Research Article

Probiotic Powder Supplementation in Haematology and Biochemistry Blood Late-Phase Laying Hens

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Abstract | The probiotic powder can improve the value of hematology, reduce blood fat and increase the production of late-phase laying hens. The study was conducted to assess the impact of probiotic powder on the different physiological activities system of late-phase laying hens measured with hematological values and serum biochemistry. The research was carried out from March until April, 2022 at the Layer Farm Sukarapi Village, Universitas Padjadjaran, Indonesia. A total of 40 laying hens 90 weeks were randomly allocated to four treatments and five replications, with experimental Completely Randomized Design (CRD). The treatments were, T0: probiotic powder free-control diet. T1: ration and 2 % powder probiotic, T2: ration and 3% powder probiotic, T3: ration and 4% powder probiotic. Statistically, there was no significant differences (P>0.05) in hemoglobin, hematocrit, erythrocyte, leukocyte, cholesterol, and triglyceride blood levels. Meanwhile, our data showed statistically significant difference in glucose blood levels. However, the administration of powder probiotics of 3% and 4 % has improved the hemoglobin, hematocrit, erythrocyte, and leukocyte levels, and decreased cholesterol, and triglyceride blood levels.

Keywords | Probiotic powder, Late-phase laying hens, Hematology, Biochemistry, Lipid profile, Physiological traits, Immunostimulant, Gut micro flora

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INTRODUCTION

Egg production and quality will decrease with the increasing age of laying hens after the peak of egg laying (Liu et al., 2013). It makes restricted economic benefit of layer production. The lower productivity as a result of decreased physiological system function, particularly intestinal function. Supplementation of probiotics in feed is one effort to increase late-phase laying hen production. Probiotics play an important role in the gut, contributing significantly to performance and health (Adriani, 2005; Feng and Liu, 2022).

Probiotics are microorganisms that improve the balance of the intestinal flora ecosystem, it has an impact on improving intestinal health (Nangpal et al., 2012; Hill et al., 2014). *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, according to Adriani (2005), do not belong to the reliable probiotic group because they are reduced in the digestive tract, specifically the large intestine. Several probiotics such as *Lactobacillus acidophilus* and *Bifidobacterium bifidum* as used in this study were able to reach the large intestine, thereby decreasing the total pathogen in the large intestine (Adriani, 2005; Adriani et al., 2019). Probiotics are an alternative antibiotic wich can

increase livestock productivity. In this study, the probiotic powder was used to laying-hen because it was easy to apply and store long time. However, probiotic is very sensitive to heat because not all probiotic bacteria are resistant to high temperatures, so it is necessary to apply the appropriate method to produce the probiotic powder. In the study Adriani et al. (2020, 2021a, b, 2022) and Kumalasari et al., (2020), probiotic powder in broiler chickens or laying hens can efficiency ratio consumption, reduce feed conversion ratio, increase body weight gain, reduce abdominal fat weight, increase immunity, blood hematology, blood lipids, and other blood biochemistry.

Novelty of this study is probiotics in powder form for late-phase laying hens. Based on these findings, we hypothesize that the results of supplementation probiotic powder have a beneficial effect on the physiological system, especially the function of the digestive system, in order to increase laying hen production at 70 and 90 weeks. Therefore, in this study, we provided probiotic powder with cultures of *Lactobacillus bulgaricus, Lactobacillus acidophilus, Streptococcus thermophilus,* and *Bifidobacterium bifidum* in laying hens feed on haematology and blood biochemistry. The novelty in this study is the use of probiotic powder as a feed additive on hematological and biochemical blood of late-phase laying hens. This study planned to prove that the administration of up to 4% probiotics improves livestock health, as seen from hematology and blood biochemistry.

MATERIALS AND METHODS

Bird and ration

The study was conducted at Layer Farm Sukarapi Village in Sumedang, Indonesia, from March to April 2022. Analysis was carried out in Physiology and Biochemistry Nutrition Laboratory, Animal Husbandry Faculty, Universitas Padjadjaran, Indonesia. Forty layers of Lohman Brown strain at 70 and 90 weeks with an average weight of 1.8 kg were allocated randomly to four treatments and five replicates using an experimental Completely Randomized Design (CRD). The treatments were T0: probiotic powder free-control diet. T1: ration and 2 % powder probiotic, T2: ration and 3% powder probiotic, T3: ration and 4% powder probiotic (Adriani et al., 2022). The rations used in this study consisted of corn, rice bran, concentrate commercial (KSL-SP SINTA), and mineral mix. The rations were given to livestock as much as 120 grams/head/day plus probiotics according to the treatments. Table 1 shows the nutrient content and metabolic energy of the study ration.

