Turmeric (Curcuma domestica) and Garlic (Allium sativum) towards broiler immune system infected by Salmonella pullorum bacteria as a feed additive

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Abstract. The use of synthetic antibiotics as feed additive causes residues and drug resistance in bacteria. Synthetic antibiotics can be obtained from fitobiotik. This study is conducted to determine the effects of phytobiotic Turmeric Extract, Garlic Extract, and the combination of Turmeric and Garlic Extracts as a feed additive on broiler immune system which is infected by Salmonella pullorum. One hundred of d.o.c were reared intensively for 38 days and divided into five treatments and four replications, with five chicks in each replicate. The treatments consisted of R0 to R4. R0 is basal diet without phytobiotic or antibiotic as a negative control; R1 is basal diet + 0.015% tetracycline (Salmonella pullorum infection as a positive control); R2 is basal diet + 2.5% TWE (Salmonella pullorum infection); R3 is basal diet + 2.0% GWE (Salmonella pullorum infection); and R4 is basal diet + 2.5% TGWE + Salmonella pullorum infection). The results show that feeding fitobiotik Turmeric Extract, Garlic Extract, and the combination of Turmeric and Garlic Extracts to maintain the immune responses of broiler does not show the effect on the H/L ratio (P>0.05), but it significantly affects the percentage of the bursa fabricius weight and percentage of the spleen weight (P<0.05). As the conclusion of this study, turmeric and garlic phytobiotic extracts diet as feed additive can maintain broiler immune responses which infected by Salmonella pullorum.

1. Introduction
Natural antibiotics can help the poultry's immune system to deal with various infections. The immune system is body's defense system carried out by the lymphatic system by producing antibodies. Antibodies function as a natural defense of the body against foreign substances that enter the body such as bacteria, virus, fungi, and other infectious substances. Indonesia is a country which is very rich in flora and fauna. Related to the richness of the flora (plants), many of the florals belong to the category of medicinal plants which have been used by our ancestors for centuries. The use of traditional medicine has increased and is no longer an alternative medicine. The development of the traditional medicine is not only used in humans, but also used in the field of animal husbandry, known as phytobiotics.

Phytobiotics is a feed additive derived from pure plants which can improve the performance of livestock [1]. Fitobiotics are able to stimulate animal nutrition, act as antimicrobial, coccidiostatic, and antihelmintic [2]. Fitobiotics can be obtained by utilizing various herbal plants such as garlic and turmeric. Garlic contains various levels of alliin, rich vitamins (vitamin C, thiamine, riboflavin and niacin), selenium, and potassium. The addition of garlic powder to chicken feed has a beneficial effect
on the growth and meat. Garlic powder is used in multicomponent phytosupplement to stimulate chicken production [3].

Turmeric is a type of plant from the ginger family that contains active substances such as essential oil and curcumin compounds. Curcumin is a diarylhatanoid which gives yellow color. Turmeric has great potential in pharmacological activities, namely anti-inflammatory, anti-immunodeficiency, anti-virus (bird flu virus), antibacterial, anti-fungal, anti-oxidant, anti-carcinogenic, and anti-infectious [4].

Pulmonary disease is a disease caused by the infection of the Salmonella pullorum bacteria. The transmission of this disease can occur both vertically and horizontally and has a high mortality rate. [5] stated that the combination of phytobiotic extract of turmeric and garlic water can act as an antibacterial agent against Salmonella, Lactobacillus, and Escherichia coli at the level of 2.5 percent. Based on the description above, it is necessary to conduct a study to determine the effectivity of turmeric and garlic extracts as feed additives on the resistance of broiler bodies infected with Salmonella pullorum bacteria seen from the weight of bursa fabricius, spleen, and heterophyl lymphocyte ratio (H/L ratio).

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 extract ingredients. Turmeric and garlic extracts are produced by CV. Lansida, Yogyakarta. Turmeric extract and garlic extracts were obtained through a series of processes. At first, the fresh turmerics were washed until they were clean from the soil that was attached. Then, they were drained and sliced thinly. Meanwhile, the garlics were stripped of the outer skin and then also sliced thinly. Garlics and turmerics were separately mixed with water (in a ratio of 1:5). Then, they were blended and stirred using ultra turax. Furthermore, the solution of turmerics and garlics was filtered and evaporated. The turmeric and garlic water extracts are ready to be used and mixed according to the level of treatments.

