

Blood Lipid Profile and Yolk Cholesterol Content of Coturnix coturnix japonica Receiving African Leaf (Vernonia amygdalina) Extract in Drinking Water

Handling of digested slurry from bio-digester with initial substrate dairy cow manure and papaya seed

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ABSTRACT

The aim of this study was to determine the effect of supplementation of African leaf (*Vernonia amygdalina*) extract on quail blood lipid profile (comprising total cholesterol, triglyceride, HDL cholesterol, LDL cholesterol concentrations) and yolk cholesterol. One hundred twenty female quail aged 8 weeks were fed a supplementary *Vernonia amygdalina* for 6 weeks. The study was of completely randomized design with 3 treatment and 5 replications. The treatment were no supplementation of African leaf extract to the control and supplementation of African leaf extract at doses of 3 and 6 ml quail/day. The observed variables were those of blood lipid profile (comprising total cholesterol, triglycerides, HDL cholesterol, and LDL cholesterol concentrations) and egg yolk cholesterol. Blood lipid profiles data were analyzed of variance and yolk cholesterol concentrations were analyzed descriptively with 1 sample (composite from each replication) for each treatment. The study results showed that African leaf extract significantly ($P < 0.05$) reduced quail blood cholesterol and yolk cholesterol. Yolk cholesterol was reduced by 5.01% and 11.38, respectively, at doses of 3 and 6 ml quail/day as compared to controls. Blood triglyceride, HDL cholesterol, and LDL cholesterol concentrations were not significantly different from those in controls. From these study results it was concluded that supplementation of African leaf at a dose of 6 mL quail/day was capable of reducing quail blood cholesterol and yolk cholesterol.

ABSTRAK

Tujuan penelitian ini adalah untuk mengetahui suplementasi daun Afrika (*Vernonia amygdalina*) yang diekstrak terhadap profil lemak darah yaitu kolesterol, trigliserida, High Density Lipoprotein (HDL) dan Low Density Lipoprotein (LDL), serta kandungan kolesterol kuning telur puyuh. Seratus dua puluh ekor puyuh betina umur 8 minggu dipelihara selama 6 minggu dan diberikan suplemen ekstrak daun Afrika. Rancangan penelitian yang digunakan adalah Rancangan Acak Lengkap (RAL) dengan 3 perlakuan dan 5 ulangan. Perlakuan yang diberikan yaitu kontrol tanpa suplemen ekstrak daun Afrika dan suplementasi ekstrak daun Afrika 3 mL dan 6 ml ekor/hari. Peubah yang diamati yaitu profil lemak darah (kolesterol, trigliserida, HDL kolesterol, LDL kolesterol) dan kolesterol telur. Kolesterol kuning telur dianalisis secara deskriptif dengan 1 sampel (komposit dari setiap ulangan) untuk setiap perlakuan. Hasil penelitian menunjukkan bahwa ekstrak daun Afrika nyata ($P < 0,05$) menurunkan kolesterol darah puyuh dan menurunkan kolesterol kuning telur. Kolesterol kuning telur menurun pada perlakuan suplemen ekstrak daun Afrika 3 dan 6 ml ekor/hari sebesar 5,01% dan 11,38% dibandingkan kontrol. Kadar trigliserida, HDL kolesterol dan LDL kolesterol dalam darah tidak berbeda nyata dibandingkan dengan kontrol. Hasil penelitian ini disimpulkan bahwa suplementasi ekstrak daun Afrika sebanyak 6 mL ekor-1 hari-1 dapat menurunkan kolesterol darah dan kolesterol kuning telur puyuh.

