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THE EFFECT OF GIVING MANGOSTEEN (*Garcinia mangostana* L.) EXTRACT WITH MINERAL SUPLEMENTATION ON BLOOD AND YELLOW EGG CHOLESTEROL LEVELS OF CHICKEN PHASE LAYER

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Abstract

The research was conducted to know the effect of mangosteen peel extract supplemented Cu and Zn on blood cholesterol and egg yolk cholesterol of Sentul chicken. The research was done from Agustus until November 2019. Samples test was held in Physiology and Biochemistry Laboratory, Animal Husbandry Faculty, Padjadjaran University, Sumedang. The methods used experimental with a Completely Randomized Design (CRD) and the effect of treatment using Analysis of Variance (ANOVA) followed by Duncan and Orthogonal Polynomial. The treatment consisted of five kind with five repetitions, P0 = basal ration, P1 = basal rations + 60 mg/kg mangosteen peel extract + Cu 0.3 mg and Zn 2.4 mg, P2 = basal rations + 120 mg/kg mangosteen peel extract + Cu 0.6 mg and Zn 4.8 mg, P3 = basal rations + 180 mg/kg mangosteen peel extract + Cu =0.9 mg and Zn 7.2 mg, P4 = basal rations + 240 mg/kg mangosteen peel extract + Cu 1.2 mg and Zn 9.6 mg. The result showed that the effect of giving 120 mg/kg mangosteen peel extract supplemented Cu 0.6 mg and Zn 4.8 mg was the significant effect ($P<0.05$) decreasing blood cholesterol levet but non-significant ($P>0.05$) decreasing egg yolk cholesterol levels of Sentul chicken.

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Key words*: blood cholesterol, Cu (copper), egg yolk cholesterol, mangosteen peel extract, Zn (zinc).*

INTRODUCTION

Sentul chicken is one type of local chicken typical of the Ciamis region of West Java which has gray feather characteristics. The advantage of sentul chicken compared to other native chickens, which has relatively rapid growth (Kurnia, 2011). Sentul chickens are categorized as dual-purpose chickens, which are capable of producing meat and eggs. Sentul chicken meat and eggs can be used as an alternative to meet the needs of community animal protein.

Lately, some people are more selective because health awareness is getting higher. People crave food from animals, especially poultry with low fat content such as cholesterol (Pogurschi et al., 2019). According to Rasyaf (1995), native chicken meat has a low-fat content, but with increasing age of the chicken, the deposition of abdominal fat is increasing as well as with subcutaneous fat. The high-fat content is identical to the high cholesterol content.

Cholesterol content in eggs is influenced by several factors such as age, genetics, nutrients, and drugs (Ketaren, 2010). Effect of fat in the feed (vegetable oil, animal oil, cholesterol, and B sitosterol) can increase liver cholesterol, serum, and egg yolks in laying hens (Han et al., 1993). Cholesterol content in egg yolks can change up to 25% by cholesterol derived from feed and fat (Hargis, 1988). Egg yolk cholesterol is higher than meat cholesterol, this is because the egg is the end of the distribution of vitellogenin which is composed of cholesterol, triglycerides, phospholipids, and proteins (Watson, 2002).

Efforts to reduce cholesterol levels in the blood and eggs can be done by using rations mixed with herbal plants, one of which is mangosteen peel extract (*Garcinia mangostana* L.). Mangosteen peel extract contains xanthone compounds as antioxidants. Xanthones are natural chemical substances that are classified as phenol or polyphenolic compounds. Mangosteen peel extract contains a lot of antioxidants of 84.6-86.3% and xanthone content is more than 90% (Diwyanto et al., 2011). Xanthones can inhibit the process of cholesterogenesis. Effect of mangosteen peel

extract can decrease Low Density Lipoprotein also increased High Density Lipoprotein (Lovita et al., 2018).

