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The Effectiveness of Sambiloto Leaf (*Andrographis paniculata*) as an Immunostimulant Phytobiotic on Erythrocyte, Hemoglobin, and Hematocrit Levels in Broiler Chickens

Meifa Rahmah Nurlita[1], Nurul Humaidah[2]*
[1]Study Program of Biologi, Faculty of Mathematics and Natural Sciences, Universitas Islam Malang,
[2]Center for Animal Biological Technology Studies, Faculty of Animal Husbandry, Universitas Islam Malang,
Jl. Mayjen Hariyono Jl. Tata Surya No 193 Dinoyo, Kec. Lowokwaru, Kota Malang, 65144, Tel. +62-341-551932, Fax. +62-341-552249, Indonesia.

Corresponding author*
nurul_humaidah@unisma.ac.id

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Abstract

Andrographis paniculata (*Sambiloto*) as an immunostimulant phytobiotic is effective on the erythrocyte, hemoglobin, and hematocrit levels of broiler chickens. The objective of this study was to determine the effectiveness of sambiloto leaves as a phytobiotic on the erythrocyte, hemoglobin, and hematocrit levels of broilers. The materials used were 50 male Day-Old Chickens (DOC) of the MB 202 strain from PT. Japfa Comfeed Indonesia, Comfeed feed, sambiloto leaves, 3 ml syringes, vacutainer tubes containing EDTA anticoagulant, 70% alcohol swabs, and a Hematology Analyzer for examining hematological parameters. The research method was experimental, utilizing a Completely Randomized Design (CRD). There were 5 treatments, with 4 replications for each treatment. The treatments consisted of P0 as a negative control without sambiloto, P1 as a positive control with vitamins but without sambiloto, P2 with 5% sambiloto, P3 with 10% sambiloto, and P4 with 15% sambiloto. The treatments were administered from day 14 for a duration of 21 days. The observed variables were erythrocytes, hemoglobin, and hematocrit. The obtained data were analyzed using Analysis of Variance (ANOVA), followed by Duncan's Multiple Range Test to determine differences between treatments. The results showed that the inclusion of sambiloto powder in the feed had a highly significant effect ($P<0.01$) on broiler erythrocytes and hemoglobin, and a significant effect ($P<0.05$) on hematocrit. The highest average erythrocyte level in broilers was found in the 5% sambiloto treatment, at 12.38 $x10^6/mm^3$. The highest average hemoglobin level was also in the 5% sambiloto treatment, at 17.12 g/dl. The highest average hematocrit percentage was in the 5% sambiloto treatment, at 67.69%. In conclusion, sambiloto leaves are effective as a phytobiotic on erythrocyte, hemoglobin, and hematocrit levels, with the 5% dose of sambiloto providing the best production value.

Keywords: Broiler; Eritrosit; Fitobiotik; Hematokrit; Hemoglobin; Sambiloto.

INTRODUCTION

Sambiloto (*Andrographis paniculata*), is a plant species belonging to the genus Andrographis and has a long history of utilization in traditional medicine. In Indonesia, this herb represents one of the most frequently utilized traditional medicinal resources. This plant possesses significant potential as a biological resource for the biopharmaceutical industry. *A. paniculata* is characterized by its highly bitter taste, which is attributed to its constituent flavonoids, saponins, and tannins. While the roots and stems are rich in flavonoids, the stems and leaves contain substantial amounts of diterpene lactones, alkanes, ketones, and aldehydes (Pranoto, 2024).

A. paniculata is a medicinal plant rich in phytobiotic constituents, particularly the active compound andrographolide. According to Mathivanan & Edwin (2012), flavonoids the primary bioactive compounds in *A. paniculata*, such as quercetin and luteolin have been shown to play a critical role in enhancing the hematological parameters of broiler chickens when administered via dietary supplementation, operating through antioxidant and anti-inflammatory mechanisms. Furthermore Ayunda & Malita (2024) reported that flavonoid, saponin, and tannin compounds mitigate oxidative stress by scavenging free radicals, thereby maintaining red blood cell membrane integrity and promoting hemoglobin and hematocrit synthesis. This process enhances oxygen transport efficiency and prevents anemia. Additionally, flavonoids modulate the immune response by inhibiting the NF-κB signaling pathway, which attenuates chronic inflammation and stimulates the proliferation of leukocytes and platelets, thereby reinforcing the resistance of broiler chickens to infections (Dinata et al., 2025). This mechanism is further supported by the activation of growth factors,

such as erythropoietin, which stimulates hematopoiesis within the bone marrow (Sirat et al., 2022).

