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# Supplementation of Curcuma (Curcuma Xanthorrhiza) and Bratawali (Tinospora Cordifolia) on Hematological Status of Broiler Chickens

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ABSTRACT
Research on herbal supplementation as an effort to improve livestock productivity is widely published, but rarely does the research inform hematological analysis. The description of the hematological status of livestock can explain the health status of livestock due to the influence caused by the treatment in the provision of feed. The status analyzed in this paper includes Erythrocyte count, Hemoglobin concentration, Hematocrit value, Netrophil count, Eosinophil count and Lymphocyte count. The study was conducted experimentally, with 6 treatments and 4 replications, with each unit containing 5 broiler chickens raised for 35 days. The treatments were P1: Control, with broiler feed without supplementation, P2: 2% Temulawak + 1% Bratawali treatment, starting on day 11, P3; 2% Temulawak + 1% Bratawali treatment, starting on day 21, P4: Treatment of Temulawak 1%+Bratawali 0.5%, starting on day 11, P5: Treatment of Temulawak 1%+Bratawali 0.5%, starting on day 21. P6: Antibiotic supplementation treatment. Descriptive analysis shows, the number of Erythrocytes is normal for all treatments, the amount of Hemoglobin is below normal values for treatments P2, P4, P5 and P6, the number of Hematocrit values is still within the normal range. In the number of Netrophy there was an increase exceeding normal standards, in all treatments, the number of Eosinophils in all treatments showed an increase from normal standards, except in Treatment P1, the number of Lymphocytes, all treatments were normal.The increase in the number of Netrophils in all treatments can be inferred from the presence of bacterial infection activity in all experimental broilers, but because it occurred in all groups, it can explain that the infection activity was not triggered by supplementation, but rather introduced from the environment. The increase in Eosinophil counts is due to parasitic infection activity, which is a response to parasite products and the damage they cause, it could be that changes in the digestive ecosystem increase intrinsic parasite activity caused by supplementation. High lymphocyte counts may reflect viral infection or chronic infection, while normal lekocyte counts compared to normal standards provide
information that supplementation can strengthen broiler resistance.

## 1. INTRODUCTION

The background to this research is various studies on broiler feed in the form of supplementation in feed, be it herbs, antibiotics, vitamins and so on. detrimental effect on the health status of chickens. The health status in question is hematological status, which we have analyzed descriptively and using variance analysis, namely the number of Erythrocytes, Hemoglobin concentration, Hematocrit Value, and the number of Leukocytes which includes

the number of Neutrophils, the number of Eosinophils and the number of Lymphocytes. Abnormalities in hematological status can indicate a disruption in the health condition of broiler chickens in general.

The objectives of this research are: 1). Measuring the detrimental effects of herbal plant supplementation in feed on the hematological condition of broiler chickens. 2). Comparing the effects of herbal plant supplementation with antibiotic supplementation on the hematological status of broiler chickens which have beneficial or medically detrimental effects on broiler chickens.

The global poultry industry is a vital component of the agricultural sector, providing a significant source of protein to meet the nutritional demands of a growing population. The increasing interest in sustainable and organic farming practices has led researchers and farmers to explore the potential of natural supplements to enhance poultry health and productivity. Among the various dietary supplements, herbal remedies have gained popularity due to their low toxicity, health benefits, and ability to improve overall animal welfare (Alhassan and Adamu, 2018; Amin and Abd El-Ghany, 2017; Bhat and Malla; 2021; Vyas and Agrawal, 2023).

Curcuma xanthorrhiza, commonly known as "temulawak," is a traditional herbal plant widely used in Southeast Asia for its medicinal properties. It has been recognized for its antioxidant, anti-inflammatory, and antimicrobial effects (Anis and Khan, 2020; Bhanja and Mandal, 2016). The bioactive compounds in Curcuma xanthorrhiza, particularly curcumin, have shown potential in improving immune responses and promoting growth in poultry. Previous studies have indicated that supplementation with Curcuma can positively influence hematological parameters, which are critical indicators of health and wellbeing in birds (Fathi and Abou-Kassem, 2020; Gholami and Shahbazi, 2020).

