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Producing chicken eggs containing isoflavone as functional food due to feeding effect of soy sauce by-product

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Abstract. The present study was aimed to verify the impact of feeding soy sauce by-product in producing isoflavone-enriched chicken eggs as functional food. Experiment used 200 laying hens of 80-week old with average body weight of $1,932.75 \pm 189.50$ g. Experimental diets were formulated using yellow corn, rice bran, soybean meal, fish meal, meat bone meal, poultry meal, premix, CaCO_3 , and soy sauce by-product (SSBP). A completely randomized design with 4 treatments and 5 replication (10 birds each), was assigned in this experiment. Inclusion levels of SSBP were the treatments, namely, none (T_0), 10 (T_1), 12.5 (T_2), and 15.0% (T_3). Parameters observed were colour, index, and weight of egg yolk, and isoflavone content. Analysis of variance was applied and continued to Duncan test at 5% probability. Results indicated that yolk colour index and weight were not affected by the treatments, but isoflavone content was significantly ($P < 0.05$) increased by feeding SSBP. Egg yolk isoflavone in T_2 (0.41 mg/g) and T_3 (0.47 mg/g) were higher than those in T_0 (0.31 mg/g) and T_1 (0.35 mg/g). In conclusion, dietary inclusion of soy sauce by-product at higher level (12.5 and 15.0%) can produce isoflavone-enriched eggs as functional food.

Keywords: laying hen, soy sauce by-product, isoflavon, egg yolk

1. Introduction

Eggs are very nutritious foodstuff and function as a source of protein, thus they play an important role in basic nutrition. Also, due to the high nutrients availability and digestibility with achievable price, eggs become a common food ingredient consumed by all levels community all over the world. However, eggs are conventionally associated with negative effects on human health due to their cholesterol content. Although eggs are known to have complete and high nutrients content, but they also contain high cholesterol level, especially in the egg yolk. Cholesterol content in the egg may varied depending on the strain of the hens and feed ingredients composition provided. Previous researcher [1] reported that one egg contains about 213 mg, but other result [2] indicated higher level was about 410 mg, the quite high amount. Considering cholesterol content in the eggs is strongly influenced by feed ingredients given to laying hens [3], thus feed modification is one of many other ways that can be applied. Exploring the possibilities of the development of functional eggs containing low cholesterol by technological methods have been proposed [2]. In the present study, the effort had been explored with special reference to feeding manipulation in laying poultry through dietary inclusion of isoflavones-containing feedstuff.

Feed ingredients containing active compound such as isoflavones can be greatly possible in lowering egg's cholesterol levels [4]. Isoflavones can be found in high concentrations in soybean and soybean products. Soybean or its by-product such as sauce by-product (SSBP) is one feed ingredient containing high enough isoflavones and also protein of about 13.68 mg/g and 27%, respectively [5]. Isoflavones derived from soy product passed the intestinal lumen and mainly accumulated into egg

in the form of daidzein [6]. Isoflavones in soy products function as antioxidant and phytoestrogenic substances (phytoestrogen/phytosterol) which were known to decrease the formation of very low density lipoprotein and to increase high density lipoprotein [5,7].

Isoflavones are plant sterols (phytosterols) that can inhibit dietary cholesterol absorption and also decrease liver fat metabolism. Isoflavones derived from soybean or soy products are structurally and functionally similar to natural estrogens and can bind to estrogen receptors and may exert antiestrogenic effect. It was very logical that isoflavones exert a depressing effect on lipid profiles of the product due to their metabolic function and physiological effects as described above. Isoflavones also known to play the role of anti-cholesterol which can lowered blood cholesterol, decreased, low density lipoprotein, triglycerides, and increased high density lipoprotein. This phenomenon was supported by the finding that the content of yolk cholesterol reduced from 13.9 to 11.0 g/mg in Isa Brown laying hens fed the increasing levels (100 up to 300 mg/kg) isoflavones daidzein and genistein [8]. It was further proved in hens fed alfalfa sprout containing high antioxidants and phytosterols of 15 to 50 times higher than control, produced eggs with much higher isoflavone daidzein (15.7 vs. 5.28 mg/100g) and decreased egg yolk cholesterol from 11.8 to 10.4 mg/g [9]. As a comparison, previously reported that dietary supplementation of genistein, a compound of isoflavones, at a level of 800 mg/kg improved performance and egg quality indicated by the increased egg yolk genistein deposition and decreased egg yolk malondialdehyde in quail [10].

