World Journal of Advanced Research and Reviews
eISSN: 2581-9615 CODEN (USA): WJARAI
Cross Ref DOI: 10.30574/wjarr
Journal homepage: https://wjarr.com/

(RESEARCH ARTICLE)

Check for updates

Effect of garlic flour (*Allium sativum*) addition on immunity and histopathology of Broilers with *Newcastle disease* virus

Ganang Rilo Pambudi [1], Rositawati Indrati [2] and Osfar Sjofjan [3] *

[1] *Postgraduate of Animal Science, Faculty of Animal Science, Universitas Brawijaya, Indonesia. 65145*
[2] *Department of Animal Production, Faculty of Animal Science, Universitas Brawijaya, Indonesia. 65145*
[3] *Department of Animal Nutrition and Feed, Faculty of Animal Science, Universitas Brawijaya, Indonesia. 65145*

World Journal of Advanced Research and Reviews, 2023, 20(03), 618–625

Publication history: Received on 27 October 2023; revised on 04 December 2023; accepted on 06 December 2023

Article DOI: https://doi.org/10.30574/wjarr.2023.20.3.2496

Abstract

Broiler chickens are reared for a short period (35 days) of time for their flesh. When producing broilers, several issues develop, including low productivity, immunological state, feed prices, and antibiotic restrictions. Antibiotics ought to be avoided as growth promoters to *avoid antimicrobial resistance* (AMR). This policy stimulates broiler livestock productivity by utilizing plant elements known as photobiotic. The use of plant materials is intended to lower the risk of antibiotic resistance. Antibiotics that fail to meet regulations might cause residue buildup. Aside from it, there are several infectious disorders, such as *Newcastle disease* (ND). ND is a disease that frequently affects poultry, resulting in decreased production value, significant morbidity, and high mortality. ND can be prevented with vaccination and treatment to boost immunity. One type of vaccine utilizes immunomodulatory supplements, such as garlic (*Allium sativum*), which has anti-inflammatory and immune-boosting properties. During the ND infection, garlic flour supplementation resulted in alterations in pathological lesions in the hearts of chicken embryos. These modifications include a reduction in the severity of the infection, which is characterized by edema, congestion, necrosis, and neutrophil infiltration. After ND infection, 1% garlic flour can improve livestock immune health, as evidenced by an increase in protective antibody titers, a drop in the number of leukocytes, and a decrease in the number of lymphocytes. In the 1% garlic flour therapy group, histopathology of the spleen organ revealed differences in the severity of pathological lesions caused by ND infection.

Keywords: Antibiotic; Broiler; Garlic flour; *Newcastle disease*; Vaccination

1. Introduction

The national poultry sector performs a significant role in the national economy's movement. This is achievable since Indonesia's chicken sector is self-sufficient in terms of eggs and meat. Apart from that, despite the fact that there are numerous obstacles in the national poultry industry that restrict the development of the poultry business, this sector performs a role in enhancing the health and intellect of the Indonesian people by fulfilling national nutrition. Poultry is livestock that is bred to provide animal protein as well as a source of sustenance for people. Poultry can be raised for eggs and meat. Broiler chickens are one sort of poultry that can be used. Broiler chickens are reared for a short period of time for their flesh. The demand for broiler meat is increasing as the population and income levels rise. Several factors, including animal health, environment, and nutrition, have a significant impact on broiler chicken productivity. Several efforts have been made to boost broiler chicken productivity, including the provision of feed additives.

Broiler animals are chickens raised for 35 days to achieve maximum weight at harvest. Several factors influence the success of broiler chicken production, including livestock condition, nutrition, and environmental management. Low production, immunological status, and excessive feed costs are all issues that arise with growing broiler chickens. As a

* Corresponding author: Osfar Sjofjan

result, there is a need for innovation in enhancing broiler chicken productivity to benefit farmers. Another issue influencing broiler chicken raising is the prohibition on the use of antibiotics as growth boosters. The use of antibiotics as growth promoters in livestock has been outlawed by the government since 2017, per Minister of Agriculture Regulation 14 of 2017. Antibiotics as growth promoters in livestock are prohibited in order to prevent the emergence of antimicrobial resistance (AMR). The existence of this guideline has resulted in various efforts to boost broiler chicken output by adopting safer, cheaper, and more beneficial plant-based substances known as photobiotic. The use of plant-based components is predicted to lessen the risk of antibiotic resistance caused by uncontrolled antibiotic usage, which causes residues to build up in poultry products. The introduction of infectious poultry diseases such as *Newcastle disease* (ND) is a frequent problem in the world of poultry, particularly livestock husbandry. ND is a poultry disease that causes low production value, high morbidity, and high mortality [1]. ND can be prevented through immunization, but it can only be treated through supportive care. Increasing the immunological state of animals is one method of preventing ND infection. One effort that can be taken is to adopt a strict vaccination and biosecurity program, as well as to provide immunomodulatory supplementation or supplementary feed.

