Histomorphometric Features of Kidney Glomeruli and Liver Sinusoids of Broiler Chicken Exposed to *Salmonella enteritidis*

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ABSTRACT
Broiler chicken is one of the preferred sources of protein in Indonesia due to its high protein content. The high consumption of broiler chickens needs to be accompanied by a good maintenance system. Starter phase broilers have a rudimentary digestive system, making them susceptible to disease. Salmonellosis is common in poultry, one of the serotypes that often attacks broiler chickens is *Salmonella enteritidis*. The toxin produced by *Salmonella enteritidis* can affect the kidneys through the blood. Red ginger has potential as antibacterial and anti-inflammatory. This study aims to determine the effect of the addition of red ginger powder in broiler feed on kidney and liver tissue damage of broiler chickens infected with *Salmonella enteritidis* $1x10^7$ CFU microscopically by looking at histopathology and histomorphometry which is then continued using ImageJ. This study was conducted experimentally with treatment groups divided into six groups, namely P1 (negative control, without treatment), P2 (Antibiotic Growth Promoter tetracycline dose 15mg/kg BW + infection), P3 (2% red ginger powder + infection), P4 (positive control group), P5 (Stimuno forte 13.5 mg/KgBB + infection), and P6 (2% red ginger powder). Standard CP 511 feed was given on days 1 to 7, while the addition of 2% red ginger powder, Stimuno® forte and tetracycline was done on days 8 to 15, in the finisher phase until day 35 then induced with *Salmonella enteritidis* at a dose of $1x10^7$ CFU. On the 16th and 36th days, necropsy was performed to see the histopathological and histomorphometric images of liver sinusoids and kidney glomeruli of starter phase broilers. Furthermore, the data obtained were analyzed using One Way ANOVA test ($P < 0.05$), which was then followed by Tukey's test ($\alpha = 5\%$) and Independent T-Test. The results showed that the addition of 2% red ginger powder (*Zingiber officinale* var. *rubrum*) in feed can affect the histopathology and histomorphometry of kidney glomerular diameter and liver sinusoids. The conclusion of this study is the addition of 2% red ginger powder (*Zingiber officinale* var. *rubrum*) in feed can play a role in reducing the enlargement of kidney glomerulus size and liver sinusoids.

Keywords: *Broiler chicken, Salmonella enteritidis, Red Ginger, histopathology, histomorphometry.*

1. INTRODUCTION

Poultry, especially chicken, is known as a source of animal protein from livestock in Indonesia with a fast growth rate. Starting from laying hens and broilers, with a relatively cheap price, it is easy to obtain and process [1]. Statistics on Animal Husbandry and Animal Health explained that there was a continuous increase in the production of poultry products, especially chicken, from 2017 to 2019. In addition, it was recorded that the average consumption of broiler meat has a higher value than beef, lamb, duck, and pork. The high level of broiler consumption must be accompanied by a good maintenance system. Broiler chickens are susceptible to diseases that can be caused by pathogenic bacteria such as *Salmonella sp., Mycoplasma sp., and Escherichia coli.*

The kidneys work by controlling fluid volume and electrolyte concentration in the body, excreting metabolic waste, regulating metabolic activity, and controlling acid-base balance. Any disturbance in the kidneys can cause disruption of the electrolyte fluid balance [2]. While the liver is the largest organ in the abdomen and plays a role in the body's metabolism, the liver also has the function of detoxifying substances in the body and excreted through bile. The liver can also process nutrients from the intestines through the portal vein. The liver also receives blood through the hepatic artery and exits through the hepatic vein. Because the liver plays such an important role, it is an organ that is easily damaged. Liver damage

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is mainly caused by infection or inflammation. Chicken livers infected with Salmonella enteritidis underwent pathological changes, and heterophilic infiltration and lymphocytes around blood vessels were observed [3].

Salmonella sp. is a pathogenic bacteria that belongs to the gram-negative bacteria group, which can cause mortality rates of up to 80%-100% in broilers. This bacterial infection can affect chickens of all ages. Common symptoms that can be seen in starter chickens include lethargy, lack of appetite, diarrhea, and dirty anus. Chicks less than 4 weeks old are susceptible to *S. enteritidis*, and infection of these bacteria in chicks can cause high mortality rates [4]. *Salmonella enteritidis* also has toxins that can cause metabolic disorders including in the kidneys of chickens, ranging from inflammation, congestion, hemorrhage that can disrupt the work of the kidneys [5].

The form of control that can be done is the use of antibiotics such as tetracycline. However, the resulting residues can cause resistance to bacteria that can harm livestock and humans who consume. Thus, natural materials such as herbs are currently widely used as a substitute for antibiotics such as red ginger (Zingiber officinale Rubra), turmeric (Curcuma domestica) and meniran (Phyllanthus niruni) [6]. Red ginger (Zingiber officinale) is one of the plants that are widely consumed in Indonesia. Red ginger has properties as an antioxidant, some studies show that red ginger acts as an anti inflammatory, antioxidant, antihypertensive, antimicrobial, and immunomodulator [7].

This study was designed to determine the effect of adding 2% red ginger powder (Zinger officinale var. rubrum) on the histopathology and histomorphometry of kidney glomeruli and liver sinusoids of broiler chickens that have been exposed to *Salmonella enteritidis*.

2. MATERIALS AND METHODS

2.1. Study Period and Location

This research was conducted from August to December 2019 at the Agricultural Development Polytechnic of Malang for broiler rearing facilities. Then in September 2021 - January 2022 research was carried out at the Histology Laboratory of the Faculty of Veterinary Medicine, Brawijaya University for histopathology and reading the results of Hematoxylin Eosin staining.

2.2. Research Sample

This study used the liver and kidney organ of broiler chickens DOC CP 707 (starter phase aged 1-16 days and finisher phase aged 17-35 days). The experimental animals came from PT Charoen Pokphand Pasuruan with a total of 150 birds and an average weight of 1500-2000 grams. Animals adjusted to the environmental conditions of the cage for seven days in Federer's calculation, so that the broilers used were 30 chickens and had obtained a certificate of ethics in their use. The study used 150 broiler chickens DOC CP 707 which were divided into 6 groups with 5 replicates and each replicate of 5 chickens, namely P1 (negative control), P2 (tetracycline + infection group), P3 (2% red ginger powder + infection group), P4 (positive control), P5 (stimuno + infection group), P6 (2% red ginger powder addition group). On days 1 to 7, standard CP 511 feed was given, on days 8 to 15, feed with 2% red ginger powder, stimuno, and tetracycline was added, in the finisher phase until day 35, then induced with *Salmonella enteritidis* at a dose of $1x10^7$ CFU. On the 16th and 36th day, necropsy was performed to see the histopathological and histomorphometric features of the liver sinusoids and kidney glomeruli of starter phase broilers. Data were analyzed using One Way Analysis of Variance (ANOVA) test and followed by Tukey test ($P<0.05$).

2.3. Preparation of Histopathology Preparations

The first step of making preparations is to immerse the liver using 10% formalin solution within 24 hours. Immersion is done to prevent organ damage. Stages of histopathology preparation include fixation, trimming, dehydration, clearing, impregnation, and embedding. The function of Hematoxylin Eosin staining is so that the nucleus of the cell can appear blue or basophilic and the cytoplasm and connective tissue are pink or eosinophilic. The stages of this coloring include deparaffinization, rehydration, staining, dehydration, clearing, and mounting.

2.4. Histopathology and Histomorphometry Observations

Histopathology was observed using optilab and 40x, 100x, and 400x magnification microscopes. Histomorphometry is a method of measuring the thickness, diameter, length, width, and volume of cells or tissues to study the activity and changes in the shape of these cells or tissues [3]. Histomorphometry of sinusoid cells and kidney glomeruli was seen using Image J. Observations of sinusoid width and glomerular diameter were seen from five different fields of view for all treatment groups and then analyzed using ANOVA.

