International Journal of Biological Research, 6 (1) (2018) 1-4

International Journal of Biological Research

Website: www.sciencepubco.com/index.php/IJBR
doi: 10.14419/ijbr.v6i1.9181
Research paper

Hematological status of rats (Rattus norvegicus L.) in the lactation period after giving supplements organic quail eggs

Hasan Basri [1] *, Tyas Rini Saraswati [2], Sri Isdadiyanto [2]

[1] Department of Master Biology, Faculty of Science and Mathematics, Diponegoro University, Semarang 50275, Indonesia
[2] Department of Biology, Faculty of Science and Mathematics, Diponegoro University, Semarang 50275, Indonesia
**Corresponding author E-mail: hasanbasri7491@gmail.com*

Abstract

Organic quail eggs contain high nutrients and low cholesterol, so it is needed in the process of hematopoiesis that occurs in the bone marrow. The purpose of this study was to determine hematological status of rat (Rattus norvegicus L.) in the period of lactation after supplementation of organic quail eggs. This study is an experimental study using a completely randomized design with 4 treatments and 5 replications. Some treatments applied are: T0: Control; T1: The lactation period of (Rattus norvegicus L.) with giving quail eggs supplement of commercial feed; T2: The lactation period of (Rattus norvegicus L.) with giving quail eggs supplement of standard organic feed; T3: The lactation period of (Rattus norvegicus L.) with giving quail eggs supplement of given organic feed. The results showed that feed intake, weight femur, long bone of the femur, erythrocytes, and hemoglobin levels were not significantly different ($p > 0.05$), blood pH significantly different ($p < 0.05$). Supplementation of organic quail eggs could be maintain the condition hematological status of erythrocytes, hemoglobin and blood pH in the lactation period of (Rattus norvegicus L.).

Keywords: *Hematological Status; Rattus norvegicus L.; Organic Quail Eggs.*

1. Introduction

Hematological status had an important role in the circulation and the body's defense system. It is also play a role in the regulation of acid-base conditions, electrolyte balance, and body temperature. The changes in hematologic status can be used to detect the presence of metabolic disorders, diseases, damage to the structure of the organ functions, influence agents or medications, and stress (NseAbasi et al., 2014 and Ihedioha et al., 2014). Hematologic status is also useful to assess the health condition (Togun et al., 2007).

The process of formation and development of blood cells called hematopoiesis. It is occurs in the bone marrow begin the first week of pregnancy (Hoffbrand et al., 2008). While the process of hematopoiesis after birth is concentrated in bones framework of the central and proximal end of the humerus and femur. Impaired hematopoiesis can cause anemia. Anemia in pregnancy is a maternal condition with hemoglobin (Hb) levels < 11.0 g/% or circumstances red cell count lower than normal as a result of a deficiency of one or more substances blood cell formation (Cunningham et al., 2005).

Pregnant and lactating period are more susceptible to anemia. Therefore, in that period it was requiring red blood cells in large numbers. The factors that influence the occurrence of anemia include nutrition, environment, age, gender and genetic (Tras et al., 2000; Addass et al., 2012). Quail eggs are one nutrient that is very good for consumption because it contains protein for about 15.7%–16.6%, fat 31.8%–35.5%, carbohydrates 0.2%–1.0% and ash 1.1%. Quail eggs also contain vitamin A of 543 µg (per 100 g), 0.44% omega-3 and omega-6 1.62% (Stadelman and Cotteril, 1995; Suripta and Astuti, 2016). It was also contain quite high cholesterol for about 3.640 mg/100 g. High cholesterol levels in the body can lead to coronary heart disease and stroke (Félix-Redondo et al., 2013).

One of alternative to supply nutrients with low levels of cholesterol is by consuming organic quail eggs. Organic quail eggs are eggs produced by quail fed organic (it was derived from living organisms). Quail egg yolk cholesterol levels by turmeric powder 108 mg/quail/day of 767.77 mg/dl (Saraswati and Tana, 2016). Quail eggs also contain vitamin A, protein, carbohydrates, minerals and essential fatty acids that are good for health, with cholesterol levels lower (Saraswati and Tana, 2016), the quail eggs and nutrients that are urgently needed in the process of hematopoiesis that occurs in bone marrow. Based on this background, it was conduct the research on the hematological status of white rats (Rattus norvegicus L.) of lactation period after supplementation of organic quail eggs.

