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## Histology of the liver and small intestine broiler using phytobiotic in the ration infected *Salmonella pullorum*

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**Abstract.** Phytobiotic contains bioactive substances that function as antibacterial substances so that its use as Natural Growth Promoters substitute antibiotics continue to be suggested. This study aims to observe the histopathological changes of liver and small intestine in broilers infected with *Salmonella pullorum* with Turmeric Extract (TWE), Garlic Extract (GWE) and the combination of Turmeric and Garlic extract (1TWE: 3GWE) added to the feed. One-hundred-day old chick was kept intensive for 38 days and divided into five treatments and four replications, with five chicks in each replicate. The treatments were R0 (no phytobiotic and antibiotics as a negative control), R1; 0.015% tetracycline and infected *Salmonella pullorum* as a positive control, R2; 2.50% TWE (*Salmonella pullorum* infected), R3; 2.00% GWE (*Salmonella pullorum* infected), R4; 2.50% GWE (*Salmonella pullorum* infected). The results showed a very significant effect ( $P < 0.01$ ) reduced the presence of inflammatory nodules, congestion and haemorrhages in liver and small intestine broilers due to *Salmonella pullorum*. The addition of TGWE phytobiotic to the feed can reduce damage in the liver and small intestine infected *Salmonella pullorum* compared with the use of tetracycline, TWE, and GWE.

### 1. Introduction

Diseases that often attack broiler farms are Pullorum (bacillary white disease) caused by *Salmonella pullorum* bacteria. *Salmonella pullorum* bacteria is a type of Gram-negative bacteria that is detrimental because it is transmitted from mother to egg and DOC (Day Old Chick). The mortality rate can reach 85% in chicks, whereas in adult chicken cause infections in the digestive tract and swelling in the liver. In the chicken digestive tract there are bacterial communities such as *Salmonella thyphimurium* and *Escherichia coli* that compete in obtaining nutrients and producing metabolite products that are harmful to their hosts.

Giving additive feeds is very important to improve the health and productivity of poultry. Feed additives commonly used as growth boosters are antibiotics. However, the use of antibiotics to spur the growth of poultry is increasingly being abandoned since the EU's ban on the use of antibiotics in livestock. Giving antibiotics as an additive feed which in principle reduces the bacterial population in the digestive tract, but its use provides a problem with the discovery of antibiotic residues in livestock carcasses which are consumed by humans and increase the resistance of pathogenic bacteria.

One ingredient that has the potential to be utilized as a natural antibiotic is the addition of turmeric water extract (TWE) and garlic water extract (GWE). Turmeric can increase the body's resistance to *Salmonella pullorum* bacteria at the same time without causing damage to the liver as a center of metabolism or damage to the intestine as a place for absorption of feed nutrients.



Turmeric and garlic are herbs that have many known benefits for human and animal health. Curcuminoid compounds in turmeric have antibacterial properties by killing harmful bacteria [1]. Garlic contains allicin is one of the plants that are widely used for treatment in humans. The allicin compound in garlic functions as a broad-spectrum antimicrobial and is able to fight infections caused by parasites, bacteria, fungi, or viruses [2]. Based on the results of [3] of turmeric extract, garlic extract and a combination of turmeric and garlic extract had anti-bacterial activity against *Lactobacillus*, *Salmonella* and *E. coli* bacteria and optimal at 2.5% TE, 2.0% GE and 2.5% TGE with a ratio of 1TE:3GE. Giving TE, GE or TGE phytobiotics as feed additive can improve duodenal histomorphology by observing villi length, villi surface area, crypt depth and a ratio of villous length to a cryptic depth and ability to secrete digestive enzymes by looking at pancreatic enzyme activity.

Looking at the potential possessed by turmeric and garlic, further studies need to be done through research on the application of phytobiotic extract of turmeric and garlic extract to the level of damage to the liver and small intestine of broilers infected with *Salmonella pullorum*.

## 2. Material and methods

### 2.1. Material

The study was conducted in October to November 2016 in Tamalanrea and histology test at the Histology Laboratory of the Faculty of Veterinary Medicine, Hasanuddin University, Makassar. The material used by broiler as many as 100 tails is maintained until the age of 35 days, turmeric, garlic, tetracycline, filler, a culture of *Salmonella* bacteria, and basal ration ingredients.