Garlic (*Allium sativum*) is a component that can be employed as a photobiotic agent and has immunomodulatory characteristics. *Allium sativum* contains anti-inflammatory and immune-boosting properties [2]. Aside from garlic, the high immunomodulatory effects of *Allium sativum* have been shown to improve the health condition of broiler chickens, as measured by the immunological status and histology of the broiler spleen. Apart from its immunomodulator characteristics, garlic flour has strong antibacterial capabilities and can be utilized as an antimicrobial agent to replace synthetic antibiotics in situations where the substance is no longer useful as a growth promoter. According to the statement above, there is a need for solutions connected to attempts to boost broiler chicken output. It is thought that administering a combination of garlic flour (*Allium sativum*) may improve animal health and the immune system in broiler chickens.

2. Material and Methods

2.1. 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. The material has ethical approval from ethical commission of animal care and use committee Universitas Brawijaya, Indonesia [No 112 – KEP UB 2023]. Day-Old Chick has received a vaccine against NCD with safe and close housekeeping.

2.2. 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:

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

Meanwhile, the materials used in this research are:

- 200 broiler chickens aged 35 days;
- BR 511 standard broiler feed;
- Drinking water;
- Garlic flour extract powder;

Garlic flour extract obtained from traditional markets in Kediri. The garlic is washed clean and cut into thin pieces, then dried in the sun for one day, followed by drying in the oven. The next step is to grind the garlic until it becomes garlic powder.

- Alcohol 70%;
- Atellan;

- PBS;
- Hematoxylin Eosin;
- Formaldehyde 10%;
- Aquadest;
- Object glass;
- Cover glass.

2.3. Research Procedure

The work procedures in this research consist of several stages, which are explained in the treatment design scheme. This research uses field treatment and testing of research results carried out in the laboratory. The procedural scheme of this research can be seen in Figure 1 as follows:

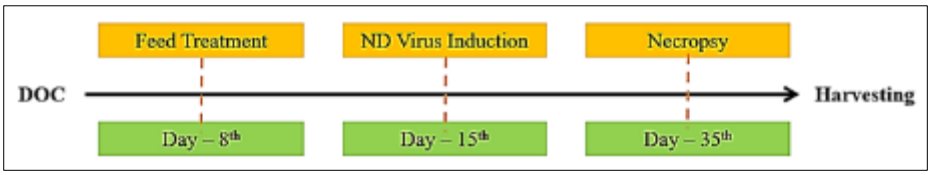

Figure 1 Schema of Research Procedure

2.4. Day Old Chick (DOC) Maintenance

This research used 175-day-old chicks with the Ross strain type. Day-old chicks aged 0–7 days undergo acclimation treatment by receiving standard broiler feed to adjust their body condition. Preparation for day-old chicks is done by giving them drinking water in the form of sugar water to increase energy and reduce the level of dehydration in the livestock. The sugar water contained 2% granulated sugar, which was then diluted with plain water and given to each group for one day in 2 liters. Water with sugar is given at 1-2 days of age, while at 3–35 days of age, they are given regular drinking water, which is replaced every afternoon. Chickens aged 8–35 days are reared by adding onion flour in various concentrations (0%, 0.25%, 0.5%, 0.75%, and 1%) to the feed. Flour is mixed directly into the feed by feeding it twice per day start at 6 AM and 4 PM Indonesia time.

2.5. *Newcastle disease* (ND) Infection

The antigen utilized in this research is the natural infectious agent of the ND virus, which was obtained from sick chickens with ND. Organs from sick chickens' spleens, brains, and livers can be isolated and implanted in embryonated chicken eggs to produce fatal dosage 50 (LD 50). Previously, the antigen was evaluated with the hemagglutinin (HA) test to ensure that the antigen to be included was the 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.

2.6. Antibody Titer Testing for *Newcastle disease*

To evaluate the protective antibody titer against ND, an antibody titer test is performed. The hemagglutinin inhibition (HI) method was used to examine antibody titers in serum samples collected from blood via inject before necropsy. The blood taken was 5 mL and was collected in an EDTA-free inject. The examination was conducted at the East Java Livestock Service's Animal Diagnostic Disease Laboratory. The findings of the agglutination inhibition test, which indicate an antibody titer of 2^4, can be used to assess the protective antibody titer value against ND [4].

