



The Effectiveness of Giving Marsh Fleabane (*Pluchea Indica L.*) Water Extract on Broiler Hematology and Blood Glucose

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Abstract | Marsh fleabane leaf is a medicinal plant that can be used as a natural alternative feed to increase the production of poultry such as broilers. This study was conducted to determine the effect of marsh fleabane leaf extract on the blood profile and glucose concentration of broilers. This study used a completely randomized design (CRD). Treatment is carried out as follows: P0: Control / without adding Marsh fleabane leaf water extract in drinking water, P1: Drinking water plus marsh fleabane leaf extract at a dose of 4ml/litre, P2: Drinking water plus Marsh fleabane leaf extract at a dose of 6ml/litre, P3: drinking water added Marsh fleabane leaf extract with a dose of 8ml/litre, P4: Drinking water plus marsh fleabane leaf water extract with a dose of 10ml/litre. Data were analyzed using analysis of variance (ANOVA), if the results were significant, continued with the LSD test. The value is considered significant if $P < 0.05$. Blood collection from the axillary vein of broiler chickens which is put in an EDTA tube. The parameters measured were: the number of leukocytes, erythrocytes, hemoglobin levels, hematocrit, platelets, lymphocytes, monocytes, granulocytes, types of leukocytes and blood glucose levels in broilers. The addition of different of Marsh fleabane leaves water extract in the feed had a significant effect on the levels of Hb, leukocytes and erythrocytes were within the normal range while all blood glucose was in a normal state apart from P4 (decrease), possibly due to the high active compounds that had an effect. The conclusion of this study is that the active compound in *Pluchea indica L.* support the hematology of broiler chickens in the normal range, while blood glucose in p4 has decreased.

Keywords | Broiler, Marsh Fleabane (*Pluchea Indica L.*), Hematology, Type of Leukocyte and Blood Glucose.

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INTRODUCTION

The need for consumption of animal protein (meat) in the community has increased every year from 3.41 g/capita in 2012 and 3.64 g/capita in 2013 (Ditjen Bina Pro-

duksi Peternakan, 2014). The demand for animal protein is currently very increasing. This is indicated by the increasing population of chickens, especially broilers/broilers. Broiler chickens are livestock commodities that have an important role as a source of animal protein (Wulandari et al., 2014). Technology that can increase chicken productivity can

be influenced by the condition of the blood in the body. Blood has an important function in regulating the body's physiology, because blood fulfills about 12% of the body weight of newly hatched chicks and about 6-8% in adult chickens (Bell, 2022). Ardiansyah et al. (2003) added that one strategy to improve broiler production performance can be done by giving plant active compounds. Ashrafi et al. (2022) stated that Marsh Fleabane leaf (*Pluchea indica Less*) is a herbaceous plant of the Asteraceae group which is widely used by Indonesian people as traditional medicinal plants.

According to Isroli et al. (2009), blood plays a role in thermoregulation to adjust the body to environmental conditions. Blood has an important function to regulate environmental influences on the physiology of livestock, including maintaining water balance in the body, buffering systems and as a means of transporting O₂ and CO₂. Ali et al. (2013) stated that the blood profile is a parameter used to indicate the health of the animal's body. Baihaqi et al. (2020) stated that the health status of livestock affects the blood hematology of these animals. Another thing that can indicate the health of the animal's body is glucose levels. Glucose level is a picture of livestock nutrient metabolism in producing quality meat. The picture of glucose in the blood is the end result of carbohydrate metabolism circulating in the blood (Purba et al., 2021). The display of blood glucose is one way to see the condition where the livestock are in good health or under stress. Wasti et al. (2020) when chickens experience stress, they will experience physiological disorders and productivity.