2.5. Data Analysis

Data were analyzed quantitatively using One Way Analysis of Variance (ANOVA) with a confidence level of 95% to determine differences in data, and if there were significant differences in ANOVA, it was continued with the Tukey test to determine between each treatment obtained using SPSS software. Liver histopathology was analyzed descriptively

qualitatively using a light microscope and then photos were taken using optilab at 40x, 100x, and 400x magnification.

3. RESULT AND DISCUSSION

3.1. Histopathologic Examination of Chicken Kidney and Liver

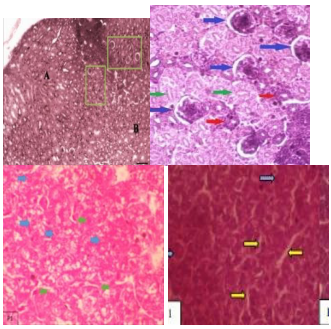

Figure 1 Microscopic images of the kidneys (top) and liver (bottom) of broiler chickens in the starter and finisher phases of group P1 (negative control). Description: Normal glomerulus (green box) in the cortex (A) and medulla (B) layers. Glomerulus (blue arrow); Proximal cortical tubule (green arrow); Distal cortical tubule (red arrow). Sinusoids (green arrow); Hepatocytes (blue arrow). (HE staining; 100x).

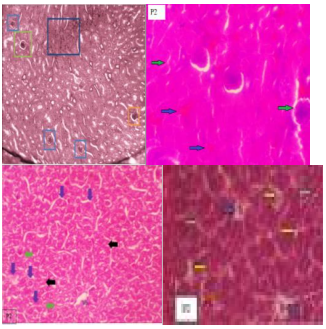

Figure 2 Microscopic images of the kidneys (top) and liver (bottom) of starter and finisher phase broiler chickens in group P2 (standard feed added Antibiotic Growth Promoter tetracycline). Description: Normal glomerulus, glomerular hypertrophy, glomerular atrophy, and tubular coagulative necrosis area, fused glomerulus (green arrow), congestion (blue arrow). Sinusoids (green arrow); Hepatocytes (blue arrow), Hydropic degeneration (purple down arrow); Cell walls between hepatocytes not clearly visible (black arrow); VS (Central Vein). (HE staining; 100x).

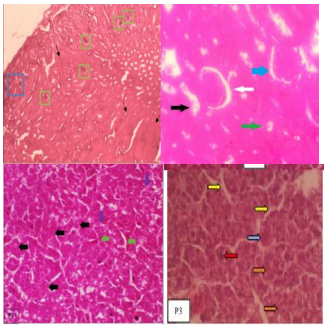

Figure 3 Microscopic images of the kidneys (top) and liver (bottom) of broiler chickens in the starter and finisher phases of group P3 (standard feed added 2% red ginger powder). Description: normal glomerulus (green box), glomerular hypertrophy (blue box), and coagulative necrosis area of tubule (black arrow), glomerulus (white arrow), distal cortical tubule (green arrow), proximal cortical tubule (black arrow), hypertrophy in glomerulus (blue arrow) Sinusoid (green arrow); Hepatocytes (blue arrow), Hydropic degeneration (purple down arrow); Cell walls between hepatocytes are not clearly visible (black arrow). (HE staining; 100x).

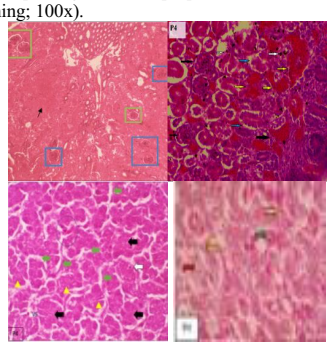

Figure 4 Microscopic images of kidney (top) and liver (bottom) of starter and finisher phase broiler chickens of group P4 (Chicken infected with *Salmonella enteritidis* without addition of 2% red ginger powder). Description: normal glomerulus (green box) glomerular hypertrophy (blue box), tubular coagulative necrosis (black arrow) and glomerular liquefactive necrosis (blue arrow). Sinusoids (green arrow); Hepatocytes (blue arrow), Hydropic degeneration (purple down arrow); Cell walls between hepatocytes are not clearly visible (black arrow); VS (Central Vein), Karyolysis necrosis (yellow triangle); Karyopic necrosis (white arrow); Inflammatory cells (blue circle). (HE staining; 100x).

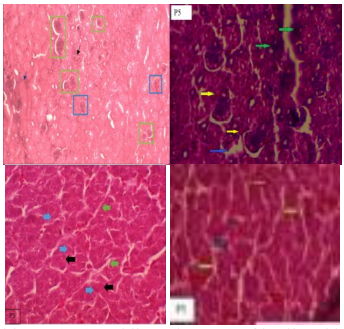

Figure 5 Microscopic images of the kidneys (top) and liver (bottom) of broiler chickens in the starter and finisher phases

of group P5 (standard feed added Stimuno forte). Description: glomerular hypertrophy (blue box), glomerular coagulative necrosis (black arrow), tubular coagulative necrosis (blue arrow), normal glomerulus (green box). Accumulation of inflammatory cells (green arrow); congestion (yellow arrow); detachment in glomerulus (blue arrow). Sinusoids (green arrow); Hepatocytes (blue arrow), Cell walls between hepatocytes are not clearly visible (black arrow). (HE staining; 100x).

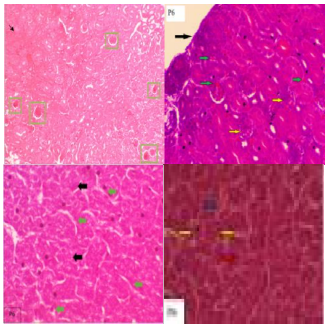

Figure 6 Microscopic images of kidney (top) and liver (bottom) of starter and finisher phase broiler chickens of group P6 (standard feed added 2% red ginger powder without *Salmonella enteritidis* infection). Description: normal glomerulus (green box), and coagulative necrosis of tubules (black arrow). inflammatory cell accumulation (black arrow); congestion (green arrow); glomerular hypertrophy (yellow arrow). Sinusoids (green arrow); Hepatocytes (blue arrow), Cell walls between hepatocytes are not clearly visible (black arrow). (HE staining; 100x).

Group P1 as a negative control or without treatment was found to have no pathology changes. Group P1 with standard feed treatment according to SNI quality requirements is used as a reference with other groups given additional feed treatment and S. enteritidis bacterial infection. The glomerulus was normal with an average size of 35.44 µm. Histopathological observations of the kidneys of P2 group chickens found inflammation after *Salmonella enteritidis* infection. This inflammation can trigger cells to remain in a state of homeostasis through adaptations such as atrophy, hypertrophy, hyperplasia, and metaplasia. Observations of group P3 obtained an average glomerular diameter of 36.30 µm, which is greater than the average glomerular diameter of group P1. Some glomeruli appear hypertrophied, this can occur due to toxins from *Salmonella enteritidis* which cause damage to the structure of kidney tissue. Cell damage due to toxin compounds is seen in the enlargement of glomerular size (hypertrophy) to lysis of the nucleus which indicates necrosis. As in the microscopic appearance of group P3, the cytoplasm looks more acidophilic (red) due to protein denaturation. Tubular damage that occurs can be caused by the function of tubules in filtering so that they are vulnerable to *Salmonella enteritidis* toxins, but when viewed from the microscopic picture of the glomerulus and glomerular diameter, it can be concluded that the addition of 2% red ginger powder to standard feed can have a good effect on chicken kidneys. Red ginger has a protective effect on the kidneys, by conducting an average score of kidney damage images against paracetamol induction, where the smallest average score was obtained in the group given red ginger [7]. Red ginger can inhibit arachidonic acid so that the cyclooxygenase and lipoxygenase pathways are inhibited in the inflammatory process [8]. Observations of group P4 found necrosis with formations such as empty spaces due to the failure of inflammatory cells to fight toxin compounds.