Low level of cholesterol-containing eggs would be categorized as functional food to ensure the believe and confidence of the consumers. In recent decades, there has been an increasing demand for functional foods, especially eggs, which is possibly to be a main attractive foodstuff protein sources in the future. Egg yolk enrichment with isoflavones provided egg to be a functional food that could potentially benefit human health [10]. Whether dietary fortification with isoflavone derived from SSBP in laying chicken can be transformed and deposited into the yolk has not been clearly verified yet. Therefore, the present research was conducted in order to clarify the beneficial effect of isoflavones on egg quality, based on egg yolk characteristics and isoflavone concentration, in 80-week laying hens fed soy sauce by-product (SSBP).

2. Materials and methods

2.1. Experimental animal and feed

The experimental animals of the present study were 200 birds of 80-week old laying chickens of Lohm strain with initial body weight of 2.02 ± 1.17 kg. The experimental rations were formulated using yellow corn, rice bran, soybean meal, fish meal, meat bone meal, poultry meat meal, premix and soy sauce by-product (SSBP), and completed with lysine and methionin. Composition and nutritional content of the experimental ration after the inclusion level of SSBP (Table 1) were created as treatments, namely none (T_0), 10% (T_1), 12.5% (T_2), and 15% (T_3).

2.2. Procedures and parameter

Dietary treatments were started to be fed when the birds were 560 day old and terminated on day 595. The birds were provided free access to feed and drinking water. The SSBP was obtained from the company of Mirama soy sauce producer in Semarang, Central Java. Desalted processing was performed [11] to minimize salt residue of the soy sauce prior to mixing it with other ingredients for feed formulation (Illustration 1). Parameters observed were colour, weight and index of egg yolks, and isoflavone content in egg yolk. Determination of isoflavones content in egg yolk was performed [12] with slight modification.

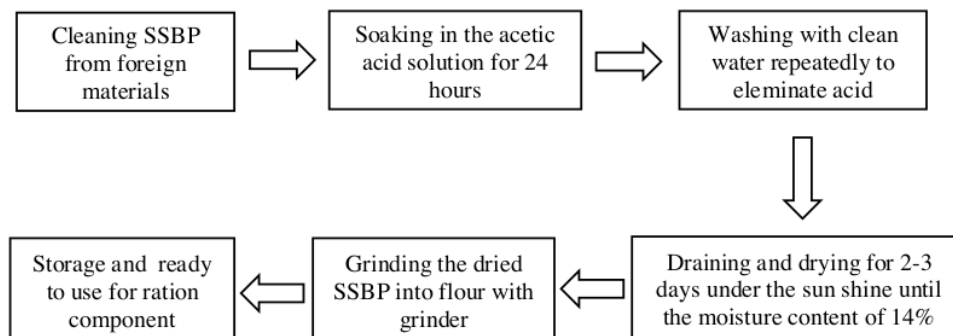


Figure 1. Flow chart of soy sauce by-product (SSBP) meal processing procedure

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2.3. Experimental design and statistical analysis

Experiment was conducted using completely randomized design (CRD) with 4 treatments and 5 replications and each replication consisted of 10 birds of laying hens. Dietary treatments tested were as follows:

- T₀: ration without soy sauce by-product
- T₁: ration with soy sauce by-product 10%
- T₂: ration with soy sauce by-product 12.5%
- T₃: ration with soy sauce by-product 15%

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Data of all parameters were statistically evaluated based on analysis of variance and continued to Duncan test at 5% probability level [13].