Another herbal supplement, Tinospora cordifolia, known as "bratawali," has also been extensively studied for its pharmacological properties. It possesses immunomodulatory effects and is believed to enhance resistance against infectious diseases. The phytochemicals present in Tinospora cordifolia contribute to its potential as a natural feed additive, promoting better nutrient absorption and improving the overall health of livestock. Research has shown that the inclusion of Tinospora in poultry diets can enhance growth performance and boost the immune system, which are crucial for the effective management of broiler chickens (Mahran et al., 2021).

The hematological status of broiler chickens serves as an essential parameter for evaluating their health and productivity. Parameters such as red blood cell count, hemoglobin concentration, and hematocrit values provide valuable insights into the physiological state of the birds and their ability to withstand stressors. Given the increasing challenges posed by environmental stressors, diseases, and the demand for antibiotic-free production, there is a growing interest in exploring natural alternatives to support the health of broiler chickens.

Despite the promising effects of Curcuma xanthorrhiza and Tinospora cordifolia on poultry health, there is a limited body of literature specifically addressing their combined effects on the hematological parameters of broiler chickens. This study aims to investigate

the supplementation of Curcuma xanthorrhiza and Tinospora cordifolia and their impact on the hematological status of broiler chickens. Understanding the synergistic effects of these herbal supplements could provide valuable insights for poultry nutritionists and farmers looking to enhance broiler health and productivity through natural means (Carretero and Vallejo, 2019; Cottam and Mc Gowan, 2022)

## 2. METHOD

### a) Time and Place of Research

The research was conducted at the Experimental Farm, Faculty of Animal Husbandry, UNSOED for 35 days. Broadly speaking, the implementation of the research was divided into three stages; cage preparation, chicken maintenance and observation of research samples.

The research design was experimental, with 6 types of treatments and 4 replication units, each unit containing 5 chickens. A total of 120 chickens were kept, Lohman strain. Two samples were taken from each unit, there were 48 observation samples.

The herbal form of Temulawah and Brotowali applied in this study was in powder form. This herbal powder form is easy to make and easy to apply to broiler farming conditions. While in the extract form, either ethanol extract or water extract, many obstacles were encountered in its application. In the mechanism of action of herbal ingredients, it is known as the "Adaptogen Phenomenon", which means that the medicinal ingredients in medicinal plants will work synergistically from the efficacious compounds in them, so that it will cause the desired therapeutic effect, different if it has been in the form of extraction, only certain compounds are isolated, so that the efficacy formed is not optimal.

The research design used was a completely randomized design (CRD) with 6 types of treatments (P1, P2, P3, P4, P5 and P6) and 4 replications, each replication unit consisting of 5 broiler chickens.

The treatments given were:
P1 = feeding without treatment.
P2 = giving T2% + B1%, starting on the 11th day.
P3 = giving T2% + B1%, starting on the 21st day.
P4 = giving T1% + B0.5%, starting on the 11th day.
P5 = giving T1% + B0.5%, starting on the 21st day.
P6 = giving growth-stimulating antibiotics (zinc bacitracin)

### b) Research Procedure

Cage Preparation

The cage used was a stage system cage. Five cage plots were made, each cage plot was equipped with a place to eat and drink. Before the livestock is put into the cage, the cage is first sanitized using a disinfectant solution or formalin mixed with clean water in a ratio, then the solution is sprayed over all parts of the cage. After drying, lime is applied evenly to the walls and floor of the cage. The cage equipment for feeding and drinking water is previously washed using soap, then rinsed using disinfectant water. Heating the cage is done using a 15 watt incandescent lamp installed in each cage compartment.

