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Addition of Garlic Extract in Ration to Reduce Cholesterol Level of Broiler

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Abstract. The purpose of this research is to know the effect of garlic extract (GE) in reducing cholesterol level of broiler chicken by analyzing cholesterol level of broiler chicken blood. Two hundred one day broiler age were used in this study for 35 days. The chickens were randomly divided into four treatments, each treatment consist of five replications and each repetition consist of ten chickens. This research is used completely randomized design, such as: T0: 0% EBP, T1: 2%, T2: 4% and T3: 6%. Furthermore, at age 35 days each chicken was taken blood to be analyzed cholesterol levels, low density lipoprotein (LDL), high density lipoprotein (HDL) and calculated the ratio of LDL and HDL levels. The data obtained were analyzed using software from Statistical Product and Service Solution (SPSS 16.0). The results of significant analysis continued by Duncan's New Multiple Range Test. Addition of GE from the 2% level decreases (P <0.05) of LDL and total cholesterol, and increases HDL and HDL-LDL ratio. The conclusions is obtained garlic extract plays an important role in lowering cholesterol levels of broiler meat.

Key words: garlic extract, cholesterol, broiler

1. Introduction

Cholesterol is a component of fat that plays a role in the formation of hormones. Normally, the human body produces cholesterol synthesized in the liver in the right amount, but the number can increase due to the addition of food derived from animal fat [1].

2 In recent years, the education and research on food safety, increasing public awareness of health. Food of animal origin, such as egg yolk, meat, liver, and brain of farm animal contains high cholesterol. Several studies have already reported the increasing of metablc disease because the consumption of high cholesterol, such as fatty liver, coronary heart disease, pancreatitis, atherosclerosis, hypertension and stroke [2].

The potential of Indonesian medicinal plants is very high, about 1260 species of plants that live in tropical rainforests as medicinal plants. Depend on these number, about 180 species are used for the medicine industry and herbal medicine industries, and only a few species have been intensively cultivated [3].

Garlic contains of high sulfur levels, including allicin, diallyl disulfide (DDS), and diallyl trisulfide (DTS), which is a volatile oil and 3-allyl cysteine (SAC), a water soluble amino acid. Sulfur-containing compounds are responsible for the taste, aroma, and pharmacological properties of garlic [4]. The majority of the sulfur-containing compounds in garlic are γ - glutamyl-S-allyl-L-cysteines and S-allyl-L-cysteine sulfoxides (aliin) which are the major compound of amino acids. All sulfoxides, exception for cycloalliin, are converted to thiosulfinates so that no thiosulfinates are found in the intact garlic. Continued γ -glutamyl-S-allyl-Lcysteines be subsequently converted to S-allyl-Lcysteines (SAC) through enzymatic transformation with γ -Glutamyltranspeptidase when garlic extracted with a liquid solvent. The SAC, which is the main product of γ -Glutamyl-S-allyl-L-cysteines, is a detectable sulfur of amino acids in the blood, proven to be biologically active and bioavailable [5].

The compound of γ -glutamate-S-alk (en) il-L-cysteine compound is an intermediate of biosynthesis compound of other organosulfurs, including alliin. This compound is formed from amino acids biosynthesis pathways. γ -glutamyl-Salk (en) il-L-cysteine enzymatic reaction will produce many derivatives, by two branches of the reaction, those are the formation pathways of thiosulfinat and S-allyl cysteine (SAC).

The process of slicing, changed γ -glutamyl-S-alk (en) il- L-cysteine took place that be held by the enzyme γ - glutamyl - transpeptidase and γ -glutamyl-peptidase oxidase, and also produce alliin. At the time of garlic bulbs sliced and mashed in the process of making the extracts or seasoning, allinase enzyme becomes active and hydrolyze alliin produces allyl sulfenic. Condensation of the acid produces allicin, pyruvic acid, and NH_4^+ ions. One milligram of alliin is equivalent to 0.45 mg allicin [6]. Heating can inhibit the activity of allinase enzyme. At temperatures above 60°C , this enzyme will convert alliin into allicin [7]. Therefore, it easy to to lead into further reactions, depending on processing conditions or other external factors such as storage, temperature, and others.

In fresh form, the biological activity of garlic is very low, as it is known to several kinds of garlic preparations, such as garlic powder, garlic oil, and garlic extract (GE) [7]. Furthermore, based on several studies that have been done, there were no side effects and toxicity of garlic, as a result it is safe to use without causing undesirable effects [8]. The safety of using GE has been recommended based on several studies that have been done. Extraction of garlic bulbs with ethanol at temperatures below 0°C , produce alliin. Extraction with ethanol and water at 25°C produced allicin and does not produce alliin, while extraction by steam distillation method (100°C) causes all ingredients of alliin turned into allyl sulfide compounds [6]. Futhermore, the extraction process need to be done at room temperature. Garlic extract in ethanol with concentration of 15-20% can be stored up to 20 months [9]. In the form of GE, all alisin is converted to alylsulfide, the sequence based on the percentage is DTS of 73%, 8% DDS, and dialylsulfide (DS) 8% [10].