3. Results and discussion

Color, weight, and index of egg yolks were not affected by feeding soy sauce by-product (SSBP) at whatever levels (Table 2). However, dietary inclusion of soy sauce at higher levels either at 12.5 or 15% significantly ($P < 0.05$) increased isoflavone content of egg yolk. The extent of egg yolk color is commonly correlated with the ingredients of the diet provided especially those containing carotenoids and/ or xanthophylls.

3.1. Egg yolk color

Feed formulation used in the present study composed of yellow corn in equal amount (Table 1), this means that the contribution containing carotenoids and/or xanthophylls also at the same level. Since feed consumption was not affected by the dietary inclusion of soy sauce (unpublished data), and soy sauce does not contain xanthophyll, thus the same intake of xanthophyll had no different effect on egg yolk color. However, the present result was quite different from that of laying hens given additional paprika extract increased yolk color by 20.4% [14], and those fed red pepper either normally processed or fine powder produced higher yolk color by 57.7 and 70.5%, respectively [15]. It has been discussed previously that the difference in yolk color was due to the content of additional ingredient fed to the chicken with special reference to carotenoids and/or xanthophylls. The intensity of yolk color is influenced by the presence and role of xanthophyll, a pigment that could be transferred from the feed. Egg yolk color is a quality indicator that can be altered by manipulating hen's diet. It is well

documented that the color of yolk is dependent on the amount of xanthophylls, fat soluble pigments, in hen's diet, but this capacity mechanism was not different in the present study since dietary fat content was the same (Table 1).

Table 1. Composition and nutrient content of experimental ration

Ingredient	Dietary treatment			
	T ₀	T ₁	T ₂	T ₃
	----- % -----			
Yellow corn	55.00	55.00	55.00	55.00
Rice bran	15.00	12.00	11.00	10.00
Soybean meal	16.00	12.00	10.50	9.00
Soy sauce by-product	0.00	10.19	12.50	15.00
Fish meal	4.00	2.00	2.00	2.00
Meat bone meal	3.00	2.00	2.00	2.00
Poultry meat meal	3.00	2.00	2.00	2.00
Lysine	0.10	0.10	0.10	0.10
Methionine	0.10	0.10	0.10	0.10
CaCO ₃	3.80	3.80	3.80	3.80
Premix	1.00	1.00	1.00	1.00
Total	100.00	100.00	100.00	100.00
Nutritional content ^a (%)				
Metabolisable energy				
(Kcal/kg) ^b	2,837.38	2,895.63	2,909.56	2,923.48
Crude protein	19.17	19.08	19.03	18.98
Crude fiber	5.95	6.16	6.16	6.17
Ether extract	5.26	6.29	6.57	6.86
Calcium	4.15	4.03	4.03	4.04
Phosphorus	1.47	1.16	1.09	1.03
Lysine ^c	1.09	0.96	0.94	0.92
Methionine ^c	0.41	0.50	0.53	0.57
Feed cost (IDR/kg)	5,664.00	5,081.00	4,964.50	4,848.00

^a Determined value based on proximate analysis¹⁶ of all ingredients used in feed formulation

^b Calculated value based on Balton formula (EM (kcal/kg) = 40.81 [0.87 (PK + 2.25 x LK + BETN) + k].[19]

^c Calculated value based on Berdasarkan Tabel Kandungan Nutrisi Bahan Pakan [20]

3.2. Egg yolk weight and index

Since feed consumption was the same, as it has been described in the previous paragraph, so that protein intake was also not different. Protein is a basic constituent of yolk, thus, the same protein intake contributing the impact to produce relatively similar egg yolk weight. This is in accordance with the result reported previously [16] that the yolk index was unchanged with the same yellow color score. It appears that the egg yolks are predominantly composed of proteins transferred from the feed, so the same feed protein (Table 1) produces the same weight of the egg yolk, which was supported by the fact that fat in egg did not affected by dietary inclusion of isoflavones [8]. Fat in egg yolk varied between 4.25 and 4.30 g/yolk in isoflavones treated groups compared to 4.27 g/yolk in control group. Therefore, it is clear that yolk weight is closely related to the dietary protein supply because isoflavones are much more effective when it bound to protein rather than fat. The size of the egg was strongly influenced by protein and amino acids in the diet, and 50% of egg dry weight is protein [17]. Previously has been reported [9] that yolk weight (between 17.0 and 17.3 g) was not affected by

