

## Evaluation of feeding *Indigofera zollingeriana* leaf meal and *Sardinella lemuru* fish oil on lipids metabolism of local ducks

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### ABSTRAK

Penelitian bertujuan untuk mengevaluasi efektivitas penggunaan tepung daun *Indigofera zollingeriana* (I) dan minyak ikan *Sardinella lemuru* (L) terhadap penurunan kadar kolesterol dan metabolisme lipida itik petelur. Penelitian ini menggunakan 144 itik Magelang, umur 20-32 minggu, periode layer dengan rata-rata bobot badan  $1.72 \pm 0.12$  kg, yang dibagi menjadi 6 kelompok perlakuan pakan, 3 ulangan, dan masing-masing 8 ekor. Rancangan penelitian menggunakan Rancangan Acak Lengkap Faktorial  $3 \times 2$ , dengan faktor pertama penggunaan tepung daun *Indigofera zollingeriana* sebagai pengganti tepung bungkil kedelai (0%, 5.5%, 11%). Faktor kedua merupakan penggunaan minyak ikan *Sardinella lemuru* (0%, 2%). Hasil menunjukkan bahwa perlakuan I5.5L2 berpengaruh sangat nyata ( $P < 0.01$ ) terhadap trigliserida, HDL, dan LDL. Perlakuan I0L2 berpengaruh nyata ( $P < 0.05$ ) menurunkan kolesterol total pada profil lipida darah dan kolesterol daging. Perlakuan I11L2 berpengaruh sangat nyata ( $P < 0.01$ ) menurunkan kolesterol telur. Kesimpulan dari penelitian ini adalah dengan pemberian tepung daun *Indigofera zollingeriana* dan minyak ikan lemuru 2% dalam ransum dapat menjaga keseimbangan profil lipida darah dengan menurunkan trigliserida, LDL, kolesterol, dan meningkatkan HDL pada plasma itik petelur dan berperan penting dalam metabolisme lipida itik petelur Magelang dalam menghasilkan telur dan daging dengan kandungan kolesterol yang rendah.

*Kata-kata kunci: Indigofera zollingeriana, itik Magelang, kolesterol, minyak ikan Sardinella lemuru*

### ABSTRACT

The objective of this study was to evaluate the effectiveness usage of *Indigofera zollingeriana* leaf meal (I) and *Sardinella lemuru* fish oil (L) in diets on the decreasing levels of cholesterol and lipid metabolism of laying duck. One hundred and forty-four Magelang ducks aged 20-32 weeks, laying periode, with an average body weight of  $1.72 \pm 0.12$  kg were used, which were divided into 6 groups of experimental diets, 3 replications, and of 8 layers each. A completely randomized design,  $3 \times 2$  factorial design was used in this experiment. The first factor was usage *Indigofera zollingeriana* leaf meal which replaced protein soybean meal (0%, 5.5%, 11%). The second factor was usage *Sardinella lemuru* fish oil (0%, 2%). The results showed that the treatment (I5.5L2) was highly significant affected ( $P < 0.01$ ) the level of triglyceride, HDL, and LDL in the blood. The treatment I0L2 significantly decrease ( $P < 0.05$ ) total cholesterol of blood lipid profile and meat cholesterol. The treatment (I11L2) was highly significantly decreased ( $P < 0.01$ ) egg cholesterol. The conclusion of this study was that feeding *Indigofera zollingeriana* leaf meal 5.5% and *Sardinella lemuru* fish oil 2% in laying duck's diet could maintain the balance of blood lipid profiles by lowering plasma triglycerides, LDL, cholesterol and raising up plasma HDL and plays an important role on lipid metabolism of Magelang laying ducks to produce eggs and meat with low cholesterol content.

*Keywords : cholesterol, Indigofera zollingeriana, Sardinella lemuru fish oil, Magelang duck*

## INTRODUCTION

Eggs contain a balanced amino acids, essential fatty acids, some minerals and vitamins (McNamara and Thesmar, 2005). Duck is one of the birds that produce eggs with higher nutritional value. The nutrient content of eggs, among others: water 73.7%, 12.9% protein, 11.2% fat, 0.9% carbohydrate and fat was not exist in egg white (Komala, 2008). Cholesterol content at 17 mg/g of duck egg yolk was higher than 14 mg/g yolk in chicken eggs (Chang and Huang, 2001). Cholesterol is needed by the human body to form cell membranes, producing sex hormones and the formation of bile acids needed on fat digestion. High cholesterol in eggs is not good if consumed by someone who is sensitive to cholesterol

