Influence of *Tenebrio molitor* L Supplementation on Egg Quality and Omega-3 Content

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ABSTRAK

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Tenebrio molitor L merupakan salah satu bahan pakan alternative karena kaya dengan kandungan nutrien, yaitu protein, vitamin, mineral (kalsium), energi dan lemak. Tenebrio molitor L juga mengandung omega-3 sebesar 33.64±0.22%, sehingga diharapkan telur yang dihasilkan mengandung omega-3. Penelitian ini bertujuan untuk mengetahui pengaruh Tenebrio molitor L sebagai bahan pakan sumber protein pengganti meat bone meal (MBM) terhadap kadar omega-3 dan kualitas telur. Dalam penelitian ini digunakan 300 ekor ayam petelur strain Lohman Brown umur 20 minggu. Rancangan acak lengkap (RAL) digunakan dalam penelitian ini dengan 3 perlakuan dan 10 ulangan, masing-masing ulangan 10 ekor. Perlakuan adalah P0= Pakan mengandung 5% MBM, P1= Pakan mengandung 2.5% MBM + 2.5% *Tenebrio molitor* L, dan P2= Pakan mengandung 5% *Tenebrio molitor* L. Perlakuan pakan selama 6 bulan. Peubah yang diamati adalah produksi telur, berat telur, indeks telur, berat kerabang, tebal kerabang, haugh unit, skor yolk, dan omega-3. Perlakuan tidak mempengaruhi kualitas fisik telur, namun berpengaruh nyata terhadap berat telur. Perlakuan P0 menghasilkan berat telur yang paling rendah yaitu 59.02±0.53 g. Perlakuan P2 mengandung omega-3 lebih banyak daripada P0 dan P1 yaitu 88.18±0.12mg 100 g^{-1}. Kesimpulan penelitian ini bahwa *Tenebrio molitor* L dapat menggantikan MBM hingga 5% dalam pakan ayam petelur, meningkatkan kualitas telur, dan menghasilkan telur omega-3.

Kata Kunci: *Tenebrio molitor* L, Kualitas Telur, Omega-3

ABSTRACT

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Tenebrio molitor L is one of the alternative feed ingredients because it is rich in nutrients, namely protein, vitamins, minerals (calcium), energy, and fat. *Tenebrio molitor L* also contains 33.64±0.22% omega-3, so it is hoped that the eggs produced contain omega-3. In this study 300 Lohman Brown laying hens of 20-week-old were used. Completely randomized design (CRD) was applied in this study with 3 treatments and 10 replications, each replication contained 10 laying hens. Treatments were: P0= Feed containing 5% MBM, P1= Feed containing 2.5% MBM + 2.5% *Tenebrio molitor L*, and P2= Feed containing 5% *Tenebrio molitor L*. This research was conducted for 6 months. The variables observed were egg production, egg weight, egg shape index, shell weight, shell thickness, Haugh unit, yolk index, and omega-3. Treatment had no influence on egg physical quality but had a significant influence on egg weight. Treatment P0 prodeced the lowest egg weight that was 59.02±0.53 g. Treatment P2 had higher omega-3 contents than P0 and P1 that was 88±0.12 mg 100 g^{-1}. It was concluded that *Tenebrio molitor* L could replace MBM up to 5% in laying hens feed, improve eggs quality, and omega-3 content in eggs.

Key Words: *Tenebrio molitor* L, Egg Quality, Omega-3

INTRODUCTION

The production of laying hens has only met the needs of the domestic market by 65%, the rest is met by kampong chicken, ducks, and quail. In the livestock business, laying hens need expensive protein feed raw materials such as meat bone meal (MBM). The feed in the poultry business accounts for 70% of the production cost. Therefore, alternative steps are needed to reduce feed costs but still maintain feed quality and livestock production. An alternative source of protein to be used as feed is insects.