Flavonoids present in *A. paniculata*, such as quercetin and kaempferol, exhibit a positive correlation with hematological development in broiler chickens when incorporated into the diet. These bioactive substances improve blood profiles, specifically hematocrit and hemoglobin levels, by suppressing oxidative stress and inflammation (Prihambodo et al., 2020). Atmaja et al. (2023) demonstrated that this mechanism involves antioxidant activity that protects the erythrocyte cell membrane from free radical-induced damage, thus preserving the structural integrity and normal physiological function of red blood cells. Moreover Choy et al. (2019) indicated that flavonoids stimulate erythropoiesis in the bone marrow by enhancing the absorption of iron and vitamin B12. This action promotes the proliferation of erythrocyte progenitor cells through the modulation of signaling pathways such as NF-κB, thereby facilitating the efficient production of healthy erythrocytes. Concurrently, Paremadjangga et al. (2024) documented up to a 10% improvement in hematological profiles in broiler chickens, suggesting that these flavonoids serve as a viable natural alternative to enhance bone marrow performance and sustain normal blood cell counts under stress conditions. To date, research evaluating the efficacy of *A. paniculata* leaves as a phytobiotic agent on the erythrocyte, hemoglobin, and hematocrit levels of broiler chickens at inclusion levels of 5%, 10%, and 15% remains unconducted. Therefore, the objective of this study was to determine the efficacy of *A. paniculata* leaves as a phytobiotic on the erythrocyte, hemoglobin, and hematocrit profiles of broiler chickens.

MATERIALS AND METHODS

Study area

The experimental animals used in this study comprised 50 male day-old chicks (DOCs) of broiler chickens, strain MB 202, sourced from PT. Japfa Comfeed Indonesia. The materials and equipment utilized included commercial Comfeed feed, *Andrographis paniculata* leaves, 3 mL syringes, vacutainer tubes containing ethylenediaminetetraacetic acid (EDTA) anticoagulant, 70% alcohol swabs, and a hematology analyzer for blood profile examination.

Procedures

Broiler Management

Broiler management was conducted in two sequential phases: the adaptation phase and the data collection phase. The adaptation phase lasted for three days to allow the broilers to acclimatize to the environment, particularly the housing and diet. The data collection phase was subsequently carried out over a 21-day period to evaluate the effects of the experimental treatments on the measured variables.

Preparation of Andrographis paniculata Leaf Extract

The preparation of the *A. paniculata* leaf extract was performed via the maceration extraction method for 24 hours using distilled water (aquades) as the solvent. The resulting mixture was filtered and pressed to separate the filtrate from the residue. The residue was then remacerated for an additional 24 hours. Finally, the combined filtrates were concentrated using a rotary evaporator to obtain a crude viscous extract (Mardiana & Handayani, 2016).

Blood Sampling Preparation

The apparatus prepared for blood collection included 3 mL syringes, vacutainer tubes containing ethylenediaminetetraacetic acid (EDTA) anticoagulant, 70% alcohol swabs, and a hematology analyzer. Broiler chickens used as blood samples were selected using a randomized sampling method. Blood collection was performed via the brachial vein (located in the wing region). Prior to venipuncture, the insertion site was sanitized using a cotton swab moistened with 70% alcohol (Afifudin & Widiastuti, 2019).

Blood Collection and Analysis

Blood was drawn using a syringe and immediately transferred into an EDTA-containing vacutainer tube to prevent coagulation. The blood samples were then processed for the evaluation of hematological parameters, including total red blood cell (erythrocyte) count, hemoglobin concentration, and hematocrit percentage. The hematological analysis can be conducted utilizing an automated hematology analyzer or via manual methods, such as a hemocytometer for erythrocyte quantification (Hidayat et al., 2013).

Data analysis

The research method employed was an experimental study utilizing a Completely Randomized Design (CRD). The experiment consisted of five treatments with four replications, and each replication contained five broiler chickens. The experimental treatments were designated as follows: P0 = negative control (without *A. paniculata* supplementation), P1 = positive control (0% *A. paniculata* + vitamins), P2 = 5% *A. paniculata*, P3 = 10% *A. paniculata*, and P4 = 15% *A. paniculata*. The administration of *A. paniculata* leaves was initiated at 14 days of age and continued for a duration of 21 days. Data were analyzed using Analysis of Variance (ANOVA), followed by Duncan's Test to determine significant differences among the treatments. The variables observed in this study were total erythrocyte count, hemoglobin concentration, and hematocrit value in broiler chickens.

