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The effect of organic quail egg supplementation on the blood lipid profile of white mice (*Rattus Norvegicus L.*) during the lactation period

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Abstract.Background: Quail eggs contain a considerable amount of complete nutritional sources such as carbohydrates, proteins, fats, and micronutrients. However, they also have a high cholesterol level, which can potentially cause atherosclerosis and chronic heart diseases. The response of the body to foods containing is influenced by factors such as ethnicity, genetics, and hormonal and nutrient status of the consumer. The cholesterol level of quail eggs can be reduced by manipulating the feed using supplemental organic feed. Organic quail eggs have been believed to correct the lipid profile of white mice during the lactation phase. Purpose: The aim of this study was to analyze the effect of feed containing organic quail eggs on the blood lipid profile of white mice (Rattus norvegicus L.) during the lactation phase. Materials and Methods: This experimental study was conducted using a completely randomized design with four experiments and five repetitions. Experimental mice: T0 mice were used as control; T1 mice were supplemented with quail eggs produced by quails that were fed with standard feed; T2 mice were supplemented with eggs produced by quails fed with standard organic feed; and T3 mice were supplemented with eggs produced by quails fed with organic feed with the addition of cassava leaf flour, mackerel flour, and turmeric powder. Quail egg supplementation was administered to the mice from the early pregnancy period till the end of the lactation phase. The acquired data were analyzed using ANOVA. SPSS version 16.0 software for Windows was used for data analyses. Results and summary: Feeding the white mice with different compositions of organic quail egg supplements had no effect on the consumption of feed and water, body weight, and lipid profile (including total cholesterol, LDL, HDL, and triglyceride) during the lactation phase (P > 0.05).

Keywords: lactation, lipid profile, supplement, organic quail egg, fatty acid

1. Introduction

Quail (*Coturnix coturnix japonica* L.) eggs are potential sources of animal protein and are largely consumed by the Indonesian people. The nutritional composition of a quail egg is as follows: carbohydrate (4.01 g/dl), protein (12.7 g/dl), fat (9.89 g/dl), and ash (1.06 g/dl) [1]. Quail eggs also contain a high cholesterol content that implies a risk of causing degenerative diseases such as cardiovascular disease. The level of cholesterol in the yolk is dependent on the quail species and age and also on the nutrition content of the feed [2]. The saturated fat level in a quail egg is ± 3 g/100 g and the cholesterol level are $\pm 200-300$ mg/100 g [3]. Besides the adverse effects, cholesterol serves as the precursor for steroid hormones, bile acid synthesis, tissue generation, cell membrane components, and nutrients for the formation of milk (breast milk) [4].

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The cholesterol level of quail eggs can be reduced by manipulating the feed by replacing the standard feed with organic feed. Quails fed with organic feed will produce eggs that are rich in omega-3 fatty acids, especially DHA. Organic eggs exert a metabolic effect that is similar to that provided on consuming mackerel flour, which thereby increases the HDL level and reduces the risk of metabolic syndrome [5]. The addition of fish oil and linseed oil to quail feed can increase the omega-3 fatty acid levels in the eggs [6,7]. Carotenoid is a natural pigment found in the yolk that imparts a yellow color, ranging from pale yellow to orange. $\pm 1\%$ Carotenoid present in the yolk consists of carotenes and xanthophylls [8-10]. This compound is also present in plants, primarily those with dark green leaves [11]. The cholesterol level of an egg of a quail that has been fed with feed mixed with turmeric powder at 108 mg/quail/day has been reported to be 767.77 (mg/dl) [12].

Quails that are fed with organic feed supplemented with cassava leaf flour, mackerel flour, and turmeric powder can produce organic eggs rich in unsaturated fatty acids, especially EPA and DHA. Grifin [13] stated that omega-3 fatty acids can inhibit cholesterol biosynthesis and lower VLDL-cholesterol and triglyceride levels. Mice can be used as experimental animals as they are highly sensitive to nutrients and hormonal and environmental changes occurring during pregnancy and lactation phases, which can cause physiological changes [14]. Based on this information, it is necessary to conduct additional research to understand the effect of the administration of organic quail egg supplements rich in unsaturated fatty acids on the lipid profile of lactating mice.