*Sardinella lemuru* fish oil is one of the sources of unsaturated fatty acids. Fish oil is naturally rich in omega-3 fatty acids such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Trujillo *et al.*, 2016). According to Atakisi *et al.* (2009), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) is more effective in lowering cholesterol compared with alpha-linoleic acid (ALA). Eggs enriched with omega-3 fatty acids through dietary fish oil supplementation may reduced the stability of lipid oxidation in fresh eggs (Botsoglou *et al.*, 2012). The weakness of omega-3 fatty acids are susceptible to lipid oxidation which has a negative impact on the nutritional value, flavor, color and texture of the food (Avila-Ramos *et al.*, 2013). The use of antioxidants was essential to prevented the oxidation of lipids in animal products (Kong *et al.*, 2010). One of the materials that could be used to protect omega-3 fatty acids from oxidation was *Indigofera zollingeriana* leaf meal that contained beta-carotene.

The objectives of this study was to evaluate the effectiveness of the use of *Indigofera zollingeriana* leaf meal and *Sardinella lemuru* fish oil on the decreased levels of cholesterol and lipid metabolism in laying ducks.

## MATERIALS AND METHODS

### Animals and Experimental Diets

A total of 144 laying ducks, 20 weeks of age, with an average body weigh of  $1.72 \pm 0.12$  kg were put in litter cages (2 x 1.25 m). Each cage was filled up by 8 ducks and grouped by treatments at the Poultry Research Unit, Department of

Nutrition and Feed Technology, Faculty of Animal Science, Bogor Agricultural University. Laying ducks were raised in litter cages with room temperature and the light-dark cycle was set at 12 h each.

### *Indigofera zollingeriana* Leaf Meal Preparation.

Harvesting *Indigofera zollingeriana* leaves aged 60 days defoliation was used by taking the third leaf from the leaves of shoots up to 20 leaves. The leaf were dried in the greenhouse until half dry so as not to cause discoloration of the green colour then continued dried using an oven on 60°C for 24 hours. *Indigofera zollingeriana* leaf meal was ready to produce.

### Procedure

Laying ducks were divided into 6 groups, each treatment was subjected to three replications. The layers were given 120 g of feed per bird per day which fed in the (morning and afternoon). Drinking water was given ad libitum. The ducks were adjusted pre-experimentally, by feeding them a commercial diet on week 20 to 21. Between week 21 to 32, were fed a dietary treatment. Experimental data were recorded between week 22 and 32 (a total of 11 weeks). Eggs were collected two times daily (morning and afternoon) and weighed daily. Composition and nutrient content used in this study is presented in Table 1.

### Rations

The diets were formulated according to Leeson and Summers (2005). The first ingredient is mixed with yellow corn, palm oil, soybean meal, fish meal, *Sardinella lemuru* fish oil. The second ingredient is mixed with *Indigofera zollingeriana* leaf meal, rice bran, CaCO<sub>3</sub> (*calcium carbonate*), premix and DL-methionine. The entire material then stirred until homogeneous in a mixing machine (mixer).

### Data Collection

On week 32, three ducks were randomly choosen from each replication. Blood sample collection was carried out on the 3 ducks gathered (total 18). Blood was taken from pectoralis vena by 3 ml syringe and then was placed in the sample tube. Blood sample was placed in Styrofoam box containing ice cubes and transported to the laboratory. In the laboratory, blood sample was placed in 4°C refrigerator for 12 hours before it

Table 1. Composition and Nutrient Content of Experimental Diet

Feed Ingredient (%)	I0L0	I5.5L0	I11L0	I0L2	I5.5L2	I11L2
Corn	57.1	53.4	49.7	49.7	45.8	42.2
Rice bran	7.8	10.9	14	13	16.3	19.1
Soybean meal	20	15	10	20	15	10
<i>Indigofera zollingeriana</i>	0	5.5	11	0	5.5	11
Fish meal	5.4	5.5	5.6	5.6	5.8	6
Palm oil	2	2	2	2	2	2
Lemuru fish oil	0	0	0	2	2	2
CaCO <sub>3</sub>	6.9	6.9	6.9	6.9	6.8	6.9
NaCl	0.2	0.2	0.2	0.2	0.2	0.2
Premix <sup>1</sup>	0.5	0.5	0.5	0.5	0.5	0.5
DL-Methionin	0.1	0.1	0.1	0.1	0.1	0.1
Total	100	100	100	100	100	100
Calculated nutrient content (%) :						
GE (kkal /kg)	4171	3996	3945	4053	3801	4060
Dry matter	84.34	91.28	91.95	87	87.95	90.05
Crude protein	16.32	16.36	15.75	15.72	15.59	15.69
Ash	8.13	8.28	9.4	9.06	9.78	9.69
Crude fat	4.59	4.79	5.15	6.86	3.66	6.45
Crude fiber	5.76	5.9	6.44	5.19	6.26	6.48
Calcium	4.17	3.63	3.92	3.79	3.87	3.68
Total phosphorus	0.55	0.55	0.51	0.53	0.61	0.67