RESULTS AND DISCUSSION

The research data showed that the administration of *Andrographis paniculata* leaves had a highly significant effect ($P<0.01$) on hemoglobin and a significant effect ($P<0.05$) on erythrocytes and hematocrit. The average values of erythrocytes, hemoglobin, and hematocrit can be observed in Table 1.

Table 1. Effects of *Andrographis paniculata* on broiler blood profiles.

Variabel	P0 (-)	P1 (+)	P2 (5%)	P3 (10%)	P4 (15%)	Range*
Eritrosit (x10^6/mm^3)	7,69 ± 0,61^{dD}	9,41 ± 1,31^{cC}	12,38 ± 0,38^{aA}	11,23 ± 0,34^{abB}	10,32 ± 34,17^{bcBC}	8,83-11,72
Hemoglobin (g/dl)	16,38 ± 0,41^{aA}	13,82± 0,46^{cC}	17,12 ± 0,26^{aA}	15,54 ± 0,24^{bB}	14,76 ± 1,02^{bcB}	12,7-18,4
Hematokrit (%)	61,76 ± 10,64^{ab}	53,89 ± 3,65^{b}	67,69 ± 0,32^{a}	61,44 ± 0,29^{ab}	56,41 ± 0,27^{bc}	49,60-66,00

Note: ^{abcd} Different superscripts within the same row indicate a significant difference ($P<0.05$) and different superscripts ^{ABCD} within the same row indicate a highly significant difference ($P<0.01$). * Source : Jannah *et al*. (2024)

Discussion

Eritrosit

Based on the ANOVA data, the inclusion of *Andrographis paniculata* leaf powder in the diet exerted a highly significant effect ($P<0.01$) on the total erythrocyte count of broiler chickens. The average erythrocyte count for each treatment group was as follows: P0 (7,69 ± 0,61^{dD} x10^6/mm^3), P1 (9,41 ± 1,31^{cC} x10^6/mm^3), P2 (12,38 ± 0,38^{aA} x10^6/mm^3), P3 (11,23 ± 0,34^{abB} x10^6/mm^3), dan P4 (10,32 ± 34,17^{bcBC} x10^6/mm^3), with the reference normal physiological range for broiler erythrocytes being 8,83-11,72 x10^6/mm^3. These data indicate that in the negative control group (P0), the erythrocyte count did not reach the ideal reference threshold. Conversely, *A. paniculata* supplementation in the P1, P3, and P4 groups successfully maintained the erythrocyte counts within the normal physiological range. Notably, the P2 treatment (5% dose) yielded the highest erythrocyte count, surpassing the standard upper limit for broiler chickens.

The attainment of optimal erythrocyte values in this treatment group demonstrates that *A. paniculata* supplementation actively stimulates the hematological system. This phenomenon is strongly suspected to be triggered by the primary phytochemical components of *A. paniculata*, namely andrographolide and flavonoids, which play a strategic role as both immunostimulants and agents inducing erythropoiesis in the bone marrow. Flavonoids, in particular, function to enhance the production of new red blood cells, while their antioxidant properties protect the integrity of the erythrocyte membrane from lysis caused by free radical exposure. Furthermore, these bioactive compounds provide comprehensive protection to cell membranes against oxidative damage, effectively preventing a premature decline in the number of circulating red blood cells. Consequently, the dual role of *A. paniculata* in both accelerating new cell formation and extending cell lifespan through membrane protection serves as a key factor behind the increased erythrocyte count in broilers throughout the study.

Andrographolide, the dominant diterpenoid lactone compound in *A. paniculata*, triggers lymphocyte proliferation and the production of cytokines such as interleukin-2, which subsequently activates macrophages and natural killer (NK) cells. This process supports the homeostatic production of new erythrocytes (at a rate of 2 million erythrocytes/second) in the bone marrow, while maintaining a balance with the destruction of damaged cells in the liver and spleen. Flavonoids act as potent antioxidants that protect the integrity of these vital organs. These compounds ensure optimal iron storage from hemoglobin within the liver to sustain the erythropoiesis cycle. Consequently, the synthesis of new hemoglobin in the bone marrow proceeds efficiently, thereby preventing a decline in erythrocyte counts. Furthermore, the antimicrobial and anti-inflammatory effects of *S. paniculata* inhibit bacterial infections and ameliorate damage to the liver and spleen, both of which are crucial to the erythrocyte life cycle. This aligns with a study by Sirat *et al*. (2022), which demonstrated that the administration of aqueous *A. paniculata* extract via drinking water successfully maintains erythrocyte counts within normal physiological limits.