Mangosteen peel extract also contains flavonoid compounds that can reduce blood cholesterol levels by reducing the absorption of cholesterol and bile acids in the small intestine which causes increased excretion through feces. Liver cells increase the formation of bile acids from cholesterol so that they can reduce fat due to being converted into energy (Sucipto, 2008).

Miryanti et al. (2011) presented mangosteen peel extract results from the analysis of Gas Chromatography Mass Spectrometry (GCMS) that mangosteen peel extract contains methyl esters of unsaturated organic acids that are easily oxidized. Supplementation of Cu and Zn plays a role to temporarily activate the bioactive contained in the mangosteen peel extract which is reactive, thus making the ionization in the digestive tract higher and can be optimally utilized right on target. Copper (Cu) is an essential mineral micro element that is cationic. Copper is one of the mineral elements that are needed in the process of metabolism, hemoglobin formation and physiology in the animal's body (Burns, 1981). The form of copper metal that is given into the feed mixture is in the form of copper salt compounds, such as copper sulfate, copper oxide, copper carbonate, and copper proteinate. Usually sulfate and copper oxide are often added to ruminant feed (Baker et al., 1991; Johnson and Engle, 2003).

MATERIALS AND METHODS

The material used in this study was 40-tail chicken, maintained from the age 28 weeks to 35 weeks. Chicken is divided into 5 treatments and each treatment is repeated 4 times, each cage contains 1 tail.

Sample Collection Stage

a. Blood sampling

Blood was taken at the end of the study, and eggs samples were taken from one chicken for each test in each treatment. Total blood samples were taken in the last week are 20 samples. The sample selection is based on the average body weight close to the same. Blood is drawn through the 2 cc chicken wing pectoralis vein. Blood samples were taken using a 5 ml syringe, and were collected in vaccumtube Ethylene Diamine Tetra Acetyl Acid (EDTA).

b. Chicken egg sampling

Egg sampling was carried out at the end of maintenance ie from each treatment 2 egg samples were taken from each cage, so that 10 egg samples were obtained from 5 treatments and 4 replications. Each egg is broken and then separated between egg whites and yolks. Egg yolks that have been separated and then placed in a plastic container, then roasted until dry, then ready to be tested for cholesterol levels. Chicken weighed initially according to treatment. The procedure for making mangosteen peel extract, namely, a sample of 7,000 g of fresh mangosteen peel, dried and cut into small pieces. The dry sample weighed 5,000 g. Then macerated with 96% ethanol for 24 hours. 96% ethanol extract from maceration was filtered with filter paper and the filtrate was collected. The filtrate was then evaporated using a rotary evaporator at ± 62°C in order to obtain concentrated ethanol extract 4.471 g. After that, the thick material is taken to the oven with a temperature of 600^0C and the mangosteen peel extract powder is obtained at 3.621 g. Mangosteen peel extract is then supplemented using Cu and Zn.

Based on the composition of the ration, the nutrient content and metabolic energy of the basal ration are presented in Table 1.

Table 1. Nutrient content and basal energy metabolism of ration

Nutrient Content and Metabolism Energy	Amount	Nutritional Needs of Local Chicken
Matabolizable Energy (ccal/kg)	2757	2750
Protein (%)	15.63	15-16
Fat (%)	5.14	8**
Crude Fiber (%)	4.16	8**
Calcium (%)	3.28	3.25-4.25
Phosphor (%)	1.39	0.3**
Lysine (%)	1.06	0.9**
Methionin (%)	0.37	0.35**

Source: Widjastuti (1996);
**NRC (1994) for light type laying hens

Observed variables include:
a. Chicken Blood Cholesterol Levels Layer Phase;
b. Chicken Egg Yolk Cholesterol Levels Layer Phase.
This study uses an experimental method with a Completely Randomized Design (CRD). There are 20 treatment units consisting of 5 treatments and 4 replications. The experimental diet consisted of P0 = basal ration, P1 = 60 mg/kg ration of mangosteen peel extract + Cu 0.3 mg and Zn 2.4 mg, P2 = 120 mg/kg mangosteen peel extract ration + Cu 0.6 mg and Zn 4.8 mg, P3 = 180 mg/kg of mangosteen peel extract + Cu 0.9 mg and Zn 7.2 mg, and P4 = 240 mg/kg of mangosteen peel extract + Cu 1.2 mg and Zn 9.6 mg. The data obtained were analyzed by ANOVA variance test and the effect of treatment using Duncan's and Polynomial Orthogonal test.

