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High Antioxidant Egg Production Through Substitution of Soybean Meal by *Indigofera* sp., Top Leaf Meal in Laying Hen Diets

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Abstract: The aim of this research was to produce functional eggs that high antioxidant through substitution of soybean meal with *Indigofera* sp., top leaf meal in the laying hen diets. One hundred and sixty laying hens of Isa Brown strain, 30 weeks of age were kept into individual cages. Completely randomized design was used in this experiment, with 4 treatments and 4 replications. The treatment diets contained four kind combination of soybean meal (SBM) and *Indigofera* sp., top leaf meal (ITLM): T0 = diet contained 20% SBM and 0% ITLM; T1 = diet contained 17% SBM and 5.2% ITLM; T2 = diet contained 14% SBM and 10.4 ITLM; T3 = diet contained 11% SBM and 15.6 ITLM. The parameters observed were feed consumptions, egg production, egg quality and antioxidant activity. The results showed that substitution of soybean meal with *Indigofera* sp., top leaf meal not significantly ($p>0.01$) affected to feed consumptions, but significantly ($p<0.05$) increased eggs production (83.63 to 92.65%), high significantly ($p<0.01$) increased yolk colour, beta-carotene as well as vitamin A and decreased cholesterol content of the yolk. It was concluded that *Indigofera* sp., leaf meal could be used until 15.6% or substitute 45% soybean meal protein in the laying hen diets.

Key words: Antioxidant activity, cholesterol, egg production, *Indigofera* sp., vitamin A, beta-carotene

INTRODUCTION

Increased prevalence of degenerative diseases in Indonesia, motivate researchers to explore the antioxidant compounds derived from natural sources. One common disease is vitamin A deficiency (VAD). Lack of vitamin A can cause keratinization of the mucous membranes lining of the respiratory tract, gastrointestinal tract, urinary tract, skin and epithelium of the eye (Mahan and Stump, 2004). Based on research conducted by the World Health Organization (WHO) declared that the VAD affects approximately 40% of the world population, especially pregnant or breast-feeding women and children under five years old. More than 127 million children in the world having insufficient intake of vitamin A (West, 2002). One indicator of VAD prevalence according to WHO is if xerophthalmia (X1B) = 0.5% of the population had serum retinol levels below 20 ug/dL and if the prevalence of blindness xerophthalmia = 1.0% of the population, the population is said to have health problems. The study of micronutrient problems in Indonesia at 2006 showed that the serum levels of vitamin A toddler on average only 11 mg/dL with a prevalence of blindness Xerophthalmia Twilight of 1.18% (Herman, 2007). This shows that VAD is a public health problem in Indonesia.

Given the importance of vitamin A, the adequacy of vitamin A and its precursor must be met. One of its efforts is the addition of carotenoids as precursors of vitamin A to the diet as a source of provitamin A as high beta-carotene. Food sources of beta-carotene that is needed is a material with good quality and have good digestibility and can be obtained at an affordable price. One of the food sources that contain beta-carotene and vitamin A is a reliable chicken egg. Increase the content of beta-carotene and vitamin A in the egg can produce functional eggs that contain antioxidants, as beta-carotene and vitamin A is an antioxidant forming compounds.

The process of egg formation requires feedstuffs which is rich in protein. So far, some ingredients used as source of protein are fish meal, meat meal and soybean meal. These ingredients are imported, leads to the high price of its, including soybean meal. Some efforts are needed to solve this problem and one of these is to look for the alternative potential ingredient to substitute of imported ingredients. One of potential ingredient that can substitute soybean meal is *Indigofera* sp., leaf meal. It is a fodder crop of the tree legume group. Akbarillah *et al.* (2002) reported the nutrient composition of *Indigofera* sp., as follows: 27.97% crude protein, 15.25% crude fiber, calcium 0.22%, phosphorus 0.18% and contains xanthophyll and carotenoid. Carotene content in legume leaves can be relied upon as a good source of carotenoids in the diet of laying hens.

