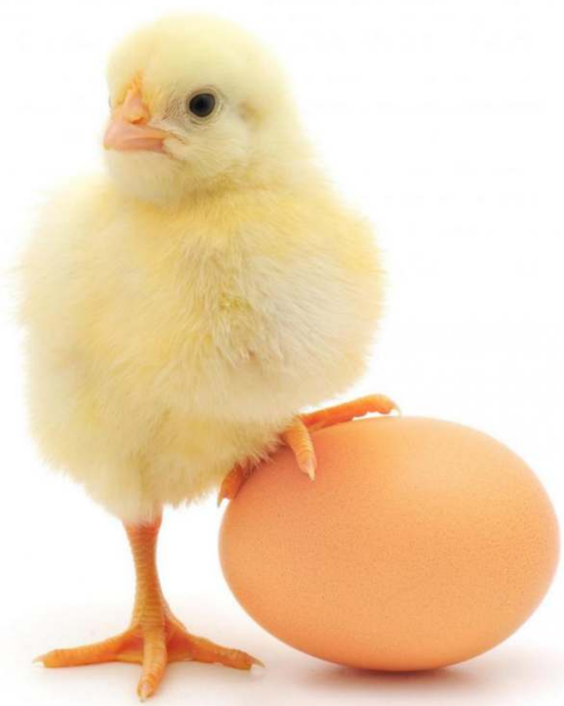
ISSN 1682-8356
ansinet.org/ijps



INTERNATIONAL JOURNAL OF POULTRY SCIENCE

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Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorijps@gmail.com

OPEN ACCESS International Journal of Poultry Science

ISSN: 1682-8356
DOI: 10.3923/ijps.2016.448.453

Research Article
Egg Quality and Blood Hematology of Magelang Laying Duck Fed with Diets Containing Different Ratios of Omega 3 and Omega 6 Fatty Acids and Organic Zn

Sumiati, A. Darmawan and K.G. Wiryawan

Department of Nutrition and Feed Technology, Faculty of Animal Science, Bogor Agricultural University,
Jl. Agatis Dramaga Campus IPB-Bogor, Indonesia

Abstract

Objective: The aim of this study was to investigate the effect of feeding diets containing different amounts of omega-3 and omega-6 fatty acids and organic zinc on egg quality and blood hematology. **Methodology:** Ninety Magelang laying ducks of 21 weeks old were randomly divided into 18 experimental units in a completely randomized design with 6 treatments and 3 replications. The treatment diets included the following: R0 = Diet without palm oil, fish oil or organic zinc with ratio of 1:5 omega-3 and omega-6, R1= Diet with ratio of 1:1.5 omega-3 and omega-6+200 ppm organic zinc, R2 = Diet with ratio of 1:3 omega-3 and omega-6+200 ppm organic zinc, R3 = Diet with ratio of 1:4.5 omega-3:omega-6+200 ppm organic zinc, R4 = Diet with ratio of 1:6 omega-3 and omega-6+200 ppm organic zinc, R5 = Diet with ratio of 1:7.5 omega-3 and omega-6+200 ppm organic zinc. **Results:** The R2 treatment decreased significantly ($p<0.01$) yolk cholesterol but the highest value of vitamin A was resulted by R5. The best ratio of omega-3 and omega-6 was produced by R2 (1:3.4). The R1 treatment resulted the lowest heterophil to lymphocyte ratio. **Conclusion:** Conclusively, feeding diets with ratio of omega-3:omega-6 fatty acids of 1:3+200 ppm organic zinc produced the best ratio of omega-3:omega-6 in yolk as well as increased vitamin A content, decreased yolk cholesterol content and did not interfere ducks physiologically.

Key words: Cholesterol, duck egg, fatty acid, organic zinc, vitamin A

Received: September 29, 2016 Accepted: October 06, 2016 Published: October 15, 2016

Citation: Sumiati, A. Darmawan and K.G. Wiryawan, 2016. Egg quality and blood hematology of Magelang laying duck fed with diets containing different ratios of omega 3 and omega 6 fatty acids and organic Zn. Int. J. Poult. Sci., 15: 448-453.

