



Effects of Dietary Fermented Soy Isoflavones on Egg Quality of Laying Hens

Wehandaka Pancapalaga^{1*}, Abdul Malik¹, Rahmad Wijaya², and Javaindi Syahrani^{1*}

¹Department of Animal Husbandry, University of Muhammadiyah Malang, Jln. Raya Tlogomas 246, Malang 65144 Indonesia

²Department of Management, University of Muhammadiyah Malang, Jln. Raya Tlogomas 246, Malang 65144 Indonesia

*Corresponding author's Email: pancapalaga1966@gmail.com; ORCID: 0000-0001-9859-1221

Received: 09 Jul. 2020
Accepted: 28 Aug. 2020

ABSTRACT

The present study aimed to examine the effects of fermented soy isoflavones on the poultry feed towards the quality of eggs. A total of 100 Isa Brown chickens aged 32 weeks were divided into 4 groups and 5 replicates, including T0 (control feed without the provision of fermented soy isoflavones), T1 (feed with 4% of fermented soy isoflavones), T2 (feed with 8% of fermented soy isoflavones), and T3 (feed with 12% of fermented soy isoflavones). The treatments were given for 10 weeks. The observed chemical qualities of eggs included HDL, LDL, isoflavones in egg yolks, physical quality (e.g., their weight and eggshell thickness). All the data were analyzed by using analysis of variance. The results showed that the administration of fermented soy isoflavones in poultry feed had no significant effect on egg weight and eggshell thickness ($p > 0.05$), but had a significant effect on HDL, LDL, and isoflavones in egg yolk ($p < 0.01$). Based on the results, it can be concluded that 80 mg/100 g of the fermented soy isoflavones (equivalent to the addition of 12%) in laying hens' feed would increase the content of isoflavones and egg yolks' HDL, reduce egg yolks' LDL, and lead to no significant change in the weight and thickness of eggshells.

Keywords: Feed, Fermented, Isoflavones, laying hens, Soybean

INTRODUCTION

The isoflavone content is found in many vegetables, fruits, grains and nuts, and especially in soybeans (Yousef et al., 2004). Soybeans and most soy products contain large amounts of isoflavone genistein and daidzein (Helen Kim et al., 1998). Typically, isoflavone content is around 0.2-1.6 mg /100 g of the balanced diet. According to Izumi et al. (2000), in soy and their processed products, isoflavone is in the form of glycoside, while fermented soy isoflavone is in the form of aglycone which is more quickly absorbed in the small intestine. Furthermore, Messina (2010) declared that soy and its processed products contain phytoestrogens as it is healthy food components which may prevent certain types of cancers, reduce the risk of osteoporosis, decrease plasma cholesterol, act as an antioxidant agent and may increase immunity for both humans and livestock.

Research on soy isoflavone for poultry has been extensively carried out by some researchers, such as the

provision of isoflavone in laying hens that were reported by Abdelghani et al. (2019), Malik et al. (2019), Lu et al. (2017); to quail by Akdemir and Sahin (2009); and to mojosari ducks (anas javanica) by Jayanti et al. (2017) and Saputro et al. (2018).

Performance of laying hens with isoflavone added at 59 weeks showed an increased in egg production (Lu et al., 2017), likewise Abdelghani et al. (2019) reported that egg yolk color and Haugh units increased, and lipoprotein cholesterol levels dropped, but added isoflavone to the diet of hens aged 80 weeks was not be able to improve egg quality (Kusumaningrum et al., 2018).

Isoflavones have been proven as compounds that have the ability to influence enzymes in the liver in the process of lipid metabolism (Yilmaz et al., 2008). Consumption of soy isoflavone at a dose of 5 mg / kg will reduce lipid peroxidation in vivo, and increase Low-Density Lipoprotein (LDL) resistance (Wiseman H et al., 2000). Isoflavones will be hydrolyzed by beta-glucosidase, and produce aglycone, daidzein, genistein and glycitein

which will then be absorbed and bounded with glucuronic acid. Then, it reaches the enterohepatic cycle, and are secreted through the gallbladder (Malik et al., 2019).

Processing and fermentation of Soybean are known to affect the performance of isoflavones. Soy sauce by-product sauce is a fermentation process of soybean that produces a source of Isoflavones in the feed for poultry (Susanti, 2006).

Present study aimed to examine the effects of fermented soy isoflavones on Isa Brown's feed towards the quality of eggs.

MATERIALS AND METHODS

One hundred of 32-week-old Isa brown strain were used in the current study. Laying hens were reared in closed house experimental-farm in University of Muhammadiyah Malang, Indonesia (Figure 1).