2. Materials and methods

2.1. Study area

This study was conducted at the Laboratory of Animal Structure and Function Department of Biology, Faculty of Science and Mathematics, Diponegoro University, Semarang, Indonesia.

2.2. Research design

Test animals used in this study was (Rattus norvegicus L.), females age 2 months and body weight ±200 g. A number of 20 (Rattus norvegicus L.), grouped into 4 treatments and 5 iterations. (Rattus norvegicus L.), females mated with males until the occurrence of pregnancy.

2.3. Quail egg collection

The eggs were used in this study is manufactured by quail eggs by commercial feed (P0); quail eggs produced from a given standard organic feed (P1) with a feed composition comprising: bran, yellow corn flour, soybean meal and flour; quail eggs produced from a given organic feed (P2) with a feed composition comprising: bran, yellow corn flour, soybean meal flour, cassava leaves, mackerel and turmeric powder. Eggs collected is produced by quail eggs at the age of 60 days.

2.4. Giving treatment and blood sample collecting

Rats was treated with 1 egg every day at 16:30 strating from early pregnancy until the end of the lactation period. Food and drink are given by ad libitum. A blood sample is taken through the heart of rat sample (Parasuraman et al., 2010).

2.5. Research variable

Variable observed is the erythrocyte count, the haemoglobin (Hb), blood pH, femur length and femur bone weight. The erythrocyte was determined by the haemocytometer method (Thrall and Weiser, 2002). The haemoglobin (Hb) was determined by the cyanomethaemaglobin method (Higgins et al., 2008).

2.6. Statistical analysis

Data were analyzed by variance One-way ANOVA if there is a significant difference continued Duncan test at the level of 95% ($\alpha = 0.05$). Analysis with the software program SPSS (version 16.0).

3. Results

Organic quail egg is an egg produced by quail fed organic feed, sourced from a living organism (plant or animal). The chemical content of quail eggs are organic and non-organic produced from quail (P0), (P1) and (P2) are presented in Table 1.

Table 1: The Chemical Composition of Quail Eggs

Chemical composition	P0	P1	P2
Protein (%)	12.63	12.67	13.02
Fat (%)	11.12	11.26	11.31
Carbohydrate (%)	0.87	0.91	0.88
Water (%)	74.33	74.29	74.77
Abu (%)	0.75	0.69	0.73
Ca (mg/100 g)	51.89	52.12	54.03
Fe (mg/100 g)	2.65	2.73	2.70
Cu (mg/kg)	0.104	0.109	0.103
Zn (mg/kg)	1.04	1.08	1.08

Note: P0: Eggs produced by quail with commercial fed. P1: Eggs produced by quail with standard organic fed. P2: Eggs produced by quail with fed which contain organic cassava leaves, mackerel, and turmeric powder.

The results of the analysis of feed consumption, weight and length of femur white rat (Rattus norvegicus L.) in the lactation period was shown in Table 2.

Table 2: The Rate of Feed Consumption, Weight, and Length of the Femur of White Rat (Rattus Norvegicus L.) During Period of Lactation

Variables	T0	T1	T2	T3
Feed consumption (g)	21.49 ± 0.64	21.06 ± 1.28	21.66 ± 0.47	21.88 ± 1.08
Weight of the femur (mg)	0.67 ± 0.17	0.72 ± 0.09	0.59 ± 0.04	0.66 ± 0.13
Length of the femur (cm)	3.26 ± 0.18	3.18 ± 0.15	3.28 ± 0.13	3.20 ± 0.25

Note: Figures followed by the same superscript in same row showed no significant difference ($p > 0.05$). T0: (control). T1: (Rattus norvegicus L.) with comercial quail eggs (P0). T2: (Rattus norvegicus L.) with standard organic quail eggs (P1). T3: (Rattus norvegicus L.) with supplement organic quail eggs (P2).