Corresponding Author: Sumiati, Department of Nutrition and Feed Technology, Faculty of Animal Science, Bogor Agricultural University, Jl. Agatis Dramaga Campus IPB-Bogor, Indonesia

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Duck egg is one of the sources of animal protein that has important function in human nutrition due to its high nutrient content. It also contains high cholesterol and saturated fatty acid. According to Kazmierska *et al.*[1], duck egg contains 13% protein, 14% fat, 10.81 mg cholesterol g^{-1} yolk with 12.8 omega-6 fatty acids and omega-3 ratio. Dietary intake of high levels of saturated fats and cholesterol result in increasing cholesterol content, as well as low density lipoprotein-cholesterol in total serum. This leads to an increase in atherosclerosis and coronary heart disease[2]. Therefore, it is important to create egg as a functional food with low cholesterol, balance ratio of omega-6 fatty acids and omega-3 and high vitamin A content. Omega-6 and omega-3 fatty acids play an important role in brain function, immune system and in preventing cardiovascular diseases (atherosclerosis and coronary heart disease)[3]. Linoleic and α-linolenic acids are the simplest members of each family of Poly Unsaturated Fatty Acid (PUFA) and essential fatty acids that cannot be synthesized in the body[4]. Furthermore, increasing the ratio of omega-6 and omega-3 could initiate inflammatory processes and exacerbate many inflammatory diseases[4]. Increased consumption of omega-6 under stress condition can increase oxidative stress and increase proinflammatory state which may increase the risk of atherosclerotic heart disease[5]. Some researches recommend the optimal ratio of 1-4 of omega-6 and omega-3 in food for human consumption[4,6]. Zinc is essential for human health having a structural and functional role in a large number of macromolecules and in over 300 enzymes reactions[7]. Zinc mineral is a cofactor of retinol binding protein which functions in vitamin A metabolism and absorption[7,8]. There are many studies showing that concentrations of omega-3 and omega-6 increased in meat and eggs due to dietary enrichment with oils rich in C18:3n-6 and C18:3n-3 fatty acid[9,10]. There are limited data how the different omega-6/omega-3 ratios in diets affect egg quality and hematology in ducks. Therefore, this study was conducted to investigate the effect of different omega-6 and omega-3 fatty acid in the diets on egg quality and hematology in ducks. Blood hematology test was used to provide valuable information on the immune status of animals[11,12], with the aim to know the normal physiological values under local conditions for proper management, feeding, breeding, prevention and treatment of diseases[12].

MATERIALS AND METHODS

Ninety magelang laying ducks of 21 weeks old were raised in cages of 1×1×1 m in size. The ducks were distributed into a completely randomized design with 6 treatments and 3 replications. Drinking water was provided *ad libitum* and feeding was given twice a day in the morning (07.00 am) and afternoon (04.00 pm). This study was carried out for nine weeks. Dietary treatments applied in the present study were: R0 = Diet without palm oil, fish oil or organic zinc with ratio of 1:5 omega-3 and omega-6, R1 = Diet with ratio of 1:1.5 omega-3 and omega-6+200 ppm organic zinc, R2 = Diet with ratio of 1:3 omega-3 and omega-6+200 ppm organic zinc, diet with ratio of 1:4.5 omega-3:omega-6+200 ppm organic zinc, R4 = Diet with ratio of 1:6 omega-3 and omega-6+200 ppm organic zinc, R5 = Diet with ratio of 1:7.5 omega-3 and omega-6+200 ppm organic zinc. Dietary treatment was grouped into iso-caloric and iso-protein, which were 16% and 2850 kcal kg^{-1}, respectively based on the research of Darmawan *et al.*[13] as well as the balance of omega-3 and omega-6 fatty acid. The composition and nutrient contents of the dietary treatments are presented in Table 1.