2.7. Histopathological Staining

Histopathological staining is a stain used to observe the microanatomical appearance of organs. Histopathology can be used with hematoxylin-eosin (HE) staining. In histopathological staining, hematoxylin color is the basic color that will color the acid structure so that a purple or blue color will be produced in the target organ and will appear basophilic. The basophilic color will color the nucleus or cell nucleus and the endoplasmic reticulum, while the eosin color will look eosinophilic or red, which colors the cell cytoplasm [5]. Observations can be made by looking at the microanatomical display on a microscope with 100x magnification to observe necrosis and lymphocyte depletion [6].

2.8. Data Analyst

This study used a completely randomized design (CRD) 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)]. The data that will be obtained includes blood profile values for lymphocytes and heterophils. The data that has been obtained is analyzed using SPSS to determine the level of significance. The data was also processed using the least significant difference (BNT) test method to determine real differences between treatments [7].

3. Results and discussion

3.1. Potential of Garlic Flour as an Antivirus

The activity of garlic ethanol extract has antiviral potential. The research was carried out using ND virus planting media in embryonated chicken eggs (TAB). This research found that administering garlic ethanol extract provides effective results. The examination is based on white blood cell infiltration observed under a microscope with 1000x magnification and a 20x field of view. Embryonated chicken eggs (TAB) that are 11 days old and have been inoculated with the ND virus are observed until the embryos die, then histopathological tests are carried out to take the heart and spleen.

Samples were stored in 10% neutral buffer formalin and stained using hematoxylin-eosin (HE) staining. The results of the observations were analyzed descriptively, and it was found that treatment with an ethanol extract of garlic provided effective results for exposure to embryonated chicken eggs infected with ND. This examination shows the presence of inflammatory cell infiltration in the form of neutrophils in the tissue. Inoculation or implantation of the ND virus in embryonated chicken eggs causes death of the embryo due to apoptosis because the ND virus can cause cell death, including chicken embryo fibroblasts and peripheral blood mononuclear cells [8]. Organs that can become sites of replication for this virus are the brain, lungs, spleen, heart, intestines, and liver. The ND virus can cause myocarditis, which indicates a systemic infection in the embryo.

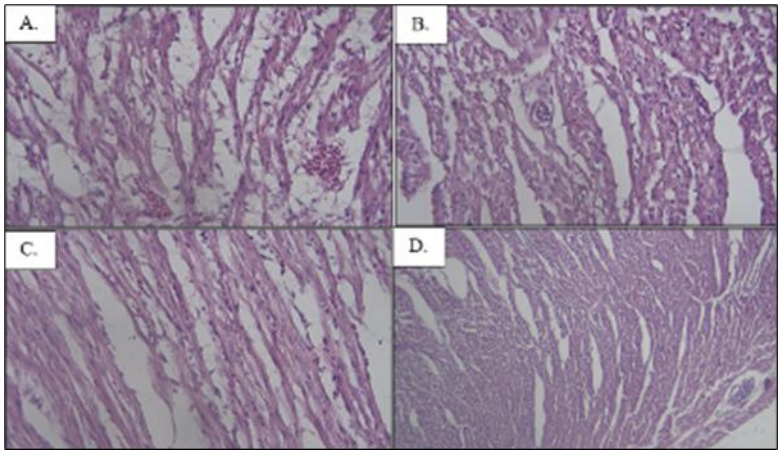

Figure 2 Histopathology of the heart in various treatment groups [A] Negative Control Group (CG-) [B] Positive Control Group (CG+) [C] Garlic Flour Treatment Group (CG1) [D] Other Flour Treatment Group (CG2)

This study was divided into 4 treatment groups, including the negative control group (CG-), namely embryonated chicken eggs that were only given ND virus inoculation; the positive control group (CG+), namely embryonated chicken eggs without ND virus inoculation; the garlic flour group (CG1), namely chicken eggs, embryonated chicken eggs with ND virus inoculation and white flour; and the other flour group (CG2), namely chicken eggs, embryonated chicken eggs with ND virus inoculation and other flour.

Figure 2 shows that when white bottom powder extract was applied to the heart organs of chicken embryos, various pathological abnormalities developed, including neutrophil infiltration, congestion, bleeding, necrosis, and edema. The image above clearly demonstrates that there is an influx of neutrophil inflammatory cells that dominate the heart organ. Table 1 shows the frequency of microscopic lesions of the heart organ.