Probiotic powder

Probiotics were used based on cow's milk with *Lactobacillus bulgaricus, Lactobacillus acidophilus, Streptococcus thermophilus,* and *Bifidobacterium bifidum* 5% (v/v). The probiotic powder was created by combining liquid fermented milk with skimmed milk. Maltodextrin was used for the encapsulation of microorganisms. Following homogenization, the mixture of probiotics was dried in a spray dryer at 160°C (inlet temperature) and 65-70°C (outlet temperature) (Juniawati and Ayu, 2019). In this study, probiotics were dry using the spray dryer method with coating maltodextrin and skimmed milk, so the viability of probiotic bacteria remained good (Adriani et al., 2021a). After drying, the total lactic acid bacteria count was $1.6x10^7$ CFU/g.

Detection method

Hematology blood measurements

The physiological system is measured with hematological values to check the health status and stress levels from erythrocyte, leukocyte, hemoglobin, and hematocrit levels. Blood samples (3ml) were collected from 40 laying hen per replicate, then placed in a heparin vacuum tube. Blood was taken from the wing vein (vena pectoralis externa). The blood samples were used for the analysis of erythrocytes, hematocrit, hemoglobin, and leukocytes. The levels of erythrocytes and leukocytes were analyzed using the hemocytometer method (Habiyah, 2015). Hematocrit levels were analyzed using a microcapillary hematocrit reader. Hemoglobin levels were analyzed using the Sahli method (Alfian et al., 2017).

Biochemistry blood measurements

The physiological system checks from the biochemical blood include cholesterol, triglycerides, and glucose which impact by the age factor. Blood samples (3ml) were taken

Table 1: Nutrient content and metabolic energy of study ration.

Treatment	Moisture	Ash content	Protein	Crude fiber	Ether extract	Carbohydrate	ME (kkal/kg)
T0	11.20	14.43	17.06	5.58	10.53	52.40	3867
T1	10.83	15.80	17.00	5.76	8.44	53.00	3733
T2	10.83	15.45	17.82	6.17	8.08	52.48	3743
T3	10.45	15.31	17.47	6.40	9.13	51.69	3811
Probiotic	9.77	5.58	27.50	3.44	13.66	49.82	4051

Note: T0= basal ration without powder probiotic; T1= basal ration + 2 % powder probiotic; T2= basal ration + 3% powder probiotic; T3= basal ration + 4% powder probiotic.

from 40 broilers in each replicate. The cholesterol and triglyceride levels were then determined at the Physiology and Biochemistry Laboratory, Animal Husbandry Faculty, Universitas Padjadjaran. To analyze the cholesterol, the Cholesterol Oxidase Phenylperoxidase Amino Phenozonphenol (CHOD-PAP) method was used. The triglycerides were analyzed using the Glycerol-3-Phosphate Oxidase (GPO) method. The glucose was measured using the glucose assay kit (Adriani et al., 2020).

Statistical analysis

The data were statistically analyzed using a univariate general linear model to see if there were any interactions or significant differences between treatments. Duncan's Multiple Distance Test was performed if the results were significantly different (Loh et al., 2014). The SPSS was used to analyze the data.

RESULTS

Haematological measurements

Hemoglobin levels

Table 2 shows the hemoglobin levels in this study ranged from 8.56-8.92 g/dl. This study showed that haemoglobin levels were not significantly different (P>0.05). Based on Duncan's test T2 and T3 treatment with 3% and 4% probiotics significantly increased compared to control at 1.86% and 4.2%.

Hematocrit levels

Table 2 shows the hematocrit in late-phase laying hens ranged from 22.2 to 23.8%. The results showed the hematocrit was below normal. This study showed that haematocrit levels were not significantly different (P> 0.05). Based on Duncan's test. T2 and T3 treatment with 3% and 4% probiotics significantly increased compared to control at 6.25% and 3.57%.

Erythrocyte levels

Table 2 shows the average erythrocytes in late-phase laying hens (Table 3) ranged from 1.73–1.85×10^6/mm^3. The results showed erythrocyte levels were below normal levels. This study showed that erythrocyte levels were not significantly different (P> 0.05. Based on Duncan's test, T2 and T3 significantly increased compared to T0 with increase each of 5.11% and 3.97%.

