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# Fatty Acid Profile and Cholesterol Contents in Egg Yolk of Hens Fed Variable Energy to Protein Ratio Diet Using *Hermetia Illucens* Meal.

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## Abstract

The impact of changing the energy-to-protein ratio using *Hermetia illucens* meal as a protein source on the fatty acid profile and cholesterol content in egg yolk for 90 days was evaluated in this study. Diets were formulated with levels of normal (155), low (140), and high (170) energy-to-protein ratio using *Hermetia illucens* meal at 0%, 5%, and 1% to form three treatments, respectively. A total of 54 nine-month-old local Arabian strain laying hens were randomly assigned to nine-floor cages. The analysis results of *Hermetia illucens* revealed that the total saturated fatty acid content was higher than the total unsaturated fatty acid content. Also, there was lower omega-3 polyunsaturated fatty acid content than omega-6 polyunsaturated fatty acid content. The finding of this experiment revealed that birds fed the low energy-to-protein diet containing *Hermetia illucens* at 5% meal increased ($P<0.05$) total unsaturated fatty acid content and depressed ($P<0.05$) total saturated fatty acid content in egg yolk compared to the birds fed the control diet and birds fed the high energy-to-protein diet. The total omega-3 polyunsaturated fatty acid content ($P<0.05$) and omega-6 polyunsaturated fatty acid content ($P<0.01$) were lowered by *Hermetia illucens* meal diets. There was a similar effect ($P>0.05$) on cholesterol content in the egg yolk of the hens fed dietary treatments. In conclusion, formulation of the diet with the low energy-to-protein ratio using *Hermetia illucens* meal had a positive effect on the fatty acid profile of the egg yolk.

***Keywords:*** *Fatty acid, Hermetia illucens, village chicken, cholesterol*

## Introduction

Globally, it is estimated that around 821 million people suffer from starvation every year (FAO, 2017; Ridge and Smith, 2018). It is expected that there will be 8.5 billion people on the planet by 2030 (Desa, 2019; Cleland, 2013). Along with a quickly increasing population, the demand for healthy food is growing due to an incline in health awareness among people. The impact of the quality foods such as their fatty acid composition, on people's health status has been well-evaluated (Borges et al., 2020; Lichtenstein et al., 2006; Hassan, 2009; Al-Rubae'i, 2010). The increased polyunsaturated fatty acid (PUFA) content in people's diets has had a positive health effect on common diseases (Jump et al., 2012; Yashodhara et al., 2009; Aljuboori et al., 2020). In addition, low levels of essential fatty acids in food such as linoleic (18:2), linolenic (18:3), and arachidonic (20:4) increase the possibility of getting growth retardation, skin diseases, and obesity (De Cerqueira and De Souza, 2017; Takic et al., 2022; European Food Safety Authority, 2008; Giménez-Arnau et al., 1997). Therefore, the current scientific interest tends towards healthy animal food origin products to meet consumer requirements.

*Hermetia illucens* is a fly (Diptera) belonging to the Stratiomyidae family, and they are spread in tropical and hotter temperate regions (Diener et al., 2011). The larvae meal of *Hermetia illucens* can be used in the broiler diet (Alqazzaz et al., 2019), and laying hen diet (Al-Qazzaz et al., 2016). Inclusion *Hermetia illucens* as alternative feed ingredients in poultry diets should take into consideration the alternative feed quality, especially cholesterol content and fatty acid profile (Eckel et al., 2014; Cherian, 2015; Lee et al., 2019). Changing dietary fat type in larvae growing media may alter the fatty acid profile of *Hermetia illucens*s thus the larvae meal can be employed as a source of saturated fatty acid (SFA), monounsaturated (MUFA), and PUFA in the animal diets (Li et al., 2022). Despite many works that evaluated the effect of *Hermetia illucens* in laying hen diets, the quality of the lipid in the egg yolk of hens fed *Hermetia illucens* meal is not established yet. This study aimed to evaluate the formulation of the laying hen diet by changing the level of energy-to-protein ratio (EPR) diet using *Hermetia illucens* meal as a protein substance in the laying hen diet on cholesterol content and fatty acid profile of egg yolk.

