Liver histological structure of rats (*Rattus norvegicus*) in the lactation period after supplemented with organic quail eggs

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Abstract. Lactation period requires more nutrients and energy to meet the needs of lactating rats and pups. Increased energy and nutrient requirements during lactation have an effect on increasing metabolic rate as well. Lactation energy deficiency may affect the histology of the liver. This study was conducted to examine and analyze the effect organic quail eggs supplementation on hepatocytes diameter, hepatic lobules diameter, liver weight, and body weight in the lactating test animal. This study used 20 females Wistar rats consisted of 5 treatments with 4 replications. The treatment of this study consisted of T0: control rat; T1: rat supplemented with commercial quail eggs; T2: rat supplemented with standard organic quail eggs; T3: rat supplemented with organic quail eggs contained cassava leaves, mackerel, and turmeric powder; and T4: rat supplemented with organic quail eggs contained cassava leaves, seaweed, and turmeric powder. This research used Completely Randomized Design. The variables measured in this study were hepatocytes diameter, hepatic lobules diameter, liver weight, and body weight in the test animal. The obtained data were analyzed using Analysis of Variance (ANOVA) at 5% significance level and continued with Duncan test with 5% significance level if there is a significant difference. The result of data analysis showed that supplementation of organic quail eggs give the significant difference in hepatocytes diameter and hepatic lobules diameter, but had no significant difference in liver weight and body weight so that it can be concluded that quail eggs supplementation in the lactation period affects rats’ liver histological structure.

1. Introduction
Nutrition is very important from the period of pregnancy to lactation for embryo growth and development. In its feed, rats need 20-25% protein, 5% fat, 45-50% starch, 5% crude fibre, 4,000 IU / kg vitamin A, 1,000 IU / kg vitamin D, and 30 mg / kg vitamin E [1]. The consumption of metabolized energy in rats that is pregnant is 9.6%; whereas during lactation it increased to 45.2% [2]. The nutritional needs of the rats in the lactation period are greater than in the pregnancy period because nutrients are needed to restore postpartum conditions besides milk production. Postpartum milk production is generally very much so that the lactating rats will be thirsty and hungry faster. The number of calories released must be balanced with nutritional intake so that the energy obtained from the feed can be reprocessed for milk formation [3].

The liver is an organ that has various functions that are important for the body [4]. Various liver functions are synthesizing and secreting bile; plays a role in the metabolism of carbohydrates, proteins, and lipids; synthesize hormones, detoxify, and store various kinds of vitamins and minerals [5]. Increased energy and nutritional needs during pregnancy and lactation affect the increased metabolic
rate [6]. Hepatocyte cells are cells that have high activity so that they are susceptible to damage [7]. High metabolism can increase the production of reactive oxygen species (ROS) [6].

Quails which are supplemented with organic ingredients produce organic quail eggs, that have higher nutrient and essential fatty acids than non-organic quail eggs [8]. Feeding organic quail eggs to pregnant and lactating rats is expected besides to meet the needs of the rats and its pups, it can also maintain the liver structure under normal conditions. Based on the background above, it is important to conduct research of organic quail eggs supplementation to Rattus norvegicus in lactation period so that the effects of organic quail eggs on liver histological structure can be known.

2. Materials and methods
Research and data collection was carried out at the Animal Structure and Function Laboratory of the Biology Department, Faculty of Science and Mathematics, Diponegoro University, Semarang. The tools and materials used in this study are digital scales, dissecting sets, microtomes, photomicrograph microscopes (Olympus BX51), 20 females and 5 males Wistar strain rats (Rattus norvegicus) aged 2 months with ±200 g body weight, commercial feed, organic quail eggs, 10% BNF solution, and Hematoxylin-Eosin dye.

The test animals used were obtained from the Ngaliyan Permai Rat Farm in Semarang and then it was acclimated for two weeks. This study used a Completely Randomized Design consisting of 5 treatments, each replicated 4 times. The treatments included T0: control rats (given commercial feed), T1: rats which were given commercial egg supplement; T2: rats which were given a standard organic quail egg supplement; T3: rats which were given supplements of organic quail eggs containing cassava leaves, mackerel, and turmeric; and T4: rats which were given supplements of organic quail eggs containing cassava leaves, seaweed, and turmeric.

