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Cholesterol, HDL, and LDL Content in Quail Egg Yolk with Probiotics and Acidifier Feeding

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Abstract

The higher cholesterol content in quail eggs compared to chicken and duck eggs needs to be taken into account, so there is a need for research focused on this issue. The aim of this study was to prove that the use of probiotics (*Pediococcus pentosaceus* ABY 118, *Lactococcus lactis*) and acidifiers in quail can give positive results on reducing cholesterol and LDL and increasing HDL content in egg yolk. Thirty quails were randomized into three treatments with 10 replicates. The treatments studied were: P0 = Control (no probiotic and no acidifier); P1 = Probiotic 20 ml/liter drinking water + Acidifier 5 g/kg feed; P2 = Probiotic 40 ml/liter drinking water + Acidifier 10 g/kg feed. The results of this study showed that there was a significant difference between cholesterol from the P0 with P1 and P2 treatments. Cholesterol in P1 and P2 was significantly lower than P0. HDL in the P1 and P2 treatments was significantly higher than the P0 treatment. LDL in the P1 and P2 treatments was lower than the P0 treatment. The conclusion that can be drawn from the results of this study is that the use of probiotics and acidifiers can reduce cholesterol, increase HDL, and reduce LDL.

Keywords

Atheroschlerosis, *Coturnix coturnix japonica*, *Lactococcus lactis*, lipoprotein, *Pediococcus pentosaceus*

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Introduction

The main products of poultry are meat and eggs. Quail egg yolk contains 15.7%-16.6% protein, 31.8%-35.5% fat, 0.2%-1.0% carbohydrate, and 1.1% ash. However, the problem is the higher cholesterol content compared to chicken eggs and duck eggs, namely in chicken eggs 7.65 mg/g, duck eggs 10.36 mg/g, and quail eggs 16.05 mg/g (Zarina Aziz *et al.*, 2012). To overcome this, feed additives that can be used are probiotics. Besides probiotics, other feed additives also have the potential to improve growth are acidifiers, as they have beneficial effects on gut morphology, growth, and production in poultry. It is necessary to conduct research to produce a combination of probiotics and acidifiers on cholesterol, HDL, and LDL content in quail egg yolk.

Probiotics are non-pathogenic live microorganisms that produce organic acids that lower the pH of the digestive tract and inhibit the growth of pathogenic bacteria. Several types of probiotics have been used to improve growth performance (Lokapirnasari *et al.*, 2017; 2019; 2020b; 2022; Yulianto *et al.*, 2020) and to modulate immune response (Yulianto *et al.*, 2021). Several studies have proven the positive effect of using probiotics on total cholesterol, LDL, HDL in broilers (Andriani *et al.*, 2020).

Cholesterol, triglycerides, and high-density lipoprotein are important parts of the body's lipid fraction. Cholesterol is a structural component that forms cell membranes and plays an important role in regulating cell functions (Cox and García-Palmieri, 2011; Craig *et al.*, 2018). Lipids circulate to various parts of the body in the form of lipoproteins. There are seven classes of lipoproteins in the blood plasma, including chylomicrons, chylomicron remnants, very low-density lipoprotein (VLDL), intermediate density lipoprotein (IDL) also called VLDL remnants, low density lipoprotein (LDL), high density lipoprotein (HDL), and lipoprotein (a) (Heurtault *et al.*, 2003; Lee and Siddiqui, 2019; Feingold, 2024).

Chylomicrons carry lipids from the diet into them at the beginning of the exogenous lipoprotein pathway. Triglycerides carried in chylomicrons in the blood circulation are further metabolized by lipoprotein lipase enzymes in muscle and adipose tissue, releasing free fatty acids, which are then metabolized to form chylomicron remnants. The remaining chylomicrons are then taken up by the liver (Cox and García-Palmieri, 2011; Lu *et al.*, 2012; Feingold, 2024). The endogenous lipoprotein pathway begins in the liver with the formation of VLDL. Lipoprotein lipase enzymes in muscle and adipose tissue metabolize triglycerides in VLDL to release free fatty acids and form IDL, which is then metabolized into LDL, which is taken up by LDL receptors found in various tissues, including the liver, which is the main absorption site (Feingold, 2024).

