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The Effect of Kepok Banana (*Musa Paradisiaca* L.) on Immunoglobulin, Vitamins, and Cholesterol Content of Eggs in Laying Hens

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ABSTRACT

Eggs contain all the proteins, lipids, vitamins, minerals, and growth factors necessary for the development of the embryo. Egg white and yolk proteins are considered functional food substances since they have biological activities, such as metal-chelating, antimicrobial, anticancer, antioxidant, and immunomodulatory activities. The current study aimed to determine the effect of banana kepok on the production levels of immunoglobulin Y (IgY), vitamins, and cholesterol of eggs in laying hens. A total of 200 laying hens (medium brown 402) were used at 80 weeks of age with 5 treatments and 5 replications and each entailed 8 chickens. The treatment groups included the use of kepok banana flour (KBF) as R0 (no KBF), R1 (95 Basal feed + 5% KBF), R2 (90 basal feed + 10% KBF), R3 (85 basal feed + 15% KBF), and R4 (80 basal feed + 20% KBF). A total of 50 eggs were used in egg yolk sampling. The investigated variables were egg IgY, vitamins (A, B1, B6, D2, D3), and cholesterol content. The results of the study indicated that the administration of kepok bananas at different levels could provide a significant difference in IgY, vitamins (A, B1, D2, D3), and cholesterol of eggs. However, it did not significantly affect Vitamin B6. The study concluded that KBF can positively affect IgY and vitamins in eggs. Moreover, it could decrease the cholesterol in eggs.

Keywords: Egg cholesterol, Egg IgY, Egg vitamins, Kepok banana flour

INTRODUCTION

Egg production is an integral part of the livestock industry and has increased by 119% (35.5 vs 76.8 million tons) from 2018 to 2019 (FAO, 2020). Global egg production is going to increase by 24% from 7.8 tons in 2020 to 9.7 tons in 2050 (Macelline et al., 2021). Eggs are one of the primary nutrient sources comprising of protein, vitamins A, D, E, and B12, and amino acids. Chicken meat and eggs are rich sources of nutrients, including proteins, vitamins meaning that it includes all nutrients (Leke et al., 2019). Eggs are also a source of macro and micronutrients that meet all the requirements to support food needs.

Complete and balanced nutrition as well as high digestibility and affordable price are contributing factors to eggs being a staple of human beings (Réhault-Godbert et al., 2019). Eggs are one of the most significant antibody producers with low production costs. Immunoglobulin Y (IgY) is an important therapeutic source of resistance to antibodies against several viral diseases for which there is no treatment (Pereira et al., 2019). Egg quality is closely related to the feed and age of laying hens (Leke et al., 2019). Passive antibody production is common in chicken egg yolk.

In laying hens, IgG in the blood is efficiently transferred across the follicular epithelium for storage in the yolk during oogenesis. The IgG is the dominant class of Ig transferred to the yolk called IgY (Pereira et al., 2019). Egg yolk is a relevant alternative source of antibodies. The main immunoglobulin in poultry blood is hereditary and accumulates in the egg yolk, allowing large amounts of antibodies to be uptake. Immunoglobulins are formed in the blood due to the transfer of antigens into the yolk and are called IgY, which has two heavy (H) and two light (L) chains.

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Vitamins are essential nutrients in specific foods and are very important for health because a lack of vitamins can cause disease. Vitamin A deficiency can cause blindness, especially in children, and miscarriage in women. Folate deficiency causes an increase in plasma homocysteine concentrations associated with plasma homocysteine concentrations and an increase in cardiovascular disease (Zang et al., 2011). Antioxidants are widely used for poultry production, especially in the form of vitamins (Poungpong et al., 2019; Sharma et al., 2020).

