

EFFECTIVENESS OF AVERRHOA BILIMBI L. LEAF EXTRACT ON TOTAL LEUKOCYTES, LYMPHOCYTES, AND MONOCYTES OF FREE-RANGE CHICKEN

(Efektifitas Ekstrak Daun *Averrhoa Bilimbi L.* Terhadap Total Leukosit, Limfosit, dan Monosit Ayam Kampung)

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Abstract

Star fruit leaves contain the active compound tannin. Tannin extracts in star fruit leaves have effectiveness as antibacterials and may also have anthelmintic activity. The utilization of belimbing wuluh leaves as a traditional medicine is beneficial to reduce the use of synthetic antibiotics that have a negative impact on native chickens, which cause resistance and residues for consumers. Blood picture is one of the parameters of animal health status because blood has an important function in the physiological regulation of the body. This study aims to determine the total leukocytes, lymphocytes, and monocytes in *Gallus domesticus* with *Averrhoa bilimbi* leaf extract treatment. The research was conducted from April-November 2023. A total of 200 grams of *Averrhoa bilimbi* leaf powder was dissolved with 1,400 mL aqua Proinjection. Then continued with evaporation to produce concentrated extracts and treatment using concentrations; P1: 25%, P2: 30%, and P3: 35% of the extract. The study with 5 treatments and 5 replicates was administered by daily feeding with a duration of 21 days. Blood samples were examined at the Animal Health Laboratory, Department of Animal Husbandry, Politani Negeri Kupang using the Hematology Analyzer VH-22 Labomec Inc. Data analysis using Analysis of Variance (ANOVA), if there are real differences then proceed with the Duncan Test. It can be concluded that the use of star fruit leaf extract concentrations of 25%, 30%, and 35% increases the total value of leukocytes and monocytes, while the value of lymphocytes is below normal values. The best and most effective is at 25% concentration. It is hoped that further research will be carried out on chickens of the same age and body weight so that the pharmacokinetic effects can be seen.

Keywords: *Averrhoa bilimbi*; leukocytes; lymphocytes; monocytes.

Abstrak

Daun belimbing wuluh memiliki kandungan senyawa aktif tanin. Ekstrak tanin pada daun belimbing wuluh memiliki efektivitas sebagai antibakteri kemungkinan juga mempunyai aktivitas antelmintik. Pemanfaatan daun belimbing wuluh sebagai obat tradisional bermanfaat untuk mengurangi penggunaan antibiotika sintetik yang berdampak negatif terhadap ayam kampung, yaitu menimbulkan resistensi dan residunya bagi konsumen. Gambaran darah merupakan salah satu cara untuk mengukur kesehatan hewan karena darah memainkan peran penting dalam mengatur fisiologis tubuh. Penelitian ini bertujuan untuk mengetahui total leukosit, limfosit, dan monosit pada *Gallus domesticus* dengan perlakuan ekstrak daun *Averrhoa bilimbi*. Penelitian dilaksanakan pada April-November 2023. Sebanyak 200 gram bubuk daun *Averrhoa bilimbi* dilarutkan dengan aqua Proinjection 1.400 mL. Kemudian dilanjutkan dengan evaporasi sampai menghasilkan ekstrak pekat dan perlakuan menggunakan konsentrasi; P1: 25%, P2: 30%, dan P3: 35% dari ekstrak tersebut. Penelitian dengan 5 perlakuan dan 5 ulangan diberikan dengan cara dicekok setiap hari dengan durasi 21 hari. Pemeriksaan sampel darah dilakukan di Laboratorium Kesehatan Hewan, Jurusan Peternakan, Politani Negeri Kupang menggunakan Hematology Analyzer VH-22 Labomec Inc. Data dianalisa dengan Analisis of Varians (ANOVA), bila terdapat beda nyata maka dilanjutkan dengan Uji Duncan. Berdasarkan hasil

penelitian dapat disimpulkan bahwa penggunaan konsentrasi ekstrak daun belimbing wuluh 25%, 30%, dan 35% meningkatkan nilai total leukosit dan monosit, sedangkan nilai limfosit berada dibawah nilai normal. Konsentrasi yang terbaik dan yang paling efektif adalah 25 %. Pada penelitian selanjutnya diharapkan ayam dengan umur dan berat badan yang seragam sehingga secara farmakokinetik sangat berpengaruh terhadap hasil hematologinya.

