Effects of cellulase, carnitine and fish supplementations on lipid and fatty acid levels of Muscovy duck eggs

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Abstract. The research aimed to investigate the effect of cellulase, carnitine and Lemuru fish oil supplementation on lipid and fatty acid contents of Muscovy duck eggs. A total of 120 Muscovy duck was allocated randomly to 4 dietary treatments. The basal diet consisted of corn, rice bran and layer concentrate, defined as P1. The remaining treatments were basal diet + 0.1% cellulase enzyme (P2), basal diet + 0.1% cellulase enzyme + 40 ppm L carnitine (P3), basal diet + 0.1% cellulase enzyme + 40ppm L-carnitine + 4% Lemuru fish oil (P4). Result shows supplementation of cellulase enzyme did not influence lipid, cholesterol, and fatty acid contents in eggs resulted, while supplementation of carnitine decreased cholesterol content (P<0.01) without affecting lipid and fatty acid contents. Furthermore, supplementation of Lemuru fish oil in diet containing cellulase enzyme and carnitine enhanced lipid, high-density lipoprotein, and unsaturated fatty acid contents in the eggs (P<0.01). Accordingly, supplementation of Lemuru fish oil decreased low-density lipoprotein, cholesterol, and saturated fatty acid contents (P<0.01). It is concluded that supplementation of carnitine and fish oil in the diet produced Muscovy duck eggs with considerably high unsaturated fatty acids and low cholesterol contents.

1. Introduction
Muscovy ducks have been raised mainly in natural environment of rural areas in Indonesia [1]. They have low performance and reproductive rate; varying dependent on the rearing systems [1,2]. A hen daily gains approximately 3g per day in extensive rearing system, while it can produce daily gain 6g and 8 g respectively in semi-intensive and intensive rearing systems [2]. The basic feed materials to Muscovy duck in Indonesia are still maize and rice bran, despite indigestible property of rice bran associated with its low fiber-degrading enzyme useful to be a nutritional strategy to raise the diet’s nutrition [3]. The carbohydrase enzyme addition improves nutrient utilization [4], one of which is cellulase usable to increase the ability of digesting nutrient by converting cellulose into glucose beneficial to produce energy [3,4] and to mitigate the effect of undigested nutrient on environment [5]. The administration of β-glucanase, β-xylanase, cellulase and phytase enzyme complex can increase egg production and feed efficiency, leading to the better nutrient utilization [3].

Animals, plants and microorganisms produce a water-soluble quaternary amine called L-carnitine (β-OH-γ-N-trimethylaminobutyric acid) [6] that contributes to lipid metabolism by transporting the free fatty acids to pass through internal mitochondrial membrane for β-oxidation leading to the decreased lipid supply for peroxidation and free fatty acid supply to be esterified into triacylglycerols...
and retention in adipose tissue [7,8]. Therefore, carnitine administration is associated with the energy production and the improved energy use through β-oxidation of fatty acids [7].

Maize, soybean and etc constituting plant-based poultry feed ingredients have low content, while animal-derived feedstuffs usually have high content of carnitine. As such, poultry feed with high cereal content may result in carnitine deficiency [9]. Furthermore, an attempt of minimizing rancidity process can be taken through supplementing carnitine with antioxidative property to the diet with high content of fat or oil [6]. Supplementing carnitine to poultry feed is associated with the improved poultry output and the modified chemical structure in the poultry output [8,10,11]. A strategic approach can be taken to enrich poultry products, i.e., through supplementing unsaturated fat sources (menhaden oil, Lemuru fish food, Lemuru fish oil etc) to its feed. Alpha-linolenic acid and linolenic acid are unsaturated fatty acids usually discovered in saltwater fish and fish oil [11]. In addition to containing omega-3 (33.6 to 44.85%), Lemuru fish oil also contains docosahexaenoic acid (14.64%), fat (5.8%) and 8260 kcal/kg total Metabolism Energy [12]. The positive contribution of the administration of unsaturated fat sources in poultry has been studied previously [13,14,15]. However, only very few studies have investigated the addition of cellulase enzyme, carnitine and fish oil to the lipid and fatty acid structures of egg, particularly in Muscovy duck. For that reason, the objective of current research is to study the influence of cellulase, carnitine and Tuna fish oil on lipid, cholesterol, and fatty acid contents supplementation on Muscovy duck eggs.

