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The Effects of Prebiotic Mannan Oligosaccharide (MOS) Supplementation on Hematology Profile of Native Chickens (*Gallus gallus domesticus*)

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ABSTRACT

Native chickens (*Gallus gallus domesticus*) are an important source of animal protein due to their adaptability and relatively simple management. However, their productivity remains limited, which poses a significant challenge in poultry farming. One promising strategy to improve the health and productivity of native chickens is supplementation with Mannan Oligosaccharides (MOS), a prebiotic known to enhance gut microflora balance, strengthen immune responses, and support overall health. This study aimed to evaluate the impact of MOS supplementation on the hematological profile of native chickens. A true experimental method with a control group pre-test post-test design was employed. The results demonstrated that MOS supplementation significantly improved the hematological profile of native chickens. Notable increases were observed in the red blood cell (RBC) count to 2.54×10^6 cells/mm³, white blood cell (WBC) count to 20.30×10^3 cells/mm³, hemoglobin concentration to 11.4 g/dL, and hematocrit value to 31%. These findings highlight the potential of MOS supplementation to enhance the health and productivity of Native chickens through improvements in key hematological parameters.

Keywords: native chicken, mannan oligosaccharides, prebiotics, hematological profile

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INTRODUCTION

Free-range chicken is one of the sources of animal protein that is sought after and in demand by the public, because it has the advantage of easy and simple maintenance with high nutritional value. The native chicken population in 2019 reached 301,761,386 heads with a contribution to national poultry meat production of 8.33% or equivalent to 292,329 tonnes. Based on statistical data, the native chicken population is only about 9.52% of the broiler population (3,169,805,127 birds). Meanwhile, the total egg production of native chickens in Indonesia is only 251 tonnes or contributes 4.44% to poultry egg production (Directorate General of Livestock and Animal Health, 2020). Based on Livestock Statistics data from the Directorate General of Animal Husbandry of Indonesia, it is known that the consumption of native chicken meat increased from 4.797 kg in 2015 to 5.110 kg in 2016. Meanwhile, the increase in local poultry population in one year, between 2015 and 2016, only increased from 1,528,329 to 1,632,568 or about 0.07% (Kilatsih and Daryono, 2020)

Compared to exotic commercial breeds, both broilers and layers, native chickens have lower production and reproduction, very slow growth rates and poor feed efficiency (Asnawi *et al.*, 2023). However, from the market point of view, Kampung chicken products have better prices and consumer preferences. This market condition is certainly an opportunity to fill and develop the Kampung chicken market. In addition, this condition also motivates farmers to increase Kampung chicken production as their livelihood (Prabowo *et al*., 2023).

Therefore, efforts to improve feed quality to increase chicken productivity are carried out through feed manipulation using Antibiotic Growth Promoters (AGPs) to increase efficiency and productivity. However, the widespread use of AGPs has led to the emergence of antibiotic-resistant pathogens and poses a significant threat to food safety due to residues, so the government currently prohibits the use of AGPs for livestock (Rahman *et al*., 2022; Wen *et al*., 2022). To overcome this problem, efforts to provide safe supplements to livestock without causing residues continue to be developed, one of which is through the provision of Mannan Oligosaccharide prebiotics. Prebiotics added to native chicken feed will increase the growth of good bacteria in the digestive tract. Prebiotic Mannan Oligosaccharide contains Saccharomyces cerivisiae fungal wall which functions in improving the balance of intestinal microflora, enhancing immune response, and providing health benefits for its host (Xu *et al*., 2021).

Evaluation of the effectiveness of Mannan Oligosaccharide (MOS) prebiotics on the health of native chickens needs to be done through blood profile examination. This is important in evaluating body health in chickens because blood reflects the physiological status and response of the body to various conditions, including nutrition, infection, stress, and supplementation such as prebiotics (Shao *et al*., 2021). Haematological parameters such as erythrocyte counts, leucocytes, haemoglobin levels, and haematocrit values can provide important information on oxygen transport capacity, immune status, disease-fighting ability, and metabolic balance of the body, making blood tests very important as they can be used to monitor the presence of disease (Okoruwa *et al*., 2023)

Blood test to evaluate the effectiveness of MOS supplementation consists of several parameters, namely white blood cells (WBC), red blood cells (RBC), haemoglobin (HGB), haematocrit (HCT). Therefore, research on the effect of Mannan Oligosaccharide (MOS) prebiotic supplementation on the haematological profile of native chickens needs to be conducted to evaluate the haematological health condition of native chickens after MOS administration.