Low cholesterol and high levels of vitamins and IgY in egg production can be achieved by formulating feeds with a high level of beta-carotene and antioxidants. One source of beta-carotene and antioxidants is banana kepok (*Musa paradisiaca*), which is a type of processed banana. It also contains pyridoxine compounds or Vitamin B6 and can be used as an alternative to the main ingredient due to high levels of carbohydrates and vitamins. Bananas are rich in B6 (pyridoxine) content, which acts as a coenzyme for metabolism reactions as well as a facilitator of protein synthesis and metabolism. Several studies have confirmed that kepok bananas in the form of juice effectively reduce LDL cholesterol, total cholesterol, and blood serum triglycerides, and increase blood serum HDL cholesterol. The performance of laying hens and egg quality is influenced by genetics, environmental factors, and nutrition. Feed ingredients are required to contain Vitamin D3 and Vitamin A as a source of beta-carotene as well as minerals, calcium, phosphorus, sodium, and chlorine (Çalişlar, 2020). Beta-carotene source effectively increases egg yolk, eggshell, laying hens immunity, and the chicken endocrine system. Production can be improved in case the nutritional quality of feed ingredients is high (Ilona et al., 2020).

The purpose of the study was to determine the effect of laying hens' diet supplemented by pyridoxine compounds from kepok banana on IgY, vitamins, and cholesterol of eggs.

MATERIALS AND METHODS

Materials

Prydoxine compound was obtained from dried kepok bananas. The dried kepok bananas were processed from fresh banana kepok ingredients obtained from Bunaken Island, Tongkaina Village, North Sulawesi, Indonesia. Fresh kepok bananas were cut into small pieces and dried in the sun for 3-4 days. Proximate analysis of kepok bananas was carried out in the PAU laboratory of the Gadjah Mada University Faculty of Animal Husbandry, Indonesia. The results of the analysis indicated that dry matter content, ash, crude protein, crude fat, crude fiber were 89.51%, 23.80%, 4.15%, 3.77%, 5.11%, respectively. The metabolism energy was estimated as 3407.54 MJ/Kg.

Treatment diet

The kepok banana flour (KBF) was used as R0 (no KBF), R1 (95 Basal feed + 5% KBF), R2 (90 basal feed + 10% KBF), R3 (85 basal feed + 15% KBF), and R4 (80 basal feed + 20% KBF). The diets were mixed with 41% corn, 15% rice bran flour, 2% Ca CO3, and 42% layer concentrate. The diets were given for 8 weeks, including 100 g of feed per day. Drinking water was given *ad libitum* and the chickens were fed in the morning (8 am) and afternoon (2 pm). The environmental factors included a 110 cm × 35 cm × 56 cm cage size at the temperature of 32-35°C and a humidity of 60-70%. The Newcastle disease vaccine was given at 3 days of age and repeated at 2 weeks of age. The chemical composition of the feed is given in Table 1.

Table 1. Nutritional content of treatment diet

Nutritional content \ 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
Phosphorus	1.04	1.03	1.02	1.01	1.01
Metabolism energy (Kcal/kg)	2844	2825.55	2807.10	2788.65	2770.20
Vitamin A (mg/100 g)*	69.69	118.93	104.71	98.43	87.39
Vitamin C (mg/100 g)*	1.2	1.85	1.73	1.38	1.71
Vitamin B6 pyridoxine (mg/100 g)*	0.47	0.37	0.40	0.41	0.43
Beta-carotene (ppm)	0.36	0.48	0.44	0.41	0.41

R0: No kepok banana flour (KBF), R1: 95 Basal feed + 5% KBF, R2: 90 basal feed + 10% KBF, R3: 85 basal feed + 15% KBF, R4: 80 basal feed + 20% KBF

A total of 200 medium brown 402 were used in the current experiment. The research design used 5 treatments and 5 replications and each replicate consisted of 8 laying hens. A total of 50 eggs were sampled for examination of IgY, vitamins, and cholesterol in eggs.

