Pharmacogn J. 2023; 15(3): 270-273
A Multifaceted Journal in the field of Natural Products and Pharmacognosy
www.phcogj.com

Original Article

The Effect Supplementation of Microbiota Inoculant in the Early Laying Hens Feed on High Density Lipoprotein (HDL) and Low-Density Lipoprotein (LDL) in Egg Yolk

Bodhi Agustono[1]*, Rini Apriliawati[1], Sunaryo Hadi Warsito[6], Maya Nurwartanti Yunita[2], Widya Paramita Lokapirnasari[6], Sri Hidanah[6], Emy Koestanti Sabdoningrum[6], Mohammad Anam Al-Arif[6], Mirni Lamid[6] Gandul Atik Yuliani[3], Shekhar Chhetri[4], Sarasati Windria[5]

ABSTRACT

Laying hens (*Gallus gallus*) are one of the poultries kept for their eggs. Eggs produced by chickens are used as a source of human food that contains a lot of nutrition and is affordable. Eggs also contain high levels of cholesterol in the yolk, so if consumed in large quantities, they will pose a risk of disease. This study aims to determine the effect of giving probiotics in the early layer laying hen feed on the levels of high-density lipoprotein and low-density lipoprotein in egg yolks. This study has four treatments, namely P1 = control, P2 = feed + AGP, and P3 and P4 = feed + probiotics. The probiotic used is a combination of three bacteria, namely Lactobacillus plantarum, Lactobacillus acidophilus, and Bifidobacterium sp. The sample used in this study was egg yolk, which was collected in the last week of the study. Testing for HDL and LDL levels in egg yolks was carried out using the Quantitative Colorimetric/Fluorometric Determination of HDL and LDL/VLDL method. Testing HDL and LDL levels in this study showed that the administration of probiotics had an effect in the form of increasing HDL levels but did not have an effect in the form of a significant decrease in egg yolk LDL levels during the four-week treatment period in laying hens in the early layer phase, aged 18–22 weeks. The best probiotic dose used among the three probiotic doses is 3 ml/kg of feed.

Key words: Laying hens, High Density Lipoprotein, Low Density Lipoprotein, Probiotics.

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[1]Division of Animal Husbandry, School of Health and Life Sciences (SIKIA), Universitas Airlangga, Surabaya 60115, Indonesia

[2]Division of Pathology Veteriner, School of Health and Life Sciences (SIKIA), Universitas Airlangga, Surabaya 60115, Indonesia

[3]Division of Basic Veterinary, Department of Veterinary Science, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya 60115, INDONESIA.

[4]Department of Animal Science, Royal University of Bhutan, BHUTAN.

[5]Department of Biomedical Science, Faculty of Medicine, Universitas Padjajaran, Bandung, INDONESIA.

[6]Division of Animal Husbandry, Department of Veterinary Science, Faculty of Veterinary Medicine Universitas Airlangga, Surabaya 60115, Indonesia

Correspondence

Bodhi Agustono

Division of Animal Husbandry, School of Health and Life Sciences (SIKIA), Universitas Airlangga, Surabaya 60115, Indonesia

E-mail: bodhiagustono@fkh.unair.ac.id

History

- Submission Date: 31-03-2023;
- Review completed: 27-04-2023;
- Accepted Date: 06-05-2023.

DOI : 10.5530/pj.2023.15.73

Article Available online

http://www.phcogj.com/v15/i6

INTRODUCTION

The poultry farming industry in Indonesia is increasingly showing improvement, along with the high demand of the community for sources of animal protein.[1] Livestock is classified as a business that has good prospects if it is done optimally.[2] Poultry is a type of livestock that is widely kept for its meat and eggs. One type of poultry that is mostly used is chicken; there are two types of chicken that are often used, namely layers and broilers.[3] Laying hens are poultry that have the potential to be reared in Indonesia with the specific aim of producing eggs commercially.[4] Chicken eggs are popular as a nutritious food and have affordable prices for the community when compared to animal-based foodstuffs such as meat and fish.[5] There are three growth phases in laying hens, namely the starter, grower, and layer phases.[6] The pre-layer phase is the initial layer phase. The chickens experience body weight gain and the development of reproductive organs before entering the layer phase. The maintenance of laying hens in the initial layer phase requires attention to their health and growth, especially stress. According to Carstens and Moberg (2000),[7] stress is a biological response that can cause homeostatic disturbances in animals and have a negative impact. With intensive maintenance, it is likely to cause more stress on the chickens so that it can reduce the level of production of laying hens.[8] So that laying hens that are in the pre-layer phase or the initial phase of this layer must pay great attention because it greatly determines the quality of the hens' production.[9]