Kata kunci: *Averrhoa bilimbi*; leukosit; limfosit; monosit

INTRODUCTION

Star fruit contains many chemical compounds that are efficacious for humans and is also expected to be used as medicine in native chickens. Star fruit leaves (also called *Averrhoa bilimbi* or BWAB) contain triterpenoid and flavonoid active compounds known to have antimicrobial, especially antibacterial properties (Wikanta *et al.*, 2011; Alisiya, *et al.*, 2018). According to Hembing (2008), Phenol is another active compound in star fruit that has antibacterial properties. Studies of star fruit leaf extraction using water solvents and chloroform have shown that the extract can stop the growth of Gram positive and Gram negative bacteria, such as *B. cereus*, *S. aureus*, *Citrobacter freundii* and *Aeromonas hydrophila* (Zakaria *et al.*, 2007).

Chicken is a favorite source of animal protein for many people and has high economic potential and value (Tana and Djaelani, 2015). Chicken is one type of poultry that is widely consumed by consumers because the price is relatively cheap compared to other types of poultry. (Nurindah, *et al.*, 2017). Free-range chickens are non-purebred chickens or local chickens in Indonesia (Krista and Harianto, 2013). This type of chicken is widely bred because it is more resistant to the threat of disease, extreme weather and its meat has a rich texture, and it also produces eggs (Yaman and Balikci, 2010). Because of its advantages, its maintenance and consumption need special attention to the food safety, and the quality of the products. According to Sinurat *et al.*, (2009) in raising chickens, many chemicals, antibiotics and hormones are used to stimulate chicken growth and maintain its health. One way to reduce the use of

synthetic antibiotics and ensure safe meat consumption is to use herbs such as lawang flowers which can be use as natural antibiotics.

One way to determine the resistance of native chickens is to check the number of leukocytes in the blood and the leukocyte differential (neutrophils, eosinophils, basophils, lymphocytes, and monocytes). In general, the total number of leukocytes and leukocyte differential can provide an overview of animal health status (Sugiharto, 2016). Blood picture is one of the parameters of animal health, because blood plays an important role in regulating the body's physiology (Age, 2010). Sufficient nutrition improves the defense system of the chicken's body. The function of transport and immunity can be seen in blood variables in the form of erythrocytes and leukocytes and differentiation of blood leukocytes (Isroli *et al.*, 2009).

Leukocytes are blood cells that protect the body from pathogens that invade the body with the help of phagocytes and produce antibodies. Leukocytes consist of lymphocytes, monocytes, basophils, neutrophils/heterophils, and eosinophils. Changes in the number of leukocytes in the bloodstream can be interpreted as the appearance of pathogens, inflammation, autoimmune diseases, or allergic reactions. (Wulandari, *et al.*, 2016).

Leukocytes and their differentiation are indicators that can be used to indicate the health status of animals, including chickens (Sugiharto, 2016). According to Ardana, *et al.*, (2016) each individual animal has a different number of leukocytes, these differences can be caused by several factors including physiological conditions, age, nutritional status. An abnormal number of leukocytes is associated with the health

status of the animal. Therefore, based on the background this study aims to analyze the effect of star fruit leaf extract on native chickens (*Gallus domesticus*) as indicated by the total number, percentage of lymphocytes and monocytes.

