.

Parameters: Quantitative Analysis of Egg Protein, Egg Fat using the Kjeldahl method (Magomya et al., 2014; Villarreal et al., 2004).

Table. 1. Chemical composition of the diets

	R0	R1	R2	R3	R4
Crude protein (%)	17.94	17.71	17.48	17.24	17.01
Crude fat (%)	7.91	7.90	7.89	7.89	7.88
Crude fiber (%)	5.64	5.80	5.95	6.11	6.26
Calcium	1.88	1.89	1.91	1.93	1.94
Phosphor	1.04	1.03	1.02	1.01	1.01
Metabolisme energy (Kcal/kg)	2844	2825.55	2807.10	2788.65	2770.20
Beta-carotene (ppm)	0.36	0.48	0.44	0.41	0.41

ME = 70 %x GE

A total of 3 ml aliquots of blood were transferred into sterile tubes without anticoagulant and sent to Medistar Clinical Laboratory for blood cholesterol, blood HDL and Blood LDL. The blood cholesterol of laying hens consisted of total cholesterol, high density lipoprotein (HDL) and low density lipoprotein (LDL). A total of 2 ml of chicken blood samples were taken, put into a blood tube containing ethylene-diamine-tetraacetic acid (EDTA) to avoid blood clots, then stored in an ice flask for analysis in the laboratory. The procedure for determining complete cholesterol levels in chicken blood uses a photometer with the cholesterol oxidase-peroxidase amino antipyrine phenol (CHOD-PAP) method with a wavelength of 546 and a temperature of 37°C.

Data Analysis

The completely randomized design allocated data was analyzed for ANOVA; further analysis was conducted with Duncan's multiple distance test if there was a highly significant difference (Steel and Torrie, 1995).

Results and Discussion

The results of feeding 20% *kepok* banana flour as a substitute for corn on the egg components are shown in Table 2. The average egg protein was 15.36 – 18.40. According to *Standar Nasional Indonesia* (SNI 3926: 2008) the standard weight of eggs is 65 grams with egg protein levels range of 8.10 -13.40 %. According to Ratih and Qamariyah (2017), ripe *kapok* bananas contain 25 % of pyridoxine compounds. These compounds play very important role in amino acid metabolism so that they can increase the absorption of amino acids in the small intestine which can be used for various purpose in the body (Liberman and Burning, 2007).

The protein content of feed was 17 % which was arranged based on the need of laying hens to increase of growth and immunity of laying hens (Farag and Dhame, 2016). Protein is the main component and important nutrient in chicken feed (Beski et al., 2015). The protein in the digestive tract is broken down and hydrolyzed to produce amino acids providing the role of blood plasma proteins, enzymes, nucleotides and antibodies and it promotes growth (Abbasi et al., 2014; Van Emous et al., 2015).

Eggs as a whole are considered a good source of protein and lipids. Still, egg whites mainly consist of water (88%) and protein (11%) and lack fat. Ovomucin is a highly glycosylated protein. About 33% of ovomucin consists of carbohydrates (Omana et al., 2010). Therefore, ovomucin is considered a good source of both proteins and carbohydrates. Ovalbumin is the main protein of egg whites, and it has a balanced composition of amino acids and thus can be used as an excellent source of protein for many food types. Other egg white proteins are also considered a good source of essential amino acids. Pharmaceutical uses egg white protein ovotransferrin, which can bind with iron and easily release the bound iron at pH < 4.5 (Ko et al., 2008).

Table 2. Effects of *kepok* banana flour on nutritional content of eggs and lipid profile of layers

	Treatments				
	R0	R1	R2	R3	R4
Egg protein (%)	$15.36^{a}\pm0.92$	$15.22^{a}\pm0.91$	$16.83^{b}\pm0.71$	$17.00^{d}\pm1.15$	$18.40^{e}\pm0.42$
Egg lipid (%)	$28.23^{e}\pm2.19$	$27.76^{d}\pm0.50$	$26.33^{c}\pm0.64$	$24.98^{b}\pm0.67$	$25.08^{a}\pm0.71$
Egg cholesterol (mg/100g)	$218.6^{a}\pm1.95$	$216.2^{b}\pm6.42$	$205.6^{d}\pm2.88$	$207.2^{c}\pm2.17$	$203.4^{e}\pm2.19$
Blood cholesterol (mg/dl)	$109.5^{a}\pm5.00$	$98.15^{b}\pm1.74$	$89.8^{d}\pm11.04$	$88.22^{d}\pm5.84$	$90.45^{c}\pm2.76$
HDL (mg/dl)	$33.04^{a}\pm5.93$	$33.98^{a}\pm4.59$	$24.69^{c}\pm1.65$	$27.24^{b}\pm5.00$	$23.99^{d}\pm1.07$
LDL (mg/dl)	$70.1^{a}\pm10.32$	$46.68^{d}\pm3.19$	$53.08^{C}\pm4.07$	$60.72^{b}\pm11.46$	$44.50^{d}\pm0.99$
Triglyceride(mg/dl)	$63.66^{d}\pm3.67$	$57.72^{b}\pm8.12$	$60.12^{c}\pm1.60$	$64.28^{d}\pm2.41$	$49.50^{a}\pm4.67$