Figure 1. The conditions of study area

Feed treatments were given for 12 weeks starting from the age of 20 weeks to 32 weeks. During that time, all of the laying hens were given Gumboro vaccine and Newcastle Disease (ND) vaccine. They were also clinically examined routinely during the study. The feed ingredients for the research were as follows: fish meal, meat and bone meal, Corn Gluten Meal (CGM), Distillers Dried Grains with Solubles (DDGS), corn, rice bran, oil, grit, amino acid lysine, methionine and fermented soy Isoflavone (Malik et al., 2019). The study used an experimental method with a Completely Randomized Design (CRD). The treatments were divided into 4 groups (each group contained 25 hens), with 5 replicates (each replicate contained 5 hens). They were T0 (control feed without the provision of fermented soy isoflavones), T1

(feed with 4% fermented soy isoflavones), T2 (feed with 8% fermented soy isoflavones) and T3 (feed with 12% fermented soy isoflavones). The composition and nutrient content of the treatment diets are presented in table 1. The feed was given measuredly in the form of mash and drinking water was prepared *ad libitum*. The diets were balanced and used according to the treatments (120 grams/head/day).

Soybeans fermentation process

Soybeans fermentation process was using Malik et al. (2019) method. Firstly, provides 1 kg of black soybean (*Glycine soja* (L) Merrit) purchased at the Malang market, Indonesia sorting was done with the aim of selecting good and dense soybeans. Secondly, washing was aimed to remove impurities that were attached or mixed in the soybeans. The next was boiling the beans for 1 hour. It was aimed to soften the soybeans, and make it easier to peel the skin was stripped, and then, the soybeans were allowed to get cool to the temperature of $\pm 30^{\circ}\text{C}$. Furthermore, the yeast inoculum was sprinkled in a ratio of 1:2 (0.5% of the number of soybeans) on the cooled and dried soybeans' surface to let them be fermented. After being fermented, the second boiling process was done at 100°C for 20-30 minutes, so that the soybeans became soft. After that, they were being cooled for the last filtering. The result of the filter pulp was used as a fermented soy isoflavone feed.

Measurement of observed variables

Measurement of the egg yolk High-Density Lipoprotein (HDL) and Low-density lipoprotein (LDL) levels was made by using the enzymatic-calorimetry method (Bursteinetal, 1970). On the other hand, the egg yolk isoflavone levels were measured using High-Performance Liquid Chromatography (HPLC) that is according to a modified procedure by Harborne (1992). The eggshell's thickness was measured using a micrometer, and measurements were made on the blunt end, middle and sharp end of the eggs, then the measurements were being averaged (Kul and Seker, 2004). In addition, the eggs' weight measurement was done by weighing the eggs' weight every day using a digital scale with units (g).

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 (LSD) (Steel and Torrie, 1991).

Table 1. Composition and nutrition content of the treatment diets in laying hens, strain isa Brown aged 32 weeks

Feed ingredients %	T0 (0%)*	T1 (4%)*	T2 (8%)*	T3 (12%)*
Rice Bran	15.65	15.80	15.40	15.50
Corn	58.5	57.6	55.4	55.2
Fish oil	0.20	0.90	0.90	0.90
Destillers Dried Grains with Solubles	5.65	3.1	3.5	2.0
Corn Gluten Meal	4.4	4.0	3.1	2.0
Meat and Bone Meal	2.5	2.0	1.0	0.7
Fishmeal	10	10	10	10
Grit	2.9	2.4	2.5	1.5
Lysine	0.1	0.1	0.1	0.1
Methionine	0.1	0.1	0.1	0.1
Fermented soy Isoflavone	0.2	4.0	8.0	12.0
Nutrient 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
Calcium (%)	1.80	1.60	1.59	1.22
Phosphorus Total (%)	0.52	0.51	0.52	0.51
Sodium (%)	0.05	0.05	0.05	0.05
Energy Metabolism (Kcal/kg)	3060.39	3000.00	3020.75	3002.00

*T0: Treatment 0 is 100 kg feed without fermented soy isoflavone. T1 (4%): Treatment 1 is 100 kg feed added with 4kg fermented soy isoflavone. T2 (8%): Treatment 2 is 100 kg feed added with 8 kg fermented soy isoflavone. T3 (12%): Treatment 3 is 100 kg feed added with 12kg fermented soy isoflavone. **Results of the laboratory analysis on the nutrition of each food

RESULTS

Data on the results of average eggs’ weight, eggshell’s thickness, HDL, LDL and Isoflavones in egg yolks can be seen in table 2.

