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# The effect of administering synthetic protein signal transducers and activators transcription 5a on total leukocytes, leukocyte differentials and erythrocyte indices on broilers is significant

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## Abstract

Chicken meat itself has a high nutritional content of protein and energy. STAT5a is an important protein in the Janus Kinase-Signal Transducer and Activator of Transcription (JAK-STAT) signal pathway. This is particularly relevant given the growing global market demand for healthy, safe, and antibiotic-free chicken meat. By understanding how STAT5a affects total leukocytes, differential leukocytes, and erythrocyte indices this study is expected to make an important contribution in developing new strategies to improve the health and productivity of broiler chickens. This study was an experimental study using a complete randomized design with The Post-test Only Control Group Design which consisted of five experimental groups (treatments) using broiler chicken as a test animal. The administration of the synthetic protein STAT 5a can increase the total blood leukocytes of broiler chickens, but still within normal limits. The administration of the synthetic protein STAT 5a can increase the heterophyil of broiler chickens, but still within normal limits. The administration of the synthetic protein STAT 5a can increase broiler chicken lymphocytes, but still within normal limits. The administration of the synthetic protein STAT 5a can increase the monocyte of broiler chickens, but still within normal limits. The administration of STAT-5a proteins at doses of 3.5%, 7% and 14% increased the Mean Corpuscular Volume (MCV) value and the dose of 3.5% was optimal. The administration of the 3.5% dose of STAT-5a protein increases the mean corpuscular hemoglobin (MCH) value and is the optimal dose. The administration of the 3.5% dose of STAT-5a protein increases the mean corpuscular hemoglobin concentration (MCHC) value and is the optimal dose.

**Keywords:** Chicken; Erythrocyte; Leukocytes; Healthy; STAT-5a

## 1. Introduction

The broiler chicken industry has grown rapidly in recent decades as a major provider of animal protein for human consumption. Chicken meat itself has a high nutritional content of protein and energy. The need for chicken meat, especially race chicken, in Indonesia itself in 2018 reached 11.5 kg/capita/year (Wibowo et al., 2020). The Central Bureau of Statistics (BPS) noted that the production of broiler chicken in Indonesia was 3.77 million tons in 2022. This

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number increased by 18.20% compared to the previous year's 3.43 million tons. However, the challenges faced in this industry continue to increase, including the increasing need for production efficiency, disease resistance, and the adaptability of chickens to various environmental pressures. The challenge required the development of an innovative new approach to ensure better health, durability, and productivity of poultry. One approach that attracts scientists is the use of synthetic protein molecules, such as Signal Transducers and Activators of Transcription 5a (STAT5a) (Cui et al., 2004).

STAT5a is an important protein in the Janus Kinase-Signal Transducer and Activator of Transcription (JAK-STAT) signal pathway. This pathway is a major molecular mechanism that regulates a variety of biological processes, including cell proliferation, differentiation, hematopoiesis, and immune responses. The activation of STAT5a can affect the expression of genes governing cytokine production and growth factors, such as interleukin-2 (IL-2), interleukin-7 (IL-7), and erythropoietin, which play an important role in the formation and function of leukocytes and erythrocytes. In the context of broiler chickens, the regulation of hematology and immune function by STAT5a opens up opportunities to increase metabolic efficiency and resistance to infection (Ahmad et al., 2019).

Hematology, particularly parameters such as total leukocytes, differential leukocytes, and erythrocyte indices, are important indicators for evaluating poultry health. Total leukocytes represent overall immune activity, while differential leukocytes (heterophiles, lymphocytes, monocytes, eosinophils, and basophils) provide more insight into immunological adaptation to specific stressors or infections. In addition, erythrocyte indices such as Mean Erythrocyte Volume (MCV) and mean Erythrocyte Hemoglobin Concentration (MCHC) can indicate hematopoiesis conditions, oxygen transport capacity, and body response to certain treatments. including the administration of synthetic proteins (Schindler & Plumlee, 2008).

The provision of the signal transducer of the synthetic protein STAT5a is believed to have a significant effect on these hematological parameters. In addition, histological observations on tissues allow visual evaluations to understand how STAT5a affects the immune system and blood circulation at the cellular level. Several previous studies have shown that the modulation of the JAK-STAT pathway in poultry can improve the immune response to vaccination, improve hematopoiesis, and improve resistance to oxidative stress. However, specific research on the impact of synthetic STAT5a giving on broiler chicken hematology parameters is still limited and requires further exploration. (Sundaresan & Pillai, 2016; Ganesan & Noh, 2017).

