

Cholesterol and Malondialdehyde Contents of Broiler-Chicken Meat Supplemented with *Indigofera zollingeriana* Top Leaf Meal

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ABSTRACT

This research aimed to increase functional value of broiler-chicken meat containing high antioxidant and low cholesterol through substitution of soybean meal (SBM) with *Indigofera zollingeriana* top leaf meal (ILM). The experiment used 160 day old broiler chicken (Cobb strain). The experimental chicken were provided dietary treatments when they were 15-days old (initial body weight of 460.5±1.56 g/bird) and terminated on day 35. A completely randomized design (CRD) with four treatments and four replications and ten birds in each replication was used in this experiment. Dietary treatments were: R1= diet containing 20% soybean meal (SBM) without *I. zollingeriana* top leaf meal (ILM); R2= diet containing 16% SBM and 5.9% ILM; R3= diet containing 12% SBM and 11.8% ILM; R4= diet containing 8% SBM and 17.74% ILM. Variables measured were performances (feed consumption, body weight gain, and feed conversion) and the quality of broiler meat (cholesterol, fat content, and malondialdehyde [MDA] concentration). The results showed that supplementation of 17.74% ILM (R4) as the substitution of 60% soybean meal protein produced the same performances of broilers as those of control diet (R1). Supplementation of 11.8% ILM as the substitution of 40% soybean meal protein (R3) decreased meat cholesterol by 34.70%, meat fat content by 52.93%, and MDA concentration by 62.52%. The conclusion of this study was that supplementation of 17.74% ILM produced the same performances as that of control diet, increased antioxidant content of the meat, indicated by a lower MDA concentration, and decreased cholesterol, as well as fat content of broiler-chicken meat.

Key words: broiler performance, cholesterol, Indigofera zollingeriana, malondialdehyde (MDA)

ABSTRAK

Penelitian ini bertujuan untuk meningkatkan nilai fungsional daging ayam broiler yang tinggi antioksidan dan rendah kolesterol melalui substitusi bungkil kedelai dengan tepung pucuk *Indigofera zollingeriana*. Penelitian menggunakan 160 ekor DOC broiler strain Cobb, yang diberi ransum perlakuan mulai umur 15 hari (bobot badan awal 460±1,56 g/ekor) sampai umur 35 hari. Penelitian menggunakan rancangan acak lengkap dengan 4 perlakuan dan 4 ulangan dengan 10 ekor broiler setiap ulangan. Ransum perlakuan yang diberikan adalah: R1= Ransum mengandung 20% bungkil kedelai (BK) tanpa tepung pucuk *I. zollingeriana* (TPI); R2= Ransum mengandung 16% BK dan 5,9% TPI (substitusi 20% protein BK oleh protein TPI); R3= Ransum mengandung 12% BK dan 11,8% TPI (substitusi 40% protein BK oleh protein TPI); R4= Ransum mengandung 8% BK dan 17,74% TPI (substitusi 60% protein BK oleh protein TPI). Peubah yang diamati adalah performa (konsumsi ransum, penambahan bobot badan, dan konversi ransum) dan kualitas daging (kolesterol, kadar lemak, dan konsentrasi malondialdehid [MDA]) ayam broiler. Hasil penelitian menunjukkan bahwa penggunaan TPI sebanyak 17,74% sebagai substitusi 60% bungkil kedelai menghasilkan performa ayam broiler yang sama dengan pemberian ransum kontrol. Penggunaan 11,8% TPI sebagai substitusi 40% protein bungkil kedelai menurunkan kadar kolesterol daging sebanyak 34,70%, kadar lemak daging 52,93%, dan menurunkan MDA daging 62,52%. Kesimpulan hasil penelitian adalah penggunaan 17,74% TPI dalam ransum ayam broiler menghasilkan performa yang sama dengan ransum kontrol dan dapat meningkatkan kandungan antioksidan yang ditandai dengan penurunan konsentrasi MDA, serta menurunkan kolesterol dan lemak pada daging ayam broiler.

Kata kunci: performa broiler, kolesterol, Indigofera zollingeriana, malondialdehid (MDA)

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INTRODUCTION

The effort to meet the requirements of poultry's feed-protein source is still a major problem. Soybean meal has been used as a main source of plant protein for poultry diet, due to the fact that soybean meal contains about 48% protein (Leeson & Summers, 2008). However, since soybean meal is mostly imported and the price is relatively expensive, the use of this feedstuff contributes to high cost of feed component. The utilization of other sources of protein for broiler chickens such as *Jatropha curcas* meal, soybean husk, and shrimp head waste to replace soybean meal has been carried out by Mirzah (2007), Mairizal (2009), and Sumiati *et al.* (2011). However, problems of unstable production, inconsistent quality, anti-nutrition contents, and high cost of processing technology are the main barriers of using this feedstuff. An alternative way that possible to be applied to overcome the problem is to use unconventional protein-rich feedstuff derived from the plant of legume group such as indigofera (*Indigofera zollingeriana*).