Leukocyte levels

Table 2 shows that the leukocyte levels in late-phase laying hens range from 77.14 – 79.96 x 10^3/mm^3. This study showed that leukocyte levels were not significantly different (P> 0.05). Based on Duncan's test, T2 and T3 treatment with 3% and 4% probiotics significantly increased compared to control at 3.65% and 2.88%.

Biochemistry blood measurements

Cholesterol blood levels

Table 3 showed cholesterol levels at week 70 range from 137.8-149.3 mg/dl, while at week 90 it ranges from 136.8-157.2 mg/dl. Statistical analysis showed that the results for lowering blood cholesterol levels were not significantly different (P>0.05). Based on Duncan's test, at week 70 treatment T1 was significantly different compared control with decrease at 7.7%. While at week 90, treatment P3 was significantly different compared control with decrease at 12.97%.

Table 2: Average of haematological values on late-phase laying hens.

Variable	Treatments			
	T0	T1	T2	T3
Hemaglobin (gr/dl)	8.56 ± 0.33 ^{a}	8.68 ± 0.72 ^{ab}	8.72 ± 0.13 ^{bc}	8.92 ± 0.57 ^{bc}
Hematocrit (%)	22.4 ± 0.89 ^{a}	22.2 ± 1.79 ^{ab}	23.2 ± 0.45 ^{bc}	23.8 ± 1.10 ^{bc}
Erythrocyte (10^6/mm^3)	1.76 ± 0.09 ^{a}	1.73 ± 0.14 ^{ab}	1.85 ± 0.09 ^{bc}	1.83 ± 0.08 ^{bc}
Leukocyte (x 10^3/mm^3)	77.14 ± 2.28 ^{a}	78.84 ± 5.05 ^{ab}	79.96 ± 6.28 ^{bc}	79.36 ± 5.77 ^{bc}

Note: T0 = basal ration without powder probiotic; T1 = basal ration + 2 % powder probiotic; T2 = basal ration + 3% powder probiotic; T3 = basal ration + 4% powder probiotic.

Table 3: Average of biochemistry blood on late-phase laying hens.

Variable	Weeks	Treatments			
		T0	T1	T2	T3
Cholesterol (mg/dl)	70	149.3 ± 12.50 ^{b}	137.8 ± 24.45 ^{a}	148.5 ± 8.58 ^{bc}	141 ± 8.73 ^{ab}
	90	157.2 ± 49.42 ^{b}	143.6 ± 30.85 ^{ab}	144.7 ± 16.71 ^{bc}	136.8 ± 29.72 ^{a}
Trygliceride (mg/dl)	70	740 ± 163.54^{c}	688 ± 135.67 ^{bc}	640 ± 211.66^{a}	673 ± 257.29 ^{ab}
	90	770 ± 311.23^{c}	750 ± 87.47 ^{bc}	565 ± 52.70 ^{a}	672 ± 256.37^{ab}
Glucose (mg/dl)	70	245 ± 15^{bc}	243 ± 7 ^{bc}	232 ± 12 ^{ab}	227 ± 13 ^{a}
	90	245 ± 8 ^{c}	239 ± 6 ^{c}	220 ± 8 ^{b}	203 ± 16 ^{a}

Note: T0 = basal ration without powder probiotic; T1 = basal ration + 2 % powder probiotic; T2 = basal ration + 3% powder probiotic; T3 = basal ration + 4% powder probiotic.

Triglyceride blood levels

Table 3 showed triglyceride levels range from 640-740 mg/dl. Statistical analysis showed that the results for lowering blood triglyceride levels were not significantly different (P> 0.05). Triglyceride levels with probiotic powder treatments showed a decrease compared to control at weeks 70 and 90. Based on Duncan's test, at weeks 70 and 90 treatment T2 significantly decreased compared to T0 at 13.5% and 26.62%.

Glucose levels

Table 3 showed glucose levels at week 70 range from 227-245 mg/dl, while at week 90 range from 203-245 mg/dl. Statistical analysis showed that lowering glucose levels at week 70 was not significantly different (P > 0.05). Based on Duncan's test, T3 significantly decreased compared to control and T1 with a decrease each 7.35 and 6.58%. Meanwhile, at week 90, statistical analysis showed significantly different (P<0.05) reduced glucose levels. The results of T3 with 4% probiotic powder reduced blood glucose levels the highest compared to other treatments. Based on Duncan's test, T3 was significantly lower than P0, P1, and P2 with a decrease each of 17.14%, 15.06%, and 7.72%.