In addition to its scientific value, this study also has significant practical implications. Improving the quality of poultry health through molecular approaches such as the use of STAT5a can support sustainable livestock productivity. This is particularly relevant given the growing global market demand for healthy, safe, and antibiotic-free chicken meat. By understanding how STAT5a affects total leukocytes, differential leukocytes, and erythrocyte indices this study is expected to make an important contribution in developing new strategies to improve the health and productivity of broiler chickens.

## 2. Material and methods

### 2.1. Research Design

This study was an experimental study using a complete randomized design with The Post-test Only Control Group Design which consisted of five experimental groups (treatments) using broiler chicken as a test animal. Environmental conditions, age, and sex of chickens are homogeneous, and sample treatment is performed randomly. Treatment is from the 14th to the 25th day.

### 2.2. Try Animals

The trial animal used is a day-old broiler chicken, a male Cobb strain derived from PT. Panca Patriot Prima Malang has a healthy condition and has been vaccinated against ND, IB Kill, and IBD, and has never been treated. The number of retries or numbers of animals per group (n) is calculated using Federer's formula (t) as the number of groups. From the formula above, the number of trial animals per group is five. This research requires 25 male broiler chickens.

### 2.3. Samples and Number of Deuteronomies

The samples required in this study were 30 days old broiler chicken blood from each treatment and test totaling 25 samples. The required blood samples are whole blood or complete blood and subsequently a total examination of leukocytes, leukocyte differentials, indices and images of erythrocytes.

### 2.4. The Place and Time of Research

This study was conducted from August to September 2023. Preparation of the synthetic protein STAT 5a is carried out at the Molecular Biology Laboratory of Airlangga University's Faculty of Veterinary Medicine. Animal maintenance is being carried out at the 4th floor of the Lecture Building with Campus B 25 of Airlangga University. The examination of blood samples was carried out at the Faculty of Medicine Faculty of Brawijaya University's Faal Science Laboratory.

### 2.5. Research Materials

The study included Cobb-style male DOC broiler chicken, standard BR1 and BR2 chicken feed, drinking clean water and Vitachick, newspaper and chaff, cotton and alcohol, 10%, aquadest, 0.1%, STAT 5a synthetic protein. The synthetic protein STAT 5a in solution is administered in three treatment groups with doses of 3.5%, 7%, and 14% as much as 0.5 mL per head respectively. These three doses were obtained by mixing 14 mg of STAT 5a synthetic protein powder with 0.1% formic acid, resulting in an 100mL solution, then a level dilution (Appendix 2).

### 2.6. A Research Tool

The tools required in this study were test animal cages, 15 and 40 watt lights, hand sprayers, feed and drink containers, 1mL of spout, hull probes, and cell phone cameras for documentation. The equipment used for blood sampling is a 3mL spout, a 23G needle, and a 3mL EDTA tube. The equipment used for the total leukocyte and differential examination of chicken blood leukocytes is a complete blood test device, a hematology analyzer with 26 types of "Vet Medical Equipment Full Auto Vet Blood Analyzer Device Animal Use YSTE320V".

### 2.7. Klirens Eticle

This study has been conducted an ethical qualification test with an ethical certificate number NO: 1.KEH.113.07.2023. A certificate of ethical conduct is attached to the Attachment.

### 2.8. Triial Animal Preparation

Pet breeding starts from a day to 30 days old chicks. 25 chickens are placed in a 1 m x 1 m x 50 cm postal cage that has been sprayed with disinfectant with 10% benzalkonium chloride using a hand sprayer, with a 40 watt light illumination. On the 10th day, chickens were randomly divided into five groups and moved into a 40 cm x 40 cm x 50 cm battery enclosure with 15 watts of light and one chicken per enclosure. The base of the cage is a newspaper. Chicken is given standard feed in the form of BR1 feed in the starter phase (ages 1 - 21 days) and BR2 feed in the finisher phase (22-35 days) twice a day at 08.00 WIB and 16.00 WIB. Drink clean water and add Vitachick administered adlibitum.

### 2.9. Treatment of Tried Animals

A day-old broiler chicken of the Cobb strain was adapted for 14 days. Chicken is randomly divided into five groups, namely groups P0, P1, P2, P3, and P4. In one group of chickens, each consists of five chickens. Chicken is treated as follows: (P0): Chicken is aquadest, (P1): Chicken is given formic acid 0.1%, (P2): Chicken is given a STAT 5a synthetic protein in the form of a 3.5% solution, (P3): Chicken is given a STAT 5a synthetic protein in the form of a 7% solution, (P4): Chicken is given the synthetic protein STAT 5a in the form of a 14% dose solution. Treatment is given from the 14th to the 25th day, then chicken is rested and harvested on the 30th day. All treatments are administered orally using probes in 0.5 mL per head per day.