Eggs are a source of high-quality protein at an affordable price.[10] The nutritional content contained in chicken eggs can be obtained from the egg white and yolk, including 73.7% water and 12.9% protein. 11.2% fat and 0.9% carbohydrates. The fat content in the yolk reaches 32%, while the egg white has very little.[11] High cholesterol levels in egg yolks are a problem that is often faced by Indonesian people. The effects of consuming excess fat and cholesterol will result in disease, for example, coronary heart disease.[11] Cholesterol is an arrangement of essential components in the external layer of cell membranes and acts as a plasma lipoprotein that is amphipathic. Lipoprotein has 4 main groups, namely triglycerides, very low-density lipoprotein (VLDL), low density lipoprotein (LDL), and high-density lipoprotein (HDL). Low-density lipoprotein (LDL) is a type of lipoprotein that has the most function in carrying cholesterol into the blood circulation.[12] Low-density lipoprotein, or LDL, is also called bad cholesterol because if you experience an increase in LDL levels in the blood, it will cause blood cholesterol levels to increase, or hypercholesterolemia. When cholesterol levels in the blood increase, it can cause several diseases due to the narrowing of the arteries. LDL can be found in egg yolks and is produced from the hydrolysis of IDL (intermediate density lipoprotein) by the enzyme lipoprotein lipase. The biggest factor causing high levels of chicken egg yolk cholesterol

Cite this article: Agustono B, Warsito SH, Yunita MN, Lokapirnasari WP, Hidanah S, Sabdoningrum EK, et al. The Effect Supplementation of Microbiota Inoculant in the Early Laying Hens Feed on High Density Lipoprotein (HDL) and Low-Density Lipoprotein (LDL) in Egg Yolk. Pharmacogn J. 2023;15(3): 270-273.

is LDL, which reaches 60%. If the LDL level in chicken egg yolks is high, you can be sure that chicken egg yolk cholesterol levels are also high.[13] Efforts to reduce egg yolk cholesterol levels can be done by using probiotics in feed. Probiotics are live microorganisms that can provide health to other organisms if administered through the digestive tract.[11] Giving probiotics will reduce feed conversion, increase the amount of egg production, and reduce egg yolk cholesterol levels.

MATERIALS AND METHOD

Ethical approval

The study was approved by the Ethical Committee of Universitas Airlangga, Indonesia, with number 15/HRECC.FODM/IX/2022.

Study period and location

The research was carried out for 36 day (June to July 2022). The research locations were carried out, namely: Treatment of experimental animals at Layer Farm of Universitas Airlangga,

Material and materials

The material used in this study was strain is a brown chicken aged 17 weeks with a body weight of 1.5 kg (±0.5). probiotics *lactobacillus acidophilus*, *bifidobacterium and lactobacillus plantarum* (source of probiotics from A.B. Yulianto's and W.P. Lokapirnasari's collection) with a concentration of $1.2x10^9$ CFU/ml. Layer layer used is commercial feed with crude protein content of 18-19%. The tools used in this study were 20 cages, sealed ration boxes, drinking water containers, scale's capacity 10 kg with an accuracy of 100 g to weight gain and feed, micrometer scrub, thermohygrometer to measure temperature and humidity of the cage, vacutainer tube with EDTA, plain vacutainer tube, ice gel, container and cleaning tools.

Preparation of LAB isolate culture

Bacteria were isolated in MRSB media, under aerobic facultative conditions, 18-24 hours, 37^0C.

Experimental design

This study used a completely randomized design consisting of five treatments, where each treatment consisted of 4 replications, each replication 5 animals for a total of 100 animals. The treatments used in this study were five treatments (T1,T2,T3,T4 and T5) each treatment consisted of four replications, consisting of (T1) 100% basal feed, (T2) basal feed + 2.5 grams of AGP/kg feed, (T3) basal feed + probiotics 1 ml / kg feed, (T4) basal feed + probiotics 3 ml / kg feed, (T5) basal feed + probiotic 5 ml / kg feed.

Egg yolk sampling

The sample used was an egg yolk sample taken in the last week of the study. Egg yolk samples came from each treatment group, taken collectively for one week. The collected eggs will be broken and placed in a petri dish to collect the yolk using a 0.1 ml syringe. The egg yolk samples obtained were labeled according to the time of collection and the treatment group to facilitate the process of collecting research data.