Hens are not able to synthesize colour pigments, but have the ability to transport pigments to the yolk from the

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ingested feed and, therefore, carotenoid profile of yolk reflects carotenoid profile of diets (Karadas *et al*., 2006). Intensity colour of the yolk is very closely related to the high carotene in the laying hen diets. The addition of sources of carotenoids in the diet of laying hens is expected to produce eggs that contain antioxidants, rich in vitamin A and has a high colour score of egg yolks. Vitamin A found in plants in the form of a precursor (provitamin A). Provitamin A consists of alpha, beta and gamma-carotene. Beta-carotene is a yellow pigment and a type of antioxidant that plays an important role in reducing free radical chain reactions in the tissue.

Indigofera sp., crop production capability and its nutrient content of the feed can be used as a source of protein feed ingredients to reduce the use of soybean meal in the diet of laying hens. Then the content of vitamin A and beta-carotene of *Indigofera* sp., top leaf meal can be used as a source of antioxidants for laying hens. Based on the description above, it is necessary to evaluate the use of *Indigofera* sp., leaf meal as feed material that can substitute soybean meal in the laying hen diets to produce functional eggs are high in antioxidants.

MATERIALS AND METHODS

Animal, diet and management: This study used 160 Isa Brown laying hens (30 weeks old). Laying hens housed in battery cages individually measuring 22 x 40 x 40 cm. Each repeat consists of 10 pieces of laying hens housed in group cages. Cages were equipped with dining and drinking, as well as incandescent lamps for lighting at night. Feed ingredients used to formulate of diets consisting of corn, rice bran, fish meal, soybean meal, *Indigofera* sp., leaf meal, $CaCO_3$, coconut oil, premix, DCP and DL-methionine and NaCl. Composition of treatment diets are presented in Table 1. Diets has given 19% crude protein content and metabolizable energy 2900 kcal/kg (Leeson and Summers, 2005).

Prior to blending all feedstuffs, first performed *Indigofera* sp., top leaf meal manufacture, through several stages:

1: Harvesting *Indigofera* sp., which has a stem diameter<0.5 cm
2: Drying *Indigofera* sp., top leaf in the green house to dry so as not to cause changes in the colour's green
3: *Indigofera* sp., top leaf ground up into flour (meal)

Research methods: This study was conducted using Completely Randomized Design (CRD) that consist of 4 treatments diet containing the proportion *Indigofera* sp., top leaf meal to substitute soybean meal in the treatment diets. Each treatment was repeated 4 replications and each replication consisted of 10 laying hens. Treatments in the study as follows:

Table 1: Composition of experiment diets

	(%)			
Feed ingredients	T0	T1	T2	T3
Corn	57	54.3	53.5	52.1
Rice bran	3.5	3.2	1.6	1.3
Fish meal	7.5	8	8.5	8.8
Soybean meal	20	17	14	11
Indigofera sp top leaf meal	0	5.2	10.4	15.6
$CaCO_3$	8	7.75	7.23	6.5
Coconut oil	2.8	3.5	3.7	3.81
Premix	0.47	0.35	0.5	0.29
DCP	0.5	0.4	0.27	0.2
NaCl	0.2	0.2	0.2	0.2
DL-methionine	0.03	0.1	0.1	0.2
Total	100	100	100	100
ME (kcal/kg)	2904	2904	2916	2913
Crude protein (%)	19.02	19.02	19.03	19.02
Fat (%)	5.3	6.1	6.4	6.6
Crude fiber (%)	2.52	2.9	3.17	3.58
Lysine (%)	1.31	1.28	1.32	1.26
Methionine (%)	0.54	0.61	0.66	0.7
Methionine+sistine (%)	0.74	0.77	0.73	0.79
Calcium (%)	3.7	3.9	3.9	3.9
Phosphor (%)	0.54	0.54	0.52	0.52
Natrium (%)	0.19	0.19	0.19	0.19
Chloride (%)	0.02	0.16	0.16	0.16
Vitamin A (IU)	1368	1502	1682	1847
Linoleat (%)	1.28	1.21	1.14	1.09
beta-carotene (mg/g)	0	26.4	52.79	79.19