### 2.10. Chicken Blood Sample Collection

On the 30th day, a sample of chicken blood was taken from each tail in all treatment groups, namely groups P0 to P4. The chicken blood sampling phase starts with satisfying the chicken for approximately 12 hours. In the next step, before blood sampling, the blood vessel area is sterilized using 70% alcohol-supplied cotton to prevent contamination. Blood samples are taken through the brachial veins of 3 mL each and inserted into a 3 mL EDTA vacutainer tube. An EDTA vacutainer tube containing chicken blood samples is put in a coolbox for storage and complete blood chemistry check is performed through a hematology analyser.

### 2.11. Chicken Blood Profile Check

To determine the total leukocytes, the differential leukocytes, the erythrocyte index and the description of erythrocytes in poultry blood can be done electronically by using a blood cell counting machine (hematology analyser). The working principles of the machine include impedance or electrical resistance and light scattering (light scattering/optical scatter). The principle of impedance is based on the detection and measurement of changes in electrical resistance produced by blood cells as they pass through a flow cell through which light travels. The results of the leukocyte and

erythrocyte count with an analyser are then displayed on the result sheet as WBC (White Blood Cell). The hematology analysis is used to examine complete blood by automatically measuring and calculating blood cells based on the impedance of electricity or light beams to the cells they pass through. The hematology analysis can be used for routine hematological examinations that include the leukocyte cell count and the leukocyte differential count.

### 2.12. Data Analysis

The data of the study results are blood profiles listed in them total leukocytes and differential leukocytes, the data are then statistically analyzed with One Way Analysis of Variance (ANOVA) and then continued with Duncan and Games-Howell's follow-up tests to find out the differences in each treatment group. The data analysis application used is Statistical Product for the Service Solutions (SPSS) 20.

## 3. Results and discussion

### 3.1. Total Broiler Chicken Blood Leukocytes

The total result of the blood leukocyte of broiler chicken given the synthetic protein STAT 5a was further tested by Duncan to determine the difference in each treatment across the group. The standard mean and deviation (Standard Deviation = SD) of total broiler chicken blood leukocytes administered with the synthetic protein STAT 5a can be observed in table 1.

**Table 1** Average total leukocytes ($10^3/mm^3$) and standard deviation of broiler chicken blood administered with STAT 5a protein

| Group (Treatment) | WBC ($10^3/mm^3$) Mean Rank ± SD |
|---|---|
| P0 | $9,22^a$ ± 0,95 |
| P1 | $11,21^a$ ± 1,03 |
| P2 | $22,82^c$ ± 2,57 |
| P3 | $16,75^b$ ± 1,70 |
| P4 | $18,50^b$ ± 0,40 |

Different superscripts (abc) in the same column indicate a real difference ($p<0.05$). P0: chicken is given aquadest; P1: chicken is given formic acid 0.1%; P2: chicken is given STAT 5a synthetic protein 3.5% dose, and P3: chicken is given formic acid 0.15% dose, chicken is given formic acid 14% dose.

