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Hematology profile of Pengging duck (*Anas javanica*) in starter period in response to *Lactobacillus Salivarius* I-11 probiotics as a feed additive and an alternative to replace antibiotics

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ABSTRACT

The study was conducted with the purpose of studying the hematology profile of Pengging Duck (*Anas javanica*) in starter period given with probiotic *Lactobacillus salivarius* I-11 as feed additives as an alternative to replace antibiotics. The completely randomized design with 4, 5 replications and 10 units. There were 200 DOD male Pengging Ducks that are classified into 4 different treatments, that were: Treatment P0= Control, P1= Antibiotics, P2= 1 ml Probiotics *Lactobacillus salivarius* I-11; P3= 2 ml Probiotics *Lactobacillus salivarius* I-11. The parameters were duck blood profiles (in terms of the number of erythrocytes, leucocytes, hemoglobin and hematocrit) and lymphocyte cell types (lymphocytes, heterophil and heterophil-lymphocytes ratio). Statistical analysis showed that control ducks had significantly lower amounts of erythrocytes, leucocytes, hemoglobin and hematocrit ($P < 0.05$) than those that were given antibiotics and probiotics. The result of statistical analysis showed that duck treated with *Lactobacillus salivarius* I-11 had percentage of lymphocyte cell and ratio of real lymphocyte heterophil ($p < 0.05$) lower than the control treatment group, but it is not significantly different in comparison to the antibiotics treatments. Providing I ml of *Lactobacillus salivarius* I-11 probiotics as feed additives can replace the use of antibiotics in ducks during the starter period which was observed from their hematological profile.

Introduction

Efforts to increase duck productivity should start from raising the ducklings in the starter period (early period). Raising the duck in starter period faces various problems, including the high mortality rate. The main factor causing the high mortality rate of duckling in starter period is the non-optimal function of gastrointestinal tract and low body endurance from the pathogen bacteria attack. Pathogenic bacteria that often cause infection and death to poultry are *Escherichia coli* and *Salmonella pullorum* (Charlton *et al*., 2000).

When the broiler ducklings are intensively raised in the starter period, feed additives are used to increase productivity, to reduce mortality, and to improve feed use efficiency. Feed additives that are often used on a commercial scale are subtherapeutical, and pharmaceutical-type antibiotics as growth promoters. The types of antibiotics that are often used as antibiotic growth promoters are: zinc-bacitracin, monensin, Chloroxytetracycline, virginiamycin, benzyl penicillin, and tetracycline (Etebu and Arikekpar, 2016). The use of antibiotic growth promoter causes disadvantageous effects as it is absorbed and buried in duck meat causing antibiotic residue in duck meat. The use of antibiotics leads to the development of bacterial populations that are resistant to antibiotics and thus require continuous doses to reach the expected effect (Gunal *et al*., 2006). Provision of probiotics as feed additives is an alternative to replace the antibiotics.

Lactobacillus salivarius I-11 is an isolate of lactic acid bacteria (BAL) which is isolated from the intestine and potentially functions as probiotics (Sumarsih *et al*., 2014). Probiotic bacteria given orally can affect the body's metabolic system (Hattingh and Viljoen, 2001), as well as blood hematologic status (Aboderin and Oyetayo, 2006). Giving probiotics is beneficial for the livestock in balancing the intestinal microflora (Mountzouris *et al*., 2007; Kabir, 2009), improving the immune system (Shahani and Walker, 2000) and can improve blood profile (Astawan *et al*., 2011). Blood is one of the parameters of animal health status because blood is a component that has an important function in the physiological arrangement of the body. Giving the Isolate Probiotics *Lactobacillus salivarius* I-11 is expected to maintain the ducks in starter period, observed from its hematological profile.

The study was conducted with the purpose of studying hematology profile of Pengging Duck (*Anas javanica*) in starter period supplemented with probiotic *Lactobacillus salivarius* I-11. The benefit of this study is to provide the hematological information of Pengging Duck (*Anas javanica*) in starter period with probiotic *Lactobacillus salivarius* I-11 as feed additives is an alternative to replace the antibiotics.

Materials and methods

Research materials

The research was conducted at Feed Technology Laboratory of Livestock Department of Animal Science and Agriculture Faculty of Diponegoro University. The materials used are: Probiotic isolates of *Lactobacillus salivarius* I-11, isolated from the caecum of healthy adult Pengging Duck, 200 Day Old Ducks (DOD) of male Pengging Duck, fumigation equipment, battery cage, Antibiotics (zinc-bacitracin) and feed BW 1 with Crude Protein content = 21% and Metabolic energy = 3000 kcal / kg.