Female rats that have entered the estrus cycle (4-5 days estrus duration) mated with male rats. Commercial feed and drinking are given ad libitum. Female rats were given one organic quail egg every day at 4.30 p.m. starting from the beginning of the pregnancy to the end of the lactation period [9]. At the end of the study, these rats were anesthetized using chloroform and dissected. The liver organ was isolated, washed with physiological saline solution, then fixed using BNF 10% [10]. Liver organ made histological preparations with paraffin method and Hematoxylin-Eosin staining (HE).

Histology observation was carried out descriptively and quantitatively using a photomicrograph microscope (Olympus BX51) by observing 3 lobules and 10 hepatocyte cells around the central vein with 1.000x magnification. Measurement of hepatocytes diameter and lobules diameter was done by divide the hepatocyte and lobule cross sections perpendicularly based on the closest distance (y) and the farthest distance (x) using the formula of \( \frac{x + y}{2} \) [11].

The variables observed in this study were hepatocyte diameter, lobules diameter, liver weight, and body weight of rats that is pregnant. The data obtained were analyzed using Analysis of Variance (ANOVA) at a significance level of 5% and if there were significant differences, it was continued with the Duncan test at a significance level of 5%. Data analysis was performed using SPSS software for Windows version 24 [12].

3. Results
The analysis results of the effect of organic quail egg supplementation on the average hepatocyte diameter, lobules diameter, liver weight, and body weight of rats were presented in Table 1.

The results of ANOVA analysis with the effect of organic quail eggs supplementation on the diameter of hepatocytes showed significantly different results (P<0.05). This shows that the feeding of organic quail egg supplements affects the diameter of hepatocytes. The results of further analysis with the Duncan test showed that there were significant differences between T0 with T2, T0 with T3, T0 with T4, T1 with T2, T1 with T3, T1 with T4, and T2 with T4, but there were no significant difference between T0 and T1, T2 with T3, and T3 with T4 (Table 1).

The results of ANOVA analysis about the effect of organic quail eggs supplementation on the diameter of liver lobules showed significant results (P<0.05). This shows that organic quail eggs supplementation affects the diameter of the liver lobules. The results of further analysis of Duncan's test showed that there were significant differences between T0 with T1, T0 with T2, T0 with T3, T0
with T4, T1 with T3, T1 with T4, T2 with T3, and T2 with T4, but there were no significant differences between T1 with T2 and T3 with T4 (Table 1).

**Table 1.** Analysis results of average hepatocyte diameter, lobules diameter, liver weight, and body weight of lactating rats after the supplementation of organic quail eggs.

| Variable          | Treatment | T0  | T1  | T2  | T3  | T4  |
|-------------------|-----------|-----|-----|-----|-----|-----|
| Hepatocyte Diameter (µm) |           | 18.35±0.90 | 18.97±1.15 | 20.74±0.38 | 21.87±0.64 | 22.37±0.85 |
| Lobules Diameter (µm) |           | 619.20±13.21 | 707.63±24.74 | 701.31±18.37 | 788.26±7.41 | 796.02±16.62 |
| Liver Weight (g)   |           | 9.50±1.74 | 9.14±1.25 | 9.12±1.68 | 9.07±2.22 | 8.18±1.95 |
| Body Weight (g)    |           | 220±35.59 | 207±30.96 | 195±12.91 | 190±27.08 | 180±24.49 |

(P>0.05). T0: Control, T1: Egg supplementation from quail that fed with commercial feed, T2: Egg supplementation from quail that given standard organic feed, T3: Egg supplementation from quail that fed with organic feed containing cassava leaves, mackerel, and turmeric, and T4: Egg supplementation from quail that given organic feed containing cassava leaves, seaweed, and turmeric.

