

## Profile of Total Leukocytes and Differential Leukocytes in Broilers Using Herbal Feed Additives

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(Submitted: 19-08-2021; accepted: 25-11-2021)

### ABSTRACT

This study aims to determine the effect of herbal plant-based feed additives, namely *Aloe vera* and turmeric flour on the appearance of total leukocytes and leukocyte differential in broilers. The material used for this research is 64 Day old Chick (DOC). The method used a Completely Randomized Design (CRD) with 4 treatments and 4 replicates which contained 4 heads. Total leukocytes profile and leukocyte differential including eosinophils, basophils, heterophils, lymphocytes, and monocytes were observed in this study. The results showed that the combination of *Aloe vera* and turmeric flour had a significant effect ( $p < 0.01$ ) on total leukocytes ( $10.73 \pm 0.43$ ). Another result in this study was that the combination of herbal feed additives had no effect ( $p > 0.05$ ) on eosinophils ( $0.27 \pm 0.25$ ) and basophils ( $0.03 \pm 0.05$ ), however heterophile ( $3.42 \pm 0.45$ ), lymphocytes ( $6.48 \pm 0.16$ ), and monocytes ( $0.54 \pm 0.16$ ) showed a significant effect ( $p < 0.05$ ). The conclusion is that the addition of herbal feed additives from *Aloe vera* flour, turmeric flour, and their combination can improve and enhance immune conditions in terms of the total leukocytes and leukocyte differential profiles in broilers.

**Keywords:** *Aloe vera* flour, turmeric flour, leukocyte profile, antimicrobial

### INTRODUCTION

The animal feed industry, supported by modern production equipment, continues to innovate to create complete feeds and feed additives to accelerate livestock productivity and reduce mortality rates. Feed additives are additional feed ingredients that are given to livestock through mixing in feed (Wahju, 2004). Additional feed in the form of antibiotics as Antibiotic Growth Promoter (AGP) is currently widely produced to be used for broilers. It has the potential to be absorbed in livestock products and indirectly consumers will get antibiotics in low concentrations which can increase resistance to bacteria and chemical residues and can cause allergic effects in humans and livestock (Kompiani, 2009).

This fact indicates that the emergence of sustainable broiler diseases was caused by one of the causes of pathogenic bacteria that are resistant to antibiotics used so that broilers are easily infected with the disease. These resistant diseases can be treated for a while but the disease chain will reappear at a certain time which results in high stress levels which ultimately affect the health of broilers. These problems need to be solved as early as possible by finding alternative additional feed made from natural compounds so that they have a positive effect on improving the number of white blood cell antibodies (leukocytes) in an early indicator to determine the health condition of broiler. According to Purnomo et al. (2016) suggested that one of the methods used to assess the



health status of broiler through hematological assessment of the blood profile to determine the level of broiler immunity, one of which is based on total leukocytes and leukocyte differential. Generally, total leukocytes and differential leukocytes can provide an overview and health status of animals (Sugiharto, 2014). The immunity system cannot be separated from the role of white blood cells (leukocytes) which are highly sensitive to infectious agents. The function of leukocytes is to protect the body against various diseases by way of phagocytes and produce antibodies. Differential leukocytes are a unit of white blood cells that are divided into two groups such as granulocytes consisting of heterosinophils, eosinophils, and basophils.

One of the innovations as an alternative herbal feed additive that does not cause resistance to pathogenic bacteria for broilers and there is no chemical residue in the meat consumed is derived from plants that are widely cultivated in Indonesia, including *Aloe vera* and turmeric (*Curcuma domestica*) which has many benefits for human and animal health. Selection of *Aloe vera* as an additional feed because it has many benefits and contains ingredients that are important for the body. The active substances in *Aloe vera* include saponins and anthraquinones that can stimulate the absorption of feed nutrients and prevent pathogenic bacteria. According to Linda et al. (2015), she stated that in low concentration, saponin can increase the permeability of intestinal mucosal cells. It can increase absorption of nutrients in the intestine. Anthraquinone functions as an anti-bacterial. Turmeric (*Curcuma domestica*) is thought to have pharmacological effects, namely increasing immunity, anti-inflammatory, antimicrobial, antioxidant, antidote, preventing excessive gastric acid secretion, reducing intestinal peristalsis, and disinfecting. Turmeric is used to add a bright or reddish yellow color to egg yolks, if mixed in broilers rations, it can reduce the smell of broiler manure and increase broiler body weight, turmeric essential oil is also antimicrobial (Regar et al., 2014). Revent excessive gastric acid secretion, reduce intestinal peristalsis, and disinfect.