Kata kunci:

Daun Afrika,
Profil lipid darah
Kolesterol telur puyuh
Vernonia amygdalina



INTRODUCTION

Herbals possess active compounds that may play the role of antimicrobials, improve the immune system, and reduce the cholesterol content of poultry products. Indonesia has diverse herbals that have frequently been utilized by the community as medicine or to improve the immune system. Low cholesterol poultry products are one of the alternatives of healthy animal meat substitutes for the elderly or individuals at high risk of coronary heart disease. African leaf (*Vernonia amygdalina*) is a plant that grows readily in Indonesia, although originating from Africa. This annual shrub has a height of 2-5 m. The leaves are utilized as herbals in traditional medicine. The plant grows readily in tropical and subtropical regions and can be propagated by stem cuttings (Adaramoye et al. 2018).

African leaf is relatively inexpensive, is rich in a number of nutrients, such as carotenes and vitamin C, and has a high content of minerals such as Fe, P, Ca, and K (Shewo and Girma 2017). African leaf contains 145 mg Ca, 0.7 mg P, 5 mg Fe, 85 mg Zn, and 710 mg Mn, and also 5.1 mg vitamin C (mg/100 dry weight) (Shewo and Girma 2017). According to Hasan et al. (2018) *Vernonia amygdalina* has a high antioxidant content, in the form of flavonoids, vitamin C, and the sesquiterpene compounds vernodaline and vernomygdin. In addition, it contains alkaloids, tannins, saponins, and steroid glucosides that confer it a bitter taste when ingested. According to Sukmawati et al. (2017), flavonoids in African leaf have the potential as antioxidants. A study conducted by Adaramoye et al. (2018) was aimed at testing the effect of a methanolic extract of *Vernonia amygdalina* in lowering the blood lipid concentration. The results of this study showed that African methanolic extract at doses of 100 mg/kg/BW and 200 mg/kg/BW had the effect of lowering the high density lipoprotein (HDL cholesterol) concentration by 10%. Another study using African leaf methanolic extract at a dose of 100 mg/kg/BW was able to reduce the total cholesterol concentration in male Wistar rats by 36.01% (Hasan et al. 2018). Saponins can reduce total cholesterol levels by binding bile salts to form an inabsorbable compound or cause the bile salts to bind to polysaccharides in dietary fiber and to be subsequently excreted in

the stools, with the result that the bile salts cannot bind to cholesterol, such that the cholesterol cannot be reabsorbed. Flavonoids and saponins are widely distributed in plant tissues in the form of glycosides that are polar in character. The use of African leaf extracted in the form of juice given through drinking water for quail during the production period has not been studied.

Quail eggs are a source of animal protein but have high cholesterol levels (Ukachukwu et al. 2017; Putri et al. 2022; Aygün and Olgun 2019; Grigorova et al. 2014). Therefore, many studies have tried to reduce egg cholesterol by adding herbs like binahong (Kismiyati et al. 2020), red mold rice (Pengnoi et al. 2018) and *Indigofera zollingeriana* top leaf (Faradillah et al. 2015). African leaf has the potential to be used as a herb that can reduce blood cholesterol and egg cholesterol because it has several bioactive substances. So that the present study aimed to determine the effectiveness of supplement of *Vernonia amygdalina* leaf extract via drinking water on the blood lipid profile of layer quail (comprising total cholesterol, triglycerides, HDL cholesterol and LDL cholesterol) and on quail egg cholesterol content.

MATERIALS AND METHODS

Birds and Housing

This study used a total of 120 8-week-old layer quail (*Coturnix coturnix japonica*) that were placed in colony cages that were 60 cm long, 50 cm wide, and 25 cm high. Each cage was equipped with a feeder and a drinker. Layer quail were divided into 3 treatments with 5 replications, 8 birds each. Sanitation of the cage was carried out before the quail chick in. Antistress medication was given after one week quails enter cage. There were no vaccinations or other medicines.

Research Procedure

Profile of African leaf (*Vernonia amygdalina*)

The African leaf plant has roundish leaves and a woody stem, grows easily in tropical regions and may be used as hedge plant. The leaves that are used in this study are the upper 3-5 leaves from the shoots, are light-green in color, and have a non-rigid texture. The leaves were obtained from Cibuntu, Bogor.