According to Asiamaya (2004), marsh fleabane leaves contain alkaloids, tannins, sodium, essential oils, calcium, flavonoids, magnesium, phosphorus, amino acids (leucine, trintophan, threonine), vitamins A and C. Hanif et al. (2020) added that marsh fleabane leaves extract given into drinking water (10% marsh fleabane leaf extract) affected on feed consumption and body weight gain. According to Sudarman et al. (2011), the flavonoid substance in marsh fleabane leaves can improve chicken performance, namely the digestive tract that can function optimally, as well as maximize the digestive process and absorption of nutrients, especially protein. The active compounds contained in *Pluchea indica* are 0.316% alkaloids, essential oils, 2.351% tannins, and 4.18% flavonoids. According to Lisnanti et al. (2019), the use of additives derived from natural ingredients is expected to produce good performance, final weight and carcass production in broiler chickens. Based on the condition, an effort to increase broiler productivity is by utilizing natural additives, one of them is marsh fleabane leaves. This study uses the water extract method because it is expected to be efficient when applied by the community. Salsabila and Sukei (2022) stated that the advantages of

the water extract method are that it is cheaper and easier to apply in the community compared to the extract method. Those substances function as antibacterial compounds, where these compounds can affect the blood profile of broilers. This study aims to determine the effect of giving Marsh fleabane leaves water extract on broiler hematology and concentration of blood glucose.

MATERIALS AND METHODS

ETHICAL CONSIDERATION

The research was approved by animal care and use committee, universitas brawijaya with no. 013-kep-ub-2022.

STUDY PERIOD AND LOCATION

This research was conducted on December 2021-February 2022 and located in the chicken coop in Sumberejo Village, Kandat District, Kediri Regency and Pathology Laboratory, Veterinary Clinic, Faculty of Veterinary Medicine, Universitas Airlangga, Indonesia.

PLANT WASTE COLLECTION AND WATER EXTRACT

Marsh fleabane leaves are collected from community gardens on Kediri City, East Java Province. The method of making Marsh fleabane leaf water extract is that the Marsh fleabane leaves are washed, withered and added with water in a ratio of 1: 2. The Marsh fleabane leaves and boiled to a temperature of $\pm 70^{\circ}$ C. The resulting liquid is filtered and cooled (Setiaji, 2004).

IN VIVO EXPERIMENT

Pluchea

Giving Marsh fleabane solution starts at the age of the chicken on the 13th day and is slaughtered at the age of 36 days. The type of broiler chicken strain used is Cobb. Giving the extract every day was carried out for 2 hours by giving the mixture to water according to the research dose. Blood collection from the axillary vein of broiler chickens which is put in an EDTA tube and Blood samples were analyzed with Sysmex KX-21 hematology analyzer. The in vivo study of Marsh fleabane (*Pluchea Indica L.*) water extract on Broiler Hematology, Type of Leukocyte and Blood Glucose used 5 treatments and 5 replications, where each unit consisted of 5 broiler chickens.

P0

Control / without adding Marsh fleabane leaves water extract in the drinking water

P1

Drinking water added by Marsh fleabane leaves water extract with 4 ml/litre dosage

P2

Drinking water added by Marsh fleabane leaves water extract with 6 ml/litre dosage

P3

Drinking water added by Marsh fleabane leaves water extract with 8 ml/litre dosage

P4

Drinking water added by Marsh fleabane leaves water extract with 10 ml/litre dosage

STATISTICAL ANALYSIS

The results of in vivo broiler hematology were recorded and analyzed by SPSS 21.0. We performed using analysis of variance ANOVA followed by LSDtest to detect significant differences ($p < 0.05$). The result was expressed as the mean \pm standard deviation.

RESULT AND DISCUSSION

Secondary plant metabolites synthesised by organisms of the plant kingdom and contribute to the defence against infections, pests, stressful conditions, and physical damage. They are abundant in all plant parts and include several compounds, such as alkaloids, flavonoids, tannins, cyanogenic glycosides, and flavanones (Robbins, 2003). Kamboh et al. (2018) stated that phitochemical regulate mucosal and cellular immunity and modulate the endocrine and circulatory markers of health; dietary supplementation with flavonoids can be, therefore, used for improving immunity and health of broiler chickens.