Some reports from clinical studies of using garlic found a cholesterol-lowering effect [7]. Several studies also reported that garlic inhibits the oxidation of cholesterol, thereby reducing the atherosclerotic, inhibiting the calcium deposits that harden the arteries, lower blood pressure, inhibit platelet aggregation to form blood clots, and reduce homocysteine (an amino acid that increases the risk of cardiovascular disease and stroke).

The research of [11] showed that giving of garlic with dose of 2 - 3mg /broiler/day was able to decrease cholesterol level contained in the meat and increase the carcass percentage of broiler better than broiler without garlic. Decreasing cholesterol levels in broiler meat is caused by the hypocholesterolemic effect of the active compound of garlic is allicin.

Based on explanation above, it is necessary to produce nutritious broiler meat but low cholesterol, with the addition of garlic extract on ration of broiler. The purpose of this study is to comprehensively examine the extent to know the effectiveness of the addition of garlic extract on ration to reduce cholesterol levels of broiler meat. The specific benefit of this research is giving recommendation the utilization of garlic extraction, especially applied to broiler.

2. Method

The research was divided into two steps: the first step was extraction of garlic, and the second step was in vivo test with the addition of garlic extract in ration and tested the cholesterol level of broiler. Equipment that used in this research include: shaker, autoclave, blender, vacuum rotary evaporator, Erlenmeyer, and beaker glass. Extraction steps of garlic were as follows: 250 grams of garlic mixed with 96% ethanol as much as 500 mL. Garlic solvent obtained was filtered with 2 layers of gauze, then filtered again with whittman paper number 2 obtained filtrate (crude extract). The filtrate is accommodated in an erlenmeyer tube. Subsequently entered it into a vacuum rotary evaporator for to evaporate ethanol and obtain garlic extract. Garlic extract was obtained and taken it as much as 100 mL. The concentration of garlic extract obtained through the above process is 280 mg / 1 mL.

Garlic extract obtained by the process with immune response of broiler. A total of 200 chickens were used in this study. Chicken grouped according to treatment as much as four treatment groups. Each treatment consisted of five replicates and each replication consisted of 10 chickens. The treatments were T1 = control, T1 = 2% garlic extract, T2 = 4% garlic extract and T3 = garlic extract 6%.

Blood sampling test was done on day 35, each replication was taken 3 blood samples, then each chicken was taken as much as 10 ml from axillary vein in the central nerve by using disposable syringe with volume of 10 mL. Blood sample inserted into vacuum tubes containing anticoagulant ethylene diamine tetra acetic acid (EDTA). Procedure of determining total cholesterol level of chicken blood using photometer with method of cholesterol oxidase-peroxidase amino antipyrine phenol (CHOD-PAP) with wavelength 546 and temperature 37°C, while the procedure of calculating white blood cell (leucocytes) by hemocytometer method using Turk solution. The nutrient feed composition is shown in Table 1.

Table 1. Nutrient composition of feed treatment

Ingredients	Experimental Diets			
	1	2	3	4
Energy Metabolism (Kcal.Kg)	3200	3200	3200	3200
Protein (%)	23	23	23	23
Lipid (%)	4	4	4	4
Crude Fiber (%)	4.6	4.6	4.6	4.6
Calcium (%)	0.99	0.99	0.99	0.99
Phosphor (%)	0.35	0.35	0.35	0.35
Garlic Extract (%)	0%	2%	4%	6%

The parameters observed in this research were high density lipoprotein (HDL), low density lipoprotein (LDL), HDL and LDL ratio, and total cholesterol.

2

3. Results and Discussion

The results of the measurement of total cholesterol, HDL, LDL are presented in Table 1. The results of statistical analysis showed that GE addition in ration had significant effect ($P < 0,05$).

Table 2. The level of LDL, HDL, and cholesterol

Parameters	Level of Garlic Extract			
	1	2	3	4
LDL (mg/dl)	101,44 ^c	86,21 ^b	78,24 ^a	72,13 ^a
HDL (mg/dl)	38,21 ^a	55,84 ^b	60,43 ^c	63,91 ^c
Kolesterol total (mg/dl)	186,66 ^c	173,31 ^b	151,11 ^a	146,22 ^a

Liver produces bile salts that play an important role in the process of digestion of fat. Bile salts are stored in the gallbladder. During digestion, the gallbladder contracts and rapidly transfers bile salts into the duodenum through the ductus choledocus. Bile salts are synthesized in the liver from cholesterol, secreted into bile and usually back to the liver through reabsorption in the small intestine (enterohepatic cycle). The use of GE in the diet significantly decreased ($P < 0.05$) total cholesterol levels compared to controls.