2. Method

2.1. Data collection technique

| Table 1. Nutrient content and composition of assay diets |
|----------------------------------------------------------|
| Components | P1   | P2   | P3   | P4   |
| Rice bran (%) | 50   | 50   | 50   | 50   |
| Yellow maize (%) | 25   | 25   | 25   | 25   |
| Layer concentrate (%) | 25   | 25   | 25   | 25   |
| Cellulase enzyme (%) | 0.1  | 0.1  | 0.004| 0.004|
| L-carnitine (%) | 0    | 0    | 0    | 0    |
| Tuna fish oil (%) | 0    | 0    | 0    | 0    |
| Lemuru oil (%) | 0    | 0    | 0    | 0    |
| **Nutrient contents** |      |      |      |      |
| Metabolizable energy (kcal/kg) | 2763.75 | 2760.99 | 2760.91 | 2985.22 |
| Crude protein (%) | 17.23 | 17.21 | 17.21 | 16.55 |
| Crude fat (%) | 6.85 | 6.84 | 6.84 | 6.81 |
| Crude fiber (%) | 5.15 | 5.14 | 5.14 | 4.98 |
| Calcium (%) | 3.02 | 3.02 | 3.02 | 2.90 |
| Phosphorus (%) | 0.23 | 0.23 | 0.23 | 0.22 |

A total of 120-layer Muscovy ducks were put onto four different dietary treatments. Each of treatment consisting of 6 hens was conducted with five repetitions. The treatments consisted of P1: Basal diet composed of maize, rice bran and layer concentrate; P2: basal diet + 0.1% cellulase enzyme; P3: basal diet + 0.1% cellulase enzyme + 40 ppm L-carnitine, and P4: basal diet + 0.1% cellulase enzyme + 40 ppm L-carnitine + 4% Tuna fish oil. Table 1 presents composition and nutrient content of assay diets. Hens were sheltered in a housing system intended specifically for egg-laying hens and were raised using standardized management practice. The treatments were administered for 90 days. Then, the hens were given free access to water during the treatments. The eggs produced were gathered every day. Lipid, cholesterol, and fatty acids contents analyses were conducted by collecting ten eggs per replicate. The Association of Official Analytical Chemist procedure was used
to analyze lipid and fatty acid contents [16] and Assmann’s [17] guideline to analyze bad cholesterol (low-density lipoprotein or LDL), good cholesterol (high-density lipoprotein or HDL), and other cholesterols.

2.2. Data analysis

Analysis of variance was employed to analyze the data, followed with orthogonal contrast test if the analysis of variance indicates significant output (α = 0.05) [18]. The set contrasts implemented were as follows: P1 vs P2, P3, P4 (to compare the feed supplemented and the one not supplemented with cellulase enzyme); P2 vs P3, P4 (to compare the feed supplemented and the one not supplemented with L-carnitine); and P3 vs P4 (to compare the feed supplemented and the one not supplemented with Tuna oil).

3. Result and discussion

3.1. Cholesterol contents

Cellulase supplementation exerts not effect on LDL, HDL, and cholesterol levels of eggs (Table 2 and Table 3). Cellulase or called exocellulase did not lower cholesterol contents because it is employed primarily to increase nutrient digestibility, particularly in highly fibrous feed [3,19]. Confirming this finding, previous observation found no effect of cellulase enzyme supplementation on HDL and LDL of Muscovy duck eggs [14].

| Treatment                      | P1       | P2       | P3       | P4       |
|-------------------------------|----------|----------|----------|----------|
| Egg’s cholesterol (mg/dl)     | 867.42^a | 839.94^a | 832.77^a | 712.33^b |
| Eggs LDL contents (mg/dl)     | 28.33^a  | 28.23^a  | 27.23^a  | 22.12^b  |
| Eggs HDL contents (mg/dl)     | 71.67^a  | 71.77^a  | 72.77^a  | 77.88^b  |

Table 2. LDL, HDL and cholesterol levels of Muscovy duck eggs.

| Treatment  | LDL | HDL | Cholesterol |
|------------|-----|-----|-------------|
| P1 vs P2, P3, P4 | NS  | NS  | NS          |
| P2 vs P3, P4    | NS  | NS  | *           |
| P3 vs P4        | *   | *   | *           |

α = 0.05; NS = non-Significant, * = Significant

Carnitine’s role in lipid metabolism makes it potentially induce desirable modifications in poultry performance and products. This research reveals that carnitine supplemented to the cellulase enzyme-containing feed lowers cholesterol content in the eggs, but has no impact on lipid, HDL, and LDL levels. This confirms the previous study finding the contribution of carnitine to lipid metabolism, particularly in transporting the free fatty acids to pass through mitochondria membrane for β-oxidation, leading to lowered lipid and cholesterol storage [20]. As a result, carnitine supplementation in the diets diminishes the volume of lipid and cholesterol deposition [8,20]. This result of research confirms the result of previous studies revealing that cholesterol level of chicken and quail eggs can be decreased through adding carnitine [8,21]. In addition, previous studies revealed that the addition of carnitine to the feed of laying hen diminished the cholesterol level of blood but had no impact on cholesterol content of its egg [10,22]. A previous study also found that supplementing carnitine to quails’ feed on the levels of good and bad cholesterols (HDL, LDL and VLDL) in the blood [8].