Macrophages function as Antigen Presenting Cell (APC) by presenting antigens to lymphocytes resulting in the secretion of IL- 2, TNF and IFN-γ which will then stimulate transforming growth factor beta (TGF-β1). Necrosis is characterized by damage to cell death, in the histopathological picture of coagulative necrosis occurs in tubules with nuclei that look karyopyknosis, while in liquefactive necrosis the nucleus is seen to have disappeared and the tissue leaves empty space like liquid and leaves persistent structures such as connective tissue. This necrosis can also be said to be a manifestation of the final phase of necrosis. The lysis of tissue or turning into liquid occurs due to macrophages that phagocytize necrotic tissue [9]. Group P5 has the same average diameter as group P3 which is 36.00 µm, where it can be said that Stimuno forte gives the same effect as the addition of 2% red ginger powder. The content of meniran (Phyllanthus niruri Linn) in stimuno forte can work as an immunostimulant [10]. This can be seen from the average phagocytosis index value in the group given meniran, where the higher the dose of meniran, the higher the phagocytosis index. Most of the glomeruli in the P6 group were normal with an average value of 34.82 µm which was not much different from the negative control group (P1). The necrosis found can be caused by the content of red ginger which can be a toxin, namely, sesquiterpenes. The cessation of blood flow causes hypoxia so that cells experience pathology. In addition, no other tissue damage was found. Red ginger works effectively to protect the kidneys from nephrotoxic substances [7].

In histopathological observations of broiler liver organs, normal conditions in liver microscopy are characterized by having clearly visible hepatocyte cells, round nuclei, centrally located, and homogeneous red cytoplasm. The cell walls are firmly bounded and sinusoids are clearly visible and the central vein as the center of the lobule appears round and empty [11]. Histopathology of group P1 looks normal with no damage or inflammatory cell infiltration, clear hepatocyte cells, round nuclei, pink cytoplasm. Histopathology of group P2, group P3, group P5, and group P6 showed hydropic degeneration, sinusoid dilatation, and cell walls between hepatocyte cells were not clearly visible but in groups P5 and P6 there was no hydropic degeneration. Hydropic degeneration is a reversible degeneration. Hydropic degeneration is caused by the presence of water-filled vacuoles in the cytoplasm. When the permeability of the cell membrane is disturbed, the liquid outside the cell will easily enter the intracell. When excessive water enters, small and numerous vacuoles will form. The collection of vacuoles will merge into a large and fill the cytoplasm so that the cell swells and will appear cloudy [12]. Hydropic degeneration will cause swelling of the hepatocyte cell membrane resulting in narrowing of the sinusoids [13]. Histopathology of group P4 showed the presence of inflammatory cells, necrosis in the form of karyolysis, karyopic necrosis, sinusoidal dilatation. Dilatation of sinusoids in the liver is a sign of damage to the liver. Sinusoidal dilatation is caused by the presence of hepatocyte cells that undergo necrosis. The necrotizing hepatocyte cells have an irregular shape, so the arrangement of hepatocyte cells in the lobules becomes irregular. As a result, the sinusoids bordering the hepatocyte cells are dilated. Dilatation of sinusoids can be caused by high levels of toxins in the blood that pass through the sinusoids to the central vein [14]. The process of hepatocyte damage begins with a degenerative process. The degeneration process is thought to be due to an increase in free radicals in the liver tissue. One of the changes caused by free radicals is lipid peroxidation which causes changes in the properties of cell membranes and cytoplasmic membranes of cellular components such as mitochondria and lysosomes. After damaging the cell membrane, the toxin effect can also reach the nucleus and destroy it, resulting in abnormal cell structure and eventually necrosis [15]. Inflammation in the liver occurs due to hepatocyte damage so that hepatocyte macrophages (kupffer cells) will quickly eat dead cells and form an accumulation of normal parenchymal inflammatory cells [16]. Necrosis is the death of cells caused by trauma, metabolic disorders, or infection. In the P4 group, cells showed karyopic necrosis and karyolysis. The stages of cell death can be indicated in the core. In karyopyknosis the nuclei usually appear condensed, with irregular borders, and dark (hyperchromatic). Karyorhexis when the cell nucleus is fragmented will leave fragments of chromatic substance in the cell nucleus. Karyolysis when the cell nucleus dies will appear empty or pale because the ability to color is lost so that the remaining cell membrane appears [17]. The addition of 2% red ginger powder in starter phase broiler feed can reduce the occurrence of liver damage as seen from the width of sinusoids in groups P3 and P6 compared to group P4 (positive control). This is because the P4 group was characterized by hydropic degeneration reaction, inflammatory cell infiltration, and necrosis. The addition of 2% red ginger powder proves that when exposure occurs, the flavonoid content will prevent bacteria from replicating so as not to trigger chronic inflammation by reducing pro-inflammatory cytokines so as to prevent damage that occurs in the tissue [18].

3.2. Histomorphometric Examination of Chicken Kidney and Liver

Table 1. Mean Kidney Glomerular Diameter of Starter Phase Broiler Chickens.

Treatment Group	Sinusoid Diameter Mean ± SD (µm)
P1(Negative control)	35,44±2,08 ^{a,b}
P2 (AGP + bacteria)	33,77±3,36 ^{a}
P3 (Red ginger powder 2% + bacteria)	36,30±3,38 ^{a,b}
P4 (Positive control)	39,11±2,56 ^{b}
P5 (Stimuno + bacteria)	36,00±2,42 ^{a,b}
P6 (Red ginger powder 2%)	34,82±0,58 ^{a,b}

Table 2. Mean Kidney Glomerular Diameter of Finisher Phase Broiler Chickens.

Treatment Group	Sinusoid Diameter Mean ± SD (µm)
P1 (Negative control)	43.54 ± 1.09^{c}
P2 (AGP + bacteria)	31.26 ± 0.87^{a}
P3 (Red ginger powder 2% + bacteria)	43.72 ± 1.42^{c}
P4 (Positive control)	56.07 ± 2.32^{d}
P5 (Stimuno + bacteria)	34.54 ± 2.34^{ab}
P6 (Red ginger powder 2%)	36.82 ± 2.01^{b}

Table 3. Mean Liver Sinusoid Diameter of Starter Phase Broiler Chicken.

Treatment Group	Sinusoid Diameter Mean ± SD (µm)
P1 (Negative control)	4,08 ± 0,33[a]
P2 (AGP + bacteria)	4,33 ± 0,70[a]
P3 (Red ginger powder 2% + bacteria)	4,30 ± 0,26[a]
P4 (Positive control)	5,20 ± 0,37[b]
P5 (Stimuno + bacteria)	4,66 ± 0,62[a,b]
P6 (Red ginger powder 2%)	4,37 ± 0,24[a]

Table 4. Mean Liver Sinusoid Diameter of Finisher Phase Broiler Chicken.

Treatment Group	Sinusoid Diameter Mean ± SD (µm)
P1 (Negative control)	4,05 ± 0,44[a]
P2 (AGP + bacteria)	3,84 ± 0,34[a]
P3 (Red ginger powder 2% + bacteria)	3,93 ± 0,43[a]
P4 (Positive control)	5,39 ± 0,28[c]
P5 (Stimuno + bacteria)	4,41 ± 0,32[ab]
P6 (Red ginger powder 2%)	4,81 ± 0,55[bc]