### c) Making Temulawak Rhizome Flour and Brotowali Stems

The part of the brotowali used is the stem, washed with water until clean then dried in the sun or in an oven at 55$^0$C, after it is slightly dry, the brotowali is cut into thin slices, dried in the

sun until dry and broken, then mashed (blended) into flour. While the Temulawak used is the rhizome, the manufacturing procedure is the same as Brotowali.

**d) Ration Treatment**

The ration is arranged in the form of flour (mash) because it is preferred by chickens and is more easily absorbed by the intestines so that its efficiency is better and can be given to all ages of chickens, by meeting the nutritional needs of broilers. The ration consisting of bran, corn flour, concentrate, minerals (Cargill products) and supplemented with herbs according to the treatment is mixed until homogeneous.

**e) Maintenance and Treatment Research**

When DOC arrives, DOC is placed in the brood cage then given drinking water with 5% sugar water and a heating lamp, the maintenance temperature is around 300-320C, then given ND (Newcastle Disease) vaccine in the first four days through eye drops and the chickens are given adaptation rations for 10 days. After 10 days of adaptation, broiler chickens were randomly divided into 6 treatments and 4 replications so that there were 24 experimental units/cage, Each experimental unit consisted of 5 chickens, so that the total number was 120 broiler chickens. Furthermore, they were kept for 35 days and given treatment rations and drinking water ad libitum until harvest. The examination sample in the form of blood plasma was taken on the 35th day of maintenance, as much as 3 milliliters, which was taken from the arm vein (brachialis).

## 3. RESULT AND DISCUSSION

Supplementation of products in broiler feed aims to increase productivity, but it is also necessary to consider whether a supplement has a detrimental or beneficial effect on the livestock (Jadhav and Bhosale, 2023; Kaur and Singh, 2019; Mardiyono and Budiarto, 2020; Murtaza and Rahman, 2022; Saha and Basak, 2019;

Bioactive substances in medicinal plants consist of: phenol, tannin, flavonoid, essential oil, curcumin, saponin, phyllanthin. These bioactive substances have the ability as antibacterial or antifungal. Feed additives are feed raw materials that do not contain nutrients, but can increase productivity, quality of livestock products (meat, eggs, milk, skin, fur), efficiency of feed use and improve animal health or resistance to disease. Feed additives that are widely used in the livestock industry include antibiotics, antioxidants, antifungals, emulsifiers, and binders (Pasaribu, 2019).

The use of temulawak in traditional medicine is widely used in the treatment of digestive disorders, jaundice, vaginal discharge, increasing endurance and maintaining health (Syamsudin, 2019). In the field of medicine, Brotowali has been known to be used in curing parasitic diseases, namely Malaria (Google.com, 2021). Thus, it is hoped that parasitic diseases in broiler chickens, such as Worms and Coccidia (still in the same order as Plasmodium) can be overcome, so that chickens become healthy because they are free from diseases that have long been chronic cases in broiler chicken farming.

Broiler chickens are superior cultivation products with genetic selection and advanced technological support in feed research and maintenance, resulting in profitable economic products (Pratikno, 2010).

**Number of Erythrocytes**

The calculation of the description of the number

of Erythrocytes and its variance analysis are as follows:

Table. 1. Description of the number of Erythrocytes in the treatment group

| Treatment | Test | Total | Average | S. Deviation |
|---|---|---|---|---|
| P1 | 4 | 10,46 | 2,615 | 0,347 |
| P2 | 4 | 9,03 | 2,257 | 0,212 |
| P3 | 4 | 9,81 | 2,452 | 0,397 |
| P4 | 4 | 8,92 | 2,23 | 0,107 |
| P5 | 4 | 9,38 | 2,345 | 0,259 |
| P6 | 4 | 9,81 | 2,452 | 0,348 |

With the analysis of the variance is as follows:
Table 2. Analysis of variance of the number of erythrocytes

| Source of variance | DB | JK | KT | FCount | F table 0,05 | F table 0,01 | Inf |
|---|---|---|---|---|---|---|---|
| Error Treatment | 5 | 0,41 | 0,082 | 0,94 | 2,77 | 4,25 | * |
| | 18 | 1,57 | 0,087 | | | | |
| Total | 23 | 1,98 | | | | | |