Chylomicron remnants, VLDL, IDL, LDL, and Lp (a), are pro-atherogenic, while HDL is anti-atherogenic. Atherosclerosis that develops progressively due to inflammation and accumulation of cholesterol-rich TRL (triglyceride-rich lipoproteins) remnants, including LDL particles, VLDL remnants, and chylomicron remnants that infiltrate into the subendothelial space of the arterial wall, becomes the first stage of atherosclerosis (Athanasiou, 2017; Feingold, 2024). These particles can interact with the arterial subendothelium through a non-receptor-mediated process and accumulate to form fatty streaks (Huggins *et al.*, 2022; Yan and Gotlieb, 2023).

LDL that enters the subendothelial space undergoes oxidation to oxLDL by various oxidation enzymes cannot be recognized by the LDL receptor but instead becomes a scavenger receptor ligand found on most macrophages (Yoshida *et al.*, 1998; Levitan *et al.*, 2010; Hadj Ahmed, 2018; Boren *et al.*, 2020; Charla *et al.*, 2020). Macrophages phagocytize oxLDL to remove it but instead turn it into foam cells. This leads to further activation of the immune response and is responsible for the failure to relieve the inflammation (Moore *et al.*, 2013; Charla *et al.*, 2020).

Materials and Methods

The materials used in this study include probiotics (*Pediococcus pentosaceus* ABY 118, *Lactococcus lactis*) (concentration 1.2 x 10^9 CFU/ml), female quail, acidifiers, and cholesterol analysis materials. The research procedure was conducted in three stages. The first stage was the culture of LAB isolates. Probiotic isolates were grown on MRSB media, facultative anaerobic, 18-24 hours, temperature 37°C. Probiotic isolates used in this study were obtained from isolation and identification results based on the researcher's roadmap. The second stage is the formulation of acidifiers. Acidifiers consist of fumaric acid, D-L malic acid, citric acid monohydrate, and lactic acid. All ingredients, according to the composition of the formulation, were weighed according to the dose and mixed until homogeneous. The third stage is the preparation of experimental animals. Cage disinfection was carried out one week before the quail arrived.

Egg samples were analyzed in two stages: yolk filtrate extraction and cholesterol level examination. Extraction of egg yolk filtrate was done by taking two eggs from each treatment and replicating in the last week of the study. Egg yolk was separated from egg white with a yolk separator into egg yolk filtrate. The filtrate was taken in 5 ml and then put into a test tube plus 5 ml of solution (Alcohol + Aceton = 1: 1). The test tube was covered with aluminum foil as soon as possible, then shaken until the filtrate clotted, then heated in hot water until the alcohol solution and acetone boiled, after which it was left for 3 minutes, centrifuged for 1 minute, and then filtered with filter paper (Jaya *et al.*, 2019). Furthermore, the egg yolk filtrate was used for the examination of cholesterol levels with a spectrophotometer.

This study used a completely randomized design (CRD). Thirty quails were randomized into three treatments with 10 replicates. The treatments studied were: P0 = Control (no probiotics and no acidifier); P1 = Probiotics (*Pediococcus pentosaceus* ABY118 1% and *Lactococcus lactis* 1%) through drinking water + Acidifier 0.5% of feed; P2 = (*Pediococcus pentosaceus* ABY118 2% and *Lactococcus lactis* 2%) through drinking water + Acidifier 1% of feed. Data were analyzed with an analysis of variance (ANOVA) factorial pattern. If there was a significant difference, Duncan's multiple range test was conducted with a significant level of 5% to determine the best treatment.

Results

The results showed that there were significant differences ($p<0.05$) between treatments on egg yolk cholesterol content (Table 1 and Figure 1).

Table 1. Cholesterol content of quail egg yolk with probiotics and acidifiers.

Treatments	Cholesterol (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
P0	$202.45^{a} \pm 51.62$	$41.39^{a} \pm 0.001$	$126.53^{b} \pm 21.60$
P1	$88.34^{b} \pm 16.52$	$68.68^{c} \pm 5.19$	$74.42^{a} \pm 20.51$
P2	$84.53^{b} \pm 14.89$	$58.73^{b} \pm 4.82$	$76.95^{a} \pm 7.04$

Note: $^{(a,b)}$ Different superscript letters within a column indicate statistically significant differences between treatment groups ($p < 0.05$).

