Effect of probiotic *Lactobacillus paracasei* on hematology and relative weight of lymphoid organs of broiler

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Abstract. One alternative approach can be done to replace the antibiotic growth promotant function (AGP) in broiler, which is the use of probiotic microorganisms. The aim of this study was to look at the effects of probiotics on *Lactobacillus paracasei* on hematomal conditions and lymphoid organs. This study used a completely randomized design consisting of 4 treatments with 5 replications, each replication using 4 broiler chickens. Treatment consists of controls (0 mL/day), probiotics 1 mL/day, probiotics 3 mL/day, and probiotics 5 mL/day. The probiotics used in this study, namely *Lactobacillus paracasei* (5.8 x 10⁷ CFU/mL) were given through drinking water. The results showed that the treatment had no significant effect (p>0.05) on the number of erythrocytes, hematocrit percentage, hemoglobin level, number of leucocytes, and erthrocyte index. Likewise, treatment had no significant effect (p>0.05) on the relative weights of bursa fabricius, thymus, and spleen exchanges. The values shown in hematology parameters are all within the normal range. Whereas in the lymphoid organ parameters there is a tendency for the relative weight of lymphoid organs to be greater in the treatment of *Lactobacillus paracasei* than in controls. Probiotics *Lactobacillus paracasei* used in this study did not respond poorly to hematological features and can be used as an immunomodulator in broilers.

1. Introduction

Antibiotics have been successfully used as growth promotant at sub-therapeutic doses in poultry feed to improve health status and performance in broilers by reducing the presence of pathogenic bacteria in the digestive system. But along with the development of food requirements, antibiotics have been banned from being used in poultry feed as a promoter of growth due to the emergence of antibiotic resistance and the presence of residues in poultry products. Therefore an alternative approach can be used to replace the function of antibiotic growth promotant (AGP), which is the use of probiotic microorganisms [1]. In 2001, an Expert Consultation of international scientists working on behalf of the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) debated the emerging field of probiotics. One output was a reworking of the definition of probiotics to the following: “live microorganisms which when administered in adequate amounts confer a health benefit on the host. Since then, this definition has become the most widely adopted and accepted version worldwide [2].
The immune system of broilers, especially in the first month, is not well developed and they are susceptible to bacterial infections caused by *Campylobacter jejuni*, *C. perfringens*, *Salmonella enterica* and *Escherichia coli* [3]. Probiotic administration is an effort to reduce pathogenic bacterial colonies in the digestive tract, so that the health condition of the digestive tract will be better. The results of the study were given *Lactobacillus sp.* through drinking water has proven to reduce the amount of *Escherichia coli* in broiler excreta [4]. Moreover, probiotics help in metabolism of minerals and synthesis of vitamins (Biotin, Vitamin-B1, B2, B12 and K), which are responsible for proper growth and metabolism [5].

Generally, microorganisms that are widely used as probiotics include lactic acid bacteria, such as *Lactobacillus*, *Streptococci*, *Bifidobacteria*, *Bacillus spp.* and fungi *Saccharomyces cerevisiae*, *Saccharomyces boulardii* and *Aspergillus oryzae*. The results showed that administration of probiotics can improve immune function in broilers maintained for 42 days [6]. The potential health benefits associated with using a probiotics include improved digestion, stimulation of gastrointestinal immunity and increased natural resistance to enteric disease [7]. One indicator that can be used to determine the health status of livestock, namely to see the description of livestock hematology. Therefore, this study was designed to evaluate the hematological response and the relative weight of broiler lymphoid organs against the administration of probiotics *Lactobacillus paracasei*.

2. Methods

2.1. Research procedure
A total of 80 strains of DOC broiler Cobb 500 were placed randomly in the experimental unit using a Completely Randomized Design (CRD). There are 4 treatments consisting of 5 replications, each replication containing 4 DOC broiler. Probiotics used as a treatment contain *Lactobacillus paracasei* (5.8 x 10^7 CFU/mL). Probiotic treatment dose consisted of control (0 mL/day), 1 mL/day, 3 mL/day, and 5 mL/day. Probiotic treatment is given for 35 days through drinking water. Rations and drinking water are given ad-libitum. The composition of starter and finisher rations is presented in table 1.