Preparation of African leaf Extract

Fresh African leaf (*Vernonia amygdalina*) that had been cleaned in water were weighed to the amount of 100 g, then soaked for 24 hours. The leaves were juiced by adding 200 mL drinking water to 100 g the leaves (2:1) and mashed in a blender (modified from Halimah et al. 2019). The mashed leaves were then strained and placed in a container. The leaf extract may be stored for 2-3 days in the refrigerator. The leaf extract was administered via the drinking water at a dose according to the treatment. The drinking water containing the leaf extract was given from morning to noon, then in the afternoon the water was replaced with drinking water without the extract. African leaf extract was given daily to the quail. The daily volume of drinking water administered was 65 mL/quail/day. Drinking water with African leaf extract was given in the morning, then washed in the afternoon and replaced with new water without treatment. The phytochemical analytical results of the *Vernonia amygdalina* leaf extract are shown in Table 1.

Table 1. Phytochemical analytical results of African leaf extract

Type of phytochemic	African leaf extract
Alkaloids	+
Flavonoids	+++
Phenolhydroquinones	+
Steroids	+
Triterpenoids	-
Tannins	++
Saponins	+++

Notes: not present, +: low, ++: moderate, +++: high . Analytical chemistry laboratory, IPB University

Table 2 Nutrient content of commercial diet (as feed)

Nutrient content (%)	Analytical results
Water	8.00
Crude protein	22.80
Crude fat	5.69
Crude fiber	2.97
Ash	12.47

Note: Center for Biological Resources and Biotechnology Research., IPB University

Diets Treatment

Quail ration used commercial layer quail crumble (PT Sinta Feedmill). The nutrient content of ration shown in Table 2. Commercial quail ration contains 22.8 % crude protein and 2.97% crude fiber. The Rations were given ad libitum.

Design Experiment and Data Anaysis

The treatment used in each experimental unit (quail/day) in this study consisted of administration of drinking water without African leaf extract (65 mL drinking water) (P0) and administration of drinking water supplemented with African leaf extract to a total of 3 mL (P1) and 6 mL (P2), respectively, of African leaf extract.

This study used a completely randomized design with 3 treatment and 5 replications (Steel dan Torrie 1993). The blood lipid profile data were analyzed by means of analysis of variance (ANOVA). If the obtained data were significantly different (P<0.05), the analysis was continued with the Duncan Multiple Range Test (DMRT). Yolk cholesterol data were analyzed descriptively.

Blood Sampling

Prior to the drawing of the blood samples, the quail were put on a fast for 24 hours. The blood was drawn from the jugular vein into a syringe to a volume of 1 ml, after the area around the jugular vein had been cleaned using 70% alcohol. Any feathers in this area were removed beforehand using a pair of scissors. The blood sample was placed into a tube containing an anticoagulant (heparin). The blood sample was stored in a thermos flask filled with ice cubes (Hidayat et al. 2017). Blood samples were drawn in the 6th week after the start of the treatments. The number of blood samples drawn was 9, consisting of 3 treatment and 3 replications, each replication using 1 quail. The blood was drawn from the jugular vein located in the neck or from a wing vein using a 1 mL syringe. The blood samples were placed in Ethylene Diamine Tetra Acetic Acid (EDTA) tubes that were stored in a cooling box containing ice cubes. The obtained blood was centrifuged at 8000 rpm for 15 minutes. The obtained blood plasma samples were subsequently placed in labeled eppendorf tubes and analyzed.

