

THE EFFECT OF ADDING GARLIC FLOUR (*Allium Sativum*) AND OREGANO LEAVES (*Oreganum Vulare*) ON THE HISTOPATHOLOGICAL QUALITY OF THE LIVER OF BROILER CHICKENS

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Abstract

*This study aims to determine the results of adding garlic flour (*Allium sativum*) and oregano leaves (*Oreganum Vulare*) to feed on the liver histopathology of broiler chickens. This research consisted of 2 stages, the research material for stage I was a descriptive analysis of the histopathological changes in the liver of embryonated chicken eggs (TAB). The research material for phase II was the 200 Day Old Chick (DOC) Ross strain, unsex. Average body weight 38.485 ± 0.90 g. The research method used laboratory experiments and field experiments using a completely randomized design (CRD) with 5 treatments and 5 replications. The treatments given were P0: Basal feed (control feed), P1: Basal feed plus 0.25% garlic flour, P2: Basal feed plus 0.5% garlic flour, P3: Basal feed plus 0.75% white onion flour, P4: Basal feed plus 1 % garlic flour. The variables measured in stage I were liver histopathological changes. The data obtained will be analyzed descriptively. The variables measured in stage II, namely the enzymes ALT, AST, and ALP, liver weight, and abdominal percentage obtained will be statistically analyzed using analysis of variance (ANOVA) from a Completely Randomized Design (RAL) and followed by an honest significant difference test (BNJ) or Tukey's test. The results of the research were that giving garlic flour in feed influenced the parameters used. The use of garlic flour in feed had a very significant effect ($P < 0.01$) on histopathological changes, changes in ALT, AST and ALP enzymes. The conclusion obtained in this research is that the addition of 1% garlic flour to broiler chicken feed provides the best productivity.*

Keywords: Oregano Flour, Garlic Flour, ALT Enzyme, AST Enzyme, ALP Enzyme, Liver Weight, Abdominal Fat Percentage, Broiler

INTRODUCTION

Broiler meat is one of the strategic commodities that is growing rapidly, produced by the livestock subsector apart from beef, eggs and milk. Based on data from the September 2018 National Social Economic Survey, household consumption of purebred chicken meat nationally is an average of 6.36 kg/capita/year or an average increase of 3.92 percent compared to 2017, and is predicted to continue to increase (Brar et al., 2017). The poultry sector contributes to meeting the need for animal protein in Indonesia, namely 65 percent. The economic value of Indonesian poultry is quite large. According to 2019 data from the Association of Poultry Breeding Companies (GPPU), this value was 428 trillion (Węglarz et al., 2020).

The high demand for broiler chickens needs to be accompanied by monitoring of product quality. Chicken meat is one of the foodstuffs of animal origin that has a risk of transmitting disease. The quality of food ingredients from livestock must provide a guarantee of Safe, Healthy, Whole and Halal (ASUH), so that it will provide peace of mind for consumers. Food products of animal origin consumed by the public must comply with ASUH principles and be guaranteed by quality standards obtained through a series of

laboratory tests. One of the problems in the poultry industry that can affect meat quality from an animal health aspect is the spread of potentially infectious diseases through the supply chain. The large number of problems becomes an obstacle in controlling the disease, making it difficult to achieve the desired results (Ismail et al., 2019).

Many different diseases attack chickens and often the symptoms are almost the same. Newcastle Disease (ND), caused by the paramyxovirus strain, is an infectious disease that attacks chickens of all ages with a high mortality rate, especially at young ages. The economic losses caused by ND disease are very high because mortality can reach 100%, and the ND virus can survive for up to 2 months in piles of dry husks used as bedding for cages (Wiedosari & Wahyuwardani, 2015). Apart from that, conditions affecting broiler chicken farming include the ban on the use of antibiotics as growth promoter agents since 2017 through Minister of Agriculture Regulation Number 14 of 2017. The prohibition on the use of antibiotics as growth promoters in livestock is aimed at preventing the occurrence of antimicrobial resistance (AMR).

The incidence of the disease is acute to chronic and can attack all types of poultry, especially chickens, both purebred and non-breed chickens. Therefore, the ND case is a serious threat to the livestock industry in Indonesia. Losses due to ND disease are caused by the very high morbidity and mortality rates in poultry. Mortality and morbidity can reach 50-100% due to VND infection with velogenic strains, especially in susceptible chicken groups, 50% for mesogenic strains, and 30% for velogenic infections (Kencana et al., 2012). In Indonesia, ND disease is still endemic, as shown by the discovery of cases throughout the year. It occurs due to several factors such as poor vaccine quality, treatment of vaccines that do not meet standards such as temperature during storage, and vaccinator errors (Kurnianto et al., 2016).

