



Effect of Adding Garlic Flour (*Allium sativum*) on Complete Blood Count Case Study Newcastle Disease in Broilers

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ABSTRACT: The purpose of this study is to find out how garlic flour (*Allium sativum*) affects the synthesis of hemoglobin, erythrocytes, hematocrit, and heterophils/lymphocytes in the blood of broiler animals infected with the Newcastle disease (ND) virus. The Duncan follow-up test was used in this study's analysis, which used a completely randomized design. According to the results of this study, the consumption of garlic flour (*Allium sativum*) had no significant influence on hemoglobin, erythrocytes, hematocrit, and heterophils/lymphocytes in the blood in the spleen organ of broiler. However, garlic flour (*Allium sativum*) only protects broilers' immune systems when they are infected with the ND virus. In addition to the ND virus, extrinsic factors such as nutrition, environmental humidity, and temperature around the rearing drum all have an impact on blood output.

KEYWORDS: Erythrocytes, Garlic flour, Hematocrit, Hemoglobin, Heterophils.

INTRODUCTION

Broiler hens are raised for 35 days for their maximum weight at harvest. Livestock condition, nutrition, and environmental management are all aspects that influence the success of broiler livestock. Low productivity, immunological condition, and excessive feed costs are some of the issues that arise with broiler rearing. As a result, there is a need for innovation in enhancing broiler livestock productivity in order to help farmers. Other conditions affecting broiler farming include the ban on the use of antibiotics as growth promoter agents. Since 2017, the government has prohibited the use of antibiotics as growth promoters in livestock through Minister of Agriculture Regulation Number 14 of 2017. The prohibition of antibiotics as growth promoters in livestock is aimed at preventing the occurrence of antimicrobial resistance (AMR). This policy is an effort to increase the productivity of broiler livestock by using safer, cheaper, and more useful plant-based ingredients, or what are commonly called phytobiotics. The use of ingredients of plant origin is expected to reduce the danger of antibiotic resistance due to the uncontrolled use of antibiotics, which causes residues to accumulate in products originating from poultry. A problem that often arises in the world of poultry raising is the infectious poultry disease Newcastle disease (ND). ND is a disease that often attacks poultry and causes a decrease in production value, high morbidity, and high mortality (Hewajuli and Dhamayanti, 2012). Prevention of ND can be done through vaccination and strict biosecurity, as well as providing feed supplements with immunomodulatory ingredients. One ingredient that can be used as a phytobiotic agent and has immunomodulatory properties is garlic flour (*Allium sativum*). Garlic (*Allium sativum*) possesses anti-inflammatory and immune-boosting effects (Arreaola et al., 2015). The potential of garlic's high immunomodulatory qualities to improve the health status of broiler cattle can be seen by a complete blood count in the spleen organ. This material has significant antibacterial capabilities in addition to being an immunomodulator, therefore it can be employed as an antimicrobial agent to substitute synthetic antibiotics where the material can no longer be used as a growth promoter. Garlic flour (*Allium sativum*) is believed to boost broiler health and increase blood output in broiler spleen organs.

MATERIALS AND METHODS

Day Old Chick (DOC)

The material used in the research was 200 Day Old Chick (DOC) Ross strain broilers with a rearing period of 35 days. Day-Old Chick has received a vaccine against NCD with safe and close housekeeping.



Cage

This research used an open house cage and used litter from rice husks, which functions to absorb chicken droppings. The cage consists of twenty units with dimensions of 70 cm long, 80 cm wide, and 70 cm high. Each cage contains 8 chickens and is equipped with equipment for research, such as:

1. 40-watt incandescent lamps used for lighting and heating (brooder).
2. Feed and drink containers made of plastic, with 20 containers each placed in each cage unit.
3. A set of minor surgical tools (dissecting set) used for necropsy procedures on chickens.
4. A set of organ pieces used to accommodate necropsy organs used for histopathology sample preparation.
5. A room thermohygrometer is used to determine temperature and humidity.
6. 3 ml syringe, micropipette, water bath, microscope, object glass, cover glass, and incubator.

Meanwhile, the materials used in this research are:

1. The materials used were 200 broilers aged 35 days;
2. BR 511 standard broiler feed
3. Drinking water;
4. Garlic flour extract powder;
5. Alcohol 70%;
6. Entellan;
7. PBS;
8. *Hematoxylin Eosin*;
9. Formalin 10%;
10. Aquadest;
11. Object glass;
12. Cover glass.

