Jurnal Agripet
p-ISSN: **1411-4623** | e-ISSN: **2460-4534**
available online at http://jurnal.usk.ac.id/agripet

Vol 23 (1): 91-97, April 2023
Accredited: 28/E/KPT/2019

Effect of Using Black Garlic Instead of Fresh Garlic in The Ration on The Performances and Blood Cholesterol Properties of Quail

(Pengaruh penggunaan bawang hitam sebagai pengganti bawang putih dalam ransum terhadap performa dan kadar kolesterol darah puyuh)

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ABSTRACT. This study aimed to determine the effects of using black garlic (BG) instead of fresh garlic (FG) in the ration on the performances and quail blood cholesterol properties. Two hundred 3-week-old female quails were fed five experimental diets for 8 weeks. Each treatment was repeated four times with ten quails each. The treatment diets were: T0= commercial feed (CF) without FG and BG supplementation, T1= CF + 3% FG, T2= CF + 2% FG + 1% BG, T3= CF + 1% FG + 2% BG, and T4= CF + 3% BG. The parameters measured were feed consumption, egg production, egg weight, egg mass, feed conversion ratio, meat quality, and blood cholesterol properties. This study found no significant (P>0.05) difference among treatment groups on feed conversion, egg weight, egg mass, total cholesterol, LDL, and HDL. Meat fat decreased significantly (P<0.05) by increasing levels of dietary black garlic. A diet with black garlic supplementation significantly increased feed consumption, egg production, and meat protein (P<0.05). It is concluded that substituting 100% garlic with black garlic in the diet could increase quail performance without adverse effects on blood cholesterol properties.

Keywords: black garlic, cholesterol, garlic, meat quality, quail performance

ABSTRAK. Penelitian ini bertujuan untuk mengetahui pengaruh penggunaan bawang hitam (BG) sebagai pengganti bawang putih (FG) dalam ransum terhadap performa dan kolesterol darah puyuh. Dua ratus ekor puyuh betina berumur 3 minggu diberi pakan perlakuan selama 8 minggu dan diulang sebanyak empat kali. Setiap unit perlakuan terdiri dari sepuluh ekor puyuh. Pakan perlakuan adalah: T0= pakan komersial (CF) tanpa suplementasi FG dan BG, T1= CF + 3% FG, T2= CF + 2% FG + 1% BG, T3= CF + 1% FG + 2% BG, dan T4= CF + 3% BG. Parameter yang diukur adalah konsumsi pakan, produksi telur, berat telur, massa telur, rasio konversi pakan, kualitas daging, dan sifat kolesterol darah. Hasil penelitian memperlihatkan bahwa tidak ada pengaruh yang nyata (P>0,05) antar kelompok perlakuan terhadap konversi pakan, bobot telur, massa telur, kolesterol total, LDL, dan HDL. Lemak daging menurun secara signifikan (P<0,05) dengan peningkatan kadar bawang hitam dalam ransum. Pakan yang disuplementasi dengan bawang hitam nyata meningkatkan konsumsi pakan, produksi telur, dan protein daging (P<0,05). Disimpulkan bahwa penggantian 100% bawang putih dengan bawang hitam dalam pakan dapat meningkatkan performa puyuh tanpa memberikan efek negatif terhadap kolesterol darah.

Kata kunci: bawang hitam, bawang putih, kolesterol, kualitas daging, performa puyuh

INTRODUCTION

Quail (*Coturnix-coturnix Japonica*) eggs are well known poultry product has containing high cholesterol of which human prevent not to consume those excessively even though they are rich in protein. Cholesterol especially low-density lipoprotein (LDL) in poultry's meat and eggs can be decreased by feeding the birds with the diet containing feed additives willingly herbs or natural feed additive ingredients such as garlic. Previous research proved that garlic could reduce cholesterol in the blood (Lonkar *et al.*, 2009; Khan *et al.*, 2008; Dehkordi *et al.*, 2010) and in the meat (Dehkordi *et al.*, 2010; Kim *et al.*, 2009). However, garlic also has some impacts in decreasing body weight (Abdullah *et al.*, 2010), nutrient content of meat, aroma, and taste. Therefore, it is necessary to do pre-treatment such as heating and fermentation on the garlic before giving it to the animal, especially poultry. This treatment is known as black garlic (Sasaki *et al.*, 2007; Kang, 2016).

