

## Substitution of Soybean Meal with *Indigofera zollingeriana* Top Leaf Meal on Egg Quality of *Cortunix cortunix japonica*

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(Received 06-08-2015; Reviewed 07-09-2015; Accepted 16-10-2015)

### ABSTRACT

This research aimed to study the substitution of soybean meal (SBM) with *Indigofera zollingeriana* top leaf meal (ILM) in the diet on egg quality of Japanese quails. The experiment used a completely randomized design with five treatments and four replications (ten quails of each replication). The dietary treatment contained five combination of SBM and ILM, R0= diets contained 18% SBM without ILM, R1= diet contained 16.2% SBM and 2.66% ILM, R2= diet contained 14.4% SBM and 5.32% ILM, R3= diet contained 12.6% SBM and 7.98% ILM, R4= diet contained 9% SBM and 13.3% ILM. The results showed that the use of 13.3% ILM (R4) significantly ( $P < 0.05$ ) increased feed consumption, egg weight, yolk colour score, egg cholesterol, and reduced malondialdehyde level. The conclusion of this study was *I. zollingeriana* top leaf meal could be used as much as 13.3% in the diets. The use of *I. zollingeriana* top leaf meal could improve the quality of eggs physically and chemically.

**Key words:** eggs quality, *Indigofera zollingeriana*, soybean meal, quail

### ABSTRAK

Tujuan penelitian ini adalah untuk mempelajari substitusi protein bungkil kedelai (BKL) dengan protein tepung pucuk *Indigofera zollingeriana* (TPI) dalam ransum untuk meningkatkan kualitas telur puyuh. Penelitian ini menggunakan rancangan acak lengkap dengan lima perlakuan dan empat ulangan (10 ekor puyuh tiap ulangan). Perlakuan percobaan terdiri atas, R0= ransum mengandung 18% BKL tanpa TPI, R1= 16,2% BKL dan 2,66% TPI, R2= 14,4% BKL dan 5,32% TPI, R3= 12,6% BKL dan 7,98% TPI, R4= 9% BKL dan 13,3% TPI. Hasil penelitian menunjukkan bahwa penggunaan 13,3% TPI (R4) memberikan pengaruh nyata ( $P < 0,05$ ) terhadap konsumsi ransum, meningkatkan bobot telur, warna kuning telur, kolesterol telur, dan menurunkan kadar *malondhyaldehide* telur. Kesimpulan penelitian ini adalah tepung pucuk *I. zollingeriana* dapat digunakan sebanyak 13,3% dalam ransum. Penggunaan tepung pucuk *I. zollingeriana* dapat meningkatkan kualitas telur secara fisik dan kimia.

**Kata kunci:** kualitas telur, *Indigofera zollingeriana*, bungkil kedelai, puyuh

### INTRODUCTION

The quail egg is one of highly nutritious animal protein source that most efficient in providing animal protein sources (Handarini *et al.*, 2008) with an average weight of 6-16 g (Sezer, 2007). A quail egg composed of 56.83% of albumin, 34.61% yolk and 8.56 % eggshell (Kumari *et al.*, 2008). The quail egg was rich in essential amino acids and minerals such as Ca, P, and Fe (Tolik *et al.*, 2014).

In quail formulated ration, source of feed protein is usually derived from meat meal, fish meal and soybean meal at a quite expensive price. In 2013, Indonesia has imported soybean meal up to 3.53 million tons (Ditjen PPHP, 2014). Condition seems to continue to increase in accordance with increasing livestock population. This problem might be overcome by substituting the conventional protein source e.g soybean meal with legume forages. *Indigofera zollingeriana* is one of the legume tree that has high production and available throughout the year. Total production of *I. zollingeriana* is 7-10 kg/ha/crop. The production is based on dry matter of the *I. zollingeriana* top leaf meal defoliation harvested at 68 days of 1805 kg/ha/cutting with an estimated produc-

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tion reached 10830 tons/ha/year (Abdullah & Suharlina, 2010).