RESEARCH METHODS

Sampel

The material used is 25 free-range chickens with various ages (2 – 12 weeks). Chickens were placed in 25 plots cages with a size of 70x70x70 cm and each unit contains 1 chicken. Chickens were given commercial feed (BR2 CP 11) during the treatment. Equipment that are used in this research included cage equipment (drinking and eating places, lamps, sprayers, brooms, buckets) and blood sampling equipment (syringes, cotton, label paper, blood tubes containing EDTA, Styrofoam, and ice flasks).

The research phase started from cage preparation, extraction of star fruit leaf, preparation of free-range chickens. Cage preparation included cleaned and manufactured 25 cage plot units. Then continue with dried of 200 grams of star fruit leaf powder then dissolved it with 1400 mL Proinjection aqua and macerated for 8 days. After that proceed with evaporation using the Rotatory Vacuum Evaporator until a concentrated extract is produced and then make the treatment concentration P1: 25%, P2: 30%, and P3: 35% of the extract.

Methods and Treatment

The study was conducted with 5 treatments and 5 repetition. Treatment given directly by feeding. The treatments are P1: 25%, P2: 30%, P3: 35%, P4: (Positive control) oxytetracycline, and P5: (Negative control) mineral water, administered daily with a duration of 21 days. Data is taken on day 22. 1–1.5 ml of blood sample is taken from the pectoralis vein under the wing using a syringe, then the blood is collected in an EDTA tube, homogenized and stored in a storage area previously filled with ice.

Blood sample examination was carried out at the Animal Health Laboratory, Department of Animal Husbandry, Kupang State Polytechnic using Hematology Analyzer VH-22 Labomec Inc. Data analysis using Analysis of Variance (ANOVA), if there are real differences then proceed with the Duncan Test.

RESULTS AND DISCUSSION

Total number of leukocytes

The results of the examination showed that the administration of star fruit leaf extract to native chickens in vivo showed an increase in the number of leukocytes in the blood. The average leukocyte counts of native chickens ranged from 53.66 – 65.62 x 10³/μL as presented in Table 1. The results of the analysis showed that there was a significant difference (P<0.05) between concentrations of 30%, 35%, and mineral water with a concentration of 25% and oxytetracycline.

According to Saputro, *et al.*, (2014) the normal leukocyte count in native chickens is in the range of 12 - 30 X 10³/μL. The increase in the number of leukocytes may be due to the appearance of an immune reaction to the active ingredients of saponins, flavonoids, and tannins contained in star fruit leaves. In accordance with the statement of Hartoyo *et al.*, (2015) that the role of leukocytes is to protect the body from pathogens through phagocytosis and antibody production. Factors that determine the number of leukocytes are biological activity, stress, environmental conditions, age, nutrition, physiological activity and drugs. According to Moenek, *et al.*, (2019) an increase in total leukocytes indicates that the immune system is under pressure, resulting in an inhibiting effect on the immune system.

Leukocytes are active blood cell units that play a role in the body's defense system against disease and can be used as indicators of health level and health status (Purnomo, *et al.*, 2015). The content of secondary metabolites of star fruit leaves is saponins according to Francis *et al.*, (2002),

has the ability to stimulate immune cells to increase antibody production so that it can act as an immunostimulant. Meanwhile, flavonoids play a role in preventing the development of microorganisms by acting as enzyme inhibitors, so that a decrease in the number of leukocytes due to microorganism attacks can be prevented.

Lymphocytes

The calculation of the percentage of lymphocytes carried out in this study was in the range of 5.22% - 11.19% as presented in Table 2. The percentage of lymphocytes found to be below the normal threshold. Scanes (2015) states that lymphocyte levels in poultry blood range from 45.5% - 57.6%.

This shows that star fruit leaves may be able to ward off free radicals and can suppress infectious agents in the chicken's body. The presence of antioxidants, which can reduce the number of bacteria infecting chickens, can cause a decrease in infectious agents (Maulana, *et al.*, 2019). Lymphocytes usually make up the largest part of leukocytes in the bloodstream. Lymphocytes will respond to bacteria or antigens that enter the body by producing antibodies that circulate in the blood or during the development of the immune system.