Egg yolks’ isoflavone

According to table 2, the results showed that the provision of fermented soy isoflavones in the feed had a significant effect ($P < 0.01$) on the content of egg yolks’ isoflavone. The content of isoflavone in the egg yolk increased from 0.164 mg / g (T0) to 0.267 mg / g (T3). This increase reached 62%.

HDL in the egg yolks

Table 2 shows that the provision of fermented soy isoflavones in the feed had a remarkable effect ($P < 0.01$) on HDL in the egg yolks. High Density Lipoprotein or as known as HDL’s level in the egg yolks without being given fermented soy isoflavone to the birds was 42.5

mg/dl. However, after being given 12% (T3) of fermented soy isoflavone in the feed, the HDL level in the egg yolks became 130.4 mg / dl.

LDL in the egg yolks

LDL level in the egg yolks decreased by approximately 48.9% after being given fermented soy isoflavone to the birds. This means that the provision of fermented soy isoflavone in feed had a notable effect ($P < 0.05$) on LDL in the egg yolks.

Eggs’ weight and eggshell’s thickness

The eggs’ weight and eggshell’s thickness slightly changed after isoflavone was added to the feed. Therefore, the provision of fermented soy isoflavones in the feed had no important effect ($P > 0.05$) on the eggs’ weight and eggshell’s thickness.

Table 2. The effect of diets with different levels of fermented soy isoflavones on egg quality of layer hens aged 32 weeks

Variable	T0 (0%)	T1 (4%)	T2 (8%)	T3 (12%)
Isoflavon (mg/100 g)	0.16 ^b	0.25 ^a	0.24 ^a	0.26 ^a
High-Density Lipoprotein (mg/dl)	42.50 ^b	125.50 ^a	116.80 ^a	130.40 ^a
Low-density lipoprotein (mg/dl)	6.85 ^a	5.08 ^{ab}	5.16 ^{ab}	3.50 ^b
Eggs’ weight (g)	59.93 ^a	60.57 ^a	61.57 ^a	62.40 ^a

Different superscript letters on the same row show significant differences ($P < 0.05$). *T0: Treatment 0 is 100 kg feed without fermented soy isoflavone. T1 (4%): Treatment 1 is 100 kg feed added with 4kg fermented soy isoflavone. T2 (8%): Treatment 2 is 100 kg feed added with 8 kg fermented soy isoflavone. T3 (12%): Treatment 3 is 100 kg feed added with 12kg fermented soy isoflavone.

DISCUSSION

The provision of fermented soy isoflavones in Isa Brown would increase the content of isoflavones and HDL in egg yolks. The results of present study were similar to the findings of Cai et al. (2013) on quails’ eggs, Malik et al. (2019) on the blood of laying hens and et al (2018) on Mojosari Ducks’ eggs. Isoflavones from fermented soybeans can increase functional components such as aglycone and active peptide which are very beneficial for health (Jiang et al., 2014). Whereas, aglycone isoflavone had a faster absorption rate in greater amounts than glucoside (Izumi et al., 2000). In addition, according to Saitoh et al. (2001), isoflavone in the blood turned into a soluble form of conjugate, so that it would facilitate the

transfer of soy isoflavones into egg yolks. Therefore, adding high concentration of isoflavones in laying hens would affect the concentration of cholesterol in the egg yolk produced.

On the other hand, the results of current study found a decrease in LDL content of egg yolk. This was in line with the LDL found in ducks' eggs (Saputro *et al.*, 2018; Abdelghani *et al.*, 2019) and the blood in laying hens (Malik *et al.*, 2019). Potter *et al.* (1998) suggested that isoflavones in the feed have estrogenic effects that cause a decrease in LDL, and an increase in HDL's concentrations which may be the result of stimulation of bile acid excretion. Furthermore, this estrogenic effect can inhibit the absorption of cholesterol which causes inhibition of VLDL formation that consequently leads to decrease of LDL levels.

Meanwhile, according to Saitoh *et al.* (2001), Fermented soy isoflavones in animals' feed had hypocholesterolemia effects. Isoflavones are plant-derived sterols (phytosterols) which can inhibit the absorption of cholesterol when are consumed. These phytosterols compete and replace the position of cholesterol in micelles. Because of those mechanism, the cholesterol absorbed by the intestine would be reduced. Moreover, when VLDL is inhibited, LDL levels fall.

Eggs' weight and eggshell's thickness in this study showed a slight change compared to the control group (without provision of isoflavones). The results of present study were different from those reported by Lu *et al.* (2017) who found that providing isoflavones in the final quails' diet will increase eggs' weight and eggshell's thickness. Chicken eggs taken from laying hens aged 32 weeks. Current study used laying hens with a productive period of 32 weeks, so that the absorption of calcium and phosphor was not for the formation of thick eggshells, but for the formation of bones that are still productive. This was reinforced by Gjorgovska *et al.* (2016) who states that isoflavones are effective supplements for increasing body weight, body length and calcium content in bones during the final period.