2. Methodology

This study was conducted in the Laboratory of Animal Structure and Function at the Biology Department of the Science and Mathematics Faculty of Universitas Diponegoro Semarang. The tools used in this study included a photomicrograph microscope (Olympus BX51), digital analytics scales (MN series digital pocket scale), a centrifuge, and a spectrophotometer diagnosis method. The study material included female *Rattus norvegicus* L. aged 2 months and weighing ±200 g, Giemsa stain solution, and the DSI reagent (Diagnostika System Indonesia) kit for assessing the serum levels of triglycerides, cholesterol, HDL, and LDL.

A completely randomized design was used in this study conducted using 20 female *R. norvegicus* L. and 5 male *R. norvegicus* L. that were obtained from Ngalian Permai Mice Breeding in Semarang. The mice were acclimatized for 2 weeks and randomly categorized for four experiments and five repetitions. The experimental mice were as follows: T0 mice were fed with standard feed; T1 mice were fed with quail egg supplements that were produced by feeding the quail with common feed; T2 mice were fed with quail egg supplements that were produced by feeding the quail with standard organic feed; T3 mice were fed with quail egg supplements that were produced by feeding the quail with organic feed mixed with cassava leaf flour, mackerel flour, and turmeric powder.

Female mice, which were in the estrus period (estrus duration 4–5 days), were mated with male mice. Each mouse was fed with one egg every day at 16:30 h for 49 days (until weaning). Feed and water were provided *ad libitum*. The standard feed and quail egg organic supplements were analyzed proximately at Wahana Semarang Laboratory (Analysis-Chemical Distributor-Consultant). On the final day of the experiment, the parent mice were anesthetized using chloroform and dissected. Blood was collected from the heart using a 5-ml syringe into a vacutainer tube and left for 2 h.

Lipid profile analysis: Serum samples were collected and centrifuged for 20 min at 10,000 rpm. Using a micropipette, $10~\mu$ l of the serum was added into a test tube, followed by the addition of reagents for assessing cholesterol and triglyceride levels. The serum was incubated for 10~min at 37° C. For HDL and LDL analysis, 0.5~ml of serum was taken using a pipette and added into a test tube, followed by the addition of 1~ml of reagents for HDL or LDL analysis. Then, the tube was centrifuged for 10~min at 10,000~rpm, $10~\mu$ l of the supernatant was collected using a pipette and added into another test tube, and 1~ml of cholesterol reagent was added and incubated for 5~min at 37° C. The lipid profile was analyzed using a spectrophotometer at a wavelength of 546~m and f = 676. The observed variables included cholesterol levels, HDL, LDL, triglyceride, feed consumption, water consumption, and final body weight. The data were analyzed by ANOVA with a probability of P > 0.05). SPSS

version 16.0 software for Windows was used for the statistical analyses.

3. Results

The results of the observation of the estrus period of the white mice are shown in Fig. 1, which were determined by observing the vaginal smears and the color of the Giemsa staining. Female mice, which were in the estrus period (estrus duration 4–5 days), were mated with male mice. The proestrus period is characterized by the oval form of epithelial cells with a blue color and a pink cell core. Estrus is the mating period with a high probability of copulation and is characterized by the keratinization of epithelial cells without a core and with pale color. The metestrus period starts immediately after the ovulation and is characterized by the keratinization of epithelial cells and the presence of leukocytes. The diestrus period occurs after the regression of the functional corpus luteum and is characterized by the presence of epithelial cells with a core, leukocytes, and the mucus.

The results of the consumption of feed and water and the final body weight of the white mice that were fed with organic quail egg supplements in the lactation phase are shown in Table 1.

