The Effect of Combination of Meniran (*Phyllanthus niruri*), Moringa (*Moringa oliefera*) and Surgery (*Curcuma domestica*) Extracts on Total Leukocytes and Differential Leukocyte of Broiler Chicken

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Abstact

This study aims to determine the effect of the combination of *Phyllanthus niruri*, moringa and turmeric extract on total leucocytes and defferential leucocyte of broiler chickens. The study was conducted in March - May 2021. The research used 60 broiler chickens with an initial weight of 41.48 ± 0.99 g. The feed given was commercial feed containing 22% protein. The study used a completely randomized design (CRD) with 4 treatments and 3 replications, each of which consisted of 5 DOCs. The treatments consisted of T0 (100% water), T1 (50% *Phyllanthus niruri* + 25% moringa + 25% turmeric), T2 (25% *Phyllanthus* + 50% Moringa + 25% turmeric), T3 (25% *Phyllanthus* + 25% Moringa + 50% turmeric. The observed variables were total leucocytes and differential leucocytes (lymphocytes and heterophils). The results showed that the combination of Phyllanthus, moringa and turmeric extract increased the number of leucocytes, the percentage of lymphocytes and decreased the percentage of heterophils. but still within normal limits. The results showed that the combination of Phyllanthus extract, moringa and turmeric increased the number of leucocytes, the percentage of lymphocytes and decreased the percentage of heterophils. but still within normal limits.

Keywords : *Phyllanthus niruri*, *Moringa oleifera*, Curcuma domestica extract, leucocyte, differential leucocyte, broiler

Introduction

Health is a factor that determines the success of broiler farming business. Healthy chickens will easily convert feed into meat, because the energy obtained from feed can be fully used for growth. One of the methods used to assess the health status of broiler chickens is through Hematology assessment. In general, total leukocytes and leukocyte differential can provide an overview and health status of animals (Sugiharto, 2014). Leukocytes are cells that play a role in the body's defense system that is very responsive to infectious agents. Leukocytes function to protect the body against various diseases by means of phagocytes and producing antibodies (Sugiharto et al., 2015). Normally, the number of leukocytes in broiler blood is in the range between 6 – 40 × 10^3/ml. Differential leukocytes are a unit of white blood cells consisting of two groups, namely granulocytes consisting of heterocytes, eosinophils, and basophils. The rate of increase and decrease in the number of leukocytes in the circulation describes the responsiveness

of white blood cells in preventing the presence of disease and inflammation genes (Sugiharto et al., 2015)).

The development of the use of traditional medicines, especially from plants to help improve the health status of broilers is quite widespread. Types of plants that can be used as natural feed additives include meniran, moringa and turmeric. Meniran is a herbaceous plant originating from the Phyllanthus genus with the scientific name *Phyllanthus niruri linn*, this plant has antibacterial and antioxidant properties (immunomodulators) and contains flavonoid compounds, tannins, alkaloids, lignin and saponins. Moringa leaves (*Moringa oleifera Lamk*) have many ingredients and various benefits. The results of the study (Rohyani et al.. 2015) showed that Moringa leaves contain secondary metabolic compounds, namely flavonoids, alkaloids, tannins, saponins, and terpenoids. Saponins function as immunostimulants, namely stimulating leukocyte activity to increase immunity (Sari et al., 2014).

Moringa leaves contain antioxidants that can be used as medicine and substitute food for livestock (Talha, 2013). The protein content of Moringa leaves is equivalent to two times higher than the protein found in milk. The crude protein content of Moringa leaves is high enough so that it is good for animal feed or a mixture that makes up a complete feed (Sumadi et al, 2017). Turmeric has the active substance curcuminoids which function to maintain digestive health and anti-virus. Curcuminoids in turmeric are efficacious as antihepatotoxic, enthelmintic, antiedemic, analgesic. The active substance in turmeric also has anti-inflammatory and antioxidant properties (Kusbiantoro and Purwaningrum, 2018). In addition, curcumin can also function as an anti-inflammatory and antioxidant (Suharsanti et al., 2020). Curcumin is also efficacious to kill germs and relieve bloating because the bile walls are stimulated more actively to secrete fat-breaking fluid. The essential oil in turmeric can be useful for reducing strong bowel movements so as to treat diarrhea. In addition, it can also be used to relieve coughs and anticonvulsants. Several studies have proven that meniran leaves, moringa and turmeric have properties in antibodies, especially meniran which contain flavonoids and tannins.

