Effect of Liquid Probiotic Supplementation in Drink Water on Blood Cholesterol and Immune Response in Japanese Quails (Coturnix coturnix japonica)

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Abstract. The aim of this research was to understand the effect of liquid probiotic supplementation in drink water on blood cholesterol (HDL, LDL, Triglyceride) level, hemoglobin level (Hb), plasma hematocrit level and total of plasma protein (TPP) of quails. Prohibition of antibiotics in poultry, resulting in increased probiotic offers on the market. Each probiotic has an advantage in increasing productivity and immunity of quails. The research was conducted as an experimental research and used completely randomized design. Four treatments were done in this research, which was control (drink water without probiotic), drink water added by probiotics A (containing Lactobacillus sp., Rhodopseudomonas sp., Streptococcus sp., Saccharomyces sp.), probiotic B (containing Bacillus careus, Azotobacterpaspalii, Bacillus laterosporu, Bacillus lentus, Bacillus licheniformes, Bacillus pumilusCorynebacterium, Pseudomonas fluorescensSarcinalutea Staphylococcus epidermis Staphylococcus thermophyllus Lactobacillus sp. Saccharomyces cerevisceae and Phicia anomola) and probiotic C (containing Lactobacillus casei, Saccharomyces cerevisceae, Rhodopseudomonas palustris, Molases, water). The obtained all data were then analyzed by analysis of variance and if the result showed a significant effect, further analysis will be done by honestly significant difference test. The analysis of variance showed that variety of fluid probiotic supplementation in drink water showed had no significant effect (P>0.05) on the on blood cholesterol, HDL level, LDL level, triglyceride, but had significant effect (P<0.05) on Hb, plasma hematocrit and TPP level. The research concluded that liquid probiotics supplementation in drink water will increase immune response but not able to reduce blood cholesterol of quails.

Keywords : antibiotics, robiotics, drink water, cholesterol, quails.

1. Introduction
Prohibition of use of Antibiotic Growth Promotors (AGP) in livestock feed including poultry, because antibiotic residues become toxic to consumers [1]. In addition antibiotics can create microorganisms that are resistant to the body of livestock and humans, especially pathogenic bacteria such as Salmonella, Eschericia coli and Clostridium perfinens [2]. Alternative AGP substitute products are now easy to obtain and widely available on the market. The products consist of enzymes, probiotics,
prebiotics, essential oils, acidifiers/organic acids, symbiotic, bioactive plants/herbs [3]. Probiotics that can be used are usually derived from bacteria, yeast or mold. Probiotic functions generally in addition to regulating microbial balance in the gastrointestinal tract, also serves to increase immunity, support growth, increase efficiency, feed conversion as well as help optimize absorption of nutrients, including fat [4]. Probiotics circulating in the market at this time many use bacteria from the species of Lactobacillus and Bifidobacterium. Commonly used Lactobacillus species include Lactobacillus acidophilus, Lactobacillus johnsonii, Lactobacillus casei, Lactobacillus rhamnosus, Lactobacillus gasseri, Lactobacillus reuteri, while the species of Bifidobacterium is Bifidobacterium bifidum, Bifidobacterium longum, Bifidobacterium breve and Bifidobacterium Infantis. The Genus Lactobacillus, Bifidobacterium and others have different roles on the health of the digestive organs, performance and immunity of livestocks [4,5].

Quail eggs are a good source of protein for humans, but quail eggs are included in the list of foods that can raise the level of human blood cholesterol. The effort to produce high quality quail eggs with higher productivity, always related to quail blood profile. Based on these descriptions it is necessary to be examined more deeply the effectiveness of probiotics that are widely circulated in the market against the cholesterol levels and livestock immunity.

2. Methodology
The research used 100 brood off-female quail material (3 weeks old), commercial quail feed, anti stress supplement, ND1 vaccine. The tools used in this study included a 30x40x45 cm battery enclosure with 20 boxes of cages, feeders and drinkers, light bulbs, balance weighing machines, thermohygrometer, syringes, a sets of routine blood measuring and a sets of cholesterol measuring.