## Materials and Methods

A total of 54 local nine months old Arabian strain hens were assigned to nine-floor cages (length 200 cm × width 300 cm/cage) at the University Putra Malaysia farm in Serdang. The physical traits, weight, and health factors of the birds were standardized. The six hens that made up each treatment replicate were placed in one cage according to a completely random design. The basal ingredients were formulated to form the first treatment (Table 1), which was based on the EPR= 155 (2777.16 kcal/kg energy and 17.86% protein). *Hermetia illucens* meal (5%) was included in the basal diet to form the second treatment based on EPR= 140 (2668.93 kcal/kg

energy and 19.05% protein). The third treatment included 1% *Hermetia illucens* meal to the basal diet based on EPR= 170 (2836.14 kcal/kg energy and 166.4% protein). Birds received 80 g of grain and ad libitum access to water during the experiment period. The *Hermetia illucens* meal was prepared by drying the fresh *Hermetia illucens* using an electric oven at 60 °C for five days.
The samples (egg yolk, diets, and *Hermetia illucens* larvae meal) were conducted for lipid extraction using a Trichloromethane: methyl alcohol solution (2 volume:1 volume) based on the method of Folch et al. (1957).
The fatty acid methyl esters of extracted samples were conducted using the method described in AOAC (2007).
Gas-liquid chromatography was used to measure the fatty acid methyl esters using an Agilent 7890A GC system (Agilent, Palo Alto, California, USA). By comparing the peak retention durations of the fatty acid methyl esters in the samples to the standards received from Sigma, fatty acid identification was accomplished (St. Louis, MO, USA). The internal normalization (area %) was used to express the results.
Egg yolk samples were saponified according to the method described by Sim and Bragg (1977) before measuring the cholesterol content using the colorimetry method according to Abell et al. (1952).
The experimental design was completely randomized. The Statistical Analysis System software (version 9.3) was used to analyze the collected data. Also, Tukey's test was used to compare the differences among treatment means.

Table 1. Composition of the experimental diets

| Ingredients (%) | Control 0% *Hermetia illucens* | Low EPR 5% *Hermetia illucens* | High EPR 1% *Hermetia illucens* |
|---|---|---|---|
| Rice bran | 14 | 10.35 | 4.8 |
| Fish meal | 3 | 0 | 0 |
| Corn | 20.2 | 20 | 26.5 |
| MBW | 4 | 4 | 6 |
| RW | 30 | 31 | 35 |
| Limestone | 9 | 9 | 8.5 |
| Di calcium phosphate | 0.5 | 0.95 | 0.5 |
| Soybean | 18.5 | 19 | 17 |
| BSFL meal | 0 | 5 | 1 |
| Sodium chloride | 0.5 | 0.3 | 0.3 |
| Methionine | 0.2 | 0.2 | 0.2 |
| Lysine | 0.1 | 0.15 | 0.2 |
| Calculated analysis | | | |
| Crude protein (%) | 17.86 | 19.05 | 16.64 |
| ME (kcal/kg) | 2777.16 | 2668.93 | 2836.14 |
| EPR | 155.49 | 140.03 | 170.35 |
| Phosphors (g kg-1) | 4 | 4 | 4 |
| Calcium (g kg-1) | 4 | 4 | 4 |
| Linoleic acid (g kg-1) | 25.8 | 28.8 | 27.9 |

ME = Metabolizable Energy; EPR= energy-to-protein ratio; RW= rice waste; MBW= meat and bone waste.