Fatty acids and vitamin A were measured from six egg samples of one day production at the end of the 9 week. Yolk cholesterol was determined at the end of the study by using one egg for each replication. This was taken randomly and analyzed using Liebermann Burchard' method[14]. The absorbance was read using spectrophotometer (Hitachi U-2001, Japan) at a wavelength of (λ) 420 nm. Fatty acid and vitamin A content of the yolk were obtained using a gas chromatography and a High Performance Liquid Chromatography (HPLC), respectively. One bird from each replicate was collected at the end of the treatment and 2 mL of blood was withdrawn from the wing vein. The hematology of the blood samples were analyzed. Blood smear was carried out by smearing blood on glass object, fixated with methanol, colored with Giemsa, washed with water and dried at room temperature. It was then observed under microscope in order to count the percentage of heterophil and lymphocyte[15].

The data obtained were subjected to analysis of variance, where significant effects of treatment was found, Duncan's multiple range test was used to determine the significant difference between the mean values.

RESULTS AND DISCUSSION

Yolk cholesterol: The yolk cholesterol of birds fed with diet of omega-3:omega-6 in the ratio of 1:1.5+200 ppm organic zinc decreased significantly ($p<0.05$) (7.29 mg g^{-1}), while the highest cholesterol content was obtained in the dietary treatment without fish oil and palm oil (9.65 mg g^{-1}, Table 2). The result of this study is consistent with the report of

Table 1: Experimental diets composition

	Experimental diets					
Ingredients	R0	R1	R2	R3	R4	R5
Yellow corn (%)	68.7	44.5	45.7	47	47.2	46
Rice bran (%)	3.5	24	22.5	21	21	20.5
Soybean meal (%)	13	10.7	12	12.7	11	12
Fish meal (%)	7	8	7	7	8	9
Palm oil (%)	0	0	3.2	4	4.6	5
Fish oil (%)	0	5	1.8	1	0.4	0
$CaCO_3$ (%)	7	7	7	6.5	7	6.7
NaCl (%)	0.2	0.2	0.2	0.2	0.2	0.2
Premix (%)	0.5	0.5	0.5	0.5	0.5	0.5
DL-methionine (%)	0.1	0.1	0.1	0.1	0.1	0.1
Organic Zn (ppm)		200	200	200	200	200
Nutrient contents (as fed)						
Crude protein (%)	16.28	16.36	16.13	16.04	16.02	16.35
Metabolizable energy (kcal kg^{-1})	2766.60	2878.25	2660.75	2905.8	2772.4	2786.175
Calcium (%)	3.11	3.22	3.16	3.01	3.22	3.17
Phosphorus available (%)	0.43	0.60	0.56	0.55	0.59	0.62
Fiber (%)	3.18	4.72	4.21	3.74	4.1	4.19
Fat (%)	4.24	7.63	7.09	8.00	8.76	8.07
Omega-6 (%)	1.43	1.94	2.17	2.26	2.37	2.39
Omega-3 (%)	0.28	1.32	0.75	0.49	0.40	0.31
Omega-3:Omega-6	1:5	1:1.5	1:3	1:4.5	1:6	1:7.5
Lysine (%)	0.98	0.98	0.98	0.98	0.98	1.03
Methionine (%)	0.46	0.47	0.46	0.46	0.46	0.46
Methionine+cystine (%)	0.71	0.70	0.70	0.71	0.71	0.73

Premix provided (mg kg^{-1} premix) = vit A 500,000 IU, vit D 100,000 IU, vit E 150 mg, vit K 50 mg, vit B1 50 mg, vit B2 250 mg, vit B12 250 mcg, niacinamide 375 mg, Ca-d-panthotenate 125 mg, folic acid 25 mg, choline cloride 5,000 mg, glysine 3,750 mg, Dl-methionine 5,000 mg, Mg sulphate 1,700 mg, Fe sulphate 1,250 mg, Mn sulphate 2,500 mg, Cu sulphate 25 mg, Zn sulphate 500 mg, K iodine 5 mg, R0: Diet without palm oil, fish oil or organic zinc with ratio of omega-3:omega-6 = 1:5, R1: Diet with ratio of omega-3:omega-6 = 1:1.5+200 ppm organic zinc, R2: Diet with ratio of omega-3:omega-6 = 1:3+200 ppm organic zinc, R3: Diet with ratio of omega-3:omega-6 = 1:4.5+200 ppm organic zinc, R4: Diet with ratio of omega-3:omega-6 = 1:6+200 ppm organic zinc, R5: Diet with ratio of omega-3:omega-6 = 1:7.5+200 ppm organic zinc