Based on the One Way ANOVA test, in the starter phase kidney glomerular diameter, the significance value is 0.061 so it can be concluded that there is no significant difference between treatment groups. The results of the Tukey test analysis also showed the same results where group P1 was not significantly different from the other groups. Group P1 has an average diameter value of 35.44□m. The normal glomerular value of chickens ranges from 24 - 134 µm. Group P2 has an average diameter value of 33.76□m [19]. The results of histomorphometric calculation of the glomerular diameter of group P2 obtained values of 17,84□m, 25,28□m, and 26,30□m. Based on statistical tests, group P2 is not significantly different from groups P1, P3, P5, and P6 but significantly different from group P4 (positive control). This is also supported by the Independent T-Test. between groups P2 and P4, the results obtained were 0.016 which means significantly different, indicating a difference between the AGP addition group and the positive control. Independent T-test is performed when two groups are compared with unrelated or different variables, used in groups with different treatments such as P2 and P4 [20]. The glomerulus with a size of 17,84□m in the picture looks atrophied, where atrophy occurs as a cell adaptation mechanism when exposed to *Salmonella enteritidis* toxin. Tetracycline residues are known to have high concentrations in the kidneys and can be nephrotoxic. Thus, it can be concluded that atrophy in group P2 can also be caused by the nephrotoxic properties of tetracycline residues. Based on this, it can be concluded that the addition of tetracycline acts as an antibacterial against the kidneys of starter phase chickens, but can also have a nephrotoxic effect by obtaining glomerular diameter values that are below the average normal glomerular diameter. Group P3 has an average diameter value of 36.30□m, not significantly different from the other groups, but has a smaller average diameter when compared to group P4, and greater than groups P2 and P5. Histomorphometric calculation of glomerular diameter obtained results 36,75□m and 43,23□m. Based on the observations and statistical tests that have been carried out, it can be said that the addition of 2% red ginger powder to the feed can have an effect on glomerular diameter. The enlargement of the glomerulus so that the bowman spatium narrows can be caused by an inflammatory process that causes vasodilation due to the process of cytokine chemotaxis, in P3 obtained an average diameter smaller than the P4 group. This shows that flavonols, which are included in one type of flavonoids in red ginger, can work as anti-inflammatory to prevent glomerular swelling. Flavonols work by suppressing the expression of VCAM-1, ICAM-1, MCP-1 induced by cytokine IL-1 which can cause atherosclerosis in the inflammatory process if it occurs continuously [21]. Group P4 has the highest average renal glomerular diameter compared to other groups, which is 39.11□m. This group is significantly different from group P2, and not significantly different from the other groups. Toxin from *Salmonella enteritidis* after passing through the intestine until finally entering the kidneys through the blood will be nephrotoxic, if continuous inflammation that occurs will cause damage to the tubular epithelium and glomerulus. Based on the observation of the histopathological picture, the glomerular diameter shows glomerular enlargement, to other damage such as epithelial erosion, hypertrophy, atrophy, to necrosis [22]. Group P5 has an average glomerular diameter of 36,00□m which is not significantly different from the other groups. The results of histomorphometric calculations obtained glomerular diameters with sizes 29,02□m, 40,188□m, 48,11□m, and 69,67□m. This group is not significantly different from the other groups, as well as the P3 group. However, in group P5 the average diameter obtained was smaller than that of group P3. So it can be said that the addition of 2% red ginger powder has the

same potential as Stimuno forte. The content of meniran in Phyllanthus niruri in Stimuno forte such as flavonoids which are the same as red ginger, work as immunomodulators by helping to stimulate specific and nonspecific immunity, which has the same function as red ginger. Red ginger, like stimuno, has the potential as an immunostimulant by helping to increase antibodies through measuring serum IgA concentrations [23]. Group P6 has an average glomerular diameter of 34.81□m and is not significantly different from the other groups based on statistical tests conducted. Therefore, based on histopathological and histomorphometric observations of group P6, it can be concluded that the addition of 2% red ginger powder has a good effect on the kidneys of starter phase chickens. Red ginger contains proteolytic enzymes such as proteinase thiol and zingibain that can improve protein digestion so as to help the body's metabolism [24].

The highest mean glomerular diameter of finisher phase broiler chickens can be seen in the P4 group (56.07µm), P3 (43.72 µm), P1 (43.54 µm), P6 (36.82 µm), P5 (34.54 µm), P2 (31.26 µm). Group P1 is a negative control that is given standard feed and not given the induction of *Salmonella enteritidis* bacteria. Group P1 is used as a reference to see an increase or decrease in the mean glomerular diameter of groups P2, P3, P4, P5, P6. Group P1 has a higher mean than groups P2, P5, P6, but lower than groups P4 and P3. Group P2 is a group that is given additional antibiotics, namely tetracycline at a dose of 15 mg / kg BW mixed with standard feed on day 8 to day 35 and induced *Salmonella enteritidis* bacteria on day 15. The average results of group P2 have the lowest results of groups P1, P3, P4, P5, P6. This proves that tetracycline at a dose of 15 mg/kg BW has a very good effect on killing *Salmonella enteritidis* bacteria. The antimicrobial effect of tetracycline is bacteriostatic and works by inhibiting bacterial protein synthesis. The antimicrobial spectrum of tetracycline shows a broad antibacterial spectrum that includes gram- negative and positive bacteria. Tetracycline itself is very often used in the treatment of respiratory tract infections, skin wounds, and bacterial infections in the intestine [25]. Group P3 is a group that added 2% red ginger powder mixed in the feed from day 8 to day 35, and induced *Salmonella enteritidis* bacteria on day 15. Group P3 had a lower mean glomerular diameter than P4 but higher than P1, P2, P3, P5, P6. Red ginger powder 2% has pharmacological activities that include antibacterial and immunomodulatory. One of the red ginger content that functions as an antibacterial is shogaol and gingerol and the immunomodulator is flavonoids. The active compounds shogaol and gingerol have moderate antibacterial activity against gram-positive and gram- negative bacteria. Red ginger phytobiotics mixed in chicken feed can spur the immune response of antibody titers. In addition, it is reported to have anti-inflammatory, antioxidant, antihypertensive[26] .Group P5 was given stimuno forte at a dose of 13.5 mg/kg BW mixed in standard feed on day 8 and induced *Salmonella enteritidis* bacteria on day 15. Group P5 had lower mean results than P1, P3, P4, P6 but higher than P2. Stimuno forte contains Phyllanthus niruri L which contains flavonoids, steroids, phenolics, saponins, and alkaloids. Flavonoids are compounds that can interfere with the constituent components of peptidoglycan in bacterial cells so that the cell wall layer is not formed perfectly which causes cell death. Alkaloids in Phyllanthus niruri L content function as antibacterials by inhibiting nucleic acid synthesis and inhibiting energy metabolism while the mechanism of action of saponin compounds as antibacterials by causing leakage of proteins and enzymes in cells[27] . Group P6 is a group that added 2% red ginger powder to standard feed without being induced by *Salmonella enteritidis* bacteria. Group P6 had a lower mean than P1, P4, P3 and higher than P2, and P5. The active compounds of red ginger can function as antibacterial and immunomodulatory, one of the active compounds is flavonoids, and phenol derivative compounds, namely shogaol and gingerol. The phenol active substances will interact with bacterial cells through the adsorption process involving hydrogen bonds. Inhibition of microbial growth by 2% red ginger powder phenol is due to the ability of phenol to denature proteins where these compounds react with porins (transmembrane proteins) and will damage cell membranes by dissolving fats contained in bacterial cell walls. Damaged porins will reduce cell wall permeability, resulting in nutrient deficiencies and inhibiting cell growth [28]. Red ginger powder 2% added to feed can be useful as a phytobiotic that can have the effect of increasing feed consumption in livestock, increasing body weight and converting feed into muscle, as well as developing the condition of the hepatic organs, kidneys, and proventriculus [26]. The content of red ginger, namely flavonoid compounds, has an inhibitory effect on the activity of angiotensin-converting enzyme (ACE) which causes the formation of angiotensin II from angiotensin I to decrease so that vasodilation occurs, then will result in lower blood pressure in the blood vessels. ACE inhibition can also increase nitric oxide and decrease superoxide anion which can also cause vasodilation [29]. The results of histopathology and tukey tests that have been carried out, found no significant difference between groups P1 with P3, but significantly different from groups P2, P4, P5, P6. The absence of differences between P1 and P3 indicates that the treatment of adding 2% red ginger powder to feed infected Salmonella *enteritidis* bacteria can prevent damage to the glomerular diameter of the broiler kidneys. Red ginger has flavonoid compounds that have antioxidant activities that can reduce free radicals that are produced in the process of glomerular damage so that further damage can be prevented and towards extension [30]. Red ginger contains phenol compounds, namely shogaol and gingerol which have antimicrobial effects by damaging the outer and inner membranes of intracellular targets, phenol compounds will interact with bacteria through the absorption process involving hydrogen bonds, these bonds are decomposed and will cause protein coagulation so that the bacterial cell membrane will experience lysis [31]. The P2 treatment was not significantly different from the P5 treatment, but significantly different from the P1, P3, P4, P6 treatments. P2 and P5 groups are significantly different from P1, it can be said that there is a significant change in the size of the glomerular diameter which can be due to the use of AGP and stimuno from day 8 to day 35. In the P2 histopathology