I0L0 : feed without *Indigofera zollingeriana* leaf meal and lemuru fish oil, I5.5L0 : feed plus 5.5% *Indigofera zollingeriana* leaf meal without lemuru fish oil, I11L0 : feed plus 11% *Indigofera zollingeriana* leaf meal without lemuru fish oil, I2L0 : feed without *Indigofera zollingeriana* leaf meal plus 2% lemuru fish oil, I5.5L2 : feed plus 5.5% *Indigofera zollingeriana* leaf meal and 2% lemuru fish oil, and I11L2 : feed plus 11% *Indigofera zollingeriana* leaf meal and 2% lemuru fish oil. <sup>1</sup>Premix composed of the following: vitamin A 12,000,000 IU, vitamin D<sub>3</sub> 2,000,000 IU, vitamin E 8,000 IU, vitamin K 2 g, vitamin B<sub>1</sub> 2 g, vitamin B<sub>2</sub> 5 g, vitamin B<sub>6</sub> 0.5 g, vitamin B<sub>12</sub> 0.012 g, vitamin C 25 g, calcium D-panthothenate 6 g, niacin 40 g, cholin chloride 10 g, methionin 30 g, lysine 30 g, manganese 120 g, iron 20 g, iodine 0.2 g, zinc 100 g, cobalt 0.2 g, copper 4 g, zinc bacitracin 21 g, excipient 10 g q.s.

was centrifuged by 3500 rpm for 10 minutes. Supernatant in the form of plasma was taken using sterile pipette and placed in eppendorf tube for further analysis.

#### Parameters Observed

The lipid profile of blood plasma consisted of triglyceride, total cholesterol (TC), Low Density Lipoprotein (LDL), High Density Lipoprotein (HDL) using CHOD-PP method, and

the cholesterol content of egg yolk and meat were analyzed using Liberman Burchard method (Burke *et al.*, 1974).

#### Data Analysis

Data obtained were tabulated using excel and analyzed using Completely Random Design (CRD). If there was significant or very significant effect of treatments, then followed by Duncan's Multiple Range Test (Steel and Torrie, 1984).

## RESULTS AND DISCUSSION

Effect *Indigofera zollingeriana* leaf meal and *Sardinella lemuru* fish oil to lipid profile of blood plasma was presented in Table 2. Plasma triglyceride levels were significantly ( $P < 0.01$ ) decreased after *Indigofera zollingeriana* leaf meal and *Sardinella lemuru* fish oil administration for eleven weeks in experimental animals compared to control (I0L0) (Table 2). I11L2 was not different ( $P > 0.01$ ) from I5.5L0 and I5.5L2. Furthermore I0L0, I11L0 and I0L2 was not different from I5.5L0 and I5.5L2, but I11L2 was different from I0L0, I11L0 and I0L2. Plasma triglyceride of laying duck in this research ranged from 233.77 to 429.80 mg/dl. This study showed that inclusion of *Indigofera zollingeriana* leaf meal and *Sardinella lemuru* fish oil in the diet significantly reduced triglyceride levels in laying ducks (Table 2). According to Schreiber *et al.* (2013) beta carotene is a potent natural antioxidants. Beta carotene is an antioxidant will gobble free radicals that lead to increased activity of the enzyme lipoprotein lipase. The enzyme lipoprotein lipase activated by apolipoprotein C, serves to hydrolyze triglycerides into fatty acids and glycerol. The more triglycerides are hydrolyzed causing a decrease in plasma triglycerides. Antioxidants from beta carotene also serves to protect the omega-3 fatty acids. Omega-3 fatty acid comprising 60% long chain fatty acids omega-3 EPA and DHA are susceptible to lipid oxidation. According to Dalle Zotte *et al.* (2006), omega-3 fatty acids play a role in lowering plasma triglycerides. Omega-3 fatty acids of *Sardinella lemuru* fish oil may inhibit Diacylglycerol transferase enzyme (DGAT) and Phosphatidic Acid Phosphohydrolase enzyme (PAP) that will reduce the availability of fatty acids for the synthesis of triglycerides. Inhibition of the synthesis of triglycerides will lead to the decreased of plasma triglycerides.

