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Research Article
Effect of Garlic (*Allium sativum* L.) Powder on the Cholesterol Content, HDL, LDL and TG in Duck Eggs and Blood

I Nyoman Sukartha Jaya, Ni Ketut Dewi Haryani and Budi Indarsih

Department of Animal Husbandry Production, Faculty of Animal Husbandry, Mataram University, Mataram, Indonesia

Abstract

Objective: This study was conducted to investigate the effect of garlic powder supplementation on the total cholesterol, high-density lipoprotein (HDL) fraction, low-density lipoprotein (LDL) fraction, triglyceride (TG) concentration in egg yolks and blood as well as the feed consumption, feed conversion, egg production and egg weight. **Materials and Methods:** One hundred laying ducks were used in this study. The proportions of garlic powder were0, 3, 6, 9 and 12 g duck^{-1} day^{-1}. Each treatment was consisted of four replicates, resulting in twenty experimental units, with five laying ducks per unit. The data were subjected to analysis of variance testing and further testing was conducted with Duncan's multiple range tests using general linear model procedures and analyses. Analyses were carried out at the end of the study using the ether extraction method and cholesterol oxidase para aminophenazone or enzymatic color tests. **Results:** The results showed that the total cholesterol level in the egg yolk decreased by 54.75 mg dL^{-1} (38.22%) with the administration of 6 g duck^{-1} day^{-1} of garlic powder, the HDL fraction increased by 43.75 mg dL^{-1} (140%) with the administration of 12 g duck^{-1} day^{-1} of garlic powder and the LDL fraction and TG concentration decreased by 67.50 mg dL^{-1} (58.70%) and 182.25 mg dL^{-1} (37.17%) with the administration of 9 g duck^{-1} day^{-1} of garlic powder, respectively. The total blood cholesterol level decreased by 51.00 mg dL^{-1} (35.17%) with the administration of 3 g duck^{-1} day^{-1} of garlic powder; the HDL fraction increased by 14.25 mg dL^{-1} (47.50%) and the TG concentration decreased by 125.00 mg dL^{-1} (35.97%) with the administration of 12 g duck^{-1} day^{-1} of garlic powder and the LDL fraction decreased by 58.75 mg dL^{-1} (57.32%) with the administration of 9 g duck^{-1} day^{-1} of garlic powder. Feed consumption, egg weight, egg production and feed conversion were not affected by the supplementation of garlic powder in feed. **Conclusion:** The results of this study suggest that 6 g duck^{-1} day^{-1} garlic powder supplementation in duck feed can be used to promote specific traits, such as low cholesterol and fat contents.

Key words: Cholesterol, duck eggs, duck blood, garlic powder, duck feed

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Corresponding Author: I Nyoman Sukartha Jaya, Department of Animal Husbandry Production, Faculty of Animal Husbandry, Mataram University, Mataram, Indonesia

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Duck eggs have been used since ancient times; they have become popular because they are widely used as a mixing ingredient for herbs. Eggs can be used in herbal medicines, such as high-strength, youth, beauty and skin care medicines. Duck egg as a food ingredient contains complete and balanced substances and has a higher protein content than eggs of other types of poultry[1] but duck eggs contain high cholesterol. This likely leads consumers to choose livestock products that contain low cholesterol levels to maintain the health of the body because consuming foodstuffs with high cholesterol levels can cause diseases such as atherosclerosis, stroke, coronary heart disease and liver cancer[1]. To overcome these obstacles, certain innovations are needed to reduce the cholesterol level in duck eggs; one of the ways this can be done is by supplementing feed with garlic (*Allium sativum* L.) powder because garlic contains an active compound called allicin (thiopropensulfinic acid allyl ester), which is a compound that is known to inhibit the biosynthesis of cholesterol. Based on the results of research by Jaya *et al.*[2,3], the administration of fresh garlic is difficult due to a very sharp sense of smell in ducks; therefore, supplementing feed with garlic powder will be attempted.