Table 1 Frequency of microscopic heart organ lesions in the research treatment group

No	Histopathological Lesions	CG-	CG+	CG1	CG2
1.	Congestion	4/5	0/5	1/5	3/5
2.	Neutrophil Infiltration	5/5	0/5	1/5	4/5
3.	Necrosis	5/5	0/5	3/5	4/5
4.	Edema	4/5	0/5	4/5	3/5

During these observations, it was found that there were several lesions in the spleen, including necrosis and depletion of lymphocytes. Necrosis indicates cellular damage to the tissue that makes up the spleen, while leukocyte depletion indicates a different area marked by a decrease in the number of lymphocytes formed. The negative control treatment (CG-) was characterized by clearly visible lymphocyte depletion compared to treatment group 1 (CG1), treatment group 2 (CG2), treatment group 3 (CG3), and treatment group 4 (CG4).

Lymphocyte depletion indicates a decrease in the bird's immune response due to ND. Lymphocyte depletion causes the work of the spleen, which functions as an organ that produces lymphocytes, to be disrupted and reduces the immune response. The presence of a good immune system can be observed from the condition of the lymphoid organs, one of which is the spleen, which plays a role in producing large numbers of lymphocytes as a form of the body's defense. Apart from its function in producing lymphocyte cells, the poultry spleen also plays a role in phagocytizing old erythrocytes and maturing lymphocyte cells.

The presence of changes in pathological lesions indicates that ND infection affects the livestock's immune system, causing the livestock to experience a decreased immune system. A damaged spleen will cause a decrease in lymphocyte production. This can be proven by the results of blood tests in the negative control group (CG-), which had uncontrolled lymphocyte production compared to the treatment group given garlic flour, which showed a decrease in infection after exposure. The treatment group given 1% garlic flour appeared to have lymphocyte deletions, but it was different from the other groups, which were clearly visible and numerous in all observation areas. Lymphocyte deletion indicates excessive lymphocyte production due to infection after exposure to ND in broilers.

In cases of embryonic infection with the ND virus, it is always followed by hemorrhage or bleeding in the dead embryo. ND virus infection in embryos causes hemorrhage, which is characterized by generalized bleeding in dead embryos [9]. The histopathology results showed that necrosis occurred in all treatments. Necrosis is cellular damage characterized by damage to cell structure. In the negative control group (CG-) all heart organs experienced necrosis. This is because replication by the ND virus can cause cell death [10].

Another histopathological lesion that can be found is congestion. Chickens infected with ND experience congestion in the heart organ. In the positive control group (CG+), there was no cardiac congestion. Several other organs that are sites of viral replication besides the heart include the lungs, intestines, liver, and kidneys [11]. These results showed that the garlic group (CG1) showed fewer pathological lesions compared to the negative control group (CG-) and the other flour treatment group (CG2). This shows that garlic flour has the potential to fight ND. The activity of allicin in garlic can be a good antiviral agent to reduce tissue damage to the embryo. This first stage of research shows that garlic flour is an antiviral agent that can be used for in vivo research.

3.2. The Effect of Garlic Flour on Histopathology of the Spleen Organ

The researchers carried out a histopathological examination of the spleen. The spleen is an organ that functions as a lymphoid organ, forming the body's immune system. Poultry infected with ND can cause lymphoid tissue depletion and necrosis.