The results of ANOVA analysis of the effect of organic quail eggs supplementation on liver weight showed no significant differences (P>0.05) (Table 1). This shows that organic quail eggs supplementation has no effect on the weight of the liver of lactating rats. The average weight of the rats’ liver is T0 which is 9.50 g; T1 is 9.14 g; T2 is 9.12 g; T3 is 9.07 g; and T4 is 8.18 g.

The results of the ANOVA analysis of the effect of organic quail eggs supplementation on the body weight of lactating rats showed results that there were no significant differences (P>0.05) (Table 1). This shows that the supplementation of organic quail eggs does not have an effect on the body weight of the lactating rats. The average body weight of lactating rats is T0 which is 220 g, T1 is 207 g, T2 is 195 g, T3 is 190 g, and T4 is 180 g.

**4. Discussion**

Factors that cause the diameter of hepatocytes in T2, T3, and T4 are higher than controls and T1 is a change in metabolism [13]. Metabolic changes are characterized by an increase in the nutritional needs of the lactating rat for the growth of its pups [14]. Increased nutritional requirements can be fulfilled from the content of organic quail eggs in the form of fatty acids, proteins, carbohydrates, β-carotene, vitamin A, vitamin B12, and antioxidants [8, 9, 15, 16] which will then pass through the metabolic process in the liver to produce energy and nutrients for the mother and pups.

The increase in the diameter of hepatocytes at T1, T2, and T3 after supplementation with organic quail eggs in the lactating rats was still at a normal level. Based on Rosioru et al. (2012) [17], the normal diameter of white rat hepatocytes ranged from 17.01 to 21.93 µm. T4 treatment showed a higher increase in hepatocyte diameter compared to other treatments, namely 22.37 µm.

Descriptions of the liver histological structure of lactating rats fed with organic quail eggs showed a normal representation of hepatocytes. The normal representation of hepatocytes is shown by the lobules which are composed of hepatocytes with the nucleus streaked in dark red and have scattered and clear chromatin and absorbs dyes strongly. Hepatocytes arranged radiate in the lobules. The cell plate is free anastomosed and has clear intercellular boundaries. Hepatocytes have one or more nuclei found in cell plates. Sinusoid contains blood from the hepatic portal vein and hepatic artery [18]. Hepatocytes in T4 experience a higher hepatocyte diameter than other treatments. Based on the
analysis of the structure of the liver microanatomy on T4, the increase in hepatocyte diameter caused by glycogen accumulation Figure 1. Glycogen is a glucose polymer that acts as an energy reserve in the cytoplasm of hepatocytes. The accumulation of glycogen is histologically characterized by the presence of vacuoles in the cytoplasm of the hepatocytes, so that it has a clear color of cytoplasmic vacuoles.

Figure 1. The liver histological structure of lactating rats after the supplementation of organic quail eggs. Description: Hematoxylin-Eosin staining and 1.000x magnification. H: hepatocytes, S: sinusoids, BC: binucleated cell, CD: cell division, ME: metaphase, AN: anaphase, GL: glycogen accumulation. T0: Control, T1: Egg supplementation from quail which fed with commercial feed, T2: Egg supplementation from quail which given standard organic feed, T3: Egg supplementation from quail which fed with organic feed containing cassava leaves, mackerel, and turmeric, and T4: Egg supplementation from quail which given organic food containing cassava leaves, seaweed, and turmeric.

The nutritional content of organic quail eggs can also maintain the liver structure of lactating rats. This is because organic quail eggs contain fatty acids which then form phospholipid which is an important component of cell membranes [20], besides that the content of β-carotene in organic quail eggs acts as an antioxidant that can balance free radicals caused by an increase in liver metabolism. Carotenoids can improve animal health because they act as antioxidants [21]. β-carotene can inhibit free radical production, reduce nuclear damage, and inhibit lipid peroxidation [22]. Rat’s liver histological structure supplemented with organic quail eggs in T3 (Figure 1) showed cells that
regenerate in metaphase and anaphase stage because organic quail eggs contained an unsaturated fatty acid that induced hepatocyte regeneration [23].