DISCUSSIONS

Hemoglobin level in this study showed 8.56-8.92 g/dl. These results are relatively similar to Agboola et al. (2017) who reported that laying hens had hemoglobin levels of 6.90–8.02 g/dl. The results of this study are lower than Marono et al. (2017) of 10.1–11.1 g/dl and Abdel-Kareem and El-Sheikh (2017) who reported the hemoglobin level of laying hens as 9.81–12.86 g/dl. This is due to the difference in the age of the laying hens during the treatment. The study of Agboola et al. (2017) used older chickens than the study of Marono et al. (2017) and Abdel-Kareem and El-Sheikh (2017).

T3 showed the highest increase compared to the control of 4.2%. However, the administration of probiotic powder tends to increase hemoglobin levels. The more doses of probiotic powder add to the ration the more beneficial microbes can thrive and inhibit the growth of pathogenic bacteria. According to a study by Lutfiana et al. (2015), administration probiotics at 2% and 3% increased hemoglobin total in laying hens compared to 0% and 1% treatments. The administration of probiotic powder can produce protease enzymes that convert protein into amino acids in the digestive tract. The more amino acids in the body make the synthesis hemoglobin and the formation of erythrocytes fast. Protein especially the amino acid glycine and the mineral Fe is the main component of hemoglobin.

The proportion of red blood cells to total blood volume is known as hematocrit. The results showed the hematocrit was below normal. However, the administration of probiotic powder tends to increase hematocrit. According to Aderinola et al. (2013), the normal range of hematocrit in broilers is between 24–43%. The administration of probiotic powder does not have a toxic effect to inhibit nutrient absorption. The hematocrit indicates the presence of toxic factors which affect the formation or decrease of red blood cells not proportional to components of the blood. According to Hidayat (2011), pathogenic microbes in the digestive tract can produce toxins that enter the bloodstream and causes hemolysis of red cells. Besides that, hematocrit is proportional to erythrocytes and hemoglobin levels. In the P3 and P4, hematocrit levels were proportional to the erythrocyte levels and hemoglobin levels.

The main function of the erythrocyte is to carry oxygen from the lungs to the tissues. Erythrocyte was low indicates the capacity of the blood to carry oxygen is reduced (Aderinola et al., 2013). Erythrocyte levels in this study were below the normal range. According to (Çetin et al., 2010) the erythrocyte levels of laying hens ranged from 2.34-3.30 $10^6/mm^3$, while Agboola et al. (2017) reported that laying hens blood erythrocyte levels ranged from 2.17-2.69 $10^6/mm^3$. This is because laying hens in the study. The number of erythrocytes is influenced by age, gender, hormone, hypoxia, activity, nutrition, egg production, environmental temperature, and climatic factors. This study was used laying hens aged 90 weeks that have passed the second phase of production at the age of 42-72 weeks. The administration of probiotic powder tends to increase erythrocytes. This is because probiotics improve the health of the digestive tract which affects protein fulfillment. The result of protein catabolism in the digestive tract will increase the formation of erythrocytes and hematocrit. Besides that, erythrocyte is affected by hemoglobin concentration and hematocrit. A high erythrocyte followed by a high hemoglobin level. The erythrocytes and hemoglobin will increase when the oxygen content in the blood is low. The oxygen content can stimulate the increase in the number of erythrocytes and hemoglobin levels. High activity will increase erythrocytes because consume a lot of oxygen.

Leukocytes are the body's immune system at the site of damage, providing cellular and humoral defense against foreign substances. This study showed that the laying hens had high leukocyte levels. Marono et al. (2017) that the leukocyte level of laying hens is between 20.9 to 21.1 $10^3/mm^3$. In another study Agboola et al. (2017) found blood leukocyte levels in laying hens ranged from 22.27 to 25.27 $10^3/mm^3$. According to Hrabáková et al. (2014) the level of leukocyte count, heterophile, lymphocyte, and basophil, heterophile to lymphocyte ratio and concentration of aspartate aminotransferase at

the end of egg laying period (84 weeks) were increased. The physiological system of laying hens is affected by age, affecting the immune system. The administration of probiotic powder tends to increase leukocyte. According to this study, high doses of probiotic powder can improve the body's immune system and cause chickens to produce more leukocytes. Paryad and Mahmoudi (2008) observed that probiotic supplementation highly increased leukocytes in broiler chicks than without probiotics. An increase in leukocyte count denotes a humoral and cellular response to pathogenic agents that cause disease in the body. The probiotic powder can reduce pathogenic microbes in the digestive tract while increasing beneficial microbes. High leukocyte levels are caused by a disease response, including infections and foreign substances. This process will result in the formation of antibodies to protect the body from pathogens through phagocytosis and the production of antibodies. Based on the study, late-phase laying hens at 90 weeks with probiotics T3 (3%) and T4 (4%) showed great haematological values.