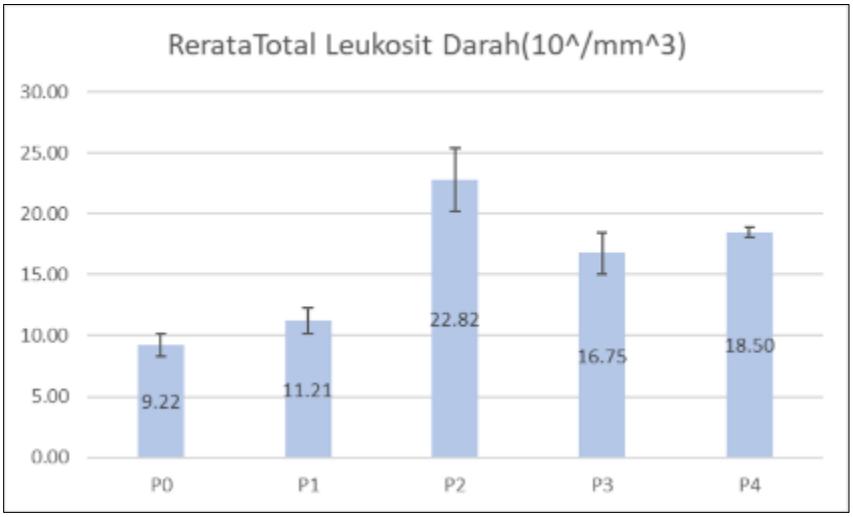


**Figure 1** Graph of the average total leukocyte chicken broiler on each treatment

Duncan's follow-up test of total leukocytes (WBC) of broiler chicken blood showed the P0 group was not real different from the P1 group, but was real different from the P2, P3 and P4 groups. The P2 group shows the real difference with the entire treatment group and shows the highest mean value. The P3 group and the P4 group are not real different but

are real different from the treatment groups P0, P1, and P2. Based on the above description, it can be interpreted that the administration of the synthetic protein STAT 5a can have an effect on the total blood leukocytes of male broiler chickens. The difference in the average dispensing of STAT 5a synthetic proteins in each group is shown in Figure 1.

Figure 1 shows the difference in the total blood leukocytes in each treatment group. The total mean of blood leukocytes in the P0 group was 9.22 ± 0.95 ($10^3$/$mm^3$), the P1 group was 11.21 ± 1.03 ($10^3$/$mm^3$), the P2 group was 22.82 ± 2.57 ($10^3$/$mm^3$), the P3 group was 16.75 ± 1.70 ($10^3$/$mm^3$), and the P4 group was 18.50 ± 0.40 ($10^3$/$mm^3$). The highest averages are shown in the P2 group at 22.82 ± 2.57 ($10^3$/$mm^3$) and the lowest averages at the P0 group at 9.22 ± 0.95 ($10^3$/$mm^3$).

### 3.2. Chicken Broiler Blood Leukocyte Differential

The differential results of chicken broiler blood leukocytes given the synthetic protein STAT 5a were carried out a Games-Howell follow-up test to determine the difference in each treatment across the group. The standard mean and deviation (Standard Deviation = SD) of the differential blood leukocytes of the broiler chicken given the synthetic protein STAT 5a are observed in table 2.

**Table 2** Differential average of leukocytes (%) and standard deviation of broiler chicken blood given the synthetic protein STAT 5a

| Group (Treatment) | Heterophile (%) Mean Rank ± SD | Lymphocytes (%) Mean Rank ± SD | Monocytes (%) Mean Rank ± SD |
|---|---|---|---|
| P0 | $25,73^{a}$ ± 4,26 | $65,36^{c}$ ± 2,71 | $6,44^{b}$ ± 1,21 |
| P1 | $26,63^{a}$ ± 2,46 | $64,04^{c}$ ± 1,88 | $6,97^{b}$ ± 0,88 |
| P2 | $38,49^{c}$ ± 1,64 | $54,47^{a}$ ± 1,35 | $5,19^{a}$ ± 0,63 |
| P3 | $33,25^{b}$ ± 1,92 | $58,25^{b}$ ± 1,28 | $6,19^{ab}$ ± 0,19 |
| P4 | $34,04^{b}$ ± 1,77 | $58,04^{b}$ ± 0,80 | $5,95^{ab}$ ± 0,70 |

Different superscripts (abc) in the same column indicate a real difference ($p<0.05$). P0: chicken is given aquadest; P1: chicken is given formic acid 0.1%; P2: chicken is given STAT 5a synthetic protein 3.5% dose, and P3: chicken is given formic acid 0.15% dose, chicken is given formic acid 14% dose.

The results of the Games–Howell follow-up test show the heterophilic percentage in the P0 group is not real different ($p>0.05$) from the P1 and P3 groups, but is real different ($p<0.05$) from P2 and P4. Furthermore, the group P1 shows no real difference ($p>0.05$) with P0, but real difference ($p<0.05$) with P2, P3, and P4. Next, group P2 shows a real difference with the entire treatment group ($p<0.05$). Then for the group P3 it shows a real difference ($p<0.05$) with the groups P1 and P2, but no real difference ($p>0.05$) with the groups P0 and P4. In the treatment group P4 the real difference ($p<0.05$) with P0, P1, and P2, but not real difference ($p>0.05$) with the group P3. Based on the above description, it can be inferred that the administration of the synthetic protein STAT 5a is influential in heterophilic percentage in white blood cells of broiler chickens.