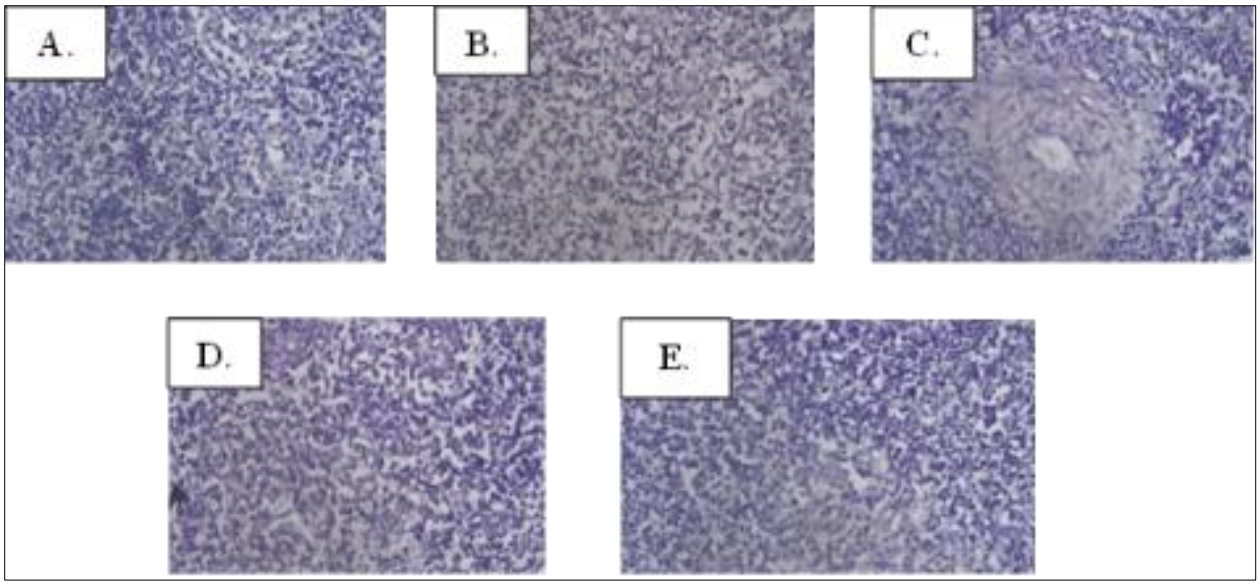

Figure 3 Histology of spleen organs in various treatment groups [A] Negative Control Group (CG-) [B] 0.25% Garlic Flour Treatment Group (CG1) [C] 0.5% Garlic Flour Treatment Group (CG2) [D] 0.75% Garlic Flour Treatment Group (CG3). [E] 1% Garlic Flour Treatment Group (CG4).

3.3. Effect of Garlic Flour on Animal Immunity

This research obtained several measurement variables regarding livestock immunity, including antibody titers, leukocytes, and lymphocytes. This measurement is intended to determine the response of garlic flour given to livestock infected with ND to the value of livestock immunity.

Table 2 Effect of giving garlic flour on antibody titter, leukocytes, and lymphocytes in broilers

Group	Antibody Titter (Average ± SD)	Leukocytes (Average ± SD)	Lymphocytes (Average ± SD)
Control Negative	1.4 ± 0.547^{a}	253.08 ± 1.758^{a}	246.90 ± 1.874^{a}
1st Treatment (0,25%)	4.8 ± 1.788^{ab}	236.46 ± 1.653^{b}	223.86 ± 1.342^{b}
2nd Treatment (0,5%)	9.6 ± 3.577^{bc}	195.12 ± 2.779^{c}	205.06 ± 2.668^{c}
3rd Treatment (0,75%)	14.40 ± 3.577^{cd}	145.42 ± 3.490^{d}	183.24 ± 3.637^{d}
4th Treatment (1%)	28.80 ± 7.155^{e}	93.88 ± 2.409^{e}	143.52 ± 2.685^{e}

Note: Notation $^{a, b, c, d, e}$ showed a significant difference ($P<0.05$) between treatments

The effect of ND infection is known to reduce the immune system in infected poultry, so the addition of garlic flour is expected to improve the immune status of poultry. The leukocyte or white blood cell variable shows a decrease in the increase from the normal physiological value of chickens, namely 3.10–23.04. The increase in leukocyte values was caused by infection in poultry. Treatment group 4 (CG 4) showed a decrease in leukocyte values compared to other groups. The negative control showed the highest leukocyte value compared to the treatment group, with garlic flour treatment in each treatment showing a significant decrease in the leukocyte value. Leukocytes play a role in infections caused by infectious agents [13].

A similar phenomenon happened with lymphocytes; adding garlic flour to the treatment group reduced lymphocyte levels. When compared to the treatment group, the negative control (CG-) had a higher lymphocyte value. This is due to the fact that lymphocytes grow as the infection proceeds. Lymphocytes contribute to immunity by suppressing immune cells' humoral responses. The presence of lymphocytes alters the phagocytic response of macrophages to antigens. The lymphocyte and leukocyte levels of chickens afflicted with ND increased [12].

The antibody titter variable is used to measure the body's immune response after being infected with the ND virus. Titer measurements can be carried out using serum samples obtained from the blood. The purpose of measuring antibody titers is to determine the immunity status of livestock after exposure to the ND virus. These results showed that the control group was negative (CG-). Broiler chickens infected with ND and given garlic flour supplementation showed higher antibody titers compared to broiler chickens infected with ND without garlic flour supplementation treatment. Antibody titers are formed from humoral responses, which can provide specific antibody responses to disease [13].