Sasaki *et al.* (2007) found that black garlic contained higher methionine and arginine than fresh garlic. Methionine is an amino acid containing sulfur that plays an important role in protein synthesis. Garlic contains 31.56 mg/100 grams of methionine while the black garlic is twice with approximately 78.11 mg/100 grams (Choi *et al.*, 2014). L-arginine is an additive and its supplementation into the ration was able to inhibit lipogenesis and lipase enzyme activity and could increase lipolysis in the body, thus, the fat deposition would also decrease (Wu *et al.*, 2011; Fouad *et al.*, 2013). Besides, black garlic also is rich in organosulfur (Bae *et al.*, 2012), phenol, and flavonoid (Kim *et al.*, 2013), and antimicrobial

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Diterima: 16 Agustus 2022
Direvisi: 9 Maret 2023
Disetujui: 29 Maret 2023
DOI: https://doi.org/10.17969/agripet.v23i1.27604

activity compounds and its cysteine amino acid is 5 times higher than fresh garlic (Bae *et al.,* 2014) which is beneficial to reduce fat content in the blood (Kim *et al.,* 2011). With these active compounds and nutrients, black garlic was expected to improve quail performances and the quality of egg production, blood cholesterol properties (total cholesterol, LDL, and HDL), and meat quality (fat and crude protein content). There is still a lack of information on using black garlic as a natural feed additive in quail ration. Therefore, it is important to determine the effect of black garlic supplementation into the ration to replace garlic on quail egg production, blood cholesterol and meat quality.

MATERIALS AND METHODS

Animals and Housing

This study was conducted in the Farm and Business Unit of the Faculty of Animal Science, Universitas Jambi, and was supervised by The Committee of Ethical Clearance for Health Faculty of Medicine and Health Sciences, Universitas Jambi. Two hundred 3-week-old female quails (*Coturnix coturnix Japonica*) were used in this study and kept for 8 weeks in the colony cages in 1 ×1 × 0.75 m size. Each cage was equipped with a feeder, drinker, and 5 watts lighting lamp. The main cage was supplied with 40 watts lighting lamp. The temperature of the housing was maintained at 27 – 30˚C.

Diets and Treatments

The feeding trial was designed in a completely randomized design with 5 groups of treatment diets, 4 replications, and 10 birds each. The birds were fed the diets which were commercial feed supplemented with fresh garlic (FG) and black garlic meal (BG) at the level of 0, 1, 2, and 3%, respectively; T0= a control diet, commercial feed without FG and BG supplementation, T1= 3% FG + 0% BG, T2 = 2% FG + 1% BG, T3 = 1% FG + 2% BG, and T4= 0% FG + 3% BG. Black garlic was produced based on Nelwida *et al.* (2019). Garlic was sorted from broken and dirty materials, and then wrapped using aluminum foil and fermented in a rice cooker with the temperature of 65-70°C and kept warm for 17 days. Thereafter, black garlic was harvested and milled to be mixed into the ration. The nutritional composition of the treatment diets is shown in Table 1.

Table 1. Nutrition Composition of Treatment Diets

Nutrient	T0	T1	T2	T3	T4
Dry Matter (%)	91.29	92.36	92.32	92.29	92.25
Crude Protein (%)	18.50	19.00	18.99	18.98	18.97
Fat (%)	4.43	4.58	4.56	4.55	4.54
Crude Fibre (%)	3.00	3.06	3.06	3.05	3.05
Ash (%)	11.17	11.35	11.33	11.31	11.29
Ca (%)	3.00	3.42	3.41	3.40	3.39
Metabolizable Energy (kcal/kg)	2900.00	2952.50	2949.10	2945.70	2942.30

Note: T0 = control diet, commercial feed, T1= T0 + 3% garlic, T2 = T0 + 2% garlic + 1% black garlic, T3= T0 + 1% garlic + 2% black garlic, and T4= T0 + 3% black garlic.

Parameter Observed

The observed parameters were feed consumption, egg production, egg weight, egg mass, feed conversion ratio, blood cholesterol (total cholesterol, LDL, and HDL), and meat quality (fat and crude protein). The quails were weighed at the beginning and at the end of the experiment. Feed consumptions were recorded every week. Eggs were collected and weighed daily. Egg mass was computed by multiplying the percentage of egg production by average daily egg weight and feed conversion ratio (FCR) was calculated by dividing feed consumption by egg mass (Zulfan *et al*., 2020).

Eight weeks after feeding trial, two quails were randomly chosen and slaughtered for blood collection to measure blood cholesterol profile. Before being slaughtered, the quails fasted for 6 hours. Total cholesterol, LDL, and HDL were determined using the cholesterol oxidase - para amino antipyrine (CHOD-PAP) method described by Valtek diagnostics (1997).