2.2. Hematological features
Taking blood samples was done at the end of the study directly on the axillary veins of the wing as much as 3cc using spoite containing anticoagulants. Red blood cell count (erythrocytes) and hematocrit percentage and leukocytes were calculated based on the method of [8]. Hemoglobin levels are calculated based on the method of [9]. MCV (Mean Corpuscular Volume), MCH (Mean Cell Haemoglobin), MCHC (Mean Corpuscular Haemoglobin Concentration) Calculated based on methods [10].

2.3. Lymphoid organs
Measurement of lymphoid organs, namely fabricius bursa, thymus, and spleen is done by means of broilers cut, then the internal organs are separated from the body of the chicken that has been cut. The relative weight of lymphoid organs is calculated based on the comparison between the weight of the lymphoid organs (g) with the final weight of the broiler (g) multiplied by 100%.

2.4. Statistic analysis
All data collected were subjected to analysis using one way ANOVA procedure of SPSS (SPSS version 21). The effect of treatment difference will compared using Duncan’s multiple area test at 5% level (p<0.05).
Table 1. Ingredients and chemical composition of basal ration (%).

| Ingredient          | Basal ration |  
|---------------------|--------------|
|                     | Starter  | Finisher |
| Yellow corn         | 53   | 60     |
| Rice bran           | 6    | 5      |
| Soy meal            | 28   | 21.2   |
| Vegetable oil       | 3    | 3.3    |
| CaCO3 (%)           | 0.80  | 1.0    |
| Dicalcium phosphate | 0.3   | 0.2    |
| DL-Methionin        | 0.1   | 0.2    |
| L-Lysin             | 0.3   | 0.5    |
| Vitamix*            | 0.5   | 0.3    |

Calculated composition:
- Crude protein: 22.75\% in Starter, 20.11\% in Finisher
- Energy Metabolism (kcal/kg): 3030 vs. 3105
- Crude fiber: 3.6\% vs. 3.3\%
- Fat: 6.7\% vs. 6.9\%
- Phosphor: 0.79\% vs. 0.71\%
- Calcium: 1.43 vs. 1.43
- Metionin (%): 0.35 vs. 0.32
- Lysin (%): 1.17 vs. 1.00

*Each one kilogram contains Vitamin A 4000,000IU, Vitamin D3 800,000IU, Vitamin E 4,500mg, Vit K3 450mg, Vitamin B1 450mg, Vitamin B2 1,350mg, Viamint B6 480mg, Vit B12 6mg, Ca-dP 2,400mg, As folate 270mg, Nicotinic acid 7,200mg, choline chloride 28,000mg, DL-Met 28,000mg, L-Lys 50,000mg, Fe 8,500mg, Cu 700mg, Mg 18,500mg, Zn 14,000mg, Co 50mg, I 70mg, Se 35mg, and antioxidants.

3. Results and discussion

3.1. Hematological

The hematological picture of broilers treated with *Lactobacillus paracasei* through drinking water for 35 days is presented in Table 2.

Table 2. Hematological features of broilers maintained for 35 days.

| Variables                  | Control | 1 mL/day | 3 mL/day | 5 mL/day | P-Value |
|----------------------------|---------|----------|----------|----------|---------|
| Erythrocytes (10⁶/mm³)     | 2.50 ± 0.06 | 2.52 ± 0.06 | 2.62 ± 0.09 | 2.67 ± 0.09 | 0.39    |
| Hemoglobin (g/100 mL)      | 8.44 ± 0.3  | 8.58 ± 0.2  | 8.84 ± 0.2  | 8.72 ± 0.4  | 0.90    |
| Hematocrit (%)             | 27.4 ± 0.8  | 28.0 ± 0.5  | 28.4 ± 0.5  | 29.2 ± 0.5  | 0.16    |
| Leukocyte (10³/mm³)        | 18.4 ± 0.3  | 18.6 ± 0.6  | 20.7 ± 0.8  | 21.1 ± 0.87 | 0.49    |
| MCV (fl)                   | 109.82 ± 4.6 | 111.53 ± 4.4 | 109.17 ± 4.9 | 109.7 ± 4.5 | 1.00    |
| MCH (Pg)                   | 33.74 ± 0.9  | 34.11 ± 0.9  | 33.91 ± 1.3  | 32.77 ± 2.0  | 0.90    |
| MCHC (%)                   | 30.92 ± 1.4  | 30.67 ± 0.7  | 31.14 ± 0.8  | 29.96 ± 1.9  | 0.92    |