RESEARCH PROCEDURE

Broiler Cage Preparation

The bedding of the cage or litter is filled with rice husks, which have been disinfected using disinfectant chemicals, and liming is carried out with the aim of preventing infection by microorganisms. Each treatment group was given a 40-watt incandescent lamp to keep the cage temperature more stable, especially at night. A thermometer is placed in each cage to maintain the temperature and humidity of the cage.

Virus Inoculation in Embryoated Chicken Eggs

The embryonic chicken eggs used must have characteristics that can be seen when candling, namely that the embryo is alive so that it can be seen moving and is surrounded by blood vessels. The requirements for embryonated chicken eggs are that they come from healthy parents, have never been vaccinated or have specific antibody-negative (SAN), have never been infected with a disease, or are specific pathogen-free (SPF) (Ernawati et al., 2008). In this virus inoculation, embryonated chicken eggs (TAB) aged 9–11 days are used because, at that stage, they contain a lot of chorioallantois. 0.1 ml of the suspension was taken using a 1cc tuberculin syringe. The suspension is then injected into the TAB into the allantoic fluid or chorioallantoic membrane (Figure 1). Cover the injection hole with tape, then label the TAB. TAB was incubated at 37°C for 5 days. Candling is done every day to determine the condition of the embryo. If a dead embryo is found, the TAB is stored at 4°C.

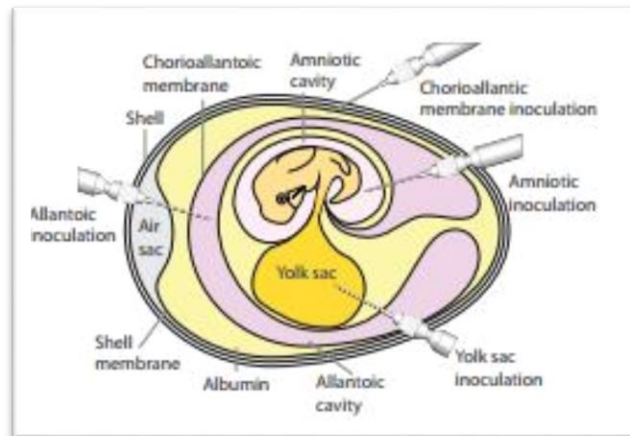


Figure 1. Chorioalantois Membrane

Day Old Chick Care (DOC)

This study involves 200 docs from ross strains. Acclimatization treatment is performed on DOC aged 0–7 days by providing a regular broiler diet to correct the body condition. Doc is prepared by providing drinking water in the form of sugar water to improve energy and minimize animal dehydration. Sugar water is made by diluting 2% sugar with ordinary water. Each group receives up to 2 liters of sugar water per day. Water with sugar is provided at the age of 1-2 days, while ordinary drinking water is given at the age of 3–35 days and is refreshed every day. The feed of the chickens aged 8–35 days is supplemented with onion flour in various concentrations (P0, P1, P2, P3, and P4). Flour is directly spoken in feed, with feeding time scheduled twice a day.

Newcastle Disease (ND) Infection

The antigen utilized in this study is a naturally infectious agent of the ND virus isolated from chickens suspected of having ND. Organs from infected chickens' spleens, brains, and livers can be isolated and implanted in embryonated chicken eggs to produce fatal dosage 50 (LD 50). Previously, the antigen obtained was evaluated with a hemagglutinin (HA) assay to confirm that it was an ND antigen. The virus is then reproduced in embryonated chicken eggs, and each chicken subjected to this treatment is infected orally using a syringe. On day 15 of the finisher phase, virus induction was performed once.

Collecting Blood and Necropsies

Blood can be collected from chicken brachialis veins that have been treated with a 70% alcoholic antiseptic solution (Kirkipinar et al., 2011). On the 35th day, a small surgery is used to do a necropsy. The necropsy action is carried out by murdering animal welfare through euthanasia. Euthanasia is carried out through neck dislocation or cervical dislocation (AVMA, 2020). When animals are put to sleep, euthanasia is used to alleviate their agony. Necropsies are performed to obtain organs such as the spleen, which are subsequently histopathological analyzed. Taking five chicken samples from each test. Following sampling, the sample is processed in the organ pot with a 10% formalin concentration.