Indigofera, categorized as an unconventional feedstuff for poultry, has been reported to have numerous advantages for animal due to its rich content of certain nutrients. Some studies had reported that crude protein content of *I. zollingeriana* top leaf meal (ILM) ranged from 25.50%-28.98% (Hassen *et al.*, 2007; Hassen *et al.*, 2008; Akbarillah *et al.*, 2008; Abdullah, 2010; Abdullah & Suharlina, 2010; Tarigan *et al.*, 2010; Palupi *et al.*, 2014a). Considering its high nutritional content, especially protein, it is greatly possible that *I. zollingeriana* is potential to be used as a protein- contributing feedstuff for broiler chickens. In addition to its protein content, *Indigofera* leaf meal also contains high pigment such as xantofil and carotenoids (Akbarillah *et al.*, 2010). Palupi *et al.* (2014a) reported that β -carotene of *I. zollingeriana* top leaf meal contains as much as 507.6 mg/kg, and the usage of ILM in laying hens diet as much as 15.6% as a substitution of 45% protein of soybean meal increased egg production by 11%, increased the antioxidant content by 59.17%, as well as decreased cholesterol in egg yolks as much as 54.13%.

Carotene content in *Indigofera* can be a good source of carotenoids for broiler chickens. Addition of dietary carotenoids source in broiler chickens is expected to produce meat containing high antioxidant and low cholesterol levels because β -carotene is one of antioxidants and functions as an important role in reducing free radical chain reaction on tissue. Accordingly, feeding this feedstuff to broiler chickens is expected to produce meat with high antioxidants and low in cholesterol levels because β -carotene is one of antioxidant function as an important role in reducing free radical chain reactions on tissue, and carotenoid content significantly inhibited lipid oxidation (Tavares *et al.*, 2011; Kim *et al.*, 2013). The level of cholesterol was decreased by feeding of carotenoids and β -carotene decreased the lipid peroxidation in skin, and the antioxidant activity of β -carotene was stronger (Lee *et al.*, 2010). Carotenoids (β -carotene) are important sources of antioxidant (Bertram & Vine, 2005; Panovskai *et al.*, 2005). The antioxidant activity of this plant was also reported in many publications (Kaur *et*

al., 2006; Ordon *et al.*, 2006; Alviano *et al.*, 2008; Doddola *et al.*, 2008).

The objective of this experiment was to study the effects of *I. zollingeriana* top leaf meal as substitution of soybean meal protein in the diet on cholesterol, fat content, and antioxidant concentration of broiler meat.

MATERIALS AND METHODS

The experiment was assigned in a completely randomized design (CRD) with four treatments and four replications with ten broiler chicken for each replication, and the birds were placed in cage of 1.5 x 1.5 m in size. The experimental animals were 160 day-old Cobb chicks. The birds were fed dietary treatment when they were at day 15 (initial body weight was 450 \pm 1.56 g/bird) and terminated on day 35. Dietary treatments were: R1= diet containing 20% SBM and without ILM (no substitution of SBM protein by ILM protein); R2= diet containing 16% SBM and 5.9% ILM (substitution of 20% SBM protein by ILM protein); R3= diet containing 12% SBM and 11.8% ILM (substitution of 40% SBM protein by ILM protein); R4= diet containing 8% SBM and 17.74% ILM (substitution of 60% SBM protein by ILM protein). The diets were formulated isocalori and isoprotein according to the recommendation of Leeson & Summers (2008) (Table 1). Variables measured were feed consumption, body weight gain, feed conversion, and meat quality (cholesterol, fat content, and malondialdehyde concentration). Sixteen chickens were slaughtered by cervical dislocation. Muscles from right thigh part were isolated and mashed to analyze its cholesterol, fat, and MDA contents. Meat cholesterol content was measured according to Liebermann-Burchard method by using spectrophotometer (Hitachi U-2001, Japan) at 420 nm (Burke *et al.*, 1974), meat fat content was assessed by using AOAC 2005 method, and malondialdehyde concentration was measured based on the method of Rice-Evans & Anthony (1991).