picture, there is blood accumulation in the lumen and necrosis which causes the glomerulus to fuse or become fused with other glomeruli. AGP has toxic properties to the kidney organs, The nephrotoxic mechanism of antibiotics is the accumulation of drugs and phospholipids mediated by lysosomes [32]. Lysosomes filled with phospholipids become unstable and break, these fragments will produce acid hydrolases and when the concentration of antibiotics in the cytoplasm is high, the cell will be structurally and functionally impaired. The mechanism of nephrotoxicity from antibiotics consists of 4 stages, namely vasoconstriction in renal blood vessels which causes a decrease in blood flow velocity, intralobular obstruction, leakage filtration, and decreased permeability of the glomerulus [33]. Stimuno has an antioxidant effect that is possessed by most flavonoids due to the presence of phenolic hydroxyl groups in its molecular structure as well as through its capture of free radicals and its activity as a metal attractant. According to the mechanism of action, antioxidants have 2 functions, namely, as a provider of hydrogen atoms and slowing down the rate of autoxidation which inhibits the formation of lipid radicals, by providing hydrogen atoms to lipid radicals, the lipids will change into a more stable form and not cause further damage [34]. Stated that stimuno made from Phylanthus niruri plants can increase phagocytosis activity in bacterial infections from macrophages. P. niruri significantly increases lysosomal enzymes and the release of TNF-α which also releases nitric oxide which can mean the phagocytosis activity of intracellular bacteria from macrophages. Treatment P6 was not significantly different from treatment P5, but significantly different from treatment P1, P2, P3, P4. This proves that 2% red ginger powder added to standard feed has the same effect as treatment P5. 2% red ginger powder has bioactive contents such as oleoresin and essential oil that can provide anti-inflammatory and anti-bacterial effects [35].

The highest mean diameter of broiler liver sinusoids in the starter phase was shown in groups P4 (5.20), P5 (4.66), P6 (4.37), P2 (4.33), P3 (4.30), and P1 (4.08). Group P1 is a negative control that is only given standard feed without induction of *Salmonella enteritidis* bacteria. This group is used as a reference for the increase or decrease in the average sinusoid diameter of groups P2, P3, P4 (positive control), P5, and P6. Group P1 (negative control) has the lowest mean sinusoid diameter among other groups. Supported by the histopathological picture in group P1 there is no pathology, so it can be said that the standard feed gives a good effect on the liver of broiler chickens aged 16 days of maintenance. In group P4 which was given standard feed then induced *Salmonella enteritidis* bacteria (positive control) had the highest mean value of sinusoid diameter among other groups. Group P4 is significantly different ($P < 0.05$) with groups P1, P3, P2, and P6, but not significantly different ($P > 0.05$) with group P5. This shows that when chickens are exposed to *Salmonella enteritidis* bacteria, the chicken's body will experience inflammation. Incoming bacteria will invade the intestines and produce enterotoxin toxins that cause ulcers and bleeding, then flow to the liver. Enterotoxins are passed through the blood from the digestive tract. Enterotoxins are hepatotoxic to the liver in addition to the intestinal epithelium. High levels of toxins in the blood flowing through the sinusoids to the central vein can cause dilation of the sinusoids. The sinusoid wall consists of endothelial cells lined with hepatocyte cells bordered by subendothelial gaps containing microvilli. This facilitates the exchange of compounds including toxins [36] [37].In the P2 group, tetracycline antibiotics were added as AGP at a dose of 15 mg/kg BW to the feed and induction of *Salmonella enteritidis* bacteria had an average sinusoid diameter lower than the P4, P5, P6 groups but higher than P1, and P3. Group P2 was significantly different from P4 ($P < 0.05$), but not significantly different ($P > 0.05$) from groups P1, P3, P6, and P5. This shows the addition of tetracycline antibiotics works better than the addition of stimuno® forte, but not better when compared to the addition of 2% red ginger powder induced bacteria seen from the average diameter of sinusoids. This is because the addition of tetracycline antibiotics as AGP is effective against Salmonella sp. Similar to the addition of 2% red ginger powder, the addition of tetracycline antibiotics can inhibit the growth and development of these bacteria. Tetracycline works bacteriostatic ally which inhibits protein synthesis. Tetracycline penetrates bacteria by passive diffusion. After entering the cell, tetracycline binds reversibly to the 30S subunit of the bacterial ribosome, thus inhibiting bacterial growth [38]. Giving antibiotics to animals can increase the average growth rate by 4-8% and feed efficiency by 2-5%. Antibiotics can also reduce the likelihood of animals dying. Antibiotics act as growth promoters because they protect the animal's food from damage caused by bacteria. Tetracycline has a relatively low toxin effect. The presence of antibiotics consumed by chickens can increase the absorption of food nutrients due to the thinning of the small intestinal barrier. Antibiotics can reduce toxins produced by bacteria in the gut and reduce the chance of intestinal infections [39].The P3 group that added 2% red ginger powder to the feed and induced *Salmonella enteritidis* bacteria had a lower mean sinusoid diameter than the P2, P4, P5, P6 groups but higher than the P1 group. Group P3 was significantly different from P4 ($P < 0.05$), but not significantly different ($P > 0.05$) from groups P1, P2, P6, and P5. This indicates that the addition of 2% red ginger powder has worked well in reducing damage to the liver of starter phase broilers when induced by *Salmonella enteritidis* bacteria. The high flavonoid content in 2% red ginger can function as an anti-inflammatory, antibacterial, and immunomodulator. Red ginger powder can increase T helper cells, macrophage cytokines, and can increase lymphocyte activation which plays an important role in immune disorders. Red ginger acts as an immunomodulator and works by enhancing the immune response in the body and preventing salmonella infection [40]. When macrophages are activated due to exposure to *Salmonella enteritidis* bacteria, there will be expression by APC (Antigen Presenting Cells) which is accepted by MHC II (Major Histocompatibility Complex class II) to produce TCD4 lymphocyte cells. TCD4 cells will produce antibodies when there is an antigen stimulus and then will proliferate into Th2 cells. Th2 cells interact with APC and induce IL-2 as a means of intercellular communication to induce the

