Research Article

Effect of Feed Supplementation with Liquid and Powdered Probiotic Yogurt on the Lipid Profile of Chicken Egg Yolk

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Abstract | Probiotics can alter lipid metabolism and biosynthesis in the body, which affects the reduction of triglyceride, cholesterol, and fat levels in chicken egg yolk. This study evaluated the effect of supplementation of liquid and powdered probiotic yogurt at various levels of administration in reducing triglyceride, cholesterol, and yolk fat levels. The research was conducted at the Test Farm, Universitas Padjadjaran, from September to November 2023. Analyses were carried out at the Laboratory of Animal Physiology and Biochemistry, Faculty of Animal Husbandry, Universitas Padjadjaran. Thirty-five 40-week-old Isa Brown laying hens were randomized to seven experimental treatments and five replicates using a Completely Randomized Design (CRD). Treatments included T0: basal diet without probiotic yogurt (control); T1: diet and 2% liquid probiotic yogurt; T2: diet and 3% liquid probiotic yogurt; T3: diet and 4% liquid probiotic yogurt; T4: diet and 2% powdered probiotic yogurt; T5: diet and 3% powdered probiotic yogurt; T6: diet and 4% powdered probiotic yogurt. Supplementation of liquid and powdered probiotic yogurt resulted in a significant effect in reducing yolk cholesterol levels in 42- and 44-week-old hens and reducing yolk fat and triglyceride levels in 44-week-old hens ($p < 0.05$).

Keywords | Laying hens, Yolk, Probiotic, Yogurt, Lipid, Metabolism

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INTRODUCTION

Eggs are a relatively inexpensive food source and provide a variety of nutrients beneficial to the health of the human body, including proteins, fats, carotenoids, vitamins, essential compounds, and minerals (Alaqil *et al*., 2020). Eggs are a high source of protein, but the lipid content is also high, particularly in the yolk. Although eggs are nutritionally perfect, some consumers, especially those with degenerative diseases, may limit their consumption due to their high lipid content. The nutritional quality of eggs can be influenced by strain, age, diet, and environmental conditions. Improving superior egg quality such as lowering lipid levels through genetic selection or nutritional strategies in laying hens has been of recent interest. One effort to improve the nutritional quality of eggs is to reduce triglyceride, cholesterol, and fat levels through the supplementation of probiotics in laying hen diets (Adriani *et al*., 2023).

Probiotics are cultures of living microbiota that, once supplemented in feed will benefit animal health (Alaqil *et al*., 2020). Probiotics supplementation containing microbiota cultures of *Lactobacillus bulgaricus, Streptococcus* *thermophilus*, *Lactobacillus acidophilus*, and *Bifidobacterium bifidum* into broiler and layer diets can improve production performances, reduce oxidative stress, improve gut health, help the digestive processes, and suppress harmful microorganisms (Kumalasari *et al*., 2020; Adriani *et al*., 2021a). Probiotic supplementation in animal feed can result in the breakdown of feed nutrients into smaller and more easily absorbed forms (McSweeney and Sousa, 2000). On the other hand, probiotic supplementation in animal feed can alter lipid metabolism and biosynthesis in the body of livestock through various mechanisms, including a decrease in acetyl-CoA carboxylase activity (Cavallini *et al*., 2009; Adriani *et al*., 2018), cholesterol assimilation by probiotics (Tomaro-Duchesneau *et al*., 2014), production of bile salt hydrolase (BSH) enzyme by probiotics leading to bile salt deconjugation (Klaver and van der Meer, 1993; Begley *et al*., 2006), and inhibition of 3-hydroxy-3-methylglutaryl CoA reductase (HMGCR) (Adriani *et al*., 2023).