The average feed consumption was 19.14–21.87 g/day. The lowest feed consumption was found in T1 mice that were fed with standard egg supplement containing saturated fatty acid, whereas the highest feed consumption was found in T3 mice that were fed with organic egg supplement containing unsaturated fatty acids. The average daily consumption of water was 2.6820–3.13 ml/day, and the highest water consumption was found in T0 mice. The final body weight of the lactating mice was 172–212 g. Administration of different feed compositions had no effect on the final body weight of the lactating mice (Table 2).

The cholesterol level of the lactating mice that were fed with organic egg supplements was 62.75–87.80 mg/dl. The T0 control mice had higher cholesterol levels than those of the experimental mice. Similarly, the highest HDL and the highest triglyceride levels were found in the T0 control mice, with the average values being 16.75–26.40 mg/dl and 182.40 mg/dl, respectively. The T2 mice that were fed with egg supplements showed a triglyceride level of 78.00 mg/dl. The T1 experimental mice had a higher LDL level (43.30 mg/dl) than that of the T0 control mice (24.9 mg/dl).

4. Discussion

The estrus cycle of the mice was confirmed by comparing the nucleated epithelial cells, keratinized epithelial cells, leukocytes, and the mucus of vaginal smear, as shown in the results depicted in Figure 1. In general, the estrus cycle in mice is divided into four phases, which include proestrus, estrus, metestrus, and diestrus. During the estrus phase, the treated mice underwent fertilization and were supplemented with one organic quail egg per day (from their pregnancy until the weaning period) [15].

Supplementation with the organic quail eggs had no significant effect on the feed intake (P > 0.05). This suggested that the appearance, smell, and taste of the quail eggs did not affect the palatability. The average feed intake in the lactation period was 19.14–21.87 g/day, according to the feed intake of the mice in the lactation phase [16-17]. Supplementation with the organic quail eggs to the white mice daily also had no significant effect on the water consumption during the lactation phase (P > 0.05). Water consumption was found to be influenced by temperature and humidity. The average temperature and humidity during the study period were 26.40°C \pm 26.69°C and 76.89% \pm 78.55%, respectively, which was an optimal environmental condition.

If the humidity in the mice cage is high, it can prevent the evaporation of body moisture through sweat, which accumulates heat in the body. The mice will then release the body heat through the urinary tract and feces. This water loss in the body needs to be replaced by drinking water. Water functions as a regulator of body temperature because it absorbs the heat generated from body metabolism. Body heat can be generated in various ways, such as dispensing the body water through the digestive tract, the skin, and through breathing. To overcome these processes, the lactating mice must drink moderate amounts of water.

Quail egg supplementation had no significant effect on the body weight of the parent mice (P > 0.05). An increase in body weight is related to daily feed intake and feed composition. Body weight

increases as the feed intake increases; the feed intake results in this study were relatively similar. The different compositions of the quail egg supplements also had no effect on the body weight of the lactating mice. Quail egg supplements administered to T1 rats contained saturated fatty acids, whereas supplements containing unsaturated fatty acids were administered to T2 and T3 rats. Feed intake and energy needs can affect the body weight of the lactating mice to produce breast milk. Lipids stored during pregnancy are used to produce milk [8].

Results of the analysis of the effect of organic quail egg supplementation on the serum levels of total cholesterol, LDL, HDL, and triglyceride of the lactating white mice showed some differences, though not significant (P > 0.05). This could be due to the presence of exogenous cholesterol amount in the feed that affected the functions of the heart and intestines in synthesizing endogenous cholesterol. The relatively similar feed intake caused the lack of organic quail egg supplementation effect on blood cholesterol level of the lactating mice. Cholesterol synthesis in the liver and the small intestine will increase when the cholesterol amount in the food is low and the vice versa [4]. Unsaturated fatty acids tend to lower the total cholesterol, not only LDL but also HDL, whereas the intake of saturated fatty acids will increase the lipid profile [19].