Determination of immunoglobulin concentration in egg yolk

A total of 50 eggs were analyzed for IgY content at the Integrated Laboratory, Department of Nutrition Science and Feed Technology, Bogor Agricultural University, Indonesia. The ELISA method was used to isolate IgY in eggs and determine the concentration of IgY in egg yolk (Selvaraj and Cherian, 2004). As much as 150-200 mg of egg yolks were diluted 1:6 (v/v) with acidified deionized water (pH 2.5), vortexed well, and stored at 4°C. After overnight cooling, the samples were centrifuged at 10.62 g at 4°C for 15 minutes and the supernatant was collected and the egg IgY content was measured by the Elisa method.

Egg vitamin content analysis

Egg yolk vitamin analysis determined the vitamins in egg yolk using the high-performance liquid chromatography method (Staffas and Nyman, 2003). Five samples were used for the analysis in the current study (each treatment was used as a composite of four replications). The analytical procedure was as follows, the sample weighed 0.5 g, then put into a flask and 30 ml of 95% ethanol was added. The flask was shaken for having an even mixture, then heated for 30 minutes at 80°C using a water bath and reverse cooling. After the completion of heating, the condenser was rinsed with 20 ml of water. The sample was extracted with diethyl ether. The sample was then filtered using folded filter paper to remove the remaining water present. The solvent extract of the vitamin was evaporated using a rotary evaporator. The residue was dissolved with ethanol and then injected into the HPLC apparatus. The results of egg yolk vitamins were analyzed descriptively with the analysis of vitamins A, B1, B6, D2, D3 in the Integrated Laboratory of the Department of Nutrition Science and Feed Technology, Bogor Agricultural University, Indonesia.

Egg cholesterol analysis

Measurement of egg cholesterol was based on the Liebarmann Burchard method (Kenny, 1952). Egg samples obtained were analyzed at week 6 of the study. Each sample treatment had 2 replications so 50 samples were obtained. The cholesterol content of egg yolks was determined using the Liberman Burchard method using a spectrophotometer at a wavelength of 530 µm.

Statistical analysis

A completely randomized design was applied in this experiment. Data were analyzed by Analysis of variance (ANOVA) using the Minitab 16 software program. If there was a significant difference ($p \leq 0.05$), then Duncan's test was further used with multiple distance tests.

RESULTS AND DISCUSSION

Egg immunoglobulin

The average egg immunoglobulin was 19.24-23.26 mg/100 g/egg (Tabel 2). The kepok banana flour (KBF) led to a significant effect on increasing egg immunoglobulin ($p < 0.05$). The highest effect was in R4 treatment followed by R3, R1, and R2 treatments. These results were related to the previous research indicating that egg IgY level produced by chickens with active virus antigens was 10-100 mg IgY (Rollier et al., 2000). The IgY antibodies in an egg were 10-20 mg/ml egg yolk (Carlander, 2002). Chicken egg yolk can be used for the production of IgY in large quantities. The reason is that chickens have a high sensitivity to foreign antigen exposure and their immune system is also very responsive so that it persists to produce IgY (Zang et al., 2011). The high level of IgY in the blood is not always the same as the level of IgY in the egg yolk because the transfer of IgY into the yolk is known to occur in 2 stages. Each stage takes a certain amount of time. In the early stages, IgY is transferred from the serum to the yolk in a process analogous to the transfer of antibody (IgY) in the fetus across the placenta in mammals. The next stage is the transfer of antibodies (IgY) from the embryo sac to the developing embryo in chicken eggs (Kim et al., 2004; Senggagau and Bond, 2020). A previous study indicated that IgY level with pyridoxine supplementation treatment in laying hens via drinking water was 2.15 g/100 ml, it was 2.13 g/100 ml for a treatment mixed with feed ingredients was, and 2.18 g/100 ml for treatment by intravenous injection (Silitonga et al., 2013).

These results showed that different pyridoxine supplementation methods (via drinking water, mixing in rations, or intravenous injections) do not significantly affect the obtained IgY levels. Pyridoxine compounds contain vitamins that are used as a defense in the body of laying hens. This vitamin plays a role in forming the body's defense system against invading microorganisms (Senggagau and Bond, 2020).