Notes: Values indicated with a different superscript are significantly different

Ovotransferrin undergoes thiol-associated automatic cleavage after reduction and produces a partially hydrolyzed product with a very strong anticancer effect on colon and breast cancer cells (Ibrahim and Kiyono, 2009).

The current study showed that average egg fat was 25.08 - 28.23%. The average egg fat content was reported at 25.19 – 27.88% (Purnayasa et al., 2018). This shows that similar results are obtained from feeding *kepok* bananas up to 20% and feeding corn flour. The most common fatty acids found in chicken egg yolks are palmitic, oleic, and linoleic (Rehault-Godbert et al., 2019; Suci et al., 2017). Duncan's distance test analysis showed that crude fat with 20% *kepok* banana flour as a substitute for corn had a very high significant effect (P < 0.01).

The yolk lipid profile can be influenced by the genetics and age of the chicks and modification of the composition of the feed given to the chickens (Franczyk-zarow et al., 2019; Orczeeska_Dudek et al., 2020). Older chickens have higher fat content. Changes in the accumulation of abdominal fat, is strongly influenced by the multiplication of fat cells and volume enlargement. Changes in fatty acid synthesis and fat oxidation, especially occurs in the liver. Fatty acids in liver are the main factors that cause changes in the synthesis of triglycerides in the liver. This change in triglyceride synthesis will affect both the concentration of triglycerides in the serum (Leenstra 1982; Leenstra 1986). The regulation effectively lowers blood cholesterol levels and lipid metabolism (Bayliak et al., 2021; Guo et al., 2017; Tekwe et al., 2019).

Fatty Acids (FA) are mainly metabolized in the liver from acetyl coenzyme A (acetyl-CoA). Alpha-ketoglutarate (RDA; 2-oxoglutaric acid, 2-Ox) is a glutamate and glutamine derivative, a precursor to arginine and other active compounds important in regulating protein metabolism in skeletal muscle (Zdzisinka et al., 2017). RDA is an intermediate in the pathway of FA synthesis from acetyl-CoA. When RDA is added as a supplement, a file increase is observed in the reaction between alanine and RDA, which produces glutamate and pyruvate; the molecules required for the formation of acetyl-CoA, from which FA is synthesized. The synthesized FA is transported to the ovary by the bloodstream like any other lipid and stored in the egg yolk (Scanes, 2014).

The results of the nutritional content of *kepok* banana flour treatment on the beta-carotene content of feed ingredients were 0.36 -0.41 ppm. This shows that beta-carotene in feed can reduce blood cholesterol content. Beta-carotene can also function as an antioxidant, so it can prevent the oxidation of unsaturated fatty acids and produce a good fatty acid composition product (Dorisandi et al., 2017).

Palmitic fatty acid undergoes beta-oxidation, the process of breaking down fatty acids and converting them into energy. The palmitic fatty acid is broken down repeatedly for seven times, producing 8 molecules of acetyl CoA, a molecule required for cholesterol synthesis. The mechanism for lowering cholesterol is quite difficult because poultry will continue to synthesize cholesterol in their bodies to meet their needs. Consumption of beta-carotene ration of 7.26 mg per day per head has not been able to inhibit the action of the enzyme HMG-CoA (hydroxymethyl glutaryl-CoA) reductase which plays an important role in the formation of mevalonate in cholesterol biosynthesis. The inhibited formation of mevalonate inhibits the formation of squalene and lanosterol. These two molecules are the molecules that undergo a series of reactions to form cholesterol. This causes the increased content of beta-carotene and crude fiber in the ration, which has no impact on decreasing the cholesterol content of egg yolks. As a result, the yolk cholesterol yield in this study was lower than 0.939 g/100 g (Rehaut-Godbert et al., 2019). In addition, there are saponins in banana peels that can increase cholesterol excretion, but in this study, there was no effect of saponins on reducing egg yolk cholesterol (Anhwange, 2008).