Statistical Analysis

The data were statistically analyzed by using One-way Analysis of Variance (ANOVA) and identifications of any significant effect on the parameter were tested by Duncan's Multiple Range Test (Steel and Torrie, 1989)

RESULTS AND DISCUSSION

Quail Performance

The mean values of feed consumption, egg production, egg weight, egg mass, and feed conversion ratio of laying quails fed the treatment diets were shown in Table 2. Analysis of variance showed that the supplementation of fresh garlic meal (FG) and black garlic (BG) had a significant effect ($P<0.05$) on feed consumption, egg production and egg mass but no significant effect on egg weight and feed conversion ratio ($P>0.05$). Duncan multiple range tests resulted in significant higher feed consumption of quails fed a ration containing 3% BG (T4) than those fed either a control diet (T0), diet with 3% FG (T1), or diet with 2% FG + 1% BG (T2). Egg production and egg mass were higher in the T4 than in the T0 and T1. The average feed consumption in this study ranged from 21.05 – 22.68 grams/head/day. Egg production was found ranging from 68.37 – 76.61%, egg mass was ranged from 6.92 – 7.63 g/head while the feed conversion ratio was between 2.95 and 3.05.

Table 2. Effect of Supplementation Garlic and Black Garlic on Quail Performance (mean ± SD)

Parameter	Treatments				
	T0	T1	T2	T3	T4
Feed Consumption (g/head/day)	21.05 ± 0.4^{c}	21.44 ± 0.8^{c}	21.55 ± 0.6^{bc}	22.31 ± 0.5^{ab}	22.68 ± 0.2^{a}
Egg Production (%)	68.37 ± 1.4^{d}	72.32 ± 3.4^{bc}	74.94 ± 3.1^{ac}	75.72 ± 1.8^{ac}	76.61 ± 1.3^{a}
Egg weight (g/egg)	10.118±0.28	9.720±0.22	9.768±0.21	9.765±0.43	9.968±0.37
Egg mass (g/head)	6.92 ± 0.31^{b}	7.03 ± 0.34^{b}	7.32 ± 0.18^{ab}	7.39 ± 0.37^{ab}	7.63 ± 0.27^{a}
Feed Conversion Ratio	3.045±0,13	3.053±0,08	2.945±0,08	3.022±0,14	2.973±0,11

Note: Different superscripts on the same line means significantly different ($P<0.05$). T0 = control diet, commercial feed, T1= T0 + 3% garlic, T2 = T0 + 2% garlic + 1% black garlic, T3= T0 + 1% garlic + 2% black garlic, and T4= T0 + 3% black garlic.

Quail feed consumption in the current study was higher in the group of quails fed a ration supplemented with 3% black garlic than in other treatment groups. This indicated that black garlic can increase appetite supposedly due to the several active compounds in the black garlic higher than in the fresh garlic (Bae *et al.*, 2012; Kim *et al.*, 2013). Besides, heated garlic could reduce a bad aroma of garlic and made it tasty. Even though, the increase in feed consumption was still in a normal condition for the quail. This finding was contrary to Lim *et al.* (2006) who found that the addition of garlic until 5% did not affect feed consumption of laying hens. It means that supplementation of garlic and black garlic into the ration has an effect on quail appetite and feed palatability. It indicated that black garlic could be used to improve bird performances because increasing feed consumption could increase body weight gain, egg production and egg mass.

Egg productions and egg mass of the quails fed a ration containing 3% BG were higher than that of other quail treatment groups. It is due to the active compounds and cysteine amino acid of heated garlic being higher up to 5 times than those in the fresh garlic and richer in organosulfur (Bae *et al.*, 2012), phenol, and flavonoid (Kim *et al.*, 2013) compounds. It was in line with Saraswati *et al.* (2013) who found that quail egg production is influenced by several factors such as maintenance management, ration quality, ration consumption, environmental conditions, and quail genetics. Berliana *et al.* (2018) stated that the egg production and egg weight of quail were affected by the quality of the ration, especially the protein content and the addition of black garlic in the quail ration had not been able to increase the weight of the egg. Thereafter, the similar result was also reported by Saraswati *et al.* (2013) that feeding ration contained organic herbs as natural feed additives such as turmeric which is containing curcuminoid compounds increased quail egg production. Female quail started to lay eggs at 40-50 days of age with the egg weight ranging from 8-12 grams and can produce as many as 300 eggs/head/year..