The purpose of this study was to determine the leukocyte count and leukocyte differential in broiler chickens treated with extracts of meniran (*Phyllanthus niruri*), Moringa (*Moringa oleifera*) and turmeric (*Curcuma Domestica*) which were extracted with water.

Materials and methods

The research was carried out for 32 days in the Animal Practice Unit of the Karanganyar Animal Husbandry Academy. The study used 60 broilers aged 5 days which were divided into 4 treatments and 3 replications, each replication consisted of 5 tails. The treatment applied was the administration of a combination of meniran extract, moringa and turmeric as much as 10 ml/liter of drinking water. The following composition : Control 1 (T0) : Chickens were given water without extract, treatment 2 (T1) : Chickens were given drinking water with extracts of meniran, moringa and turmeric in a ratio of 50:25:25%, treatment 3 (T2) : Chickens were given water drinking meniran extract, moringa and turmeric in a ratio of 25:50:25%, and treatment 4 (T3) : Chickens were given drinking water with meniran extract, moringa and turmeric in a

ratio of 25:25:50 %. Parameters observed include: total leukocytes and differential leukocytes (lymphocytes and heterophils). The study used a completely randomized design (CRD). The data collected (day 28 after the vaccine) were analyzed by analysis of variance.

Research procedure

Total leukocytes and leukocyte differential are the results of the calculation of blood samples taken through veins in the wing area (brachial veins) as much as ± 2 ml / head, blood samples are inserted into the EDTA tube with the aim of keeping the blood fresh and then shaking it so it doesn't clot . The total leukocyte count and leukocyte differential were carried out at the Banyumas Animal Health Laboratory. Blood was drawn once a week. The vaccine was carried out at the age of 4 days using the ND IB vaccine with the trademark "Medivac ND Hitchner B1". The dose of vaccine given is 1 drop or 0.03 ml/head by eye drop application.

Results and Discussion

Total Leukocytes

The results of observations of total leukocytes in chickens given meniran, moringa and turmeric extracts at D-0 to D+28 can be seen in Table 1.

Table 1. Total leukocytes H-0 to H-28 (10^3/ml)

Days to	Treatments			
	T0	T1	T2	T3
D-0	13.83	16.70	14.55	15.76
D+7	20.85	19.50	20.38	19.31
D+14	23.58	23.93	19.37	27.50
D+21	16.96	15.70	19.50	20.13
D+28*)	23.43	26.70	32.93	30.08

Note : *) not significant (P<0.05)

Total leukocytes on D-0 ranged from 13.83 – 16.70 × 10^3/ml. Normally, the number of leukocytes in broiler blood is in the range of 6 – 40 × 10^3/ml (Mangkoewidjojo, 1998). The total number of leukocytes in chickens ranges from 12 – 30 × 103 /mm3 (Komalasari, 2014). The total number of leukocytes in young birds is relatively low and will increase in adult birds. (Zinki, (1986) cited by Hamzah et al. (2012).