MCV (Mean Corspuscular Volume), MCH (Mean Cell Haemoglobin), MCHC (Mean Corpuscular Haemoglobin Concentration)

There is a tendency for an increase in the number of erythrocytes as the dose of probiotic *Lactobacillus paracasei* increases. The lowest erythrocyte values obtained in this study were found in
the control treatment \((2.5 \times 10^6/mm^3)\) and the highest at treatment 5 mL/day \((2.67 \times 10^6/mm^3)\). These values are still in the normal range of chicken erythrocytes, which is 2.5–3.5 \(10^6/mm^3\). The results of variance showed that the treatment had no significant effect \((p>0.05)\) on the value of erythrocytes. Probiotics \textit{Lactobacillus paracasei} given as a treatment in this study were able to improve the hematological picture of broilers. This can be seen in the value of erythrocytes which increases with increasing doses of \textit{Lactobacillus paracasei}. Therefore \textit{Lactobacillus paracasei} indirectly contributes to the absorption of food substances needed in the formation of erythrocytes. However, the value of erythrocytes in the study is still low compared to the results of a study on 42-day-old broilers who were given multi-strain probiotics showing their erythrocyte values \(2.9 \times 10^6/mm^3\). The difference in the results of the two studies showed that broiler age and number of strains (probiotic microbes) were used to influence the value of broiler erythrocytes [11]. One of the factors that influence the hematological value in animals, namely age. Further said, the age of poultry erythrocytes averaged 28 to 35 days [12].

Hemoglobin in erythrocytes functions to carry oxygen and cause red blood to appear. The lowest hemoglobin value in the study, namely in the control treatment \((8.44 \text{ g/dL})\) and the highest at 3 mL/day treatment \((8.84 \text{ g/dL})\). The results of variance showed that the treatment had no significant effect \((p>0.05)\) on the value of hemoglobin. The results of this study are in line with the research of which showed no difference \((p>0.05)\) between controls and the administration of probiotic \textit{Pediococcus acidilactici} \(10^9 \text{ CFU/g}\) to hemoglobin values in one week, four weeks broiler age and six weeks [13]. The value of hemoglobin in this study was lower than the results of a study in broilers who were given multi-strain probiotics for 42 days, namely \(9.7 \text{ g/dL}\) [11].

Hemoglobin is an oxygen transportation device that is located in erythrocytes so that a decrease in the amount of hemoglobin can occur due to a disturbance of erythrocyte formation (erythropoiesis). Previously it was explained that the value of erythrocytes in this study in the treatment of \textit{Lactobacillus paracasei} was still within the normal range. Therefore an increase in the amount of hemoglobin in the treatment of \textit{Lactobacillus paracasei} is also still in the normal range. According to [11], the higher hemoglobin concentration in the chicks received probiotics which resulted in better iron salt absorption from the small intestine.

The percentage of hematocrit values tends to increase with increasing dosage of \textit{Lactobacillus paracasei} treatment. The lowest hematocrit value was found in the control treatment \((27.4\%)\) and the highest at treatment 5 mL/day \((29.2\%)\). These values are still within the normal range. The results of variance showed that the treatment had no significant effect \((p>0.05)\) on the hematocrit value. Increased hematocrit values are associated with increased erythrocyte values. Hematocrit values are positively correlated with erythrocyte size but negatively correlated with the fluid concentration in the chicken body. According [14] an increase in hematocrit values has the little benefit because the viscosity (thickness) of blood will increase then it will slow blood flow in the capillaries and increase the work of the heart.