Leukocytes

The result of research showed that the effect of giving different Marsh fleabane leaves water extract gave a significant impact ($P > 0.05$). According to Swenson (1997), the normal leukocytes count ranged from $20-30 \times 10^3/\text{mm}^3$. Leukocytes play an important role in the immune response (Rosmalawati, 2008). Hartoyo et al. (2015) stated that the function of leukocytes is to protect the body from pathogens by phagocytosis and producing antibodies.

It seems that there has been a decrease and an increase in the number of leukocytes, which are still in the normal range. The increase and decrease in leukocytes in the blood is a mechanism for the body's response to invading pathogens. Moyes and Schutle (2008) stated that, high leukocyte values cannot be assumed to be sick/stressed, because the increased number of leukocytes aims to respond quickly against pathogenic agents that cause disease in the body in a humoral and cellular manner.

The active compound of *Pluchea indica* Less leaves caused high number of leukocytes, such as flavonoid. Flavonoids has benefits such as reduce the immunosuppressive leukocytes. Flavonoids has anti-inflammatory and reduce the immobilization of leukocytes. Flavonoids reducing the absorption of Fe (ferrum/ iron) and prevent free radicals

by inhibiting the release of peroxidase. Nijv et al. (2001) stated that the number of leukocytes, the formation of hemoglobin, hematocrit and total erythrocyte affected low Fe. The number of leukocytes which was within the normal range indicates that the active substances contained in the *Pluchea indica* Less leaf extract did not interfere with broilers leukocyte content during the maintenance period. Beehbodi et al (2022) stated that The impact of drinking water supplementation with a mixture of peppermint, coneflower (*Echinacea purpurea*), thyme, propolis, and prebiotics showed a significant increase in Hb, RBC and WBC in broiler chickens compared to controls.

ERYTHROCYTES

The results showed that the application of different Marsh fleabane leaves water extract gave a very significant effect on the erythrocytes of chicken blood samples. The results of the analysis of variance showed that the F test value was greater than the F table ($P < 0.05$). These results indicate that the total chicken erythrocytes are still classified as normal. Mangkoewidjojo and Smith (1988) explained that the normal erythrocyte level in broilers is 2.0 – 3.2 million/ μl . This statement is reinforced by Habibi et al. (2019), the total erythrocytes of broiler chickens are still relatively normal in twigs, the total erythrocyte is 2.26-3.32 $10^6/\text{mm}^3$.

This condition indicates that the process of nutrient metabolism in the body of chickens is normal and the nutrients needed in the formation of red blood cells are sufficient for the needs of chickens, because the high and low levels of erythrocytes indicate the ability to transport oxygen. Red blood cells (erythrocytes) have important functions in the body, namely helping transport nutrients from the digestive tract to tissues, transporting oxygen and removing carbon dioxide, transporting hormones and regulating water content in body tissues (Satyaningtjas et al., 2010).

The number of erythrocytes can differ based on feed, age, maintenance patterns, environmental temperature, altitude, and other climatic factors (Alfian et al., 2017). Togun et al. (2007) explained that if there is a decrease in blood erythrocyte levels, it can be said that the livestock is anemic.

The substance contained in marsh fleabane leaves such as tannins can affect the formation of erythrocytes. Francis et al. (2002) stated that tannins can bind proteins. Tannins causes protein binding and coats the intestinal wall thereby interfering with protein absorption. This can cause a delay in the manufacture of the hormone erythropoietin and reduce the formation of erythrocytes. The process of formation of red blood cells (erythrocytes) occurs in the bone marrow (Altan et al., 2000).