Edible insects such as mealworms (*Tenebrio molitor* L) have potential as feed ingredients. This is because, first, *Tenebrio molitor* L is rich in nutrients, namely protein (Makkar et al. 2014), vitamins, minerals (calcium), energy, fat (Van Huis 2013; Bosch *et al.* 2014), and can be readily available sustainable (Veldkamp et al. 2012). Second, *Tenebrio molitor L* can utilize organic waste as a breeding substrate, so that two benefits are obtained at the same time, namely environmental cleanliness and production of *Tenebrio molitor* L as feed material (Mutafela 2015). Third, chickens naturally consume insects at various insect life cycles from the wild (Bovera et al. 2016). Fourth, *Tenebrio molitor* L is fast-growing and easy to reproduce, as well as high feed efficiency (Józefiak et al. 2016). Fifth, the need for animal protein source feed ingredients in the chicken feed will always exist, because the amino acid composition of animal protein is better than that of vegetable protein source feed ingredients, especially the content of essential amino acids containing sulfur (Bovera et al. 2016).

Tenebrio molitor L farm is a promising business opportunity considering the very conducive market share in Indonesia. The need for *Tenebrio molitor* L in Jakarta, Bogor, Depok, Tangerang, and Bekasi (Jabodetabek) is around 73 tons per month, where Bogor needs 12 tons per month, Jakarta needs 20 tons per month, Bekasi needs 15 tons per month, Depok and Tangerang each need 13 tons per month (Hapsari et al. 2018). *Tenebrio molitor* L are an interesting protein source in feeding fish, poultry, and pig (Veldkamp et al. 2012). The larva and puppa *Tenebrio molitor* are rich in protein (46 to 60%) and easy to breed (Bovera *et al.* 2016). One adult female beetle aged 2-3 months can produce 200-300 eggs or can reach 100 kg in one harvest (22-137 days) (Manullang et al. 2018).

Tenebrio molitor L is a mealworm larva belonging to the genera Tenebrio and Tribolium (Ordo Coleoptera) experiencing 15 molts. *Tenebrio molitor* L larva stage has a positive impact because it can be bred and used as a food source for fish, reptiles, amphibians, and birds. Adult Tenebrio molitor has a negative impact because it spoils grain and stored food (Hapsari et al. 2018). The life phase starts from the egg, then hatches into a larva, pupa, or cocoon, and the last phase becomes an adult *Tenebrio molitor* L (Hartiningsih & Sari EF 2014). The potential of larvae *Tenebrio molitor* L for livestock, especially as animal feed is quite good, because cultivation is easy, has a short life cycle, high production and contains high nutrients needed by livestock.

A study on the use of larvae *Tenebrio molitor L* as a protein source substitute for Meat Bone Meal (MBM) in broiler farms showed that *Tenebrio molitor* L could replace MBM up to 50% without affecting the performance of broilers reared for 35 days (Purnamawati 2015). This will be beneficial for broiler chicken farms because most of the protein source feed for poultry in Indonesia are imported such as soybean meal, fish meal, and MBM. The high price of MBM has an impact on the use of MBM so that the use of larvae *Tenebrio molitor* L can reduce feed costs (Purnamawati 2015).

Larvae *Tenebrio molitor* L contains 33.64±0.22 % omega-3 so it is expected that the eggs produced contain omega-3. The addition of omega-3 fatty acids in poultry feed is becoming increasingly important because it can affect the immunity of chickens, improve the quality of poultry products and benefit human health (Lee et al. 2019). The strategy in preparing feed formulations for poultry can increase the nutritional content of the eggs produced (Dhama *et al.* 2014), can increase the quality and quantity of eggs (Sihvo et al. 2014), and can increase the use-value of eggs for humans (Sujatha dan Narahari 2011). Eggs do not naturally contain omega-3, so omega-3 supplementation in poultry feeds is necessary to obtain omega-3 eggs (Chen et al. 2012); (Maroufyan et al. 2012). Omega-3 eggs are beneficial for health and fulfill human nutrition (Kassis et al. 2012).

MATERIALS AND METHODS

This study was carried out at the Field Laboratory of the Faculty of Animal Husbandry, Institut Pertanian Bogor University, West Java. The use of experimental animals has obtained approval from Komisi Kesejahteraan Hewan Coba Balitbangtan (KKHB), Ministry of Agriculture with registration number: Balitbangtan/BBP2TP/A/01/2021. In this study 300 heads of 20-week-old Lohman Brown laying hens were used with 3 treatments and 10 replications, each replication with 10 laying hens. The study was carried out for 6 months. Before treatment, laying hens were adapted to feed contain *Tenebriomolitor* L (P1 and P2) for 7 days.