Table 2: Egg yolk content of magelang laying duck fed experimental diets for 9 weeks experiment

Parameters	R0	R1	R2	R3	R4	R5
Cholesterol (mg g^{-1})	9.65 ± 12^{c}	7.29 ± 0.52^{a}	8.04 ± 0.14^{b}	8.31 ± 0.34^{b}	8.43 ± 0.11^{b}	9.42 ± 0.27^{c}
Vitamin A (IU/100 g)	1345	1351	1359	1453	1468	1675
Arachidate (20:4)	0.03	0.01	0.03	0.03	0.03	0.03
Linoleic (18:2)	0.74	0.8	0.79	0.71	0.99	1.12
α-linolenic (18:3)	0.02	0.05	0.09	0.02	0.09	0.09
Eicosapentaenoic acid (20:5)	0	0.03	0	0	0	0
Docosahexaenoic acid (22:6)	0.09	0.65	0.15	0.12	0.02	0.01
Saturated fatty acid	41.84	39.15	40.58	39.6	40.4	40.61
Mono unsaturated fatty acid	57.28	59.31	58.36	59.52	58.56	58.14
Poly unsaturated fatty acid	0.88	1.54	1.06	0.88	1.13	1.25
Total of omega-3	0.11	0.73	0.24	0.14	0.11	0.1
Total of omega-6	0.77	0.81	0.82	0.74	1.02	1.15
Ratio of omega-6:omega-3	7	1.11	3.42	5.29	9.27	11.5

Means in the same row with different superscripts differ significantly ($p<0.05$). R0: Diet without palm oil, fish oil or organic zinc with ratio of omega-3:omega-6 = 1:5, R1: Diet with ratio of omega-3:omega-6 = 1:1.5+200 ppm organic zinc, R2: Diet with ratio of omega-3:omega-6 = 1:3+200 ppm organic zinc, R3: Diet with ratio of omega-3:omega-6 = 1:4.5+200 ppm organic zinc, R4: Diet with ratio of omega-3:omega-6 = 1:6+200 ppm organic zinc, R5: Diet with ratio of omega-3:omega-6 = 1:7.5+200 ppm organic zinc

Kehui *et al.*[16], who stated that omega-3 fatty acids in laying hens ration can decrease cholesterol content of eggs. Yolk cholesterol content of this study was lower than the value reported by Aziz *et al.*[17] and Kazmierska *et al.*[1] which were 10.36 and 10.81 mg g^{-1}, respectively. Continual decreased in yolk cholesterol content resulted from the use of fish oil, containing omega-3 fatty acid. Omega-3 fatty acids can decrease the concentration of triglyceride by inhibiting the secretion of Low Density Lipoprotein (LDL) in the liver[18] and regulating gene expression in the liver[18,19].

Vitamin A: Vitamin A content in the yolk increased with supplementation of 200 ppm organic zinc and palm oil. The highest content of vitamin A was obtained from eggs of birds fed with diet of omega-3 and omega-6 in the ratio of 1:7.5+200 ppm organic zinc (1675 IU/100 g). β-carotene and zinc mineral contributed to the increase of vitamin A content in yolk. The results obtained were in line with the findings of Palupi *et al*.[20] who reported that the content of vitamin A increased significantly due to the higher content of β-carotene in the ration. According to Leeson and Summers[21], the vitamin A content of yolk increases with increasing pro-vitamin A content in ration. β-carotene content in diet can greatly affect vitamin A of animal products, because β-carotene has the largest pro-vitamin A activity compared to other carotenoids. Furthermore, the increase of vitamin A in yolk is also caused by zinc mineral content in the plasma. According to Rahman *et al*.[22], zinc mineral has effect on vitamin A metabolism, absorption and transportation. Munoz *et al*.[8] stated that zinc deficiency can decrease vitamin A in plasma and decrease synthesis of Retinol Binding Protein (RBP) in the liver as well as in the plasma.