The average egg cholesterol is 203,4 -218.6 mg/100g. A previous study reported cholesterol levels of 198-212 mg/100 g by giving papaya skin flour in laying hens rations (Leke et al., 2020). Duncan's test analysis showed that the substitution of corn with 20% *kepok* banana flour resulted in a highly significant difference ($P > 0.001$) in egg cholesterol. These results indicate that beta-carotene contained in banana flour can reduce chicken egg cholesterol. The ability of beta-carotene to lower cholesterol is related to the hydroxymethyl glutaryl-CoA enzyme. This enzyme plays a role in the formation of mevalonic in cholesterol biosynthesis. Synthesis of cholesterol and beta-carotene together through mevalonic and derived from acetyl CoA, beta-carotene consumption is better than saturated fatty acids. The biosynthesis process through the HMG-CoA enzyme will be directed at synthesizing beta-carotene to convert saturated fatty acids into cholesterol. High consumption of beta-carotene can reduce cholesterol levels in egg yolks because beta-carotene can inhibit the enzyme HMG-CoA reductase (Hydroksi methyl glutaryl-CoA), which plays a role in the formation of mevalonic. Mevalonic is needed in the cholesterol synthesis process by inhibiting enzymes, thereby inhibiting the formation of cholesterol (Wang and Keasling, 2002).

Chicken can produce 10 times more cholesterol per kg of liver than humans (Bertechini, 2003). The concentration of yolk cholesterol is very resistant to change because egg yolk cholesterol is required for embryo development. However, hens can substitute egg yolks for polyunsaturated fatty acids in response to dietary fat sources. This substitution is a result of the ability of birds, in contrast to mammals, to absorb dietary fat through the portal system as portomicrons, which are directly absorbed into the blood and transported to the liver, the main site of lipogenesis, thereby allowing direct fat absorption by the liver (Van Elswyk et al., 1994).

The current study results showed average blood cholesterol of 88.22 – 109.5 mg/dl, the average blood HDL of 23.99 – 33.04 mg/dl, and the average blood LDL of 44.50 – 70.1 mg/dl. A previous study reported that blood cholesterol is 98.76 – 112.40, blood HDL is 112.7 -120.2 mg/dl, blood LDL is 102.5 – 109.3 mg/dl (Leke et al., 2019). The normal blood cholesterol of laying hens is 52-148 mg/dl (Basmacio et al., 2005). The effect of probiotics on laying hens resulted in the average blood cholesterol of 116 – 137 mg/dl, blood HDL of 35.80 – 59.40 mg/dl, and LDL of 39.20 -100.20 mg/dl (Sumardi et al., 2016).

The results of Duncan's test analysis showed that feeding *kepok* bananas up to 20% could have a highly significant effect ($P > 0.01$) on blood cholesterol, blood HDL, blood LDL. These results indicate a decrease in blood cholesterol to the level of 20% and can emphasize the occurrence of a reduction in LDL, which is bad cholesterol and can maintain good HDL cholesterol. High HDL levels prevent the risk of atherosclerosis by transporting cholesterol from peripheral tissues to the liver and reducing excessive cholesterol (Harini and Astirin, 2009). HDL is a lipoprotein that transports lipids from the periphery to the liver and reduces the excessive accumulation of cholesterol (Murray et al., 2003). *Kepok* banana flour contains pectin compounds which allow it to lower blood cholesterol levels and lower LDL cholesterol. The ability of pectin in lowering cholesterol in the blood of rats is due to the effect of pectin on binding bile acids in the small intestine (Abidah et al., 2014). Bile acids bound by pectin will not form micelles with fat so that they are wasted into the large intestine and are wasted with feces.

Conclusions

Kepok banana flour can be used as feed for layers. The usage of *kepok* banana flour up to 20% can increase the nutrition value of eggs. Egg protein can increase and can reduce level of egg fat, egg cholesterol, blood cholesterol, blood HDL, blood LDL and blood triglycerides. It can be recommended that substituting *kepok* banana flour in layer chicken feed up to 20% can increase the nutrition and blood profile of layer.

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