*I. zollingeriana* has potency as a source of poultry feed ingredient. The leaves of *I. zollingeriana* had a 27.68% crude protein, 0.08% tannin, 0.41% saponin, 1.16% calcium (Ca), 0.26% phosphorus (P), 3.70% crude fat (CF) and crude fiber (CF) 15.25% (Abdullah, 2010; Akbarillah *et al.*, 2008). Vitamin content of *I. zollingeriana* top leaf meal (ILM) were: vitamin D as much as 42.46 mcg/100g, vitamin K 1.149 ppm,  $\alpha$ -tocopherol 148.74 mg/kg and  $\beta$ -carotene 507.6 mg/kg that was not found in soybean meal (Palupi *et al.*, 2014b). Beta carotene is a carotenoid that was also found in ILM for yolk pigmentation. Based on the potency of *I. zollingeriana*, this research was conducted to study the effect of substitution of soybean meal with *I. zollingeriana* top leaf meal in the formulated quail ration.

## MATERIALS AND METHODS

### Experimental Design, Bird Management, and Experimental Diets

*I. zollingeriana* top leaf meal (ILM) was obtained from Bogor Agricultural University Plantations Education and Research in Jonggol, West Java. *I. zollingeriana* top leaf meal was processed into flour by sun-drying for 30 min and then was ground to become ILM powder. The protein content of the ILM powder was 28.41%. The content of crude protein in *I. zollingeriana* was 27.68% or 28.98% (Palupi *et al.*, 2014b).

This study used nine weeks old laying quail (*Cortunix Cortunix Japonica*) with an average egg production of 50%. A completely randomized design of five treatments and four replications consisted of 10 birds in each replication was used in this study. The birds were distributed into 20 units of cages (60 x 40 x 20 cm). The dietary treatments contained five combinations of soybean meal (SBM) and ILM, R0= diets contained of 18% SBM without ILM, R1= 16.2% SBM and 2.66% ILM, R2= 14.4% SBM and 5.32% ILM, R3= 12.6% SBM and 7.98% ILM, R4= 9% SBM and 13.3% ILM. Each cage was equipped with a lighting bulb. Drinking water was supplied *ad libitum* and the feed was given twice, once in the morning and once in the afternoon. Prior to the experiment, the birds were acclimatized to the experimental rations for one week before and the data collection was done for 8 weeks after acclimatization. Rations were formulated according to the standard of nutrient requirements for quail (NRC, 1994), It composed of 20% crude protein and 2900 Kcal/kg of metabolizable energy. Composition and nutrient content of the treatments are showed in Table 1.

### Variables Measured

**Feed consumption.** Feed consumption was measured every week by subtracting the amount of rations given with the amount of leftover.

**Physical eggs quality.** The eggs physical quality analysis began in the second week of observation. Egg collection

was carried out for six consecutive weeks. There eggs per experimental replication were sampled randomly once a week. Egg weight (g), eggshell weight (g), yolk weight (g), and albumen weight (g) were measured by using digital scales (Osuka-HWH®, Japan). The thickness of the shell and the height of egg white (mm) were measured by using 150 Digital Caliper of Nankai®, Japan). Haugh unit was measured according to the methods of Buckle *et al.* (1986), and egg yolk score was determined by comparing standards color of Egg yolk on Roche Yolk Colour Fan (Ovo Color, Aktiengesellschaft BASF, Germany).

**Yolk cholesterol.** The determination of yolk cholesterol levels was carried out at the end of the observation. One egg from each replication was sampled at random for analysis. Analysis of egg cholesterol was conducted in accordance with the method of Liebermann Burchard (Burke *et al.*, 1974) and the absorbance was read using spectrophotometer reading of Hitachi U-2001, Japan at 500 nm wavelength ( $\lambda$ ).

**Yolk malondialdehyde (MDA).** Malondialdehyde was analyzed by using method of Rice-Evans *et al.* (1991). MDA measurement was carried out at the end of the study. About one gram of egg yolk, PBS (phosphate buffered saline) with pH 4, KCl 11.4 g/L, containing cold KCl with the ratio of 1: 2 was added and then homogenized by using centrifuge at a speed of 10000 rpm for 20 min at a temperature of 40°C. 0.5 mL of supernatant formed was added into a mixture of concentrate HCl (2.23 mL) with 15 g TCA (trichloroacetic), 0.38 g TBA (thiobarbituric acid) and 100 mL of distilled water. Homogeneous samples were then stored in the oven with a temperature of 80°C for one hour, centrifuged with a speed of 3000 rpm for 15 min and cooled before absorbance reading. Absorbance was read using spectrophotometer Hitachi U-2001, Japan in wave ( $\lambda$ ) of 523 nm.