Animal experiment and feeding

There are 200 DOD male Pengging Ducks that are treated into 4 different treatments, that are: Treatment P0 = Control, P1 = Antibiotics, P2 = 1 ml Probiotics *Lactobacillus salivarius* I-11; P3 = 2 ml Probiotics *Lactobacillus salivarius* I-11. One g of antibiotics is given and it is mixed in feed since the age of 1 day on treatment P1. The provision of *Lactobacillus salivarius* I-11 probiotic dropped by mouth 1-2 ml (depending on treat-

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ments) / duck / day with BAL concentration of 109 CFU/ml during maintenance period. Preparation of the cage is conducted by cleaning the cage then disinfecting, liming and fumigation. The heating lamp is used for the maintenance of early ducklings. Feeding BW 1 and drinking water is done in *ad libitum*. The maintenance is done up to 14 days.

Blood sampling and hematology analysis

At the end of maintenance or starter period, duck blood profiles and lymphocyte cell types were observed. Blood samples were taken by syringe through the lower wing vein (brachialis), after which the wing veins were covered with 70% alcohol cotton to avoid infection. Blood is then put into a container tube with anticoagulant ethylene diamine tetra acetic acid (EDTA). The EDTA tube is inserted into an ice flask and taken to the clinical pathology laboratory of the Faculty of Veterinary Medicine of Gadjah Mada University for observed duck blood profiles and lymphocyte cell types.

Experimental design and data analysis

The experimental design used was complete randomized design (RAL) of unidirectional pattern, with 4 treatments and 5 replications with 20 experimental units each. The data were tested by various analyses and if the result showed effect of treatment, then in order to know the difference between treatments, it followed by Duncan multiple range test (Steel and Torrie, 1994).

Results

Effect of Lactobacillus salivarius I-11 to the blood profile of duck in starter period

The probiotics treatment gave a positive role in the blood profile (the number of erythrocytes, hemoglobin and hematocrit) and it was higher ($p < 0.05$) than other treatments, but it is not significantly different in comparison to the antibiotic treatments (Table 1). The statistical analysis shows the result that control group had significantly lower amounts of erythrocytes, leucocytes, hemoglobin and hematocrit ($P < 0.05$) than those that were given antibiotics and probiotics. Duck groups treated with probiotics *Lactobacillus salivarius* I-11 showed a better blood profile than the control, but it is not significantly different in comparison to the antibiotic treatments.

Effect of probiotic Lactobacillus salivarius I-11 to blood type leucocytes of the duck starter period

The result of *in vivo* test of the effect of *Lactobacillus salivarius* I-11 on type of Leukocyte of post hatch ducks can be seen in Table 2. The result of statistical analysis showed that duck treated with *Lactobacillus salivarius* I-11 had lymphocyte cell and heterophil to lymphocyte ratio ($p <0.05$) lower than the control treatment group, but it is not significantly different in comparison to the antibiotic treatments. The control ducks with no probiotics and antibiotics had a significant percentage of lymphocytes ($p <0.05$) which is higher than the other group of ducks with different treatments.

Discussion

The Probiotic treatment of *Lactobacillus salivarius* I-11 improved blood profile (in terms of the number of erythrocytes, leucocytes, hemoglobin and hematocrit) during the starter period (Table 1). Probiotics that have antimicrobial properties can protect cell membranes. Cell membrane damage can be caused by the adhesion of pathogenic bacteria that can cause cell wall leakage and reduced the number of erythrocyte cells (Aboderin and Oyetayo, 2006). Erythrocytes count of ducks ranged from 1.80 to 3.82×10^6/µl (Mitruka and Rawnsley, 1981).

The Probiotic-treated ducks had significantly higher number of leukocyte ($p <0.05$) than control treatments, but it is not significantly different in comparison to the antibiotic treatments. The range of normal leukocytes is 13.40 -33.20 $\times 10^3$/µl (Mitruka and Rawnsley, 1981). The treatment of probiotics in ducks shows that the probiotics are capable to act as an immuno modulator. Some strains of lactic acid bacteria can stimulate the immune response by activating antibodies, and improving macrophage activity (Bodera and Chcialowski, 2009).