Blood Lipid Profile Analysis

The blood plasma samples were analyzed for cholesterol, triglyceride, HDL cholesterol, and LDL cholesterol levels. Blood total cholesterol (mg dL-1) was determined by the cholesterol-oxidase-p-aminophenazone (CHOD-PAP) method with cholesterol oxidase phenol amino phenazone (CHOD-PAP) (Rodriguez et al. (2000) with kit number 101592. The blood cholesterol

concentration was obtained by means of the following formula:

$$\text{Cholesterol (mg/dL)} = \frac{\text{absorbance of sample}}{\text{absorbance of standard}} \times \text{standard cholesterol concentration}$$

The triglyceride concentration (mg dL-1) was determined with the glycerol phosphate oxidase-p-aminophenozone (GPO-PAP) method Rodriguez et al. (2000) kit number 116392. The blood triglyceride concentration was obtained with the following formula:

$$\text{Triglyceride (mg/dL)} = \frac{\text{absorbance of sample}}{\text{absorbance of standard}} \times \text{standard triglyceride concentration}$$

HDL cholesterol was determined using the cholesterol oxidase-p-aminophenazone (CHOD-PAP) method (Gordon et al. (1977) kit number 101592. The HDL cholesterol concentration was obtained with the following formula:

$$\text{HDL cholesterol (mg /dL)} = \frac{\text{absorbance of sample}}{\text{absorbance of standard}} \times \text{standard cholesterol concentration}$$

LDL cholesterol concentration (mg/dL) was obtained using Friedewald’s formula (1972):

$$\text{LDL cholesterol concentration} = \text{Total cholesterol} - \text{HDL cholesterol} - \frac{1}{5} \text{Triglyceride}$$

Determination of yolk cholesterol

The eggs were taken in the 6th week after the start of the treatment. The part of the eggs used was the yolk. The yolk samples used consisted of one sample for each composite treatment (two or more samples that are combined or mixed). Yolk cholesterol analysis was carried out at the Center for Quality Testing and Certification of Animal Products, Cimanggu, Bogor.

RESULTS AND DISCUSSION

The Effect of the Treatments on Blood Plasma Lipid Profile of Layer Quail

The results showed that administration of 6 mL/quail/day of African leaf extract in drinking water produced the lowest (P<0,05) blood cholesterol concentration as compared with the controls and the administration of 3 mL/quail/

day, but did not differ with respect of the blood triglyceride, HDL cholesterol, and LDL cholesterol concentration of the layer quail. Data on the average blood plasma lipid profile of Quail is shown in Table 3.

Cholesterol is required as precursor for the production of important steroid hormones and bile salts in the body. The quail blood lipid profile in this study was higher than that in the study results of Salman et al. (2019). According to Dosoky et al. (2021) quail cholesterol triglyceride, HDL cholesterol, and LDL cholesterol concentrations were 167.67 mg/dL, 218 mg/dL, 75 mg/dL, and 50.33 mg/dL, respectively. Sahara et al. (2019) reported that blood lipid profile comprising cholesterol, triglyceride, HDL cholesterol, and LDL cholesterol concentrations differed with those of the present study, being 318.72 mg/dL; 837.64 mg/dL; 56,78 mg/dL, and 12.54 mg/dL, respectively. The reduction in blood cholesterol in productive quail affected the yolk cholesterol content (Figure 1).

The yolk cholesterol was significantly reduced due to the presence of bioactive substances that play a role in reducing blood cholesterol, namely the saponins, tannins, and flavonoids that are present in African leaf extract in adequate to large concentrations (Table 1). Sahara et al. 2019 and Meirindasari et al. (2013) stated that saponins, tannins, and flavonoids that are present in papaya seeds may reduce total cholesterol. Flavonoids are present at high concentrations in African leaf extract (Table 1) and can act as antioxidants. Flavonoids decrease blood cholesterol concentrations by inhibiting the activity of acyl-CoA cholesterol acyl transferase (ACAT) and play a role in reducing cholesterol esterification in the intestines and liver, and in inhibiting the activity of 3-hydroxy-3-glutaryl CoA that results in inhibiting cholesterol synthesis (Hasan et al.