2.2.2. Salmonella pullorum bacteria culture. The culture of Salmonella pullorum bacteria was taken by 1 loop colonies. The bacteria were planted on broth media of brain heart infusion (BHI) and incubated at 37°C for 18-24 hours [6]. This was then centrifuged (500 g, 10 minutes) so that the pellets were formed. To obtain the dose of inoculum, the pellets were diluted with a sterile physiological NaCl solution, and its turbidity was compared with the McFarland No. 1 standard which was equivalent to 108 colony forming units (CFU)/ml [7].

2.2.3. Experimental diet. Broiler The experiment diet was given to broiler chickens from d.o.c (day old chick) until the age of 5 weeks after randomization. Feeding ingredients consist of fine yellow corn, rice bran, meat and bone meal, vegetable oil, chicken feather meal, soybean meal, CaCO3, Dicalcium phosphate, premix, salt, L-lysine, and DL-methionine. Treatment feed consisted of basal rations added with TWE, GWE, and TGWE as feed additives. The composition of the research ration is presented in table 1. Group I was offered a basal diet served as a negative control, group 2 was offered a basal diet with 0.015% tetracyclin served as positive control, group 3, 4 and 5 were offered a basal diet with 0.25% turmeric extract (TWE), 0.20% garlic extract (GWE), and 0.25% turmeric and garlic extracts (TGWE) with Salmonella pullorum infection respectively, during a six-week experimental period.
2.2.4. Broiler Maintenance. Broiler chickens were divided randomly into 5 treatments and 4 replications so that there were 20 cages. Broiler chickens were kept in a colony cage for 5 weeks randomly before the period of adaptation. Feed and drinking water were given ad libitum. Vita stress was given before and after vaccination. Newcastle Disease (ND) Vaccination was given at the age of 4 days through eye drops, and ND/AI vaccine was given at 21 days through subcutaneous. Broiler chickens were weighed once a week to find out the weight gain every week and the weight of the remaining feed to find out the food consumed when a 3-week-old chicken is infected with Salmonella pullorum orally at a dose of $10^8$ CFU/ml [6].

2.2.5. Sampling for H/L. On day 38, blood was taken through the pectoralis vein on the underside of 2–3 ml with syringe. Blood samples were collected in a vacuum tube containing EDTA anticoagulants. The tube was shaken slowly so that the blood did not freeze to be immediately sent and inspected at the Unhas Educational Animal Clinic Laboratory. The underside of the chicken wings was cleaned with cotton that has been moistened with 70% alcohol before and after the blood collection. The percentage of heterophil and lymphocytes was then multiplied by the number of leukocytes, so that the number of heterophilies and lymphocytes was obtained. The number of leukocytes counted through the improve neubeur count chamber after blood was diluted with Turk solution.

2.2.6. Sampling for Lymphoid Organs. Lymphoid organ harvesting was carried out after the blood sampling. The organ harvesting was carried out by randomly taking 1 chicken from each experimental unit (as much as 20%). A total of 20 chickens were cut for the whole treatment. The methods of taking lymphoid organs consisted of several steps. Right after the chicken was slaughtered, it was then dissected with a scalpel in its abdominal part. The bursa of Fabricius and the spleen of the chicken which has been dissected were then taken to be weighed. Lymphoid organ weight (bursa of Fabricius and spleen) was observed by means of organs separation through the surgical process to be weighed.

2.3. Statistical analysis
Data for H/L ratio and lymphoid organ were subjected to an analysis of variance. The treatments’ means with significant differences at $p<0.05$ were compared using orthogonal contrast procedure.