In lymphocyte percentage, it is seen that the P0 group shows no real difference ($p>0.05$) from P1 but real difference ($p<0.05$) from the P2, P3, and P4 groups. Furthermore, the group P2 shows a real difference ($p<0.05$) with the entire treatment group. Then, for group P3 it shows a real difference ($p<0.05$) with the groups P0, P1, and P2 but not a real difference ($p>0.05$) with the group P4. Based on the above description, it can be inferred that the synthetic protein STAT 5a is influential in the percentage of lymphocytes in the white blood cells of broiler chickens.

In the monocyte percentage the group P0 looks no different in real ($p>0.05$) from the entire treatment group. Furthermore, for group P1 it shows a real difference ($p<0.05$) with group P2 but not a real difference with the groups P0, P3, and P4. Then the P3 and P4 groups look no different in real ($p>0.05$) from the entire treatment group. Based on the above description, it can be inferred that the administration of the STAT 5a synthetic protein at a dose of 3.5% has an effect on the percentage of white blood cell monocytes of broiler chickens. The average of the entire treatment group for the heterophilic percentage of broiler chicken blood given the synthetic protein STAT 5a is shown in Figure 2.

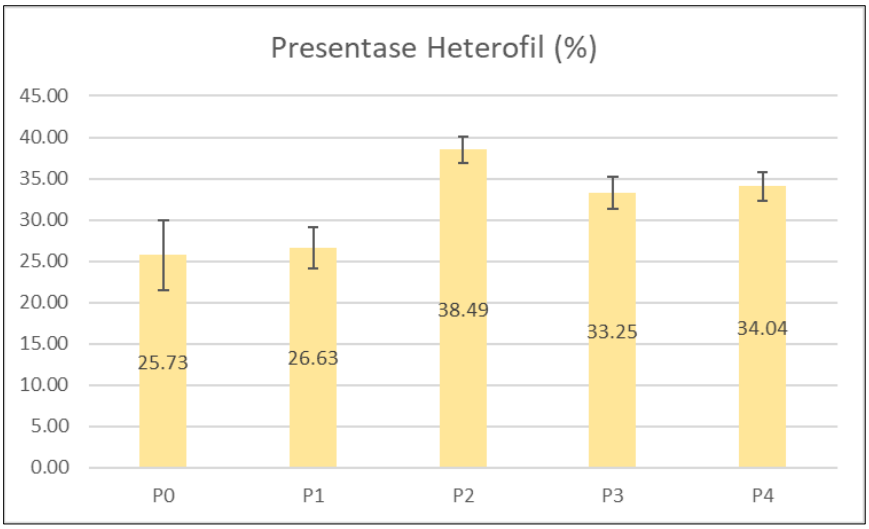


**Figure 2** Average – average heterophilic percentage for each treatment

Figure 2 shows the average difference from the heterophyil percentage of broiler chicken blood on each treatment. In heterophilic percentages group P0 had an average of 25.73 ± 4.26%, group P1 had an average of 26.63 ± 2.46%, group P2 had an average of 38.49 ± 1.64%, group P3 had an average of 33.25 ± 1.92%, and group P4 had an average of 34.04 ± 1.77%. The highest percentage of heterophiles is derived from the treatment group P2 and the lowest heterophile is derived from the treatment group P0. Furthermore, the average of the entire treatment group for the blood lymphocyte percentage of broiler chickens given the synthetic protein STAT 5a is shown in Figure 3.

