Impact of the Feeding With the Black Soldier Fly (*Hermetia Illucens*) on Egg Physical Quality, Egg Chemical Quality and Lipid Metabolism of Laying Hens

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Abstract. BSF larvae have a high growth rate and optimal feed conversion and can be well utilized of various types of material as a food source including decomposed organic matter. BSF larvae have amino acid compositions that resemble the amino acid composition of soybean meal or fish meal so that they can function as (antibiotic growth promoters-AGP). AGP aims to maintain the health of the digestive tract of poultry so that feed conversion becomes more efficient and growth performance can be optimal. This study aims to obtain the best type of BSF protein to improve egg quality physically and chemically and the effect of BSF on lipid metabolism. The results of this study indicated that treatment of T3 was the best physically egg quality and chemically egg quality of-laying hens with the use of BSF because it contained protein extract was quite high compared to other treatments. The results of the analysis of blood lipid metabolism that the treatment of live BSF (T1) has the lowest average value, this proved that live BSF was able to reduce the fat content in the blood of laying hens.

1. Introduction
AGP (antibiotic growth promoter) improves chicken performance. It is estimated that the use of AGP increases chicken growth by 4-8% and feed conversion by 2-5%. The role of AGP can kill pathogenic bacteria in the digestion of chickens, such as. *Salmonella* sp., *Campylobacter* sp., *Enterococci* sp. and *Escherichia coli*. An uncontrolled and inappropriate use of antibiotics leads to a microbial resistance to these antibiotics. This problem requires a solution in the form of a product that can boost livestock growth without causing resistance. AGP problems are also associated with local Indonesian fishmeal whose quality does not meet the standards of quality fish meal requirements for animal feed ingredients and whose price is expensive. Alternative solutions for animal protein are needed at affordable prices and as a replacement for AGP, namely Black Soldier Fly (BSF). BSF larvae (Black Soldier Fly) have a relatively high protein content of 40-50% and a fat content of 29-32% [8]. According to [35], BSF flour has the potential role to replace fishmeal up to 100% for
broiler feeds, without affecting the digestibility of the dry matter (57.96-60.42%), energy (62.03% - 64.77%) and protein (64.59-75.32%). Calcium minerals contained in BSF flour by 88% [16]. Another advantage is having antimicrobial and antifungal properties so that it can increase the body's resistance from bacterial and fungal diseases. The supply of high quality animal feed is one of the critical success factors in the poultry industry, which is 50-70% [22]. Protein is the most expensive feed component compared to other feed components. The impact, economically, farmers or businesses in meeting the source of protein can burden production costs. According to [7] stated that the protein component has an important role in an animal feed formula because it is actively involved in the process of body tissue formation and vital metabolism such as enzymes, hormones, antibodies and so on. The current feed study is focused on finding alternative sources of protein by utilizing insects. The substitution of soy flour in whole or in whole with BSF flour does not affect feed intake, egg performance, egg weight and feed efficiency in laying hens when compared with standard feeding of [30].

2. Methodology

The method of this treatment used an overall system of self-mixing, all feed ingredients were divided into rations according to the nutrient requirements of the laying hens. The composition of the feed ingredients consisted of corn, rice bran, coconut oil, fish meal, soybean meal, CaCO3, salt, premix, methionine and lysine uniformly mixed in the base ration for the control treatment, while for the composition of the feed ingredients the percentage of fishmeal consumption was reduced to 5% and 8% fresh BSF. Fresh BSF was administered after the preparation of the basal ration has been spread on the ration (topping).

The steps for producing the dried BSF production was to separate pupa from the cocoon layer by washing and steaming at 90°C for 10 minutes. The larvae were then heated to 55°C for 24 hours to remove the water. Then the larva was ground to flour with a blender and put in airtight plastic. The steps for producing the BSF extract were carried out according to the modified method of [13]. BSF larvae were washed and steamed for 10 minutes at 90°C, then the larvae were heated at 55°C for 24 hours to remove water, then the larvae were ground to flour with a blender and then with a denanol ratio of 1:10 (b/v) for 24 hours at room temperature, then the solution was filtered out twice with Whatman paper. The extract was then evaporated using an evaporator rotary vacuum at 40°C.