From various research findings, it is known that pyridoxine deficiency conditions in humans and various animal species lead to abnormalities in the body's defense system, such as fewer antibody-producing cells, fewer lymphocytes, and decreased immune system function, compared to normal conditions (Kumar and Axelrod, 1968; Debes and Kirksey, 1979). Efforts to increase the production of IgY in egg yolk have been made and it was demonstrated that oral/feeding pyridoxine supplementation at a dose of 3.0 mg/kg ration could result in the production of IgY of 106.1 mg/egg. This means that it has undergone an increase of about 6%, compared to the IgY content of another study (Li et al, 1998). Table 2 tabulates the results of the levels of egg IgY, vitamins, and cholesterol.

The effect of giving kepok bananas up to 20% in laying hens diets on IgY was significant ($p < 0.05$). This showed that adding 20% KBF could increase egg IgY because the nutrient content in the diet is protein, fat, and vitamins. The nutritional content of the feed affects the yolk and as a result the IgY of the egg. Setiani (2016) reported that the IgY concentrations of 7.89, 10.07, and 26.31 mg/ml in layer eggs, native chicken eggs, and duck eggs, respectively. Nutrition treatment feed contains protein, vitamins, essential amino acids that affect egg yolks. The quality of the vitelline membrane and egg yolk is influenced by diet protein. The IgY is transferred from maternal blood to egg yolk via oocyte cytoplasmic membrane receptors from development to maturity (Nimmerjahn and Ravetch, 2007).

Table 2. Egg immunoglobulin, vitamins, and cholesterol contents of laying hens in Indonesia

Nutritional content \ Diets	R0	R1	R2	R3	R4	SEM	P Value
Immunoglobulin (IgY; mg/100 g)	19.24 ± 0.09[d]	20.28 ± 0.47[c]	20.20 ± 0.1[c]	22.38 ± 0.26[b]	23.26 ± 0.13[a]	0.113	0.00
Egg Vitamin A (µg/100g)	0.47 ± 0.04[c]	0.54 ± 0.05[b]	0.63 ± 0.01[a]	0.61 ± 0.01[a]	0.62 ± 0.01[a]	0.014	0.00
Egg Vitamin B1 (µg/100g)	123.8 ± 4.76[c]	138.6 ± 6.87[b]	149.8 ± 1.64[a]	132.6 ± 9.28[bc]	150.8 ± 1.10[a]	2.53	0.00
Egg Vitamin B6 Pyridoxine (µg/100g)	295.2 ± 4.81	294 ± 12.25	299 ± 7.44	296 ± 4.47	302.2 ± 4.91	3.306	0.347
Egg Vitamin D2(µg/100g)	4.88 ± 0.09[c]	5.38 ± 0.61[bc]	5.82 ± 0.52[ab]	5.92 ± 0.16[ab]	6.06 ± 0.21[a]	0.170	0.00
Egg Vitamin D3(µg/100g)	2.88 ± 0.19[b]	2.98 ± 0.24[ab]	3.38 ± 0.22[ab]	3.40 ± 0.51[ac]	3.56 ± 0.47[a]	0.158	0.027
Cholesterol (mg/100g)	199.52 ± 0.43[a]	193.04 ± 5.53[ab]	188.42 ± 6.93[b]	188.78 ± 0.57[b]	188.90 ± 0.41[b]	1.783	0.001

R0: No kepok banana flour, R1: 95 basal feed /bf+ 5% KBF, R2: 90 bf + 10% KBF, R3: 85 bf + 15% KBF, R4: 80 bf + 20% KBF; Significant differences amongst the treatments were indicated by different superscript letters in a row ($p < 0.05$)

Egg vitamin

The average yields of egg vitamins A, B1, B6, D2, D3 were 0.47-0.63,123.8-150.8, 294.0-302.2, 4.88-6.06, 2.88-3.56 µg/100g, respectively. In another study, the contents of egg vitamins were reported as vitamin A precursor or beta-carotene of 88 g/100g, Vitamin B1 (Thiamin) of 17688 g/100g, Vitamin B2 (Riboflavin) of 528 g/100g, Vitamin B6 350 of g/100g, and Vitamin D of 5.4 g/100g (Réhault-Godbert et al., 2019).

