The quality of Isa-Brown chicken eggs given isoflavone from soy sauce pulp in feed

W Pancapalaga¹, A Malik¹ and R Wijaya²

¹Department of Animal Husbandry, University of Muhammadiyah Malang, Malang 65144
²Department of Management, University of Muhammadiyah Malang, Malang 65144 Jl. Raya Tlogomas 246, Kampus III UMM, 65151, Indonesia

E-mail: pancapalaga1966@gmail.com

Abstract. This study aims to examine the effect of giving isoflavones from soy sauce pulp in feed to the Haugh Unit value, yolk index, yolk color, and yolk cholesterol level. 100 laying of Isa-Brown hens aged 32 weeks were included in this study. The experimental design used was Complete Random Design involving 4 levels treatments repeated 5 times namely T0 (control feed without giving isoflavones from soy sauce), T1 (feed with 4% isoflavones from soy sauce), T2 (feed with 8% isoflavones from soy sauce), and T3 (feed with 12% isoflavones from soy sauce). The treatments were given for 10 weeks. Then, the quality of eggs was observed from their Haugh Unit (HU) value, egg yolk index, egg yolk color, and egg yolk cholesterol level. The obtained data were analyzed using variance analysis. The results indicate that the administration of isoflavones from soy sauce in the feed had no significant effect (P>0.05) on HU value, egg yolk index, and egg yolk color. However, it had a significant effect (P<0.05) on the egg yolk cholesterol level. Thus, the administration of isoflavones from fermented soybean as much as 80mg/100g (equivalent to the addition of 12%) in laying hens feed can maintain the HU value, yolk index, and yolk color as well as reduce egg yolk cholesterol level.

1. Introduction
Isoflavones are additive compounds that work to increase the productivity and quality of laying hens. Isoflavones function as pitoestrogenic, which has a role as a substitute for estrogen which functions as a trigger for the development of follicles produced by the ovaries.

Mainly, isoflavones exist in vegetables, fruits, grains, and nuts, especially in soybeans [1]. Isoflavones are found in soy protein and processed products made from soybeans in the form of daidzein, genistein, and glycitein [2]. Furthermore, it is said that consumption of soy isoflavones can reduce cardiovascular risk, due to the low lipid in the blood for its nature as a source of antioxidants [1].

One of the processed soy products commonly consumed by humans is soy sauce. Whereas soy sauce pulp is a waste in the form of solid filtering and pressing results from the process of making soy sauce that can be used as additional animal feed. The provision of isoflavones from soy sauce pulp in poultry feed for quail caused a significant decrease in total lipids, cholesterol, and triglycerides in blood [3]. Meanwhile, a study by Malik et al (2019) found that giving soy sauce pulp in laying hens by 80/100 mg can reduce the LDL ratio and increase the HDL of oxy and SOD of chicken blood [4]. Provision of 10% soy sauce pulp in duck feed significantly increased levels of isoflavone, LDL, and HDL of duck yolk...
Isoflavones provide a powerful antioxidant effect in preventing lipid peroxidation to reduce the rate of LDL [6].

Soy sauce pulp has 27% crude protein content, 19% ash, 0.39 calcium, 0.33% phosphorus, 12% crude fat, and 11% crude fiber [7]. If it is used as a feed ingredient to prepare feeds for laying hens, it is expected that it might maintain egg quality.

Therefore, this study aims to examine the effect of giving isoflavones from soy sauce in Isa-Brown laying hens feed on egg quality.

2. Method

This study involved 100 laying hens, layers of Isa-Brown strains hens, aged 32 weeks. Feed treatment was given for 12 weeks, starting from the age of 20 to 32 weeks. The feed was given an average of 110 grams, with drinking water ad libitum, lighting for 18 hours. This study was conducted in the enclosure closed house UMM PUPPIK Experimental Farm, Universitas Muhammadiyah Malang. The feed ingredients for the study were as follows: fish meal, meat meal, corn gluten meal (CGM) distillers dried grains with solubles (DDGS), yellow corn, rice bran, fish oil, clam shell, lysine, methionine, and isoflavones from fermented soybean. The experimental method with a completely randomized design was employed in this study. The treatments were divided into 4 levels repeated 5 times namely T0 (control feed without giving isoflavones from soy sauce), T1 (feed with 4% isoflavones from soy sauce), T2 (feed with 8% isoflavones from soy sauce), and T3 (feed with 12% isoflavones from soy sauce). The composition and nutrient content of the treatment ration is presented in table 1.