METHODS

The materials used in this study included 4 native chickens, anticoagulant substances, distilled water, EDTA (Ethylene Diamine Tetraacetic Acid) anticoagulant tubes, Nat and Herrick reagents, 0.1N HCL, basal feed, 70% alcohol, and Mannan Oligosaccharide (MOS) powder prebiotics containing Saccharomyces cerevisiae. Equipment used for taking and checking blood samples were 3 mL syringe, micropipette, EDTA tube, cooling box, cotton swab, haemocytometer to determine the number of red blood cells, leukocyte thoma pipette, microscope, digital balance, neubauer counting chamber, sahli tube, centrifugator, and haemoglobinometer to calculate the amount of haematocrit.

The method used in this study was true experimental with a control group pre-test post-test design by dividing it into 2 groups, each group consisting of

2 native chickens with the following design: E1: the treatment group that was checked for blood profile before the experiment. E2: treatment group with blood profile checking after the experiment, K1: control group with blood profile checking before the experiment, K2: control group with blood profile checking after the experiment.

The implementation of this study consisted of research activities, sampling, and sample testing. The prebiotic treatment of Mannan Oligosaccharide as much as 3% (w/w) in 120gram of basal feed was carried out every day for 7 days. Blood samples were collected on the 8th day from each chicken using a syringe through the brachial vein. Then the blood samples were put into test tubes that had been given EDTA anticoagulant for analysis. Parameters observed in this study were haematological profile consisting of the number of red blood cells (RBC), white blood cells (WBC), haemoglobin, and haematocrit values.

Red blood cell counts were performed using a Neubauer counting chamber. The procedure started with sucking blood using a haemocytometer pipette tip that had been cleaned with a tissue until the 0.5 mark. Next, Nat's and Herrick's reagent solution was sucked in quickly and carefully until there was 101 on the pipette. The pipette was then homogenised in a figure 8 motion for 15-30 seconds, the first 3-4 drops of solution were discarded. The liquid was then put into the counting chamber. Erythrocytes were allowed to settle for about one minute, after which the counting chamber was observed under a microscope with a 10x lens, then continued with a 40x lens. All erythrocytes in 5 boxes consisting of 16 small boxes were counted (Merlina *et al.*, 2024). The calculation of the number of poultry erythrocytes can be done with the formula of Benjamin (1978) as follows:

$$RBC = (E1 + E2 + E3 + E4 + E5) \times 5 \times 200 \times 10mm^3$$

Description:

Σ (E1-E5) = Number of erythrocytes in each room counted

Factor 5 = Calculated field

Factor 200 = 200x dilution

Factor 10 = Depth of counting chamber

Leukocyte counts were performed using a leucocyte thoma pipette and aspirator. Blood was aspirated to a level of 0.5, then Nat and Herrick's diluent solution was aspirated to a level of 11 in the leucocyte pipette. The unshaken liquid was discarded, then a drop of liquid was put into the counting chamber and allowed to settle. The blood granules are then counted using a microscope with 100x magnification (Aji *et al.*, 2023). The number of leucocytes can be calculated using the following formula:

$$\text{Leukocyte count} = (\text{Volume area}/\text{Number of cells}) \times \text{Dilution factor}$$

Description:

Cell count: Total leucocytes in a specific area on the haemocytometer.

Area volume: The volume of the room in the haemocytometer

Dilution factor: The blood dilution used during sample preparation

The Sahli method is used to calculate blood haemoglobin levels. This method relies on the colour change of the solution after blood is mixed with hydrochloric acid (HCl) which causes hemolysis and the release of hemoglobin into the solution so that it will give a yellowish-red colour to the solution when released (Channa Basappa *et al.*, 2024).

Hematocrit in chickens is measured using blood centrifugation, where blood that has been drawn from the chicken's body is put into a centrifuge tube. After centrifugation, the blood separates into three layers: red blood cells (erythrocytes) at the bottom, a thin layer consisting of white blood cells and platelets in the middle (buffy coat), and blood plasma at the top. The haematocrit value is calculated by measuring the height of the red blood cell column and dividing it by the total volume of blood (Quirynen *et al.*, 2024).

RESULT AND DISCUSSION

The results showed that the administration of Mannan Oligosaccharide (MOS) affected the haematological profile of native chickens after 7 days of treatment. Before prebiotic administration (Table 1), the average erythrocyte counts for the control group (K0) and treatment group (K1) were 2.45 x 10^6 cells/mm^3 and 2.47 x 10^6 cells/mm^3, with an average of 2.46 x 10^6 cells/mm^3. This indicates that the initial conditions of both groups were relatively equivalent in erythrocyte levels. In addition, the erythrocyte counts were within the normal range. This is in accordance with Nathaniel *et al* (2022) who stated that normal erythrocyte levels in adult chickens vary between 2.4 to 3.5 million cells per microlitre of blood so all chickens were in good health prior to the experiment.