The supplementation of organic quail eggs to lobules diameter showed significant differences compared to controls which given commercial feed. The real difference is shown in the diameter of the hepatocytes between T0 and T2. T0 with T3, T0 with T4, T1 with T3, T1 with T4, and T2 with T4 followed by a significant difference in the diameter of the liver lobules. The supplementation of organic quail eggs between T3 and T4 to the diameter of the liver lobule also showed no significant difference, this was indicated by the absence of a significant difference in T3 with T4 in the diameter of hepatocytes. The provision of organic quail egg supplements to the lactating rats was actually able to increase the diameter of the hepatocytes. By the increase in the diameter of the hepatocyte then it was actually also able to increase the diameter of the liver lobules. This is because the liver lobules are composed of many hepatocytes, so the increase in the diameter of the hepatocytes affects the liver lobules. The liver lobules are mainly composed of hepatocytes arranged radiate from the central vein to peripheral [24]. The increase in liver lobules is evidenced by an increase in the size of the liver cell which is caused by an increase in the accumulation of metabolic results [11].

The provision of commercial quail egg supplement (T1) with control namely commercial feeding (T0) and supplementation of standard organic quail eggs (T2) with organic quail eggs (T3) on lobules diameter showed significant differences, despite the provision of commercial quail egg supplements (T1) compared to controls (T0) and supplementation of standard organic quail eggs (T2) with organic quail eggs (T3) showed no significant differences in the diameter of hepatocytes. It is suspected that the diameter of the liver lobules is affected by the number of hepatocytes.

The supplementation of commercial quail eggs (T1) with standard organic quail eggs (T2) on lobules diameter showed no significant differences, although the provision of commercial quail eggs (T1) supplements compared to standard organic quail eggs (T2) showed a significant difference in hepatocytes diameter. It is suspected that the diameter of the liver lobules is influenced by the number of the rat’s pups. Saraswati et al. (2016) stated that the number of rat pups in T2 was more than T1, which amounted to 54 while the number of pups in T1 was 41. In the pregnancy and lactation period, the mother does not only need energy for her own body but also to provide energy for the pups [25]. The lactation period plays an important role in the process of mobilizing fat reserves that accumulate during the pregnancy period [26] so that with more pups than other treatments, the energy needs that must be provided are also greater and the energy reserves stored in the mother T2 rat hepatocytes become more quickly mobilized.

The results of ANOVA analysis of the effect of organic quail eggs supplementation on liver weight showed no significant differences (P>0.05). This shows that organic quail eggs supplementation has no effect on the weight of the liver of the lactating rats. It is suspected that the liver weight is not only affected by the diameter of the hepatocytes but also influenced by the number of hepatocytes and metabolic activity. The increase in liver weight is affected by the size of the hepatocytes and the number of hepatocytes, but it is also influenced by the rate of biochemical activity to maintain optimal functional weights. The liver is also affected by several conditions such as pregnancy and lactation; hormonal fluctuations; and the composition of feed in the form of fat, carbohydrate, and protein [27]. Liver weight is affected by fat content in the liver. When entering the lactation period, the mobilization of fat used for the formation of rats’ breast milk can also affect liver weight [28]. In addition, liver weight is not only affected by the proliferation of hepatocytes; but also affected by liver non-parenchymal cells such as sinusoid endothelial cells, stellate cells, and Kupffer cells [29].

The supplementation of organic quail eggs to the lactating rats affected the diameter of the hepatocytes and the diameter of the liver lobules, but did not affect the weight of the liver and body weight, presumably because body weight is not only affected by liver weight but also influenced by the weight of other organs and metabolic activity. The mother weight in the lactation period is affected by feed consumption and energy use. Mothers rats whose nutritional needs are sufficient will increase feed consumption and reduce physical activity to meet the needs during lactation. During the pregnancy period there was an increase in fat deposition, but in the period of lactation, the lipid reserves in adipose tissue stopped and lipid reserve into the mammary tissue increased because lipids were used for milk formation [26].
5. Conclusion

Based on the results of the study it can be concluded that quail eggs supplementation in the lactation period affects rats’ liver histological structure.

Suggestions

Observation of liver histological structure using Periodic Acid Schiff (PAS) dye is needed to show glycogen accumulation in the hepatocyte.

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