The physical quality of the eggs was checked once a week. From each of four eggs egg samples were taken per repeated treatment. Egg weight was measured by daily weighing during the study and then averaged according to treatment and replication. Egg yolk was separated from egg whites with a spatula and weighed. The calculation was made according to the following formula: Egg Yolk percentage = (egg yolk weight (g) / Weight of whole eggs (g)) x 100%. Egg white weight is the difference from the weight of whole eggs with eggshell weight and egg yolk, can be calculated by the formula: Percentage of egg white = (egg white weight (g) / weight of whole eggs (g)) x 100%. Eggshell was separated from egg white using a spatula, then weighed, can be calculated using the formula: Weight percentage of eggshell = (eggshell weight (g) / weight of whole eggs (g)) x 100%. To get the Haugh Unit (HU) value, the height of egg white (mm) was transformed into the correction value of the egg weight function, namely: HU = Log 100 (H - 1.7 W^{0.37} + 7.57). Information : H = protein height (mm), W = egg weight (g egg^{-1}) Results of the HU value: > 72 = AA quality, 60-72 = quality A, 31-60 = quality B, <31 = quality C. Egg yolks were measured in color by comparing the yolk color to the standard fan color egg roche yolk color fan on a scale of 7 to 14.

Chemical quality of laying hens: egg yolk cholesterol content was analyzed using the Liebermann Burchard Color Reaction method [26], egg yolk fat was analyzed using the Sochlet method AOAC 2005 [2] and MDA (Malondialdehyde) was analyzed using the method [10]. The metabolism of blood lipids (cholesterol, triglycerides and HDL) were analyzed using the Kit method AOAC 2005 [2] and serum LDL were analyzed using a calculation method known as the Friedwald method [17].
3. Results and Discussion

Based on Table 1, the data showed that the provision of fish meal containing different types of BSF larvae had a significant effect on the average weight of the egg yolk. The highest egg yolk weight value in T1 was 17.65 g (Table 8). The average value of egg yolk weight in this study ranged from 17.26 – 17.65 g (28.69 – 31.62%), higher than the [11] study of 14.60 - 15.20 g (24.30 - 25.00%), higher than of [36] amounted to (23.19%) and was within the normal range compared to the standard percentage of egg yolk which was 25 - 33% [32]. The quality of egg yolk is determined by the color, texture, suppleness and odor of the yolk [5].

The highest egg white weight value at T3 was 36.65 g (Table 1). The average value of egg white weight in this study ranged from 36.31 - 36.65 g (60.94 - 65.16%), which was below the [11] research of 38.70 - 40.30 g (63.10 - 64.60%), which is lower than [36] research, amounted to (64.63%) and was within the normal range compared to the standard percentage of egg whites ie 57 - 65% [32]. Egg white weight is influenced by egg white protein content, age, genetic and hormones [46], while the percentage of egg white is influenced by the protein content of the feed [36].

The average weight value of eggshell in this study ranged from 7.62 - 7.82 g (12.94 - 13.65%), was above the [11] research of 6.63 g (11.00%), below that of [20]. The brown color of the eggshell is influenced by pigments which are the result of transportation through the blood from the liver to the uterus, namely pigment phorphyrin, composed of protophorphyrin, coprophorphyrin, pentacarboxyl phorphyrin, urophorphyrin and several types of phorphyrin that have not been identified, pigment, genetic, stress level, age, and infectious bronchitis [4].

| Variable             | T0                        | T1                        | T2                        | T3                        |
|----------------------|---------------------------|---------------------------|---------------------------|---------------------------|
| egg weight (g)       | 54.82±4.15a               | 53.56±4.76b               | 54.76±4.41a               | 57.17±3.76a               |
| egg yolk weight (g)  | 17.59±0.13a               | 17.65±0.15a               | 17.48±0.07ab              | 17.26±0.08b               |
| egg yolk weight (%)  | 30.63±0.02a               | 31.62±0.03b               | 30.56±0.03a               | 28.69±0.02a               |
| egg white weight (g) | 36.31±0.09b               | 36.41±0.13b               | 36.45±0.07ab              | 36.65±0.10ab              |
| egg white weight (%) | 63.29±0.05a               | 65.16±0.06b               | 63.70±0.05ab              | 60.94±0.04a               |
| egg Shell Weight (g) | 7.82±0.05a                | 7.62±0.05c                | 7.72±0.05c                | 7.78±0.03abc              |
| egg Shell Weight (%) | 13.64±0.01a               | 13.65±0.01ab              | 13.48±0.01a               | 12.94±0.01ab              |
| Haugh Unit (HU)      | 80.53±1.75a               | 80.05±1.55a               | 80.16±0.65a               | 79.15±1.09a               |
| egg index (%)        | 0.77±0.002a               | 0.77±0.002a               | 0.77±0.003a               | 0.77±0.001a               |
| egg yolk index       | 0.40±0.004c               | 0.41±0.006b               | 0.42±0.004b               | 0.43±0.006b               |
| egg white index      | 0.13±0.004ab              | 0.13±0.005b               | 0.14±0.005a               | 0.13±0.005ab              |
| egg yolk color score | 4.86±0.51ab               | 5.43±0.58ab               | 4.71±0.56b                | 4.14±0.51b                |