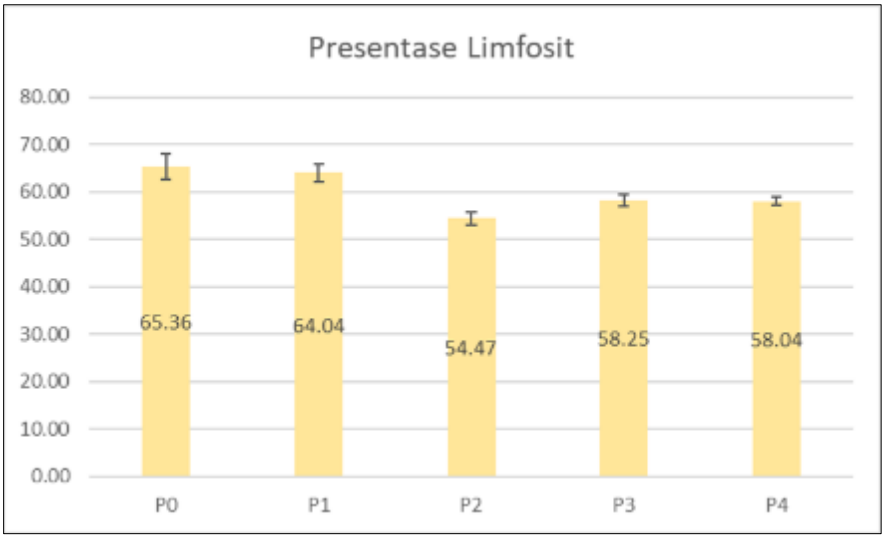


**Figure 3** Average percentage of lymphocytes per treatment

In the percentage of lymphocytes seen in treatment group P0 has an average of 65.36 ± 2.71%, group P1 has an average of 64.04 ± 1.88%, group P2 has an average of 54.47 ± 1.35%, group P3 has an average of 58.25 ± 1.28%, and group P4 has an average of 58.04 ± 0.80%. The highest average percentage of lymphocytes is from the P0 treatment group and then the lowest percentage of lymphocytes is from the P2 treatment group. Furthermore, the average of the entire treatment group for the blood monocyte percentage of broiler chickens given the synthetic protein STAT 5a can be seen in Figure 4.4 of the following.

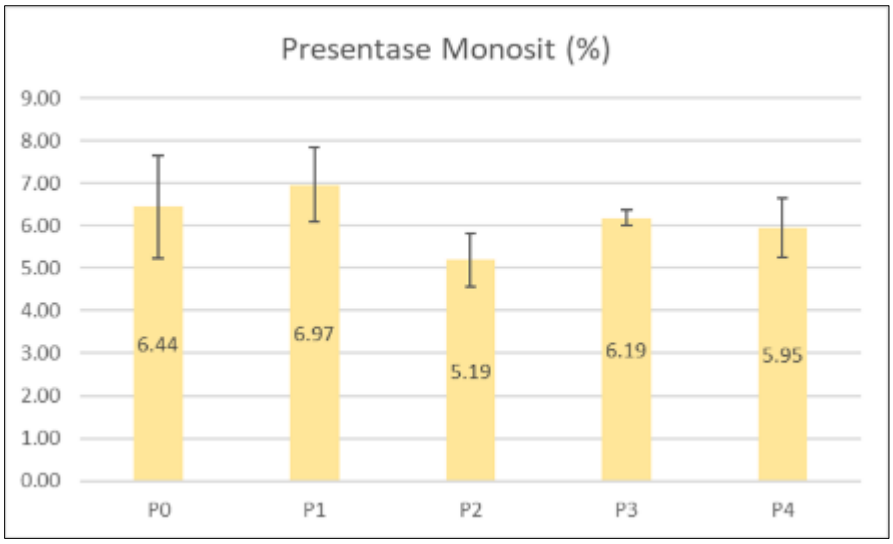


**Figure 4** Average monocyte percentage per treatment

In the percentage of monocytes, the average of the treatment group P0 was 6.44 ± 1.21%, the group P1 had an average of 6.97 ± 0.88%, the group P2 had an average of 5.19 ± 0.63%, the group P3 had an average of 6.19 ± 0.19%, and the group P4 had an average of 5.95 ± 0.70%. The highest mean in monocyte percentage comes from the treatment group P1 and the lowest mean comes from the treatment group P2.

The increase in total leukocytes and differential leukocytes in this study in line with the results of Schuringa et al (2004), the study proved that the activation of STAT 5a could facilitate hematopoietic differentiation. Activation of STAT 5a can result in several CFU-GM colonies that can activate the formation of granulocyte leukocyte cells.

In rats lacking STAT 5a there is a deficiency in hematolymphoid that eventually lowers T cells and B cells significantly, lowers monocyte survival progenitors, and there is a decrease in neutrophil maturity in the bone marrow (Feldman et al., 1997). Rats lacking the STAT 5a gene show a reduced ability for cells to assemble DNA-protein complexes that are responded by GM-CSF. This defect indicates a reduced response to GM-CSF and decreased gene expression (Feldman et al., 1997). In a study by Snow et al (2002) in which mice were exposed to radiation and caused hematopoietic failure, administering STAT 5a in vivo was able to signal antiapoptosis and maintain survival and maintain GM - CSF proliferation. Sustainable activation of STAT 5a is required to maintain strong hematopoietic reserves and contribute to host viability by improving the survival of early progenitor cells (Snow et al., 2002).