T0: Control ration (0% *Indigofera* sp., top leaf meal)
T1: Substitution 15% of soybean meal protein with protein *Indigofera* sp., top leaf meal in the diets, which is equivalent to 5.2% of *Indigofera* sp., top leaf meal
T2: Substitution 30% of soybean meal protein with protein *Indigofera* sp., top leaf meal in the diets, equivalent to 10.4% of *Indigofera* sp., top leaf meal
T3: Substitution 45% of soybean meal protein with *Indigofera* sp., top leaf meal protein in the diets, equivalent to 15.6% of *Indigofera* sp., top leaf meal

The composition of feed ingredients and nutrient composition of experiment diets constituent metabolizable energy content during the study are presented in Table 1.

Observation and statistical analysis: The adaptation of feed was 8 days prior of the study. Observations made during 10 weeks. Parameters measured were feed consumption, consumption of nutrients (protein and fat), beta-carotene consumption and consumption of vitamin A, hen day production, egg mass production and feed conversion, followed by measuring the quality of egg yolk, such as egg yolk colour, beta-katoten content on the yolk, the vitamin A content in the yolk, yolk cholesterol and antioxidant activity measured by IC_{50} DPPH (2,2-diphenyl-1-picrylhydrazyl) method with 517 nm wavelength spectrophotometry. The data obtained were analyzed statistically by analysis of variance according to the design (CRD) and the difference between treatments were analysis by Duncan 's Multiple Range Test (Steel and Torrie, 1993).

RESULTS AND DISCUSSION

Treatment effect on feed consumption: The average of feed consumption and the consumption of nutrients and consumption of active compound in the diet of the hen are presented in Table 2.

Feeding *Indigofera* sp., top leaf meal did not influence the feed consumption of the laying hen. Average feed consumption of laying hens during the study ranged from 108.44 to 110.89 g/head/day. Based on the analysis of variance, substitution of protein soybean meal with *Indigofera* sp., top leaf meal in the diet was not significantly different ($p>0.01$) in the feed consumption of laying hens. This is because the ration palatability same treatment has a bunch of the ration. Palatability is determined by the phytochemical content of feed ingredients. Phytochemicals that are found on *Indigofera sp*. top leaf meal very low: 0.29% tannins and saponine 3.64 ppm. so did not affected to the palatability. Tolerance limit of tannins in poultry diets of 2.6 g/kg (Kumar *et al*., 2005). The use of *Indigofera* sp., top leaf meal up to 15.6% in the diets containing tannins of 0.045 g/kg, while the saponins content of 0.57 g/ kg. Tolerance limit of saponins in poultry diets of 0.37% which is equivalent to 3.7 g/kg diet (FAO, 2005). The addition of *Indigofera* sp., top leaf meal in diets containing active substances saponins and tannins did not affect palatability in laying hens.

Feed consumption of each treatment was relatively similar causes increased fat consumption, beta-carotene consumption and vitamin A consumption in laying hens fed *Indigofera* sp., top leaf meal. Increased provision of *Indigofera* sp., top leaf meal in the diets causes an increase in fat consumption, beta-carotene consumption and consumption of vitamin A in laying hens. This is due to the fat content in diets, beta-katoten in diets and vitamin A in diets also increased along with the increase in the provision of *Indigofera* sp., top leaf meal.

Treatment effect on egg production: The average of egg production, egg mass and feed conversion of the hens are presented Table 3.

