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Effect of *Indigofera zollingeriana* Top Leaf Meal Supplementation as Natural Antioxidant Source on Production and Quality of Pegagan Duck Eggs

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ABSTRACT

This study aims to produce Pegagan duck eggs which is high in antioxidants to be used as functional comestibles. Efforts to achieve these goals can be done by adding a source of natural antioxidants in the form of legume leaves of Indigofera zollingeriana which contain active compounds of β-carotene. The study used a completely randomized design (CRD) consisting of 5 treatments (4 replications each), which were levels of *Indigofera zollingeriana* top leaf meal supplementation (R0 = 0%, R2 = 1%, R2 = 2%, R3 = 3% and R4 = 4%) in Pegagan duck rations. The observed variables are production performance; egg quality, both physically and chemically. Physical quality includes egg weight, eggshell percentage, egg white weight percentage (albumen), and weight percentage of egg yolk and egg yolk color. The quality of egg chemistry includes the content of vitamin A, β-carotene, cholesterol, and antioxidant activity of eggs. The data obtained were analyzed by variance analysis, followed by the Duncan Multiple Range Test. The results showed that supplementation of *Indigofera zollingeriana* top leaf meal in rations could improve egg quality and contain antioxidants. Supplementation of *Indigofera zollingeriana* top leaf meal with a dose of 4% in rations can increase the color of the yolk by 43.40%, reduce the inhibition rate by 11.38%, with an increase in the content of β-carotene by 75.23% and can maintain normal cholesterol in eggs.

Keywords: Antioxidant, *Indigofera zollingeriana*, Pegagan duck eggs

Introduction

Ducks are one of the many poultry commodities that are kept in Indonesia as producers of meat and eggs. Local ducks are poultry producing eggs, meat and feathers (Ismoyowati, 2008). Indonesian local ducks, especially those in South Sumatra, known as Pegagan ducks from Ogan Ilir Regency, are genetic resources that have become the choice of business for the supply of eggs and meat by the local community. The Pegagan duckling business often finds obstacles with the problem of providing feed.

Feed ingredients that are often used in composing rations generally come from industrial waste, in the form of onggok from cassava to tapioca processing, rice bran and tofu dregs. Efforts to produce eggs that contain antioxidants, need to add an antioxidant source to duck rations. One source of feed ingredients containing antioxidants that can be used is *Indigofera zollingeriana* top leaf meal containing ß-carotene 507.6 mg/kg (Palupi *et al*., 2014a). Increasing the content of β-carotene and vitamin A in duck eggs can produce functional eggs which contains antioxidants, because β-carotene and vitamin A are antioxidant-forming compounds. Palupi *et al*. (2014b) reported that the addition of *Indigofera zollingeriana* top leaf meal to 5.2% in the rations had increased the content of vitamin A, β-carotene and the antioxidant activity of *Isa Brown* strain chicken eggs. Supplementing carotenoid sources in laying duck rations can produce eggs that contain antioxidants, are rich in vitamin A and have a thicker yolk color. Hammershøj *et al*. (2010) reported that the carotenoid content in the ration had a positive effect on the color of the yolk.

Materials and Methods

Livestock and research rations

This study used 60 Pegagan ducks in the egg production phase with a body weight of 1,500 - 1,800g. The enclosure used is a litter system with a size of 60 x 60 x 60 cm made of bamboo. Each replication consists of 3 Pegagan ducks. Basal rations have a protein content of 18 percent and metabolizable energy of 2,700 kcal/kg (Sinurat, 2000). The feed ingredients used to prepare Pegagan duck rations and composition of treatment rations are presented in Table 1.

Research procedure

The Treatments of addition of *Indigofera zollingeriana* top leaf meal in Pegagan duck rations observed for 8 weeks. Measurement of ration consumption is carried out every week. Feeding is done twice a day, at 07.00 Western Indonesia Time and at 17.00 Western Indonesia Time. Drinking water is given ad libitum.

The parameters observed in this study were the performance of duck production, including: ration consumption (g/head/day), daily egg production (%) and ration conversion. Observations on the physical quality of eggs, namely: egg weight (g/egg), eggshell percentage (g/egg), weight percentage of egg white (albumen), weight percentage of egg yolk, and duck egg yolk color measured by comparing standard egg yolk color in Egg Roche Yolk Color Fan. Measuring the chemical quality of eggs is done by analyzing the content of ß-carotene and vitamin A in egg yolks analyzed by High Performance Liquid Chromatography, measuring the content of yolk cholesterol (mg), analyzed by Gas Chromatography, and antioxidant activity using the DPPH (*2,2-diphenyl-1-pikrilhidrazil*) method.