Note: Different superscripts on the same row showed significant differences (P <0.05); T0: Ration contains 8% fish meal; T1: Ration containing 5% fish meal + 8% fresh BSF; T2: Ration containing 5% fish meal + 8% dried BSF; T3: Ration containing 5% fish meal + 8% BSF extract.

The highest haugh unit value at T0 was 80.53 (Table 1). The average value of haugh units in this study ranged from 79.15 - 80.53, which was lower than [37] of 88.3 - 90.8, in the same range as the research of [14] to 79.3 - 80.8, which was lower than of [1] amounted to 94.80, and that was lower than of [33] amounting to 91.40. The HU value in this study was within the normal range according to the United States Department of Agriculture Standards [42], namely the AA-quality egg category HU value: > 72, A-quality egg HU value: 60-72, B-quality egg HU value: 51-60 and C quality eggs HU value: <31. The HU value was an indicator of egg quality, the higher the albumen value the better the egg quality [37], the high HU value indicates the level of freshness of the egg [21]. The average of egg index value was 0.77 %, the average of egg yolk index value was 0.42, and the average of egg white
index value was 0.13. The average egg index results were higher than the results of research by [37] in the amount of 74.1 - 76.7. The egg index test aims to determine the egg’s quality physically, because it affects the egg’s shape and reproductive function. The shape of the egg is determined by the diameter of the isthmus [45]. The index value yolk is determined by the content of protein in the ration, because protein serves to membrane formation of vitelin and khalaza, while the index value of the egg white is determined by the level of viscosity egg whites, because the content of CO₂ and H₂O in the eggs can evaporate when the longer the eggs are stored [34]. A low egg white index value indicates ovomucin has been damaged [19]. Some factors that influence the color of egg yolk are the ratio of eggs and ration, where the rate of egg production causes the diversity of the color of the yolk [3], β-carotene, xantopil [18].

Based on Table 2, the average of egg yolk cholesterol of this study ranged from 5.61-7.10 μg/g (Table 2). The aim of the analysis test of malondialdehyde content on egg yolk was to see the antioxidant activity of [12]. The malondialdehyde content test measured the lipid peroxidation process due to an increase in free radicals resulting in oxidative stress [44]. Malondialdehyde was a free radical product from lipid peroxidation [15]. The average of egg yolk fat was lower than [23] in quail of 31.30-32.30% and below the standard according to [11], that was 31.48-32.33%. Some factors that influence the quality of egg chemistry are the type of feed, types of livestock, genetic and hormone [32].

| Variables | T0 | T1 | T2 | T3 |
|-----------|----|----|----|----|
| Malondialdehyde (µg g⁻¹) | 7.10±0.02ᵃ | 6.71±0.05ᵇ | 5.61±0.07ᶜ | 6.43±0.18ᶜ |
| Cholesterol (mg g⁻¹) | 5.20±0.14ᵃ | 5.90±0.07ᵃ | 5.59±0.05ᵇ | 5.40±0.16ᵇ |
| Fat (%) | 22.67±0.04ᵇ | 23.71±0.05ᵃ | 22.49±0.06ᶜ | 22.38±0.07ᶜ |

Note: Different superscripts on the same row showed significant differences (P<0.05); T0: Ration contains 8% fish meal; T1: Ration containing 5% fish meal + 8% fresh BSF; T2: Ration containing 5% fish meal + 8% dried BSF; T3: Ration containing 5% fish meal + 8% BSF extract.