A decrease in hematocrit values can be found in anemic conditions, namely due to a lack of erythrocyte production, erythrocyte damage, and erythrocyte size [15]. In this study, although there was an increase in hematocrit value along with the increase in the dose of \textit{Lactobacillus paracasei}. However, it does not have a negative effect on the physiological conditions of poultry, because the value is still within the normal range for chickens \((22–35\%)\). If the value of erythrocytes, the hematocrit value, and the hemoglobin value are normal, then it shows that the animals are physiologically healthy.

The lowest leukocyte value in this study was found in the control treatment \((18.4 \times 10^3/mm^3)\) and the highest in the treatment of 5 mL/day \((21.1 \times 10^3/mm^3)\). However, these values are still in the normal range of chicken leukocyte values, namely \(12-30\ (10^3/mm^3)\). The results of variance showed that the treatment had no significant effect \((p>0.05)\) on the value of leukocytes. The increase in leukocyte value along with the increasing dose of \textit{Lactobacillus paracasei} showed that isolates obtained in this study could function as immunomodulators. According to [16], probiotics of lactic acid bacteria are one of the immunomodulators that play a role in improving the body's mechanisms both specifically and nonspecifically. The average leukocyte count in this study tends to increase with increasing doses of \textit{Lactobacillus paracasei}, but not statistically significant \((p>0.05)\). Results of the study using probiotics
*Lactobacillus acidophilus* and commercial multi-strain probiotics also showed higher leukocyte counts in the probiotic treatment of *Lactobacillus acidophilus* 18.96 x 10^3/mm^3 compared to controls 15.39 x 10^3/mm^3 [17]. Similarly, the results of the study compared the provision of probiotics (*Saccharomyces cerevisiae*) 18.45 x 10^3/mm3 with commercial enzymes (Zyme®) 13.22 x 10^3/mm^3 and the combination showed higher leukocyte counts than controls [18].

The MCV value in this study is in the range of 109.82-111.53 fl. The results of variance showed that the treatment had no significant effect (p>0.05) on the MCV value. The MCV values in all treatments in this study are still within the normal range. This is in accordance with [19], that the average value of MCV 90-140 fl. Therefore the nutritional needs of broilers in this study are sufficiently available for erythrocyte formation. According [20], MCV will be of high value when anemia occurs which indicates folic acid deficiency, while MCV which is lower when anemia indicates iron deficiency.

The MCH value in this study is in the normal range of 32.77–34.11 Pg. The results of variance showed that the treatment had no significant effect (p>0.05) on the value of MCH. According [19] the average value of MCH in chickens is 33-47 Pg. The results of this study are lower than the reports from the results of value of MCH in the 35th day old broiler of strain Cobb 53 (Pg) [21]. According to [22], large (macrocytic) erythrocytes usually have a high MCH value and on the contrary small erythrocytes have a low MCH value.

The MCHC value is a measure of the mass of hemoglobin contained in erythrocytes or the amount of hemoglobin per erythrocyte. The MCHC value in this study is in the range of 29.96% -31.14%. This value is still in the normal range. This is according to [19], that the MCHC value is normal, which is 26%-35%. The results of variance showed that the treatment had no significant effect (p>0.05) on the value of MCHC. The MCHC value obtained in the study can be an indicator, that the broiler has a hemoglobin concentration that corresponds to the number of erythrocytes in the blood fluid. This means that hemoglobin performs its function well, namely the amount of hemoglobin that carries oxygen (O₂) from the lungs or in the bloodstream, which is carried by erythrocytes for sufficient tissue needs and carries carbon dioxide (CO₂) from tissue to the lungs. This condition certainly helps the body's metabolic activities better.

Hematological conditions of livestock will experience changes along with physiological changes internally and externally. Internal changes can be caused by increasing age, nutritional status, health, body heat, and stress. External changes can be caused by diseases of microorganisms and changes in environmental temperature [23]. The hematological parameters measured in this study, namely erythrocytes, hemoglobin, hematocrit, leukocytes and the erythrocyte index in whole shows normal conditions. Therefore the probiotic treatment of *Lactobacillus paracasei* used in the study gave a positive response to the physiology of the blood so that it could be said to be a good broiler health condition.