HDL and LDL test

Eggs obtained in each treatment group on days 1 - 7 in the last week were separated between egg whites and egg yolks. The egg yolks obtained collectively on days 1 - 7 in the last week were then placed in microtubes which were labeled according to the treatment group and stored in the freezer. Then the total egg yolk sample obtained was homogenized according to the treatment group and taken in the same amount to test HDL and LDL levels. The method used to test HDL and LDL cholesterol levels is the Quantitative Colorimetric/Fluorometric Determination of HDL and LDL/VLDL method, BioAssay Systems EnzyChrom AF HDL/LDL Assay Kit (E2HL-100). This method is used because it can measure directly, and is ready to automation measures the desired HDL and LDL/VLDL concentrations. This method is a precipitation method in which HDL and LDL/VLDL are separated, then the cholesterol concentration is determined using a Working Reagent that combines cholesterol ester hydrolysis, oxidation, and color reactions. The kit used contains PBS: 2 x 1.5 ml, precipitating reagent: 1.5 ml, assay buffer: 20 ml, enzyme mixture: 120 ul, coloring reagent: 120 l, standard: 1 ml 300 mg/dL cholesterol.

RESULT

The effect of probiotics on the levels of High-Density Lipoprotein and Low-Density Lipoprotein in the yolk of laying hens tested using BioAssay Systems is shown in Table 1. The HDL results tested using ANOVA showed that the data were normally distributed ($p<0.05$) and then continued using Duncan's test. which showed the results that the addition of probiotics to the feed of laying hens in the early layer phase showed a significant difference ($p <0.05$) between the treatment group and the control group. Meanwhile, the LDL results showed no significant difference ($p<0.05$) between the treatment group and the control group. The average data of HDL and LDL concentrations of egg yolk in the early layer phase of each treatment can be seen in Figure 1.

DISCUSSION

Egg yolk HDL levels

High Density Lipoprotein is a lipoprotein which has a lipid component in the form of cholesterol and phospholipids, but has a small number of triglycerides. HDL lipoprotein is formed in the liver, intestine and blood circulation.[14] Based on the results of the research data analysis conducted, it was found that the provision of probiotics as a feed additive for laying hens in the early layer phase had an effect on egg yolk HDL levels. The results showed that the average yield of the T4 treatment had the highest HDL level of 437.28 ml/dL compared to the T1, T2, and T3 treatments. Based on the results obtained from this study, it can be concluded that giving probiotics mixed into the feed can increase HDL levels in the egg yolks of laying hens in the early layer phase.

Table 1: Concentration of HDL ml/dL levels in yolk eggs of laying on probiotic treatment.

Treatment	HDL concentration ml/dL (Mean ± SD)	LDL concentration ml/dL (Mean ± SD)
T1	$307,64^a$± 46,36	$197,65^a$±71,39
T2	$311,22^a$±41,32	$195,16^a$±68,38
T3	$349,25^a$±41,40	$174,27^a$±54,87
T4	$437,28^b$±21,44	$168,21^a$±30,23

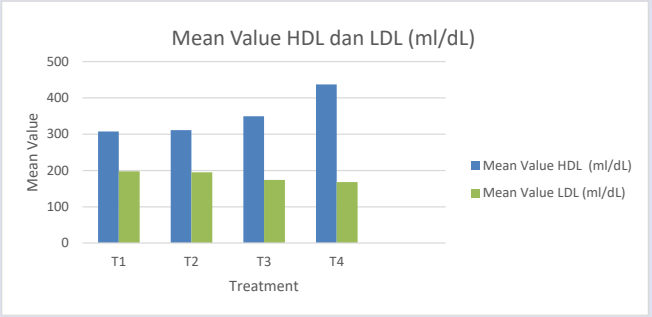

Figure 1: Mean of concentration HDL dan LDL (ml/dL) levels in yolk eggs of laying on probiotic treatment

Probiotics are live microorganisms that can produce cellulase, amylase and protease enzymes which play a role in increasing the digestibility of nutrients in the digestive organs.[15,16] The higher the concentration of probiotics given, the higher the bacteria which will increase the efficiency of livestock in consuming feed.[17] Supplementation of probiotics in poultry will maintain the normal flora of the digestive tract of poultry by changing metabolism and increasing the activity of bacterial enzymes, and can suppress serum cholesterol concentrations and poultry egg yolk.[18,19]