The group of ducks with probiotic treatment had significantly higher Hb ($p <0.05$) than control. The amount of Hb depends on the absorption of iron (Fe) (Kullisaar *et al*., 2001). The presence of pathogen bacteria such as *Salmonella pullorum* infection which damages the intestinal wall causes the intestinal lumen to be unable to absorb Fe, thus it inhibits the Hb formation. The range of normal Hb ducks is 9.00-21.00 g/dl (Mitruka and Rawnsley, 1981). The control group of ducks had significantly less hematocrit ($p <0.05$) doses than other treatment groups. The decline in hematocrit number of the Control ducks group was caused by intestinal mucosal damage due to attachment of pathogenic bacteria that causes the nutrients not to be absorbed properly. The hematocrit is the percentage of erythrocytes in 100 ml of blood (Astawan *et al*., 2011). The range of

Table 1. The mean of blood profile of posthatch duck with various treatments for 14 days.of maintenance.

Treatments	Erythrocytes (x 10^6/µl)	Leukocytes (x10^3/ µl)	Hb (g/dl)	Hematocrit (%)
Control	1.66±0.01[b]	15.10±0.06[a]	8.20±0.07[b]	28.00±0.12[b]
Antibiotic	2.13±0.02	13.60±0.04[b]	9.80±0.08[a]	35.21±0.14[a]
Probiotic 1 ml	2.84±0.04[a]	13.50±0.02[b]	11.20±0.09[a]	41.56±0.16[a]
Probiotic 2 ml	2.44±0.08[3]	13.40±0.08[b]	10.20±0.07[a]	36.44±0.11[a]

Different superscripts in the same column showed significant differences ($p <0.05$)

Table 2. Duck lymphocytes and heterophil counts in starter period with various treatments for 14 days of maintenance.

Treatments	Lymphocytes (Cell/ µl)	Heterophil (Cell/ µl)	H/L
Control	4690±7.02[b]	4720±6.12[b]	1.01±0.06[a]
Antibiotic	4375±511[a]	3650±4.35[a]	0.83±0.06[b]
Probiotic 1 ml	4430±5.06[a]	3613±3.34[a]	0.81±0.06[b]
Probiotic 2 ml	4326±6.07[a]	3630±5.11[a]	0.84±0.06[b]

Different superscripts in the same column showed significant differences ($p <0.05$)

normalducks hematocrit is 32.60 - 47.50% (Mitruka and Rawnsley, 1981).

The treatment of probiotic *Lactobacillus salivarius* I-11 gives a positive role in post-hatch duck leukocytes. The giving of probiotics decreases the percentage of lymphocytes and the lymphocyte heterophil ratio (Table 2). Lymphocytes are cells that are often found in leukocytes and have a primary function of response to foreign substance by forming antibodies (Baratawidjaja, 2002). Lymphocytes are an important element in the immune system that has the function of responding to antigens by forming antibodies (Yalcinkaya *et al*., 2008). Heterophils are the largest component of leukocytes and play a major role in phagocytic and bacteriocidal. The percentage ranges of heterophil and normal duck lymphocytes, are 1930 – 4980 and 1300 – 7350 cell/ Cell/ µl (Mitruka and Rawnsley, 1981). The indicator of body resilience in poultry livestock can be seen from ratio of lymphocyte heterophil. The higher the ratio number of the lymphocyte heterophil, the higher the stress level of the duck as a form of adaptation to the environment (Huff *et al*., 2005).

The giving of *Lactobacillus salivarius* I-11 probiotics is able to enhance the cellular and humoral immune systems such as the increased number canand proliferation of lymphocyte cells, interferonin-12 (IL-12), interleukin-12 (IL-12), IL-10 and Immunoglobulin (Ig) A, E, G and M (Gackowska *et al*., 2006; Bodera and Chcialowski, 2009). The treatment of probiotic gave a positive role in the higher percentage of real lymphocyte cells ($P < 0.05$) and the ratio of real lymphocyte heterophil ($p < 0.05$) was lower than the control treatment, but it is not significantly different in comparison to the antibiotic treatments. The results of the study showed that the treatment of administering 1 ml of probiotics was more efficient because all blood profile parameters (in terms of the number of erythrocytes, leucocytes, hemoglobin and hematocrit) were not significantly different from the treatment of administering 2 ml of probiotics and could replace the administration of antibiotics in ducks during the starter period. This proves that giving probiotics as a feed additive can replace the use of antibiotics in ducks during the starter period.

Conclusion

Providing I ml of *Lactobacillus salivarius* I-11 probiotics as feed additives can replace the use of antibiotics in ducks during the starter period which was observed from their hematological profile.

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Conflict of interest

The authors declare that they have no conflict of interest regarding the publication of this paper.

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