Probiotics used in this study are a consortium of microbiota *Lactobacillus bulgaricus, Streptococcus thermophilus, Lactobacillus acidophilus*, and *Bifidobacterium bifidum* in liquid and powder form derived from fermentation of milk into yogurt. Probiotic supplementation in liquid form in animal feed tends to be less effective and risks cross-contamination (Adriani *et al*., 2021a). Currently, powder-form probiotics are often used because of their easy application and longer shelf life (Kumalasari *et al*., 2020). However, the probiotic powder prepared by oven drying had low bacterial viability, but still beneficial when supplemented in animal feed (Adriani *et al*., 2021a; Kumalasari *et al*., 2020). Previous studies have shown that liquid and dry probiotic supplementation in feed tends to have a significant effect in reducing blood triglyceride, cholesterol, and total lipid levels in broiler chickens and laying hens (Mateova *et al*., 2009; Adriani *et al*., 2018; Alaqil *et al*., 2020). However, other studies have shown that probiotic supplementation in feed does not significantly affect lipid profile (Ramasamy *et al*., 2009; Kumalasari *et al*., 2020). Therefore, research into liquid and powdered probiotic yogurt supplementation in laying hen diets needs to be evaluated in terms of its effect on egg yolk lipid profile.

This study aimed to evaluate two forms of probiotic yogurt, liquid and powder, and their effect on egg yolk lipid profile. We hypothesized that probiotic yogurt supplementation in laying hen diets could reduce triglyceride, cholesterol, and fat levels in egg yolks.

MATERIALS AND METHODS

Bird and treatments

The research was carried out at the Test Farm, Universitas Padjadjaran, from September to November 2023. Analyses were carried out at the Laboratory of Animal Physiology and Biochemistry, Faculty of Animal Husbandry, Universitas Padjadjaran. Thirty-five Isa Brown laying hens, aged 40 weeks, and weighing 1.51±0.16 kg were obtained from a commercial distributor, PT Sapta Karya Megah, Indonesia. The study used an experimental Completely Randomized Design (CRD). Laying hens were randomized to seven treatments and five replicates. The list of treatments can be seen in Table 1.

Table 1: List of treatments.

Category	Treatment
T0	basal diet without probiotic yogurt (control)
T1	diet and 2% liquid probiotic yogurt
T2	diet and 3% liquid probiotic yogurt
T3	diet and 4% liquid probiotic yogurt
T4	diet and 2% powdered probiotic yogurt
T5	diet and 3% powdered probiotic yogurt
T6	diet and 4% powdered probiotic yogurt

The diet used in the study was commercial (EH 711) obtained from PT East Hope Agriculture Indonesia. Laying hens were reared for four weeks in an open house using individual battery cages with a cage size of 40 cm × 35 cm × 30 cm. The rearing cage environment has a temperature of 22-27 °C with relative humidity of 60-70%. The diet supplemented with probiotic yogurt according to the treatment was given to laying hens twice a day, in the morning and evening, as much as 120 g/head/day and drinking water was provided ad libitum. Feed and water containers and the housing environment are cleaned daily for disease prevention. Table 2 presents the nutrient content of the diets and probiotic yogurt analyzed based on the international method of chemical analysis (AOAC, 2005).

Liquid probiotic yogurt

The probiotics used contained a consortium of bacteria, including *L. bulgaricus, S. thermophillus, L. acidophilus*, and *B. bifidum*, obtained from PT Agritama Sinergi Inovasi, Indonesia. Bacterial consortium at 5% (v/v) was inoculated into 250 mL growth media de Man Rogosa and Sharpe (MRS), then incubation was carried out at 37 °C for 24 hours. Fresh cow milk obtained from North Bandung Dairy Cooperative (KPSBU Lembang) was pasteurized through heating at 70-80 °C. Furthermore, 5% of bacterial consortium in liquid form was added and homogenized into the pasteurized milk that had been cooled (37-40 °C). The milk fermentation process is then carried out for 14 hours at 40 °C (Rosiyanti *et al*., 2023). The liquid probiotic yogurt made in this study has a pH of 4.20 with a total of 3.82×10^7 CFU/mL lactic acid bacteria.