Laying hens were placed in individual battery cages and randomized to 3 treatments × 10 replications × 10 individuals. Feeding was carried out at 07.00 WIB and 15.00 WIB. Vitamin was also given in drinking water to reduce stress in laying hens. Feed treatment for 6 months was carried out after laying hens was adapted to the cage for 2 weeks and adapted to feed contain *Tenebriomolitor* L (P1 and P2) for 7 days. During rearing, laying hens were vaccinated with ND, IB, and AI vaccines. ND and IB vaccinations were carried out every 3 months through clean and chlorine-free drinking water following procedure or SOP described by Center of Veterinary Research, Ministry of Agriculture. Before being given the vaccine, the laying hens fasted for 2 hours so that the vaccine in drinking water could be consumped immediately and evenly distributed throughout the laying hens. In addition, drinking water must also be protected from the sun (Wiyono et al. 2019).

The feed formulation was prepared based on the nutritional needs of laying hens with crude protein content of 22% each treatment (Table 1). The feed formulation was prepared using the Pearson Square method (Table 2) with 3 treatments are P0: Feed contain 5% meat bone meal, P1: Feed contain 2.5% meat bone meal + 2.5% *Tenebrio molitor* L, P2: Feed contain 5% *Tenebrio molitor* L. A hundred fourty gram

Table 1. Nutritional content of feed for each treatment

Nutrional Content	P0 (%)	P1 (%)	P2 (%)
Crude protein	22	22	22
Metabolic energy	3.08	3.21	3.35
Calcium	0.1	0.1	0.1
Phospor	0.5	0.5	0.5
Methionine	0.3	0.4	0.3
Lysine	1.1	1.2	1.1
Omega-3	0	0.84	1.68

P0= Feed containing 5% MBM, P1= Feed containing 2.5% MBM + 2.5% *Tenebrio molitor L*, and P2= Feed containing 5% *Tenebrio molitor L*

Table 2. Feed Composition of each treatment based on NRC (1994)

Feed Material	P0	P1	P2
Corn	52	52	52
Fine barn	10	10	10
Soybean meal	31	31	31
Meat bone meal	5	2.5	0
Tenebrio molitor L	0	2.5	5
Coconut oil	1	1	1
Salt	0.3	0.3	0.3
Premix	0.5	0.5	0.5
Total	100	100	100

P0= Feed containing 5% MBM, P1= Feed containing 2.5% MBM + 2.5% *Tenebrio molitor L*, and P2= Feed containing 5% *Tenebrio molitor L*

feed per head of laying hens per day and drinking water was provided ad libitum. Variables observed included (1) egg production, eggs were collected every day at 09.00 WIB, then weighed and counted for 6 months, (2) egg weight (g hen-1), Eggs were weighed digitally with an accuracy of 0.1 g then calculated the average of each treatment and replication, (3) egg shape index. The egg shape index is the quotient between the width and length of the egg and then multiplied by 100 (Ledvinka et al. 2012). The measurement of the egg length and width used a caliper with an accuracy of 0.05 m, (4) eggshell weight, Eggshell was weighed with a digital scale, (5) eggshell thickness (mm), eggshell thickness was measured using a caliper at the center (equator), the blunt end, and the pointed end of the egg to then calculate the average, (6) Haugh Unit (HU), the eggs were cracked on a smooth and flat surface (glass), then the egg white height was measured using a caliper. According to (Rahmawati & Irawan 2021), (7) egg yolk color, egg yolk color was tested using a yolk color fan by comparing the yolk color with a standard egg yolk fan, on a scale of 1 - 15 (light yellow to dark yellow), (8) omega-3, the omega-3 fatty acids content in egg yolk was analyzed by the Park and Goins method (1992). The Haugh Unit formula is:

$$HU = Log\ 100\ (H - 1.7\ W0.37 + 7.57)$$

where: H = Egg white height (mm), W = egg weight (g egg-1). HU >72 is grouped to grade AA, HU= 60-72 grouped to grade A, and HU= 31-60 grouped to grade B. The data were analyzed using variance (ANOVA) and if they were significantly different (P<0.05) followed by the Duncan Multiple Range Test (Steel and Torrie 1993). The descriptive analysis results are presented in tabular form.