**Results**

The fatty acid profile of *Hermetia illucens* larvae meal and dietary treatments is shown in Table 2. The majority of fatty acid contents in *Hermetia illucens* larvae meal were C14:0 (27.17%), C16:0 (25.4+8%), C 16:1-7 (7.39%), C18:0 (1.76%), C18:1 n-9 (28.59%), and C18:2 n-6 (8.15%), while the minor of fatty acid contents were C18:3 n-3 (0.58%), C20:4 n-6 (0.11%), C20:5 n-3 (0.59%), C22:5 n-3 (0.08%), and C22:6 n-3 (0.09%). The total SFA content (54.41%) was higher than the total unsaturated fatty acid (USFA) content (45.59%). The total monoene content was 35.98. Also, the total polyunsaturated fatty acid n-6 (PUFA n-6) (8.26%) was higher than the total polyunsaturated fatty acid n-3 (PUFA n-3) (1.35%).
The fatty acid profile in the egg yolk of hens fed the variable EPR diets using *Hermetia illucens* larvae meal is shown in Table 3. The lowest ($P<0.05$) total SFA content was reported in the egg yolk of hens fed a low EPR diet among treatments, although the C14:0 content was the highest ($P<0.01$) in the same treatment. The C16:0 content dominated the SFA was declined ($P<0.01$) in the egg yolk of hens fed *Hermetia illucens* diets. A similar ($P<0.05$) result was reported with C 22:6 n-3 content. Also, hens fed the high EPR diet reported the highest content of C18:0 ($P<0.05$), and C 22:5 n-3 ($P<0.01$) in the egg yolk. However, there was a similar effect between hens fed the low EPR diet and hens fed the control diet in terms of the C18:0 content. The hens fed the low EPR diet reported the highest ($P<0.05$) total USFA content in the egg yolk among treatments. Also, the contents of C18:2 n-6 and C 20:4 n-6 were decreased ($P<0.05$) by the low EPR diet compared with the hens fed the control diet. However, the C 18:1 n-9 content was increased by the low EPR diet. The content of total monoene fatty acid was high ($P<0.01$) with increasing the *Hermetia illucens* larvae meal in the diet. The total PUFA n-3 content was decreased ($P<0.05$) in the



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egg yolk of hens fed *Hermetia illucens* larvae meal diets. Also, hens fed the low EPR diet had depressed (P<0.05) total PUFA n-6 content in egg yolk compared with hens fed the control diet. The Poly: Sat ratio was decreased (P<0.01) with an increase of *Hermetia illucens* larvae meal content in the diet. In addition, the Unsat: Sat ratio was increased (P<0.05) in birds fed the low EPR diet compared with other treatments.
The effect of changing the EPR diet using *Hermetia illucens* larvae meal on the cholesterol content of egg yolk is shown in Table 4. There is no significant effect (P>0.001) of *Hermetia illucens* larvae meal on cholesterol content in egg yolk.

Table 2. Fatty acid profile (%) of experimental diets and *Hermetia illucens* meal

| | Dietary treatments | | | *Hermetia illucens* |
|---|---|---|---|---|
| Fatty acid | Control | Low EPR | High EPR | |
| C14:0 | 1.21 + 0.02 | 4.38 + 0.14 | 2.14 + 0.10 | 27.17 + 0.14 |
| C16:0 | 25.28 + 0.15 | 25.27 + 0.47 | 28.48 + 1.06 | 25.48 + 0.19 |
| C 16:1-7 | 1.42 + 0.48 | 2.38 + 0.08 | 1.63 + 1.15 | 7.39 + 0.09 |
| C18:0 | 4.89 + 1.68 | 5.79 + 0.02 | 3.07 + 0.41 | 1.76 + 0.22 |
| C18:1 n-9 | 38.96 + 0.28 | 38.14 + 0.22 | 40.03 + 2.21 | 28.59 + 0.17 |
| C18:2 n-6 | 23.23 + 0.20 | 21.28 + 1.06 | 21.42 + 0.51 | 8.15 + 0.10 |
| C18:3 n-3 | 1.60 + 0.01 | 1.49 + 0.06 | 1.65 + 0.04 | 0.58 + 0.43 |
| C20:4 n-6 | 0.44 + 0.22 | 0.42 + 0.08 | 0.50 + 0.04 | 0.11 + 0.01 |
| C20:5 n-3 | 0.65 + 0.35 | 0.57 + 0.17 | 0.36 + 0.03 | 0.59 + 0.48 |
| C22:5 n-3 | 0.24 + 0.08 | 0.16 + 0.05 | 0.31 + 0.01 | 0.08 + 0.01 |
| C22:6 n-3 | 0.16 + 0.03 | 0.12 + 0.05 | 0.41 + 0.08 | 0.09 + 0.01 |
| Total SFA | 32.29 + 0.27 | 35.44 + 0.61 | 33.69 + 1.41 | 54.41 + 0.11 |
| Total USFA | 67.44 + 0.01 | 64.56 + 0.61 | 66.31 + 1.41 | 45.59 + 0.11 |
| Total Monoenes | 40.72 + 0.30 | 40.52 + 0.29 | 41.66 + 1.86 | 35.98 + 0.20 |
| Total PUFA n-3 | 2.92 + 0.22 | 2.34 + 0.15 | 2.73 + 0.11 | 1.35 + 0.20 |
| Total PUFA n-6 | 23.80 + 0.35 | 21.70 + 0.99 | 21.92 + 0.48 | 8.26 + 0.10 |
| Poly:Sat Ratio | 0.83 + 0.03 | 0.68 + 0.04 | 0.73 + 0.02 | 0.18 + 0.00 |
| Unsat:Sat | 2.08 + 0.03 | 1.82 + 0.05 | 1.97 + 0.13 | 0.84 + 0.00 |
| n-6: n-3 Ratio | 8.19 + 0.48 | 9.35 + 0.96 | 8.04 + 0.29 | 6.31 + 1.14 |