Table 1: The Average of Broiler Leukocytes Level Given Marsh Fleabane Extract with The Different Level

Treatment	Average	Stdev	Notation
P0	27,70	±4,14	A
P1	26,40	±2,93	A
P2	31,62	±0,68	Ab
P3	30,20	±0,94	Bc
P4	30,20	±3	Cd

Description: The real significant difference showed by the different letter in the row and the same column (P<0,05)

Table 2: The Average of Broiler Erythrocytes Level Given Marsh fleabane leaves water extract with The Different Level

Treatment	Average	Stdev	Notation
P0	2,44	±0,17	A
P1	2,62	±0,08	A
P2	2,94	±0,27	C
P3	2,72	±0,15	Ab
P4	2,44	±0	A

Description: The real significant difference showed by the different letter in the row and the same column (P<0,05)

Table 3: The Average of Broiler Hemoglobin Level Given Marsh fleabane leaves water extract with the different level

Treatment	Average	Stdev	Notation
P0	9,74	±0,54	A
P1	10,76	±0,30	A
P2	12,23	±0,67	A
P3	10,82	±0,33	A
P4	10,48	±0	A

Description : The real significant difference showed by the different letter in the row and the same column (P<0,05)

Table 4: Average of Broiler Hematocrit Level Given Marsh fleabane leaves water extract with the different level

Treatment	Average	Stdev	Notation
P0	30,35	±0,26	A
P1	32,73	±0,65	A
P2	34,85	±1,24	A
P3	31,20	±0,61	A
P4	30,10	±0	A

Description: The real significant difference showed by the different letter in the row and the same column (P<0,05)

Table 5: The Average of Broiler Thrombocytes Given Marsh fleabane leaves water extract with The Different Level

Treatment	Average	Stdev	Notation
P0	179,25	±50,11	A
P1	213,25	±8,38	A
P2	296,00	±22,74	A
P3	239,40	±27,27	A
P4	269,00	±40	A

Description : The real significant difference showed by the different letter in the row and the same column (P<0,05)

HEMOGLOBIN

The addition of Marsh fleabane leaves water extract did not have a significant effect on the hemoglobin of broil-

er chickens. The results of the analysis of variance showed that the F test value was smaller than the F table (P > 0.05). According to Samour (2015), normal hemoglobin in chickens ranges from 10.2-15.19 g/dl., according to Sugito

Table 6: Average level of Glucose (mg/Dl) of broilers fed with Marsh fleabane leaves water extract with different levels

Treatment	Average	Stdev	Notation
P0	215,0	±15,22	A
P1	255,2	±25,19	A
P2	295,0	±10,72	Ab
P3	303,2	±8,17	B
P4	261,2	±32	Bc

Description: The real significant difference showed by the different letter in the row and the same column (P<0,05).

Table 7: Average Lymphocyte Percentage of broilers fed with Marsh fleabane leaves water extract with different levels.

Treatment	Average	Stdev	Notation
P0	80,60	±3,97	A
P1	84,00	±3,24	A
P2	82,00	±2,74	A
P3	83,00	±3,81	A
P4	1,00	±4	A

Description : The real significant difference showed by the different letter in the row and the same column (P<0,05).

Table 8: Average percentage of broiler monocytes treated with Marsh fleabane leaves water extract with different levels

Treatment	Average	Stdev	Notation
P0	9,50	±1,29	A
P1	9,40	±1,67	A
P2	10,50	±1,29	A
P3	9,00	±2	A
P4	9,40	±1	A

Description : The real significant difference showed by the different letter in the row and the same column (P<0,05).

Table 9: Average percentage of broiler granulocytes given Marsh fleabane leaves water extract with different levels

Treatment	Average	Stdev	Notation
P0	10,60	±4,51	A
P1	6,60	±2,07	A
P2	8,20	±1,92	A
P3	9,00	±2	A
P4	9,67	±2	A

Description: The real significant difference showed by the different letter in the row and the same column (P<0,05).

et al. (2007) normal hemoglobin levels of broiler chicken is 8.73+0.64 g/100 ml.

Research conducted on blood samples of broiler chickens showed a hemoglobin value of 7.0-13.0 g/dl. The factor for increasing hemoglobin levels in the blood is nutritional adequacy, the higher the hemoglobin level indicates the chicken's blood condition is in good condition and the high hemoglobin level in the blood indicates that the oxygen supply is fulfilled.