Blood Cholesterol Properties

The effect of supplementation of black garlic into the diet on the properties of blood cholesterol relating to total cholesterol, LDL, and HDL were shown in Table 3. There was no significant difference ($P>0.05$) of blood cholesterol (total cholesterol, LDL, and HDL) among the treatment groups but total blood cholesterol tended to decrease in the group with 3% FG (T1) rather than in the group with 3% BG (T4). This difference might be due to the difference of the concentration of bioactive compounds which were higher in black garlic

rather than in fresh garlic so that serum fat profiles such as triglycerides were improved (Jung *et al.*, 2011). It indicated that black garlic could be consumed by people who have problems with their blood cholesterol without any adverse effects on smell and taste.

Table 3. Mean values (±SD) of blood cholesterol properties and meat quality of quail-fed treatment diets

Parameter	Treatments				
	T0	T1	T2	T3	T4
Meat Protein (%)	21.54±0.27^{b}	21.94±0.84^{ab}	22.81±1.10^{a}	22.91±0.75^{a}	23.51±0.52^{a}
Meat Fat (%)	4.84 ± 0.69^{a}	3.92 ± 1.14^{a}	3.92 ± 0.63^{a}	3.44±0.42^{ab}	2.71±0.45^{b}
Total Cholesterol (mg/dl)	219.35±7.59	228.55±24.76	224.25±15.94	222.90±2.91	225.85±9.27
LDL (mg/dl)	44.25±7.89	48.25±7.04	39.00±8.41	37.00±2.31	37.25±3.30
HDL (mg/dl)	161.50±1.60	165.50±20.40	170.75±19.45	171.75±2.75	174.75±10.44

Note: Different superscripts on the same line means significantly different (P<0.05). T0 = control diet, commercial feed, T1= T0 + 3% garlic, T2 = T0 + 2% garlic + 1% black garlic, T3= T0 + 1% garlic + 2% black garlic, and T4= T0 + 3% black garlic.

The cholesterol content found in the present study was higher than that of in the previous studies. Blaszczyk *et al.* (2006) reported that cholesterol content in the female quail during a production period was around 180-220 mg/dl, 139 mg/dl, and 144 mg/dl (Elnagar and Abd-Elhady, 2009). Isnaeni *et al.* (2010) found that blood cholesterol content in quail at 17 weeks was 148.10 mg/dl. The high level of blood cholesterol was probably caused by the quails were still in the production period for which they needed cholesterol to form egg yolks. In addition, cholesterol was needed for making several types of hormones of reproductive organs such as estrogen, testosterone, and others. Modaresi and Taromsari (2013) stated that garlic could increase the secretion of the estrogen and progesterone in the female reproductive system. Garlic also influences the concentration of estrogen, the more follicles produced will increase the concentration of the estrogen in the blood. The lower total cholesterol level in the treatment group of black garlic supplementation was supposed black garlic had better efficacy in synthesizing cholesterol for follicle formation than garlic so that it easily stimulated an ovulation. This result was in line with Elnagar and Abd-Elhady (2009), who stated that cholesterol and triglycerides were part of the vitellogenin components. Vitellogenin would be transported to the ovaries for the growth of the follicular hierarchy. More follicles formed means more cholesterol into vitellogenin so that blood cholesterol would decrease.

Total cholesterol content in blood, the LDL content of quail blood, was also not different among treatment groups. However, the LDL content of the quails fed ration supplemented with black garlic was lower than that of other treatment groups even though they were not significant different. It could be explained that the active compounds in black garlic were much higher than in garlic (Sasaki *et al.*, 2007; Bae *et al.*, 2012; Choi *et al.*, 2014; Bae *et al.*, 2014) causing the increased unsaturated fatty acids content and the decreased saturated fatty acids so that levels of LDL in the quail blood that plays a role in providing cholesterol in body tissues. LDL is the main component for cholesterol that was distributed from the liver to the body tissues through the blood, thus, LDL levels in the blood are also influenced by cholesterol concentrations.

The HDL content of quail blood among treatment groups was not influenced either by garlic or black garlic supplementation. However, the HDL content in the quail group fed ration supplementing black garlic tended to be higher than in other groups. HDL is a high-density lipoprotein that has the function of removing excess cholesterol and carrying it to the liver to metabolize into bile salts. There was a positive correlation between cholesterol levels with LDL and HDL in the blood, an increase in blood cholesterol was influenced by increasing blood LDL and HDL. The variation of blood cholesterol levels was affected by 91.8% by blood LDL levels, increased every 1 mg/dl of blood LDL level might increase blood cholesterol level of 1,082 mg/dl. The variations of blood cholesterol levels were influenced by 26.3% by levels of blood HDL, an increase every 1 mg/dlof blood HDL levels might increase 1,741 mg/dl of blood cholesterol (Hasanuddin *et al.*, 2013). The result of the present study stated that black garlic was better than garlic to improve blood cholesterol properties; reducing total cholesterol and LDL and increasing HDL content in the blood.