Statistical Analysis

Data were subjected to analysis of variance (ANOVA) by using SPSS V21 IBM program, and the differences among treatment means were distinguished by Duncan's Multiple Range Test (Steel & Torrie, 1995).

RESULTS AND DISCUSSION

Treatment Effect on Broiler Chickens Performance

Growth performances of broilers fed the experimental diets are presented in Table 2. Supplementation of *I. zollingeriana* top leaf meal in the diet of broiler chicken did not significantly affect feed consumption, body weight gain, and feed conversion. Supplementation of broiler diet with *I. zollingeriana* top leaf meal did not affect the feed palatability. It was indicated that *I. zollingeriana* top leaf meal could be given up to 17.74% for 2 to 5-week broilers. The increased β -carotene content of diet supplemented with *I. zollingeriana* top leaf meal was able to increase endurance. Beta-

Table 1. Composition and nutrient content of experimental diet of finisher period (2-5 weeks)

| Feed ingredients (%) | R1 | R2 | R3 | R4 |
|--|--------------|--------------|--------------|--------------|
| Corn | 60.00 | 59.50 | 58.70 | 57.33 |
| Rice bran | 6.05 | 3.65 | 1.60 | 0.00 |
| Corn gluten meal | 4.00 | 4.00 | 4.00 | 4.00 |
| Soybean meal | 20.00 | 16.00 | 12.00 | 8.00 |
| <i>Indigofera zollingeriana</i> top leaf meal (ILM) | 0.00 | 5.90 | 11.80 | 17.74 |
| Fish meal | 5.75 | 6.40 | 7.00 | 7.60 |
| Palm oil | 2.35 | 2.70 | 3.00 | 3.47 |
| Dicalcium phosphate | 0.10 | 0.10 | 0.15 | 0.09 |
| CaCO ₃ | 1.25 | 1.10 | 1.00 | 0.87 |
| NaCl | 0.10 | 0.10 | 0.10 | 0.10 |
| Premix | 0.30 | 0.30 | 0.30 | 0.30 |
| L-Lysine | 0.00 | 0.10 | 0.20 | 0.30 |
| DL-Methionine | 0.10 | 0.15 | 0.15 | 0.20 |
| Total (%) | 100.00 | 100.00 | 100.00 | 100.00 |
| Nutrient content (calculated value / %): | | | | |
| Metabolizable energy (kcal/kg) | 3101.08 | 3105.45 | 3101.2 | 3101.13 |
| Protein | 20.02 | 20.02 | 20.01 | 20.02 |
| Ether extract | 5.25 | 5.52 | 5.74 | 6.13 |
| Crude fiber | 2.98 | 3.36 | 3.78 | 4.23 |
| Lysine | 1.13 | 1.13 | 1.12 | 1.11 |
| Methionine | 0.55 | 0.58 | 0.55 | 0.57 |
| Meth + cystine | 0.88 | 0.88 | 0.82 | 0.82 |
| Calcium | 0.92 | 0.91 | 0.93 | 0.91 |
| Phosphor | 0.45 | 0.46 | 0.47 | 0.46 |
| Sodium | 0.12 | 0.11 | 0.11 | 0.11 |
| Chloride | 0.14 | 0.14 | 0.14 | 0.14 |

Note: R1= diet containing 20% SBM without *I. zollingeriana* top leaf meal (ILM) (no substitution of SBM protein by ILM protein); R2= diet containing 16% SBM and 5.9% of ILM (substitution of 20% SBM protein by ILM protein); R3= diet containing 12% SBM and 11.8% ILM (substitution of 40% SBM protein by ILM protein); R4= diet containing 8% SBM and 17.74% ILM (substitution of 60% SBM protein by ILM protein).

carotene has antioxidant activity that acts to improve the immune system. In addition, β -carotene serves as a barrier to free radicals, so the animal health can be improved and the feed consumption is maintained to support the optimum growth. Carotenoid supplementa-

tion would improve immune condition (Cucco *et al.*, 2007; Lee *et al.*, 2010; Vrolijk *et al.*, 2015). *I. zollingeriana* top leaf meal could be given to laying hens up to 15.6% as a substitution of 45% soybean meal protein without affecting feed consumption (Palupi *et al.*, 2014b). Fresh leaves of *Indigofera* could be given in a diet of duck up to 10% without affecting the performance (Akbarillah *et al.*, 2010).