Ulupi and Ihwantoro (2014) reported that protein and iron are involved in the formation of hemoglobin. Proteins, especially the amino acid glycine and the mineral Fe, are the

main components of hemoglobin formation (Duka et al., 2015). Habiyah (2015) added that hemoglobin is a complex organic compound consisting of four red porphyrin pigments, each containing an iron atom plus globin, which is a globular protein. sex, feed nutrition, muscle activity, psychological condition, season, air pressure and living habits of the species (Kusumasari et al., 2012). Hemoglobin in the blood functions to bind oxygen into oxyhemoglobin and then circulates it throughout the body to carry out metabolic processes (Rini et al., 2013).

HEMATOCRIT

The test results showed that the effect of adding Marsh fleabane leaves water extract did not have a significant ef-

fect on the hematocrit levels of broiler chickens. The results of the analysis of variance showed that the F test value was smaller than the F table ($P > 0.05$). The hematocrit levels listed in Table 4. are in the abnormal range, according to Kusumawati (2000) the hematocrit value in chickens ranges from 25.0-55.0%. Sugito (2009) stated that the hematocrit average in normal broiler chickens was 29.5+1.8 %. A reduction in the hematocrit value to below the normal range indicates anemia due to iron deficiency, hemolytic reactions, leukemia, cirrhosis, excessive blood loss, and hyperthyroidism (Depkes RI, 2011).

Hematocrit value is influenced by disproportionate changes in the volume of erythrocytes and blood plasma in circulating blood. High environmental temperatures will reduce the hematocrit value due to a lack of erythrocytes. This is in accordance with the statement of Winarsih (2005) that the hematocrit level is highly dependent on the number of erythrocyte cells, because erythrocytes are the largest cell mass in the blood.

Several factors that affect the hematocrit value are the breed and type of livestock, age and production phase, gender, disease, and climate. According to Guyton and Hall (2010), the indicator that causes chickens to become anemic when tested for erythrocyte index is a low hematocrit value under the normal level because the hematocrit value is positively correlated with the number of erythrocytes (Scanes, 2015).

Reduction and addition in hematocrit values out of the normal range in animals can cause changes in body homeostasis. This is due to animals experiencing dehydration, disease, or stress. Mursito (2000) which states that the essential oil contained in marsh fleabane leaf extract can help improve the performance of livestock when experiencing stress, namely by helping to increase the absorption of food substances. Chickens under heat stress try to reduce feed consumption, in an effort to reduce the accumulation of more heat (Cooper & Washburn 1998).

THROMBOCYTES

The results in Table 5. show the blood platelet/ thrombocytes levels. Mitruka and Rawnsley (1997) reported the normal range of platelets in healthy chickens was $60.4 \times 10^6/\text{mm}^3$, while Handayani et al. (2013) stated that chicken platelets amounted to 200,000-500,000/ mm^3 .

The results of statistical analysis showed that the addition of *Marsh fleabane* leaves water extract had a significant effect ($P < 0.05$) on platelet levels. The content of flavonoids in marsh fleabane leaves can increase the number of platelets. Sudaryono (2011) explained that flavonoid compounds can increase platelet levels. According to Handayani et al

(2013), platelet plays a role in inflammation in the repair of body tissues. Platelets in chickens are used for protein lammatory cytokines, and are strongly influenced by various environmental conditions, including: stress, feed, air, and micro-organisms, and are used as system phagocytes.

LYMPHOCYTE PERCENTAGE

The results showed that the effect of giving Marsh fleabane leaves water extract had no significant effect on lymphocyte levels. According to Harahap (2014), the percentage of lymphocytes in poultry blood ranges from 42 to 66%. Lymphocytes are an important element in the immune system, which functions to respond to antigens by forming antibodies (Yalcinkaya et al., 2008). Fathurrahman et al. (2021) stated that the increase of leukocyte cells along with their differential indicates a health improvement through immunity with better immune response.

The increase and decrease in lymphocytes is related to the body's immune response. Most of the lymphocytes are produced in the lymphoid glands, especially the lymph nodes, spleen, thymus, tonsils and various other lymphoid glands scattered throughout the body. The main function of lymphocytes is as an immune system (Hall, 2011). Lymphocytes are responsible for responding to antigens and stress by increasing circulating antibodies in the development of the immune system (Salasia and Hariono 2010). The high number of lymphocytes in the blood may be due to the presence of foreign objects in the form of bacteria, viruses, and parasites that enter the body so that the lymphocytes respond by producing antibodies.