### Data Analysis

Data were analyzed by using the Statistical Package for Social Sciences (IBM®SPSS® version 21.0). Significantly different means level was determined by one-way analysis of variance (ANOVA). The mean value differences were considered significant at the level of  $P < 0.05$ , the data were further analyzed by using Duncan multiple range test (Mattjik & Sumartajaya, 2006)

## RESULTS AND DISCUSSION

### Feed Consumption and Physical Quality of the Eggs

Results of this study indicated that the use of ILM as much as 13.3% (R4) as the substitution of 50% protein soybean meal significantly ( $P < 0.05$ ) increased feed consumption (Table 2). This condition indicated that the diet containing ILM had good palatability. Phytochemical content in the constituent material ration affected the palatability of diets, ILM had a low phytochemical content as much as 0.29% tannin and 0.036% saponin (Palupi *et al.*, 2014a). Saponin tolerance

Table 1. Feed ingredients and nutrient compositions of diets with substitution of protein soybean meal (SBM) by *I. zollingeriana* top leaf meal (ILM) (%)

Feed ingredients	Treatments				
	R0	R1	R2	R3	R4
Yellow corn	52.72	51.87	51.8	50.72	51.00
Rice bran	7.66	7.71	6.91	6.98	4.89
Soybean meal	18.00	16.2	14.4	12.60	9.00
ILM	0.00	2.66	5.32	7.98	13.30
Fish Meal	9.50	9.50	9.50	9.50	9.50
CaCo3	5.00	5.00	5.00	5.00	5.00
Oil	2.50	2.50	2.50	2.50	2.50
Premix	0.30	0.30	0.30	0.30	0.30
DL-Methionine	0.05	0.09	0.00	0.00	0.09
L-Lysine	0.10	0.00	0.10	0.20	0.10
NaCl (salt)	0.07	0.07	0.07	0.07	0.07
Dicalcium phosphate	0.10	0.10	0.10	0.15	0.25
Corn gluten meal	4.00	4.00	4.00	4.00	4.00
Total	100.00	100.00	100.00	100.00	100.00
Crude protein	20.55	20.49	20.39	20.32	20.10
Crude fat	5.24	5.47	5.68	5.89	6.32
Crude fiber	2.97	3.43	3.81	4.27	4.98
Calcium	2.59	2.58	2.58	2.56	2.60
Phosphor	0.55	0.55	0.54	0.55	0.55
Methionine	0.48	0.45	0.46	0.45	0.51
Lysine	1.36	1.16	1.24	1.28	1.05
Chlorine	0.14	0.14	0.14	0.14	0.13
Sodium	0.12	0.12	0.12	0.11	0.11
Meth + cystine	0.72	0.89	0.77	0.75	0.77
Metabolizable energy (kcal/kg)	2971.37	2953.38	2945.23	2919.97	2908.33

Note: R0= diets contained of 18% soybean meal (SBM) without *I. zollingeriana* top leaf meal (ILM), R1= 16.2% SBM and 2.66% ILM, R2= 14.4% SBM and 5.32% ILM, R3= 12.6% SBM and 7.98% ILM, R4= 9% SBM and 13.3% of ILM.

Table 2. Feed consumption and physical qualities of eggs produced by quails fed diets with substitution of protein soybean meal (SBM) by *I. zollingeriana* top leaf meal (ILM)

