The effect of fish meal protein substitution with Black Soldier Fly (BSF) larva meal protein in quail feed on the chemical quality of eggs

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Abstract. The purpose of this study was to determine the effect of fish meal protein substitution with BSF larva meal protein on the chemical quality of quail eggs. This study used 120 female quails aged 6 weeks and maintained for 7 weeks. This research method uses an experimental method with a completely randomized design (CRD). In this study using BSF larva meal consisting of 4 different treatments with 5 replications in the treatment. namely P0: rations without the addition of BSF larva meal; P1: 3.18% BSF larva meal (1.70% crude protein BSF larva meal); P2: 6.37% BSF larva meal (3.41% crude protein BSF larva meal); P3: 9.56% BSF larva meal (5.12% crude protein BSF larva meal). The variable observed was the chemical quality of quail eggs. The results of the study showed that the treatment was significantly different (P<0.05) on egg fat and cholesterol levels. However, the treatment was not significantly different (P>0.05) on egg protein content. Substitution of fish meal protein with BSF larva protein meal at a level of 6.37% can reduce the percentage of total egg cholesterol content.

1. Introduction
Quail eggs are one of the animal proteins that contain nutrients that are good for human life. Egg consumption is higher than the consumption of other livestock products, because it is easy to obtain and the price is relatively cheap. As a food ingredient, eggs must be maintained in physical and chemical quality so that they are safe for consumption. Quail eggs are used as a food source because they have a high protein content (13.05 g), higher than chicken eggs (12.58 g). Cholesterol contained in quail eggs is also greater, namely 844 mgdL-1, while chicken eggs are only 423 mgdL-1, this makes some people feel hesitant to consume quail eggs [1]. LDL levels that are too high in the body can cause accumulation of fatty tissue or plaque in the arteries that interfere with circulation, which can lead to strokes and coronary heart disease [2]. The availability of high-quality feed that is both healthy for livestock and adequate throughout the year is part of an initiative to improve quail livestock productivity and quality. Owing to the growing cost of imported raw materials such as fish meal and meat and bone meal, the cost of high-quality protein source feed ingredients is increasing (MBM).
This reliance on imports raises the cost of quail feed in the long run. Feed costs, on the other hand, are known to account for 70 percent to 80 percent of total production costs. The rising cost of protein sources, as well as the threat of animal feed shortages, environmental pressures, rising human population, and rising market demand for protein, have pushed up the cost of animal-based protein. [3]. As a result, current feed research is focusing on insects as an alternative protein source. Van Huis (2013) claims that protein derived from insects is more cost-effective, plays an important role in nature, and is environmentally friendly [4]. Insects are said to have a high feed conversion rate and can be easily maintained and mass produced. Insect production can also help to minimize organic waste, which can pollute the atmosphere [5].

Black Soldier Fly (BSF) is one of the insects whose characteristics and nutritional content are starting to be studied. This BSF originates from America and then spreads to tropical and subtropical regions around the world [6]. The tropical climate in Indonesia is very suitable for BSF cultivation. In terms of cultivation, BSF does not require special equipment and is very easy to develop on a mass production scale. From the various existing insects, it can be developed as a source of animal feed protein, because the protein content of BSF is quite high, around 40% –50% and fat content ranges from 29%–32% [7]. The use of BSF larva meal can replace fish meal up to 100% in quail rations in the starter and finisher phases and can increase the body weight gain of white cattle [8].

According to the above potential, research can be carried out on utilizing BSF larvae flour for poultry feed as an alternative protein source that is conventional, how much influence is BSF larva protein in replacing fish meal protein on the chemical quality of quail eggs.

2. Research method

In this study, the material used was 120 female quails aged 6 weeks. The quail used came from Gowa Regency. The quail is put into a battery cage made of wire mesh measuring 30 × 35 × 26 cm³. Each cage is filled with 6 quail, equipped with a place to feed, a place for drinking water and incandescent lamp (60 watts) placed on the cage for lighting, soxhlet extraction, 30 ml kjedahl flask, distillation flask, Erlenmeyer flask, centrifuge, water bath, UV visible spectrophotometer, ovens and glassware.