The role of STAT 5, which consists of STAT5a and STAT5b, has been heavily involved in various malignant diseases. STAT 5 can aid cell proliferation and melanoma survival by activating the Bcl-XL antiapoptosis protein (Hassel et al., 2008). Activation of the STAT 5 protein also results in good prognosis in breast cancer and nasopharyngeal cancer (Hsiao et al., 2003; Nevalainen et al., 2004). The excessively activated STAT5 signaling is thought to promote tumor growth and epithelial transition to mesenchymes in head and neck squamous cell carcinoma that causes resistance to chemotherapy (Koppikar et al., 2008). Excessive activation of STAT5 proteins also results in poor prognosis in prostate cancer patients (Li et al., 2005).

The expression STAT 5a correlates with infiltration of CD4+ T cells, B cells, macrophages, neutrophils, and dendritic cells. The close relationship between STAT 5a expression levels and infiltration of various types of immune cells further suggests that STAT 5 can be interpreted as an immune regulator in tumors. Expression of STAT 5 can also be a predictor of further immunotherapy treatment (Li et al., 2022). This is in line with this study which shows that the broiler chicken treatment group given the synthetic protein STAT 5a has an increased total leukocyte and differential leukocyte but is still within normal thresholds.

**Table 3** The results of different Superscript erythrocyte indices in the same column show a real difference ($p<0.05$) with different treatments

| Group (Treatment) | Blood MCV level (fL) (Mean ± SD) |
|---|---|
| P0 | $90,09^{a}$ ± 2,71 fl |
| P1 | $90,64^{a}$± 1,79 fl |
| P2 | $109,58^{c}$ ± 4,76 fl |
| P3 | $92,19^{ab}$ ± 2,02 fl |
| P4 | $95,0^{b}$ ± 3,15 fl |

Different superscripts in the same column show a noticeable difference ($p<0.05$); P0; chicken aqueducted; P1: chicken supplied 0.1% formic acid solution; P2: chicken supplied synthetic protein STAT-5a dose 3.5%; P3: chicken given a STAT-5a synthetic protein of 7% dose; P4: chicken given a STAT-5a synthetic protein of 14% dose; administering the treatment orally using a probe with a volume of 0.5 mL 1 per day for 12 days (14th-25th day); repeat: 5.

The provision of aquades to the control treatment group has the lowest value compared to other treatments and the treatment group 1 given formic acid has a result not much different from the control treatment group. Based on the data, formic acid has no significant effect on the MCV level of broiled chicken. The effect of formic acid on the physiology of blood according to Isrol (2020), suggests that formic acid has no significant effect on the blood of broiler chickens, but formic acid has an effect on increased Feed Conversion Ratio (FCR). It maintains microbes in the digestive organs and improves the digestive efficiency of proteins and amino acids in chickens (Raga et al., 2016).

The dose administration of treatment group 2 has the highest value with a dose of STAT-5a synthetic protein of 3.5%. This suggests that this dose has a significant effect on the MCV levels of other treatment groups. An increase in the normal range of Mean Corpuscular Volume (MCV) indicates the optimal distribution of nutrients and oxygen transport and has a positive effect on the physiological conditions of broiler chickens (Lukito et al., 2020). An increasing value means increase in volume size of erythrocytes with sufficient oxygen and nutrient capacity. Subnormal levels of mean corpuscular volume (MCV) can be indicated by chickens having microcytic anemia and vice versa with macrocytic anemia (Parwati et al., 2017). High ambient temperature causes stress and fluid deficiency in the body of broiler chickens can cause abnormal Mean Corpuscular Volume (MCV) volumes (Londok et al., 2018).

**Table 4** Hemoglobin Mean Corpuscular Level (pg; average ±SD) of Stats-5A Synthetic Protein-Powered Beef Chicken

| Group (Treatment) | Blood MCH level (pg) (Mean ± SD) |
|---|---|
| P0 | $28,02^{a}$ ± 1,04 pg |
| P1 | $28,24^{a}$ ± 0,75 pg |
| P2 | $34,49^{b}$ ± 3,77 pg |
| P3 | $28,48^{a}$ ± 1,26 pg |
| P4 | $29,63^{a}$ ± 1,50 pg |

Different superscripts in the same column show a noticeable difference ($p<0.05$); P0; chicken is aquaded; P1: chicken is given a 0.1% formic acid solution; P2: chicken is given a STAT-5a synthetic protein of dose 3.5%; P3: chicken given a STAT-5a synthetic protein of 7% dose; P4: chicken given a STAT-5a synthetic protein of 14% dose; administering the treatment orally using a probe with a volume of 0.5 mL 1 per day for 12 days (14th-25th day); repeat: 5.