As can be seen in Table 2, supplementation of chickens' diet by KBF led to significant differences ($p < 0.05$) in the levels of vitamins A, B1, D2, and D3, compared to the control. The results showed that the addition of 20% KBF could increase egg vitamins. The average contents of vitamins A, B1, B6, D2, and D3 in eggs were highest at R4. This difference is possible because the vitamins used by reproduction organs require the same amount of vitamins so that the vitamin deposition in egg yolks is different. Vitamins A and D are fat-soluble vitamins, which are stored in the liver or adipose tissue in poultry. These vitamins are excreted in the bile and then feces.

According to the results, the kepok banana contains vitamins and beta-carotene, which can increase egg vitamins. The beta-carotene content is one of the major sources of vitamins in the feed. Eggs contain almost all vitamins except vitamin C. The vitamins are grouped into fat-soluble vitamins (A, D, E, and K) and water-soluble vitamins (thiamin, riboflavin, pantothenic acid, niacin, folic acid, and Vitamin B12, Çalişlar, 2020).

The Vitamin A content of egg yolks is influenced by beta-carotene consumption (Suci et al., 2020). Consumption of beta-carotene was higher in the KBF diets than in the control diets (Table 2). Beta-carotene is a carotenoid that can act as pro-vitamin A which will be converted into vitamin A in the intestinal mucosa and absorbed in the form of vitamin A. Beta-carotene consumed is catabolized through an oxidation reaction by the enzyme beta-carotene dioxygenase which produces Vitamin A. The higher beta-carotene in the ration increases the levels of Vitamin A in eggs (Wiradimadja et al., 2010).

Egg yolk is a food that is rich in most vitamins (Réhault-Godbert et al., 2019). Chickens transfer vitamin D from feed to egg yolks so that they are able to produce high levels of 25-hydroxy vitamin D3 (Macelline et al., 2021).

Egg cholesterol

The average egg cholesterol of chickens fed with KBF is 188.42-193.04 mg/100 g The papaya leaf flour treatment ration to medium brown 402 laying hens on egg yolk reduced egg cholesterol level to 118-212 mg/100g (Leke et al., 2019). The treatment of KBF had a highly significant effect on egg cholesterol ($p < 0.05$). The results showed that the higher the level of KBF, the lower the egg cholesterol. This is due to the fiber content in the kepok banana. The small intestine of hens can not digest the fiber. One of the properties of dietary fiber is that it can bind cholesterol from food and is excreted with feces so that the absorbed cholesterol is reduced. Kepok bananas contain resistant starch, inulin, calcium, phenolic compounds, beta-carotene, vitamins A, B1, B2, B3, B6, C, and minerals. The increase in beta-carotene and crude fiber content due to the addition of KBF levels affects a decrease in egg cholesterol. Bananas that can reduce the concentration of cholesterol, free fatty acids, and triglycerides in serum and tissue are found in flavonoid and phenol compounds (Rusdaina and Syauqy, 2015). The cholesterol related to the hydroxymethyl glutaryl-CoA (HMG) enzyme in cholesterol can be reduced by Beta-carotene (Bidura et al., 2017). This enzyme has an important role in the formation of mevalonic in cholesterol biosynthesis. The cholesterol and beta-carotene synthesis are derived from acetyl CoA. According to the obtained results of the current study, supplementation of diet with up to 20% KBF can lower egg cholesterol.

CONCLUSION

In conclusion, the obtained results of the current study indicated that supplementation of poultry diet with kepok banana flour at different levels (5%, 10%, 15%, and 20%) can enhance immunity and vitamin (A, B1, D2, D3) contents. It can also decrease the level of cholesterol in eggs. Therefore, kepok banana flour can be used in the laying chickens' feed although the obtained results should be also confirmed by future studies.

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