Experimental design and statistical analysis

This research was carried out using an experimental method using a completely randomized design (CRD) with 5 (five) treatments of rations added with *Indigofera zollingeriana* top leaf meal as a supplement. Each treatment was repeated four times. Each replication or research unit consisted of the 3 Pegagan ducks with 56 weeks of age placed on the battery enclosure.

The treatment in this study was the addition of *Indigofera zollingeriana* top leaf meal in Pegagan duck rations, namely: R0 = without addition of *Indigofera zollingeriana* top leaf meal in rations, R1 = 1% addition of *Indigofera zollingeriana* top leaf meal in rations, R2 = 2% *Indigofera zollingeriana* top leaf meal in rations, R3 = addition of 3% *Indigofera zollingeriana* top leaf meal in rations and R4 = 4% addition of *Indigofera zollingeriana* top leaf meal in rations. The data obtained were analyzed statistically by analysis of variance and if the effect of treatment was significant effect, followed by Duncan multiple range test (DMRT) according to Steel and Torrie (1995).

Result and Discussion

Effect of treatment on the performance of Pegagan duck production

Ration consumption. Pegagan duck production performance data during the research are shown in Table 2. Supplementation of *Indigofera zollingeriana* top leaf meal in the ration did not significantly effect on ration consumption. The average consumption of rations ranged from 153.67 to 158.15 g/head/day. The metabolizable energy content of the ration after supplementation with *Indigofera zollingeriana* top leaf meal in all treatments was almost the same, namely: without supplementation (R0) 2,704.19; supplementation of 1% (R1) 2,705.06; supplementation of 2% (R2) 2,705.92; supplementation of 3% (R3) 2,706.80 and supplementation of 4% (R4) 2,707.60 kcal/kg. Ration consumption is influenced by the energy content of rations, poultry fed rations with low energy content, causing more ration consumption compared to those fed rations with higher energy content (Wu *et al*., 2005).

In addition to the same metabolic energy content in all treatments, supplementation with *Indigofera zollingeriana* top leaf meal did not cause changes in the palatability of the treatment ration. One of the rationability is determined by the phytochemical content of the feed ingredients. Phytochemicals found in *Indigofera zollingeriana*

Table 1. Composition and nutritional content of experimental diet

Feed ingredients	R0 (%)	R1 (%)	R2 (%)	R3 (%)	R4 (%)
Groundcorn	56.67	56.67	56.67	56.67	56.67
Rice bran	4.44	4.44	4.44	4.44	4.44
Meat meal	3.48	3.48	3.48	3.48	3.48
Soybean meal	23.25	23.25	23.25	23.25	23.25
Bone meal	1.35	1.35	1.35	1.35	1.35
Clamshell meal	5.08	5.08	5.08	5.08	5.08
Dry noodles waste	3.98	3.98	3.98	3.98	3.98
Premix	0.25	0.25	0.25	0.25	0.25
DCP	1.50	1.50	1.50	1.50	1.50
TOTAL :	100.00	100.00	100.00	100.00	100.00
Basal feed	100.00	99.00	98.00	97.00	96.00
I. zollingeriana top leaf meal	0.00	1.00	2.00	3.00	4.00
Metabolizable energy (kcal/kg)	2700.19	2701.10	2702.01	2702.91	2703.80
Crude protein	18.75	18.85	18.95	19.05	19.16
Fat (ether extract)	3.09	3.09	3.09	3.10	3.10
Crude fiber	2.45	2.51	2,57	2,63	2,69
Lysine	1.05	1.05	1.06	1.06	1.07
Methionine	0,39	0,39	0,39	0,39	0.39
Calcium	3.15	3.12	3.10	3.07	3.05
Phosphorus	0.87	0.87	0.86	0.85	0.85
Natrium Chloride	0.04	0.04	0.04	0.04	0.04
Linoleic acid	0.05	0.05	0.05	0.05	0.05

Table 2. Production performance of Pegagan duck given dietary supplementation of *Indigofera zollingeriana* top leaf meal

Parameter	R0	R1	R2	R3	R4
Ration consumption (*feed intake*) g/head/day	153.67±3.17	156.82±2.55	158.15±3.60	155.55±3.24	155.75±2.35
Egg production (*hen day production*) %	46.87±3.17	45.82±2.55	58.32±3.60	62.50±2.94	58.32±2.76
Ration conversion (*feed conversion*)	2.25±0.16	2.33±0.14	2.23±0.04	2.17±0.06	2.20±0.04