Flavonoids play a critical role in erythrocyte formation and function as potent antioxidants that protect the body from the detrimental effects of free radicals, which can otherwise induce erythrocyte damage. Upon absorption, the flavonoids contained in *A. paniculata* enter the bloodstream. Once in circulation, these compounds stimulate the kidneys specifically the plasma globulin cells to synthesize the hormone erythropoietin. Erythropoietin is a glycoprotein hormone present in the blood that acts to stimulate the bone marrow to upregulate red blood cell production, a process known as erythropoiesis. This mechanism is strongly supported by Atmaja *et al*., (2023) who stated that flavonoids are bioactive compounds with antioxidant effects capable of enhancing erythropoiesis in the bone marrow while simultaneously exerting immunostimulatory properties. Furthermore, flavonoids scavenge free radicals to form stable, non-reactive compounds, thereby effectively inhibiting the oxidation process (Artati *et al*., 2024).

Antioxidants play a pivotal role in maintaining erythrocyte membrane stability, thereby preventing cellular damage. According to Mahdalena (2020), the antioxidant activity exhibited by plants can prevent the destruction or lysis of red blood cells. In addition to flavonoids, *A. paniculata* also contains andrographolide as one of its primary compounds. This andrographolide possesses potent antioxidant activity that effectively protects erythrocytes from free radical-induced damage.

Hemoglobin

ANOVA results indicated that the administration of *A. paniculata* leaves had a highly significant effect ($P<0.01$). On the total hemoglobin levels of broiler chickens. The average hemoglobin concentration for each treatment group was as follows: P0 ($16,38 \pm 0,41^{aA}$ g/dl), P1 ($13,82\pm 0,46^{cC}$ g/dl), P2 ($17,12 \pm 0,26^{aA}$ g/dl), P3 ($15,54 \pm 0,24^{bB}$ g/dl), P4 ($14,76 \pm 1,02^{bcB}$ g/dl). The values across all treatment groups remained within the normal physiological range for broiler chicken hemoglobin, which is 7,00-15,1 g/dl. Dietary supplementation with *A. paniculata* leaves resulted in the highest hemoglobin concentration in the P2 group (17,12 ± 0,26 g/dl). This elevated average of total hemoglobin in the P2 treatment is presumably associated with the optimal dosage of sambiloto, which effectively increases flavonoid intake. This finding aligns with Atmaja *et al.* (2023) who demonstrated that the administration of liquid *A. paniculata* extract via drinking water exerted a positive effect on hemoglobin levels. Flavonoids contained in *A. paniculata* play a vital role in elevating hemoglobin concentrations. According to Aini *et al*. (2022), flavonoids and morin can enhance hemoglobin levels by inhibiting the activity of heme enzymes, such as cytochrome and cyclooxygenase. These lipophilic polyphenols can penetrate the erythrocyte plasma membrane and oxidize heme within the cytoplasm, thereby preventing erythrocyte membrane damage (hemolysis). Additionally, polyphenols preserve the heme ion and prevent heme degradation induced by membrane disruption.

Andrographis paniculata also contains vitamin C, which plays a critical role in hemoglobin synthesis. Vitamin C is a water-soluble vitamin capable of providing protection against various diseases. According to Fatmawati *et al*. (2025) vitamin C accelerates the absorption of iron (Fe) from the mucosa of the small intestine, facilitates its transport into the bloodstream, and directs it to the bone marrow for hemoglobin synthesis. Vitamin C is widely known to enhance the absorption of iron, which is essential for hemoglobin production in the bone marrow. Furthermore, Fitria et al. (2023) stated that disruptions in iron transport can lead to a decrease in hemoglobin levels.