Salasia and Hariono (2010) stated that in the process of forming the immune system, lymphocytes increase the circulation of antibodies to respond to the presence of antigens and stress. Purnomo, *et al.*, (2015) report that heat or environmental stress and stress are the two biggest factors affecting lymphocyte count, because heat stress reduces the weight of thymus lymphoid organs and bursa phabrisius, which has an impact on decreasing lymphocyte count. Moenek, *et al.*, (2019) also reports that the presence of antigens in the body (endoparasites) will also affect the value of Lymphocytes.

Monocytes

The calculation of the percentage of monocytes obtained is in the range of 13.16% - 24.04% (Table 2). According to

Eroschenko and Di Fiore, (2013) the normal limit for the number of monocytes in the blood of chickens is 3-10%. The content of saponin and tannin compounds in star fruit leaf extract is suspected to be responsible for the high number of monocytes in this study. According to Zahro and Agustini (2013), the antimicrobial properties of saponins work by disrupting the stability of bacterial cell membranes, which causes bacterial lysis. Pambudi, *et al.*, (2016) reported that tannins act as antimicrobials by inhibiting cell membrane permeability, disrupting the exchange of substances required by bacterial cells, stopping development and causing bacterial cell death. In addition, Jackson, (2013) argues that the increase in monocyte values occurs in several conditions such as acute stress reactions, inflammatory diseases, and chronic infections, especially if many cell impurities must be removed.

Monocytes are one type of white blood cell that develops in the bone marrow. After developing very quickly, these cells move into the bloodstream before entering the tissue through capillary membranes. There, these cells swell until they become enormous in size to become tissue macrophages (Guyton, 2010). Monocytes only survive a few days in the blood, but when they leave the blood vessels and enter the tissues, they can survive for months (Senapati *et al.*, 2015).

One of the factors that causes the results of blood tests to vary is the age of various chickens which affects body weight (the concentration treatment of each tail is the same) so that pharmacokinetically it greatly affects the hematological results.

CONCLUSION AND SUGGESTION

Conclusion

Based on the results of the study, it can be concluded that the use of star fruit leaf extract concentrations of 25%, 30%, and 35% in vivo in free-range chickens increases the total value of leukocytes and monocytes, while the value of lymphocytes is below

normal values. The best and most effective is at 25% concentration. Statistically there is a significant difference ($P < 0.05$) between concentrations of 25% and 35% and 35%.

Suggestion

It is hoped that further research will be carried out on chickens of the same age and body weight so that the pharmacokinetic effects can be seen.

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Table 1. Average leukocyte count ($10^3/\mu\text{L}$) of native chickens given star fruit leaf extract in vivo.

Deuteronomy	Treatment					Up to Normal ($10^3/\mu\text{L}$)
	25% (BWAB)	30% (BWAB)	35% (BWAB)	Oxytetracycline	Air mineral	
1	62,2	56,3	59,6	70,7	69	
2	65	52,4	52,9	52,5	69,3	
3	64,2	59,5	60	61,9	52,2	
4	62,4	54,5	53,3	63,7	69,8	
5	62	51,6	59,8	59,3	60	
Total	315,8	286,3	275,6	328,1	268,8	
Average	63,16 ^a	53,66 ^b	55,12 ^b	65,62 ^a	53,76 ^b	12 - 30

Note : Different lowercase superscripts on the same line indicate significant differences ($P < 0,05$).

Table 2. The average number of lymphocytes, and monocytes in native chickens given star fruit leaf extract wuluh in vivo.

Treatment	Lymphocytes (%)	Monocytes (%)
(BWAB) 25%	4,626 ^a	22,978 ^a
(BWAB) 30%	17,1 ^b	21,948 ^b
(BWAB) 35%	11,19 ^c	21,7 ^c
Oxytetracycline	5,22 ^d	24,04 ^d
Air mineral	6,936 ^e	13,16 ^e

Note : Different lowercase superscripts in the same column indicate significant differences ($P < 0,05$).