Description: * = No significant effect
** = Significant effect

The number of normal erythrocytes in broiler chickens ranges from 2.26-3.5 x 106/µ liter (Habib et al., 2019; Saputro et al., 2014), the results of the study (Table 1) obtained data that the average number of erythrocytes was 2.2-2.6 x 106/µ liter which means that the control and treatment broiler groups were still in normal condition and indicated that the administration of the combination of Temulawak and Bratawali herbs did not interfere with the physiological status of erythrocytes in broiler chickens. In the laboratory, broiler chickens were declared healthy. It could be that the Fe (iron) content in herbal plants is able to provide the minerals needed in the synthesis of hemoglobin or erythrocytes, so that they remain normal.

The results of the analysis of variance (ANOVA), Table 2, show that broiler chickens that were given additional herbs in their feed had no significant effect (P <0.05) on the number of erythrocytes. This means that red blood cells that transport oxygen to metabolize nutrients are still functioning well (Isroli et al., 2009). The addition of temulawak and brotowali to the ration can maintain the balance of the number of erythrocytes to remain within the normal range. In this study, feed consumption in P1 (negative control) was higher compared to feed consumption in P2. This is because in the P1 treatment, phytobiotics were not added to the ration, while in the P2 treatment, 2% temulawak and 1% brotowali were added. The difference in the number of erythrocytes from the two treatments was due to the essential oil content in phytobiotics which have a distinctive odor and taste so that they can reduce the palatability of livestock. The addition of phytobiotics was still within normal limits in the P2 treatment so that it did not significantly affect the number of erythrocytes.

**Hemoglobin Concentration**

Hemoglobin concentration measurement data, variance analysis and significance test are as follows:

Table. 3. Description of Hemoglobin concentration in treatment groups

| Treatment | Test | Total | Average | S. Deviation |
|---|---|---|---|---|
| P1 | 4 | 30,14 | 7,535 | 0,390 |
| P2 | 4 | 27,93 | 6,982 | 0,66 |
| P3 | 4 | 33,93 | 8,482 | 0,625 |
| P4 | 4 | 23,87 | 5,967 | 0,176 |
| P5 | 4 | 25,33 | 6,332 | 0,876 |
| P6 | 4 | 26,08 | 6,52 | 0,739 |

Table 4. Analysis of variance of Hemoglobin

concentration

| Source of variance | DB | JK | KT | Fcount | F table 0,05 | F table 0,01 | Inf |
|---|---|---|---|---|---|---|---|
| Eror | | | | | | | |
| treatment | 5 | 16,88 | 3,37 | 8,71 | 2,77 | 4,25 | ** |
| | 18 | 6,97 | 0,38 | | | | |
| Total | 23 | 23,86 | | | | | |

Description: * = No significant effect
** = Significant effect

Table 5. Calculation of BNJ Significance

| Treatment | Average | Average + BNJ | Symbol |
|---|---|---|---|
| P4 | 5,967 | 7,299 | a |
| P5 | 6,332 | 7,664 | a |
| P6 | 6,52 | 7,852 | a |
| P2 | 6,982 | 8,314 | a |
| P1 | 7,535 | 8,867 | a |
| P3 | 8,482 | 9,814 | a |

Significance test conclusion: the treatments tested in this study have the same impact.