The results of the analysis of the grant supplements organic quail egg against the consumption of feed, the femur bone weights and bone the femur length shows real results did not differ (P 0.05 >), both on the treatment of T0, T1, T2 and T3.

The results of the analysis of hematological status: the number of erythrocytes, hemoglobin levels and blood pH of white rat (Rattus norvegicus L.) in the period of lactation was shown in Table 3.

Table 3: The Rate of Hematological Status Includes the Number of Erythrocytes, Hemoglobin Levels, and Blood Ph of White Rat (Rattus Norvegicus L.) in the Period of Lactation

Hematological status	T0	T1	T2	T3
Erythrocytes ($\times 10^6/mm^3$)	7.09 ± 0.56^a	$6.89^a \pm 0.06^a$	$7.19^a \pm 0.26^a$	6.92 ± 0.74^a
Hb (mg/dL)	12.87 ± 0.27^a	12.42 ± 0.16^a	12.97 ± 0.23^a	12.56 ± 0.68^a
Blood pH	6.87 ± 0.27^{bc}	6.84 ± 0.07^c	7.13 ± 0.06^a	7.08 ± 0.05^{ab}

Note: Figures followed by the same superscript in same row showed no significant difference ($p > 0.05$). T0: (control). T1: (Rattus norvegicus L.) with comercial quail eggs (P0). T2: (Rattus norvegicus L.) with standard organic quail eggs (P1). T3: (Rattus norvegicus L.) with supplement organic quail eggs (P2).

The research grant of the supplement organic quail egg against erythrocytes and haemoglobin levels shows real results did not differ (P 0.05 >). While the results of the analysis of the grant supplements organic quail egg on a white rat (R. norvegicus L.) lactation against blood pH showed different results real ($P < 0.05$). Further test results by test Duncan there is a real difference between T0 with T2; T1 with a T2; T1 with T3, but there is no real difference between the T0 by T1; T0 with T3.

4. Discussion

The results of chemical composition of quail eggs showed that the highest composition was proteins (12.02%), and then fat (11.31%), Ca (54.03 mg/100 g) and Zn (1.08 mg/kg) contained in the treatment of P2. Carbohydrates (0.91%), Fe (2.73 mg/100 g) and Cu (0.109 mg/kg) was highest composition in the treatment P1, but relatively similar to the P2 treatment. The results of the analysis supplementation of organic quail eggs in a white rat (Rattus norvegicus L.) in the lactation period showed results that was not significantly different ($p > 0.05$). Feed consumption was still at the stage of the normal range, namely (21.06 – 21.88 g) (Table 2), feed intake in the rat (Rattus norvegicus L.) on the period of lactation 15–30 g/day (Krinke, 2000; Hubrecht and Kirkwood, 2010). It was caused by environmental factors (temperature and humidity) in the research is still in a normal condition, that is the temperature of 25–28 ºC and the humidity 60–80%. Organic quail eggs supplementation did not affect feed intake. Along with no difference in feed intake led to no significant difference in the weight of the femur, the long bone of femur and hematological status of white rat (Rattus norvegicus L.) lactation.

The fed was one of the materials used for the growth and development of bone. Bone growth occurs at the initial growth phase. The bone growth of white rat (Rattus norvegicus L.) on the period of lactation in the research was 83 days. Other factors that affect bone growth is the composition of the mineral calcium (Ca) found on the quail egg supplement specifically contributes to the synthesis of bone, so that the length of the femur bone and femur bone weights did not differ significantly.

Analysis on supplementation of organic quail eggs in the lactation of white rat (Rattus norvegicus L.) against the red cell count showed that there were not significantly different ($p > 0.05$). The number of erythrocytes in lactation of white rat (Rattus norvegicus L.) were in normal conditions, which is 6.89 –7.19 $\times 10^6/mm^3$ (Table 3). The number of normal red cells in the lactation of white

rat (Rattus norvegicus L.) were 7.71 $\times 10^{12}$/L (Urasoko et al., 2012), while the normal range of erythrocytes of white rat not in the period lactation (Rattus norvegicus L.) were 6.76–9.2 $\times 106/mm^3$ (Lindstrom et al., 2015).