Table 1. Erythrocyte count (cells/mm^3) before prebiotic administration for 7 days

Treatment	**Eritrosit x x 10^6 (cell/mm^3)**
K0	2,45 x 10^{6}*
K1	2,47 x 10^{6}*
Average	2,46 x 10^{6}*

Table 2. Erythrocyte count (cells/mm^3) after prebiotic administration for 7 days

Treatment	**Eritrosit x x 10^6 (cell/mm^3)**
K0	2,46 x 10^{6}*
K1	2,63 x 10^{6}*
Average	2,54 x 10^{6}*

Table 3. Leukocyte count (cells/mm^3) before prebiotic supplementation for 7 days

Treatment	**Leukocyte x 10^3 (cell/mm^3)**
K0	18,72 x 10^{3}*
K1	17,59 x 10^{3}*
Average	18,15 x 10^3

Table 4. Leukocyte count (cells/mm^3) after prebiotic supplementation for 7 days

Treatment	**Leukocyte x 10^3 (cell/mm^3)**
K0	19,24 x 10^{3}*
K1	21,37 x 10^{3}*
Average	20,30 x 10^3

Table 5. Haemoglobin level (g/dL) before prebiotic administration for 7 days

Treatment	**Haemoglobin (g/dL)**
K0	10,2*
K1	9,4*
Average	9,8

Table 6. Haemoglobin level (g/dL) after prebiotic administration for 7 days

Treatment	Haemoglobin (g/dL)
K0	10,3*
K1	12,5*
Average	11,4

Table 7. Haematocrit value (%) before prebiotic administration for 7 days

Treatment	Haematocrit (%)
K0	29*
K1	29*
Average	29

Table 8. Haematocrit value (%) after prebiotic administration for 7 days

Treatment	Haematocrit (%)
K0	29,5*
K1	32,5*
Average	31

After giving prebiotic cell for 7 days (Table 2), the control group (K0) did not experience a significant increase in the number of erythrocytes, while the Treatment group (K1) showed a greater increase, namely 2.63 x 10^6 cells/mm^3, with an average of 2.54 x 10^6 cells/mm^3. The increase in the number of erythrocytes in chickens in both the control group and the group given MOS Treatment is still within the normal range so that the administration of MOS can increase the number of erythrocytes but does not interfere with the health of chickens because the number of erythrocytes is still within normal limits, namely in the range of 2.4 x 10^6 (cell/mm^3) to 3.5 x 10^6 (cell/mm^3) (Nathaniel *et al.*, 2022). These results are in accordance with the research of Abate (2023) which showed that the increase in the number of erythrocytes in the Treatment group after the administration of MOS was proven to stimulate an increase in the number of erythrocytes. This increase in the number of erythrocytes proves that the role of MOS as a prebiotic is able to improve intestinal health, improve nutrient absorption, and have a positive impact on the body's metabolism. In this case, the increase in erythrocyte count indicates that MOS supplementation can support increased red blood cell production, which is important for optimal oxygen distribution in the chicken body (Do-Huu *et al.*, 2023).

Based on the data in Table 3 and Table 4, there is an effect of prebiotic Mannan Oligosaccharide (MOS) on the number of leucocytes in chicken cells for 7 days. Before prebiotic administration, the average number of leucocytes was 18.15 x 10^3 cells/mm^3 or 18,150 cells/mm^3. This shows that both groups have normal leucocyte levels, which means the chickens are in good health. This is in accordance with the statement of Jumadin and Samai (2020) that chickens in normal conditions the number of erythrocytes ranges from 10,000 to 30,000 cells per microlitre of blood.

The increase in leucocyte count in group K1 after MOS administration suggests that this prebiotic may function as an immunomodulator that stimulates the immune system (Yilmaz *et al.*, 2022). Although this difference is not very large, the significant increase after treatment indicates that there is a change in the immune response of the body of chickens given MOS. In the control group (K0) the number of leucocytes also increased, but not as much as in the group given MOS. This is due to other factors such as natural variations or adaptation of the chicken body to the environment or stress, which can affect the number of leukocytes (Hofmann *et al.*, 2020). Significant differences occurred in group K1, which proves that MOS has a positive effect on chicken health, especially in modulating the number of immune cells (Bełdowska *et al.*, 2024). The increase in leucocyte counts in group K1 also indicates that MOS can improve the balance of gut microflora, which affects the immune system and the ability of chickens to fight infection. This is very important, because the digestive system and the immune system interact, and the maintenance of a healthy microflora can support a better immune response (Obayomi *et al.*, 2024). Based on previous studies, MOS can enhance cellular, humoral, and mucosal immunity in chickens by strengthening immunological capacity. Mannan, which is present in yeast cell walls, interacts with PAMP receptors on GALT defence cells, which activates the chicken immune system (Reddyvari, 2020).