The average cholesterol of this study ranged from 5.20-5.90 mg/g (Table 2). The average of egg yolk cholesterol in this study was lower than the results of the study of [11] that the egg yolk cholesterol was 8.34-9.24 mg/g, [20] in the amount of 12.05-14.54 mg/g and [43] in the amount of 10.25-12.86 mg/g. The cholesterol ester content in the ration was positively correlated with the egg yolk cholesterol content and the VLDL (very low density lipoprotein) concentration in the blood plasma of laying hens. The content of cholesterol and LDL in the blood can affect cholesterol levels in egg yolks [32].

| Variables | T0 | T1 | T2 | T3 |
|-----------|----|----|----|----|
| Triglycerides (mg/dl) | 424.44±48.22ᵃ | 409.73±67.62ᵃ | 421.40±103.88ᵃ | 412.04±34.69ᵃ |
| Cholesterol (mg/dl) | 134.78±11.09ᵃ | 133.02±7.55ᵃ | 135.05±19.32ᵃ | 133.56±5.68ᵃ |
| HDL (mg/dl) | 13.84±6.27ᵃ | 15.96±3.92ᵃ | 16.30±7.11ᵃ | 16.03±2.37ᵃ |
| LDL (mg/dl) | 36.06±11.13ᵃ | 35.11±12.54ᵃ | 34.47±12.11ⁿ | 35.12±11.45ᵃ |

Note: Different superscripts on the same row showed significant differences (P<0.05); T0: Ration contains 8% fish meal; T1: Ration containing 5% fish meal + 8% fresh BSF; T2: Ration containing 5% fish meal + 8% dried BSF; T3: Ration containing 5% fish meal + 8% BSF extract.
The average triglyceride values in this study ranged from 409.73 - 424.44 mg/dl, it was lower than [36] amounting to 522.63-753.09 mg/dl. The normal standard for laying hen triglycerides is <150 mg/dl [6]. The cause of high triglyceride values is the amount of excess fatty acids then converted to triglycerides to be transported and stored. Endogenous lipid metabolism formed by the mechanism of lipogenesis, lipid export, synthesis and oxidation of fatty acids that occur in the liver [24], is an important mechanism for controlling the accumulation of triglycerides in the liver [45].

The average of cholesterol value of this study ranged from 133.02-135.05 mg/dl, it was lower than the study of [38] amounting to 152.00 mg/dl with laying hens treatment at heat stress temperatures and higher on laying hens treatment at thermoneutral zone temperatures of 104.20 mg/dl. The normal standard for laying chicken cholesterol values is 52-148 mg/dl [6]. Data in this study indicated that the causes of high cholesterol values were low HDL values and high triglyceride values in the blood of laying hens [25]. The intestine has the capacity to absorb cholesterol derived from food nutrients, but the intestine does not have the ability to absorb the total amount of cholesterol in the lumen [28].

The average HDL value of this study was around 13.84-16.30 mg/dl, lower than study from [40] amounting to 40.5-50.4 mg/dl and being in the same interval as [36] in the amount of 14.00-17.58 mg/dl. The normal standard of HDL in laying hens is> 22 mg/dl [6]. The low value of HDL content is caused by the use of HDL for the synthesis of steroid compounds such as hormones or bile salts in the liver and cholesterol entering HDL into the cell membrane [31]. HDL is a lipoprotein that functions to maintain and manage the balance of cholesterol so it does not accumulate in cells, by transporting sterols from the membrane at the same level as the amount of cholesterol synthesized to the liver [41]. High levels of HDL can prevent LDL oxidation, HDL molecule size is smaller than other lipoproteins so that HDL can transport cholesterol collected in macrophages by passing through vascular endothelial cells and into intestinal tunica [9].

The average LDL value of this study ranged from 34.47-36.06 mg/dl. A low LDL value is positively correlated with a low value of triglycerides in plasma, caused by the residual hydrolysis of triglycerides to be metabolized in the liver which will become LDL [45]. According to [29], LDL and chylomicrons are transported in the bloodstream, aiming to metabolize lipids and hydrolyze triglycerides with the help of lipoprotein lipases and provide free fatty acids for absorption by tissues for storage or oxidation.

4. Conclusion
BSF extract (T3) has amino acid content close to fish meal so that it can improve the physical and chemical quality of eggs. BSF extract has the lowest average of chemical test value compared to other treatments, this proved that BSF extract can reduce fat content in egg yolks. The results of the analysis of blood lipid metabolism that the treatment of live BSF (T1) has the lowest average value, this proved that live BSF was able to reduce the fat content in the blood of laying hens.

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