Feed consumption affects the process of erythropoiesis (Sadowska and Kuchlewska 2011). The nutrients contained in the feed is protein, fat, and carbohydrates. The nutrients contained in the feed consumed in the same quantity, causing no difference to the number of erythrocytes. Other factors affecting were environmental conditions such as temperature and humidity. Temperature and humidity during the study is still in normal condition for survival of white rat (Rattus norvegicus L.), which is 26 ºC and 78%. Because of the temperature and humidity normal in the rat (Rattus norvegicus L.), which is the temperature of 25–28 ºC and the humidity 60–80% (Njoya et al., 2009). This does not affect the metabolic activity of white rat (Rattus norvegicus L.) during the period of lactation.

Along with the absence of significant differences in the number of erythrocytes, so it cause no difference in the levels of hemoglobin. The formation of hemoglobin occurs in the erythrocytes. The results of supplementation of organic quail eggs against hemoglobin levels showed results that are not significantly different ($p > 0.05$). Hemoglobin levels of T0, T1, T2 and T3 are in the normal physiological. The normal range of hemoglobin levels of white rat (R. norvegicus L.) lactation was 15.5 (mg/dL) (Urasoko et al., 2012). The normal range of haemoglobin levels in white rat not lactation period (Rattus norvegicus L.) were 11.5–16.1 (g/dL) (Lindstrom et al., 2015). The hemoglobin in lactation of white rat (Rattus norvegicus L.) was caused by feed consumption which are not significantly different and environmental conditions that include temperature and humidity which are also in normal conditions.

The mineral content in organic quail eggs supplements such as iron (Fe), copper (Cu) and zinc (Zn) are specifics contributed greatly in the synthesis of erythrocytes. The process of formation of red blood cells (erythropoiesis) and of hemoglobin synthesis occurs in red bone marrow (Palis and Yoder, 2001). Iron (Fe) contained in the supplement organic quail eggs suspected to have a role as the main constituent of hemoglobin chemical bonds that became one component of red blood cell formation. Kullisaar et al., (2001) explain that the iron (Fe) is absorbed from the intestinal lumen will be attached directly to apotransferrin that carries an iron (Fe) to the liver cells in the formation of hemoglobin. Hemoglobin is composed of molecules heme and globin (Hoffbrand et al., 2008).

Micromineral Zn has a direct role to the conformation of membrane proteins and interaction between proteins in the cell membrane (Chakravorty et al., 2013). Micromineral Zn also stabilizes membranes by supporting the relationship between skeletal membrane cytoskeletal proteins. Zinc also plays a role as one of the antioxidant nutrients, which serves to scavenge free radicals in the plasma membrane (Gropper et al., 2005). Micromineral Zn and Cu work together on an enzyme called superoxide dismutase are involved in the binding of superoxide anions were damaged and a free radical (Linder, 1991). The chemical content of quail eggs in the form of Zn and Cu could be expected to maintain the integrity of the erythrocyte cell membrane damage caused by free radicals, so that the life span of erythrocytes remains intact, while the process of formation of erythrocytes (erythropoiesis) persists.

The results of the analysis supplementation of organic quail eggs in the lactation white rat (Rattus norvegicus L.) against blood pH showed significantly different ($p < 0.05$). The test results further by Duncan test there is a real difference between T0 to T2; T1 to T2; T1 to T3; but there is no real difference between T0 to T1; T0 to T3. Average of all treatments had a blood pH below the normal value of 6.84 –7.13 (Table 3), whereas normal blood pH on the white rat at 7.35–7.45 (Fang et al., 2015).

Factors that cause the pH of blood on the white rat (Rattus norvegicus L.) lactation below the normal value, among others, changes in metabolism (William and Mitch, 2002). The change of metabolism is due to the increasing production process of rat milk (Picciano, 2003). It is causes the lower blood pH of the normal value.