Average daily egg production (hen day production) respectively for each treatment are listed in Table 3, shows an increase in egg production with increasing *Indigofera* sp., top leaf meal administration in laying hens rations. Results showed that egg production was significantly ($p<0.05$) affected by the use of *Indigofera* sp., top leaf meal in diets. Used *Indigofera* sp., top leaf meal 5.2% in diets significant increased egg production compared to without used *Indigofera* sp., top leaf meal in diets. This is due to the increasing content of beta-carotene in the diet along with increased use of *Indigofera* sp., top leaf meal. Beta-carotene is converted to vitamin A in the body of the chicken. Vitamin A is needed in the process of synthesis of steroid hormones, one of which is for the development of these cells into cells ootid ovum (egg yolk).

Use of *Indigofera* sp.,leaf meal 15.6% in the diets can improve the daily egg production of 3.41% compared to the diet without *Indigofera* sp., top leaf meal. This increase is due to the higher consumption of vitamin A, in the amount of 2011.15 IU. Brody (1993) stated that vitamin A use to maintenance differentiation of epithelial cells and to sustain reproduction.

Treatment effect on egg quality: Average of yolk quality egg like: the yolk colour intensity, beta-carotene content of eggs, vitamin A and cholesterol content of eggs and antioxidant activity is presented in Table 4.

Yolk colour: The average intensity of yolk color in this study ranged from 8.5 to 13.25. Results showed the intensity of yolk color in this study indicate that the use of *Indigofera* sp., top leaf meal in the diets significantly ($p<0.05$) in improving egg yolk colour. Differences in the use of *Indigofera* sp.,top leaf meal in the diets caused an increase in levels of beta-carotene than eggs produced in line with the increase in consumption of beta-carotene. Carotenoids are natural pigments and is widely recognized by its colour, especially yellow, orange and red. One of its kind to benefit the beta-carotene (Gross, 1991). Carotenoids are naturally shaped consisting of 60-90% is trans and 10-30% cis form. Carotenoids are a very effective form of trance as a pigment. Beta-carotene is a carotenoid trans form, so that beta-carotene can fund the yellow pigment in chicken eggs (Hencken and Hoffmann, 1992; Damron *et al*., 1984).

The higher use of *Indigofera* sp., top leaf meal in diets, the bright yellow colour of the eggs produced. The brighter the yolk will be higher physical quality so it is preferred by consumers. Sangeetha and Baskaran, (2010) stated that laying hens can not change all the carotenoids into vitamin A, but will partly be used to enhance the yellow colour of the yolk.

beta-carotene content of egg: The average content of beta-carotene in eggs that were given *Indigofera* sp., top leaf meal ranged between 85.95 to 124.13 mg/100 g. Results showed that beta-carotene content in eggs was significantly affected by the use of *Indigofera* sp., leaf meal ($p<0.05$) in the diets. The higher use of *Indigofera* sp., top leaf meal in the diet of laying hens will further increase the deposit beta-carotene in eggs. This is due to an increase in absorption of beta-carotene contained in *Indigofera* sp., top leaf meal. Bortolotti *et al*. (2003) stated that concentrations of carotenoids in egg yolks were strongly associated with diet and reproduction. Overall, the proportions of the major types of carotenoids in yolks were similar to those in feed.

Table 2: Consumption of feed, nutrients and active compound

Parameters	T0	T1	T2	T3
Feed consumption (g/head/day)	110.28±3.15	108.72±2.69	108.44±6.33	108.89±3.23
Protein consumption (g/head/day)	20.98±0.60	20.68±0.51	20.64±1.20	20.71±0.61
Fat consumption (g/head/day)	5.84±0.17[a]	5.97±0.35[a]	6.94±0.40[b]	7.19±0.21[c]
beta-carotene consumption (g/head/day)	0.00±0.00[a]	2.87±0.7[b]	5.72±0.33[c]	8.62±0.26[d]
Vitamin A consumption (IU/head/day)	1508.63±43.08[a]	1632.97±40.41[b]	1823.96±106.39[c]	2011.15±59.72[d]