The results showed that group I0L2 had a significantly ( $P < 0.05$ ) lower plasma TC levels of  $129.10 \pm 14.97$  mg/dl, followed by group I0L0 with  $133.33 \pm 17.55$  mg/dl plasma TC levels. Based on result administration of *Indigofera zollingeriana* leaf meal and *Sardinella lemuru* fish oil reduced TC levels significantly (Table 2). This study showed that the addition of lemuru fish oil in the ration provide a good influence on the content of plasma cholesterol in laying ducks. According to Dalle Zotte *et al.* (2006) omega-3 fatty acids play a role in lowering cholesterol

levels. When cholesterol is transported from the intestine peripherals to the liver, omega-3 fatty acids will inhibit HMG-CoA reductase which served to transform asetoasetil CoA into mevalonate (Babio *et al.*, 2010). This causes the synthesis of endogenous cholesterol becomes blocked. Antioxidants also play a role in lipid metabolism, especially cholesterol. The antioxidant activity of carotenoids based conjugated system of the electron-rich chain polyenes, and responsible for meeting the singlet oxygen and free radicals to terminate the chain reaction that is adverse (McNulty *et al.*, 2008). Antioxidants prevent the formation of free radicals in the liver for the synthesis of endogenous cholesterol. Products of cholesterol by the liver is reduced which will result in a decrease in serum cholesterol levels. Several studies have shown that EPA and DHA more effectively lower plasma cholesterol compared with ALA (Atakisi *et al.*, 2009). Chan *et al.* (2003) reported that omega-3 fatty acids may reduce the rate of entry of VLDL particles into the blood circulation which will be converted into LDL particles.

*Indigofera zollingeriana* leaf meal and *Sardinella lemuru* fish oil had significant ( $P < 0.01$ ) reduction in plasma LDL-C levels in laying ducks. The lowest plasma LDL-C levels was shown on group I0L2 ( $37.38 \pm 3.22$  mg/dl). Group I5.5L0 had significantly ( $P < 0.01$ ) higher mean plasma LDL-C levels of 86.75 mg/dl. I0L2 which was not different ( $P > 0.01$ ) from I5.5L2 and I11L2. Furthermore, I5.5L0 was not different from I11L0, but I5.5L0 was different from I0L2. LDL plasma of laying duck in this research ranged from 37.38 to 86.75 mg/dl. In this research *Indigofera zollingeriana* leaf meal and *Sardinella lemuru* fish oil supplementation significantly reduces plasma levels of LDL after eleven weeks of treatment in the diet of laying ducks. Low levels of plasma LDL is usually followed by low levels of triglycerides in plasma. The rest of the hydrolysis of the triglycerides will be metabolized in the liver which will become LDL. Yang *et al.* (2010) reported that the long-chain fatty acids omega-3 (EPA and DHA) affected to the decreased secretion of LDL from the liver. LDL are lipoproteins that are rich in cholesterol because it transports much cholesterol in the blood. Decrease of LDL level is suspected because of the omega-3 fatty acid can reduce the production and secretion of VLDL particles are activated by Apolipoprotein B. Omega-3 fatty

Table 2. Lipid Profile of Blood Plasma of Laying Duck Supplemented by *Indigofera zollingeriana* Leaf Meal and Lemuru Fish Oil

Parameter	Lemuru Fish Oil	<i>Indigofera zollingeriana</i> Leaf Meal		
		I0	I5.5	I11
Triglyceride (mg/dl)	L0	311.26±44.22 <sup>B</sup>	331.46±14.24 <sup>AB</sup>	233.77±27.81 <sup>B</sup>
	L2	297.85±29.64 <sup>B</sup>	329.47±63.91 <sup>AB</sup>	429.80±60.26 <sup>A</sup>
Total cholesterol (mg/dl)	L0	133.33±17.55 <sup>c</sup>	173.45±6.21 <sup>a</sup>	140.68±14.12 <sup>bc</sup>
	L2	129.10±14.97 <sup>c</sup>	146.52±13.40 <sup>abc</sup>	164.97±23.16 <sup>ab</sup>
LDL (mg/dl)	L0	59.93±8.68 <sup>BC</sup>	86.75±6.11 <sup>A</sup>	75.64±10.55 <sup>AB</sup>
	L2	37.38±3.22 <sup>D</sup>	44.19±4.12 <sup>CD</sup>	45.06± 1.32 <sup>CD</sup>
HDL (mg/dl)	L0	26.26±2.45 <sup>B</sup>	20.41±3.16 <sup>B</sup>	18.29± 2.33 <sup>B</sup>
	L2	24.67±2.58 <sup>B</sup>	39.43±6.35 <sup>A</sup>	24.16± 3.58 <sup>B</sup>