R0=ration control (whithout *Indigofera zollingeriana* top leaf meal), R1= Ration supplemented with 1% *Indigofera zollingeriana* top leaf meal, R2= Ration supplemented with 2% *Indigofera zollingeriana* top leaf meal, R3= Ration supplemented with 3% *Indigofera zollingeriana* top leaf meal, R4= Ration supplemented with 4% *Indigofera zollingeriana* top leaf meal.

top leaf meal are very low, namely: tannin 0.29% and saponin 0.036%, still within the limits that can be tolerated by ducks. Johri (2005) states that the tolerance limit of saponin levels in poultry rations is 0.37%. Kumar *et al*. (2005) stated that the content of phytochemical compounds (saponins, flavonoids, and tannins), which is a natural chemical found in plants or plants that have physiological functions, in large quantities can increase consumption.

Egg production. Supplementation of 1 to 4% *Indigofera zollingeriana* top leaf meal in the ration had no significant effect (P>0.05) on egg production. One of the factors that influence egg production is the consumption of proteins that are not significantly different, which ranges from 28.81 - 29.65 g/head/day. Creswell (2012) reported that duck protein requirements ranged from 25.35 g/head/day. Daily protein consumption is needed by poultry to meet the needs of the formation of an egg, basic living needs and growth of body weight and feathers.

Pegagan duck egg production during the study increased by 24.43, 33.35, and 33.35% for R2, R3, and R4 respectively. The increase of egg production because ß-carotene contained in *Indigofera zollingeriana* top leaf meal ranges from 5.07 - 20.30 mg/kg can prevent the occurrence of fat oxidation, which can resist peroxidation by converting back to fatty acids. The peroxide which is allowed to continue will result in the breakdown of the peroxide into aldehyde and ketone. ß-carotene in the ration will work to protect normal cells and neutralize free radicals, thus avoiding damage to the egg cell. Bortolotti *et al*. (2003) stated that carotenoids function as pigments and play a role in physiological processes for egg production and embryo development.

Ration conversion. Based on ration consumption and Pegagan duck egg production, the feed conversion of Pegagan duck was obtained during the study as listed in Table 2. Supplementation of *Indigofera zollingeriana* top leaf meal did not significantly affect ration conversion in Pegagan ducks. Egg production during the study was offset by consumption of the same ration in all treatments. The feed conversion of Pegagan duck ranges from 2.17 to 2.33. The results of the study of Akbarillah *et al*. (2010) who reported that the average conversion rate of duck rations given by the leaves of *Indigefera* was 5% in the ration of 3.80. When compared with the results of these studies, the value of feed conversion of Pegagan duck during the study was better, because egg production and egg weight produced in this study were higher.

Feed conversion of Pegagan ducks supplemented with 4% *Indigofera zollingeriana* top leaf meal is the best conversion rate, which is 2.17 compared to other treatments. Supplementation with *Indigofera zollingerian* top leaf meal containing β-carotene of 5.07 - 20.30 mg/kg in the treatment ration functions as a natural antioxidant in the Pegagan duck body. Antioxidants increase the work of female reproductive hormones, so that the duck can produce better if compared with livestock without supplementation with *Indigofera zollingeriana* top leaf meal. In line with the results of research by Rosa *et al*. (2012) that canthaxanthin supplementation in rations can increase egg fertilization and hatchability in broiler breeding.

Effect of treatment on the physical quality of eggs

Egg weight. The average physical quality of duck eggs during the study is presented in Table 3. Supplementation of *Indigofera zollingeriana* top leaf meal had no significant effect (P>0.05) on egg weight. Supplementation of *Indigofera zollingeriana* top leaf meal to a level of 4% in the ration did not cause a decrease in egg weight. Egg weight is influenced by the content of linoleic acid and protein ration, the protein content of the treatment ration in this study is almost the same after being supplemented with *Indigofera zollingeriana* top leaf meal. Wahju (2004) states that one of the factors that determine egg weight is the protein content in the rations consumed. Protein content of rations ranges from 18.75% to 19.16% and protein consumption 28.81 - 29.65 g/head/day and the content of linoleic acid ration in this study was 1.30% (Table 1). Supplementation of linoleic acid up to 1.6% in rations increases saturated fatty acid content and does not saturate chicken eggs (Liu *et al*., 2017).

The protein requirements for Pegagan ducks are fulfilled, and lysine to form an egg is also fulfilled, which is 1.05%. The amino acid lysine needed to produce eggs is a minimum of 0.85% (Leeson and Summer, 2005). Yuwanta (2010) states that chickens lacking lysine and methionine can each reduce the weight of egg yolk and the weight of egg white. The status of duck minerals during maintenance has met the needs of calcium and phosphorus (Table 1). Sinurat (2000) reports that the calcium requirement of laying ducks is 2.90 - 3.25% and the need for phosphorus is available 0.6%.