Based on the abovementioned research, an analysis of the content of egg from ducks given garlic (*Allium sativum* L.) powder will be conducted. Several studies on cholesterol in relation to diseases, such as atherosclerosis, stroke, coronary heart disease and liver cancer, have been reported previously[1]. The consumption of food containing high cholesterol results in high plasma cholesterol; thus, cholesterol is classified as a dangerous food ingredient. Cholesterol is a product of animal metabolism that is found in foods derived from animals, such as meat, liver, brain and egg yolks. The cholesterol content of duck eggs is quite high based on a study conducted by Jaya and Syamsuddin[4].

Previous research showed that there was a reduction in the cholesterol levels in blood and meat of laying hens[4-6], quails[7], broilers[8,9] and rats[10,11] after supplementing feed with garlic. However, studies on duck egg cholesterol levels are still limited. Therefore, efforts, such as adding garlic (*Allium sativum* L.) powder in feed, to reduce the cholesterol levels in duck eggs are needed. This study aimed to investigate the effect of garlic powder on the total cholesterol, HDL, LDL, TG concentration in egg yolks and blood as well as the feed consumption, feed conversion, egg production and egg weight of duck.

MATERIALS AND METHODS

A total of 100 laying ducks were used in this study; the ducks were randomly divided into five treatments. The first treatment (control) was local feed without garlic powder, whereas treatments two, three, four and five were local feed with garlic powder supplementation at 3, 6, 9 and 12 g duck^{-1} day^{-1}, respectively. Each treatment consists of four replicates, so there were 20 units of experiment and each unit contains five laying ducks. The animal was fed restrictedly, while drinking water was given *ad libitum*. On day 43rd, some evaluations on total cholesterol, HDL, LDL and TG content in yolk and blood and the level of consumption and conversion of feed, egg production and weight were performed.

Evaluations of total cholesterol, HDL, LDL and TG levels were carried out by separating the yolk from the white using a yolk separator and weighing the yolk. Two egg yolks were shaken until homogenized and then 5 mL of the homogenized egg yolks and 5 mL of alcohol-acetone in a 1:1 (v/v) solution were poured to the test tube. The test tube was immediately covered with aluminum foil, mixed until the filtrate aggregated, heated in hot water until the solution boiled and then rested for 3 min. The mixture was centrifuged at 8000 rpm for 10 min and then filtered.

Experimental procedure: The examination of egg yolk total cholesterol was carried out by filling test tubes with 10 µL of yolk filtrate and mixing with cholesterol reagents until the volume equaled 1000 µL; one tube that only contained cholesterol reagent and distilled water was prepared as blank solution. All the tubes were incubated for 10 min. Then, the absorbance was measured by spectrophotometer with a wavelength of 546 nm and a factor of 853 for yolk total cholesterol and a factor of 1040 for egg yolk triglycerides.

The examination of egg yolk HDL was carried out by adding 200 mL of egg yolk filtrate with 500 mL of HDL reagent and centrifuging for 10 min at 3000 rpm to obtain the supernatant. Subsequently, 100 mL of the supernatant was mixed with the HDL reagent until the volume reached 1000 mL; one tube contained only HDL reagent and distilled water was prepared as a blank solution and all the tubes were incubated for 10 min. The absorbance was then measured using a spectrophotometer with a wavelength of 546 nm and a factor of 325.1. A similar procedure was implemented to examine egg yolk LDL, except 100 mL of LDL reagents and a factor of 519.4 were used. The data were subjected to one-way analysis of variance (ANOVA). The differences between the means of the groups were identified by Duncan's multiple

range tests at a 5% significance level. Egg yolk and blood cholesterol, HDL, LDL and TG analyses were carried out at the end of the study using the ether extract method and the cholesterol oxidase para aminophenazone (CHOD-PAP) or enzymatic color tests.

RESULTS AND DISCUSSION

The average data of the total cholesterol, HDL fraction, LDL fraction and TGs of duck eggs and blood are presented in Table 1.