This study was conducted to determine the broiler immunity through the profile of total and differential leukocytes using herbal feed additives from *Aloe vera* flour and turmeric flour.

## MATERIALS AND METHODS

### Materials Design

This research was conducted for 3 months in the Farm Laboratory, Faculty of Animal Husbandry,

University of Islam Malang, Malang District, East Java by using a Completely Randomized Design (CRD). The materials were used 64 DOC (Day Old Chick) broiler strains of Cobb 500 with an average initial weight of 55 g which were kept in metabolic cages (individual cages) at a size of 60 x 50 cm for 35 days. Liter in each cage using rice husk  $\pm 5$  cm. Each unit is equipped with a feed and drink container complete with a light bulb 25 watts for heating and lighting in age of 0 to 7 days.

Adaptive feeding was applied to broilers at age of 0 to 7 days. The application of feed additives in the treatment of *Aloe vera* flour and turmeric flour were given at the age of 8 to 35 days as follows: T<sub>0</sub>: (0% feed additives), T<sub>1</sub>: (1.0% *Aloe vera* Flour), T<sub>2</sub>: (1.5% Turmeric Flour), T<sub>3</sub>: (1.0% *Aloe vera* Flour + 1.5% Turmeric Flour). Each treatment has 4 replications which contain 4 heads. The amount of ration given from the second week was 120 g/head/day, 130 g/head/day and fourth week 150 g/head/day. The ingredient and nutrient content of feed presented on Table 1.

### Preparation of Herbal Feed

Fresh *Aloe vera* is cleaned and the skin is separated from the flesh. Then mashed using a blender to form a liquid (semi-solid). The mashed *Aloe vera* was dried using an oven at a temperature of 60-70°C for 2 days so that it was obtained in dry form.

Turmeric was cleaned from remaining soil attached, peeled and cut into small pieces and thin then dried under the sun. The dried turmeric was ground and sifted into turmeric flour. These materials are obtained and processed at the Materia Medika Laboratory, Batu City, East Java.

Table 1. The Ingredients and Nutrient Contents of Feed

Ingredients	Value (%)	Nutrient Content
Corn	55	Metabolic Energy (kcal/kg)
Soybean meal	27	Crude Protein
Fish meal	9.25	Crude Fiber
Rice bran	7.75	Calcium
Potassium phosphate	0.25	Phosphor
NaCL	0.25	Lysine
Premix*	0.50	Methionine
Total	100	

\* Vitamin premix (per Kg of diet); vitamin A 10.000.000 UI; vitamin D<sub>3</sub> 1.000.000 UI; vitamin E 20 g; vitamin K<sub>3</sub> 1.5 g; vitamin B<sub>1</sub> 2 g; vitamin B<sub>2</sub> 4 g; vitamin B<sub>6</sub> 3 g; vitamin B<sub>12</sub> 15 g; D-calcium 60 g; D-pantotenic Acid 1 g; vinegaric Acid 35 g. Mineral premix (Per kg of diet); Titanium 100 mg; copper 5 g; iodine 400 mg; iron 40 mg; zinc 44 g

## Data Analysis

Data from the samples were tabulated using the Excel program. The data were analyzed using analysis of variant (ANOVA) and Duncan's multiple distance test to determine the difference in treatments.

## RESULTS AND DISCUSSION

### Total Leukocyte

Based on the analysis of variance in the study of the effect of feed additives in the form of aloe vera flour and its combination on total leukocytes, the results are presented in Table 2.