maturation of B cells, which then differentiate into plasma cells to produce antibodies. Flavonoids interfere with bacterial invasion, thus inhibiting bacterial attachment to the mucosa which affects the amount of antigens and antibodies. [41]. Red ginger contains phenolic derivative compounds that exert antibacterial effects by disrupting the outer and inner membranes of the bacterial cytoplasm. The compound activates macrophages and inhibits polymorphonuclear migration (PNM) of vascular endothelial cells by affecting lymphocyte proliferation, thereby increasing T cell production. Red ginger has a higher gingerol content than other ginger. Phenolic compounds namely gingerol, shogaol, zingerone have a function that is anti- inflammatory. These compounds can inhibit cytokines in increasing inflammatory cells through suppressing the expression of proinflammatory mediators directly where these mediators are responsible for persistent inflammation and can inhibit inflammatory components [42]. In group P5, which was added with 13.5 mg/kg BW of stimuno® forte to the feed and induced by *Salmonella enteritidis* bacteria, the mean sinusoid diameter was lower than P4 but higher than P1, P2, P3, and P6. Group P5 was not significantly different ($P>0.05$) from groups P1, P2, P3, P4, and P6. This shows the addition of stimuno® forte has worked well in reducing liver damage to starter phase broilers, but not better when compared to the addition of 2% red ginger and tetracycline antibiotics when viewed from the average sinusoid diameter. According to IAI, stimuno contains Phyllanthus niruri L (green meniran). Just like red ginger, the meniran plant can act as an antibacterial because it contains antibacterial components, namely flavonoids, alkaloids, phenols and tannins. The ingredients in this medicine can increase the activity and function of several components of nonspecific and specific immunity, including humoral and cellular. This plant can increase phagocytosis and nonspecific immune responses in the form of macrophage chemotaxis, neutrophil chemotaxis, NK (natural killer) cell cytotoxicity, and complement hemolytic activity. The content of Phyllanthus niruri L in the drug also increases the proliferation of T lymphocytes, increases the secretion of TNFα and IL4, and reduces the secretion of IL-2 and IL-10. For humoral immunity, this plant increases the production of IgM and IgG [43]. The stimuno® forte addition group was not significantly different from the other groups, this treatment group was able to reduce the dilation of liver sinusoids although the mean value was higher than the 2% red ginger powder addition group, tetracycline antibiotics, and the mean value was closer to the positive control. Similar to red ginger, the content in stimuno® forte can prevent the production of cytokines that can cause damage to the liver of chickens that have been infected with S. enteritidis. So the addition of stimuno® forte is still good to use. One of the phytochemical compounds that may be present in some types of plants in medicine is alkaloid, which states that alkaloids can damage liver cells [44]. This is likely to cause the treatment group P5 with P4 (positive control) not significantly different. In the P6 group, 2% red ginger powder was added to the feed without bacterial induction, the average was higher than P1, P2, P3 but lower than P4 (positive control) and P5. Group P6 was significantly different from P4 ($P<0.05$), but not significantly different ($P>0.05$) from groups P1, P3, P2, and P5. This shows that the addition of 2% red ginger powder is able to reduce liver damage to starter phase broilers even without the stimulation of *Salmonella enteritidis* bacteria induction. This is because red ginger contains flavonoid compounds that can increase broiler productivity by improving feed characteristics, increasing production performance and improving the quality of broiler products, so the addition of 2% red ginger powder results in optimal antibody formation. The age factor of broiler chickens affects the formation of body defenses. The defense response of young broilers is limited by specific antigen epitopes and minimal immune response. The function of the hisporespecific nature of immune cells of young broilers is immature and the formation of immunity is not perfect, so when infected with *Salmonella enteritidis* bacteria, starter phase broilers have a weak immune response [45]. Chickens infected with *Salmonella enteritidis* at the age of 2 weeks (15th day of the study), there are no antibodies produced by the chicken's immune system [23]. Chickens infected with salmonella at a young age require a longer period of antibody production, even up to about 4 weeks post-infection [46]. Red ginger also contains essential oils consisting of sesquiterpene compounds, zingiberene, zingiberol, linalol, curcumin. Volatile oils are useful for pain relief, anti-inflammatory and anti-bacterial [47].

The mean diameter of liver sinusoids of finisher phase broiler chickens was highest in the P4 (5.39), P6 (4.81), P5 (4.41), P1 (4.05), P3 (3.93), and P2 (3.84) groups. In group P1 or negative control is given only standard feed without the induction of *Salmonella enteritidis*, P1 is used as a comparison of whether there is a decrease or increase in the diameter of sinusoids from several other groups. In group P1 has a mean of 4.05 which is higher in value when compared to the mean of P3 and P2 but still lower than groups P4, P5 and P6. Group P2 is a group that is given additional tetracycline antibiotics (AGP) at a dose of 15 mg / kg BW added to standard feed and induction of *Salmonella enteritidis*, has a mean sinusoid diameter of 3.84 which is the lowest mean value when compared to other groups. This shows that the addition of tetracycline antibiotics as AGP is effective in inhibiting the growth and development of *Salmonella enteritidis* bacteria. This tetracycline antibiotic works bacteriostatic ally by inhibiting bacterial protein synthesis in its ribosomes, the cell wall will lose its strength if protein synthesis is delayed so that the bacteria will die [38]. Group P3, which is a group with the addition of 2% red ginger powder to standard feed and induced *Salmonella enteritidis* bacteria, has a mean sinusoid diameter of 3.93 which is lower than groups P1, P3, P4, P5, but higher than group P2. This indicates that the addition of 2% red ginger powder is good because it can prevent damage to the chicken liver induced by *Salmonella enteritidis* because it is proven by the lower mean sinusoid diameter of group P3 when compared to group P4 (positive control), but still higher when compared to group P2, this indicates the addition of 2% red ginger powder is good but not better than the addition of tetracycline antibiotics as AGP. Similar to antibiotics, red ginger powder also has antibacterial power. The antibacterial

ability is because it contains some of what is contained in it. Red ginger contains 3.9% essential oil. Essential oil components that act as antibacterial are sabinen, β-mirsen, α-pinen, α-tuyan, trans- caryophyllene, β-pinen. The compounds α- pinene and β-pinene are terpenoid compounds known to have antimicrobial effects. α-pinene and β-pinene have the ability to damage cellular integrity and inhibitory responses and can damage transport processes. In addition, the high flavonoid content in red ginger functions as an antibacterial, immunomodulator, and anti- inflammatory [48]. Red ginger can increase the activation of lymphocytes that play a role in immune disorders and as an antioxidant, besides red ginger as an immunomodulator by increasing the immune response and preventing infection from *Salmonella enteritidis* bacteria. The flavonoid content can inhibit bacterial attachment to the mucosa which affects the amount of antibodies and antigens. In addition, the gingerol and shogaol content in red ginger is also high which can function as antibacterial and anti-inflammatory [41]. In group P4 or positive control is a group given standard feed induced by *Salmonella enteritidis* which has a mean sinusoid diameter of 5. 39 where the average is the highest mean value of sinusoid diameter compared to other treatment groups, this proves the reaction of exposure to *Salmonella enteritidis* bacteria, bacteria that enter orally will enter through the intestines and produce enterotoxins, then enterotoxins go to the liver through blood from digestion, the liver is an organ that receives many effects from enterotoxins because it is the first organ to receive blood from the digestive tract, high toxic levels in the blood and then pass through the sinusoids in the liver which causes dilation of the sinusoids. S. Enteritidis infects the liver and will cause hyperemia of blood vessels and in small blood vessels there will be degeneration and necrotic hepatocyte cells [49].Group P5 is a group that is given an additional stimuno® forte dose of 13.5 mg / kg BW added to standard feed and induction of *Salmonella enteritidis*, has a mean sinusoid diameter of 4.41 which is lower than P4 and P6 but higher than P1, P2, P3. This suggests that the addition of stimuno forte has worked quite well but not better than the addition of tetracycline antibiotics and 2% red ginger powder. Stimuno containing meniran extract (Phyllanthus niruri L) because it contains flavonoids that can function as immunomodulators, by providing stimulation to immune cell receptors and sending intracellular signals to these cell receptors so that they can improve the work of immune cells to be better than before, meniran can also increase phagocytosis, increase T lymphocyte proliferation and increase TNFα and IL4 secretion [50]. Group P6 is a group with the addition of 2% red ginger powder to standard feed and without the induction of *Salmonella enteritidis* bacteria, has a mean sinusoid diameter of 4.81. Red ginger contains flavonoids that have various pharmacological activities such as antibacterial. Pharmacological activities on flavonoids other than antibacterial such as anti-inflammatory, anticarcinogenic, antioxidant, antiviral. Therefore flavonoids are broad-spectrum in improving health. Red ginger also contains shogaol and gingerol compounds. They have antibacterial effects by damaging the outer and inner membranes and can interact with membrane proteins and intracellular targets. Flavonoid compounds interact with bacterial cells through an absorption process involving hydrogen bonds, the bonds break down and cause protein coagulation, resulting in lysis of the cell membrane. This compound will interact with cell proteins in bacteria to form protein compounds that will cause protein coagulation and cell membrane lysis. Shogaol and gingerol work optimally to be able to activate macrophages effectively to inhibit the production of nitric oxide and prostaglandin E2 [31].