Hemoglobin concentration exhibits a direct positive correlation (directly proportional) with the total erythrocyte count; thus, an increase in the total erythrocyte count is followed by a concomitant rise in hemoglobin levels. The P2 treatment demonstrated the highest erythrocyte count compared to the other treatments, thereby resulting in the maximum hemoglobin concentration. This finding is in alignment with who stated that the red blood cell count is positively correlated with hematocrit (PCV) and hemoglobin values. Concurrently, a higher erythrocyte count leads to an increase in both hematocrit and hemoglobin levels, whereas a decrease in erythrocytes results in a subsequent decline in these parameters.

Hematokrit

ANOVA test results indicated that the administration of *A. paniculata* leaves exerted a significant effect ($P<0.05$) on the total hematocrit values of broiler chickens. The mean hematocrit values were P0 ($61,76 \pm 10,64^{ab}$ %), P1 ($53,89 \pm 3,65^{b}$ %), P2 ($67,69 \pm 0,32^{a}$ %), P3 ($61,44 \pm 0,29^{ab}$ %), P4 ($56,41 \pm 0,27^{bc}$ %) for P4, relative to the standard normal hematocrit range for broilers of 22-35%. The dietary supplementation of *A. paniculata* leaves showed that the highest hematocrit level was observed in the P2 group (17,12 ± 0,26 g/dl). This elevated mean hematocrit in the P2 treatment is presumably driven by the presence of flavonoids, andrographolide, and terpenoids in *A. paniculata*, which enhance erythrocyte levels. Vitamin C and iron also contributed to the variations in hematocrit levels. Dani *et al*. (2025) stated that essential elements such as iron, manganese, cobalt, vitamins, amino acids, and hormones are required for hematocrit formation, which critically influences the total erythrocyte count since hematocrit measures the volume proportion of erythrocytes. In the P3 treatment, which received the highest dose of *A. paniculata* compared to the other groups, the flavonoids present in the plant potentially upregulate erythrocytes. This is supported by Kurnia et al. (2022) who noted that flavonoids, acting as active antioxidant compounds, are capable of stimulating erythropoiesis in the bone marrow while simultaneously exerting immunostimulatory effects.

Treatments P1 and P4 exhibited lower hematocrit values compared to P0 and P3, which is presumably attributed to the saponin content in *A. paniculata*. Saponins are known to inhibit iron absorption, as demonstrated by Devi et al. (2024) who reported reduced iron absorption in experimental rats due to saponin exposure. This reduction in iron absorption subsequently leads to lower hematocrit levels, given that iron is an essential element for its formation. Furthermore, according to Paremadjangga et al. (2024) a decrease in hematocrit can also be triggered by erythrocyte destruction, diminished erythrocyte production, and variations in erythrocyte counts and sizes among individual broilers. Hematocrit values increase concurrently with an elevated erythrocyte count, as erythrocytes constitute the primary cellular component in blood that directly influences hematocrit volume. In the

P2 treatment, the highest mean erythrocyte count consequently resulted in the highest hematocrit value. Paremadjangga et al. (2024) stated that hematocrit is highly dependent on the number of erythrocytes, since erythrocytes represent the largest cell mass in the blood. This is corroborated by Dani et al. (2025) who noted that an increase in hematocrit is generally driven by an elevated erythrocyte count in circulation. Therefore, under normal physiological conditions, the relationship between erythrocyte count and hematocrit tends to be positive and directly proportional.

CONCLUSIONS

The leaves of *Andrographis paniculata* are highly effective as a phytobiotic in optimizing erythrocyte counts, hemoglobin levels, and hematocrit percentages. Dietary supplementation with a 5% dose yielded the most superior production values, with erythrocytes at 12,38 ± 0,38 x10^6/mm^3, hemoglobin at 17,12 ± 0,26 g/dl, and hematocrit at 67,69 ± 0,32%. Hal ini menunjukkan bahwa zat aktif *andrographolide* efektif dalam meningkatkan kapasitas angkut oksigen These findings demonstrate that the active compound andrographolide effectively enhances oxygen-carrying capacity while preserving blood cell membrane integrity. In the practical implementation of biological science, these results encourage the standardization of extraction protocols for bioactive compounds to ensure secondary metabolite stability in feed. Concurrently, this reinforces the utility of hematological parameters as accurate bioindicators for non-invasively monitoring livestock health status. The systematic application of *A. paniculata* serves as a viable substitution strategy for Antibiotic Growth Promoters (AGP) to minimize the risk of antimicrobial resistance, ultimately optimizing basal metabolic efficiency and cellular growth rates through a more sustainable feed biotechnology approach.

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Competing Interests: The authors declare that there are no competing interests.

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