3. Result and discussion

3.1. General Result.
Microscopic The heterophil/Lymphocyte ratio, weight of fabricius, and 38-day spleen broilers with the administration of turmeric water extract and garlic water extract in different levels can be seen in table 1.

| Treatments | Parameters          |                  |                  |
|------------|---------------------|------------------|------------------|
|            | H/L ratio           | Bursa Fabricius (%) | spleen(%)       |
| R0         | 0.97 ± 0.36         | 0.09 ± 0.01      | 0.06 ± 0.01      |
| R1         | 1.50 ± 0.57         | 0.10 ± 0.04      | 0.13 ± 0.02      |
| R2         | 1.43 ± 0.51         | 0.08 ± 0.02      | 0.11 ± 0.04      |
| R3         | 1.68 ± 0.24         | 0.14 ± 0.02      | 0.18 ± 0.02      |
| R4         | 1.66 ± 0.60         | 0.06 ± 0.03      | 0.11 ± 0.01      |

3.2. Heterophil/Lymphocyte Ratio.
The results of analysis of variance show that the administration of the WTE, WGE, and TGWE has no significant effect ($P>0.05$) on the ratio of H/L broilers infected with Salmonella pullorum. The highest average of the H/L ratio at GWE reaches 1.68, and the lowest in negative control was 0.97. All treatments show an average value higher than the normal value. According to [8], the level of body
resistance in poultry can be determined by the value of the H/L ratio which is about 0.2 (low), 0.5 (normal), and 0.8 (high) to environmental adaptations.

The H/L ratio in the TTWE, GWE and TGWE administration in the ration show a higher value than the negative control, but compared to the positive control of the TWE administration, it shows lower values related to bacterial infection resulting in an increase in heterophil values and a decrease in lymphocyte values. According to [9], the H/L ratio can be used to determine the presence of physiological stress due to the infection.

The H/L ratio in the addition of the TWE shows a lower value than the positive control indicating that the TWE administration is able to maintain the body's defenses against bacterial infections and is able to be used as a substitute for synthetic antibiotics. This is in accordance with the opinion of [10] that essential oil in turmerics are bactericidal, whereas curcumin is bacteriostatic. The H/L ratio in the addition of the GWE and TGWE shows higher values than positive controls indicating that the GWE and TGWE are not better than tetracycline in fighting bacterial infections, but still play a role in fighting infection. The addition of the TGWE shows that the H/L value is lower than the addition of the GWE due to the active substance content of the TWE. This is consistent with the opinion of [11] that garlics serve as antibacterial (antibiotic) with their active ingredient known as allisin. Garlics also contain antioxidants and anti-inflammatory [12] state that feed containing a single herb (turmerics or garlic) which is coupled with relative zinc is able to provide an increase in the number of erythrocytes, hematocrit values, and hemoglobin. The content of curcumin and essential oil in turmerics is thought to be able to increase the value of lymphocytes. This is supported by [13] stating that curcumin has the ability to activate T lymphocytes and B lymphocyte cells.

3.3. **Bursa Fabricius.**

The results of the analysis of variance show that the administration of the TWE, GWE and TGWE has a significant effect (p <0.05) on the fabricius stock weight. The highest average of the fabricius stock weight in the GWE reaches 0.14\%, and the lowest in TGWE is 0.06\%. The weight of fabricius stock in all treatments is below the normal range, namely 0.22 - 0.26\% of life weight. The average of the orthogonal F-contrast test results of 38-day fabricius weight with the application of turmeric water extract and garlic water extract in different levels can be seen in table 2.

| Treatments        | Average | Significant |
|-------------------|---------|-------------|
| R0 VS R1, R2, R3, R4 | 0.09 VS 0.095 | 0.837 ns     |
| R1 VS R2, R3, R4   | 0.1 VS 0.093  | 0.963 ns     |
| R2 VS R3           | 0.08 VS 0.18  | 0.016 s      |
| R2 VS R4           | 0.08 VS 0.06  | 0.485 ns     |
| R3 VS R4           | 0.14 VS 0.06  | 0.004 s      |

Note: s : Significant; ns : Not significant;

The F-Contrast test results in table 3 show that the treatment of R2 vs R3 and R3 vs R4 is significantly different (p<0.05). The weight of the fabricius stock at the GWE administration is higher than the TWE or TGWE administration. The active ingredients in garlics can increase the weight of the fabricius stock. This is in accordance with the opinion of [14] stating that garlic extract has immunomodulatory effects and reduces age-related damage from the immune response. Garlic supplements in chickens increase the relative weight of the spleen, bursa of fabricius, and thymus. [15] state that garlics can enhance the immune system to fight potential diseases and maintain health. Garlics also have the ability to stimulate
the lymphoid system which accelerates the elimination of waste products from the body and is also considered as an effective antioxidant to protect cells against free radical damage.