The raw materials for the rations used in this study consisted of corn, fish meal, BSF larva meal, coconut meal, soybean meal, rice bran, methionine, lysine, premix, DCP and CaCO₃. Treatment ration formulas in the study are presented in table 1.

| Ingredient          | Type of treatment (%) | P0 (0) | P1 (3.18) | P2 (6.37) | P3 (9.56) |
|---------------------|-----------------------|--------|-----------|-----------|-----------|
| Corn (%)            |                       | 46.90  | 50.10     | 52.50     | 52.10     |
| Rice bran (%)       |                       | 18.73  | 13.80     | 12.80     | 12.90     |
| Soybean meal (%)    |                       | 12.51  | 14.65     | 15.60     | 16.44     |
| Fish meal (%)       |                       | 10.00  | 6.67      | 3.33      | 0.00      |
| Coconut cake (%)    |                       | 6.76   | 6.10      | 4.30      | 3.50      |
| BSF larvae meal (%) |                       | 0.00   | 3.18      | 6.37      | 9.56      |
| L-lysine (%)        |                       | 0.30   | 0.30      | 0.30      | 0.30      |
| Dicalcium phosphate (%) |                   | 2.60   | 3.00      | 3.40      | 3.80      |
| Premix (%)          |                       | 0.10   | 0.10      | 0.10      | 0.10      |
| Calcium carbonate (%) |                     | 2.00   | 2.00      | 1.20      | 1.20      |
| DL-methionine (%)   |                       | 0.10   | 0.10      | 0.10      | 0.10      |

The research method used was a completely randomized design 4 treatments and 5 replications [9] based on the following treatments: P0: control ration (feed without the addition of BSF larvae meal); P1(3.18% BSF larva meal = 1.70% crude protein BSF larva meal); P2 (BSF larva flour 6.370% =
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Table 2. Nutritional content of treatment rations.

| Nutritional content      | Type of treatment | P0 (0) | P1 (3.18) | P2 (6.37) | P3 (9.56) |
|--------------------------|-------------------|--------|-----------|-----------|-----------|
| Energy metabolism (kcal/kg) |       | 2723.51 | 2743.67 | 2786.49 | 2772.86 |
| Crude protein (%)       |       | 20.46  | 20.47    | 20.16    | 20.01    |
| Crude fat (%)           |       | 5.97   | 5.80     | 6.20     | 6.47     |
| Crude fiber (%)         |       | 3.66   | 3.68     | 3.25     | 3.33     |
| Calcium (%)             |       | 3.03   | 2.42     | 1.99     | 1.84     |
| Phosphor (%)            |       | 1.14   | 1.03     | 0.96     | 0.95     |
| DL-methionine (%)       |       | 0.45   | 0.46     | 0.45     | 0.43     |
| L-lysine (%)            |       | 0.42   | 0.35     | 0.40     | 0.54     |

Note: Results of analysis of the Laboratory of Feed Chemistry, Universitas Hasanuddin Makassar (2019).

2.1. Research implementation
This study was for 7 weeks. Feed and drinking water were given ad libitum. Before giving the ND vaccine, the quail is fasted for 4 hours and then given the vaccine through drinking water during the day. Data collection was carried out every week. Taking and recording eggs is carried out every day at 04.00 PM. The collected eggs are counted and then weighed.

2.2. Research variable
The principle of protein content is the process of liberating nitrogen from protein in the material using sulfuric acid which is carried out by heating. Determination of total nitrogen and protein content using the micro-kjeldahl method. The formula for calculating protein content according to AOAC (2005) [10] is as follows:

\[
\%N = \frac{mL \text{ HCl} \times N \text{ HCl} \times (14.008)}{mg \text{ sample}} \times 100\% \quad (1)
\]

\[
\% \text{ Protein} = \% \text{ conversion factor (6.25)} \quad (2)
\]

Fat content analysis is the separation of fat from the sample by circulating the fat solvent into the sample, so that other compounds cannot dissolve in the solvent. The percentage of fat content can be calculated using the formula according to AOAC (2005) which is as follows [10]:

\[
% \text{Fat} = \frac{\text{final weight (g) - initial weight (g)}}{\text{material weight (g)}} \times 100\% \quad (3)
\]

According to Schunack et al (1990) states that cholesterol analysis uses the Liebermann Burchard method [11]. The research materials used were egg yolk, anhydrous acetic acid solution, chloroform solution, cholesterol standard solution, concentrated sulfuric acid, alcohol ether solution (3:1). The research tools used were oven and glassware, water bath, centrifuge, UV visible spectrophotometer. The working principle of cholesterol analysis is that chloroform extract containing cholesterol from the material will react with concentrated sulfuric acid and acetic anhydride, forming a colored reaction and the absorption is measured at a wavelength of 420 nm.

3. Results and discussion
The effect of substitution of fish meal protein with BSF larvae flour protein on the chemical quality of quail eggs, namely egg protein content, egg cholesterol content and quail egg fat content (table 3).
Table 3. Chemical quality of quail eggs aged 6–13 weeks.

| Variable                        | P0 (0)       | P1 (3.18)    | P2 (6.37)    | P3 (9.56)    |
|---------------------------------|--------------|--------------|--------------|--------------|
| Egg protein content (%)         | 12.27±1.06   | 13.74±2.69   | 11.79±0.66   | 11.19±0.92   |
| Egg fat content (%)             |              | 10.99±0.81\textsuperscript{a,b} | 10.46±0.66\textsuperscript{b} | 11.70±0.77\textsuperscript{a} | 11.88±0.48\textsuperscript{a} |
| Egg cholesterol content (mg/100 g) | 678.37±19.05\textsuperscript{a} | 670.30±14.10\textsuperscript{a,b} | 579.86±14.37\textsuperscript{c} | 648.65±24.29\textsuperscript{b} |

\textsuperscript{a,b}different superscript in the same row differ significantly (P<0.05).