Feeding *I. zollingeriana* top leaf meal as a feed supplementation did not affect body weight gain of broiler chicken (Table 2). The differences in body weight gain among the treatments were caused by the protein quality of *I. zollingeriana* top leaf meal that is similar to that of soybean meal. Proteins and amino acids that are sufficient in the diet will support optimal growth. The protein quality of *I. zollingeriana* top leaf meal is good, because the content of the Non Protein Nitrogen (NPN) is 1.12%, and amino acid score of *I. zollingeriana* top leaf meal was 24.56 that is similar to amino acid content of soybean meal i.e., 26.50 (Palupi *et al.*, 2014a). It was indicated that *I. zollingeriana* top leaf meal could be used in the diet of broiler chicken up to 17.74% as a substitution of 60% soybean meal protein. Soybean meal and *I. zollingeriana* top leaf meal did not have equivalent protein content, so substitution of soybean meal with *I. zollingeriana* top leaf meal was based on the proportion of soybean meal in the diet. Thus, soybean meal protein could be replaced by the protein of *I. zollingeriana* top leaf meal.

Supplementation of *I. zollingeriana* top leaf meal at the levels of 5.9%, 11.8%, and 17.74% in the diet as substitutions of 20%, 40%, and 60% soybean meal protein, respectively, did not affect feed conversion ratio, compared with control diet (Table 2). Beta-carotene in the *I. zollingeriana* top leaf meal can maintain the health of broiler chickens and feed intake can be maintained to achieve the optimum weight gain. Beta-carotene could maintain rat growth (Siqueira *et al.*, 2007). Therefore, incorporation of dietary antioxidants in poultry feed had been implemented to achieve optimal growth performance, reproduction, and meat quality (Li *et al.*, 2009; Delles *et al.*, 2014). This good effect could be related to the amino acid contents of *I. zollingeriana* top leaf meal that could replace amino acid of soybean meal. The similarity of feed conversion ratio obtained in this study was due to the fact that feed consumption and body weight gain were also similar in all treatments.

Table 2. Feed consumption, body weight gain, and feed conversion of broiler chicken during treatments (2-5 weeks)

| Variables | Treatments | | | |
|-----------------------------|----------------|---------------|---------------|---------------|
| | R1 | R2 | R3 | R4 |
| Feed consumption (g/bird) | 2043.66±108.64 | 2135.29±64.62 | 2147.67±63.08 | 2216.81±76.12 |
| Final body weight (g/bird) | 1379.50± 86.60 | 1383.61±68.17 | 1382.50±72.14 | 1382.83±45.54 |
| Body weight gain (g/bird) | 917.50± 86.89 | 923.61±68.17 | 922.50±72.14 | 922.83±45.82 |
| Feed conversion ratio (FCR) | 2.25± 0.28 | 2.32± 0.20 | 2.34± 0.20 | 2.40± 0.10 |

Note: R1= diet containing 20% SBM without *I. zollingeriana* top leaf meal (ILM) (no substitution of SBM protein by ILM protein); R2= diet containing 16% SBM and 5.9% of ILM (substitution of 20% SBM protein by ILM protein); R3= diet containing 12% SBM and 11.8% ILM (substitution of 40% SBM protein by ILM protein); R4= diet containing 8% SBM and 17.74% ILM (substitution of 60% SBM protein by ILM protein).

Meat Quality of Broiler Chicken

The averages of cholesterol, fat content, and malondialdehyde concentrations of broiler-chicken meat are presented in Table 3. Substitution of soybean-meal protein with *I. zollingeriana* top leaf meal protein significantly ($P<0.05$) decreased meat cholesterol and fat content, compared with R1. Supplementation of *I. zollingeriana* top leaf meal at the levels of 11.8% (R3) and 17.74% (R4) into the diet significantly decreased malondialdehyde concentration as compared with R1 and R2. Supplementation of 11.8% *I. zollingeriana* top leaf meal as a substitution of 40% soybean meal protein reduced meat cholesterol by 34.70% (158.31 ± 17.08 mg/100g to 103.38 ± 25.80 mg/100g), meat fat content by 52.92% ($9.31\pm 0.65\%$ to $4.38\pm 1.18\%$), and meat MDA concentration by 62.52% (7.13 ± 0.66 μ g/g to 2.67 ± 0.62 μ g/g). The present result was in accordance with the report of Palupi *et al.* (2014b) that feeding of 15.6% *I. zollingeriana* top leaf meal as a substitution of 45% soybean meal protein in laying hens decreased cholesterol content by 54.13% and increased egg antioxidant content up to 59.17%.