Variables	Treatments				
	R0	R1	R2	R3	R4
Feed consumption (g/head)	18.96±0.97 <sup>b</sup>	20.44±0.97 <sup>ab</sup>	19.47±1.07 <sup>a</sup>	20.59±1.08 <sup>ab</sup>	21.32±0.71 <sup>a</sup>
Egg weight (g)	8.83±0.11 <sup>bc</sup>	9.05±0.26 <sup>ab</sup>	8.73±0.14 <sup>c</sup>	8.92±0.06 <sup>bc</sup>	9.32±0.16 <sup>a</sup>
Yolk colour	6.24±0.91 <sup>c</sup>	8.14±0.16 <sup>b</sup>	8.65±0.12 <sup>b</sup>	9.24±0.59 <sup>a</sup>	9.46±0.44 <sup>a</sup>
Egg shape	81.34±1.32	81.74±0.40	81.72±0.47	81.25±2.81	82.34±1.17
Haugh unit	91.52±0.44	91.70±0.94	91.97±0.26	91.05±0.71	92.39±1.75
Egg shell (%)	11.94±0.68	12.16±0.35	12.26±0.35	12.02±0.46	12.13±0.12
Egg yolk (%)	31.31±1.19	31.77±0.76	31.91±1.15	33.05±1.01	33.07±0.70
Albumen (%)	56.75±0.80	56.06±0.50	55.83±1.24	54.93±0.84	54.80±0.67

Note: Means in the same row with different superscripts differ significantly (P<0.05). R0= diets contained of 18% soybean meal (SBM) without *I. zollingeriana* top leaf meal (ILM), R1= 16.2% SBM and 2.66% ILM, R2= 14.4% SBM and 5.32% ILM, R3= 12.6% SBM and 7.98% ILM, R4= 9% SBM and 13.3% of ILM.

in poultry rations was 0.37% and 0.5% tannins according to Kumar (2005) and Leeson & Summer (2005), respectively. Feed consumption was affected by environmental temperature, also the quality and quantity of ration (Leeson & Summer, 2005).

### Physical Quality of the Eggs

Substitution of soybean meal with 13.3% (R4) ILM significantly (P<0.05) increased egg weight and score of yolk colour (Table 2). The average eggs weight in this study ranged from 8.73 to 9.32 g and the results obtained were still at the normal range of 6-16 g (Sezer,

Table 3. Chemical quality of eggs (MDA and cholesterol) produced by quails fed diets with substitution of protein soybean meal (SBM) by *I. zollingeriana* top leaf meal (ILM)

Variables	Treatments				
	R0	R1	R2	R3	R4
MDA ( $\mu\text{g/g}$ )	5.40 $\pm$ 0.51 <sup>a</sup>	5.31 $\pm$ 0.73 <sup>a</sup>	3.09 $\pm$ 0.69 <sup>b</sup>	3.11 $\pm$ 0.80 <sup>b</sup>	3.02 $\pm$ 0.46 <sup>b</sup>
Cholesterol (mg/g)	6.67 $\pm$ 0.95 <sup>b</sup>	7.08 $\pm$ 1.08 <sup>b</sup>	7.85 $\pm$ 1.15 <sup>b</sup>	12.05 $\pm$ 1.39 <sup>a</sup>	10.42 $\pm$ 0.78 <sup>a</sup>

Note: Means in the same row with different superscripts differ significantly ( $P < 0.05$ ). R0= diets contained of 18% soybean meal (SBM) without *I. zollingeriana* top leaf meal (ILM), R1= 16.2% SBM and 2.66% ILM, R2= 14.4% SBM and 5.32% ILM, R3= 12.6% SBM and 7.98% ILM, R4= 9% SBM and 13.3% of ILM.

2007). Egg size is influenced by the content of protein as amino acids. *I. zollingeriana* top leaf meal has amino acid score as well as soybean meal (Palupi *et al.*, 2014b). According to Grindstaff *et al.* (2005), the process of egg formation is influenced by the nutrients in the diets consumed by poultry.

In general, actually the addition of carotenoids in the feed increased score of yolk colour (McGraw, 2006; Amo *et al.*, 2013; Jiang *et al.*, 2013; Palupi *et al.*, 2014a). In term of yolk color, substitution of soybean meal with ILM by 2.66%-13.3% in the diets improved the egg quality. The increase in score of yolk color due to the addition of ILM indicates that  $\beta$ -carotene contained in ILM is possibly deposited into the yolk. Deposition of beta-carotene in the yolk depended on the efficiency of transferring carotene from the intestine into the ovum (Hammershoj *et al.*, 2010). The content of carotenoids and xanthophyll in the ration was contributed by ILM and greatly affected the colour of egg yolk (Akbarillah *et al.*, 2010). Most of  $\beta$ -carotene was also an antioxidant that would be converted into an anti-free radical (Loetscher *et al.*, 2013).