Powdered probiotic yogurt

Probiotic powder was made using a simple and inexpensive technology: Drying with an oven equipped with an exhaust fan. Probiotic yogurt powder is made by mixing liquid probiotic yogurt with encapsulation material (maltodextrin DE 10-12) as much as 5% (v/v) as a nutrient source to keep microorganisms alive (Adriani *et al*., 2021a). The encapsulation material was first dissolved using distilled water with maltodextrin: distilled water ratio of 1:1. The mixture was then homogenized and dried using an oven at 40 °C for 48 hours. The dried probiotic yogurt was then ground into powder. The probiotic yogurt powder made in this study has a pH of 4.25 with a total of 8.82×10^4 CFU/mL lactic acid bacteria. The total number of lactic acid bacteria in probiotic yogurt powder decreased due to heating during the drying process using an oven, which affects bacterial viability (Kumalasari *et al*., 2020).

Table 2: Nutrient content of the diet used in the study.

Treat-ment	Mois-ture (%)	Ash (%)	CP (%)	CF (%)	EE (%)	NFE (%)	ME (kcal/kg)
T0	8.48	15.21	17.57	4.77	8.03	54.42	2935.80
T1	10.22	15.32	17.99	4.77	8.11	55.81	2993.19
T2	11.09	15.37	18.21	4.77	8.15	56.50	3021.88
T3	11.97	15.42	18.42	4.77	8.19	57.20	3050.57
T4	8.45	15.00	17.63	4.67	7.97	54.72	2935.72
T5	8.44	14.89	17.66	4.63	7.95	54.87	2935.67
T6	8.42	14.79	17.70	4.58	7.92	55.02	2935.63

CP: crude protein; CF: crude fiber; EE: ether extract; NFE: Nitrogen free extract; ME: Metabolizable energy; T0: basal diet without probiotic yogurt (control); T1: diet and 2% liquid probiotic yogurt; T2: diet and 3% liquid probiotic yogurt; T3: diet and 4% liquid probiotic yogurt; T4: diet and 2% powdered probiotic yogurt; T5: diet and 3% powdered probiotic yogurt; T6: diet and 4% powdered probiotic yogurt.

Detection methods

Yolk analysis

The collection of chicken eggs for analysis of yolk fat, triglyceride and cholesterol levels was carried out on the last day of week 2 and week 4 for each treatment and each replicate. Analysis of crude fat content in egg yolk was carried out using the Soxhlet Extraction method, as referred to in AOAC (2005). The procedure for determining egg yolk triglyceride and cholesterol levels requires the extraction of lipids, separating triglycerides and cholesterol from other constituents, and measuring triglycerides and cholesterol (Dinh *et al*., 2011). The egg yolk lipid extraction process is carried out by weighing 1 gram of egg yolk sample and adding 5 mL of acetone: alcohol solution (1:1), then heated for 5 minutes using a water bath at 70 °C. Then, centrifugation was performed at 3,000 rpm for 15 minutes to obtain the lipid extract. Triglyceride and cholesterol levels in egg yolk were then assayed using a specialized kit from Biolabo, France. Triglyceride analysis was assayed using a specialized kit with catalog number REF 80019 according to the GPO (Glyserol-3-Phospatase Oxidase) method. Cholesterol analysis was assayed using a specialized kit with catalog number REF 80106 according to the Cholesterol Oxidase Peroxidase Amino-antipyrine (CHOD-PAP) method.

Statistical analysis

Data, including triglyceride, cholesterol, and fat levels, were analyzed statistically by Analysis of Variance (ANOVA) using IBM SPSS Statistics software (ver. 25.0; IBM Corp., NY, USA, 2017). Furthermore, Duncan's Multiple Range Test tested significant differences between treatments. Statistical significancy was set at $P<0.05$.

RESULTS AND DISCUSSION

Triglyceride levels

The effect of probiotic yogurt supplementation on egg yolk triglyceride levels can be seen in Table 3. Egg yolk triglyceride levels in 42-week-old hens ranged from 492.74-574.35 mg/dL, and in 44-week-old hens ranged from 603.44-716.19 mg/dL. Supplementation of liquid and powdered probiotic yogurt tended to reduce yolk triglyceride levels in laying hens. The decrease in egg yolk triglyceride levels showed a significant difference ($p < 0.05$) at 44 weeks of age. At 44 weeks of age, T6 showed the highest reduction in triglyceride levels at 15.74%, followed by T4 (11.26%), T1 (4.86%), T5 (3.09%), T3 (2.97%), and T2 (1.36%), compared to T0 or control.