3.1. Egg protein content
The data analysis showed that the substitution of fish meal protein with BSF larvae meal in the ration treatment was not significantly different (P>0.05) on the protein content of quail eggs. Based on the research results, the protein content of quail eggs is 11.19–13.74% fresh egg protein. Bambang [12] stated that protein content of eggs was 13.1%. It is seen numerically that there is an increase in protein content in P1 compared to the control treatment, but statistically, the treatment has not significantly different (P>0.05).

The increasing provision of BSF larvae meal in this study had an impact on increasing chitosan levels in the ration. Chitosan does not have a bad effect, chitosan can increase the production of the hormone gonadotropin, namely, as a luteinizing hormone-releasing hormone (LHRH) which functions for ovulation and egg production. Increased gonadotropins lead to better reproductive results and thus do not cause any toxic effects [13]. Gonadotropin-releasing hormone (GnRH), also known as luteinizing-hormone-releasing hormone (LHRH), is a tropic peptide hormone that releases LH hormone from the anterior pituitary and follicle stimulant (FSH). The gonadotropin releasing hormone is degraded by proteolysis within minutes [14].

3.2. Egg fat content
Based on the analysis of the research data showed that the substitution of fish meal with BSF larvae meal on the ration treatment was significantly different (P<0.05) on the fat content of quail eggs. Based on the research, it was found that the crude fat content of quail eggs was 10.46–11.87% fresh egg protein. Stadelman and Cotterill (1995) stated the fat content of quail eggs was 11.1%. The increased provision of BSF larvae flour resulted in increased ration crude fat content [15].

Egg fat depends on the ration fat consumed by livestock, the higher the dietary fat the higher the egg fat. The increasing of BSF larvae feeding causes chitin to increase, so that lipid metabolism in the liver increases. Chitin will bind to bile acids, namely, as an emulsifier of fat so that fat does not break down into fatty acids that can be absorbed by the body. The more fiber in the feed, the more bile acids are removed [16]. The liver is the site of mobilization of fatty acids in adipose tissue when the consumption of triglycerides increases, causing an imbalance in the process of lipolysis and triglyceride synthesis [17].

3.3. Egg cholesterol content
Based on the analysis of the research data showed that the substitution of fish meal with BSF larvae meal in the ration treatment was significantly different (P<0.05) in the cholesterol levels of quail eggs. The addition of 6.37% BSF larvae can reduce the percentage of total egg cholesterol levels. The addition of 6.37% BSF larvae to the ration had the lowest mean total cholesterol, namely 579.86±14.36% mg/100 g compared to the control and addition of BSF larvae 3.18% and 9.56%, namely 678.37±19.05% mg/100 g, 670.30±14.10% mg/100 g and 68.65±24.29% mg/100 g. The cholesterol content obtained in this study was lower than research [12], namely the cholesterol content of 2138.17 mg/100 g.

Decreased cholesterol levels are likely BSF larvae contain linoleic fatty acids which can affect the cholesterol concentration in the eggs. This is also supported by the statement [18] that the concentration of oleic and linoleic affects the concentration of cholesterol in eggs. In addition, BSF larvae contain crude animal fiber (chitin) which can reduce cholesterol levels in quail egg yolk [19]. Cholesterol is a component of fat that functions for the synthesis of bile acids, precursor to steroid hormones in the body.
and the synthesis of vitamin D. Cholesterol in the body comes from exogenous and endogenous materials. Exogenous cholesterol is cholesterol that is synthesized from feed ingredients from outside the body, while endogenous cholesterol is cholesterol that comes from the body which is synthesized in several tissues, especially in the liver [20,21]. Chitin has the potential to be high hypocholesterolemia and absorption of fat in the intestinal tract interacts with the formation of mycelia or lipid emulsification in the absorption phase. Chitin will bind to bile acids, namely, as an emulsifier of fat so that fat does not break down into fatty acids that can be absorbed by the body. The more fiber in the feed, the more bile acids are removed. This causes the cholesterol or LDL excreted through the feces to increase while for HDL it is the opposite [16].

4. Conclusion
Substitution of BSF larvae meal at a level of 6.37% in quail feed can reduce the percentage of total egg cholesterol content.

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