### 2.2. Methods

**2.2.1. Making *Salmonella pullorum* bacteria culture.** The culture of *Salmonella pullorum* bacteria is taken 1 loop colony and planted in Brain Heart Infusion (BHI) broth media and incubated at 37°C for 18-24 hours. Then centrifuged (500 g, 10 minutes) to form pellets. To obtain a dose of inoculum, the pellet was diluted with a sterile physiological NaCl solution and the turbidity was likened to the McFarland standard No.1 which was equivalent to 10<sup>8</sup> colony forming units (CFU)/ml.

**2.2.2. Ration treatment.** Treatment rations were given to broilers starting from DOC (day old chick) until the age of 5 weeks. Basal ration constituent material consists of fine yellow corn, rice bran, Meat and Bone Meal, vegetable oil, chicken feather meal, soybean meal, CaCO<sub>3</sub>, Dicalcium phosphate, premix, salt, L-lysine, and DL-methionine. The treatment ration consisted of basal ration added with TWE, GWE, and TGWE as feed additives.

**2.2.3. Maintenance and research treatment.** Broiler chickens were randomly divided into 5 treatments and 4 replications so that there were 20 cage units. Broiler chickens were kept in a colony cage for 5 weeks. Feed and drinking water are given by *ad libitum*. Three weeks old chickens were infected with *Salmonella* orally at a dose of 10<sup>8</sup> CFU/ml. At the end of the study, broiler were taken randomly to each of the experimental units to be cut. The parameters observed in this study were the level of tissue damage in the liver and small intestine. The treatment composition consists of 5 kinds of rations, namely: R0 = Basal ration without phytobiotics and antibiotics and without *Salmonella* infection (negative control); R1 = Basal ration + tetracycline 0.015% + filler 2.485% + *Salmonella* infection (positive control); R2 = Basal ration + 2.50% TWE + *Salmonella* infection; R3 = Basal ration + 2.00% GWE + filler 0.50% + *Salmonella* infection; R4 = Basal ration + TGWE 2.50% + *Salmonella* infection.

**2.2.4. Sampling and histopathological preparations.** At the end of the study, each experimental unit was taken 1 randomly to cut and then the liver and small intestine were taken, then cut the part of the organ longitudinally to clean the contents. The sample preparation method for histopathological observation.

**2.2.5. Scoring method.** Assessment is done by a scoring method based on the level of damage seen from the presence of inflammatory nodules, congestion and haemorrhage in the liver and small intestine. Histopathological abnormalities were measured based on the degree of infection of infected organs, score 0 (-) = normal (damage level 0 - 10%); score 1 (+) = light (damage rate 11 - 25%); score 2 (++) = moderate (level of damage 26 - 50%); score 3 (+++) = weight (damage rate 51 - 75%).

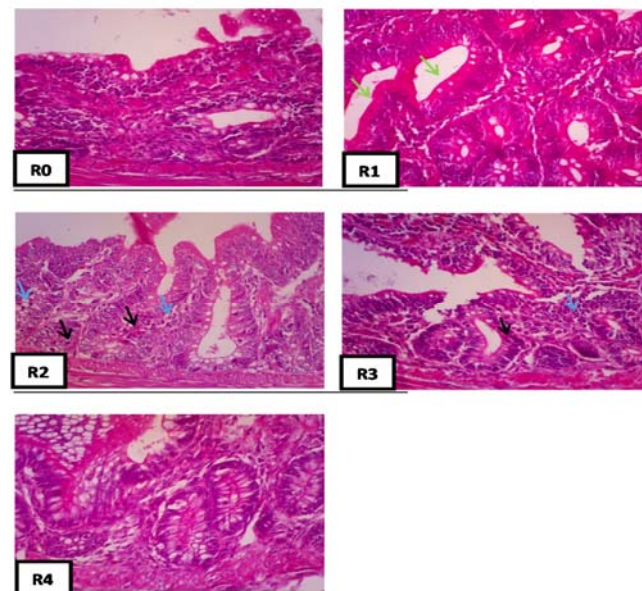
### 2.3. Statistical analysis

All data in this study are expressed as the mean  $\pm$  standard deviation of representative duplicate experiments, and statistical significance was determined by one-way analysis of variance, with  $P < 0.05$  considered significant. When  $P < 0.05$ , Duncan's multiple range tests was performed to determine the significance between mean values. All analyses were performed using the SPSS ver. 16.0.

## 3. Result and discussion

### 3.1. Microscopic picture of broiler liver organ

Microscopic images of the liver in all treatments at 40x10 magnification can be seen in fig. 1.