RESULTS AND DISCUSSION

Effect of *Tenebrio molitor* L supplementation in feed on average egg production, egg weight, physical quality, and external quality are presented in Table 3.

Egg production

Tenebrio molitor L supplementation into feed did not significantly influence (P < 0.05) egg production (Table 2). This is because the crude protein content in all treatments was not different (22%), although the omega-3 content was different. Fatty acid saturation was significantly influence production of laying hens at the end of production period (58–74 weeksold), on egg production performance, egg weight, egg production, feed efficiency, and body weight (Buitendach et al. 2014). Egg production is influenced by linoleic fatty acid and amino acid methionine. Linoleic fatty acids control proteins and lipids needed for follicle development which directly control egg production (Mulyadi 2013). Since the crude protein level was the same in the three treatments resulted in non significant influence on egg production. Egg production in this study was around 83.78%-85.40% or in normal conditions. This is because the feed was sufficient for basic living needs and egg production. Egg production is expressed as Hen Day Egg Production (HDP), influenced by the fulfillment of basic living needs (Ledvinka et al. 2012; Kingori et al. 2014; Setiawati et al. 2016; Berenjian et al. 2021).

Egg weight

Tenebrio molitor L supplementation into feed significantly influenced (P<0.05) egg weight (Table 2). The average egg weight in P1 and P2 treatments were significantly higher (P<0.05) than P0. It was suspected that the amino acid content in *Tenebrio molitor* L and MBM proteins was different, although the protein

Table 3. Average production, weight, physical quality, and external quality with *Tenebrio molitor* L supplementation in feed

Variable	Treatment		
	P0	P1	P2
Egg production (%)	83.78±0.04	84.77±0.05	85.40±0.05
Egg weight (g)	59.02±0.53[a]	60.21±0.60[b]	60.26±0.61[b]
Egg shape index	76.57±0.02	78.39±0.01	77.28±0.02
Shell weight	4.56±0.06	4.75±0.05	4.61±0.05
Shell thickness	0.39±0.01	0.33±0.01	0.38±0.01
Haugh unit	78.56±0.56	78.53±0.58	79.13±0.49
Yolk index	7.20±0.21	7.40±0.11	7.32±0.20

[ab] denotes that same line with different letters shows significant difference in P < 0.05. P0= Feed containing 5% MBM, P1= Feed containing 2.5% MBM + 2.5% *Tenebrio molitor L*, and P2= Feed containing 5% *Tenebrio molitor L*

content of the feed was adjusted to the need for feed per laying hens namely 22%. The feed quality will affect the egg weight, a good feed will produce large eggs. Egg weight is influenced by protein content, genetic, environment, and age of chickens (Rahmawati & Irawan 2021). Protein and amino acids are the most important food substances in controlling egg size. *Tenebrio molitor* L crude protein was little bit higher than (45.87%) than crude protein of MBM (42.4%). This caused eggs P1 and P2 to be heavier than P0.

Egg weight between P1 and P2 was not different because both of treatment P1 and P2 contented *Tenebrio molitor* L. In addition to protein, the Ca content in the feed also affects egg weight, the higher the percentage of calcium in the feed, the heavier the eggs will be. Good quality feed have good protein, amino acids, and linoleic acid so that it affects product output (Sjofjan et al. 2020). Egg weight is influenced by several factors such as seasonal changes, parent body weight, feed given, storage time, lineage, heredity, room temeperature, hen age, and sanitary (Ledvinka et al. 2012; Kingori et al. 2014; Kasmiati et al. 2019; Viana et al. 2020). Egg weight is a price-determining factor in the marketing aspect. Egg weight based on SNI 01-3926-2006 is divided into 5 groups, namely extra-large (> 60 g), large (56–60 g), medium (51–55 g), small (46–50 g), and extra small (< 46 g). Table 2 shows that the average egg weight P0 was in the large group while egg weight P1 and P2 was in the extra group.