The highest of blood pH was found in the treatment T2 and T3, which is 7.13 and 7.08. It was affected by the chemical composition in the form of protein, fat, carbohydrates and calcium (Table 1), which is contained in a supplement higher organic quail eggs than usually period. It could be meet the needs of nutrition in improving metabolic processes in the lactation period. The treatment given T1 quail eggs produced from quail fed commercial feed chemical compositions was lower than other.

Supplementation with organic quail eggs in the lactation of white rat (Rattus norvegicus L.) indicated that the blood of pH in treatment T3, which is 7.08 closer than the normal value. It was compared to the treatment of T1, which is 6.8 with commercial quail eggs.

In conclusions, the results of the study showed that supplementation with organic quail eggs can maintain condition hematological status of erythrocytes, hemoglobin and blood pH tends to be close to normal values in the period of lactation of rats (Rattus norvegicus L.).

References

[1] Addass, P.A., David, D.L., Edward, A., Zira, K.E. and Midau, A. (2012). Effect of age, sex and management system on some haematological parameters of intensively and semi-intensively kept chicken in mubi, adamawa state, Nigeria. Iranian Journal of Applied Animal Science. 2: 277-282.

[2] Chakravorty, D.K., Parker, T.R., Guerra, A.J., Sherrill, C.D., Giedroc, D.P. and Merz, K.M. Jr. (2013). Energetics of zinc-mediated interactions in the allosteric pathways of metal sensor proteins. J. Am. Chem. Soc. 135, 30-33. https://doi.org/10.1021/ja309170g.

[3] Cunningham, F.G., Gant, N.F., Leveno, K.J., Gilstrap, III. L. C., Hauth, J.C. and Wenstrom K D. (20050. Williams Obstetrics. 22th ed. New York, NY, USA: McGraw-Hill Medical Publishing Division. pp. 39-151.

[4] Fang, K-F., Chen, Z-J., Liu, M., Wu, P-S. and Yu, D-Z. (2015). Blood pH in coronary artery microthrombosis of rats. Asian Pacific Journal of Tropical Medicine. 8: 864-869. http://doi.10.1016/j.apjtm.2015.09.015.

[5] Félix-Redondo, F.J., Grau, M. and Fernández-Bergés, D. (2013). Cholesterol and cardiovascular disease in the elderly. Facts and gaps. Aging and Disease. 4: 154-169.

[6] Gropper, S.S., Smith, J.L. and Groff, J.L. (2005). Advanced Nutrition and Human Metabolism. 4th ed. Wardsworth, USA: Publisher Wadsworth.

[7] Higgins, T., Beutler, E. and Doumas, B.T. (2008). Measurement of haemoglobin in blood. In: Burtis C A, Ashwood E R, Bruns D E, editors. Tietz Fundamentals of Clinical Chemistry. 6th ed. Missouri: Saunders Elsevier. pp. 514-515.

[8] Hoffbrand, A.V., Moss, P.A.H. and Pettit, J.E. (2008). Erythropoiesis and general aspects of anemia. In: Hoffbrand AV, Moss PAH, Pettit JE. Essential Haematology. Wiley-Blackwell. pp. 12-27.

[9] Hubrecht, R. and Kirkwood J. (2010). The UFAW handbook of the care and management of laboratory and other research animals. Issue-8. University Federation for Animal Welfare. pp. 311-324. https://doi.org/10.1002/9781444318777.

[10] Ihedioha, J.I., Ugwuja, J.I., Noel-Uneke, O.A., Udeani, I.J. and Daniel-Igwe, G. (2012). Reference values for the haematology profile of conventional grade outbred albino mice (Mus musculus) in Nsukka, Eastern Nigeria. ARI. 9:1601-1612.

[11] Krinke, G.J. (2000). The Laboratory Rat. The Handbook of Experimental Animals. 1st ed. Novartis Corporation, Stein, Switzerland: Academic Press. pp. 3-56.

[12] Kullisaar, T., Zilmer, M., Mikelsaar, M., Vilhelm, T., Annuk, H., Kamane, C. and Klik, A. (2001). Two antioxidant Lactobacilli strains as promising probiotics. Food Microbiol J. 72:215-224. https://doi.org/10.1016/S0168-1605(01)00674-2.