Total cholesterol level in duck egg yolk: Table 1 shows that there was a significant decrease ($p \leq 0.05$) in the total cholesterol levels in duck egg yolks due to the supplementation of garlic powder in feed. The lowest amount of egg yolk cholesterol, at 88.50 mg dL^{-1}, was associated with the administration of 6 g $duck^{-1}$ day^{-1} of garlic powder, while the highest egg yolk cholesterol, at 143.25 mg dL^{-1}, was associated with no garlic powder supplementation (0 g $duck^{-1}$ day^{-1}). Notably, the level of total cholesterol in egg yolks was quite low, i.e., 54.75 mg dL^{-1} (38.22%). The administration of garlic powder at 6, 9 and 12 g $duck^{-1}$ day^{-1} did not have a significant effect on cholesterol ($p \geq 0.05$). This means that 3 g $duck^{-1}$ day^{-1} of garlic powder is enough to see effects and additional increases will not cause changes in the level of egg yolk total cholesterol. This is in line with the study conducted by Khan *et al.*[5] who showed that garlic powder supplementation can decrease cholesterol content of egg yolk. The relationship between garlic powder dose and total cholesterol in egg yolk was linear, with the equation $Y = 132.5\text{-}9.95\ X$ and a coefficient of determination (R^2) of 33.8%, meaning that a high dose of garlic powder lowered the total cholesterol level in egg yolks.

HDL fraction in egg yolk: Table 1 shows that there was a significant association between garlic powder supplementation ($p \leq 0.01$) and the HDL fraction in egg yolk. Increasing doses of garlic powder in feed will lead to increasingly high HDL in egg yolks. This is in line with the opinion of Piliang and Djojosoebagio[12,13] who suggested that an increased HDL fraction is a positive trait because HDL is considered good cholesterol. Furthermore, when analyzed by simple regression, the relationship between the dose of garlic powder and the HDL fraction in egg yolk was linear, with the line equation $Y = 19.25+11.00\ X$ and a coefficient of determination (R^2) of 84.6%, meaning that the higher the dose of garlic powder is, the higher the HDL fraction of the egg yolk produced. This egg-yolk HDL fraction amounted to 43.75 mg dL^{-1} (140%).

LDL fraction in egg yolk: Overall, this study showed that the effect of garlic powder supplementation in feed significantly ($p \leq 0.01$) decreased the LDL fraction in egg yolk. The lowest egg yolk LDL fraction (47.50 mg dL^{-1}) was associated with 9 g $duck^{-1}$ day^{-1} garlic powder, while the highest (115 mg dL^{-1}) was associated with no garlic powder supplementation (0 g $duck^{-1}$ day^{-1}). The magnitude of this decline was approximately 67.50 mg dL^{-1} (58.70%). The differences in the LDL fractions of egg yolks from ducks without garlic powder supplementation (control) and ducks that received 6, 9 and 12 g $duck^{-1}$ day^{-1} of garlic powder were very significant ($p \leq 0.01$). However, the differences between ducks that received 6, 9 and 12 g $duck^{-1}$ day^{-1} of garlic powder supplementation was not significant ($p \geq 0.05$). Nevertheless, there is a numerically visible indication of a decrease in the egg yolk fraction value. The relationship between the dose of garlic powder in feed with the LDL fraction of egg yolk was linear, with the line equation $Y = 135.50\text{-}18.50\ X$ and a coefficient of determination (R^2) of 57.3%, meaning that the higher the dose of garlic powder in the feed is, the lower the LDL fraction in the egg yolk. Overall, from the results of this study, an increased dose of garlic powder in feed will reduce the LDL fraction of egg yolk. This is in line with the study by Piliang and Djojosoebagio[12,13] who showed that LDL

Table 1: The average levels of total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL) and triglycerides (TGs) in duck egg yolks and blood according to the level of garlic powder supplementation (mg dL^{-1})