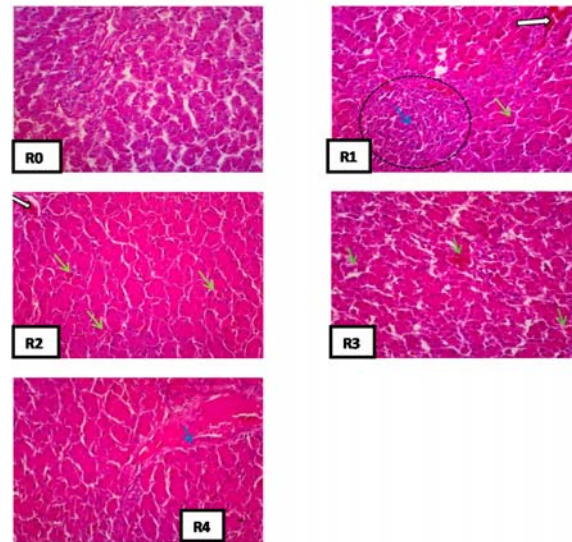


**Figure 1.** Histopathology of liver organ in all treatments (enlargement 40 x10)

➡ : Inflamed nodule : ➡ : Congestion ➡ : Haemorrhage

### 3.2. Microscopic picture of small intestinal organs

Microscopic images of the small intestine in all treatments at 40x10 magnification can be seen in fig. 2. Broiler liver with R0 treatment under normal conditions. This is characterized by liver cells and small intestine in normal conditions and the absence of inflammatory nodules in the liver, not found congestion (bleeding) and bleeding (haemorrhage). Whereas fig. 2 shows that the condition of the broiler small intestine is normal because of the absence of inflammatory nodules in the small intestine, not found congestion (bleeding) and bleeding (haemorrhage). This happened because the broiler in treatment R0 was not infected with *Salmonella pullorum*.



**Figure 2.** Histopathology of small intestine organ in all treatments (Enlargement 40 x10)

→ : inflamed nodule    → : Mitotic phase    → : Haemorrhage

According to [4] microscopic damage to organs and tissues caused by viruses, bacteria or parasites can be observed by looking at bleeding (haemorrhage) and blocking (congestion) of blood vessels around the tissue while based on research there are inflammatory nodules, congestion or bleeding in a tissue shows abnormalities of an infected tissue. Fig. 1 shows the broiler liver in treatment R1 in a damaged condition characterized by inflammatory nodules in the liver (blue arrow) which indicate the presence of *Salmonella pullorum* infection. Also found is congestion (white dam) (white arrow) and bleeding (haemorrhage) (green arrow). Fig. 2 the condition of the small intestine tissue in treatment R1 shows bleeding (green arrow). This means that the administration of tetracycline 0.015% is not able to reduce *Salmonella pullorum* infection in the liver tissue and broiler small intestine because of the amount of damage that occurs in the liver tissue and broiler small intestine. Fig. 1 shows that the broiler liver in treatment R2 showed no inflammatory nodules in the liver, but still found congestion (white dams) and bleeding (haemorrhage) (green arrow). Whereas in fig. 2 the condition of the small intestine tissue in the R2 treatment shows that there are quite a number of cells undergoing the mitotic phase (black arrows). The mitotic phase itself is a phase where there is cell division in tissue for cell regeneration in organ tissues due to cell or tissue damage or replacing dead cells. Turmeric flour in broilers was able to inhibit the growth of *Salmonella typhimurium* bacteria and the most effective concentration to inhibit bacterial growth was the concentration of 3% turmeric with an average inhibition zone diameter of 20 mm [5]. Fig. 1 shows that the broiler liver in treatment R3 shows an inflammatory nodule (blue arrow). In addition, found damages (congestion) (white arrows) and bleeding (haemorrhage) (green arrows). Whereas in fig. 2 the condition of the small intestine tissue in the R3 treatment shows that the epithelial cells and active glandular cells are characterized by the mitotic phase (black arrow) which indicates the regeneration of dead cells. In addition, there was a migration of inflammatory cells (blue arrows) which indicated that there was an effort to cleanse foreign objects such as bacteria and others. Giving garlic extract can reduce the infection of *Salmonella pullorum* compared to the use of tetracycline antibiotics.

Fig. 1 shows that the condition of broiler liver in treatment R4 suffered only a little damage because of the few inflammatory nodules found. Whereas in fig. 2, the small intestine condition in treatment R4 is in a normal condition due to the absence of tissue damage to the organ because there are no inflammatory nodules, damages, and bleeding. When compared between treatments R0 with treatment

R4, the level of damage to the liver and small intestine broiler at treatment R4 is almost the same as the condition of the liver and small intestine broiler in treatment R0 (control) which indicates the least damage to liver and broiler small intestine with TGWE.