CONCLUSION

The provision of 80 mg / 100 g fermented soy isoflavones (equivalent to the addition of 12%) in laying hens' feed will increase the content of isoflavones and egg yolks' High Density Lipoprotein (HDL), and it will reduce egg yolks' Low-Density Lipoprotein (LDL), but not change the weight and thickness of eggshells much.

DECLARATIONS

Acknowledgements

The researchers would like to thank the Rector of the University of Muhammadiyah Malang, Indonesia (Jln. Raya Tlogomas 246, Malang 65144) and the Directorate of Research and Community Service of University of Muhammadiyah Malang for facilitating this research.

Competing interests

The researchers state that there are no conflicts of financial interests or conflicts with other people or organizations that can affect or create bias in the contents of this paper.

REFERENCES

- Abdelghani E, Xing W, Li Y, Shen. D, Alsiddig MA and Li C (2019). Effects of Dietary Supplementation of Soy Isoflavones on the Performance and Egg Quality in Native Chinese Breeder Hens. *Brazilian Journal of Poultry Science*, 21(4): 1-8. DOI: <http://dx.doi.org/10.1590/1806-9061-2018-0940>
- Akdemir F and Sahin K (2009). Genistein supplementation to the quail: Effects on egg production and egg yolk genistein, daidzein, and lipid peroxidation levels *Poultry Science*, 88 : 2125–2131. DOI: <https://doi.org/10.3382/ps.2009-00004>
- Burstein M, Scholnick HR and Morfin R (1970). Rapid method for the isolation of lipoproteins from human serum by precipitation with polyanions. *Journal of Lipid Research*, 11: 583-594. Available at: <https://pubmed.ncbi.nlm.nih.gov/4100998/>
- Cai J, H Gu, Shi S and Tong H (2013). Effects of high-dose daidzein on laying performance, egg quality and antioxidation in laying hens. *Poultry Science*, 50: 237-241. DOI: <https://doi.org/10.2141/jpsa.0120118>. Available at: <http://www.jstage.jst.go.jp/browse/jpsa>
- Gjorgovska N, Filev K, Levkov V, Nastova R and Jusufi E (2016). Effects of Dietary Supplementation with Isoflavones On Exterior Development and Tibia Bone Quality of Laying Hens. *Slovak Journal of Animal Science*, 49 (3): 112-115. Available at: <https://sjas.ojs.sk/sjas/article/view/169>
- Harborne JB (1996). *The Flavonoid: Advances in Research since 1986*. London: Chapman & Hall, Inc.
- Izumi T, Piskula MK, Osawa S, Obata A, Tobe K, Saito M, Kataoka S, Yoshiro Kubota and Ki M (2000). Soy Isoflavone Aglycones Are Absorbed Faster and in Higher Amounts than Their Glucosides in Humans. *American Society for Nutritional Sciences*, 1695-1699. DOI: <https://doi.org/10.1093/jn/130.7.1695>
- Jayanti RD, Mahfudz LD and Kismiat S (2017). Pengaruh Penggunaan Ampas Kecap Dalam Ransum Terhadap Kadar Protein, Lemak dan Kalsium Kuning Telur Itik Mojosari. *Jurnal Peternakan Indonesia*, 19 (3): 126-133. Available at: <http://jpi.faterna.unand.ac.id/index.php/jpi/article/view/277>
- Jiang SQ, Jiang ZY, Lin YC, Xi PB and Ma XY (2007). Effects of Soy Isoflavone on Performance, Meat Quality and Antioxidative Property of Male Broilers Fed Oxidized Fish Oil. *Asian-Australasian Journal of Animal Sciences*, 20(8): 1252-1257. DOI: <https://doi.org/10.5713/ajas.2007.1252>
- Lu J, Qu L, Shen MM, Li SM, Dou TC, Hu YP and Wang KH (2017). Safety evaluation of daidzein in laying hens: Effects on laying performance, hatchability, egg quality, clinical blood parameters,