Supplementation with organic quail eggs showed no significant effects on the serum LDL and HDL levels of the lactating mice (P > 0.05). The factor that caused a difference in the LDL levels was the composition of the quail egg supplements. The composition of the quail egg supplements administered to T0 and T1 rats contained saturated fatty acids, whereas T2 and T3 rats were fed with supplements containing unsaturated fatty acids. Commercial eggs are rich in saturated fatty acids, whereas organic eggs are rich in unsaturated fatty acids, especially EPA and DHA [12]. PUFA (EPA and DHA) tends to lower the average LDL cholesterol; however, it may decrease HDL cholesterol [20]. PUFA plays an important role in fat metabolism and transportation, immune function, and maintaining the function and integrity of cell membranes [4]. Unsaturated fatty acids produce greater energy than that produced by saturated fatty acids. The LDL receptor on the cell surface is regulated by cholesterol that is required for the production of cell membranes, steroid hormones, and breast milk [21,4]. VLDL particles contain a high amount of triglycerides, while saturated fatty acids tend to raise the LDL levels [22]. The type of fatty acids present in the organic quail egg supplements might influence the HDL levels. Different HDL levels are affected by HDL lipid metabolism for the production of breast milk during the lactation phase [23]. Parent mice need more energy to meet their nutritional requirements for producing breast milk. The gender of the mouse pup lso affects the cholesterol level, as it has been reported that LDL and HDL cholesterol levels were higher in male mouselings than in female pups [24].

Triglyceride is the highest energy source, containing almost 9 kcal/g. The maximum level of daily total fat needed is 30% of the total energy, which includes 10% of saturated fatty acids (SFA), 10% of monounsaturated fatty acids (MUFA), and 10% of polyunsaturated fatty acids (PUFA). Cells require energy to meet the nutritional needs of breast milk production, and the enzyme lipase in fat cells breaks down triglycerides into glycerol and free fatty acids and releases them into the blood vessels [25]. Most of the free fatty acids return into circulation, allowing the absorption of the lipoprotein triacylglycerol fatty acid to produce milk fat [26]. The analysis of the lipid profile (Table 2) in this study showed that supplementation with organic quail eggs can meet the nutritional requirements of the lactating mice. However, further study is needed to determine the optimal level of organic quail egg supplementation to decrease the total cholesterol, triglyceride, and LDL levels and increase the HDL levels during the lactation phase.

5. Conclusion

Organic quail egg supplementation, feed and water consumption, and the final body weight of the lactating mice have no significant effect on the total cholesterol, LDL, HDL, and triglyceride levels in the lactation phase. Similarly for giving organic egg quail supplements did not increase feed intake, drink consumption, and body weight of mice in the lactation period.

6. Suggestions

Results of this research suggest that further study is needed to determine the optimal level of organic quail egg supplementation to decrease the total cholesterol, triglyceride, and LDL levels and increase the HDL levels during the lactation phase.

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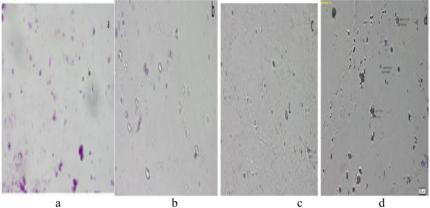


Figure 1. Illustration of the vaginal smear cytology of white mice during one estrus cycle: proestrus (a), estrus (b), metestrus (c), and diestrus (d) phases.

Table 1. Feed consumption, water consumption, and final body weight of white mice fed with organic quail egg supplements in the lactation phase

1. Variable	2. Treatment				
	3. T0	4. T1	5. T2	6. T3	
7. Feed consumption	9. 19.56 ^a ± 4.37	10. 19.14 ^a ± 4.42	11. 21.66 ^a ± 0.47	12. 21.87 ^a ± 1.01	
8. (g/day)					
13. Water consumption (ml/day)	14. $3.13^{a} \pm 0.47$	15. $2.77^{a} \pm 0.46$	16. 2.6820 a ± 0.26	$17. \ \ 2.8840^a \pm 0.20$	
18. Final body weight (g)	$19. \ \ 211.20^{\ a} \pm \\ 37.77$	$20.\ \ 212.00^{\ a}\pm 28.63$	21. $192.00^{a} \pm 13.03$	22. $172.00^{a} \pm 25.88$	

Description: T0: Rattus norvegicus L. not supplemented with quail eggs, T1: R. norvegicus L. supplemented with P0 quail eggs (produced by quails fed with standard feed), T2: R. norvegicus L. supplemented with P1 quail eggs (produced by quails fed with standard organic feed), T3: R. norvegicus L. supplemented with P2 quail eggs (produced by quails fed with standard feed with the addition of turmeric powder, cassava leaf flour, and mackerel flour).