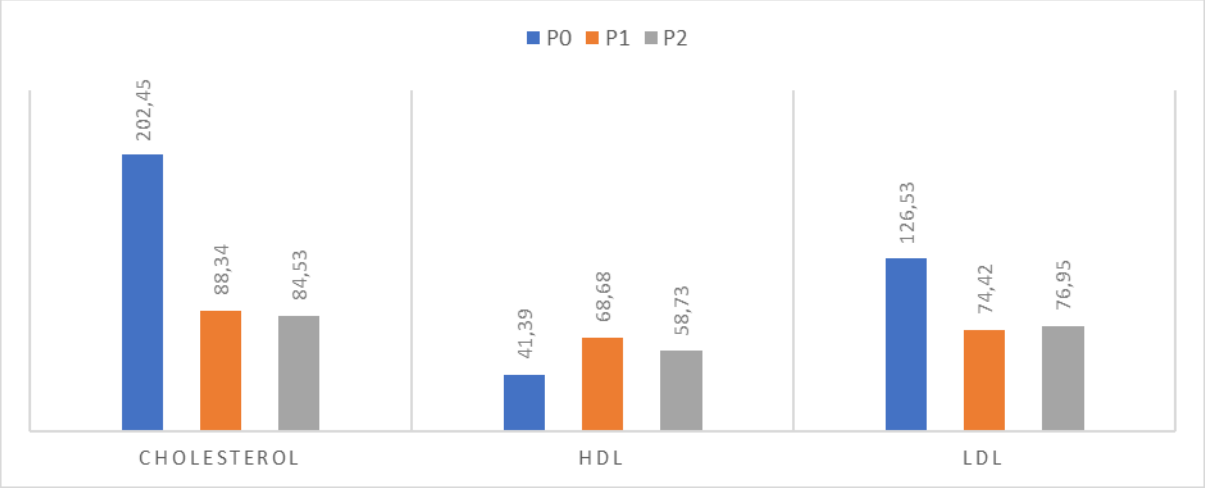

Figure 1. Cholesterol, HDL and LDL content in quail egg yolk treated with probiotics and acidifiers.

In the treatments that examined cholesterol levels in quail egg yolks, differences were seen if the control treatment (P0) levels were 202.5 mg/dl (milligrams per deciliter), then there was a decrease in both treatments with probiotics and acidifiers. Treatment 1 (P1), which used probiotics *Pediococcus pentosaceus* ABY118 1% and *Lactococcus lactis* 1% and acidifier of 0.5%, showed a significant decrease of 88.3 mg/dl. The second treatment (P2) showed a significant difference of 84.5 mg/dl but was not significant from the first treatment (P1). Treatment 2 (P2) used the same type of probiotic with a higher dose (*Pediococcus pentosaceus* ABY118 2% and *Lactococcus lactis* 2%) and an acidifier at 1%. The results of this study are in line with other researchers who have shown that the use of probiotics can lower cholesterol levels. Lactic acid bacteria are widely used as probiotic isolates to produce SCFA in the fermentation process (Lokapirnasari *et al.*, 2020a; Markowiak-Kopec and Slizewska, 2020; Yulianto *et al.*, 2021a; Cheng *et al.*, 2022; Thananimit *et al.*, 2022). *Pediococcus pentosaceus* belongs to the *Firmicutes* phylum. This phylum group can ferment undigested feed fiber into SCFA, especially butyrate (Yulianto *et al.*, 2021b).

Increased SCFA production in the intestinal lumen through dietary fiber intake can increase ApoA-I concentrations. Apolipoprotein A-I (ApoA-I) is the main protein in HDL particles. This protein can be obtained in the blood serum of chickens and most other animals, including quail. There is homology as high as 94.5% in the nucleotide

sequence comparison between ApoA-I chicken and quail, while in the amino acid sequence there is homology of 91.7%. This protein plays an important role in reverse cholesterol transport, which transports excess cholesterol in peripheral tissues such as blood vessel walls. These SCFAs enter the liver through the hepatic portal vein, which transports blood from the gastrointestinal tract. SCFAs, especially butyrate, can increase ApoA-I mRNA expression in the liver (Oku *et al*., 1997; Popeijus *et al*., 2021).