The quails were let in the one-week preliminary before treatments. Probiotics supplement was offered gradually: Day 1 = 25% treatment liquid probiotic + 75% drinking water; Day 2 = 50% treatment liquid probiotic + 50% drinking water; Day 3 = 75% treatment liquid probiotic + 25% drinking water; Day 4 onward = 100% treatment liquid probiotic. liquid probiotic was mixed with drinking according to dietary level, i.e. 2ml/l for three months. Feed was offered twice a day—at 7 am and 3 pm. Water was given ad libitum three times a day every morning, afternoon and evening.

The research was conducted as an experimental research and used completely randomized design. Four treatments were done in this research, which was control (drink water without probiotic), drink water added by probiotics A (containing Lactobacillus sp., Rhodopseudomonas sp., Streptococcus sp., Saccaromyches sp.), probiotic B (containing Bacillus careus, Azotobacterpaspladi, Bacillus laterosporu, Bacillus lentus, Bacillus licheniformes, Bacillus pumilusCorynbacterium, Pseudomonas fluorescensSarcinalutea Staphylococcus epidermis Staphylococcus thermophyllus Lactobacillus sp, Saccharomyces cereviscae and Phicia anomola) and probiotic C (containing Lactobacillus casei, Saccharomyces cerevisae, Rhodopseudomonas palustris, Molases, water).

The observed research variables are HDL, LDL, Triglyceride, hemaglobin, plasma hematocrit level and total of plasma protein of quails. The obtained all data were then analyzed by analysis of variance and if the result showed a significant effect, further analysis will be done by honestly significant difference test.

3. Results and Discussion
Blood Cholesterol
The average of total blood cholesterol, HDL, LDL and Triglyceride blood level of quails which are given liquid probiotics can be seen in Table 1. HDL quail cholesterol which was given liquid probiotics showed higher compared to controls, whereas blood triglycerides decreased compared to controls.

The analysis of variance showed that variety of fluid probiotic supplementation in drink water showed had no significant effect (P>0.05) on the on cholesterol, HDL level, LDL level, triglyceride level of quails blood. These results show that the three probiotics found on the market have not been able to significantly lower cholesterol levels. It is suspected that a much-traded probiotic traded its
content of low lipolitical bacteria so that it has not been able to increase lipase enzyme activity Consequently the process of fat breakdown is still the same as control. The probiotics used in this study contain bacteria that are effective in boosting the immune and digestibility of coarse fibers, that in general the local probiotic contains bacteria that function in optimizing function of the gastrointestinal tract to digest and absorb feed nutrients [6]. Probiotics have a working way of "competitive exclusion" which is a probiotic competing on the surface of the intestine that is worn by pathogens to cling [7], so that pathogenic bacteria cannot be attached and excreted from the intestines. Improved feed digestibility results in increased absorption.

| Table 1. Effect of liquid probiotic on total blood cholesterol, HDL, LDL and triglyceride blood level of quails |
|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|
| Total blood cholesterol (mg/dl) | HDL- cholesterol (mg/dl) | LDL- cholesterol (mg/dl) | Triglyceride (mg/dl) |
|--------------------------------|--------------------------|--------------------------|----------------------|
| P0 217.77± 15.07 | 23.32±5.95 | 194.28±25.82 | 305.71±91.81 |
| P1 253.33± 14.37 | 29.04±7.37 | 224.30±25.79 | 257.14±72.84 |
| P2 217.78±13.88 | 29.52±8.03 | 192.26±24.02 | 242.86±45.17 |
| P3 237.73±13.95 | 35.2±13.01 | 202.58±26.88 | 268.58±34.11 |

ns sign in the same column shows that the treatment has no significant effect (p> 0.05)

Probiotics can produce statins, which are 3-hydroxy-3-methyl-glutaryl-Coa reductase inhibitors (HMG-Coa reductase) which are the regulating enzymes of cholesterol, LDL, VLDL, and blood triglyceride levels [8]. All three of these probiotics have the same effectiveness in inhibiting the rate of fatty acid synthesis. Probiotic microorganism prevented the elevation of triglycerides during the protocol. The HMG-CoA reductase inhibitors have been considered effective at lowering triglyceride – or more specifically, VLDL triglyceride – levels. *Lactobacillus acidophilus* is the effective bacteria to decreasing blood cholesterol [9]. *Lactobacillus acidophilus* serves to increase the activity of lipase enzymes that function in the hydrochloric monoglycerides, digliserides and triglycerides to produce free fatty acids and glycerol. Free fatty acids will be hydrolyzed to cholesterol by 5 phase i.e. (1) Convert acetyl CoA to HMG-CoA; (2) converts HMG-CoA to Mevalonate; (3) Mevalonat is converted into a base molecule of isoprene, isopentenyl pyrophosphate (IPP) along with loss of CO2; (4) The IPP is converted into Squalen; (5) Squalen is converted into cholesterol [10,11].