[13] Linder, M.C. (1991). Nutritional Biochemistry and Metabolism with Clinical Application. 2nd ed. Prentice-Hall Int. pp. 119-225.

[14] Lindstrom, N.M., Zimmerman, K., Moore, D.M. and Smith, S.A. (2015). Hematologic assessment in pet rats, mice, hamsters, and gerbils blood sample collection and blood cell identification. Clin. Lab. Med 35: 629-640.

[15] Njoya, H.K., Ofusori, D.A., Nwangwu, S.C., Amegor, O.F., Akinyeye, A.J. and Abayomi, T.A. (2009). Histopathological effect

of exposure of formaldehyde vapour on the trachea and lung of adult wistar rats. International Journal of Integrative Biology 7: 160-165.
[16] NseAbasi N E, Mary E W, Uduak A, Edem E A O. (2014). Haematological parameters and factors affecting their values. Agricultural Science 2, 37-47. https://doi.org/10.12735/as.v2i1p37.
[17] Palis, J. and Yoder, M.C. (2001). Yolk-sac hematopoiesis: The first blood cells of mouse and man. Experimental Hematology 29: 927-936.
[18] Parasuraman, S., Raveendran, R., Kesavan, R. (2010). Blood sample collection in small laboratory animals. J. Pharmacol Pharmacother 1:87-93.
[19] Picciano, M.F. (2003). Pregnancy and Lactation: Physiological Adjustments, Nutritional Requirements and the Role of Dietary Supplements. J. Nutr. 133(6): 1997S-2002S. https://doi.org/10.1093/jn/133.6.1997S.
[20] Sadowska, J. and Kuchlewska, M. (2011). Effect of diet composition and mixture of selected food additives on the erythrocytic system and iron metabolism in peripheral blood of male rats. Acta Scientiarum Polonorum, Technologia Alimentaria. 10: 497-506.
[21] Saraswati, T.R. and Tana, S. (2016). Effect of turmeric powder supplementation to the age of sexual maturity, physical, and chemical quality of the first japanese quail's (coturnix japonica) egg. Biosaintifika. 8: 18-24. https://doi.org/10.15294/biosaintifika.v8i1.4982.
[22] Stadelman W J and Cotteril O J. (1995). Egg Science and Technology. 4th ed. New York, NY, USA: Food Products Press.
[23] Suripta, H. and Astuti, P. (2016). The effects of sardine and palm oil in rations on the ratio of omega-3 to omega-6 fatty acids in eggs of coturnix coturnix japonica. J. Indon. Trop. Anim. Agric. 32: 22-27.
[24] Thrall, M. A. and Weiser, M. G. (2002). Haematology. In: Hendrix CM. Laboratory Procedures for Veterinary Technicians. 4th ed. Mosby Incorporated, USA: Missouri. pp. 29-74.
[25] Togun, V.A., Oseni, B.S.A., Ogundipe, J.A, Arewa, T.R., Hammed, A.A., Ajonijebu, D.C. and Mustapha, F. (2007). Effects of chronic lead administration on the haematological parameters of rabbit – a preliminary study. In: Proceedings of the 41st Conference of the Agriculural Society of Nigeria. p. 341.
[26] Tras, B., Inal, F., Bas, A.L., Altunok, V., Elmas, M. and Yazar, E. (2000). Effects of continuous supplementations of ascorbic acid, aspirin, vitamin E and selenium on some haematological parameters and serum superoxide dismutase level in broiler chickens. British Poultry Science. 41:664-666. https://doi.org/10.1080/00071660020009225.
[27] Urasoko, Y., He, X., Masao, T., Kinoshita, Y., Edamoto, H., Hatayama, K., Asano, Y., Tamura. K. and Mochizuki, M. (2012). Changes in blood parameters and the expression of coagulation-related genes in lactating sprague–dawley rats. Journal of the American Association for Laboratory Animal Science. 51: 144-149.
[28] William, E and Mitch. (2002). Insights into the abnormalities of chronic renal disease Attributed to malnutrition. Journal of the American Society of Nephrology. 13: S22-S27.