Egg shape index

Tenebrio molitor L supplementation into feed was not significantly influence (P>0.05) egg shape index. This is because the protein feed content in the three treatments was not different. Protein will affect the viscosity reflecting the internal quality of the egg, thus affecting the egg shape index. The more protein content in the feed, the thicker the albumen, so the higher the egg shape index (Christina et al. 2020; Elem 2021).

The egg shape index was in the range of 76.57–78.39 or in good category (70-79) (Widyantara et al. 2017). Egg shape index can be used to physically determine egg quality because egg shape index will affect egg shape and reproductive function. The decrease in the egg shape index was caused by the evaporation of CO_2 gas and water in the egg so that the alkaline nature of the albumen increased and caused the ovomucin fibers to be damaged. The egg shape is affected by the isthmus diameter. If the diameter is wide, the egg shape produced tends to be round, and conversely, the egg shape tends to be oval if the isthmus diameter is not wide (Ledvinka et al. 2012). Besides being influenced by the isthmus diameter, the egg shape index is also influenced by the age of the parent. Young parents tend to produce small and oval eggs, while older parents tend to produce round eggs (Kasmiati et al. 2019).

Yolk index

Tenebrio molitor L supplementation into feed significantly influenced yolk index. This is because the content of β-carotene or xanthophyll pigments between MBM and *Tenebrio molitor L* was significantly different. Yolk color is affected by feed. If the feed contains more carotenoids, namely xanthophylls, the yolk index will be more reddish-orange in color (Amo et al. 2013; Kotrbáček et al. 2013).

The Yolk index was of good quality or ranged from 7.2–7.4. Poultry consuming higher carotenoid pigments will produce a higher intensity of egg yolk color. The type and amount of carotenoid pigment consumed by laying hens is a major factor in egg yolk pigmentation. Yolk index is influenced by the content of carotenoid compounds found in plants and pigments affecting egg yolk color namely carotene pigments (Kotrbáček et al. 2013; Christina et al. 2020). The Yolk index was in a good category or 7–12 and had a positive effect on consumer tastes (Mulyadi 2013).

The yolk color was influenced by xanthophyll dyes with the most abundant in the hydrolytic carotenoid group (Kotrbáček et al. 2013), and also due to the relationship between nutritional content feed or is almost the same in each treatment (Suryana et al. 2020). The high and low yolk index is influenced by a number of color pigment content in the feed called carotenoids. Carotene is contained in xanthophyll pigments, while xanthophyll pigments are abundant in corn (Christina et al. 2020).

Shell thickness

Shell thickness was measured at the blunt, middle, and taper sections, then averaged. *Tenebrio molitor L* did not significantly influence (P>0.05) influence shell thickness. This is because the Ca and P in the feed in the 3 treatments was significantly different. Shell thickness and quality depend on the calcium content (Christina et al. 2020). Shell thickness is influenced by the absorption of calcium in the small intestine. If calcium is well absorbed in the small intestine, egg weight and thickness increase in the uterus (Suryana et al. 2020). The higher the calcium quality, the better the eggshell quality. Eggshell quality is influenced by the nutritional quality of livestock, livestock health, maintenance management, and environmental conditions.

Shell is influenced by light use and laying hens weight (Mulyadi 2013), and vitamin D (Świątkiewicz et al. 2010; Rahardja et al. 2015). Adequate levels of vitamin D are needed to absorb calcium (Kannan & Lim 2014) in the shell formation process (Amo *et al.* 2013)(Kannan dan Lim 2014)(Kannan dan Lim 2014)(Kannan dan Lim 2014)(Kannan dan Lim 2014)(Kannan dan Lim 2014)(Kannan dan Lim 2014). The eggshell is the outermost part of the egg and it is important to pay attention to its quality because the eggshell serves to protect egg content from bacteria causing damage to egg content resulting in decreased egg quality (Amo *et al.* 2013). Eggshell has an important role to protect eggs from microorganisms thereby reducing spoilage (Kingori *et al.* 2014; Vakili dan R 2016). Lack of calcium and phosphorus in the feed produces a thin shell so that the eggs are easy to crack and bacteria can easily enter the eggs (Hanusová *et al.* 2015).