Data on the average total leukocytes on D+7 to D+21 after the vaccine was between 15.70-27.50 x 103/ml. These data showed an increase in total leukocytes compared to D-0. The increase in the number of leukocytes due to the administration of vaccines and the addition of herbal extracts of meniran turmeric and moringa, due to the presence of antigens that enter the body and can strengthen chicken antibodies. Hartoyo et al., (2015) stated that the factors that affect the number of leukocytes are stress, environment, activity biology, nutrition, age, sex, hormones and ultraviolet light or radiation. Yuniwarti., (2015) added that the function of leukocytes is to protect the body

from pathogens by phagocytosis and producing antibodies. The increase and decrease in leukocytes in the blood is a mechanism for the body's response to invading pathogens (Purnomo et al., 2015). Meniran and Moringa (Suhirman and Winarti, 2010) are immunomodulators that are able to stimulate the immune system, Turmeric contains anti-inflammatory curcuminoids and antioxidants that function as anti-inflammatory and antioxidant (Meiliana and Mukhtar, 2018) so it can be assumed that there are no pathogens or infections that make antibodies increase

The average data of total leukocytes at D+28 increased in each treatment. The lowest increase in total leukocytes occurred at T0 and the highest increase in total leukocytes occurred at T2. However, the results of statistical analysis showed that there was no difference between treatments ($P<0.05$). This shows that chickens given meniran, moringa and turmeric extracts can maintain the total leukocytes of broiler chickens within normal numbers so that the chickens are in a healthy condition and there is no infection or interference with pathogenic bacteria that attack the body. The increase and decrease in leukocytes in the blood is the body's response mechanism to invading pathogens. The high production of leukocytes cannot be assumed that the animal is in a state of illness.

Soeharsono et al., (2010) stated that the physical health of livestock can be measured by the number of leukocytes produced, where an increase in the number of leukocytes indicates an increase in the body's defense capability. While the decrease in the number of leukocytes can also be assumed that there is no infection or interference with pathogenic bacteria that attack the body.

Factors that determine total leukocytes include biological activity, environmental conditions, age and feed. Guyton and Hall (1997) stated that total leukocytes describe the level of health influenced by several internal factors including gender, age, disease and hormones as well as external factors such as environmental conditions, livestock activity, stress and feed provided. Hartoyo et al., (2015) added that factors that affect the number of leukocytes are stress, environment, biological activity, nutrition, age, gender, hormones and ultraviolet light or radiation.

Leukocyte Differential

Deferential leukocytes are a unit of white blood cells consisting of two groups, namely granulocytes consisting of heterophils, eosinophils and basophils and agranulocytes group consisting of lymphocytes and monocytes (Cahyaningsih et al., 2007). Lymphocytes play a role in antibody and cellular immunity so that the chicken's health system can be seen from the increase and decrease in lymphocytes (Yalcinkaya et al., 2008); Yosi and Sandi, 2014). Heterophile is a form of neutrophil in poultry which is the first line of defense against invading pathogenic agents. A rapid increase in the number of heterophils occurs during acute inflammation, while the decrease in heterophils can be caused by a decrease in the number of parasites.

Lymphocytes

The number of lymphocytes before treatment according to the standard. According to Harahap (2014) the percentage of lymphocytes in poultry blood ranges from 42% - 66%. Meanwhile, Guyton and Hall (1997) stated that normally the number of lymphocytes is in the range of 24% – 84%. On D+7 to D+14 there was an increase in

lymphocytes in all treatments and a decrease in D+21. The increase in lymphocytes due to the administration of the vaccine resulted in an increase in the number of lymphocytes due to antigens that entered the body and the possibility of giving meniran extract, moringa and turmeric. Meniran, Moringa and turmeric contain flavonoids that function as boosters of the activity of the immunomodulatory system (Suhirman and Winarti, (2010).