DATA ANALYST

This study uses a complete random design (RAL) with 5 treatments and 5 replications [P0: Feed Standard of Broilers; P1: Feed + 0.25% (Garlic Extract); P2: Feed + 0.5% (Garlic Extract); P3: Feed + 0.75% (Garlic Extract); and P4: Feed + 1% (Garlic Extract)]. Data that will be obtained include hemoglobin, hematocrit, erythrocytes, and heterophil. The data that has been obtained is analyzed using SPSS to determine the level of significance.

RESULTS AND DISCUSSIONS

A. Hemoglobin

Based on studies, the consumption of garlic (*Allium sativum*) with a variety of treatments had no significant effect ($P > 0.05$) on hemoglobin in broilers infected with the ND virus. Table 1 displays the average hemoglobin result.

**Table 1.** Average of Hemoglobin in Broiler with ND Virus

Hemoglobin (Average \pm SD)				
P0	P1	P2	P3	P4
13.36 \pm 0.709 ^a	14.34 \pm 1.383 ^a	15.44 \pm 3.649 ^a	13.86 \pm 0.999 ^a	12.72 \pm 3.706 ^a

Explanation: ^a = The same superscript on the same line shows that giving garlic flour did not have a significant effect ($P > 0.05$) on Hemoglobin in broilers.

Hemoglobin is a blood pigment that increases in metabolism and releases in accordance with the body's metabolism. Hemoglobin production begins in erythrocytes and continues through the phases of erythrocyte development. The synthesis of hemoglobin will continue as long as the nucleus remains in erythrocytes. If animals are in a condition or condition caused by the release of catecholamines (epinephrine/norepinephrine), hemoglobin, hematocrit, and erythrocytes will increase. Table 1 shows the hemoglobin analysis findings, which demonstrate that broiler chickens infected with the ND virus with P2 treatment (0.5% garlic powder) had the best results of 15.44 \pm 3.649. This is because garlic powder is used as an immunomodulator in broiler chickens. Hemoglobin levels in broiler chickens are typical, ranging from 12.7 to 18.4 g/dl on average. This suggests that while garlic flour (*Allium sativum*) helps broiler chickens maintain their immunological status, it has no effect on raising hemoglobin in the blood of broilers infected with the ND virus. This is similar to the statement (Siregar, 2017) that the addition of garlic flour to broiler chickens can accelerate growth, help improve the digestive organ working system, and maintain the animal's immune system. Another case study showed that giving garlic flour (*Allium sativum*) increased feed consumption compared to control feed. Palatability is a factor that can affect high levels of feed consumption. In broilers, palatability is used to predict feed preference. In general, chickens like food with bright colors (Nuningtyas, 2014). Age, sex, feed, and environment all have an impact on hemoglobin levels (Sturkie, 1976). Furthermore, the height of the location where animals live can impact hemoglobin levels in the blood (Atadilaga, 1979). Hemoglobin accounts for one-third of the volume of erythrocytes in several varieties of normal poultry (Campbell, 1995). Because hemoglobin is an iron-rich protein, it is found in erythrocytes and permits them to bind oxygen. Furthermore, an increase in hematocrit is followed by an increase in hemoglobin levels (Soetrisno, 1987). The synthesis of hemoglobin begins in the proerythroblast and then proceeds to the reticulocyte stage in the bone marrow, where it continues until the erythrocytes are cooked. If erythrocytes leave the bone marrow and enter the bloodstream, they will continue the formation of a little hemoglobin for several days or after (Schalm, 2010).

B. Erythrocytes

Based on the results of the research, providing garlic flour (*Allium sativum*) with a range of treatments had no significant effect ($P > 0.05$) on erythrocytes in broiler chickens infected with the ND virus. Table 2 shows the average hemoglobin results.

Table 2. Average of Erythrocytes in Broiler with ND Virus

Erythrocytes (Rata-rata \pm SD)				
P0	P1	P2	P3	P4
2.55 \pm 0.198 ^a	2.86 \pm 0.327 ^a	2.92 \pm 0.764 ^a	2.73 \pm 0.151 ^a	2.35 \pm 0.828 ^a

Explanation: ^a = The same superscript in the same line shows that garlic flour treatment had no significant ($P > 0.05$) effect on erythrocytes in broilers.