[ab]Means within a row with different superscripts different (p<0.05)

T0: control ration (0% *Indigofera* sp., top leaf meal in diets)
T1: 5.2% of *Indigofera* sp., top leaf meal in diets
T2: 10.4% of *Indigofera* sp., top leaf meal in diets
T3: 15.6% of *Indigofera* sp., top leaf meal in diets

Table 3: Data hen day egg production, egg mass production and feed conversion

Parameter	T0	T1	T2	T3
Hen day production(%)	83.63±5.75[a]	93.05±3.06[b]	91.36±3.27[b]	92.65±2.73[b]
Egg mass production(g/bird/day)	49.03±2.15	51.93±0.87	49.46±1.49	49.61±1.39
Feed conversion	2.23±0.03	2.08±0.04	2.20±0.08	2.19±0.10

[ab]Means within a row with different superscripts different (p<0.05)

T0: control ration (0% *Indigofera* sp., top leaf meal in diets)
T1: 5.2% of *Indigofera* sp., top leaf meal in diets
T2: 10.4% of *Indigofera* sp., top leaf meal in diets
T3: 15.6% of *Indigofera* sp., top leaf meal in diets

Increased content of beta-carotene in eggs in this study was 118.88% compared to diet without the use of *Indigofera* sp., top leaf meal. Most abundant carotenoids in dark green vegetables, is one type of hydrocarbon carotenoids. Increase in beta-carotene in the egg look physically on the bright yellow colour of the eggs produced are a reflection of the carotenoid content in feed (Karadas *et al.*, 2006; Na *et al.*, 2004). Liu *et al.* (2012) reported that beta-cryptoxanthin eggs increased 663.64% by administering corn fortified with beta-cryptoxanthin in the diets.

Content of vitamin A in eggs: The average content of vitamin A in eggs that were given to *Indigofera* sp., top leaf meal ranged 2536.16 to 3380.91 mg/100 g. Results showed that the content of vitamin A was significantly affected by the use of *Indigofera* sp., leaf meal ($p<0.05$) in the diets. This is due to the higher content of beta-carotene in the ration which is the contribution of *Indigofera* sp., top leaf meal was 79.19 mg/g. Tee (1992) stated that beta-carotene had 100% activity as provitamin A in the body.

Beta-carotene in *Indigofera* sp., top leaf meal content is a carotenoid naturally shaped. According to Hencken (1992), the natural carotenoids as monohydroxy and monoketocarotenoids compounds are converted to vitamin A. The use of 15.6% of *Indigofera* sp., top leaf meal in the diets may increase vitamin A in eggs reached 47.17%. The results are in line with the findings of Piliang *et al.* (2001), who reported that the higher content of beta-carotene in the diet is getting old/dark yellow eggs produced. Yellow colour of the egg is very closely related to the high content of vitamin A. Yolk color is influenced by the feed. Jiang *et al.* (1994) reported that vitamin A content of eggs increased by 19.83% with the addition of 200 mg beta-carotene/kg diets. Stahl and Sies (2003) stated that carotenoids in animals is not a synthesized in the body, but derived from feed consumed containing carotenoids. Carotenoid synthesis can only occurs in plants.

Cholesterol content of egg: The average of cholesterol content in eggs 172 mg/100 g to 280 mg/yolk. The highest cholesterol content contained in chicken eggs that are not added *Indigofera* sp., top leaf meal in diets, while chicken egg added *Indigofera* sp., top leaf meal in diets lower cholesterol. Using 15.6% of *Indigofera* sp., top leaf meal in the diet had lower cholesterol content of eggs reached 54.13% compared to the diet without the use of *Indigofera* sp., top leaf meal.