	Garlic powder supplementation (g $duck^{-1}$ day^{-1})				
Parameters	0	3	6	9	12
Egg yolk cholesterol	143.25±6.55^{a}	94.50±18.84^{b}	88.50±14.84^{b}	92.50±10.38^{b}	94.50±20.02^{b}
HDL fraction egg yolk	31.25±4.79^{a}	40.00±9.13^{a}	52.50±6.45^{b}	62.50±9.57^{b}	75.00±7.07^{c}
LDL fraction egg yolk	115.00±29.44^{a}	112.50±22.17^{a}	70.00±25.17^{b}	47.50±18.93^{b}	55.00±14.14^{b}
TG egg yolk	490.25±64.23^{a}	349.50±20.44^{b}	316.50±39.53^{b}	308.00±0.55^{b}	318.25±36.89^{b}
Blood cholesterol	145.00±9.13^{a}	94.00±15.08^{b}	97.00±23.37^{b}	110.75±17.19^{bc}	132.50±12.36
HDL blood fraction	30.75±7.23^{a}	35.00±8.16^{ab}	30.00±5.77^{a}	42.50±5.00^{bc}	44.25±1.71^{bc}
LDL blood fraction	102.50±27.54^{a}	85.00±35.12^{ab}	65.00±20.82^{ab}	43.75±17.97^{c}	55.00±20.82^{bc}
Blood triglycerides	342.50±37.75^{ab}	347.50±96.05^{a}	227.50±26.30^{c}	257.50±67.02^{ac}	222.50±17.08^{c}

^{a,b}Different superscripts within the same row indicate significant differences ($p \leq 0.05$)

(low-density lipoprotein) has the main function of depositing cholesterol in the tunica intima blood vessels to prevent plaque *atherosclerosis* in the area. Therefore, a low LDL fraction is good for the body and may result in a decrease in the total cholesterol level.

Triglycerides in egg yolk: As shown in Table 1, garlic powder supplementation very significantly ($p \leq 0.01$) lowered egg yolk triglyceride levels. The lowest egg yolk triglyceride level (308.00 mg dL^{-1}) occurred in the ducks given feed with 9 g $duck^{-1}$ day^{-1} of garlic powder, while the highest (490.25 mg dL^{-1}) was present in ducks that were not given garlic powder (0 g $duck^{-1}$ day^{-1}). The difference in magnitude was approximately 182.25 mg dL^{-1} (37.17%). The differences in the egg yolk triglyceride levels in laying ducks that were not given garlic powder (0 g $duck^{-1}$ day^{-1}) and ducks that were given 3, 6, 9 and 12 g $duck^{-1}$ day^{-1} of garlic powder were very significant ($p \leq 0.01$). However, the opposite was observed between laying ducks that were given 6, 9 and 12 g $duck^{-1}$ day^{-1} of garlic powder ($p \geq 0.05$). Nevertheless, there is a numerically visible indication of a decrease in the level of egg yolk triglycerides. This is in line with the study conducted by Khan *et al.*[5] and Sutama[6]who showed that supplementation of garlic powder can decrease triglyceride level of egg yolk. The relationship between the garlic powder dose and the egg yolk triglyceride level was linear, with the equation Y = 427.15-38.55 X and a coefficient of determination (R^2) of 51.2%, meaning that the higher dose of garlic powder in the feed is, the lower the egg yolk triglyceride level (37.17%). However, at 12 g $duck^{-1}$ day^{-1} of garlic powder, a difference in the egg yolk triglyceride level was observed. This means that laying ducks can consume up to 9 g $duck^{-1}$ day^{-1} of garlic powder but more than 9 g $duck^{-1}$ day^{-1} (e.g., 12 g $duck^{-1}$ day^{-1}) should be considered with caution. Overall, the results of this study showed that an increased dose of garlic powder reduced the egg yolk triglyceride level.