Garlic (*Allium sativum*) is one of the ingredients that can be chosen to be used as a phytobiotic agent and has immunomodulatory properties. Garlic has activities including anti-atherosclerosis, antimicrobial, hypolipidemic, antithrombotic, anti-hypertensive and anti-diabetic (Umatiya et al., 2018). Another ingredient besides garlic that can be used as a phytobiotic agent is oregano leaves. Oregano leaves (*Oreganum vulare*) have high activity as an antimicrobial agent (Simirgiotis et al., 2020). The high immunomodulatory properties of garlic (*Allium sativum*) and oregano leaves (*Oregano vulare*) are to improve the health status of broiler chickens which can be observed through blood biochemistry including SGPT, SGOT, and ALP enzymes and by observing histopathological changes in the liver organ.

The potential of the test agent as an antiviral against ND can be inoculated at 11 day old TAB. A definitive diagnosis of ND can be made by isolating and identifying the virus using various types of tissue culture. The most practical and frequently used method for isolating the ND virus is culturing in 9-10 day old embryonated chicken eggs. The materials used for virus isolation are trachea or tracheal swabs, pulmonary, feces, intestinal contents, or cloacal swabs. Viral antigens in tissue can be tracked using immunohistochemical techniques (Okoroafor et al., 2018).

Based on the problems that have been described, a solution is needed in an effort to increase the productivity of broiler chickens. The addition of garlic flour (*Allium sativum*) and oregano leaf flour (*Oreganum vulare*) in feed at certain levels can act as an immunomodulator and can improve the health status of livestock by knowing liver histopathology. Meanwhile, production aspects can be assessed from the weight of internal organs and the percentage of tissue fat. Chicken liver is an organ that has a high nutritional content compared to liver that comes from other livestock (Lutfiah et al., 2021). Giving a combination of garlic flour (*Allium sativum*) and oregano leaves (*Oregano vulare*) is expected to improve the health status of livestock and increase the productivity of broiler chickens.

METHOD

Research on the effect of adding garlic flour (*Allium sativum*) and oregano leaves (*Oreganum vulare*) on the liver histopathology of broiler chickens, namely testing the content of garlic extract and oregano leaves which consists of testing the activity of the test material, namely a combination of garlic (*Allium sativum*) and oregano leaves. (*Oreganum vulgar*) against embryonated chicken eggs (TAB)

Research purposes

Phase I research aims to determine the potential of the test material agent as an antiviral against the Newcastle disease virus which is inoculated in 11-day-old embryonated chicken eggs and organ changes in the embryos through histopathological observations.

Research Location and Time

The place for implementation of phase 1 research is the Microbiology and Virology Laboratory, Faculty of Veterinary Medicine, Brawijaya University. Meanwhile, preparations were made and histopathological analysis was carried out at the Histology Laboratory, Faculty of Medicine, Universitas Brawijaya.

Research Materials

The materials used in phase I research are as follows:

- a. Embryoated chicken eggs (TAB) obtained from the Farma Veterinary Center
- b. Newcastle Disease (ND) antigen obtained from the Farma Veterinary Center
- c. Garlic flour obtained from Materia Medika Batu, Malang
- d. Oregano leaf flour obtained from Materia Medika Batu, Malang
- e. 70% alcohol as disinfection from chemical stores
- f. Antibiotic Penicillin- Streptomycin as obtained from

Research methods

The method used in phase I research was a laboratory experiment with a Completely Randomized Design (CRD) consisting of 4 groups with the following details:

P0: TAB + Newcastle Disease Virus Infection

P1: TAB + Newcastle Disease Virus Infection + Garlic Extract 0.2 ml

P2: TAB + Newcastle Disease Virus Infection + Oregano Leaf Extract 0.2 ml

P3: TAB + Newcastle Disease Virus Infection + Garlic and Oregano Leaf Extract 0.2 m

Each treatment tested was repeated 4 times and then tested for nutritional content through proximate laboratory analysis.

Research Equipment

Equipment used in phase I research included incubators, candling, plastic bags, 1 ml syringes, Petri dishes, gloves, masks, label paper, markers, writing instruments, and cameras.

Research procedure

The 10-day-old embryonated chicken eggs that will be used are first examined in a dark room using egg binoculars. This aims to determine the fertility of the egg and to ensure that the embryo is still healthy. Observations were made by looking at the movement of the embryo, and the sac, and observing the condition of the blood vessels which still looked red. After obtaining healthy eggs, the next step is to disinfect the eggs using alcohol. Clean embryonated chicken eggs are placed in an egg incubator at a temperature of 38°C with 6% humidity. Observations on TAB were carried out after the virus and garlic powder and oregano leaves were injected, then observations were carried out after 24 hours. Dead TAB would be opened for the embryos to be cut and then histopathological preparations made. The data obtained were analyzed descriptively to observe embryo death, organ damage in TAB, and gamma interferon (IFN- γ) activity.