Measurement of erythrocyte levels, according to Putriani et al. (2012), is done by preparing the calculation room. The cover glass is placed above the counting room so that it covers the counting area. The blood that has been given anticoagulants is sucked with an erythrocyte pipette to a sign of 0.5. When exceeding the limit, blood is removed by touching the tip of the pipette with the tip of the finger. The outside of the pipette is removed with tissue paper. Immediately, the Hayem diluent solution is sucked up to the sign of 101. During suction, the pipette must be rotated along its long axis so that the blood and Hayem's solution mix well. Cover both ends of the pipette with the thumb and middle finger and shake with a perpendicular movement to its long axis for two minutes. Dissolve the diluent contained in the capillary and discard the diluent that does not contain blood by dripping three drops. The blood solution is introduced into the counting chamber by placing the pipette tip on the edge of the cover glass. Due to capillary action,



the blood solution will flow between the cover glass and the counting chamber; there should not be too much blood solution. The calculation room that already contains a blood solution is placed under a microscope, and the calculation is done using a 45x objective lens. Calculation of the number of erythrocytes found in 5 fields in the middle with an area of $1/25 \text{ mm}^2$, Cells that offend the left and lower boundary lines are not calculated. How to calculate must be systematic to avoid having one cell calculated more than once. Calculated as an example of the number of erythrocytes contained in the five fields is N. The number of the fifth volume of the field is $5/250 \text{ mm}^3$. So, each mm^3 of blood is available $(1: 5/250) \times n = (250: 5) n = 50 n$ erythrocytes. The dilution is carried out as much as 200 times; the number of erythrocytes per mm^3 of blood is $50 N \times 200 = 10,000 N$. In order for the calculation to be thorough, two calculations are carried out in the two arithmetic rooms. Errors using this method are around 7.8% (Dharmawan, 2002).

C. Hematocrit

Based on studies, the consumption of garlic (*Allium sativum*) with a range of treatments had no significant effect ($P > 0.05$) on the hematocrit in broilers infected with the ND virus. Table 3 displays the average hemoglobin result.

Table 3. Average of Hematocrit in Broiler with ND Virus

Hematocrit (Average \pm SD)				
P0	P1	P2	P3	P4
33.74 ± 2.069^a	37.20 ± 3.629^a	39.04 ± 10.778^a	35.24 ± 2.152^a	31.24 ± 11.394^a

Explanation: ^a = The same superscript in the same row shows that garlic flour administration had no significant effect ($P > 0.05$) on broiler hematocrit.

Blood with anticoagulants can be used to test hematocrit in a microhematocrit pipette of approximately 6/7 pipette. Use a specific cover or a seal to cover the end of the blood. The pipette is mounted on a microhematocrit centrifuge. Then it is confused for 5 minutes at a speed of 10,000 rpm. The acquired hematocrit value is then read using a specialized reading instrument (microhematocrit reader) (Dharmawan, 2002). Following the measurement, the data is given in Table 3. Based on the table, the broiler afflicted by the ND virus with the P2 (0.5% garlic powder) therapy achieves the greatest results of 39.04 ± 10.778 . Because the hematocrit in broilers typically ranges between 49.6 and 66.0%, the results of the preceding study are considered to be below the normal range. It may be identified that garlic flour (*Allium sativum*) treatment had no significant influence ($P > 0.05$) on hematocrit in broiler blood. Giving garlic flour (*Allium sativum*) to broilers in critical condition, on the other hand, can prolong their lives. According to the results of Tables 2 and 3, the hematocrit value and the quantity of erythrocytes are connected. The greater the number of erythrocytes, the greater the hematocrit value in the blood. Vice versa, a decrease in hematocrit values can be caused by damage to erythrocytes, a decrease in erythrocyte production, or it can also be influenced by the number and size of erythrocytes (Dawson and Whittow, 2000). Because erythrocytes are the largest cell mass in the blood, the hematocrit value is heavily dependent on their number (Virden et al., 2007). Increased or decreased hematocrit values in the blood will have an effect on blood viscosity. The higher the hematocrit percentage, the greater the viscosity of the blood. Spleen contractions or dehydration are to blame for the condition. The release of epinephrine hormones, which occurs when animals feel fear, disease, or exercise, stimulates spleen contraction. Change in the hematocrit value will have a negative influence since they affect the viscosity (thickness) of the blood. A high or low hematocrit value causes an increase, and conversely, it will slow down blood flow in the capillaries and speed up the heart's activity (Cunningham, 2002). Sodium and potassium ions in body fluids, both extracellular fluid (blood) and intracellular fluid (cytoplasmic fluid), can influence the action of epinephrine to suppress excessive spleen contraction, so that erythrocyte contraction becomes stable while the hematocrit value in the blood remains within normal limits (Von Borell, 2001).