### 3.3. Liver organ and small intestine damage level analysis

The results of the variance of the level of damage to the liver and small intestine in broilers with the administration of TWE, GWE, and TGWE infected with *Salmonella pullorum* bacteria can be seen in table 1.

Based on the variance in table 1 it can be seen that the addition of TWE, GWE and TGWE showed a very significant effect ( $P < 0.01$ ) reducing tissue damage to the liver and small intestine infected with *Salmonella pullorum*. The addition of TWE, and TGWE to broiler rations infected with *Salmonella pullorum* showed better results than the ration added by tetracycline on all parameters. As for the addition of GWE, the effect of reducing damage to liver and broiler intestines was lower compared to the ration added by TWE and TGWE.

**Table 1.** Small intestinal organ damage and liver with TWE, GWE, and TGWE infected with *Salmonella pullorum*

Treatment	Type of damage			
	Inflammatory nodules in the liver	Hemorrhage at the liver	Liver congestion	Hemorrhage at the small intestine
R <sub>0</sub>	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>
R <sub>1</sub>	1.50 ± 0.58 <sup>c</sup>	1.75 ± 0.50 <sup>d</sup>	1.75 ± 0.50 <sup>d</sup>	1.75 ± 0.50 <sup>c</sup>
R <sub>2</sub>	0.50 ± 0.58 <sup>ab</sup>	1.00 ± 0.00 <sup>bc</sup>	1.00 ± 0.00 <sup>bc</sup>	1.00 ± 0.00 <sup>b</sup>
R <sub>3</sub>	1.25 ± 0.50 <sup>bc</sup>	1.50 ± 0.58 <sup>cd</sup>	1.25 ± 0.50 <sup>cd</sup>	1.25 ± 0.00 <sup>bc</sup>
R <sub>4</sub>	0.50 ± 0.58 <sup>ab</sup>	0.50 ± 0.58 <sup>ab</sup>	0.50 ± 0.58 <sup>ab</sup>	0.75 ± 0.50 <sup>b</sup>

### 3.4. Inflammatory nodules in the liver

The higher the average score, the higher the level of damage to the liver. The best results were obtained in R2 and R4 treatments with an average score of 0.50 each. Decreased levels of liver damage seen from the presence of inflammatory nodules in the treatment of R2 and R4, allegedly caused by the presence of active ingredients contained in turmeric and garlic which play a role in suppressing the presence of *Salmonella pullorum* bacteria.

### 3.5. Heart congestion

Congestion (hyperemia/dam) is a condition accompanied by increased blood volume in vessels that are widening in a device or parts of the body. Allegedly because of the combination of active ingredients contained in turmeric and garlic which play a role in reducing liver tissue degeneration, specifically reducing the occurrence of congestion in the liver. Turmeric in the form of powder can be used for the treatment of liver damage (degeneration) [6]. [7] stated that the active ingredient in garlic (allicin) had an antihepatotoxic effect by reducing the levels of carbon tetrachloride and galactosamine. In addition, *S-allylmercaptocysteine* and *S-methylmercaptocysteine* can protect the liver from damage caused by hepatotoxins in hepatitis to the liver.

The average score in terms of haemorrhage in the small intestine treated R0 was significantly different ( $P < 0.01$ ) with R4 treatment, while treatment R4 was not significantly different ( $P > 0.01$ ) with treatment R2 and R3, but differed significantly higher compared to R1. The results obtained a score for the presence of haemorrhages in the small intestine range of 0.00 - 1.75. The best results obtained by treatment R4 with an average score of 0.75 if compared with the other three treatments.

Judging from the presence of haemorrhage in the liver and broiler small intestine, the best results were obtained in R4 treatment. This supports [4] which explains that a 2.5% TGWE (1 EAK: 3 EABP) in vitro shows the highest inhibition of pathogenic bacteria such as *Salmonella* and *E. coli*. The inhibition

of bacterial growth causes reduced infection of the digestive organs caused by bacteria. [8] report that the cause of haemorrhage or bleeding was seen in liver and small intestine tissue is an indication of damage to blood vessels or organs due to viruses or other microorganisms

#### 4. Conclusion

In general, the addition of TWE phytobiotic, GWE and TGWE as feed additives on rations can reduce tissue damage to the liver and broiler small intestine after *Salmonella pullorum* infection.

#### 5. Acknowledgment

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