RESULTS AND DISCUSSION

Effect of Treatments on Chicken Blood Cholesterol Layer Phase

Sentul chicken blood cholesterol content which has been treated with mangosteen peel extract which has been supplemented with Cu and Zn, the results of laboratory analysis are presented, presented in Table 2.

Table 2. Blood cholesterol and egg yolk levels of chicken layer phase

Parameter	Treatment				
	P0	P1	P2	P3	P4
Blood cholesterol (mg/dl)	206.28^{a}±26.25	160.42^{a}±14.43	119.31^{b}±17.44	114.81^{b}±12.11	112.67^{b}±26.74
Egg yolk cholesterol (mg/100 g)	122.25±30.51	125.62±35.38	109.48±45.93	149.48±11.54	119.23±18.63

Based on Table 2 that the highest blood cholesterol level in P0 is 206.28 mg/dL, followed by P1 which is 160.42 mg/dL, then P2 has a value of 119.31 mg/dL, then P3 is 114.81 mg/dL, and P4 has a value of 112.67 mg/dL. The results of the analysis of variance showed that the administration of mangosteen peel extract supplemented with Cu and Zn had a significant effect ($P<0.05$) on the level of layer chicken blood cholesterol levels.
The treatment of P1 (60 mg/kg ration) was not significantly different from the treatment of P0 (basal ration). P2 (120 mg/kg ration) was not significantly different ($P>0.05$) with P3 (180 mg/kg ration), and P4 (240 mg/kg), but significantly different ($P<0.05$) with P0 treatment (0 mg/kg ration) and P1 (60 mg/kg ration). These results indicate that treatment P2, P3, and P4 has the effect of reducing blood cholesterol levels
Increasing the dose of mangosteen peel extract supplemented with Cu and Zn in the ration can reduce blood cholesterol levels. Mangosteen peel extract dosage 120-240 mg/kg ration gives real results in reducing blood cholesterol chicken layer phase. This is because the active

compound contained in the mangosteen peel extract is xanthone which is able to inhibit the process of cholesterol synthesis. The process of cholesterol synthesis starts from acetyl CoA which is the result of carbohydrate or fat metabolism. The change in acetyl co-A to mevalonate until cholesterol is mediated by the enzyme HMG CoA reductase. Xanthones work through a mechanism of inhibiting the activity of the HMG CoA reductase enzyme, which can cause inhibition of cholesterol biosynthesis (Botham and Mayes, 2015; Lovita, et al., 2005). Xanthones play a role in the preparation of xanthydrol which is useful in controlling blood cholesterol oxidation Low Density Lipoprotein (LDL) (Jung et al., 2006). Xanthones reduce the concentration of cholesterol in hepatocytes and increase the performance of LDL receptors which are closely related to the components of very low density lipoprotein (VLDL) which causes cholesterol to be reduced (Grundry, 1988).

Decreased levels of sentul chicken blood cholesterol are also affected by another active compound found in mangosteen peel extract, namely flavonoids. Flavonoids contained in mangosteen peel extract can increase the activity of the lipoprotein lipase enzyme. The increase in the enzyme VLDL lipoprotein which carries triglycerides will undergo hydrolysis to fatty acids and glycerol. The released fatty acids are then absorbed by muscles and other tissues that are oxidized to produce energy and by adipose tissue and stored as energy reserves (Marks et al., 2000). Flavonoids can also act as cofactors of cholesterol esterase enzymes and inhibitors of food cholesterol absorption by inhibiting the formation of micelles so that cholesterol absorption is inhibited (Olivera et al., 2007).