Table 3. Mean blood plasma lipid profile of layer quail receiving African leaf (Vernonia amygdalina) extract in the drinking water

Blood lipid profile (mg/dL)	Administration of African leaf extract in drinking water			Normal (mg /dL)
	P0	P1	P2	
Cholesterol	173.00±4.47b	171.27±6.75b	159.73±5.43a	163.33±1.221)
Triglycerides	330.60±27.11	316.90±84.59	254.34±36.72	154.83±5.621)
HDL cholesterol	61.32 ±13.02	76.14±11.72	65.05±16.18	122.66±0.661)
LDL cholesterol	45.56±9.14	31.82±22.11	43.81±15.86	10±1.181)

Note : mean ± sd. P0: drinking water without African leaf extract, P1: drinking water with 3 mL of African leaf extract quail/day, P2: drinking water with 6 mL of African extract/quail/day; different superscripts in the same line showed significant differences (P<0.05) in Duncan’s ; 1)Salman et al. (2019)

2018). The role of saponins in the reduction of cholesterol concentrations is associated with the binding of cholesterol to bile acids to form compounds that are difficult to absorb (Hasan et al. 2018; Vinarova et al. 2014). The Saponins and tannins can also function as antinutrients with negative effect on feed intake, nutrient digestibility, and production performance (Huang et al. 2018; Hasan et al. 2020). Low concentrations of several tannin sources improved health status, nutrition and animal performance in monogastric farm animals (Hasan et al., 2020; Starcevic et al. 2015).

The effect of the administration of African leaf extract on blood triglyceride, HDL cholesterol, and LDL cholesterol concentrations is not yet apparent. Triglycerides are a form of fat that is efficient for the storage of energy obtained from enzymatic hydrolysis of lipids or carbohydrates in the digestive tract. Triglycerides are utilized by the liver and the remaining are stored in the form of adipose tissue as an energy reserve. In our study the blood triglyceride concentrations were not significantly different because the rations used were the same, and it is assumed that the digestibility of the rations was not disturbed by the presence of the African leaf extract, which contains bioactive substances that can also play a role as antinutrients. The bioactive substances present in African leaf extract do not play a significant role in reducing the activity of glycerol-3-phosphate dehydrogenase (GPDH), which is an enzyme that plays a role in the biosynthesis of triglycerides. The high blood triglyceride concentrations in the results of our study were due to the fact that productive quail require the triglycerides for the formation of egg yolk.

The HDL cholesterol concentrations in all interventions were higher than the blood LDL cholesterol concentrations (Table 3). These study results are in line with the results of the study of Luna et al. (2018), namely that HDL cholesterol and LDL cholesterol concentrations were 111.2 mg/dl and 35.5 mg/dL, respectively (intervention on control quail hens) and also in line with the study of Khalifa and Noseer (2019), but contrary to the study of Arroshicin et al. (2016) who obtained higher LDL cholesterol versus HDL cholesterol concentrations of 168.38 mg/dL and 86.6 mg/dL, respectively, and to the results of the study by Shokrollahi and Sharifi (2018) and Aetin et al. (2017). The LDL to HDL cholesterol ratio in our study with African leaf extract supplementation was much lower as compared with the controls. The LDL to HDL cholesterol ratio in all treatments was in the range of 0.42 to 0.74, whereas in the study of Luna et al. (2018)

with the addition of thymol and isoeugenol as treatment, the ratio ranged from 0.23 to 0.31. The higher HDL cholesterol concentrations as compared with the LDL concentrations point to a better condition of the quails, because HDL cholesterol functions to cleanse the blood vasculature of adhering cholesterol caused by high LDL cholesterol. LDL cholesterol acts as the principal source of cholesterol, namely 90% of blood cholesterol is in the form of LDL cholesterol. The increase in blood HDL cholesterol in the intervention groups P1 and P2 was by 24.17% and 6.08%, respectively, as compared to the controls. The high blood LDL cholesterol concentrations cause much of the cholesterol to be deposited and to form plaques on the blood vessel walls. The obstruction causes narrowing of the blood vessels and atherosclerosis in the quails. The African leaf also contains vitamin C (5.1 mg/100 dry matter) (Shewo and Girma 2017) that may act as antioxidant by binding peroxide free radicals, such that it minimizes the formation of oxidized LDL cholesterol (Hasan et al. 2018), but in the present study the amount of vitamin C entering the quail body could not show a reduction in LDL cholesterol concentrations.