Table 3: Effects of liquid and powdered probiotic yogurt supplementation on egg yolk lipid profile.

Parameters	Age	Treatments							P value
		T0	T1	T2	T3	T4	T5	T6	
Triglyceride (mg/dL)	42	574.35±16.48	517.94±29.23	558.30±34.95	497.98±35.10	492.74±18.27	506.50±32.09	496.26±21.99	0.259
	44	716.19±9.24^{a}	681.38±24.50^{ab}	706.48±15.12^{a}	694.95±11.69^{ab}	635.55±12.21^{bc}	694.09±30.97^{ab}	603.44±33.14^{c}	0.000
Cholesterol (mg/dL)	42	283.60±4.69^{ab}	300.94±8.05^{a}	272.89±4.41^{b}	271.67±7.34^{b}	273.36±4.22^{b}	244.28±7.92^{c}	247.28±4.73^{c}	0.000
	44	324.41±10.78^{a}	277.00±9.25^{b}	273.22±14.79^{bc}	266.95±8.26^{bcd}	240.78±13.60^{cd}	243.89±12.19^{bcd}	234.56±7.64^{d}	0.000
Fat (%)	42	35.85±0.83	35.38±0.56	34.62±0.58	34.21±0.26	35.02±0.44	34.77±0.35	33.78±1.24	0.424
	44	31.75±0.11^{a}	31.08±0.46^{ab}	29.86±0.77^{c}	25.65±0.41^{e}	31.25±0.26^{ab}	30.39±0.18^{bc}	27.68±0.61^{d}	0.000

Data are represented as mean ± standard error (n = 5). ^{a,b,c,d} Means in each row with different superscripts are significantly different ($p < 0.05$); Age in weeks; T0: basal diet without probiotic yogurt (control); T1: diet and 2% liquid probiotic yogurt; T2: diet and 3% liquid probiotic yogurt; T3: diet and 4% liquid probiotic yogurt; T4: diet and 2% powdered probiotic yogurt; T5: diet and 3% powdered probiotic yogurt; T6: diet and 4% powdered probiotic yogurt.

Previous studies have shown that liquid or dry probiotic supplementation in laying hen feed has a significant effect in reducing blood triglyceride levels (Kalavathy *et al*., 2003; Adriani *et al*., 2018; Alaqil *et al*., 2020). Meanwhile, other studies have shown that probiotic supplementation in layer feed tends to reduce blood triglyceride levels although not significantly different (Tang *et al*., 2017; Adriani *et al*., 2023). In this study, we observed that supplementation of liquid and dry probiotic yogurt in the diet of laying hens also tended to reduce triglyceride levels in egg yolk. The decrease in egg yolk triglyceride levels could be due to the supplementation of liquid and powdered probiotic yogurt, which affects the fatty acid synthesis process in the body of the hens. According to previous studies, microbiota in probiotics can effectively reduce the activity of acetyl-CoA carboxylase (ACC), which is an enzyme involved in the rate of fatty acid synthesis. Less secretion of ACC results in less formation of fatty acids and decreased fatty acid formation lowers blood triglyceride levels (Cavallini *et al*., 2009; Adriani *et al*., 2018; Rahmania *et al*., 2022). Thus, decreased blood triglyceride levels lead to decreased triglyceride levels synthesized for yolk formation. In addition, according to Adriani *et al*. (2018), probiotics can also assimilate cholesterol, leading to impaired micelle formation. Lower micelle formation decreases the uptake of lipids in the intestinal lumen, ultimately reducing the number of circulating triglycerides in the blood. This will lead to a decrease in the uptake of triglycerides synthesized by the laying hen's body for yolk formation.