### Table 3. Percentage of lymphoid organs in broilers aged 35 days.

| Lymphoid organs | Treatment | Control | 1 mL/day | 3 mL/day | 5 mL/day | P-Value |
|-----------------|-----------|---------|----------|----------|----------|---------|
| Bursa of Fabricius (%) | 0.152 ± 0.04 | 0.186 ± 0.03 | 0.186 ± 0.04 | 0.194 ± 0.03 | 0.17 |
| Thymus (%) | 0.31 ± 0.09 | 0.30 ± 0.06 | 0.32 ± 0.09 | 0.31 ± 0.05 | 0.99 |
| Spleen (%) | 0.17 ± 0.02 | 0.20 ± 0.06 | 0.18 ± 0.04 | 0.20 ± 0.03 | 0.62 |

### 3.2. Lymphoid organs

The biggest percentage of fabricius bursa weight was found in the treatment of 5 mL/day (0.194%) and the smallest in the control treatment (0.152%). Likewise, the largest percentage of the spleen was found in the treatment of 1 mL/day and 5 mL/day (0.20%) and the lowest was in the control treatment (0.17%). Furthermore, the largest thymus weight percentage was found in the treatment of 3 mL/day (0.32%) and the smallest treatment was 1 mL/day (0.30%). The results of variance analysis showed that the treatment
had no significant effect ($p>0.05$) on the weight percentages of fabricius bursa, thymus, and spleen. The weight percentages of lymphoid broiler organs in this study are presented in table 3.

The probiotic treatment of *Lactobacillus paracasei* used in this study tended to have higher values of the weight of the fabricius bursa and spleen than the controls. Therefore, overall treatment of *Lactobacillus paracasei* has a greater percentage of lymphoid organs (fabricius bursa and spleen) than controls. The results of this study are in line with the research conducted [24] which found that the relative weight of the fabricius bursa was not affected by the administration of probiotics *Bacillus subtilis* ($10^9$ CFU/kg). Likewise the results of [25] study showed no difference ($p>0.05$) in the administration of probiotics *Bacillus subtilis* ($2 \times 10^{10}$ CFU/g ration) to the relative weight of the fabricius bursa, except that the weight of the thymus and spleen showed differences in significantly ($p<0.05$).

Poultry that has a greater relative weight of the fabricius bursa will be more resistant to various diseases. Increased in the relative weight of fabricius bursa may be attributed to increase the number of immune cells. The bursa of fabricious in probiotic- treated group showed an increase in the number of follicles with high plasma cell reaction in the medulla [24]. The fabricius bursa will experience optimum growth when it reaches sexual maturity. The results of study showed that the relative weight percentages of the 4-week-old of fabricius bursa average of broiler chicken 0.19% of live weight [26], whereas according [27] 0.098%.

Immunosuppression will be indicated by the presence of stresses, obstacles, or disturbances in the components of the immune system, among others, directly damaging and disrupting the growth of primary lymphoid organs (fabricius bursa and thymus), as well as secondary lymphoid organs (spleen) [28]. Very small primary and secondary lymphoid organs are reactions to cases of immunosuppression that last for a long time. Animals that have a large relative lymphoid weight tend to be resistant to various diseases [12]. A relatively small spleen indicates a low appetite. The spleen that is attached to the stomach helps distribute nutrients because it also produces erythrocytes [20]. The spleen will develop rapidly during an inflammatory disease (clinical symptoms). The percentage of spleen weight from live weight is 0.18%. The percentage of thymus weight is 0.48% [27].

**4. Conclusion**

Probiotics *Lactobacillus paracasei* does not give a bad response to the hematological picture of broilers. The relative weight percentage of lymphoid organs tends to increase with increasing probiotic doses. Therefore probiotic *Lactobacillus paracasei* can be used as one of the immunomodulators in broilers.

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