The decreased of meat cholesterol and fat content was caused by β -carotene content of *I. zollingeriana*. Cholesterol biosynthesis from acetyl CoA requires the presence of HMG-CoA reductase (hydroxymethyl glutaryl-CoA) enzyme to form mevalonate. The activity of the HMG-CoA reductase will be disturbed by β -carotene contained in *I. zollingeriana* top leaf meal. Beta carotene content of *I. zollingeriana* top leaf meal was 506.7 mg/kg (Palupi *et al.*, 2014a). Carotenoid (β -carotene) contained in *I. zollingeriana* top leaf meal has an effect to reduce cholesterol in blood serum through inhibiting micelle formation in small intestine so that decreases intestinal cholesterol absorption. 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 to form mevalonate that is required for the synthesis of cholesterol (Eisenbrand, 2005). The high content of β -carotene in feedstuffs consumed decreases cholesterol content of carcass (Lee *et al.*, 2010; Syahrudin *et al.*, 2011). Reduced levels of cholesterol resulted in the decreased transport of cholesterol to tissues so that the fat content of meat is reduced. Considering that β -carotene could inhibit enzyme HMG-CoA in the formation of cholesterol, this compound could reduce the amount of fat attached to the arterial wall. Decreased amount of fat in the arteries caused the reduced fat

transport to the meat (Fki *et al.*, 2005).

Beta-carotene is an active compound that lowers blood lipid and β -carotene could reduce MDA concentration in broiler meat. Lower cholesterol and fat content resulted in lower lipid peroxidation, so the meat MDA levels also decreased. The lower meat MDA concentration indicated that meat of broiler chicken contains high antioxidant. The higher level of *I. zollingeriana* top leaf meal supplementation in broiler chicken diet would further increase deposition of β -carotene in the meat. This was due to an increase in absorption of β -carotene derived from *I. zollingeriana* top leaf meal. Xanthophyll and β -carotene are the sources of antioxidant that could increase the yolk pigmentation and improve the health of chicken (Lee *et al.*, 2010). Beta-carotene is a natural antioxidant that can protect the cellular component from the biological damage caused by Reactive Oxygen Species (ROS). High concentrations of antioxidants have been demonstrated to reduce lipid peroxidation in chicken's muscle (Jiang *et al.*, 2007).

Malondialdehyde is one of the most frequently used indicators of lipid peroxidation associated with oxidative stress (Aksu *et al.*, 2010). Lipid oxidation is one of the main factors limiting the quality and acceptability of meats and meat products (Jiang *et al.*, 2007). Free radical and lipid peroxidation have been implicated in the deterioration of many physiological functions including growth, reproduction, and immunity (Gladine *et al.*, 2007). The decreased meat MDA concentration could be due to the ability of β -carotene to affect antioxidant defense system. In the present study, the meat MDA concentration showed a significant decrease with the supplementation of 11.8% and 17.74% ILM in the diets. The mechanism of beta-carotene to counteract with free radicals is occurred via electron (Dutta *et al.*, 2005). Antioxidant supplementation improved breast quality of broiler chicken (Tavarez *et al.*, 2011). Dietary antioxidants improved meat tenderness and reduced lipid oxidation in broiler breast and thigh meat (Delles *et al.*, 2014).

CONCLUSION

Feeding 17.74% ILM as the substitution of 60% soybean meal protein in broiler produced the same performances as that of control diet, but reduced the MDA concentration, cholesterol, and fat content of broiler-chicken meat.

Table 3. Cholesterol and fat contents, and malondialdehyde (MDA) concentration of broiler-chicken meat

| Variables | Treatments | | | |
|------------------------------|---------------------------|---------------------------|---------------------------|---------------------------|
| | R1 | R2 | R3 | R4 |
| Cholesterol (mg/100g) | 158.31±17.08 ^a | 106.92±19.38 ^b | 103.38±25.80 ^b | 111.61±20.52 ^b |
| Fat (%) | 9.31± 0.65 ^a | 6.01± 1.05 ^b | 4.38± 1.18 ^c | 4.53± 0.93 ^{bc} |
| Malondialdehyde (μ g/g) | 7.13± 0.66 ^a | 6.95± 0.64 ^a | 2.67± 0.62 ^b | 4.56± 0.60 ^c |

Note: Means in the same row with different superscripts differ significantly ($P<0.05$). R1= diet containing 20% SBM without *I. zollingeriana* top leaf meal (ILM) (no substitution of SBM protein by ILM protein); R2= diet containing 16% SBM and 5.9% of ILM (substitution of 20% SBM protein by ILM protein); R3= diet containing 12% SBM and 11.8% ILM (substitution of 40% SBM protein by ILM protein); R4= diet containing 8% SBM and 17.74% ILM (substitution of 60% SBM protein by ILM protein).

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