Omega-3 and omega-6 content: Diet with ratio of 1:1.5 omega-3 and omega-6 (R1) had higher omega-3 fatty acids in yolk than the other treatments (Table 2). Diet with 5% of fish oil could improve omega-3 fatty acids content from 0.1-0.73% in yolk. Carrillo-Dominguez *et al*.[23] reported that the use of 2.5% of lemuru oil combined with 100 ppm of vitamin E in the diet can improve omega-3 fatty acids content of yolk. Diet with ratio of 1:3 omega-3 and omega-6+200 ppm organic zinc resulted in the best ratio of omega-6 and omega-3 fatty acids, which was 3.42. Some researches recommend that the optimal ratio of omega-6 and omega-3 in food for human consumption is 1-4[4,6]. Suripta and Astuti[24] reported that the use of 8% lemuru oil in diet can decrease the balance of omega-3 and omega-6 in quail yolk from 1:24 to 1:11.

Erythrocyte: Dietary treatments had no significant effect ($p>0.05$) on erythrocytes (ranged between $2.71\text{-}2.88\times10^6$ mm^{-3}, Table 3). According to Sturkie and Griminger[25] and Ismoyowati *et al*.[26] the normal range for erythrocytes was 2×10^6 mm^{-3} and $2.11\text{-}2.30\times10^6$ mm^{-3}, respectively. Ali *et al*.[27] also reported that the quantity of Magelang duck erythrocytes using probiotics in diet was $2.50\text{-}3.47\times10^6$ mm^{-3}. According to Wardhana *et al*.[28], the lack of precursors such as iron and amino acids that assist in the formation of erythrocytes will decrease the number of erythrocytes.

Hematocrit and leukocyte: The treatments had no significant effect ($p>0.05$) on the value of hematocrit, ranging between 33.92-34.9%. This value was lower than what was reported by Ismoyowati *et al*.[26] and Ali *et al*.[27] which were 36.85 and 38.4%, respectively in 28 weeks old birds. The R1 treatment decreased leukocyte significantly ($p<0.05$) than R0 treatment. This was caused by omega-3 fatty acids content which increased the immune function, thereby suppressing infection[29]. Rusmana *et al*.[30] reported that using 6% of fish oil and 200 ppm vitamin E led to give a positive response for immunomodulator with increasing antibody titers. Wajizah *et al*.[31] also reported that the use 4.5% of fish oil in diet can decrease leukocytes level from 17.99×10^3 mm^{-3} to 13.10×10^3 mm^{-3}. The R1 treatment contained eicosapentaenoic acid and docosahexaenoic which were 0.96 and 12.12%, respectively (Table 2). Eicosapentaenoic acid plays a role in the formation of eicosanoids, which subsequently function in improving the immune and anti-inflammatory system.

Hemoglobin: Dietary treatments had no significant effect ($p>0.05$) on hemoglobin level. The use of 200 ppm organic zinc did not suppress Fe function to bind to hemoglobin. According to Kelleher and Lonnerdal[32], zinc can compete

Table 3: Blood hematology of magelang laying duck fed experimental diets for 9 weeks experiment

Parameters	R0	R1	R2	R3	R4	R5
Erythrocyte (10^6 mm^{-3})	2.71±12:10	2.78±12:09	2.78±12:24	2.87±12:23	2.53±00:46	2.88±00:46
Hematocrit (%)	34.97±4.32	31.92±3.47	34.57±2:30	33.92±3:00	33.75±3:15	34.58±3:32
Hemoglobin (g%)	13.86±1.81	12.37±12:58	12.87±1:14	12.57±1.74	13.63±0.97	12.64±1.90
Leukocyte (10^3 mm^{-3})	20.13±3.35^b	13.20±3.34^a	17.53±3.06^{ab}	13.40±3.03^a	18.00±2.20^{ab}	14.53±3.00^{ab}
Heterophil (H) (%)	76.67±2.08^b	64.33±4.04^a	65.67±2.52^a	65.33±3.06^a	66.33±9.29^a	67.33±1.53^a
Lymphocyte (L) (%)	28.00±1.00^a	35.00±1.00^b	28.67±3.79^a	29.33±5.13^{ab}	29.67±1.15^{ab}	30.67±4.04^{ab}
H/L	2.74±0.07^b	1.84±0.15^a	2.29±0.37^{ab}	2.23±0.42^{ab}	2.24±0.39^{ab}	2.20±0.26^{ab}