4. CONCLUSION

Based on the research data, it can be concluded that:

1. The addition of 2% red ginger powder (*Zingiber officinale* var. *rubrum*) in feed can reduce damage to liver and kidney organs based on histopathology of liver and kidney of broiler chickens induced by *Salmonella enteritidis*.
2. Addition of 2% red ginger powder (*Zingiber officinale* var. *rubrum*) in feed reduces damage by decreasing the diameter of sinusoid width and kidney glomerulus based on histomorphometry of liver and kidney of broiler chickens induced by *Salmonella enteritidis* significantly ($p<0.05$).

5. SUGGESTION

Suggestions for this study need to do blood chemistry tests including SGPT, SGOT, albumin to find out more about the condition of the liver organs of starter phase broilers exposed to *Salmonella enteritidis* to the effect of adding 2% red ginger powder.

REFERENCES

[1] R. Rukmini, M. Mardewi, And R. Rejeki, "Kualitas Kimia Daging Ayam Broiler Umur 5 Minggu Yang Dipelihara Pada Kepadatan Kandang Yang Berbeda," *Wicaksana: Jurnal Lingkungan Dan Pembangunan*, Vol. 3, No. 1, Pp. 31–37, 2019.

[2] L. G. Setyawati[1], I. W. Sudira, I. K. Berata, And I. M. Merdana, "Perubahan Histopatologi Ginjal Ayam Kampung Yang Diberikan Jamu Daun Ashitaba Dan Divaksin Penyakit Tetelo".

[3] M. Meutia, *Zat Zat Yang Mempengaruhi Histopatologi Hepar*. Unimal Press, 2018.

[4] A. Kusumaningsih, "Patogenisitas Salmonella Enterica Serotipe Enteritidis Isolat Lokal Pada Anak Ayam Dan Mencit," *Berita Biologi*, Vol. 10, No. 4, Pp. 463–469, 2011.

[5] D. E. Swayne, *Diseases Of Poultry*. John Wiley & Sons, 2013.

[6] W. L. M. S. Mario, E. Widodo, And O. Sjofjan, "Pengaruh Penambahan Kombinasi Tepung Jahe Merah, Kunyit Dan Meniran Dalam Pakan Terhadap Kecernaan Zat Makanan Dan Energi Metabolis Ayam Pedaging," *Jurnal Ilmu- Ilmu Peternakan (Indonesian Journal Of Animal Science)*, Vol. 24, No. 1, Pp. 1–8, 2014.

[7] V. Anindita, S. Susianti, E. Cania B, And A. Janar Wulan, "Pengaruh Pemberian Jahe Merah (Zingiber Officinale Var. Rubrum) Terhadap Gambaran Histopatologi Ginjal Tikus (Rattus Norvegicus) Galur Sprague Dawley Yang Diinduksi Oleh Parasetamol," *Medula*, Vol. 9, No. 3, Pp. 552–557, 2019.

[8] M. C. Pungus, M. P. Lintong, And M. K. Sambuaga, "Efek Pemberian Ekstrak Jahe (Zingiber Officinale) Terhadap Gambaran Histopatologik Lambung Tikus Wistar Yang Diinduksi Asam Asetat," *Medical Scope Journal*, Vol. 1, No. 2, 2020.

[9] J. F. Zachary And M. D. Mcgavin, *Pathologic Basis Of Veterinary Disease5: Pathologic Basis Of Veterinary Disease*. Elsevier Health Sciences, 2012.

[10] Y. Aldi, Y. Rasyadi, And D. Handayani, "Aktivitas Imunomodulator Dari Ekstrak Etanol Meniran (Phyllanthus Niruri Linn.) Terhadap Ayam Broiler," *Jurnal Sains Farmasi & Klinis*, Vol. 1, No. 1, Pp. 20–26, 2014.

[11] R. Januar, Y. Yusfiati, And F. Fitmawati, "Struktur Mikroskopis Hati Tikus Putih (Rattus Novergicus) Akibat Pemberian Ekstrak Tanaman Tristaniopsis Whiteana Griff.," Riau University, 2014.

[12] A. Insani And I. Samsuri, "Gambaran Histopatologi Hati Tikus Putih Yang Diberikan Deksametason Dan Vitamin E," *Indonesia Medicus Veterinus*, Vol. 4, No. 3, Pp. 228–237, 2015.

[13] R. Auliyah, "Gambaran Histopatologi Hepar Ayam Pedaging Yang Diinfeksi L2 Toxocara Vitulorum," Universitas Airlangga, 2016.

[14] H. Hayati, H. Sunaryo, And T. H. Syahbandono, "Efek Hepatoprotektor Fraksi Etil Asetat Daun Sprague Dawley Sangitan (Sambucus Canadensis L.) Pada Tikus," *Media Farmasi: Jurnal Ilmu Farmasi*, Vol. 11, No. 1, 2014.

[15] H. Andreas, H. F. Trianto, And M. Ilmiawan, "Gambaran Histologi Regenerasi Hati Pasca Penghentian Pajanan Monosodium Glutamat Pada Tikus Wistar," *Ejournal Kedokteran Indonesia*, 2015.

[16] N. E. Purwatari And F. Saktini, "Pengaruh Pemberian Dosis Bertingkat Ekstrak Kulit Buah Naga Putih (Hylocereus Undatus) Terhadap Gambaran Mikroskopis Hepar Mencit Balb/C Yang Diberi Paparan Asap Obat Nyamuk Bakar," Diponegoro University, 2016.

[17] A. R. Rohmatin, E. Susetyarini, And S. Hadi, "The Damage Of Hepar Cells Of White Male Mice (Rattus Norvegicus) Which Are Induced By Carbon Tetrachloride (Ccl4) After Being Given Bawang Dayak (Eleutherine Palmifolia Merr.) Ethanol Extract," In *Proceeding Biology Education Conference: Biology, Science, Enviromental, And Learning*, 2015, Pp. 942–946.

[18] M. H. Natsir, "Penggunaan Kombinasi Kunyit (Curcuma Domestica) Dan Jahe (Zingiber Officinale) Bentuk Enkapsulasi Dan Tanpa Enkapsulasi Terhadap Karakteristik Usus Dan Mikroflora Usus Ayam Pedaging," *Buletin Peternakan*, Vol. 40, No. 1, Pp. 1–10, 2016.

[19] M. Puette And W. A. Crowell, "Histologic And Morphometric Examination Of Avian Glomeruli From Normal And Swollen Kidneys Of Broilers At Slaughter.," *Avian Diseases*, Vol. 37, No. 3, Pp. 874–879, 1993.

[20] T. K. Kim, "T Test As A Parametric Statistic," *Korean Journal Of Anesthesiology*, Vol. 68, No. 6, Pp. 540– 546, 2015.

[21] S. Zhang, X. Kou, H. Zhao, K.-K. Mak, M. K. Balijepalli, And M. R. Pichika, "Zingiber Officinale Var. Rubrum: Red Ginger's Medicinal Uses," *Molecules*, Vol. 27, No. 3, P. 775, 2022.

[22] A. A. Arimbi, R. Darsono, H. Plumeriastuti, T. V. Widiyatno, And D. Legowo, "Buku Ajar Patologi Umum Veteriner," *Airlangga University Press. Surabaya*, 2013.

[23] A. K. Anisa, "Potensi Imunostimulator Jahe Merah (Zingiber Officinale Var. Rubrum) Berdasarkan Sekresi Iga, Ifn-Dan Il-2 Pada Ayam Broiler Yang Diinfeksi Salmonella Enteritidis.," *Program Studi Magister Vaksinologi Dan Imunoterapeutika. Fakultas Kedokteran Hewan. Universitas Airlangga.*, 2021.