Yolk Cholesterol of Quail

Quail eggs are known for their higher cholesterol as compared to chicken eggs, such that the administration of herbals to layer quail is frequently conducted. The quail yolk cholesterol after administration of African leaf extract via drinking water at doses of 3 and 6 ml/quail/day is shown in Figure 1. The yolk cholesterol content data is a composite of yolk from each replicate in each treatment. Yolk cholesterol concentration was reduced by 5.01% and 11.38%, respectively, as compared with the controls. The yolk cholesterol in the present study was higher than that in the studies of Nastiti et al. 2014 at 10.62 – 14.06 mg/g and of Ukachukwu et al. (2017) at 6.79 mg/g. According to Dosoky et al. (2021) quail total yolk cholesterol was 19.15±0.28 g/100 g in the controls, but was significantly reduced by the addition of onion and cinnamon. This yolk cholesterol level is lower than the research of Febriani (2017), which is 484 mg/100 g of yolk. Cholesterol is required by the body as material for the synthesis of steroid hormones, bile salts, and vitellogenins (egg yolk precursors), but a high concentration in the eggs may also have a negative impact on consumers who are at risk of cardiovascular disease.

The formation of follicles in the ovaries will be stimulated by phytoestrogen compounds so that the number of developing follicles will increase, causing the fat and cholesterol that make up the yolk to be distributed to the development of more follicles, so that the levels of fat and cholesterol in the eggs will decrease (Saraswati et al.

2013). In our study, the administration of African leaf extract at a dose of 6 ml/quail/day resulted in reduced egg cholesterol. The use of African leaf extract containing high concentrations of saponins (Table 1) made it possible to influence the yolk cholesterol concentrations. Saponins are antinutrients that may increase the excretion of cholesterol into the digestive tract. Meirindasari et al. (2013) stated that the bioactive substance are saponins, flavonoids, and tannins. Saponins may reduce the cholesterol in the liver and increase the excretion of cholesterol through the binding of the saponins to bile acids and the cholesterol from the feed to form micelles that cannot be absorbed by the intestines.

Tannins decrease the absorption of cholesterol in the small intestine and increase the excretion of bile acids by binding to body proteins and adhering to the intestinal wall, thereby inhibiting fat absorption. Flavonoids as antioxidants can reduce the oxidation of LDL cholesterol, inhibit the activity of enzymes that play a role in reducing cholesterol esterification in the liver and intestines, and inhibit the activity of enzymes that result in cholesterol synthesis (Merindasari et al. 2013; Nuralifah et al. 2020). The African leaf extract in our study has high concentrations of saponins and flavonoids and moderate concentrations of tannins. In addition, the role of bioactive substances in the body is also affected by the administered dose (Nuralifah et al. 2020).

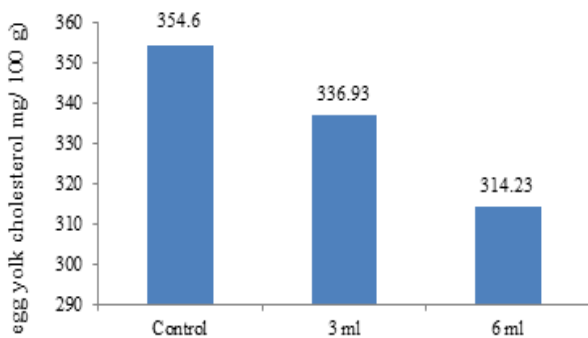


Figure 1. Supplementation of African leaf (*Vernonia amygdalina*) extract (mL/quail/day)

CONCLUSIONS

Supplementation of African Leaf (*Vernonia amygdalina*) extract to layer quail at a dose of 6 mL/quail/day could reduce quail blood cholesterol concentration by up to 7.67%. This is in line with the reduction in egg yolk cholesterol concentration by 11.38%. The triglyceride, HDL cholesterol, and LDL in the normal range.

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