Cholesterol levels

The effect of probiotic yogurt supplementation on egg yolk cholesterol levels can be seen in Table 3. Egg yolk cholesterol levels in 42-weeks old hens ranged from 244.28-300.94 mg/dL, and in 44-week-old hens ranged from 234.56-324.41 mg/dL. Supplementation of liquid and powdered probiotic yogurt tends to reduce yolk cholesterol levels in laying hens. The decrease in egg yolk cholesterol levels showed significant differences ($p < 0.05$) at 42 and 44 weeks of age. In 42-week-old hens, T5 showed the highest cholesterol level reduction of 13.87%, followed by T6 (12.81%), T3 (4.21%), T2 (3.78%), and T4 (3.61%), compared to the control. Meanwhile, in 44-week-old hens, T6 showed the highest cholesterol level reduction of 27.70%, followed by T4 (25.78%), T5 (24.82%), T3 (17.71%), T2 (15.78%), and T1 (14.61%), compared to T0 or control.

This study showed relatively normal egg yolk cholesterol levels. Egg yolk may contain 243-372 mg/dL cholesterol (Adeniyi *et al*., 2016; Zhao *et al*., 2022). In this study, we observed that supplementation of liquid and dry probiotic yogurt in the diet of laying hens tended to reduce triglyceride levels in egg yolk. In agreement with previous studies that showed that probiotic supplementation in laying hen feed has a significant effect in reducing cholesterol levels in both blood and egg yolk (Ramasamy *et al*., 2009; Tang *et al*., 2017; Alaqil *et al*., 2020). Previous research explained that the decrease in cholesterol levels in blood and eggs due to probiotic supplementation in laying hen feed can be due to probiotics being able to produce short chain fatty acids (SCFAs) and secondary bile acids to regulate lipid metabolism (He and Shi, 2017; Tang *et al*., 2017). Probiotics can produce SCFAs, including acetic, butyric, and propionic acids. Acetic acid is a significant substrate for cholesterol synthesis in the liver (Song *et al*., 2023), while butyric and propionic acid can lower cholesterol through inhibition of 3-hydroxy-3-methylglutaryl CoA reductase (HMGCR), an enzyme involved in the regulation of cholesterol biosynthesis (He and Shi, 2017; Adriani *et al*., 2021b, 2023). Another study found that microbiota in probiotics can assimilate cholesterol in the gastrointestinal tract, leading to reduced cholesterol absorption by enterocytes and more cholesterol excreted with feces (Tomaro-Duchesneau *et al*., 2014).

According to our previous study, the cholesterol-lowering effect on egg yolk can also be caused by bile salt hydrolase (BSH) produced by probiotics (Adriani *et al*., 2018). Probiotics can produce the BSH enzyme that deconjugates bile salts into the free form of cholic acid. Deconjugated bile acids are poorly soluble and less able to be reabsorbed by the gut, so they will end up being excreted. This leads to increase de novo synthesis to replace the lost bile acids and use the body's cholesterol as a precursor for bile acid formation (Tsai *et al*., 2014; Adriani *et al*., 2023). This will ultimately lead to a decrease in the accumulation and absorption of cholesterol in the chicken's body so that cholesterol synthesis for yolk formation will also be reduced. Moreover, our previous study also showed another variation of results, that probiotic supplementation in the diet of laying hens in the peak production phase could not reduce yolk cholesterol levels due to the high activity of reproductive hormones (Situmeang *et al*., 2024).

Fat levels

The effect of liquid and powdered probiotic yogurt supplementation on the yolk fat content of chicken eggs can be seen in Table 3. Egg yolk fat levels in 42-week-old hens ranged from 33.78-35.85%, and in 44-week-old hens ranged from 27.68-31.75%. Supplementation of liquid and powdered probiotic yogurt tended to reduce yolk fat levels in laying hens. The decrease in egg yolk fat levels showed a significant difference ($p < 0.05$) at 44 weeks of age. In 44-week-old hens, T3 showed the highest fat levels reduction of 19.20%, followed by T6 (12.81%), T2 (5.95%), T5 (4.28%), T1 (2.11%), and T4 (1.56%), compared to T0 or control.