**Immune Response**

Blood acts as a bioindicator of the status of nutrients, toxicity, and the body's physiological condition of the livestock [10]. Blood can be used as an indication of physiological disorders in the body of livestock because blood acts as a media homeostasis and media for the formation of antibodies related to the immune system [12,13]. Blood parameters that can indicate physiological and immune functions include hemoglobin, hematocrit and total plasma proteins. Furthermore, value of hematocrit and total plasma proteins can be used as indicators to suspect the effect of environmental stress and the quail immune system [14]. The results of the analysis of variances showed that the influence of different liquid probiotics are of real pangs (P< 0.05) to the hemoglobin, hematocrit, and total female quail plasma proteins. This is because probiotics play an important role in quail health. A healthy quail then the it is hemoglobin levels in the normal range is 11.57-12.49 g/dl [14] the normal hematocrit value is between 30-40% [15] and the total normal protein plasma is between 2.78-4.35 g/dl [16]. Normal hemoglobin indicates that the amount of oxygen that is able to be bound by normal hemoglobin, the percentage of blood volume containing red blood cells and the total plasma protein is also normal. In the process of erythropoesis requires the basic ingredients of protein, glucose and various activators. Some of the erythropoesis activators are microminerals [17]. Probiotics also produce a number of important nutrients in the immune system and host metabolism, such as vitamin B (pantothenic acid), pyridoxin, niacin, folic acid, cobalamin, and biotin as well as important antioxidants such as vitamin K [13,18]. Number of probiotic microbes produce compounds/substances necessary to aid the digestive process of certain feeding substances in the gastrointestinal tract, i.e. enzymes [19]. One is on bacteria
Bacillus sp. which produces protease enzyme. Protease enzyme is an extracellular enzyme that serves to hydrolyze proteins into amino acids that the body needs. Protease enzymes are needed to break down protein into the necessary amino acids in the process of hemopoiesis.

**Table 2. Effect of liquid probiotic on hemoglobin, hematocrit dan total plasma protein**

|          | Hemoglobin (g/dl)* | Hematocrit (%)* | Total Plasma Protein (g/dl)* |
|----------|--------------------|-----------------|-------------------------------|
| P0       | 8.12±0.3*          | 25.6±1.67*      | 3.04±0.26*                   |
| P1       | 9.23±0.22*         | 30.4±2.61*      | 3.62±0.49*                   |
| P2       | 12.34±0.49*        | 35.2±4.60*      | 4.23±0.25*                   |
| P3       | 12.18±0.51*        | 30.4±4.77*      | 4.08±0.65*                   |

*Different superscripts on the same column indicate a noticeable effect (p<0.05)

The concentration of plasma proteins or serum proteins can be a share of health indicators and physiological status, including immune and inflammatory responses [20]. The effect of probiotics gives a positive effect on the blood plasma concentration of quail proteins (P<0.05), it suggests that microbes contained in liquid probiotics prevent damage to the colon so that the process of protein absorption from the intestines is optimal. Additionally probiotics also increase the enzyme secretion of protease and enzyme activity related to protein metabolism causing the rate of protein metabolism in the liver increases so that the total plasma protein levels are increased [21]. Increasing the total levels of plasma proteins will affect the levels of globulin, especially γ-globulin that functions in body defense (antibodies) [22]. In addition, Lactobacillus sp produces bacterisine compounds that are able to increase the body's immunity to reduce the residual metabolism and stimulate system immune system [13, 23]. The total plasma protein contained in the blood of the Japanese quail at the age of 42 days amounted 4.15mg/dl [24].

4. Conclusion
Liquid probiotics that are on the market have not been able to lower blood cholesterol but are able to improve female quail blood profile.

Acknowledgement
Best appreciation to Dr. Sri Rahayu, M.Si. and Ir. Winarto Hadi, SU. for the support of liquid probiotics, and the students who have helped the implementation of the research.

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