Eggshell weight. The average eggshell weight of this study was 9.64 to 10.93 g with eggshell weight percentage 14.33 to 16.28%. The

Table 3. Physical quality of Pegagan duck eggs

Parameters	R0	R1	R2	R3	R4
Egg weight (g)	66.75±2.21	67.25±8.54	71.00±2.16	72.00±3.37	70.75±0.50
Egg shell weight (g)	10.87±0.89	9.64±0.41	10.93±0.35	10.08±0.66	10.60±0.77
(%)	16.28±0.36	14.33±0.36	15.39±0.53	14.00±0.30	14.98±0.20
Albumen weight (g)	15.00±3.21	15.23±4.71	22.33±2.20	22.59±3.32	19.87±0.88
(%)	22.47±0.62	22.64±1.14	31.45±0.34	31.37±0.30	28.08±0.53
Yolk weight (g)	40.88±2.81	42.38±4.05	37.71±2.14	39.33±2.86	40.28±0.68
(%)	61.24±0.26	63.02±0.88	53.11±0.83	54.62±0.30	56.93±0.72
Yolk color	$7.5±0.58^{a}$	$12.25±0.96^{b}$	$12.75±0.50^{b}$	$13.75±0.50^{b}$	$13.25±1.50^{b}$

[a,b] Different superscripts in the same row show highly signifcant difference (P<0.01), R0= control ration (whithout Indigofera zollingeriana top leaf meal), R1= Ration supplemented with 1% Indigofera zollingeriana top leaf meal, R2= Ration supplemented with 2% Indigofera zollingeriana top leaf meal, R3= Ration supplemented with 3% Indigofera zollingeriana top leaf meal, R4= Ration supplemented with 4% top leaf meal.

percentage of Pegagan duck eggshell in this study was higher when compared to the percentage of general eggshell weight. Pundir *et al*. (2009) stated that the percentage of eggshell was around 11% of obese weight. Supplementation of *Indigofera zollingeriana* top leaf meal up to 4% in the ration did not affect the weight and percentage of eggshell. The research ration had almost the same protein, energy, Ca and P content and ration consumption in this study was not significantly different, so the consumption of Ca and P was also not different in all treatments. Bar (2008) states that eggshell contains about 95% calcium in the form of calcium carbonate and the rest is magnesium, phosphorus, sodium, potassium, zinc, iron, manganese, and copper. If the level of Ca rations in the intestine is high, then the absorption of Ca by the intestine occurs passively, but if the Ca level is low, it is necessary to actively absorb Ca with the help of *dihydroxycolecalciferol*, a metabolite of vitamin D. *Dihydroxycolecalciferol* is formed in the kidney under the influence of the pathatiroid hormone. Supplementation of *Indigofera zollingeriana* top leaf meal does not interfere with the metabolism of calcium and vitamin D Pegagan ducks, although the top leaves of *Indigofera zollingeriana* contain tannin and saponins, but the numbers are very low. Ahmed *et al*. (2017) report that an increase in the content of antinutrients in the ration can reduce mineral availability and reduce digestibility of starch and protein.

ß-carotene in the ration results in the need for vitamin A to be fulfilled to carry out metabolic activities in the body of the animal. Surai (2003) states that the source of rations containing carotenoids that have vitamin A-forming activities function as antioxidants. Vitamin A increases the stability of epithelial tissue in the mucous membranes of the digestive tract, so that absorption of nutrients becomes better, especially protein. Protein is needed for laying hens for the formation of Calcium Binding Protein (CaBP) which is used for active calcium absorption. Wahju (2004) states that the quality of eggshell is determined by the thickness and structure of the eggshell. The content of Ca and P in the ration plays a role in the quality of eggshell, because in the formation of eggshells it is necessary to have enough carbonate ions and Ca ions to form CaCO3 of eggshell.

Egg white weight. Supplementation of *Indigofera zollingeriana* top leaf meal to 4% in rations did not affect the weight of egg white. The weight average of Pegagan duck egg white in this study ranged from 15.00 to 22.59 g. Egg white weight is strongly influenced by protein quality and amino acid ration content. Coon (2002) stated that egg size is influenced by the availability of protein, because it is needed to form albumen. The quality of *Indigofera zollingeriana* top leaf meal protein is good with an amino acid score of 64.20% and a very low protein content of 1.22% of the total protein (Palupi *et al*., 2014b). The low content of Non Protein Nitrogen indicates the high protein of *Indigofera zollingeriana* top leaf meal can be used for the formation of albumen of Pegagan duck eggs.