In addition, the administration of MOS affects blood haemoglobin levels. The results showed in Table 5 that before prebiotic treatment, the haemoglobin level in group K0 (control) before treatment was relatively normal because it was in the range between 7-13 g/dL (Horhoruw and Kewilaa, 2024).

Haemoglobin levels increased in group K1 (MOS administration) from 9.4 g/dL to 12.5 g/dL in accordance with the research of Youssef *et al* (2023) that prebiotics have a positive influence on the haematopoiesis system (blood cell formation process) in chickens. The mechanism of haematopoiesis is by increasing the absorption of nutrients such as iron and folic acid needed for haemoglobin production (Oni *et al.*, 2020). The increase in haemoglobin levels in the K1 group is also related to improved nutritional status and increased metabolism of the chicken body. This is consistent with Singh and Kim's (2021) statement that MOS prebiotics can affect the intestinal microflora, which in turn can increase the absorption of essential nutrients that play a role in haemoglobin formation. In addition, this increase in haemoglobin levels may also reflect the increased oxygen transport capacity in the chicken body, which is important to support metabolic activity and endurance, especially if the chicken is exposed to stressful or infectious conditions.

MOS administration also has an impact on haematocrit values. Based on the data provided in Table 7 and Table 8, the following are the results of changes in haematocrit values before and after prebiotic administration in the K0 (control) and K1 (treatment with prebiotics) treatment groups. Before prebiotic administration, the haematocrit value between the control group (K0) and the treatment group (K1) showed the same value, which was 29%. This shows that there is no difference in haematocrit value between groups before treatment. Haematocrit levels are still within the normal range, which ranges from 22% to 35%. (Horhoruw and Kewilaa, 2024) means that when given the treatment, the whole cell is in good health.

After administration of prebiotics for 7 days (Table 8), there were changes in haematocrit values. Group K1 (which was given prebiotics) showed a significant increase in haematocrit, from 29% to 32.5%. Whereas in the K0 group, the increase in haematocrit value was not significant because it occurred naturally without being given MOS treatment. According to Horhoruw and Kewilaa's research (2024) the haematocrit range after treatment is still within the normal range, which ranges from 22% to 35%. Haematocrit is the percentage of blood volume that consists of red blood cells. An increase in haematocrit may indicate an increase in the number or volume of red blood cells containing haemoglobin, which functions to carry oxygen to cells throughout the body (Londok *et al.*, 2022). Although there was an increase in the control group (K0), the increase was not as large as the Treatment group (K1), these results are in accordance with the research of Youssef *et al* (2023) which showed that prebiotics have the potential to modulate factors related to the formation or maintenance of red blood cells thus increasing the percentage of haematocrit in poultry.

CONCLUSION

Based on the results of the research conducted, it can be concluded that the provision of Mannan Oligosaccharide (MOS) prebiotics has a positive effect on the haematological profile of native chickens (*Gallus gallus domesticus*). MOS was shown to improve a number of haematological parameters, including erythrocyte count which increased to 2.54×10^6 cells/mm³, leucocyte count which increased to 20.30×10^3 cells/mm³, haemoglobin level which increased to 11.4 g/dL, and haematocrit value which reached 31%. The results of this study indicate that MOS supplementation can improve the haematological condition of native chickens which has the potential to improve the health and productivity of chickens, thus making a positive contribution to native chicken farming.

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Author Contribution

Fullan Ausati Putri Sri Trisna Dewi: research conceptualisation, methodology, writing the original article draft, haematology data analysis. Prima Sukma Rizky Utami: haematological testing, data curation, data collection, preparation of original article draft. Tsamara Aulia Rusyda: editing, documentation, preparation of materials, and conducting in vivo tests. Dhio Salhisya Marta Kusuma: haematological testing, data collection, sampling, and in vivo testing. Disty Putri Maharani: preparation of research tools and materials, conducting in vivo tests, sampling. Dewangga Arya Bima: acclimatisation of experimental animals, sampling, conducting in vivo tests. Kadek Rachmawati: provided guidance in the research process, supervised the entire research process, provided direction in completing the manuscript.

Competing Interest

The authors declare that they have no known financial interests or personal relationships that could influence the research reported in this article.

Ethical Approval

The present study was approved by the Ethics Committee of Faculty of Veterinary Medicine, Universitas Airlangga.

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