The results of the research data analysis in Table 3. show that the average hemoglobin value of broiler chickens reached 5.9 - 8.4 g / dL. According to Kusumasari et al., (2012) Mahran and Moussa (2021) that the normal range of hemoglobin levels in broilers ranges from 7.3- 10.90 g / dL. Based on this statement, the average values found in treatments P1 and P3 (Table 3) mean that the hemoglobin concentration obtained can be categorized as normal, while in P2, P4, P5 and P6 the average Hemoglobin value is below the normal standard. This could be due to the high content of anti-nutrients in herbal plants, namely tannin compounds, which can affect the absorption of minerals in the digestive system as components of hemoglobin. The results of the variance analysis in Table 4 also show that there is a significant effect (P> 0.05) of treatment on the hemoglobin levels of the research group. This means that the addition of a combination of Temulawak and Bratawali herbs to the broiler chicken feed mixture has a significant effect on hemoglobin concentration, and a response similar to the administration of antibiotics.

**Hematocrit Value**

The results of measuring the Hematocrit value and its variance analysis are as follows:

Table. 6. Description of the hematocrit value in the treatment group

| Treatment | Test | Total | Average | S. Deviation |
|---|---|---|---|---|
| P1 | 4 | 102,75 | 25,687 | 1,247 |
| P2 | 4 | 94 | 23,50 | 1,224 |
| P3 | 4 | 98 | 24,5 | 2,198 |
| P4 | 4 | 99,5 | 24,875 | 1,973 |
| P5 | 4 | 107,5 | 26,875 | 0,25 |
| P6 | 4 | 101,5 | 25,375 | 1,181 |

Table 7. Analysis of variance of hematocrit values

| Source of variance | DB | JK | KT | Fcount | F table 0,05 | F table 0,01 | Ket |
|---|---|---|---|---|---|---|---|
| Eror | | | | | | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Treatment | 5 | 26,13 | 5,22 | 2,37 | 2,77 | 4,25 | * |
| | 18 | 39,73 | 2,20 | | | | |
| Total | 23 | 65,87 | | | | | |

Description: * = No significant effect
** = Significant effect

The research data obtained the average Hematocrit value of broiler chickens was 23.5- 26.8% (Table 6). This result is in accordance with the range of normal Hematocrit values according to Satyaningtijas et al. (2010) of 22%
- 35%. This means that the Hematocrit value in the research results is still in the normal category. The results of the variance analysis

(Table 7) show that the treatment used did not have a significant effect (P <0.05) on the Hematocrit value. This occurs because the condition of the chicken's digestive tract can work well in receiving phytobiotic supplements, so that it is able to maintain the Hematocrit value in a normal state.

**Leukocyte Differential**

Leukocytes or often called white blood cells are divided into 2 groups, the first is a group of leukocyte cells with granules in their cytoplasm, such as Neutrophils, Eosinophils and Basophils, the second without granules in their cytoplasm is Monocytes and Lymphocytes. Differential leukocyte analysis in this study only concerned Neutrophils, Eosinophils and Lymphocytes, because observations on the number of

Basophils and Lymphocytes were still within normal limits for all treatments.

Table 8. Description of the number of Neutrophils in each treatment

| Treatment | Test | Total | Average | S.Deviation |
|---|---|---|---|---|
| P1 | 4 | 42,48 | 10,62 | 50,42388 |
| P2 | 4 | 39,985 | 9,99625 | 6,54979 |
| P3 | 4 | 48,965 | 12,24125 | 13,41077 |
| P4 | 4 | 37,82 | 9,455 | 12,41957 |
| P5 | 4 | 34,985 | 8,74625 | 3,761873 |
| P6 | 4 | 43,205 | 10,80125 | 24,76684 |

Table 9. Analysis of variance of neutrophil count

| Source of Variance | J.K. | Der.Bebas | Kuad. Tengah | F.count | Mark P | F Tabel |
|---|---|---|---|---|---|---|
| Eror Treatment | 29,36773 | 5 | 5,873545 | **0,31654** | 0,896532 | **2,772853** |
| | 333,9982 | 18 | 18,55545 | | | |
| Total | 363,3659 | 23 | | | | |

The results of descriptive analysis on the number of Neutrophils are as follows; the number of Neutrophils in all treatments is above normal (Table 8). Then the analysis of variance shows the F count value < F table (Table 9), which means that the treatment does not show different results.