Means in the same row with different superscripts differ significantly ($p<0.05$). R0: Diet without palm oil, fish oil or organic zinc with ratio of omega-3:omega-6 = 1:5, R1: Diet with ratio of omega-3:omega-6 = 1:1.5+200 ppm organic zinc, R2: Diet with ratio of omega-3:omega-6 = 1:3+200 ppm organic zinc, R3: Diet with ratio of omega-3:omega-6 = 1:4.5+200 ppm organic zinc, R4: Diet with ratio of omega-3:omega-6 = 1:6+200 ppm organic zinc, R5: Diet with ratio of omega-3:omega-6 = 1:7.5+200 ppm organic zinc with Fe, therefore increasing of Zn will suppress Fe and Fe deficiency will decrease blood hemoglobin. According to Brody[33], zinc mineral is a cofactor of many enzymes including aminolevulinic acid dehydratase enzyme that produces hemoglobin. The hemoglobin level obtained was 12.37-13.86 g%. Ismoyowati *et al.*[26] reported that hemoglobin level on layer duck was 10.81 g%.

Heterophil: There were no significant effect (p<0.05) in the number of heterophil. Heterophil function as the first line defense in response to an infection[34]. The average heterophil value in this study was 67.61%. This value is still in the normal range of 54.75-69.75% as reported by Ismoyowati *et al.*[35]. Rations with ratio of 1:5 omega-3 and omega-6 and without organic zinc (R0) resulted in a significant higher heterophil (p<0.05). The high heterophil level was probably due to insufficient zinc in the diet. According to Prasad *et al.*[36], zinc mineral determines the development of immune cells and plays an important role in maintaining the activity of the immune system.

Lymphocyte: Diet with ratio of 1.5 omega-3 and omega-6+200 ppm organic zinc (R1) resulted in a significant higher level of lymphocyte. The high level of lymphocytes was caused by fish oil and organic zinc. This increasing lymphocytes level relates to the role of lymphoid organs in producing lymphocytes cells (T-cells/B-cells) while B-cells produce antibodies. This statement was supported by Rusmana *et al.*[30] who reported that 6% of fish oil increased the number of lymphocytes and antibody titers of broiler chickens from 69.50-78.25%. Winarsi *et al.*[37] reported that the use of zinc can increase significantly the number of lymphocytes in the peripheral blood circulation. Increase in the number of lymphocytes due to zinc supplementation led to increase in the activity of catalase enzyme and superoxide dismutase enzyme (SOD), which is helpful for maintaining cell damage caused by.

Ratio of heterophil and lymphocyte (H/L): Dietary treatments affected (p<0.05) the ratio of heterophil to lymphocyte. The R1 showed the lowest ratio of H/L, which was 1.8 lower than R0 (without the use of oil and zinc). This shows that 5% fish oil can reduce stress levels of Mandalung ducks. According to Campo *et al.*[38], one indicator of stress condition in poultry is the ratio of heterophil and lymphocyte.

CONCLUSION

The feeding diet containing the ratio of 1:3 omega-3 fatty acids and omega-6 supplemented with 200 ppm organic zinc resulted in the best ratio of omega-3 and omega-6, reduced cholesterol in egg yolk and increased vitamin A content and did not interfere ducks physiologically.

ACKNOWLEDGMENTS

This study was funded by the Indonesian Ministry of Education and Culture, Directorate General of Higher Education with No. 52 /IT3.41.2/L1/SPK/2013.

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