The content of allicin and diallyl sulfide contained in garlics is thought to be able to increase the body's immune system. Allicin and diallyl sulfide inhibit bacterial growth so that the fabricius bursa does not work too hard and does not undergo immunosuppression. [16] states that the high antimicrobial power of garlic is due to the content of allicin and diallyl sulfide contained in garlic essential oil. Allicin and diallyl sulfide show an inhibitory activity for bacterial growth.

3.4. Spleen.
The results of the analysis of variance show that administration of the TWE, GWE and TGWE has a significant effect (p <0.05) on the weight of the spleen. The highest average of the spleen weight in GWE administration reaches 0.18%, and the lowest in negative control is 0.06%. All treatments are in the normal range of under 0.2% of the body weight. [17] states that the percentage of normal spleen does not exceed 0.2%. The average of the orthogonal F-contrast test results of 38-day fabricius broiler weight with the application of turmeric water extract and garlic water extract in different levels can be seen in table 3.

| Treatments          | Average    | Significant |
|---------------------|------------|-------------|
| R0 VS R1 R2 R3 R4  | 0.06 VS 0.1325 | 0.002 s     |
| R1 VS R2 R3 R4     | 0.13 VS 0.1333 | 0.857 ns    |
| R2 VS R3           | 0.11 VS 0.18  | 0.003 s     |
| R2 VS R4           | 0.11 VS 0.11  | 0.735 ns    |
| R3 VS R4           | 0.18 VS 0.11  | 0.006 s     |

Note: s : Significant; ns : Non significant;

The F-contrast test results in table 4 show that the treatment of R0 VS R1, R2, R3, and R4 is significantly different (p <0.05). The spleen weight in R0 is lower than that in R1, R2, R3 and R4. The spleen weight in R0 does not increase due to the absence of Salmonella pullorum infection, while in the treatment of R1, R2, R3 and R4 Salmonella pullorum infection causes an increase in the spleen weight due to fighting disease.

The F-Contrast test results in table 4 show that the treatment of R2 VS R3 and R3 vs R4 is significantly different (P <0.05) while the R2 VS R4 treatment is not significantly different (P> 0.05). The spleen weight in R2 and R4 treatment is lower than that in R3. The content of essential oil and curcumin in the TWE and TGWE can play a role in fighting bacterial infections and reducing inflammation, so that the spleen weight does not experience a large increase. Essential oil can be used as antibacterial because it contains hydroxyl and carbonyl groups which are derivatives of phenols [18]. Curcumin compounds contained in turmeric rhizomes are toxic to some types of bacteria and can also act as anti-inflammatory [19].

All treatments show that the spleen weight is in the normal range even though the Salmonella pullorum bacterium infects. This shows that the administration of the TWE, GWE and TGWE in feed is able to maintain the resistance of the broiler body in fighting infection. This improves the previous research by [20] that in vitro, the addition of turmeric and garlic extracts to broiler rations has antibacterial properties against Lactobacillus, Salmonella and E. coli bacteria. Purwanti further explains that the concentration of 2.5% of the TGWE combination (1TWE: 3GWE) in vitro shows the highest inhibition against pathogenic bacteria such as Salmonella and E. coli but it does not show a zone of inhibition in good bacteria such as Lactobacillus.
In all treatments with *Salmonella pullorum* bacterial infection, the weight of the fabricius bursa is smaller than that of the spleen, while the negative control of bursa fabricius weight is greater. This shows that the bacterial infection of *Salmonella pullorum* results in immunosuppression. [21] state that the ratio between the stock exchange weight and spleen can be used as immunosuppression parameters.

4. Conclusion
It is concluded that the addition of Turmeric Water Extract, Garlic Water Extract and the combination of both as feed additives in the ration is able to maintain the resistance of the broiler body to the infection of *Salmonella pullorum* bacteria.

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