enriched sprout containing daidzein in white Leghorn hens, and this is the same as found in the present study in which yolk weight was between 16.9 and 17.0 g (Table 2). No difference in yolk weight supports the unchangeable in egg yolk index due to feeding soy sauce by-product. When either egg weight in a whole or yolk weight are the same, it can be a certain of providing similar egg yolk index.

Table 2. Colour, weight, and index of egg yolks, and isoflavone content in egg yolk due to feeding soy sauce by-product (SSBP)

Parameter	Dietary treatment			
	T ₀	T ₁	T ₂	T ₃
Egg yolk colour	7.88	7.13	7.40	7.30
Egg yolk weight (g)	16.90	16.68	16.58	17.00
Egg yolk index	4.23	4.14	4.32	4.30
Isoflavone in egg yolk (mg/g)	0.31 ^b	0.35 ^b	0.41 ^a	0.47 ^a

^{a-b}Mean value in the same raw followed by different superscript differ significantly (P<0.05)

3.3. Isoflavones in egg yolk

The interesting phenomenon is that feeding soy sauce by-product at the levels 12.5% (T₂) and 15% (T₃) significantly increased isoflavones deposited in the egg yolk although they were not followed by the change in egg yolk color and weight (Table 2). It is assumed that the higher level of soy sauce was given, the much more amount of isoflavones could be consumed although with similar feed consumption. Therefore, the more isoflavones derived from soy sauce by-product consumed, the greater possibility of being transferred into the egg yolk as found in the present study. The mechanism of isoflavones transportation into the egg yolk have been partly clarified whether they changed to a conjugated form or not [12]. Isoflavones was known to reduce to 30% during their metabolic fate, suggesting that 70% of the isoflavones existed in the egg yolk in the form of conjugated. Thus, a brief conclusion can be formulated for the treatments of T₂ and T₃ that isoflavones changed to a conjugated form, a soluble isoflavones, in order to make it to be easier for the transfer into the egg yolk. Dietary supplementation of isoflavone genestein in quail increased the content of isoflavones in quail yolk [10, 18] because isoflavones from feed can be transferred to the yolk [6]. Biotransformation of isoflavones indicating the transfer from feed to blood and finally into egg yolk as indicated in the Figure 2 [21].

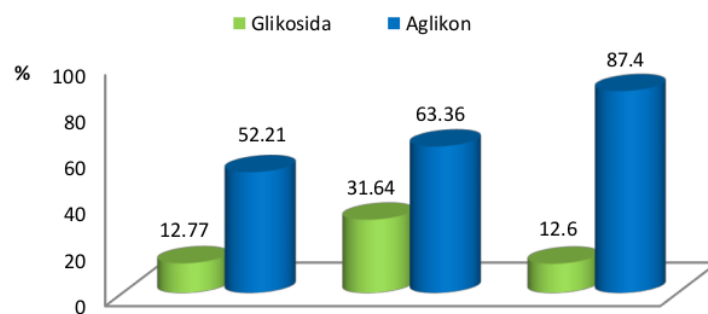


Figure 2 Comparison of isoflavones in feed, blood and egg yolk (*Japanese Poultry Science, in progress*) [21]

4. Conclusion

Dietary inclusion of soy sauce by-product meal containing isoflavones at higher levels of either 12.5% (T₂) or 15% (T₃) increase the content of isoflavones in egg yolk, and the eggs are possible to become functional food.

5. Implication

Isoflavones-enriched yolk ensures the egg can be a source of high quality food for people, and giving rise to the possibilities for production of functional eggs.

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