Treatment group 2 with 3.5% STAT-5a protein dose has higher values than other treatment groups. This suggests that a 3.5% dose of the STAT-5a protein has a significant effect on the MCH levels of other treatment groups. The provision of aquades to the control group has the lowest MCH value compared to other treatment groups and the treatment group 1 given formic acid has a result not much different from the treatment other than treatment group 2. Based on the results of this assessment, formic acid has no significant effect on the test animals.

Normal mean corpuscular hemoglobin (MCH) values are affected by hematocrit and hemoglobin values because MCH values reflect the hemoglobin content of erythrocytes. This indicates that normal mean corpuscular hemoglobin (MCH) values have sufficient oxygen transported by hemoglobin. Abnormal Mean Corpuscular Hemoglobin (MCH) values can be indicative of problems such as anemia blood disorders, Talasemia, and iron deficiency (Samour, 2015).

**Table 5** Concentration Hemoglobin Mean Corpuscular Level (g/dL; average ± SD) of Stats-5a Synthetic Protein-Powered Rooster

| Group (Treatment) | Blood MCHC level (g/dl) (Mean ± SD) |
|---|---|
| P0 | 31,14[a] ± 1,73 g/dl |
| P1 | 31,16[a] ± 0,91 g/dl |
| P2 | 34,40[b] ± 2,28 g/dl |
| P3 | 30,89[a] ± 1,23 g/dl |
| P4 | 31,24[a] ± 2,19 g/dl |

Different superscripts in the same column show a noticeable difference ($p<0.05$); P0; chicken is aquaded; P1: chicken is given a 0.1% formic acid solution; P2: chicken is given a STAT-5a synthetic protein of dose 3.5%; P3: chicken given a STAT-5a synthetic protein of 7% dose; P4: chicken given a STAT-5a synthetic protein of 14% dose; administering the treatment orally using a probe with a volume of 0.5 mL 1 per day for 12 days (14th-25th day); repeat: 5.

The resultant data of treatment group 2 with a dose of STAT-5a protein of 3.5% had the highest MCHC value compared to other treatment groups, while those of treatment groups 3 and 4 with a higher dose of STAT-5a protein showed the result of decreasing the MCHC value and treatment group 3 had the lowest value. The decreased mean corpuscular Hemoglobin Concentration value at higher dose administration can be caused by incompatibility because excess STAT-5a proteins in the body can inhibit the performance of STAT-5a proteins, resulting in increased apoptosis of erythrocytes and inhibiting progenitor erythroid cells (Socolovsky et al., 2001). The provision of aquades to the control group has an MCH value that is not significantly different from the treatment group 1 with a formic acid content of 0.1%. Based on the results of this research, formic acid has no effect on blood physiology.

A normal mean corpuscular Hemoglobin Concentration (MCHC) value indicates the concentration of sufficient hemoglobin in the erythrocytes of broiled chicken. This affects the metabolic processes of chickens that are closely related to the oxygen content that is related to the concentration of hemoglobin (Londok et al., 2018). Low levels of mean corpuscular hemoglobin Concentration (MCHC) or hypochromia indicate iron deficiency anemia, thalassemia, and lead poisoning. Hyperchromia or above normal mean corpuscular hemoglobin concentration (MCHC) values indicate hepar disorders, dehydration and genetic abnormalities in erythrocytes (Samour, 2015).

## 4. Conclusion

The administration of the synthetic protein STAT 5a can increase the total blood leukocytes of broiler chickens, but still within normal limits. The administration of the synthetic protein STAT 5a can increase the heterophyil of broiler chickens, but still within normal limits. The administration of the synthetic protein STAT 5a can increase broiler chicken lymphocytes, but still within normal limits. The administration of the synthetic protein STAT 5a can increase the monocyte of broiler chickens, but still within normal limits. The administration of STAT-5a proteins at doses of 3.5%, 7% and 14% increased the Mean Corpuscular Volume (MCV) value and the dose of 3.5% was optimal. The administration of the 3.5% dose of STAT-5a protein increases the mean corpuscular hemoglobin (MCH) value and is the optimal dose. The administration of the 3.5% dose of STAT-5a protein increases the mean corpuscular hemoglobin concentration (MCHC) value and is the optimal dose.

## Compliance with ethical standards

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### *Disclosure of conflict of interest*

All authors declare that they have no conflict of interest.

### *Statement of ethical approval*

This study protocol was approved by the Animal Ethical Committee of Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia (Number: 1.KEH.113.07.2023).

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