D. Heterophile/Lymphocytes (H/L) Ratio

In the study (Yalcinkaya et al. 2008), lymphocytes are an important component of the immune system that responds to antigens by generating antibodies. Meanwhile, Mayes et al. (1997) claim that heterophil serves as a bodily defence against external influences; if foreign particles accumulate in the cytoplasm of heterophils, the particles will enter a compartment known as phagosomes. Data on the results of the analysis are given in Table 4.

**Table 4.** Average of Heterophile/Lymphocytes in Broiler with ND Virus

Unit	Heterophile/Lymphocytes (Average \pm SD)				
	P0	P1	P2	P3	P4
Percentages	9.10 \pm 1.210 ^a	9.26 \pm 0.673 ^a	10.36 \pm 2.559 ^a	9.78 \pm 1.446 ^a	8.48 \pm 1.411 ^a
10 ⁹ /mm ³	21.70 \pm 3.277 ^a	22.60 \pm 2.234 ^a	25.94 \pm 8.116 ^a	19.02 \pm 3.795 ^a	22.67 \pm 7.432 ^a

Explanation: ^a = The same superscript in the same row shows that the administration of garlic flour does not have a significant effect ($P > 0.05$) on the percentage of 10⁹/mm³ heterophil/lymphocytes in the broiler spleen organs.

Table 4 shows that P2 (0.5% garlic flour) has the highest average percentage of heterophile, with an average of 10.36 ± 2.559 . Because the percentage of heterophile/lymphocytes in broiler spleen organs is 25.94 ± 8.116 10⁹/mm³, this average can be classified properly. Standardization of heterophil/lymphocytes in the 5.0–76.0 10⁹/mm³ range. Interleukin, granulocyte/monocyte colony stimulating factor, and granulocyte stimulating factor all played a role in the growth of these heterophils (Jackson, 2007). Heat stress significantly increases the amount of heterophil, according to a study conducted by Altan et al. (2000). When compared to other treatment groups, broilers 14 days old with P0 treatment produce an average heterophil value of (11.01 ± 5.121 10⁹/mm³) after the infection. This is caused by a *Salmonella pullorum* antigen infection. Heterophil possesses amoeboid activity and participates in phagocytosis to protect the body from infections or foreign objects such as bacteria and other tiny particles that cause inflammation. Tizard (1982) adds to this by stating that heterophil is very active in functioning and swiftly phagocytoses, but quickly fatigues and spends a short period of time in circulation. Further from that, the length of maintenance is another element that affects heterophils and lymphocytes. The length of maintenance can cause an increase in the H/L ratio. This suggests that prolonged exposure to high temperatures reduces the number of lymphocytes, resulting in a weaker immune system. As previously stated, the average temperature at the research site was relatively high compared to the comfortable temperature for broiler chickens; therefore, the birds will experience heat stress and a rise in the H/L ratio the longer they are exposed to this temperature. In poultry, the H/L ratio is the primary stress indicator. The greater the ratio, the greater the amount of stress (Kusnadi, 2009). Table 4 shows that P2 had the highest average heterophil/lymphocyte reception, with a percentage of $10.36 \pm 2.559\%$. However, the percentage in P2 is lower than expected. The typical proportion of heterophils and lymphocytes is 19.01–46.09%. According to the Rochmana et al. (2013) investigation, broiler chickens treated with bangle flour had no significant effect ($P > 0.05$). The H/L ratio at T0 is greater than the research results (Toghyani et al. 2010), which are in the range of 0.3–0.4. The flavonoid and saponin chemicals found in bangle flour can alter this by reducing stress.

CONCLUSIONS

The content of garlic flour (*Allium sativum*) is an immunomodulator; however, broiler chickens infected with the ND virus can only maintain their immunity and do not have a significant effect ($P > 0.05$) on hemoglobin, erythrocytes in the blood, hematocrit, and also heterophils and lymphocytes. This can be influenced by feed nutrition, the level of heat stress on the drum, and other factors that could possibly be the cause of research results that are below average.

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