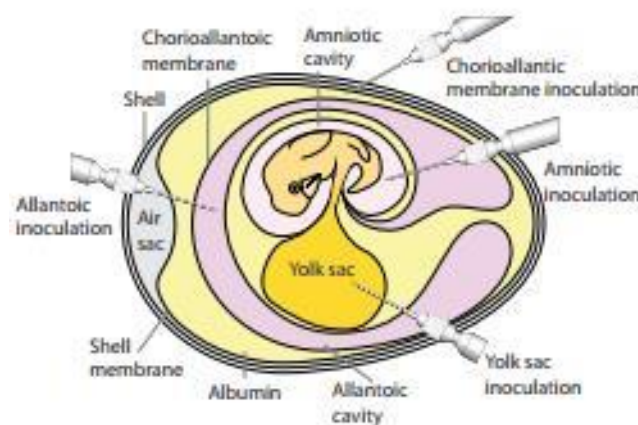


Figure 1. How to Inject Virus Inoculation in Chorioalantois (ATCC, 2012)

If dead embryos are found, TAB is stored at 4°C. Observations on TAB were carried out after the virus and garlic flour and oregano leaves were injected, then observations were carried out after 24 hours. The dead TAB would be opened for the embryos to be cut, then immunohistochemical preparations would be made and the expression of interferon-gamma (IFN- γ) would be seen.

Research variable

Variables observed include liver histopathology, histopathological staining is carried out to observe the microanatomical appearance of the organ. 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 (Gautam et al., 2017). Observations can be made by looking at the microanatomical display on a microscope with a magnification of 100 x to observe necrosis and lymphocyte depletion (Pirgozliev et al., 2019).

Data analysis

The data obtained were tabulated using Microsoft Excel and analyzed using RAL (Completely Randomized Design) and followed by the Duncan test if there were significant effects and differences.

RESULTS AND DISCUSSION

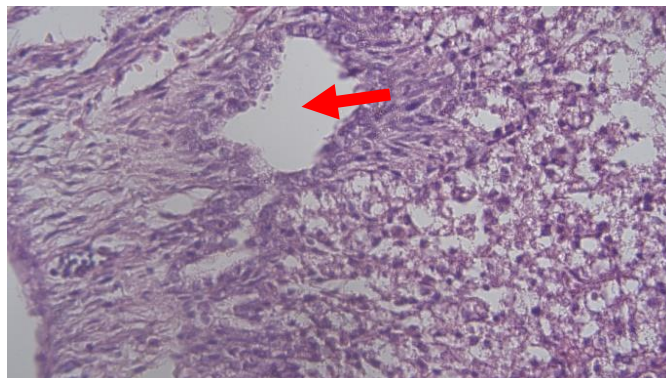


Figure 2. Histopathology of Positive Control Group

In the positive control group, cells were seen experiencing necrosis, the cytoplasm appeared cloudy.

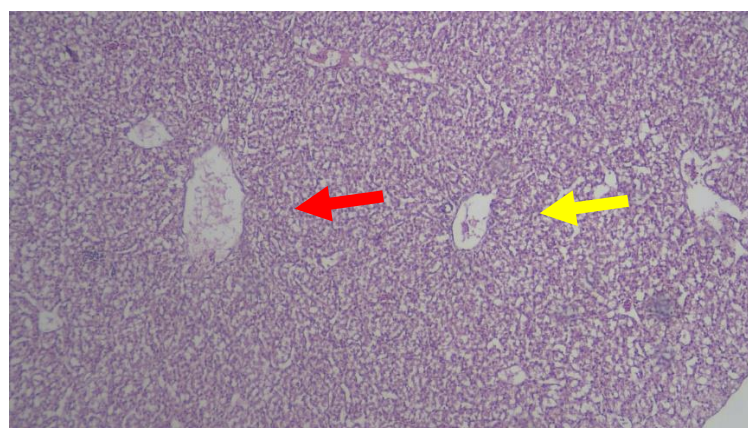


Figure 3. Histopathology of Negative Control Group

In the negative control group, the central vein (red arrow), portal vein (yellow arrow), and sinusoids appeared normal without inflammation or degeneration.