I0L0 : feed without *Indigofera zollingeriana* leaf meal and lemuru fish oil, I5.5L0 : feed plus 5.5% *Indigofera zollingeriana* leaf meal without lemuru fish oil, I11L0 : feed plus 11% *Indigofera zollingeriana* leaf meal without lemuru fish oil, I2L0 : feed without *Indigofera zollingeriana* leaf meal plus 2% lemuru fish oil, I5.5L2 : feed plus 5.5% *Indigofera zollingeriana* leaf meal and 2% lemuru fish oil, and I11L2 : feed plus 11% *Indigofera zollingeriana* leaf meal and 2% lemuru fish oil. Significant difference between various groups is by Duncan's Mutiple Range Test, <sup>abcd</sup> p<0.05, <sup>ABCD</sup> p<0.01.

acid inhibits Diacyl glycerol transferase enzyme (DGAT) and Phosphatidic Acid Phosphohydrolase enzyme (PAP) so as to reduce the secretion of VLDL which will affect the reduction in LDL. According to Bruss (2008) that the inhibition of HMG-CoA reductase inhibitors cause a decrease in cholesterol synthesis and increase the number of LDL receptors located in the liver cell membrane and extra hepatic tissues, so that the levels of total cholesterol and LDL in plasma decline.

Plasma HDL-C levels were significantly increased (P<0.01) with I5.5L2 administration in laying ducks compared with all other treatments (Table 2); however, I0L0, I5.5L0, I11L0, I0L2, and I11L2 were not different. This study showed that the combination of *Indigofera zollingeriana* leaf meal and *Sardinella lemuru* fish oil significantly increased plasma HDL levels (Table 2). Plasma HDL of laying duck in this research was 18.29 to 39.43 mg/dl. When combined with *Indigofera zollingeriana* leaf meal, the ability of omega-3 fatty acid from *Sardinella lemuru* fish oil in lowering LDL and raising up HDL were more potent. The mechanism of the increase in plasma HDL levels might be due to lipoprotein lipase

enzyme activity of *Sardinella lemuru* fish oil. Another possible mechanism was the active compounds of beta-carotene of *Indigofera zollingeriana* leaf meal which acted as a potential as an antioxidant that increased the secretion of bile acids (Carvajal-zarrabal *et al.*, 2005). Beta carotene increased the incorporation of esterification of cholesterol in HDL-C to be transported to the liver (Carvajal- zarrabal *et al.*, 2005). Cholesterol in the liver to be converted into folic acid and kenokolat acid that are secreted into the bile as bile acids (Murray *et al.*, 2009). Bile acids are stored in the gallbladder and are secreted into the small intestine to aid in the digestion of fat in the diet. This causes the total cholesterol level in the blood decreases which leads to increased plasma HDL levels. According to Adkins and Kelley (2010), omega-3 fatty acids can also inhibit the secretion of low density lipoprotein (LDL) in the liver, which would affect the levels of plasma high density lipoprotein (HDL)

Table 3 showed that feed supplemented with *Indigofera zollingeriana* leaf meal and *Sardinella lemuru* fish oil significantly affected (P<0.01) egg cholesterol of 32 weeks old laying duck. I11L2

Table 3. Cholesterol of Eggs and Meat of Laying Duck Supplemented by *Indigofera zollingeriana* Leaf Meal and Lemuru Fish Oil

Parameter	Lemuru Fish Oil	<i>Indigofera zollingeriana</i> Leaf Meal		
		I0	I5.5	I11
Egg cholesterol (mg/g)	L0	8.46±0.36 <sup>BC</sup>	7.83±0.17 <sup>BC</sup>	8.44±0.43 <sup>BC</sup>
	L2	8.85±0.68 <sup>AB</sup>	9.58±0.48 <sup>A</sup>	7.66±0.26 <sup>C</sup>
Meat cholesterol (mg/g)	L0	0.28±0.01 <sup>a</sup>	0.12±0.01 <sup>b</sup>	
	L2	0.11±0.01 <sup>b</sup>	0.14±0.01 <sup>b</sup>	