| Feed ingredients | T0(0%)* | T1(4%) | T2(8%) | T3(12%) |
|------------------|---------|--------|--------|---------|
| Rice bran        | 15.65   | 15.80  | 15.40  | 15.50   |
| Yellow corn      | 58.5    | 57.6   | 55.4   | 55.2    |
| Fish oil         | 0.20    | 0.90   | 0.90   | 0.90    |
| DDGS             | 5.65    | 3.10   | 3.50   | 2.00    |
| CGM              | 4.40    | 4.00   | 3.10   | 2.00    |
| Meat meal        | 2.50    | 2.00   | 1.00   | 0.70    |
| Fish flour       | 10      | 10     | 10     | 10      |
| Clamshell        | 2.90    | 2.40   | 2.50   | 1.50    |
| Lysine           | 0.10    | 0.10   | 0.10   | 0.10    |
| Methionine       | 0.10    | 0.10   | 0.10   | 0.10    |
| Isoflavones from fermented soybean | 0 | 4 | 8 | 12 |
| Total            | 100     | 100    | 100    | 100     |
| Nutrition content ** |
| Crude protein (%)| 17.03   | 17.02  | 17.05  | 17.04   |
| Crude fat (%)    | 4.09    | 4.59   | 4.54   | 4.47    |
| Crude fiber (%)  | 2.71    | 2.85   | 2.93   | 3.07    |
| Ca (%)           | 1.80    | 1.60   | 1.59   | 1.22    |
| P (%)            | 0.52    | 0.51   | 0.52   | 0.51    |
| Na (%)           | 0.05    | 0.05   | 0.05   | 0.05    |
| Energy metabolism (Kcal/kg) | 2,860.39 | 3,000.00 | 3,020.75 | 3,002.00 |

*without giving isoflavones from soy sauce pulp.

**Laboratory analysis results of the nutritional content of feed ingredients for each treatment

2.1. Research procedure

The procedure that was carried out in this study was egg collection after the treatment of soy sauce pulp feed for 12 weeks in the UMM PUPPIK experimental farm. The egg quality was measured at the Nutrition Laboratory, Faculty of Agriculture, Animal Husbandry, Universitas Muhammadiyah Malang. Eggs were given a number mark using permanent markers to facilitate the research process. All eggs were weighed one by one using a 10–1 gram digital scale to determine egg weight. After all the eggs...
had been weighed, the eggs were put one by one on the glass surface. Their height and diameter of the thick egg white and the height and diameter of the yolk were measured using a caliper. Then, the color of the yolk was seen and the score was determined using the egg yolk color fan instrument. The obtained data from observations were recorded for further data processing.

2.2. Observed parameters

2.2.1. Haugh unit (HU) value. Haugh unit is a unit of value from the results of egg white quality measurements, by calculating logarithmically the height of the thick white egg and then transformed into the correction value of the egg weight function. The formula used to determine the HU value was [8,9]:

\[
HU = 100 \log(h + 7.57 - 1.7W^{0.37})
\]

2.2.2. Egg yolk index. The yolk index was measured by comparing the height (h) and the yolk diameter (w). Measurements were made using calipers. The formula used to calculate the yolk index value according to Genchev et al (2008) was as follows [8]:

\[
IY = h/w
\]

2.2.3. Egg yolk color score. The yolk color score was observed by breaking the egg and comparing the yolk color with the egg yolk color fan instrument. The range of egg yolk in the egg yolk color fan was a scale of 1–15, from pale yellow to deep orange. The color of yolk close to one of the colors in the instruments is the number of the yolk color score.

2.2.4. Egg yolk cholesterol level. The total cholesterol level was analyzed according to the method of Kleiner and Dotti [10].

2.3. Statistical analysis

The collected data were analyzed using analysis of variance (ANOVA) and F-test at 5% level and continued with the Least Significant Difference [11].