4. Conclusion

During ND infection, the effect of garlic powder on pathological abnormalities in the hearts of chicken embryos was observed. These modifications include a reduction in the severity of the infection, which is characterized by edema, congestion, necrosis, and neutrophil infiltration. Giving 1% garlic flour to animals can improve their immune state, as evidenced by an increase in protective antibody titers, a decrease in the number of leukocytes, and a decrease in the number of lymphocytes following ND infection. In the 1% garlic flour therapy group, histopathology of the spleen organ revealed differences in the degree of pathological lesions caused by ND infection.

Compliance with Ethical Standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

References

[1] Hewajuli DA and Dharmayanti, NLPI. Peran Sistem Kekebalan Non-Spesifik dan Spesifik Pada Unggas Terhadap *Newcastle disease*. Jurnal Wartazoa. 2015. 25: 135-136.

[2] Arreola R., Saray QF., Lopez RRI. Immunomodulation and Anti-Inflammatory Effects of Garlic Compounds. Journal of Immunology Research. 2015. 3: 1-13.

[3] Ernawati RAP., Rahardjo N., Sianita FA., Rantam., and Suwarno. Buku Penuntun Praktikum Penyakit Viral. Laboratorium Virologi dan Imunologi: Fakultas Kedokteran Hewan, Universitas Airlangga; 2008.

[4] Office International Des Epizooties (OIE). Manual OIE, Avian Influenza Chapter 2.7.12; © 2008. [cited 2023 Sep 1st]. Available from http://web.oie.int/fr/normes/mmanual/A_00037.html.

[5] Gurina, TS and Lary S. Histology Staining. Stat Pearls; 2021.

[6] Nofantri L., IK Berata., and AAA Mirah Adi. Studi Histopatologi Limpa dan Otak Ayam Terinfeksi Penyakit Tetelo. Indonesia Medicus Veterinus. 2017; 6 (5): 417-427.

[7] Sudarwati H., MH Natsir., and VMA. Nurgiartiningsih. Statistika dan Rancangan Percobaan: Penerapan dalam Bidang Peternakan. Malang, Indonesia: Universitas Brawijaya Press; 2019.

[8] Azizah SN., and A Saifudin. Antivirus Activity of Garlic Ethanol Extract (*Allium sativum* L.) Against *Newcastle disease* Model. Usadha: Journal of Pharmacy. 2023; 2 (3): 393-404.

[9] Balachandran P., P Srinivasan., S Sivaseelan., GA. Balasubramaniam., and TRGK Murthy. Isolation and Characterization of *Newcastle disease* Virus from Vaccinated Commercial Layer Chicken. Veterinary World. 2014; 7: 457-462.

[10] Putra, HH., MH Wibowo., T Untari., and Kurniasih. A Study of Macroscopic and Microscopic Lesions of Chicken Embryos Infected by Virulent *Newcastle disease* Virus Field Isolates. Jurnal Sain Veteriner. 2012; 30 (1): 57-67.

[11] Alexander, DJ and Senne DA. *Newcastle disease*, Other Avian Paramyxovirus and Pneumovirus Infection In: Disease of Poultry. Blackwell Publishing. IOWA; 2008.

[12] Malik M., M Sohail., M Sajid., Hamidullah., M Shoaib., N Bano., and SSA. Shah. Effects of *Newcastle disease* Virus on Different Haematological Parameters in Broilers. Advances in Animal and Veterinary Sciences. 2018; 6 (4): 183-186. DOI: http://dx.doi.org/10.17582/journal.aavs/2018/6.4.183.186.

[13] Jafari, RA., M Ghorbanpoor., and SH Diarjan. Study on Immunomodulatory Activity of Dietary Garlic in Chickens Vaccinated against Avian Influenza Virus (Subtype H9N2). International Journal of Poultry Science. 2019; 8(4): 401-403.

Author's short biography

Ganang Rilo Pambudi He is the first author to contribute to writing about histology in this article. The author has almost three years of experience in veterinary science and his expertise about veterinary public health. The author has done research in biomolecular and animal diagnostics.	
Rositawati Indrati She is the second author to contribute to writing about data analysis and animal immunity in this article. Her research interests focus on animal health, parasitology, and veterinary epidemiology. The author has over thirty-five years of research experience.	
Osfar Sjofjan He is the third author to contribute to writing an abstract, an introduction, and conclusions. He has more than thirty-five years of research experience. His research interests focus on poultry nutrition and feed biotechnology.