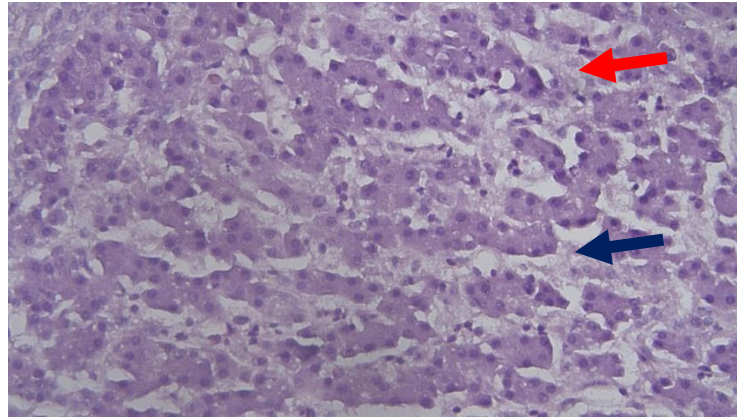


Figure 4. Histopathology of Oregano Leaf Treatment Groups
Biliary duct proliferation (blue arrow), fatty degeneration (red arrow)

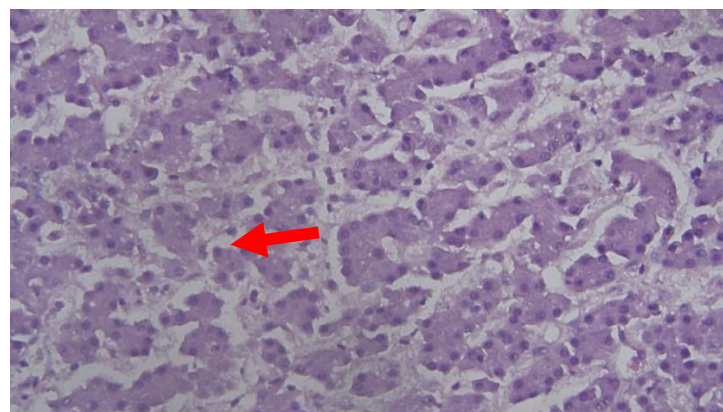


Figure 5. Histopathology of Garlic Treatment Group
When treated with garlic, histopathological changes were seen in the form of regular sinusoids.

The histopathological picture of the liver of the positive control group showed pathological changes where the liver was damaged, characterized by cells experiencing necrosis and the cytoplasm appearing cloudy. Administration of the ND virus to the positive control group resulted in increased liver activity because the liver functions as a detoxification and secretion organ. When the ND virus enters the body and replicates, the virus spreads through the bloodstream and causes secondary viremia. The virus is then phagocytosed by macrophages and secretes antibodies to protect the cells from the virus which continues to replicate. Macroscopic changes include hemorrhage, hyperemia, and swelling of the liver. The bleeding that occurs is a reaction to general inflammation due to the presence of antigens at that location. Changes caused by the Avian Paramyxovirus serotype-1 (APMV-1) virus cause organ damage ranging from hyperemia to cell necrosis and blood agglutination. The activity of viruses that agglutinate blood causes the formation of bleeding spots on organs. Necrosis or cell death in organs occurs because the supply of

cell nutrition is hampered, besides that there will also be infiltration of inflammatory cells. Inflammatory cell infiltration in interstitial tissue is the result of inflammation as a protective mechanism to isolate and eliminate injury-causing agents and repair tissue from injury. Infiltration that occurs in the liver can be caused by several factors, both infectious agents (bacteria, viruses, parasites) and non-infectious agents (Zaefarian et al., 2019). The presence of inflammatory cell infiltration in the liver will cause an increase in liver enzymes and microscopic changes in cell necrosis, cell damage, multiplication, or rapid cell regeneration (Wenno, 2018).

In the negative control group, the liver histopathology appeared normal, where the central veins, portal veins, and sinusoids were normal, and there was no visible inflammation or degeneration. The normal histological structure of the liver is characterized by the presence of single-nucleated hepatocytes in the center and transparent cytoplasm, the liver cells are distributed radially near the central vein, and the sinusoids appear clearly with a regular location (Latifah et al., 2022). The histopathological picture of the garlic treatment group showed results where the sinusoids were wide and regularly located, the cytoplasm was not turbid, and the hepatocytes had one nucleus in the middle.

In the oregano leaf treatment group, histopathological changes showed bile duct proliferation and fatty degeneration, where fatty degeneration was characterized by the presence of vacuoles that varied in size and severe cases pushed the nucleus to the edge. Fat in the cell cytoplasm can push the cell nucleus to the edge which is visible on microscopic examination. This is an initial reaction before tissue necrosis occurs. Degeneration and necrosis can occur due to various causes, including lack of oxygen. The degeneration that occurs due to a lack of oxygen supply to tissues is called hypoxia. This is related to congestion so that the tissue lacks a blood supply that contains lots of oxygen. Cells need oxygen to maintain their survival, if cells experience hypoxia then there will be damage to the cells (degeneration) and necrosis. (Siswandy et al., 2020).

CONCLUSION

The garlic flour and oregano leaf flour used had an impact on changes in the liver histopathology of Embryoated Chicken Eggs (TAB). The use of 1% garlic flour can provide the best results in numerical and qualitative terms.

It is recommended to use 1% garlic flour in feed because garlic flour has a good impact on changes in liver histopathology, AST, ALT, and ALP enzymes as well as liver weight and abdominal fat percentage in broiler chickens.

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