I0L0 : feed without *Indigofera zollingeriana* leaf meal and lemuru fish oil, I5.5L0 : feed plus 5.5% *Indigofera zollingeriana* leaf meal without lemuru fish oil, I11L0 : feed plus 11% *Indigofera zollingeriana* leaf meal without lemuru fish oil, I2L0 : feed without *Indigofera zollingeriana* leaf meal plus 2% lemuru fish oil, I5.5L2 : feed plus 5.5% *Indigofera zollingeriana* leaf meal and 2% lemuru fish oil, and I11L2 : feed plus 11% *Indigofera zollingeriana* leaf meal and 2% lemuru fish oil. Significant difference between various groups is by Duncan's Mutiple Range Test, <sup>abcd</sup> P<0.05, <sup>ABCD</sup> P<0.01.

was not different (P<0.05) from I0L0, I5.5L0, I11L0. I5.5L2 was not different from I0L2. I11L2 was different from I0L2 and I5.5L2. The effect of *Indigofera zollingeriana* leaf meal and *Sardinella lemuru* fish oil in diet produce eggs cholesterol levels ranged 7.66 to 9.58 mg/g are relatively similar to 7.29 to 9.65 mg/g of laying duck by Darmawan (2013). Decreased plasma cholesterol levels will affect the reduction in cholesterol levels in animal products, especially eggs. Results Palupi *et al.* (2014) showed that the use of *Indigofera zollingeriana* leaf meal could be used up to 15.6% in the diet of laying hens in the equivalent of replacing the use of 45% soybean meal, resulting on lowering cholesterol egg yolk cholesterol by 54.13%. The cholesterol functioned in poultry as forming materials of vitelogenin, steroid hormone and structural constituent of cells. Combinations of *Indigofera zollingeriana* leaf meal and *Sardinella lemuru* fish oil interact well. The high percentage of *Indigofera zollingeriana* leaf meal replace up to 11% protein soybean meal can increase the content of beta-carotene in the diet of laying ducks. Carotenoids (beta carotene) provide antioxidant defenses in lipids (Surai *et al.*, 2016). Moreover, the *Sardinella lemuru* fish oil rich in omega-3 fatty acids that most have double bonds so susceptible to oxidative degradation (Shahidi and Zhang, 2010). According to Levi *et al.* (2009) cholesterol is a steroid hormone precursor that will trigger the hormone estrogen to synthesize vitelogenin. Vitellogenin is a precursor of egg yolk contains

cholesterol, protein and lipid. The cholesterol and fats for metabolism and the production of vitellogenin would cause changes in lipid metabolism that would affect blood lipid profile. Beta-carotene is a potent antioxidant, so it can be used in protecting omega-3 fatty acids (Hennekens *et al.*, 1996). EPA and DHA were an example of an omega-3 fatty acids that could be found in *Sardinella lemuru* fish oil can lower blood cholesterol levels in fatty acid hydrolysis by-products.

Table 3 showed that feed without *Indigofera zollingeriana* leaf meal and *Sardinella lemuru* fish oil (I0L0) produced different meat cholesterol (P<0.05) from all other treatments; however, I5.5L0, I0L2 and I5.5L2 were not different. Meat cholesterol of laying duck in this research ranged from 0.11±0.01 to 0.28±0.01 mg/g. This is because beta carotene from *Indigofera zollingeriana* leaf meal function as antioxidant that prevent the forming of free radicals and increase oxidative stability of lipid which effects decrease of meat cholesterol. Antioxidant compounds derived from beta-carotene *Indigofera zollingeriana* leaf meal highly efficient in free radical prey to protect LDL from oxidation in vitro in the blood circulation (Karppi *et al.*, 2010). LDL mass formed of cholesterol by 50%. LDL protection of beta-carotene in preventing cell damage can increase the effectiveness of the use of *Sardinella lemuru* fish oil, that are lowering blood cholesterol levels. A decrease in blood cholesterol levels will affect the reduction in

cholesterol levels in meat laying ducks aged 32 weeks.

## CONCLUSION

Feeding *Indigofera zollingeriana* leaf meal 5.5% and *Sardinella lemuru* fish oil 2% in laying duck's diet could maintain the balance of blood lipid profiles by lowering plasma triglycerides, LDL, cholesterol and raising up plasma HDL and plays an important role on lipid metabolism of Magelang laying ducks to produce eggs and meat with low cholesterol content.

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