The main SCFAs, namely acetic, propionate, and butyric acids produced by the fermentation process in the digestive tract, are responsible for the reduction of cholesterol levels in blood plasma (Hara *et al*., 1998). SCFA can inhibit the effect of oxLDL in causing inflammatory cell injury, thereby suppressing atherosclerotic plaque formation and preventing coronary microcirculation dysfunction (Yi *et al*., 2022; Hong *et al*., 2024). SCFAs can reduce cholesterol synthesis, which can have an effect on reducing cholesterol levels in blood plasma (Hara *et al*., 1999). It can be concluded that SCFAs produced by probiotic bacteria can increase HDL levels and suppress LDL and cholesterol levels in the blood plasma, which are ultimately also transported to the egg follicles in the ovaries.

The use of acidifiers consisting of organic acids in animal feed can have beneficial effects by modulating the population of digestive tract microbiota by increasing the diversity and proportion of beneficial bacteria and suppressing the growth of harmful bacteria (Pearlin *et al*., 2020; Wei *et al*., 2021). The use of acidifiers has an influence on the structure of the GI microbiota community and the SCFAs it produces. High SCFA production is associated with low digestive tract pH, and conversely, high digestive tract pH will result in low SCFA production (Wangko, 2020; Firrman *et al*., 2022; Xie *et al*., 2024). Therefore, the use of acidifiers can increase SCFAs, which in turn will increase HDL levels and decrease cholesterol and LDL levels in blood plasma, which also means in egg yolk.

Observations on HDL levels in this study obtained the following results in the treatment groups. The control treatment (P0) without any treatment showed HDL levels as high as 41.39 mg/dl and showed significantly different from the P1 and P2 treatment. Treatment 1 (P1) showed a significant increase of 68.68 mg/dl and showed significantly different from the P0 and P2 treatment. Meanwhile, HDL levels in P2 treatment (58.73 mg/dl) also appeared significantly different from the control (P0) and P1 treatment. HDL levels in this second treatment (P2) decreased significantly compared to HDL levels in the first treatment (P1). Differences in HDL levels can be due to differences in the growth rates *of L. lacti* and *P. pentosaceus* ABY 118 at different pH. *L. lacti* can grow optimally at pH 6.3 to 6.9 and *P. pentosaceus* grows optimally at pH 6.0 to 6.5. Increasing the level of acidifiers from 0.5% to 1% will certainly lower the pH of the intestinal lumen which results in a decrease in their growth rate. These bacteria can produce SCFA which can increase expression of ApoA-I which is the main protein of HDL. A decrease in the growth rate of these two bacteria results in a decrease in SCFA levels which leads to a decrease in HDL levels (Sánchez *et al*., 2008; Pato *et al*., 2021; Tayyeb *et al*., 2019; Popeijus *et al*., 2021).

LDL levels observed in the P1 and P2 treatments in this study showed that the treatment of using probiotics and acidifiers can significantly reduce LDL levels compared with

the control treatment (P0). The LDL level in control treatment showed levels of 126.53 mg/dl, while in P1 the levels became 74.42 mg/dl and in P2 the levels were 76.95 mg/dl. LDL levels in probiotic and acidifier treatments P1 and P2 showed no significant difference. The decrease in cholesterol and LDL levels in quail egg yolk is closely related to the increase in HDL in egg yolk. HDL has cholesterol reverse transport and anti-inflammatory and antioxidant functions. HDL remove excess cholesterol from tissues and peripheral cells, including macrophage foam cells, carry it to the liver, and then channel it through bile, protecting against the development of atherosclerosis (Barter *et al*., 2004; Röhrl and Stangl, 2013; Zhou *et al*., 2015; Brites *et al*., 2017; Marques *et al*., 2018; Denimal, 2023; Feingold, 2024). The use of probiotics and acidifiers has an important role in reducing cholesterol and LDL levels in quail blood plasma due to an increase in HDL levels in quail blood plasma. Cholesterol is transported from the liver as a metabolic organ in the form of lipoprotein particles to the ovarian follicles.