[24] Herawati And Marjuki, "The Effect Of Feeding Red Ginger (Zingiber Officinale Rosc) As Phytobiotic On Broiler Slaughter Weight And Meat Quality," *International Journal Of Poultry Science*, Pp. 983–986, 2011.

[25] B. R. April Agustin, A. L. D. ,. Atma, C. D. &. Tirtasari, K., "Detection Of Antibiotic Resistance Salmonella Sp Isolated From Layer Chicken Farm In Sesaot West Lombok," *Media Kedokteran Hewan*, Vol. 33 (1), P. 18, 2022.

[26] Herawati, "The Effect Of Feeding Red Ginger As Phytobiotic On Body Weight Gain, Feed Conversion And Internal Organs Conditions Of Broiler. International Journal Of Poultry Science," *International Journal Of Poultry Sience*, Vol. 9 (10), Pp. 963–967, 2010.

[27] I. Fitri, "Efektivitas Antibakteri Ekstrak Herba Meniran (Phylanthus Niruni) Terhadap Pertumbuhan Bakteri Salmonella Sp. Dan Propionibacterium Acnes.," *Jst (Jurnal Sains Dan Teknologi)*, Vol. 6 (2), Pp. 300–310, 2017.

[28] P. Widiastuti,D N., "Terhadap Staphylococcus Aureus Antimicrobial Test Of Red Ginger Extract (Zingiber Officinale

) Against Staphylococcus Aureus.," 2018.
[29] E. A. Nadia, "Efek Pemberian Jahe Terhadap Tekanan Darah Pada Pasien Hipertensi," *Jurnal Medika Hutama*, Vol. 2 (1), Pp. 43–348, 2020.
[30] P. Ulum,K S. ,. Pratiwi,D, "Review Artikel: Potensi Jahe Merah (Zingiber Officinale Var. Rubrum) Sebagai Antibakteri. Universitas Singaperbangsa Karawang. Karawang," 2020.
[31] Awanis, M. A., Mutmainnah, A. A, "Uji Anti Bakteri Ekstrak Oleoresin Jahe Merah (Zingiber Officinale Var.Rubrub) Terhadap Bakteri Streptococcus Pyogenes. Medika Tadulako," *Medika Tadulako*, Vol. 3(1), Pp. 23–32.
[32] Kurniawan,F, "Analisis Pengobatan Antiobiotik Pada Geriatri Berdasarkan Laju Infitrasi Glomerulus Dengan Formula Modificaion Of Diet In Renal Disease Di Rumah Sakit Se-Kotamadya Yogyakarta Periode 2009.," *[Skripsi]. Universitas Sanata Dharma Yogyakarta.*, 2010.
[33] Ettinger, *Textbook Of Veterinary Internal Medicine, 6th Edition*. Elsevier Saunders, 2000.
[34] Ariputri,F,A, "Pengaruh Pemberian Ekstrak Meniran (Phyllanthus Niruri L.) Dosis Bertingkat Terhadap Gambaran Mikroskopis Ginjal.," *Universitas Diponegoro*, Vol. 6(2), Pp. 505–513, 2016.
[35] Hutomo, S., Putri, D. U., Suryanto, Y. I., Susilowati, H, "Potential Immunomodulatory Activity Of Phyllanthus Niruri Aqueous Extract On Macrophage Infected With Streptococcus Sanguinis.," Vol. 51(3), Pp. 124–128, 2018.
[36] Madihah,. Ratningsih, N, Malini, M.D, Faiza. H.A, Iskandar, J., "Uji Toksisitas Akut Ekstrak Etanol Kulit Buah Jengkol (Archidendron Pauciflorum) Terhadap Tikus Wistar Betina.," *Pros Sem Nas Masy Biodiv Indon*, Vol. 3(1), Pp. 33–38, 2017.
[37] Surasa, "Struktur Mikroanatomi Hati Dan Kadar Kolesterol Total Plasma Darah Tikus Putih Strain Wistar Pasca Suplementasi Minyak Lemuru Dan Minyak Sawit.," *Biosaintifika*, Vol. 6(2), 2014.
[38] Perdian,H,A, "Kajian Residu Tetrasiklin Hcl Dalam Daging Dan Hati Ayam Broiler Pada Beberapa Peternakan Di Kabupaten Lamongan Menggunakan Metode Spektomorfometri Ultraviolet.," *Skripsi Universitas Islam Bandung*, 2015.
[39] E. Krueger And A. C. Brown, "Inhibition Of Bacterial Toxin Recognition Of Membrane Components As An Anti-Virulence Strategy," *Journal Of Biological Engineering*, Vol. 13, No. 1, Pp. 1–14, 2019.
[40] Sharififar, F., Shirin, P., Moslem,A, "Immune Modulatory Activity Of Aqueous Extract Of Achillea Wilhelmsii C. Kock. In Mice," *Indian J Exp*, Vol. 47, Pp. 668–670, 2009.
[41] Manullang, J, R., Ardhani, F., "Efektifitas Jahe Merah (Zingiber Officinale Var. Rubrum) Sebagai Additif Pakan Dan Antimikrobia Terhadap Pertumbuhan Bakteri Anaerob Dan Coliform Secara In Vivo Pada Ayam Pedaging.," *Jurnal Peternakan Indonesia*, Vol. 17(3), 2015.
[42] Roufogalis, B,D, "Zingiber Officinale (Ginger): A Future Outlook On Its Potential In Prevention And Treatment Of Diabetes And Prediabetic States," *Science*, Vol. 15, 2006.
[43] Munasir, Z, "Manfaat Pemberian Ekstrak Phyllanthus Niruni Sebagai Imunostimulator Pada Penyakit Infeksi Anak.," *Fakultas Kedokteran Universitas Indonesia*, 2007.
[44] Fitmawati, Titrawani, Welly, S, "Struktur Histologi Hati Tikus Putih (Rattus Norvegicus Berkenhout 1769) Dengan Pemberian Ramuan Tradisional Masyarakat Melayu Lingga, Kepulauan Riau.," Vol. 3(1), 2018.
[45] T. Ariyanti, "Antigenicity And Immunogenicity Of Salmonella Enteritidis: Its Implication For Diagnosis And Development Of Local Isolate Vaccine For Poultry," *Wartazoa. Indonesian Bulletin Of Animal And Veterinary Sciences*, Vol. 18, No. 4, Pp. 187–197, 2008.
[46] R. K. Beal And A. L. Smith, "Antibody Response To Salmonella: Its Induction And Role In Protection Against Avian Enteric Salmonellosis," *Expert Review Of Anti-Infective Therapy*, Vol. 5, No. 5, Pp. 873–881, 2007.
[47] S. Dharma, E. S. Adelinda, And N. Suharti, "Uji Efek Antiinflamasi Ekstrak Etanol Rimpang Jahe (Zingiber Officinale Roscoe) Pada Tikus Putih Jantan," *Jurnal Farmasi Higea*, Vol. 1, No. 2, Pp. 79–83, 2016.
[48] A. Senthilkumar And V. Venkatesalu, "Phytochemical Analysis And Antibacterial Activity Of The Essential Oil Of Clausena Anisata (Willd.) Hook. F. Ex Benth," *Int J Integr Biol*, Vol. 5, No. 2, Pp. 116–120, 2009.
[49] W. Winarsih, B. P. Priosoeryanto, B. W. Lay, I. Wibawan, And I. P. Kompiang, "Gambaran Mikroskopis Hati Ayam Broiler Yang Diberi Probiotik Dan Infeksi Salmonella Subklinis," 2008.
[50] W. Wahyuni, M. I. Yusuf, F. Malik, A. F. Lubis, A. Indalifiany, And I. Sahidin, "Efek Imunomodulator Ekstrak Etanol Spons Melophlus Sarasinorum Terhadap Aktivitas Fagositosis Sel Makrofag Pada Mencit Jantan Balb/C," *Jurnal Farmasi Galenika (Galenika Journal Of Pharmacy)(E-Journal)*, Vol. 5, No. 2, Pp. 147–157, 2019.