Decreasing cholesterol levels due to using of GE on the diet can be explained as follows: in addition to improving the performance of digestive enzymes, GF also decreases cholesterol formation. The synthesis of cholesterol occurs in the liver, which is divided into four stages, the first stage of acetic acid is converted into mevalonate derivatives with enzyme 3-hydroxy-3 methyl glutaryl coA reductase (HMGCoA-reductase), the second stage is the change of mevalonate derivatives into squalene derivatives, the third stage is conversion of the squalene compound into demosterol by the steps of

lanosterol; zimosterol, and kholestadienol, and the fourth stage, the change of demosterol into cholesterol [12].

The effectiveness of GE in lowering total cholesterol levels follows as a mechanism as follows: the primary target of GE is the HMGCoA-reductase enzyme which is the initial enzyme for cholesterol synthesis. Alisin is able to bind to the sulfhydryl group which is the functional part of co-enzyme A in the process of formation the cholesterol [13]. Furthermore alisin, diallyl disulfide, and alilmercaptan inhibit the enzyme HMGCoA-reductase and accumulation of lanosterol [14]. Constraints on the HMGCoA-reductase enzyme are an indication of the absence of cholesterol synthesis and simultaneously inhibit the mevalonate. The last products of fat digestion in the intestine are monoglycerides, fatty acids, cholesterol, phospholipids and triglycerides.

Fat is insoluble in water, so it requires a special carrier to be able to flow with blood throughout the body, in order to soluble the fat bound by proteins, this bond is called lipoprotein. According [12] LDL contains 43% of cholesterol to be sent throughout the body. The main protein that forms LDL is apo-b (apoprotein b) that is easily attached to the blood vessels. Apo-b binds to lipoprotein b that has a high cholesterol content.

The treatments of garlic extract on ration lowers LDL levels (T1, T2 and T3). To determine the percentage decrease in LDL with using GE on ration, calculations were performed by comparing LDL levels of treatments to control. The calculation result of LDL more lower than control, decreasing LDL level 14,58% (GE 2%), 25,82% (GE 4%), and 28,89% (GE 6%). The LDL data of 4% and 6% were close to [15]. Garlic extract supplementation on ration effectively reduced LDL levels by 30% compared to controls.

From the research results can be observed a decrease in LDL levels which with the increasing use of GE on ration. This phenomenon raises the assumption that GE accelerated LDL catabolism followed by cholesterol translocation into cells. Cholesterol in-cell inhibition inhibits cholesterol synthesis [5]. Studies in rats showed that GE of 200 mg/Kg body weight decreased LDL levels significantly [16].

High Density Lipoprotein (HDL) is a high protein content lipoprotein [17] the major proteins that make up HDL are apo-a (apolipoprotein a), low fat content and high density. Statistical analysis showed a significant increase in HDL ($P < 0.005$) in EBP treatment versus control. Decreased HMG-CoA activity by GE affects LDL receptor activity that is central to cholesterol metabolism and increases the apo-a activity binding to HDL. Increased apo-a activity binding to HDL will lower LDL concentrations in the blood.

The HDL level of control was 38.21 mg/dL, while GE treatment resulted in HDL levels between 55.84-63.91 mg/dL, the results of which are close to [18] HDL chickens range from 40.5 to 50.4 mg / dl. Increased HDL levels indicate a response to the treatment. High levels of HDL prevent the risk of atherosclerosis by transporting cholesterol from peripheral tissue to the liver and reducing excessive cholesterol. According to [1], HDL is a lipoprotein that transports lipids from the periphery to the liver.

The HDL molecule is relatively small compared to other lipoproteins, so it can pass through the vascular endothelial cells and enter the intima to transport back the cholesterol collected in the macrophages. In addition, HDL also has anti-oxidant properties that can prevent the occurrence of LDL oxidation. The use of EBP affects HDL levels. In the T1 treatment (GE 2%) HDL levels increased 11.21%, followed by an increase of 20.35% (T2) and 27.28% (T3) compared to controls. According to [19], EBP can raise HDL levels up to 15%.

Based on the results of the study the higher the use of GE in the feed will increase the ratio of HDL and LDL. Reduced LDL in the blood will raise HDL, whereas HDL serves to transport cholesterol from the tissues and blood vessel walls to the liver to be metabolized. The higher level of HDL in the blood will be more cholesterol that can be taken, so that cholesterol levels in the blood will decrease. It is further explained that not only total cholesterol is taken into account but also the ratio of LDL and HDL, ideally LDL as low as possible and HDL as high as possible.

4. Conclusions

From the results of the study concluded that the application of garlic extract in the diet to reduce levels of LDL and cholesterol, raising levels of HDL in broiler chickens.

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