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The results of the blood lipid profile analysis of white mice supplemented with organic quail eggs during the lactation phase are presented in Table 2.

Table 2. The blood lipid profile analysis of white mice supplemented with organic quail eggs during the lactation phase

Variable	ariable Treatment			
	T0	T1	T2	Т3
Cholesterol (mg/dl)	$87.80^a \pm 22.016$	$83.00^a \pm 27.839$	$65.75^{a} \pm 30.192$	$62.75^{a} \pm 21.030$
LDL (mg/dl)	$24.9^a \pm 14.464$	$43.30^a \pm 16.914$	$32.90^a \pm 23.212$	$28.15^{a} \pm 4.312$
HDL (mg/dl)	$26.40^a \pm 8.877$	$18.00^{a} \pm 2.646$	$17.25^{a} \pm 6.702$	$16.75^{a} \pm 11.500$
Triglyceride s (mg/dl)	$182.40^{a} \pm 120.492$	$108.00^a \pm 47.032$	$78.00^{a} \pm 31.454$	$89.25^{a} \pm 31.542$

Description: T0: Rattus norvegicus L. not supplemented with quail eggs, T1: R. norvegicus L. supplemented with P0 quail eggs (produced by quails fed with standard feed), T2: R. norvegicus L. supplemented with P1 quail eggs (produced by quails fed with standard organic feed), T3: R. norvegicus L. supplemented with P2 quail eggs (produced by quails fed with standard feed with the addition of turmeric powder, cassava leaf flour, and mackerel flour).

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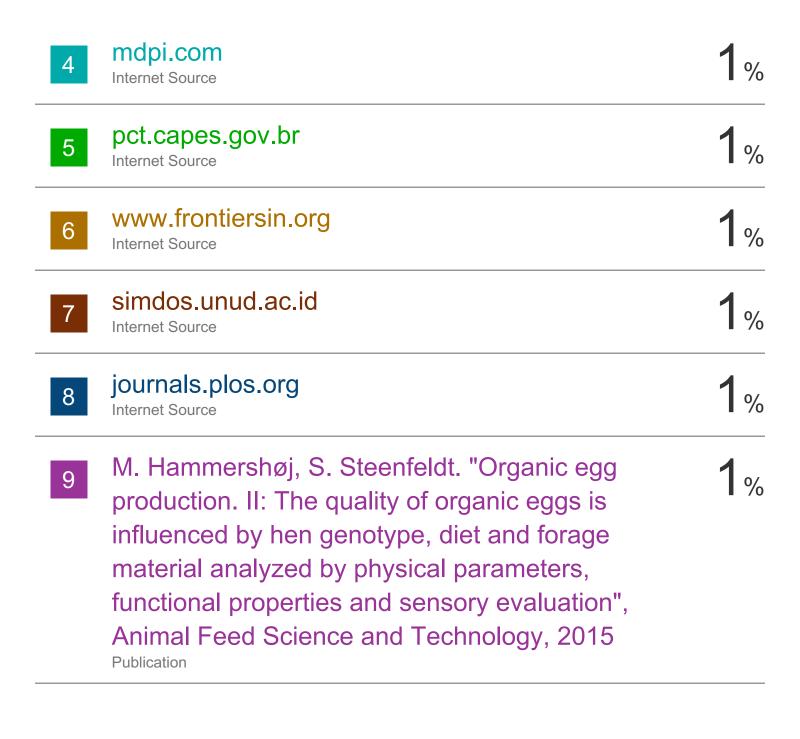
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