The average weight of Pegagan duck egg yolk in this study ranged from 37.71 to 42.38 g, with the weight percentage of egg yolk amounting to 53.11 to 63.02%. The weight percentage of Pegagan duck yolk in this study was relatively higher when compared to the standard percentage of egg yolk weight. The percentage of duck egg yolk weight in fresh condition is 33.94% from egg weight (Kaewmanee *et al*., 2009).

Egg yolk weight. Supplementation of *Indigofera zollingeriana* top leaf meal did not affect egg yolk weight. This is due to the fat content and protein of *Indigofera zollingeriana* top leaf meal did not affect the nutrient composition of treatment ration with supplementation to 4%, so that the protein and fat content of eggs is not different. According to Agro *et al*. (2013) stated that the factors that influence the weight of egg yolk are the fat and protein content in eggs, where many fatty acids found in the yolk are linoleic, oleic and stearic acids which function to increase the yolk weight. Shim *et al*. (2004) stated that egg cholesterol is generally found in egg yolk produced in the liver and sent via blood in the form of lipoproteins and deposited in developing follicles.

The yolk weight in the treatment whose ration was supplemented with *Indigofera zollingeriana* top leaf meal was relatively the same between all treatments with the control ration (without the addition of *Indigofera zollingeriana* top leaf meal). The weight of egg yolk is influenced by the content of linoleic acid in the ration to meet the needs of 1.30%. The need for a minimum standard of linoleic acid in the ration of laying hens is 1.00% (Wahju, 2004). Egg yolk weight is influenced by the fat content because most fat deposits are in the yolk.

Many fatty acids found in the yolk are linoleic, oleic and stearic. The fat content in egg yolk can be affected by the fat content of the feed (Yamamoto *et al*., 2007). *Indigofera zollingeriana* top leaf meal has a low fat content of around 3.39%, it is not dominant to be able to change the composition of linoleic acid in the ration with relatively the same fat which is 3.09%.

Egg yolk color. The yolk color intensity of Pegagan duck egg is one of the determinants of physical egg quality. The higher the yolk color score produced, the better the quality of the egg. The measurement of the quality of the yolk color in this study is presented in Figure 1.

The yolk color intensity in this study showed that supplementation of *Indigofera zollingeriana* top leaf meal in the real ration (P<0.01) increased the yolk color score (Table 3). The use of *Indigofera zollingeriana* top leaf meal increased the yolk color score for R1 (66.67%), R2 (70.00%), R3 (83.33%) and (R4) 76.67% compared to the control. This is caused by an increase in the consumption of ß-carotene originating from *Indigofera zollingeriana* top leaf meal 0.80 mg at R1 increasing to 3.16mg on R4. Carotenoids are natural pigments and are widely known for their colors, especially yellow, orange and red. These dyes will be absorbed in the digestive tract of chickens and deposited in egg yolk. The concentration of carotenoids in egg yolks is a reflection of the food consumed by these poultry (Nys and Guyot, 2011).

The higher the supplementation of *Indigofera zollingeriana* top leaf meal in duck rations, the higher the score of the yolk color produced. Rations that contain more carotene, namely xanthophyll, the color of the yolk is increasingly reddish orange (Yamamoto *et al*., 2007). Sangeetha and Baskaran (2010) state that laying hens cannot convert all carotenoids to vitamin A, but some are used to increase the color of the yolk.

Effect of treatment on the chemical quality of duck eggs

The egg chemical quality data in this study (ß-carotene content, vitamin A, cholesterol) and antioxidant activity are presented in Table 4. The results of the variance analysis showed that supplementation with *Indigofera zollingeriana* top leaf meal in rations had a very significant effect (P<0.01) on the content of β-carotene of Pegagan duck eggs. The ration supplemented with *Indigofera zollingeriana* top leaf meal has carotenoid compounds in the form of ß-carotene. The content of ß-carotene from *Indigofera zollingeriana* top leaf meal is 507.6 mg / kg (Palupi et al., 2014a). ß-carotene is one of the carotenoid compounds that functions as an antioxidant and can be stored in tissues (Winarsi, 2007).

The increase in using Indigofera zollingeriana top leaf meal in rations increases the deposit of ß-carotene in eggs. The increase of ß-carotene absorption occurs through ß-carotene compounds forming 2 molecules of vitamin A. In the body of poultry only a portion of ß-carotene is converted to vitamin A and the rest is stored as a reserve (Mayes, 2002). The proportion of ß-carotene converted is controlled by vitamin A status in the body, so it does not become vitamin A hypervitaminosis. ß-carotene plays an important biological role even though it is in the status of a provitamin. Absorption of vitamins and ß-carotene into the chicken's body requires bile salts through the upper small intestine, with a maximum absorption time of 2 to 6 hours. β-carotene is stored in fat tissue, so that it is yellowish in color and the product produced is like the yellow color of the egg (Doucha *et al*., 2009).