3. Result and discussion

Data from the results of the average Haugh Unit, yolk index, yolk color, cholesterol, and triglycerides level in egg yolk are presented in table 2.

| Variable                      | T0(0%)     | T1(4%)     | T2(8%)     | T3(12%)    |
|-------------------------------|------------|------------|------------|------------|
| Egg yolk index                | 0.43±0.12  | 0.42±0.23  | 0.41±0.21  | 0.42±0.15  |
| Egg yolk color                | 8.58±0.16  | 8.43±0.02  | 8.40±0.14  | 8.30±0.21  |
| Haugh unit                    | 74.30±13.40| 73.50±10.30| 74.50±11.20| 74.90±11.10|
| Total yolk cholesterol level  | 11.60±0.50a| 9.70±0.40b | 8.60±0.60c | 8.30±0.60c |

\(a, b, c\) Different superscript letters on the same row show significant differences (P<0.05).
3.1. Egg yolk index
Table 2 shows that giving isoflavone from soy sauce pulp had no significant effect on the yolk index. However, the value of the yolk index studied was still within the standard limit of 0.41–0.43. This is following Réhault-Godbert et al (2019) which states that the value of the yolk index ranges from 0.33 to 0.52 [12]. The index of the yolk depends on the size of the yolk. The egg yolk index is an index of freshness quality measured from the height and diameter of the yolk [13]. Data on the total weight of eggs produced in this study were not significantly different, allowing the yolk produced to be the same both height and color. This might be caused by the consumption of protein in the same feed (average 17% in Table 1). The only difference is the composition of the use of soybean pulp flour. Protein is a constituent of solids that produce the same egg yolk. Thus, the same consumption will produce relatively the same egg yolk. This is consistent with the argument of Yousef (2004) who stated that the egg yolk index is relatively the same as the feed protein content is relatively the same [1].

3.2. Egg yolk color
Table 2 shows that giving isoflavones from soy sauce pulp had no significant effect on egg yolk color. The yolk color of the results of the study ranged from 8.30 to 8.58. Meanwhile, the value of the yolk color that was much sought after by the community ranged from 9 to 11 on the Roche scale [14]. The provision of soy sauce pulp did not affect the color of the egg yolk for the color of egg yolk was influenced by the level of xanthophyll in the feed. Soy sauce pulp in the feed less contain xanthophyll enabling the yellow color produced by egg yolk can be obtained from other feed ingredients such as yellow corn, soybean meal, seaweed, and fish meal. This is under the opinion of Sikder et al (1998) which stated that the color of the yolk is produced by xanthophyll pigments obtained from feeds consumed by livestock such as yellow corn, which is an energy source and pigment supplier [15]. Egg yolk pigments are usually owned by yellow feed ingredients such as yellow corn. The difference in the percentage of feed ingredients used in each treatment can affect the xanthophyll level in the feed, which will ultimately affect the yolk color produced.

3.3. Haugh unit
Table 2 proves that the administration of soybean pulp isoflavone had no significant effect on haugh unit eggs. The Haugh unit value is one of the criteria for determining the quality of an inner egg by measuring egg albumen height and egg weight, which might be a correlation between egg weight and albumen height. A high haugh unit value indicates that the viscosity of the albumen is getting thicker. Albumen contains ovomucin which plays a role in binding water to form albumen gels. Albumen is thicker if the nodes of the ovomucin are numerous and strong with high albumen viscosity. Albumen protein consisted of fiber proteins namely ovomucin and the batter the inferior quality of the egg. The haugh value of this research unit ranges from 74.30±13.40 to 74.90±11.10. This value is relatively good but the administration of isoflavones from soy sauce residues has not affected the process of egg albumen formation as a result of the HU value remains unchanged. Ovomucin plays an important role in the binding of water to form the albumen gel structure [16]. Thus, if the nodes of the ovomucine are many and strong. Thus, the albumen might be thicker indicating high albumen viscosity which affects the HUvalue.

3.4. Cholesterol
Table 2 shows that the administration of isoflavones from soy sauce pulp had a significant effect on egg yolk cholesterol levels. Cholesterol level decreased with the administration of isoflavones from soy pulp. According to Izumi (2000) soy sauce pulp can reduce egg cholesterol isoflavones for soybean contains plant-derived sterols (phytosterols) [17]. If those active substances consumed by ducks, it will inhibit the absorption of bad cholesterol from food and the body (liver). Thus, the absorption of cholesterol in the intestine can be reduced causing the cholesterol deposited into the egg to become less.
4. Conclusion

Provision of isoflavones from soy sauce as much as 80 mg/100g (equivalent to the addition of 12%) in laying hen feed can reduce egg yolk cholesterol level. However, it still maintains a haugh unit value, egg yolk index, and egg yolk color. Thus, giving isoflavones from soy sauce pulp produces eggs that are low in cholesterol.

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