Meat Quality

The meat quality that is measured by fat and crude protein content of quail-fed ration

containing black garlic is shown in Table 3. Supplementation of garlic and black garlic into the quail ration had a significant effect ($P<0.05$) on meat quality based on crude protein and fat content. There was a significantly higher crude protein content of meat of quail group fed ration supplemented by 3% of black garlic (T4) than that of quail fed a control diet (T0). Quails fed a ration supplemented by 3% black garlic had lower meat fat content than other treatment groups; without any supplementation and with garlic supplementation (T0, T1, and T2).

Protein and fat contents within the meat were significantly affected by black garlic supplementation in the diet. This might be caused by the amino acid content, especially methionine which plays an important role in protein synthesis was higher in black garlic than in garlic (Bae *et al.,* 2012). The availability of protein as a substrate was closely related to the metabolism of the body's protein, especially protein synthesis, which results in the deposition of body proteins which ultimately affects the growth. Protein has an important role in tissue formation, the consumed protein would be broken down into amino acids then absorbed by the body and rearranged into tissue proteins. The average meat protein content obtained in this study was 21.54–23.51%. The highest increase in protein was found in the supplementation of black garlic 3%; which might be due to the black garlic containing higher protein than garlic so that higher level of supplementation of the black garlic might further increase the protein content in meat. The more ration consumed, the greater chance of protein in ration being consumed, digested, and utilized. As a result, protein deposition in meat would occur and protein content in meat increased. This statement was supported by Gultom *et al.* (2012) which stated that protein consumption was influenced by feed consumption. Increase feed consumption caused increase protein consumption and then affect protein intake that would be used in the process of proteins and amino acids deposition in meat.

Fat content in meat was highly influenced by supplementation up to 3% of black garlic and its content was lower than other treatment groups including quail groups fed a ration supplemented by garlic. These results supported previous findings that the active compound in black garlic that affects the synthesis of fat in the body was higher in garlic than in fresh garlic (Wu *et al.*, 2011; Fouad *et al.,* 2013; Choi *et al.,* 2014). The decreased meat fat content was in line with a decrease in cholesterol and LDL. According to Ismoyowati and Sumarmono (2011), meat fat content was highly influenced by blood cholesterol and triglyceride levels. When energy or fat in the blood exist excessively, it would be exposed in fat and muscle tissue as energy reserves. According to Rahmat and Wiradimadja (2011), the way to reduce meat cholesterol was to reduce blood cholesterol, because blood cholesterol and meat cholesterol have a high relationship. Levels of crude fat found in quail breast meat decrease with decreasing levels of cholesterol in the blood. This is because triglycerides, a component of cholesterol were stored in body tissues. Fat that is formed in the body is the result of food metabolism, besides excessive carbohydrates and protein might be used as a source of energy. When cells need energy, lipase enzymes in fat cells will break down triglycerides into glycerol and fatty acids and release them into the blood vessels. Lipase enzymes are a group of enzymes that generally function in the hydrolysis of monoglycerides, diglycerides and triglycerides to produce free fatty acids and glycerol.

The average fat content obtained in this study ranged from 2.71-4.84%, the lowest fat content is 2.71% obtained from the supplementation of 3 % of black garlic. This might be due to black garlic containing several active compounds better than fresh garlic, mainly scordinin (Choi *et al.,* 2014; Kang 2016), an active compound that can reduce the fat content in meat.

CONCLUSION

It is concluded that substitution 100% garlic with black garlic in the diet could increase quail performance without any adverse effect on blood cholesterol properties. It is suggested to use black garlic up to 3% in the poultry ration.

CONFLICT OF INTEREST

The author of this article declares that there is no conflict of interest with financial organizations or other parties regarding the material discussed in the manuscript. The funder (Universitas Jambi) has no right to intervene in the research design (collection, analysis, or interpretation of data), the writing of the manuscript, or the decision to publish the results.

ACKNOWLEDGEMENT

We gratefully thank the undergraduate students (Wan Syafiq Syafhan, Tamiani Andara, Soleman Hutasuhut, and Antoni Anggara) who worked with us in the field. Hope all of you will succeed in the future as animal scientists.

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