Regarding the high unsaturated fat level of Tuna oil, particularly alpha-linoleic acid, the Tuna oil supplementation to the cellulase enzyme- and carnitine-containing feed raises lipid and HDL and lowers cholesterol and LDL levels of Muscovy duck eggs. Fish oil is one of energy and unsaturated fat sources [23], so that its supplementation can increase the eggs’ lipid contents [14]. In addition, the
Hamp seed oil supplemented to the laying hens’ feed generated lipid deposition in eggs [24]. Similarly, previous study found that the polyunsaturated fatty acid supplementation to the feed increased lipid content in rats’ body tissue [25]. This experiment’s result confirms previous observation finding that the addition of fish oil lowered serum and egg yolk cholesterol level [26] and raised HDL level [27]. Then, the lipid and cholesterol transportation are facilitated by the high HDL content [23]. Furthermore, the polyunsaturated fatty acid supplementation to the feed raised HDL content of body tissue [15,28]. The increase in HDL content of chicken eggs resulting from the unsaturated fat supplementation has been studied previously [14]. Alpha-linoleic acid lowers cholesterol and LDL levels through provoking HDL to transport cholesterol to liver and to secrete cholesterol from liver to intestine [20]. Previous research also observed that the addition of alpha linoleic acid to the feed lowered cholesterol level of milk [19, 23]. For that reason, the polyunsaturated fatty acid supplementation to the feed lowers LDL content of chicken body tissue [28].

3.2. Fatty acid contents
Supplementation of cellulase enzyme and carnitine to the cellulase enzyme-containing feed affects the fatty acid (omega-3, omega-6, unsaturated and saturated fatty acids) contents of eggs (Table 4 and Table 5). The Tuna oil supplementation to the cellulase enzyme- and carnitine- containing feed raises alpha linoleic acid, linoleic acid and unsaturated fat levels and lowers saturated fat levels of eggs (P<0.01). Because of its high unsaturated fatty acid content transferred to the eggs, Tuna fish oil can increase the unsaturated fatty acids contents, particularly Omega-3 and Omega-6. Omega-3 and omega-6 contents of body tissue can be increased through supplementing omega-3 [25]. This result is corresponding to previous study finding that the Lemuru fish oil supplementation raised unsaturated fat, alpha-linoleic acid, and linoleic acid levels of chicken eggs [14,27], as well cow milk [19].

Table 4. Saturated fat levels of Muscovy duck eggs.

| Treatment                  | P1           | P2           | P3           | P4           |
|----------------------------|--------------|--------------|--------------|--------------|
| Omega-3 (Linolenic acid (%))| 5.71a        | 5.74a        | 5.81a        | 8.40b        |
| Omega-6 (Linoleic acid (%)) | 28.74a       | 28.87a       | 28.71a       | 37.30b       |
| Saturated fatty acid (%)    | 30.88a       | 32.34a       | 31.96a       | 25.02b       |
| Unsaturated fatty acid (%)  | 69.12a       | 67.66a       | 68.04a       | 74.98b       |

Table 5. Orthogonal contrast result of fatty acids contents of Muscovy duck eggs.

| Treatment                  | Omega-3 (%) | Omega-6 (%) | Unsaturated fatty acids (%) | Saturated fatty acids (%) |
|----------------------------|-------------|-------------|----------------------------|--------------------------|
| P1 vs P2, P3, P4           | NS          | NS          | NS                         | NS                       |
| P2 vs P3, P4               | NS          | NS          | NS                         | NS                       |
| P3 vs P4                   | *           | *           | *                          | *                        |

α = 0.05; NS = non-Significant, * = Significant

As one of energy and unsaturated fatty acid sources, the addition of fish oil can raise their contents in the animal products [23]. The increase in alpha linoleic acid level of the egg of laying hens fed with fish oil supplemented feed revealed the absorption of unsaturated fat in the intestine and its deposition in the eggs [29]. The Tuna oil administration raised alpha linoleic acid level of laying hens’ egg yolk [30]. Other studies also found a higher concentration of omega-3 in egg of laying hens receiving fish oil-supplemented feed compared with those receiving soybean oil-supplemented feed [31]. Additionally, the combined fish and peanut oils raised the contents of free unsaturated fatty acid [32]. Moreover, due to its high contents of unsaturated fatty acid, the Tuna oil supplemented to the cellulase enzyme and carnitine-containing feed lowers saturated fat level of eggs (P<0.05). Accordingly, the Lemuru oil supplementation lowers saturated fat level of eggs [14]. Furthermore, the unsaturated fat supplemented to the feed lowers saturated fat level of body tissue [25,27,33].
4. Conclusion
The fish oil supplementation to the cellulase enzyme and carnitine-containing feed generates a higher level of HDL and saturated fat (alpha linoleic and linoleic acids), while lowers LDL and total cholesterol levels in Muscovy duck eggs. Hence, the addition of fish oil lowers the contents of saturated fatty acid in Muscovy duck eggs.

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