Total blood cholesterol levels ducks laying: As seen in Table 1, there was a very significant ($p \leq 0.01$) effect of garlic powder on the total blood cholesterol. The highest total blood cholesterol levels were observed at 0 g $duck^{-1}$ day^{-1} (control) of garlic powder, while the lowest was observed at 3 g $duck^{-1}$ day^{-1} of garlic powder. This decline amounted to 51 mg dL^{-1} (35.17%). There was no significant difference between the control (without the supplementation of garlic powder) and 12 g $duck^{-1}$ day^{-1} of garlic powder ($p \geq 0.05$) and there was no difference between 3, 6, 9 g $duck^{-1}$ day^{-1} of garlic powder. This means that supplementation of 3 g $duck^{-1}$ day^{-1} of garlic powder in feed is efficient. This is in line with the studies conducted by Jaya *et al.*[3] and Jaya and Syamsuddin[4] who showed that supplementation of garlic powder can decrease duck blood cholesterol. The relationship between the dose of garlic powder in the feed and the total blood cholesterol levels in the ducks was linear, with the equation Y = 118.25 - 0.925X and a coefficient of determination (R^2) of 0.3%, meaning that the higher the dose of garlic powder in the feed is, the lower the level of total blood cholesterol (35.17%).

Blood HDL fraction in laying ducks: The main function of the HDL fraction is its ability to transport cholesterol from the peripheral tissues to the liver to undergo degradation. The broken-down products are excreted in the bile. According to these properties, it can be interpreted that an increased HDL fraction in the blood is good for livestock because an increased amount of cholesterol deposits in the peripheral tissues can be transported to the liver to be degraded by bile[8,9]. The HDL fractions in the blood of laying ducks are shown in Table 1.

Table 1 shows that there was a significant effect ($p \leq 0.05$) of garlic powder on the HDL fraction in blood. The HDL fraction increased by 14.25 mg dL^{-1} (47. 50%), from 30.0-44.25 mg dL^{-1}. According to the Duncan's multiple range test results, the differences between ducks that were not given garlic powder (0 g $duck^{-1}$ day^{-1}), ducks that were given 3 and 6 g $duck^{-1}$ day^{-1} of garlic powder and ducks that were given 9 and 12 g $duck^{-1}$ day^{-1} were significant ($p \leq 0.05$). This is in line with the study by Jaya *et al.*[3] and Jaya and Syamsuddin[4]which showed that supplementation of garlic powder can increase fraction of HDL in ducks' blood.

The relationship between the garlic powder supplementation dose and the blood HDL fraction in laying ducks was linear line, with the equation Y = 26.15+3.45 X and a coefficient of determination (R^2) of 38.6%, meaning that the higher the dose of garlic powder in the feed is, the higher the blood HDL fraction in laying ducks (47.50%).

Blood LDL fraction in laying ducks: LDL deposit cholesterol in the tunica intima blood vessels to prevent plaque *atherosclerosis* on the area[8,9]. Therefore, a low LDL fraction in the blood is good for the health of laying ducks. The blood LDL fraction of laying ducks in the study is shown in Table 1.

Table 1 shows that there was a significant influence ($p \leq 0.05$) associated with the blood LDL fraction in laying ducks. The lowest blood LDL fraction in laying ducks (43.75 mg dL^{-1}) was observed in the group of ducks given 9 g $duck^{-1}$ day^{-1} of garlic powder, while the highest (102.50 mg dL^{-1}) was observed in the laying ducks that did not

Table 2: The average feed consumption, egg weight, egg production and feed conversion of laying ducks according to the level of garlic powder supplementation

	Garlic powder supplementation (g duck^{-1} day^{-1})				
Parameters	0	3	6	9	12
Feed consumption (g duck^{-1} day^{-1})	152.54±3.66[a]	150.52±2.92[a]	153.13±2.76[a]	149.18±3.03[a]	149.18±3.79[a]
Egg weight (g duck^{-1})	71.50±5.51[a]	69.75±6.65[a]	73.00±3.16[a]	68.00±4.69[a]	70.75±2.63[a]
Egg production (eggs/6 week)	108.75±7.77[a]	100.50±15.76[a]	87.50±42.25[a]	93.25±25.00[a]	77.50±43.88[a]
Feed conversion	2.14±0.11[a]	2.22±0.13[a]	2.10±0.08[a]	2.20±0.12[a]	2.11±0.07[a]