This study showed relatively normal yolk fat levels.

According to Tomaszewska *et al.* (2021), chicken egg yolk can contain about 32% fat. The decrease in yolk fat content in this study was associated with a reduction in triglyceride and cholesterol levels in chicken egg yolk, since total fat in egg yolk contains 65.5% triglycerides, 28.3% phospholipids, and 5.2% cholesterol (Rosnah *et al*., 2022). A previous study showed a similar result that probiotic supplementation in laying hens' diets can significantly reduce yolk fat (Situmeang *et al*., 2024). The decrease in yolk fat levels due to probiotic supplementation was due to the inhibition of acetyl-CoA carboxylase (ACC) and 3-hydroxy-3-methylglutaryl CoA reductase (HMGCR) activities, which resulted in a decrease in cholesterol, low-density lipoprotein (LDL), and triglyceride levels. In contrast, high-density lipoprotein (HDL) levels and the expression of low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL) receptors will increase (Mushawir *et al*., 2021; Bansal and Cassagnol, 2023). The decrease in yolk triglyceride and cholesterol levels will eventually lead to a decrease in total yolk fat (Kharazi *et al*., 2022; Adriani *et al*., 2023). However, other studies have shown variations in the results that probiotic supplementation in laying hen feed has no significant effect in reducing fat content in eggs, this can be due to several factors, such as laying hen strain, age, dose, feed, and probiotic bacteria culture (Haddadin *et al*., 1996; Ramasamy *et al*., 2009).

In addition, in this study, probiotic yogurt powder supplementation tends to result in a higher reduction in cholesterol and triglycerides of chicken egg yolk compared to liquid probiotic yogurt supplementation. This can be due to adding an encapsulant (maltodextrin) to prepare dry probiotic yogurt, which also acts as a prebiotic. Prebiotics act as a source of nutrients and can be fermented by probiotics, resulting in increased production of SCFAs, strengthening of the intestinal mucosal layer and resistance to pathogen invasion, as well as promoting the reproduction and metabolism of probiotics while in the gastrointestinal tract (Ballini *et al*., 2023; Cummings and Macfarlane, 2002; You *et al*., 2022). According to Tang *et al*. (2017), supplementation of probiotics mixed with prebiotics into the diet of laying hens gave the most optimal results in improving the blood lipid profile of laying hens, compared to probiotic supplementation only.

CONCLUSIONS AND RECOMMENDATIONS

The study concluded that liquid and powdered probiotic supplementation in laying hens feed was statistically significant in reducing triglyceride and yolk fat levels in laying hens after four weeks of treatment and reducing cholesterol levels after two weeks of treatment. Overall, based on the results of this study, supplementation of probiotic yogurt powder in chicken feed in the T6 treatment tends to give the most optimum results in reducing triglyceride, cholesterol, and yolk fat levels.

Although this study showed the expected results, further research would be highly recommended regarding the use of probiotic yogurt powder through different drying methods and its addition in feed to further study its effect on the yolk lipid profile of laying hens at different strains and ages of hens during the production phase.

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NOVELTY STATEMENT

The supplementation of liquid and powdered probiotics in chicken feed and its effect on egg yolk lipid profile has been studied, and remarkable results have been shown. Supplementation of liquid and powdered probiotic yogurt containing a consortium of microbiota *Lactobacillus bulgaricus, Streptococcus thermophilus, Lactobacillus acidophilus,* and *Bifidobacterium bifidum* was effective in reducing triglyceride, cholesterol, and yolk fat levels. This study also showed that despite having lower total lactic acid bacteria, probiotic yogurt in powder form still produced beneficial effects in reducing triglyceride, cholesterol, and yolk fat levels.

AUTHOR'S CONTRIBUTION

All authors contributed equally to the writing of this manuscript.

Ethical approval

All procedures of this study have been reviewed and approved by the Ethical Review Committee for Animal Experiments, Directorate of Research and Technology, DRT with number: 2502/An.Res.ER/02/24.

Conflicts of interest

The authors have declared no conflicts of interest.

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