- and organ development *Poultry Science*, 96: 2098–2103. DOI: <https://doi.org/10.3382/ps/pew483>.
- Kim H, Peterson TG and Barnes S (1998). Mechanisms of action of the soy isoflavone genistein: emerging role for its effects via transforming growth factor β signaling pathways 1–3. *American Journal of Clinical Nutrition*, 68(suppl): 1418S–1425S. DOI: <https://doi.org/10.1093/ajcn/68.6.1418S>.
- Kul S and Seker I (2004). Phenotypic Correlations Between Some External and Internal egg quality Traits in The Japanese Quil (*Coturnix coturnix japonica*). *International Journal of Poultry Science*, 3(6): 400-405. DOI: <https://doi.org/10.3923/ijps.2004.400.405>
- Kusumaningrum DU, Mahfudz LD, and Sunarti D (2018). Pengaruh Penggunaan Tepung Ampas Kecap pada Pakan Ayam Petelur Tua terhadap Kualitas Interior Telur dan Income Over Feed Cost (IOFC). *Jurnal Sain Peternakan Indonesia*, 13 (1) 36-42. DOI: <https://doi.org/10.31186/jspi.id.13.1.36-42>. Available at: <https://ejournal.unib.ac.id/index.php/jspi/article/view/4068>
- Malik A, Suprijatna E, Yuniarto VD, Mahfudz LD, and Suthama N (2019). Egg quality and isoflavone deposition due to dietary inclusion of isoflavone soy sauce by-product (ISSBP) in laying. *Journal of the Indonesian Tropical Animal Agriculture*, 44(2): 187-194. DOI: <https://doi.org/10.14710/jitaa.44.2.187-194>
- Messina M (2010). A Brief Historical Overview of the Past Two Decades of Soy and Isoflavone Research 1,2. *J. Nutr.*, 140: 1350S–1354S. DOI: <https://doi.org/10.3945/jn.109.118315>.
- Potter SM, Baum JA, Teng H, Stillman RJ, Shay NF, and Erdman JW (1998). Soy protein and isoflavones: their effects on blood lipids and bone density in postmenopausal women 1–3. *American Journal of Clinical Nutrition*, 68(suppl): 1375S–9S. DOI: <https://doi.org/10.1093/ajcn/68.6.1375S>
- Saitoh S, Sato T, Harada H and Matsuda T (2004). Biotransformation of soy isoflavone glycosides in laying hens: intestinal absorption and preferential accumulation into egg yolk of equol, a more estrogenic metabolite of daidzein. *Biochimica et Biophysica Acta*, 1674(2):122-130. DOI: <https://doi.org/10.1271/bbb.65.2220>
- Saputro H, Mahfudz LD, dan Sarjana TA (2018). Sarjana. Pengaruh Penggunaan Ampas Kecap dalam Ransum terhadap Isoflavon LDL dan HDL Telur Itik Mojosari. *Jurnal Sain Peternakan Indonesia*. 13. (3)238-243. DOI: <https://doi.org/10.31186/jspi.id.13.3.238-243>. Available at <https://ejournal.unib.ac.id/index.php/jspi/index>
- Sirtori CR, Lovati MR, Manzoni C, Mohetti M, Pazzucconi F, and Gatt E. (2014). Soy Intake and Cholesterol Reduction. *Journal of Nutrition*, 3: 598-605. DOI: https://doi.org/10.1093/jn/125.suppl_3.598S
- Steel RGD and Torrie JH (1991). Principles and Procedures of Statistics. 2nd Ed. International Book Company, Tokyo. DOI: <https://doi.org/10.1002/bimj.19620040313>
- Susanti S (2006). Kajian Komposisi Kimia Ampas Kedelai Hasil Samping Pengolahan Kecap. *Buana Sains*, 6 (1): 59-66. DOI: <http://dx.doi.org/10.33366/bs.v6i1.93>
- Wiseman H, O'Reilly JD, Adlercreutz H, Mallet AI, Bowey EA, Rowland IR, and Sanders TA (2000). Isoflavone phytoestrogens consumed in soy decrease F2-isoprostane concentrations and increase resistance of low-density lipoprotein to oxidation in humans 1–3. *American Journal of Clinical Nutrition*, (72) : 395–400. DOI: <https://doi.org/10.1093/ajcn/72.2.395>
- Yilmaz O, Guvenc M, Cetintas B, Tuzcu M, Dayangac A, and Sahin K (2008). Effects of Isoflavones Supplementation on Cholesterol and Fatty Acid Levels of Muscle and Liver Tissues of Quail. *Journal of Animal and Veterinary Advances*, 7(11): 1444-1449. Available at: <https://medwelljournals.com/abstract/?doi=javaa.2008.1444.1449>
- Yousef MI, Kamel KI, Esmail AM, Baghdadi HH (2004). Antioxidant activities and lipid lowering effects of isoflavone in male rabbits. *Food and Chemical Toxicology*, 42 (2004): 1497–1503. DOI: <https://doi.org/10.1016/j.fct.2004.04.012>