The quality of rations supplemented with Cu can improve the metabolic system and physiological processes that are in the body of chickens (Scott et al., 1982). High concentrations of Cu are closely related to high cholesterol levels. This is consistent with Klevay's (1980) study which states that Cu minerals and cholesterol are negatively correlated with plasma cholesterol concentrations. The lack of Cu minerals does not increase cholesterol concentrations in the liver but the bile acids increase and the effect of the increase is not large to reduce blood cholesterol levels. Zn Mineral has various functions in the body, especially for the digestion process. Zn is involved in several enzyme activities and is also a cofactor of more than 70 kinds of enzymes. Minerals Cu and Zn act as protective compounds for mangosteen peel extract, which causes ionization in the digestive tract to increase.

To find out the pattern of the relationship between the effect of using mangosteen peel extract supplemented with Cu and Zn on blood cholesterol levels, an orthogonal polynomial test was performed. The results of the Orthogonal Polynomial Test showed a significant difference ($P<0.05$) in linear regression with the equation $Y = -23.282x + 212.55$, and the coefficient of determination was 0.8234 ($R2 = 0.8234$). The average blood cholesterol level drops with increasing concentration of mangosteen peel extract supplemented with Cu and Zn. The results of the analysis of the coefficient of determination (R2) showed the percentage contribution of free variables (level of mangosteen peel extract supplemented with Cu and Zn) to the dependent variable (blood cholesterol level) was 82.34%.

Effect of treatments on egg yolk cholesterol chicken layer phase

The highest egg yolk cholesterol level in P3 is 149.46 mg/100 g, followed by P1 which is 125.62 mg/100 g, then P0 is 122.25 mg/100 g, P4 is 119.23 mg/100 g, and P2 is 109.48 mg/100 g. The results of the analysis of variance showed that the administration of mangosteen peel extract supplemented with Cu and Zn was not significantly different ($P>0.05$) on egg yolk cholesterol levels.

This study was not y significantly different, but showed an improvement decrease in egg phase yolk cholesterol levels, in P2 is 17.48% compare to control. This is because the active compound extract of flavonoid mangosteen peel can inhibit cholesterol synthesis. According to Metwally et al. (2009) flavonoids reduce cholesterol synthesis by inhibiting the activity of the enzyme acyl-CoA cholesterol acyl transferase (ACAT) which plays a role in

decreasing cholesterol esterification in the intestine and liver. Flavonoids are antioxidants that can reduce cholesterol levels in the blood, the mechanism by which flavonoids inhibit cholesterol synthesis through HMG CoA reductase inhibitors (Chen et al., 2006).

Egg cholesterol is synthesized in the liver, then carried by the blood in the form of lipoproteins and stored in growing follicles and passed on to the ovaries (Hammad et al., 1996). The active compound contained in mangosteen peel extract, namely flavonoids, acts as a phytoestrogen which triggers the biosynthesis of vitellogenin in the liver. Vitellogenin is composed of cholesterol, triglycerides, phospholipids, and proteins (Watson, 2002). Phytoestrogens will stimulate the formation of follicles in the ovary which causes the number of follicles to increase so that the distribution of fat and cholesterol for the development of more follicles which in turn causes a decrease in egg cholesterol. Cholesterol levels in the blood cause the amount of cholesterol that enters the ovaries to be lower.

CONCLUSIONS

1) The administration of mangosteen peel extract supplemented with Cu and Zn minerals has can reduce blood cholesterol, but can not reduce the egg cholesterol chicken phase layer.
2) The dose of mangosteen (*Garcinia mangostana* L.) peel extract as much as 120 mg/kg of rations supplemented with Cu and Zn minerals is the best concentration.

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