Supplementation of 1% *Indigofera zollingeriana* top leaf meal was significantly different (P <0.01) with 2%, 3% and 4% in rations for the content of ß-carotene. The average content of ß-carotene in eggs given *Indigofera zollingeriana* top leaf meal ranged from 1.30 to 5.54 mg/100g. The increase in ß-carotene in duck eggs is seen physically in the brighter color of the yolk produced which is a reflection of the

R0(without ITM) R1(1% ITM) R2(2% ITM) R3 (3% ITM) R4 (4% ITM)

Figure 1. Yolk color intensity of Pegagan duck due to supplementation of *Indigofera zollingeriana* top leaf meal.

Table 4. Chemical quality of Pegagan duck eggs with supplementation of *Indigofera zollingeriana* top leaf meal

Parameter	R0	R1	R2	R3	R4
ß-carotene content (mg/100g)	1.35±0.38[a]	1.91±0.41[a]	2.92±0.07[b]	3.65±0.17[c]	5.45±0.51[d]
Vitamin A content (mg/100g)	322.24±46.29	384.75±49.17	380.50±35.05	386.25±34.35	400.00±21.79
Cholesterol content (mg/100 g yolk)	807.25±21.09	806.75±5.62	7807.25±18.61	805.50±10.66	802.75±18.31
Inhibition concentration (mg/g)	92.84±1.14[b]	89.98±0.42[b]	84.97±2.57[a]	82.40±0.44[a]	81.35±1.42[a]

[a,b] Different superscripts in the same row show highly signifcant difference (P<0.01), R0=control ration (whithout *Indigofera zollingeriana* top leaf meal), R1= Ration supplemented with 1% *Indigofera zollingeriana* top leaf meal, R2= Ration supplemented with 2% *Indigofera zollingeriana* top leaf meal, R3= Ration supplemented with 3% *Indigofera zollingeriana* top leaf meal, R4= Ration supplemented with 4% *Indigofera zollingeriana* top leaf meal.

carotenoid content in the ration (Na *et al*., 2004). Liu *et al*. (2012) reported that ß-cryptoxanthin eggs increased 663.64% by giving corn fortified with ß-cryptoxanthin. Kotrbacek *et al*. (2013) reported that chorella heteropic biomass supplementation increased total carotenoid deposition in eggs.

The average vitamin A content in Pegagan duck eggs supplemented with *Indigofera zollingeriana* top lef meal ranged from 322.25 mg/100g to 400.00 mg/ 00g. The results of the variance analysis showed that supplementation with *Indigofera zollingeriana* top lef meal had no significant effect (P>0.05) on the vitamin A content of Pegagan duck eggs, but there is an increase in line with the increasing of ß-carotene content in the duck eggs.

The average cholesterol content in egg yolk in this study ranged from 802.75 to 807.25 mg/yolk. The highest cholesterol content was found in chicken eggs which were not added with *Indigofera zollingeriana* top leaf meal in its ration (R0), while chickens fed *Indigofera zollingeriana* top leaf meal produced lower egg cholesterol. Supplementation with 4% *Indigofera zollingeriana* top leaf meal containing the highest ß -carotene with low duck egg cholesterol content compared with other treatments. The Increase of carotenoid level in egg yolk cause a decrease in cholesterol in the yolk produced (Akdemir *et al*., 2012).

The use of *Indigofera zollingeriana* top leaf meal 2% (R2) to 4% (R3) was very significant (P<0.01) on DPPH inhibition concentration. These results indicate that the higher the use of *Indigofera zollingeriana* top leaf meal in the ration, the more antioxidant content of the eggs produced to counteract free radicals. The inhibition concentration increased by 3.08, 8.48, 11.24, and 12.38% for R1, R2, R3, and R4 respectively. This situation is due to the increasing content of ß-carotene in chicken eggs supplemented with *Indigofera zollingeriana* top leaf meal. ß-carotene is a chain breaker antioxidant, lipophilic, acting on cell membranes including egg cells to prevent lipid peroxidation (LPO). The role of ß-carotene as an antioxidant to clean and reduce free radicals by giving electrons to free radicals or oxidants, so that radical compounds become stable (Mayes, 2002). In line with the results of Sangeetha and Baskaran (2010) who reported that astaxanthin can be converted to ß-carotene and retinol in retinol-deficient mice, and can also act as an antioxidant in the body. Chidambara *et al*. (2005) reported that carotenoids found in Dunaliella algae have an antioxidant effect that matches antioxidants to synthetic ß-carotene.