Oral administration of probiotics can significantly reduce cholesterol levels. The effect of reducing cholesterol levels can be observed in the activity of BSH (Bile Salt Hydroilase). Other mechanisms such as cholesterol assimilation by bacteria, binding of cholesterol to the bacterial cell wall and physiological actions that produce the end product of short-chain fermentation of fatty acids. According to Wu *et al*., (2017)[20] administering probiotics using bacteria belonging to the Lactobacillus group can increase HDL levels and reduce TG. Giving probiotics can optimize the absorption of nutrients and stimulate the production of bile salt hydrolyse (BSH) enzymes which can reduce blood cholesterol levels without leaving residues that are easily absorbed by the intestine because they can break long chain fatty acids into medium and short chain fatty acids.[21] Another mechanism is by inhibiting the reabsorption of bile acids resulting from the synthesis of cholesterol in the intestine by the intestinal epithelium so that bile acids will be immediately excreted along with the feces, then to replace the excreted bile acids, cholesterol in the serum will be converted by the liver into bile acids resulting in a decrease in cholesterol. in the blood.[22]

Unconjugated bile acids are absorbed more efficiently than conjugated bile acids, resulting in greater excretion of free bile acids in the feces. Free bile acids also have a lower efficiency of solubility and absorption in the intestine, so that unconjugated bile acids can result in a reduction in serum cholesterol levels by de novo synthesis of bile acids to replace bile acids lost in feces. Decrease activity also by reducing cholesterol solubility and absorption of cholesterol through the intestine.[23]

Probiotics work by competitive exclusion, namely by competing with pathogenic bacteria to adhere to the surface of the intestinal lumen so that pathogenic bacteria cannot adhere to the surface of the intestinal lumen and will be expelled from the intestine.[24] Digestibility of feed increases and the absorption process increases because fat and fat derivatives which are insoluble in water must be bound by proteins which become lipoprotein compounds to be soluble in water, probiotics play a role in lowering intestinal pH so that they can increase H+ ions in the intestine which causes an increase in the process of binding water with lipids *via* HDL lipoprotein.[25]

Egg yolk LDL levels

Low Density Lipoprotein is the end result of VLDL catabolism which is formed in the blood circulation and is rich in cholesterol.[14] Very Low-Density Lipoprotein (VLDL) is formed by liver parenchyma cells and functions to transport TG synthesized by the liver to extrahepatic tissues to be oxidized into energy or stored in adipose tissue and to transport cholesterol and cholesterol esters from the liver into the blood circulation.[26] The oxidation process in the blood circulation is influenced by the lipoprotein lipase (LPL) enzyme which hydrolyzes VLDL so that triglycerides (TG) will turn into fatty acids and glycerol which are then oxidized into energy.[14]

Based on the results of the research data analysis conducted, it was found that probiotics as a feed additive and AGP as a feed additive for laying hens in the early layer phase within four weeks (30 days) had no effect on LDL levels of egg yolks. The results showed that the average results for the treatments T1, T2, T3 and T4 were not significantly different, so it can be concluded that there was no specific decrease in LDL levels in egg yolks.

The non-specific effect of giving probiotics to feed can be caused by giving probiotics that are too low (Sumardi *et al*., 2016). Another factor that affects the absence of specific changes in egg yolk LDL levels is the diet factor given. Based on research conducted by Ooi *et al*. (2010)[27] explained that giving probiotics showed non-specific results on day 30, but there were results in the form of a specific decrease in LDL on days 60 and 90 of probiotic administration.

Antibiotic Growth Promoters are given as feed additives which aim to be compared with the use of probiotics. Antibiotics used as additives in feed are prohibited from being used in Article 22 Paragraph 4 letter C of the Republic of Indonesia Law of 2009. The prohibition on using AGP is because it can cause microorganisms in the digestive tract to become resistant to certain antibiotics.[28] Giving antibiotics to chicken feed only functions to suppress the presence of pathogenic bacteria in the digestive tract of chickens so that it does not affect cholesterol levels in the blood but if the administration is stopped it will affect feed consumption.[29]

CONCLUSION

The results of the research and data analysis showed that the provision of probiotics in the feed can increase the levels of High-Density Lipoprotein in egg yolk. The best dose that can be used among the three doses is 3 ml/kg of feed. Meanwhile, the egg yolk Low Density Lipoprotein levels did not have a significant effect.

ACKNOWLEDGMENTS

We thank the Chancellor of Airlangga University and the Dean of the Faculty of Veterinary Medicine, Airlangga University Indonesia for providing financial support for the implementation of this research (Grant Number 658/UN3.15/PT/2022).

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Cite this article: Agustono B, Warsito SH, Yunita MN, Lokapirnasari WP, Hidanah S, Sabdoningrum EK, et al. The Effect Supplementation of Microbiota Inoculant in the Early Laying Hens Feed on High Density Lipoprotein (HDL) and Low-Density Lipoprotein (LDL) in Egg Yolk. Pharmacogn J. 2023;15(3): 270-273.