However, the addition of ILM in quail's ration did not affect eggs shape, haugh unit, eggshell or albumen (Table 2). The egg shape is the ratio between the width to the length of the whole eggs. In this study, eggs produced tended to be rounded as was indicated by an increase in egg shape value. An ideal shape value of quail's egg was 79.90 (Kumari *et al.*, 2008). A slight increase but not statistically significant of haugh units in R4 was probably influenced by the contribution of dietary protein and amino acid. This was supported by the content of amino acid balance in ILM that was close to soybean meal amino acid balance score (Palupi *et al.*, 2014b). Albumen height was influenced by the viscosity of albumen (Zita *et al.*, 2012). The high value of haugh units would describe the quality of the eggs (Alkan *et al.*, 2010). In this study the unit ranging from 91.05 to 92.39 was considerably good. According to Zita *et al.* (2013) haugh units was influenced by the factors of age, 9-17 weeks old quails have haugh units of eggs 90.13-90.50. The percentage of egg shell in the study showed a slight increase, compared with the control diets. The balance of calcium (Ca) and phosphorus (P) in the ration played an important role in the formation of egg shell. According to the NRC (1994) laying quail ration needed 2.5% Ca and 0.55% P. The balance of the two minerals was very important because Ca and P also would affect egg production (Amoah *et al.*, 2012).

### Chemical Quality of the Eggs

Malondialdehyde (MDA) is one indicator to see antioxidant activity (Chen *et al.*, 2009). Increased free radicals would stimulate the process of lipid peroxidation and oxidative stress, which could be measured by analyzing the content of MDA (Valko *et al.*, 2006). Substitution of soybean meal with 5.32%-13.3% (R1-R4) ILM in quail diets significantly decreased ( $P < 0.05$ ) the MDA and increased yolk cholesterol (Table 3). The lower content of MDA in egg yolk in R4 was probably due to the role of antioxidants in the ILM which reduced lipid peroxidation. Antioxidants were actually stable which could reduce the levels of MDA in the yolk as well as in serum and liver (Sahin *et al.*, 2008; Ni *et al.*, 2012; Kurtoglu *et al.*, 2008). Beta carotene in ILM acted as an antioxidant so did the isoflavone. The process of lipid peroxidation was triggered by the separation of hydrogen. The presence antioxidants donates a hydrogen group to ILM substitution (Wang *et al.*, 2005).

Quail's eggs had a high concentration of polyunsaturated fatty acids with cholesterol content of only 7.78 mg/g (Mennicken *et al.*, 2005; Kazmierska *et al.*, 2005). Increased yolk cholesterol with the increase ILM substitution was influenced by the role of  $\beta$ -carotene as an antioxidant for anti-free radical as to minimize lipid peroxidation process without interfering the formation of cholesterol. Giving antioxidants and  $\beta$ -carotene could reduce the levels of SGOT (Serum transaminases Glutamix Oxaloatic) and SGPT (Serum Glutamic Pyruvic Transaminase), showing the occurrence of lipid peroxidation (Jaya, 2013). Beta carotene supplementation in the diet would affect the activation of lipid peroxidation and antioxidant enzyme that protected cholesterol from damage (Shih *et al.*, 2008). This condition would benefit the quail, because cholesterol is needed in the process of embryos development to produce DOQ (day old quail). Egg yolk is rich in lipids, vitamins A, E and  $\beta$ -carotene which will be transferred to a membrane bag by the phagocytosis to form lipoprotein particles that are released into the circulation of embryos that supports the growth and development of the embryo (McGraw, 2006).

### CONCLUSION

*Indigofera zollingeriana* top leaf meal (ILM) could be used in the diet of laying quail (*Cortunix Cortunix Japonica*) up to 13.3% as substitution of 50% protein in

soybean meal. Supplementation of ILM increased feed consumption, egg weight, egg yolk colour, egg yolk cholesterol and reduced levels of malondialdehyde in the quail's eggs.

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