Table 2. Effect of Herbal Feed Additive on Total Leukocytes Profile of Broiler

Treatments	Total Leukocytes ( $\times 10^3$ cells/mm <sup>3</sup> )
Without Feed Additive (T <sub>0</sub> )	17.70 $\pm$ 0.55 <sup>b</sup>
1.0% AVF (T <sub>1</sub> )	18.85 $\pm$ 0.81 <sup>b</sup>
1.5% TF (T <sub>2</sub> )	20.50 $\pm$ 0.37 <sup>c</sup>
1.0% AVF +1.5% TF (T <sub>3</sub> )	10.73 $\pm$ 0.43 <sup>a</sup>

<sup>a,b,c</sup> Means in the same column show a significant difference ( $p < 0.05$ ) between the means; \*Superscript showed significant difference ( $p < 0.05$ )

### Differential Leukocytes

Based on the analysis of variance in the study of the effect of feed additives form combination of *Aloe vera* and turmeric flour on differential leukocytes are presented in Table 3. The results of this study, most of the leukocyte differentials showed significantly different results. This was obtained based on the percentage of heterophils and lymphocytes which gave a significant difference ( $p < 0.01$ ) and the percentage of monocytes gave a significantly different effect ( $p < 0.05$ ). Then the study also gave results that were not significant different ( $p > 0.05$ ) in the percentage of eosinophils and basophils. The number of differential percentages of leukocytes in broilers blood circulation varies greatly, this is due to the effect of all the results of calculating the percentage of types of leukocytes because the calculation is based on

100%. The difference is based on certain factors such as season, gender, age, general condition of the organism, infectious disease.

### Eosinophils

According to Ardelli and Woo (2006) that eosinophils have the main function in secreting the contents of their granules in response to parasitic infections. Eosinophils are *polymorphonuclear-eosinophilic granulocytes* with round granules and are relatively larger than heterophils and are red in color.

The results of research had been carried out on the additional feed additive from *Aloe vera* and turmeric flours showed no significant effect ( $p > 0.05$ ) on eosinophils. The absence of a real difference is suspected because during the study the results of the calculation of the percentage of eosinophils had an average of 0.27-0.77%. Based on Douglas and Jane (2010) stated that the normal value of eosinophils is around 0 – 3%, meaning that the percentage of eosinophils in this study is still in normal conditions even though the value is small. Francis et al. (2002) that the large amounts of saponins found in *Aloe vera* and given for a long time can cause irritation of the digestive tract mucus. Irritation of digestive tract mucus thereby stimulating the formation of increased eosinophils.

### Basophils

Basophils are the most rare granulocytes found in the circulatory system (Metcalf, 2006). The results of research had been carried out on the additional feed additives from *Aloe vera* and turmeric flours showed no significant effect ( $p > 0.05$ ) on basophils. It is because during study, the results of percentage of basophils had an average of 0.03-0.15%. This percentage shows that basophil component in white blood cells is very small. The control treatment up to T<sub>2</sub> has a percentage value that continues to increase from 0.09-0.15% and there is a decrease in T<sub>3</sub> as much as 0.03%.

Table 3. Effect of Herbal Feed Additive on Broiler's Differential Leukocytes Profile

Variable (%)	Treatments			
	Without Feed Additive (T <sub>0</sub> )	1.0% AVF (T <sub>1</sub> )	1.5% TF (T <sub>2</sub> )	1.0% AVF +1.5% TF (T <sub>3</sub> )
Eosinophils	0.45 $\pm$ 0.35	0.71 $\pm$ 0.19	0.77 $\pm$ 0.31	0.27 $\pm$ 0.25
Basophils	0.09 $\pm$ 10	0.09 $\pm$ 0.11	0.15 $\pm$ 0.10	0.03 $\pm$ 0.05
Heterophile*	4.73 $\pm$ 0.52 <sup>b</sup>	5.71 $\pm$ 0.47 <sup>b</sup>	6.87 $\pm$ 0.48 <sup>c</sup>	3.42 $\pm$ 0.45 <sup>a</sup>
Lymphocytes*	11.21 $\pm$ 0.86 <sup>b</sup>	11.35 $\pm$ 0.18 <sup>b</sup>	11.79 $\pm$ 0.88 <sup>b</sup>	6.48 $\pm$ 0.16 <sup>a</sup>
Monocytes*	1.24 $\pm$ 0.22 <sup>b</sup>	1.24 $\pm$ 0.47 <sup>b</sup>	0.92 $\pm$ 0.20 <sup>ab</sup>	0.54 $\pm$ 0.16 <sup>a</sup>

<sup>a,b,c</sup> Means in the same column show a significant difference ( $p < 0.05$ ) between the means \* Superscript showed significant difference ( $p < 0.05$ ). AVF = *Aloe vera* Flour; TF=Turmeric Flour

The higher value of basophils was found in T<sub>2</sub> which is 0.15% within feed additive of turmeric flour as much as 1.5% and the lower percentage is 0.27% in T<sub>3</sub> within combination treatment of feed additive (1% *Aloe vera* and turmeric flour 1.5%). The percentage of basophils in the blood of poultry, it only ranges from 0-5% (Vinkler et al., 2010). According to Nurfaizin et al. (2014) basophils will only be detected in the blood when there are parasites that attack broilers.