Shell thickness was in the range of 0.33–0.39 mm or normal and of good quality. Good shell thickness for the market is 0.3–0.33 mm so it is not easily broken (Suryana *et al.* 2020). Shell thickness is influenced by several factors, namely age, type of chicken, nutrients, organ function, stress, and components of the eggshell layer (Mulyadi 2013). Thin eggshells are relatively more porous and large (Kasmiati *et al.* 2019), thereby accelerating the decline in egg quality due to evaporation and faster spoilage (Widyantara *et al.* 2017).

Haugh unit (HU)

Haugh unit reflects albumen condition to determine egg quality. The HU value was determined based on albumen conditions or the ratio between albumen height and egg weight. Haugh unit is used to determine egg freshness, especially in the albumen (Rahmawati & Irawan 2021).

Tenebrio molitor L did no significantly influence (P > 0.05) HU value (Table 2). The resulting Haugh unit was included in quality I with a HU > 72 (SNI, 2006). This means that the eggs are fresh and have thick albumen, so the correlation between albumen and egg weight is high. The higher the HU, the better the egg quality (Rahardja et al. 2015). The best egg quality is still new and fresh (Suryana et al. 2020). HU is influenced by ovomucin found in albumen. The higher the albumen, the higher the HU obtained. Some of the indicators determining HU in laying hens are shelf life, age, nutrients, egg storage, strain, vitamin C and or E supplementation (Ledvinka et al. 2012; Rahmawati & Irawan 2021). The albumen height was maximum at the time the eggs were released and decreased with increasing storage time. The decrease in quality is caused by long storage time, handling factors, and environmental conditions (Rahardja et al. 2015; Feddern et al. 2017). Egg quality is affected by season, ambient temperature, and age (Hanusová et al. 2015; Christina et al. 2020).

Omega-3 content

Tenebrio molitor L supplementation into feed influenced Omega-3 content.

Table 4. The influence of *Tenebrio molitor L* supplementation into feed on Omega-3 content

Treatment	Omega-3 content (mg/100 g)
P0	37.08±0.23[a]
P1	72.42±0.43[b]
P2	88.18±0.12[c]

[abc] denotes that same line with different letters shows significant difference in P < 0.05. P0= Feed containing 5% MBM, P1= Feed containing 2.5% MBM + 2.5% *Tenebrio molitor L*, and P2= Feed containing 5% *Tenebrio molitor L*

Tenebrio molitor L supplementation into feed significantly influence (P<0.05) omega-3 content. *Tenebrio molitor* L in feed serves as an energy source to produce eggs containing omega-3 fatty acids. *Tenebrio molitor L* contains 33.64±0.22 % of omega-3. Treatment P2 contains more omega-3 than P0 and P1. This is because *Tenebrio molitor L* contains omega-3 fatty acids, so the more *Tenebrio molitor L* supplementation in the feed, the higher the omega content in eggs produced. The increase in omega-3 in eggs can be manipulated by providing feeds containing omega-3 (Kassis et al. 2012; Hasyim et al. 2016; Lee et al. 2019; Berenjian et al. 2021). Egg yolks from laying hens given omega-3 supplements contain higher levels of Omega-3 than chickens not given supplements (Konieczka et al. 2016).

CONCLUSION

As much as 5% *Tenebrio molitor* L (P2) supplementation produced eggs with more omega-3 than P0 and P1 namely 88±0.12 mg. *Tenebrio molitor* L supplementation into feed did not significantly influence ($P<0.05$) egg production, egg shape index, yolk index, shell thickness, and HU, but significantly influence ($P<0.05$) egg weight. Egg production was ranged from 85.40±0.05%, egg weight 60.26±0.16 g; egg shape index was ranged from 77.28±0.02 or in good value, yolk index was of good quality or ranged from 7.32±0.204. *Tenebrio molitor* L could replace MBM up to 5% in laying hens feed, it improved eggs quality, and omega-3 content of eggs.

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