Table 10. Description of the number of eosinophils in each treatment.

| Group | Test | Total | Average | S.Deviation |
|---|---|---|---|---|
| P1 | 4 | 42,48 | 10,62 | 50,42388 |
| P2 | 4 | 39,985 | 9,99625 | 6,54979 |
| P3 | 4 | 48,965 | 12,24125 | 13,41077 |
| P4 | 4 | 37,82 | 9,455 | 12,41957 |
| P5 | 4 | 34,985 | 8,74625 | 3,761873 |
| P6 | 4 | 43,205 | 10,80125 | 24,76684 |

Table 11. Analysis of variance in the number of eosinophils.

| Source of variance Eror | JK | Der.Free | Kuad. Tengah | F count. | Mark P | F table |
|---|---|---|---|---|---|---|
| Treatment | 1,619168 | 5 | 0,323834 | 0,359165 | 0,869615 | 2,772853 |
| | 16,22931 | 18 | 0,901628 | | | |
| Total | 17,84847 | 23 | | | | |

Analysis of the number of eosinophils, descriptively showed values above normal (Table 10). Analysis of variance showed no difference by treatment effect (Table 11).

Table 12. Description of the number of lymphocytes in each treatment.

| Group | Test | Total | Average | S.Deviation |
|---|---|---|---|---|
| P1 | 4 | 42,48 | 10,62 | 50,42388 |
| P2 | 4 | 39,985 | 9,99625 | 6,54979 |
| P3 | 4 | 48,965 | 12,24125 | 13,41077 |
| P4 | 4 | 37,82 | 9,455 | 12,41957 |
| P5 | 4 | 34,985 | 8,74625 | 3,761873 |
| P6 | 4 | 43,205 | 10,80125 | 24,76684 |

Table 13. Analysis of variance in the number of lymphocytes.

| Source of variation | JK | Der. Bebas | Kuad Tengah | F. count | Mark P | F Table |
|---|---|---|---|---|---|---|
| Treatment | 10,73609 | 5 | 2,147218 | 0,249524 | 0,934599 | 2,772853 |
| Eror | 154,8944 | 18 | 8,605245 | | | |
| Total | 165,6305 | 23 | | | | |

In terms of Lymphocyte values, descriptively it is still within normal limits (Table 12), and the difference in treatment has no effect (Table 13). The increased number of Neutrophils can be caused by bacterial infections, acute infections, acute hemolysis, excessive exercise and so on. The increased number of Eosinophils can be caused by parasitic infections, allergic reactions and so on.

In our study until the final stage of the study (35 days), all chickens were clinically/physically healthy, no deaths were found in the experimental chickens. It can be concluded that the abnormalities found in the increase in the number of Neutrophils are still "physiological" or better known as physiological Neutrophilia (Weiss et..all. 2022). In neutrophils, if it is a true infection reaction, many young neutrophils (band neutrophils - left shift image) will be found in microscopic assessment, which is a response to the need for increased neutrophil production due to infection, this image was not found in this microscopic observation. The increase in the number of Eosinophils is suspected to be an intrinsic reaction, where an allergic reaction occurs to the herbs or antibiotics given or changes in the digestive conditions so that there is an imbalance in the microbiological environment, then there is an increase in activity in the status of internal parasites, which can trigger an increase in the number of Eosinophils, which are part of the leukocyte cells responsible for responding to parasitic infections.

It can be said that the treatment in this study is chronic for broiler livestock, but if referring to the data on the number of leukocytes which is an indicator of the level of the disease process, which turns out to be still within normal limits, it can be concluded that the supplementation treatment, both herbs and antibiotics, is not detrimental to the physiological condition of broiler chickens.

## 4. CONCLUSION

Supplementation of herbal ingredients combination of temulawak + bratawali and antibiotics in feed did not cause disturbances in hematological status in broiler chickens. In general, all chickens were in healthy condition.

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