Conclusions

Based on the results of the study it can be concluded that *Indigofera zollingeriana* top leaf meal supplementation in Pegagan duck ration can improve egg quality without decreasing duck egg production. The use of *Indigofera zollingeriana* top leaf meal up to 4% in rations can increase egg yolk color by 43.40%, reduce inhibition rate by 11, 38% with an increase in β-carotene 75.23% and can maintain normal cholesterol in egg yolk.

References

Agro, L. B., Tristiarti, and I. Manigisah. 2013. Kualitas ayam arab fase I dengan berbagai level *Azolla microphilla*. Animal Agricultural J. 2: 445 – 447.

Ahmed, S., A. Khalique, T. N. Pasha, S. Mehmood, K. Hessain, S. Ahmad, M. S. Shaheen, M. Naeem, and M. Shafiq. 2017. Effect of *Moringa oleifera* (Lam) pod as feed additive on egg antioxidants, chemical composition and performance of commercial layer. S. Afr. J. Anim Sci. 47: 864-874. http://doi: org/10.4314/sajas.v4716.14.

Akbarillah, T., Kususiyah, and Hidayat. 2010. Pengaruh penggunaan daun Indigofera segar sebagai suplementasi pakan terhadap produksi dan warna yolk itik. J. Sain dan Peternakan Indonesia 5: 27 – 33.

Akdemir, F., C. Orhan, N. Sahin, K. Sahin, and A. Hayirli. 2012. Tomato powder in laying. hen diets: effect on concentrations of yolk and lipid peroxidation. Br. Poult. Sci. 53: 675-680. http://doi: 10.1080/00071668.2012.729142.

Bar, A. 2008. Calcium homeostasis and vitamin D metabolism and expression in strongly calcifying laying birds. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 151: 477-490. http://doi: 10.1016/j.cbpa.2008.07.006.

Coon, N. C. 2002. Feeding commercial egg-type layers in Commercial chicken meat and egg production.. 5th edn. Springer Science, Business Media, Inc. New York.

Bortolotti, G. R., J. J. Negro, P. F. Surai and P. Prieto. 2003. Carotenoids in eggs and plasma of Red-Legged partridges: Effects of diet and reproductive output. Physiol. Biochem. Zool. 76: 367-374. http://doi: 10.1086/375432.

Chidambara, M. K. M., A. Vanitha, J, Rajesha, S. M. Mahadewa, P. R. Sowya, and G. A. Ravishankar. 2005. In vivo antioxidant anctivuty of carotenoids from *Dunaliella salina* a green microalga. J. Lifesci. 4: 1381 – 1390.

Creswell, D. 2012. Feeding meat and laying ducks for maximum performance. Proceeding *of* Poultry Feed and Quality Conference - Bangkok. July 9-10, 2012

Doucha, J., K. Livansky, V. Kotrbacek, and V. Zachleder. 2009. Production of chlorella biomass enriched by selenium and its use in animal nutrition : a review. App. Microbiol. Biotechnol. 83: 1001 - 1008. http://doi: 10.1007/s00253-009-2058-9.

Johri, T. S. 2005. Endogenous and exogenous feed toxins. In Poultry Nutrition Research in India and its Perspective. Central Avian Research Institute Izatnagar - 243 122, Distt.Bareilly (U.P.)

Hammershøj, M., U. Kidmose, and S. Steenfeldt. 2010. Deposition of carotenoids in egg yolk by short-term supplement of coloured carrot (*Daucus carota*) varieties as forage material for egg-laying hens. J. Sci. Food Agric. 90: 1163 - 1171. http://doi: 10.1002/jsfa.3937.

Ismoyowati, L. 2008. Study of detection of egg production of tegal ducks through blood protein polymorphism. Anim. Prod. 10 : 122- 128.

Kaewmanee, T., S. Benjakul, and W. Visessanguan. 2009. Changes in chemical composition, physical properties and microstructure of duck egg as influenced by salting. Food Chem. 112: 560 - 569. http://doi.org/10.1016/j.foodchem.2008.06.011.

Kotrbacek, V., M. Skrivan, J. Kopecky, O. Penkava, P. Hudekova, I. Uhrikova, and J. Doubek. 2013. Retention of carotenoid in egg yolk of laying hens supplemented with heterotropic chlorella. Czech. J. Anim. Sci. 58: 193 - 200.