### Heterophile

Heterophile is a component of white blood cells that is produced in the spinal cord with a lobulated nucleus. The results of study showed that had been made regarding the addition of feed additives from *Aloe vera* and turmeric flours showed a significant difference ( $p < 0.01$ ) against heterophiles. The difference is significant because heterophile gives an effect that is in accordance with the total number of leukocytes.

Calculation of the average heterophile increased in each treatment. The control treatment (T<sub>0</sub>) had a heterophile percentage of 4.73% and an increase in total leukocytes up to T<sub>2</sub> (TF 1.5%) with an average of 6.87%. Increasing of average number indicates the physiological of broiler in good condition. It is because heterophils act as the first base of defense in the body. Heterophile is thought to be due to animals being under stress conditions or a process of the body's immune response. Age and environmental factors, especially climate change or very extreme environmental weather, are thought to have contributed to the emergence of stress (Widhyari et al., 2009).

The factors that determine the level of heterophile include environmental conditions, stress levels in livestock, genetics and the adequacy of feed nutrients (Lucas & Marcos, 2000). Based on the statement of Moyes and Schute (2008) and Soeharsono et al. (2010) concluded that the physical animal health can be measured by the number of leukocytes produced. When leukocyte rises so that it indicates an increase in the body's defense ability. While the decrease in the number of leukocytes can also be assumed that there is no infection or interference with pathogenic bacteria that attack the body.

### Lymphocytes

Based on Yalcinkaya et al. (2008) stated that lymphocytes are an important element in the immune system, which functions to respond to antigens by forming antibodies. The results of observations that have been made regarding the addition of feed additives from *Aloe vera* and

turmeric flour showed a significant difference ( $p < 0.01$ ) to the percentage of broiler lymphocyte count. The difference is significant because the lymphocyte cells have an effect that is in accordance with the total number of leukocytes. The calculation of the mean lymphocyte increased in each treatment. The control treatment (T<sub>0</sub>) had a lymphocyte percentage of 11.21% and an increase in total leukocytes up to T<sub>2</sub> (TF 1.5%) with an average of 11.79%. The increase in the average number indicates the physiological status of broilers in good condition. Based on Lucas and Marcos (2000) reported that the biggest factors that affect the number of lymphocytes is heat or environmental stress and stress, because heat stress condition reduces weight of lymphoid organ such as thymus and bursa *fabrisius* which has an impact on the decrease in the percentage of lymphocytes.

### Monocytes

Monocytes are large cells consisting of blue-gray to blue cytoplasm that occupies most of the cell contents. The results study showed that the additional feed additives from *Aloe vera* and turmeric flour was showed a significant difference ( $p < 0.05$ ) to the percentage of monocytes. Calculation of the average total leukocytes an increase in each treatment. The control treatment (T<sub>0</sub>) had a lymphocyte count of 1.24% and there was no increase in the number of lymphocytes up to T<sub>1</sub> (AVF 1%). This study also contains results from the T<sub>2</sub> . treatment (TF 1.5%) and T<sub>3</sub> (AVF 1% + TF1.5%) which decreased by an average of 0.92% to 0.52%. However, the lowest result was still in the normal range of total leukocytes in broilers as revealed by (Salam et.al., 2000) that total percentage of leukocytes per mm<sup>3</sup> of broiler blood is ranged from 7.9-24.0%. The control treatment (T<sub>0</sub>) had an average value of total leukocytes which was still in the normal level even though the control treatment did not add treatment feed, it was assumed that the broilers were not infected with disease.

### CONCLUSION

The leukocyte profile was higher in the T<sub>2</sub> treatment (basal diet + 1.5% turmeric flour) compared with control which was indicated by an increase in the total and differential leukocyte counts such as heterophils, lymphocytes and monocytes. This indicate that an increase in broiler immunity. The use of herbal feed additives in the form of a combination of *Aloe vera* and turmeric flours did not show an adverse effect on broiler immunity condition based on the total and differential leukocyte counts.

## CONFLICT OF INTEREST

The authors whose names are listed have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

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