There are two separate phases of highly intensive lipid metabolism on which the embryonic development of the chick depends. The first phase consists of lipid synthesis by the hen's liver and transport of these lipids to the ovaries for incorporation into the maturing oocytes that occur in the hen prior to egg laying. The second phase occurs during the final trimester of embryonic life, which occurs two weeks later, when these lipids are rapidly utilized by the developing embryonic tissues (Speake *et al*., 1998). The components in egg yolk are largely derived from precursors found in blood plasma. These precursors are made in the liver under the control of the hormone estrogen. In chickens, the main precursors of egg yolk are VTG (vitellogenin) and VLDL. Vitallogenin is a metal-binding protein, while VLDL is a type of TRL that is abundant in the blood plasma of laying hens. Vitellogenin and VLDL are transported through the bloodstream from hepatocyte cells to the ovaries. A portion of the yolk protein is then split into two, lipovitellin (HDL) and phosvitin. Triglycerides are transported to the yolk as β-lipoproteins, which are then incorporated into the yolk in the form of fat globules (Vieira *et al*., 1995; Utomo, 1997; Anton, 2007; Mushawwir and Latipuddin, 2013).

The yolk is composed of 50% water; the rest is mostly protein and lipids in a ratio of 1:2. Lipids present in egg yolk are in the form of lipoproteins. Egg yolk solids contain phosphoprotein (phosvitin), lipoprotein, and lipovitellin (HDL). Polysaccharides in the form of mannose-glucosamine are carbohydrates found in egg yolk. Lipovitellin (HDL) in egg yolk is found in granules associated with phosphoprotein (phosvitin). These granules also contain calcium (Ca) and iron (Fe) and about 4% LDL. HDL is the second group of yolk lipoproteins. The dry matter of HDL is about 1/6 of the yolk, while its protein is 36% of it. Egg yolk HDL consists of 75-80% protein and 20-25% lipids. The lipids in egg yolk consist of 65% phospholipids, 30% triglycerides, and 5% cholesterol. LDL in egg yolk contains 90-95% lipids (Anton, 2007; Mushawwir and Latipuddin, 2013). LDL in poultry comes from exogenous and endogenous sources. Exogenous sources of LDL are mainly from feed absorption, while endogenous sources are from synthesis in the liver. LDL and VLDL are synthesized in the liver. Both are transported through the blood plasma from the liver to the ovaries and then into the follicles by endocytosis. VLDL is

converted into LDL, together with LDL transported from the liver to form egg yolk LDL (Zhang *et al.*, 2022). Chicken egg yolk is mostly composed of 68% LDL, while HDL amounts to 16%, the remaining 10% livetin, and 4% phosvitin (Anton *et al.*, 2003).

Conclusion

Based on the results of the study, it can be concluded that the use of probiotics (*Pediococcus pentosaceus* ABY 118 1% + *Lactococcus lactis* 1%) and acidifier 0.5% and probiotics (*Pediococcus pentosaceus* ABY 118 2% + *Lactococcus lactis* 2%) + acidifier 1% can reduce cholesterol content, increase HDL content, and reduce LDL content compared to the control treatment without the use of probiotics and acidifier. The development of this research needs to be carried out for application at the industrial level and has prospects for the use of probiotics and acidifiers for healthy food production.

Approval of Ethical Commission

This study received approval from the Animal Care and Use Committee of the Faculty of Veterinary Medicine, Universitas Airlangga with a certificate number 1.KEH.080.05.2023.

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Author's Contribution

CEAP and WS involved in the interpretation and collecting of data and editing of the manuscript. ES involved in writing and preparing the final version of the manuscript. CEAP was responsible for collecting data and submitting the manuscript. All authors reviewed the paper and approved the final version of the manuscript.

Conflict of Interest

The authors have no conflicts of interest that could influence the research

Data Availability Statement

Data used in this study are available on request from the corresponding author.

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