Kumar, V., A. V. Elangovan, and A. B. Mandal. 2005. Utilization of reconstituted high-tannin sorghum in the diets of broiler chickens. Asian-Aust J. Anim. Sci. 18: 538 - 544.

Leeson, S. and J. D. Summers. 2005. Commercial poultry nutrition. 3rd edn. Manor Farm, Churh Lane, Thrumpton, Nottingham University Press. Nottingham.

Liu, Y. Q., C. R. Davis, S. T. Schmaelzle, T. Rocheford, M. E. Cook, and S. A. Tanumihardjo. 2012. Β-Cryptoxanthin biofortified maize (zeamays) increases Β-Cryptoxanthin cincentration and enhaces the color of chiken egg yolk. J. Poult. Sci. 91: 432 - 438.

Liu, X., Y. Zhang, P. Yan, T. Shi, and X. Wei. 2017. Effect of conjugated linoleic on the performance of laying hens, lipid composition of egg yolk, egg flavor, and serum components. Asian-Australas. J. Anim Sci. 30: 417-423. http://doi: 10.5713/ajas.15.1036.

Mayes, P. A. 2002. Lipids of physiologic significance. In : Harper's biochemistry. McGraw-Hill Education (Asia). New Delhi. pp. 148-281.

Na, J. C., J. Y. Song, B. D. Lee, S. J. Lee, C. Y. Lee and G. H. An. 2004. Effects of polarity on absorption and accumulation of carotenoids by laying hens. Anim. Feed Sci. Technol. 117: 305 - 315.

Nys, Y. and N. Guyot. 2011. Egg formation and chemistry. Improving the safety and quality of eggs and egg products. Woodhead Publishing - Cambridge UK. pp. 88 -132.

Palupi, R., L. Abdullah, D. A. Astuti and Sumiati. 2014a. High antioxidant egg production through substitution of soybean meal by *Indigofera* sp., top leaf meal in laying hen diets. Int. J. Poult. Sci. 13 : 198 - 203.

Palupi, R., L. Abdullah, D. A. Astuti, and Sumiati. 2014b. Potensi dan pemanfaatan tepung pucuk *Indigofera* sp. sebagai bahan pakan substitusi bungkil kedelai dalam ransum ayam petelur. JITV. 19: 210-219.

Pundir, C. S., M. Bhambi and N. S. Chauhan. 2009. Chemical activation of egg shell membrane for covalent immobilization of enzymes and Its evaluation as inert support in urinary oxalate determination. Talanta. 77: 1688 - 1693.

Rosa, A., A. Scher, J. Sorbara, L. Boemo, J. Forgiarini and A. Londero. 2012. Effects of canthaxanthin on the productive and reproductive performance of broiler breeders. Poult. Sci. 91: 660 - 666.

Sangeetha, R. K. and V. Baskaran. 2010. Retinol deficient rats can convert a pharmacological dose of astaxanthin to retinol : Antioxidant potential of astaxanthin, lutein and β carotene. J. Physiopharma. 88: 977 - 985. http://doi: 10.1139/y10 – 074.

Shim, K. S., G. H. Park, C. B. Choi and C. S. Na. 2004. Decreased triglyceride and cholesterol levels in serum, liver and breast muscle in broiler by the supplementation of dietary *Codonopsis lanceolata* root. Asian-Australas. J. Anim. Sci. 17:511 - 513.

Sinurat, A. P. 2000. Penyusunan Ransum Ayam Buras dan Itik. Balai Penelitian Ternak, Ciawi.

Steel, R. G. and J. H.Torrie. 1995. Prinsip dan Prosedur Statistika : Suatu Pendekatan Biometrik. Terjemahan: Bambang Sumantri. PT. Gramedia Pustaka Utama, Jakarta.

Surai, P. F. 2003. Natural antioxidant in avian nutrition and production. Nottingham University Press, Nottingham.

Wahju, J. 2004. Ilmu Nutrisi Unggas. 4th edn. Universitas Gadjah Mada Press, Yogyakarta.

Winarsi, H. 2007. Antioksidan Alami dan Radikal Bebas. Penerbit Kanisius, Yogjakarta.

Wu, G., M. M. Bryant, R. A. Voitle, and D. A. Roland. 2005. Effect of dietary energy on performance and egg composition of Bovans White and Dekalb White hens during phase I. J. Poult. Sci. 84: 1610–1615.

Yamamoto, T., L. R. Juneja, H. Hatta, and M. Kim. 2007. Hen eggs: Basic and Applied Science. University of Alberta, Canada.

Yuwanta, T. 2010. Telur dan Kualitas Telur. Gadjah Mada University Press, Yogyakarta.