This study showed relatively normal cholesterol levels. In line with Basmacioglu and Ergul (2005), that normal blood cholesterol levels in laying hen range from 52–148 mg/dl. Cholesterol levels that exceed the normal threshold can lead to hypertension (Adriani et al., 2018). Cholesterol levels with probiotic treatment showed a decrease compared to control. The administration of probiotic powder has an effect in reducing blood cholesterol late-phase laying hens. Reduction of cholesterol levels by probiotic powder in several ways. The role of probiotic powder in conjugating bile salts in the intestine and preventing cholesterol synthesis. Probiotic produces the enzyme Bile Salt Hydrolase (BSH) that makes bile acids deconjugated in the form of free cholic acid. Deconjugated bile acids are less soluble and thus less absorbed in the intestine. They are more likely to excrete cholesterol and its fractions in the feces, reducing cholesterol accretion in the body. Probiotics also produce Short-Chain Fatty Acids (SCFA) like butyric acid and propionic acid. Butyrate can inhibit liver cholesterol synthesis. Propionic acid has been shown to reduce cholesterol levels by inhibiting the HMG-CoA reductase enzyme's activity in cholesterol synthesis. When cholesterol levels are low, propionic acid inhibits the incorporation of acetate into blood cholesterol, as acetate is a precursor in forming cholesterol (Kumalasari et al., 2020). Probiotic can inhibit cholesterol with produce the enzyme coprostanol which convert cholesterol into coprostanol. Coprostanol Can not (typo) absorbed by the small intestine (Adriani et al., 2017; Nosser and Khalifa, 2020; Adriani et al., 2020; Kumalasari et al., 2020).

This study showed triglyceride levels were higher than normal levels. The normal triglyceride levels in the blood of laying hens is < 150 mg/dl (Basmacioğlu and Ergül, 2005; Ambarwati and Iriyanti, 2017). Factors that affect serum triglyceride are age, diets, estrogen, fat formation, and disease. This study used 90 weeks laying hens. The highest cell growth at this age is fat cells. The growth of body tissue starts from nerves tissue, bone, muscle, and fat. The higher the age as similar to triglyceride level (Hendry et al., 2019). Triglyceride levels decrease because of HMG-CoA reductase, which lowers cholesterol levels and increases LDL receptors. The decrease in triglyceride synthesis in the liver is primarily responsible for the decrease in triglyceride concentrations in plasma (Adriani et al., 2020). Probiotic bacteria can also produce esterase and lipase enzymes, which convert free fatty acids from triglycerides into different ester forms in the digestive tract (Sumardi et al., 2016).

This study showed relatively normal glucose levels. Normal glucose levels in the blood of laying hens are around 197-299 mg/dl (Adewole et al., 2021). Glucose levels with probiotic treatment showed a tendency to decrease compared to control. Organic compounds are broken down into smaller molecules by microbial enzymes during fermentation, which has various physiological effects (Setiawan et al., 2020).

CONCLUSIONS AND RECOMMENDATIONS

The administration of probiotic powder with 3% and 4% probiotics can increase hematological values including hemoglobin, hematocrit, erythrocyte, and leukocyte. Also, improved serum biochemistry includes cholesterol, triglyceride, and glucose blood. This study shows that probiotic powder can improve the physiological system of late-phase laying hens.

This present study shoed that hematology profile are unaltered. The recommendation is that giving 3% and 4% probiotic powder to hens can improve blood lipid and the value of hematology. Finally, adding 3% and 4% probiotic powder to the feed ration is very important to be recommended.

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NOVELTY STATEMENT

Probiotics have been widely used in laying hens at a young or productive age, and this research aims to extend product life and increase production in late-phase laying hens.

Probiotics consortium powder using *Lactobacillus bulgaricus*, *Streptococcus thermophilus*, *Lactobacillus acidophilus,* and *Bifidobacterium sp.* have a beneficial effect on physiology system especially the function of digestive systems in order to increase laying hen production at 70 and 90 weeks.

AUTHOR'S CONTRIBUTION

LA: Idea and research design.
CK: writing the manuscript and editing.
LA and CK: Revision.
LA, RL, and ES: *In vivo* collection, laboratory analysis, and statistical analysis.

Conflict of interest

The authors have declared no conflict of interest.

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