Value= mean + standard deviation, SFA= Saturated fatty acids, USFA=Unsaturated fatty acids, PUFA= Polyunsaturated fatty acids

Table 3. Effect of changing energy-to-protein ratio diet using Hermetia illucens on the fatty acid profile (%) in egg yolk.

| | Dietary treatments | | | | |
|---|---|---|---|---|---|
| Fatty acid profile | Control 0% *Hermetia illucens* | Low EPR 5% *Hermetia illucens* | High EPR 1% *Hermetia illucens* | SEM | *P* -value |
| 14:0 | 0.48c | 0.95a | 0.56b | 0.06 | ** |
| 16:0 | 30.09a | 26.94b | 27.43b | 0.47 | ** |
| 16:1n-7 | 2.61b | 2.42b | 4.07a | 0.29 | * |
| 18:0 | 2.66b | 2.58b | 4.33a | 0.33 | * |
| 18:1 n-9 | 44.24b | 53.08a | 46.85b | 1.20 | ** |
| 18:2 n-6 | 11.42a | 8.38b | 9.89ab | 0.47 | * |
| 18:3 n-3 | 0.63 | 0.65 | 0.57 | 0.08 | ns |
| 20:4 n-6 | 2.59a | 1.63b | 2.19ab | 0.15 | * |
| 20:5 n-3 | 0.97 | 0.97 | 0.90 | 0.06 | ns |
| 22:5 n-3 | 0.64b | 0.48c | 0.71a | 0.03 | ** |
| 22:6 n-3 | 3.63a | 1.88b | 2.47b | 0.29 | * |
| Total SFA | 33.24a | 30.47b | 32.33a | 0.45 | * |
| Total USFA | 66.76b | 69.52a | 67.67b | 0.45 | * |
| Total Monoenes | 46.85c | 55.51a | 50.92b | 1.14 | ** |
| Total PUFA n-3 | 5.87a | 3.99b | 4.66ab | 0.33 | * |
| Total PUFA n-6 | 14.02a | 10.0cb | 12.08ab | 0.56 | ** |
| Poly:Sat | 0.60a | 0.45c | 0.52b | 0.02 | ** |
| Unsat:Sat | 2.01b | 2.28a | 2.10b | 0.04 | * |
| n-6 : n-3 | 2.48 | 2.55 | 2.79 | 0.16 | ns |

EPR= energy-to-protein ratio, SFA= Saturated fatty acids, USFA=Unsaturated fatty acids, PUFA= Polyunsaturated fatty acids

Table 4. Effect of changing energy-to-protein ratio diet using *Hermetia illucens* meal on the cholesterol content (%) in egg yolk

| Treatment | Cholesterol |
|---|---|
| Control (0% *Hermetia illucens*) | 3.39 |
| Low EPR (5% *Hermetia illucens*) | 3.17 |
| High EPR (1% *Hermetia illucens)* | 3.51 |
| SEM | 0.33 |
| *P*-Value | 0.31 |