The biggest factors that affect the number of lymphocytes are heat or environmental stress and stress, because heat stress reduces the weight of the lymphoid organs of the thymus and bursa fabrisius which results in a decrease in the number of lymphocytes (Puvadolpirod and Thaxton 2000).

MONOCYTE PERCENTAGE

Based on statistical results, it is known that the application of Marsh fleabane leaves water extract in drinking water of broiler chickens did not have a significant effect ($P > 0.05$) on the percentage of monocytes. This result was higher than the normal number of monocytes in chickens. According to Sismanto (2007), the normal number of monocytes ranges from 3-5% of the number of leukocytes in the blood.

Monocytes are the second line of defense against infection, while a decrease in monocytes below the normal range can be caused by stressed cattle (Harahap, 2014). The content of essential oil in Marsh fleabane leaves can improve the performance of livestock when experiencing stress. Mur-

sito (2000) which states that the essential oil contained in Marsh fleabane leaf extract can help improve the performance of livestock when experiencing stress, namely by helping to increase the absorption of food substances. Monocytes are white blood cells that resemble heterophils which are phagocytic, namely the ability to attack foreign materials, such as bacteria (Frandsen, 1992).

GRANULOCYTE PERCENTAGE

The results showed that the effect of giving Marsh fleabane leaves water extract had no significant effect on the levels of granulocytes. Triastuty (2006) explained that neutrophil granulocytes have the highest number compared to eosinophils and basophils. So the number of granulocytes is influenced by the number of neutrophils produced. Granulocytes are formed in the bone marrow and then released into the bloodstream to the tissues where they are needed. The life span of granulocytes will be shortened if there is a serious infection in the tissue, because the granulocytes will develop more quickly into the infected tissue to work hard to perform their functions and then self-destruct (Hall, 2011).

BLOOD GLUCOSE

The results showed that the effect of giving different Marsh fleabane leaves water extract gave a significant impact ($P > 0.05$). According to Adewole (2021), glucose levels normal blood in broilers is around 197-299 mg/dl. Chicken blood glucose levels in the P4 treatment decreased this could be due to the content contained in the leaves of Marsh fleabane. Yesiana Dwi Wahyu Werdani and Widyawati PS (2018), stated that in the Marsh fleabane plant there are flavonoid compounds thought to play an important role in significantly inhibiting the activity of the α -glucosidase enzyme, thereby reducing blood glucose levels.

Glucose is a group of simple carbohydrate compounds or monosaccharides (Purba et al. 2021). Glucose functions as an energy generator when livestock are under heat stress or stress. Glucose in the blood is formed through the process of digestion, gluconeogenesis, and glycogenolysis. The source of glucose in the blood is glucose as a result of metabolism in the liver (Tan et al., 2010). Vargas et al. (2019) added that the blood glucose level is under persistent tight regulation because of its crucial physiologic aspects, given that too much or too little glucose can cause detrimental effects.

CONCLUSIONS

Based on the results of laboratory tests, the addition of different Marsh fleabane (*Pluchea Indica L.*) leaves water extract in feed had a significant effect on the levels of

leukocytes, erythrocytes and platelets and blood glucose and had no significant effect on the levels of hemoglobin and hematocrit as well as the percentage of lymphocytes, monocytes and granulocytes in broilers.

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CONFLICT OF INTEREST

The authors have declared no conflict of interest.

NOVELTY STATEMENT

This review addresses the role of the effect of giving Marsh fleabane (*Pluchea Indica L.*) leaves water extract on blood hematology, leukocyte type and broiler blood glucose.

AUTHOR'S CONTRIBUTION

EFL and ZAB wrote the manuscript, WPL and EPH as research supervisors and editing the final version of the manuscript. All authors contributed to manuscript revisions, intellectual content, and approved the manuscript for publication.

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