Results showed that the cholesterol content of eggs was significantly affected by the use of *Indigofera* sp., leaf meal ($p<0.05$) in the diets caused by beta-carotene contained in *Indigofera sp.* meal can decreased cholesterol. Carotenoids are antioxidants that can prevent oxidation of lipids and carotenoids are able to inhibit the action of the enzyme activity of HMG Co-A reductase mevalonate so formed is not required for the synthesis of cholesterol (Eisenbrand, 2005; Sies *et al.*, 1995).

Antioxidant activity: The average of antioxidant activity in the eggs was 86.19 to 35.78 mg/g. The lower the value of antioxidant activity in the eggs, indicating that the higher the antioxidant content of eggs, meaning that given the antioxidants in egg *Indigofera* sp., leaf meal has a higher ability to counteract free radicals. Based on the analysis of the diversity of usage *Indigofera* sp., leaf meal significantly ($p<0.05$) improved the antioxidant activity of chicken eggs. The lower antioxidant activity in the treatment of *Indigofera* sp., top leaf meal at the level of 10.4 and 15.6% in the diet due to the high content of beta-carotene that accumulates in chicken eggs, which is 124.13 mg/100 g. Beta-carotene is one of the sources carotenoid in *Indigofera* sp., leaf meal. Krinsky and Johnson (2005), stated that carotenoids have provitamin A activity as a function as an antioxidant. Paiva and Russell (1999) reported that beta-carotene and others carotenoids have antioxidant properties *in vitro* and in animal models. Mixtures of carotenoids or associations

Table 4: Yolk colour, content of beta-carotene, vitamin A, cholesterol and Inhibisition concentration

Parameter	T0	T1	T2	T3
Yolk colour	8.5 ± 0.58^{a}	11.5 ± 0.58^{b}	12.15 ± 0.5^{b}	13.25 ± 0.5^{c}
beta-carotene (mg/100g)	56.71 ± 7.67^{a}	85.95 ± 9.55^{b}	109.5 ± 7.41^{c}	124.13 ± 4.59^{d}
Vitamin A (mg/100g)	2297.21 ± 78.4^{a}	2536.16 ± 44.42^{b}	2776.16 ± 37.06^{c}	3380.91 ± 54.31^{d}
Cholesterol (mg/yolk)	375 ± 11.53^{a}	280 ± 47.06^{b}	220 ± 10.6^{c}	172 ± 25.36^{c}
Inhibisition concentration (mg/g)	87.63 ± 4.09^{a}	86.19 ± 3.67^{a}	41.41 ± 9.45^{b}	35.78 ± 3.22^{c}

^{ab}Means within a row with different superscripts different ($p<0.05$)

T0: control ration (0% *Indigofera* sp., top leaf meal in diets)

T1: 5.2% of *Indigofera* sp., top leaf meal in diets

T2: 10.4% of *Indigofera* sp., top leaf meal in diets

T3: 15.6% of *Indigofera* sp., top leaf meal in diets

with others antioxidants (e.g., vitamin E) can increase their activity against free radicals.

Mayes (2002) stated that the role of antioxidants such as beta-carotene, vitamin E, vitamin C, glutathione and other antioxidants is to clean up and reduce oxidants or free radicals. Antioxidants such as carotene is a compound that can give electrons to free radicals or oxidants so radical a stable compound. These results are in line with the findings of Sangeetha and Baskaran (2010) who reported that astaxanthin can be converted into beta-carotene and retinol when it occurs in retinol-deficient mice and can also act as antioxidants in the body. Chidambara *et al.* (2005) reported that the carotenoids found in Dunaliella algae has an antioxidant effect that equal the antioxidants in synthetic beta-carotene.

Conclusion: It is concluded that *Indigofera* sp., top leaf meal can be used up to 15.6% in the diet of laying hens, means equal replace of 45% protein soybean meal in laying hen diets, which can increase 11% egg production. Used *Indigofera* sp., top leaf meal can increase 59.17% the antioxidant content and 47.17% vitamin A mean while reduced yolk cholesterol up 54.13%.

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