[a]Similar superscripts within the same row indicate no significant difference ($p \geq 0.05$)

consume garlic powder. The magnitude of the decline was approximately 58.7 mg dL^{-1} (57.32%). Overall, this study showed that garlic powder supplementation significantly ($p \leq 0.05$) lowered the blood LDL fraction of the laying ducks. This is in line with a previous study conducted by Jaya *et al.*[3] and Jaya and Syamsuddin[4] who showed that supplementation of garlic powder can decrease fraction of LDL in duck's blood. The blood LDL fraction between laying ducks who did not receive garlic powder supplementation and ducks that received 6, 9 and 12 g duck^{-1} day^{-1} of garlic powder were significantly decreased ($p \leq 0.05$). However, the differences between ducks that received 6, 9 and 12 g duck^{-1} day^{-1} of garlic powder were not significantly different ($p \geq 0.05$). The relationship between the dose of garlic powder in the feed with the blood LDL fraction in laying ducks was linear, with the equation Y = 111.125-13.625 X and a coefficient of determination (R^2) of 40.3%, meaning that the higher the dose of garlic powder is, the lower the blood LDL fraction in laying ducks. Overall, according to the results of this study, an increased dose of garlic powder in duck feed will decrease the LDL fraction of the blood in laying ducks. This is in line with the study conducted by Piliang and Djojosoebagio[12,13] who showed that LDL (low-density lipoprotein) has the main function of depositing cholesterol in the tunica intima blood vessels to prevent plaque *atherosclerosis* in the area. Therefore, a low LDL fraction is good for the body and may result in a decrease in total cholesterol.

Blood triglycerides in laying ducks: As seen in Table 1, garlic powder supplementation significantly ($p \leq 0.05$) lowered the blood triglyceride level in laying ducks. The lowest blood triglyceride level (222.5 mg dL^{-1}) was observed in ducks given 12 g duck^{-1} day^{-1} of garlic powder, while the highest (347.5 mg dL^{-1}) was observed in ducks that consumed 3 g duck^{-1} day^{-1} of garlic powder. The difference in the magnitude was approximately 125 mg dL^{-1} (35.97%). The blood triglyceride levels in the ducks that did not receive garlic powder (0 g duck^{-1} day^{-1}) and the ducks that were given 3, 9 and 12 g duck^{-1} day^{-1} of garlic powder were significantly decreased ($p \leq 0.05$). However, the differences between laying ducks given 6, 9 and 12 g duck^{-1} day^{-1} of garlic powder were not significant ($p \geq 0.05$). Nevertheless, there was a numerically visible indication of impairment of blood triglyceride levels in laying ducks that were not given garlic powder compared with ducks supplemented with 3 and 9 g duck^{-1} day^{-1}, although the difference was non-significant ($p \geq 0.05$). This is in line with the study conducted by Jaya *et al.*[3] and Jaya and Syamsuddin[4] who showed that supplementation of garlic powder can decrease TG in duck's blood. The relationship between the garlic powder dose and blood triglyceride level in the laying ducks was linear, with the equation Y = 378.5-33 X and a coefficient of determination (R^2) of 40.1%, meaning that the higher the dose of garlic powder in feed is, the lower the level of blood triglycerides (35.97%).

Feed consumption, egg weight, egg production and feed conversion: The average feed consumption, egg weight, egg production and feed conversion in the laying ducks fed garlic powder during the study are listed in Table 2.

Table 2 shows that the administration of fresh garlic did not affect ($p \geq 0.05$) the feed consumption duck^{-1} day^{-1}. Although, the results were not significantly different, numerically, there was a tendency for the increase in feed consumption. This means that the administration of fresh garlic can increase feed consumption due to the garlic content, which stimulates the appetite of laying ducks. Similar results regarding egg weight, egg production and feed conversion were also observed. Garlic powder supplementation numerically increased egg weight and production as well as feed conversion but these differences were not significantly different from the control group ($p \geq 0.05$). This finding was closely applicable to feed consumption, as increased egg weight and production showed a tendency to increase with an increase in feed consumed.

CONCLUSION

It is concluded that 6 g duck^{-1} day^{-1} garlic powder supplementation in duck feed can be used to promote specific traits, such as low cholesterol and fat contents.

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