Table 2. Chicken Blood Lymphocyte Count on D-0 – H+28

Days to	T0	T1	T2	T3
		%		
D-0	60.33	56.00	56.66	65.66
D+7	74.00	82.66	79.66	82.00
D+14	77.00	75.33	78.66	69.00
D+21	64.00	63.00	66.66	49.66
D+28*)	74.33	77.00	64.33	68.33

Note : *) not sinificant (P<0.05)

The biggest factors that affect the number of lymphocytes are heat or environmental stress and stress, because heat stress can reduce the weight of the lymphoid organs of the thymus and bursa fabrisius which has an impact on reducing the number of lymphocytes (Puvadolpirod and Thaxton, 2000). Changes in the number of leukocytes in the blood circulation can be interpreted as the emergence of disease agents, inflammation, autoimmune diseases or allergic reactions (Lestari et al., 2013)

On day 28 after the vaccine, the lymphocyte data increased in all treatments. The results of the analysis showed that there was no difference in the number of lymphocytes between treatments (P<0.05). This shows that chickens vaccinated and given meniran extract, moringa and turmeric can maintain broiler chicken lymphocytes in normal numbers so that the chickens are healthy and there is no infection or interference with pathogenic bacteria that attack the body. Factors that affect the number of leukocytes and their differential include environmental conditions, age and nutritional content of feed. Among these factors, nutritional factors (proteins) have a very important role in the process of leukocyte formation because protein is one of the components of blood (Addas et al., 2012; Etim et al., 2014)

Heterophile

Heterophiles have amoeboid activity and phagocytic properties to defend the body against foreign body infections such as viruses and other particles. At D-0 in all treatments, the number of heterophils was above the normal number except for T1 treatment. The percentage of heterophils generally ranges from 17-30% (Park et al., 2014).

At D+7 to D-14 the number of heterophils decreased in all treatments and was below the normal number. The decrease in heterophile indicates that the parasite is not attacking broiler chickens. A rapid increase in the number of heterophils occurs during acute inflammation as a result of the response received by the bone marrow, while the decrease in heterophils can be caused by a decrease in the number of parasites (Cahyaningsih et al., 2007).

Table 3. Chicken Blood Heterophiles On H-0 - H-28

Days to	T0	T1	T2	T3
	%			
D-0	29.66	36.66	29.66	22,66
D+7	18.67	11.33	13.00	16.00
D+14	11.67	12.67	11.00	19,67
D+21	23.33	17.67	16.00	21.00
D+28*)	12.00	13.33	14.33	17.00

Note : *) not significant (P<0.05)

At D+21 there was an increase in the number of heterophils in all treatments. Although there was an increase, the number of heterophils was still within normal numbers. It can be assumed that there are no parasites that attack chicken antibodies so that the heterophils are still in normal numbers. .

Heterophiles at D+28 decreased in all treatments and were below normal values. Factors that affect the percentage of heterophils are genetics, environmental conditions and nutrient adequacy of broiler chicken feed (Pulvadolpirod and Thaxton, (2000). The percentage of heterophils can be low due to a decrease in the migration of heterophils from the bone marrow to the blood circulation. It can be assumed that the percentage of heterophils is low in D+28 indicates that there are no parasites that attack chicken antibodies.

The results of the analysis of the number of heterophils showed no difference between treatments (P<0.05). The percentage of heterophile in broiler chickens given a combination of meniran extract, moringa and turmeric can maintain the number of heterophils so that it does not increase beyond the normal number. A rapid increase in the number of heterophils occurs during inflammation due to acute bacterial infection as a result of the response received by the bone marrow, while the decrease in heterophils can be caused by a decrease in the number of parasites. Baratawidjaja and Rengganis (2012) added that the work system of heterophils is to destroy pathogens through independent exogenous pathways (lysosomes, proteolytic enzymes and cationic proteins) and dependent oxygen. He et al. (2005) and Redmond et al (2011) reported that heterophiles contain antimicrobial substances associated with disease resistance in the body and is influenced by the genetic control of the animal.

Conclusion

Based on the results of the research that has been carried out, it can be concluded that the differences in the composition of the extracts of meniran, moringa and turmeric do not affect the total number of leukocytes, lymphocytes and heterophils. The number of leukocytes, lymphocytes and heterophils was in the normal range until day 28 after the vaccine.

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