**Discussion**

In the analysis *Hermetia illucens* larvae meal, the higher total SFA content and the lower total USFA content were reported than those found in Raphia, Weevil, Cricket, Grasshopper, Termites, Imbrasia, UI caterpillar. (Womeni et al., 2009). Also, the total SFA content of *Hermetia illucens* larvae meal was lower and the USFA was higher than those reported in *Hermetia illucens* larvae oil by Kim et al. (2020). This could be because of the difference in the composition of larvae feed media. Ewald (2019) reported a significant difference in the fatty acid profile of larvae meal grown on different substrates. The contents of SFA and USFA in *Hermetia illucens* larvae meal may be altered regarding their contents in the larvae feed media and larvae weight on the harvesting day. Kawasaki et al. (2019) reported 22.9% of SFA and 3.2 PUFA in *Hermetia illucens* larvae meal raised on household organic waste. Liland et al. (2017) mentioned that the contents of PUFA, iodine and vitamin E in the *Hermetia illucens* larvae meal were increased by using seaweed in the feeding media. The larvae meal can be a dietary source of UFA by adding oils rich in UFA to the larva diet (Georgescu et al., 2022). Also, Tirtawijaya and Choi (2022) reported a good source of PUFA, DHA, and EPA in *Hermetia illucens* larvae meal by feeding the larvae on squid liver oil in an appropriate amount.

The findings of this study revealed fatty acid profile variation in egg yolk when changing the EPR diets using *Hermetia illucens* larvae meal. The hens fed a low EPR diet using *Hermetia illucens* at 5% laid eggs were slightly lower in total SFA, palmitic acid, and stearic acid, but higher in total UFA content. According to the previous study, the egg yolk of hens fed *Hermetia illucens* larvae diets contained more SFA and less PUFA than eggs laid by hens fed the control diet (Heuel et al., 2021). Also, in the study of Bejaei and Cheng (2020), it was reported that laying hens fed dry and crushed non defatted *Hermetia illucens* larvae had a high content of SFA, and MUFA compared with hens fed soybean meal. Also, the author mentioned that n-3 and n-6 fatty acid content were lowered by larvae treatments in the egg yolk compared to hens fed the basal diet. However, Secci et al. (2018) did not find a significant difference in the fatty acid profile of egg yolk laid by hens fed a defatted *Hermetia illucens* larvae diet. In the current study, the content of total SFA content in the egg yolk of hens fed the low EPR diet was lower than being used in the diet. In contrast, the total USFA content was higher in egg yolk than those consumed from the diet. This showed that more of SFA, particularly myristic acid and stearic acid were either divided up into other roles such as metabolism energy of the hen body or expansion in the hen metabolism (enterohepatic de novo lipogenesis) rather than being used for integration into the egg lipids as USFA. Also, the improvement in the fatty acid profile of egg yolk could be due to the including *Hermetia illucens* larvae meal in the diet, thus total UFA content was increased and total SFA content declined compared with hens fed the control diet. However, Chatzidimitriou et al. (2016) reported an increase in SFA content and a decrease in PUFA content when replacing soybean cake with *Hermetia illucens* larvae meal in Leghorn layers diets at 12% and 24%. In the fish study, St-Hilaire et al. (2007) mentioned that including the *Hermetia illucens* larvae meal as feedstuff in rainbow trout diets led to a decrease in omega-3 fatty acid content in fish fillets. The omega-3 (n-3) fatty acid content is fundamental for man and associated with health benefits for humans upon consumption (World Health Organization, 2003). Also, the content of palmitic acid in the egg yolk was higher than the amount consumed in the diet, this may be due to utilizing some dietary palmitic acid for active purposes and synthesis of palmitic acid through enterohepatic lipogenesis(Carta et al., 2017; Ravindran et al., 2016). Secci et al. (2018) reported double palmitic acid content in the egg yolk of laying hens fed a diet containing black solder fly larvae than those found in the consumed diet. In this study, changing EPR diets using *Hermetia illucens* larvae meal had a similar effect on cholesterol content in yolk eggs. This result did not agree with Razdan et al. (1997) who reported linkage between chitin-chitosan with bile acids led to a decrease cholesterol content in blood and liver tissue. Chitin-chitosan may decrease the content of bile acids with cholesterol which are available for reabsorption by enterohepatic circulation in the lower intestine. In conclusion, the fatty acid profile of egg yolk can be modified using *Hermetia illucens* larvae meal in the laying hen diet. The low EPR diet using *Hermetia illucens* larvae meal at 5% in the laying hen diet had a positive effect on the fatty acid profile of egg yolk.

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