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Hematological Status of Broilers with Addition Pegagan (*Centella asiatica* (L.) Urban) on Feed in Maintenance Without Vaccination

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Abstract **Original Research Article**

Pegagan (Centella asiatica), is a potential herbal plant as a supplement ingredient in feed. Because Pegagan contains various bioactive compounds such as triterpenoid saponins, triterpenoid genins, essential oils, flavonoids, phytosterols, and other components such as volatile oils, tannins, amino acids, and carbohydrates. This study aimed to evaluate the effect of giving Pegagan through feed on the hematological status of broilers reared without using vaccines. This study used 100 Day Old Chicks (DOC) which were reared for 35 days in a closed-house cage system. The feed used is commercial feed and Pegagan (*Centella asiatica* (L.) Urban.) powder. Feed was given ad-libitum. This study used a completely randomized design (CRD) which consisted of 5 treatments, namely the concentration of Pegagan powder in the feed (0, 0.5, 1, 1.5, and 2%) with 4 replicates and each replicate consisting of 5 chicks. The variables observed were Haematological Status including erythrocyte quality, hemoglobin, and blood sedimentation rate consisting of 3 parts, namely MCV (Mean Corpuscular Volume, MCHC (Mean Corpuscular Hemoglobin Concentration, and MCH (Mean Corpuscular Hemoglobin)) and Leukocyte Profile including Heterophiles, Eosinophils, Basophils, Lymphocytes, and monocytes were measured using the Automatic Hematology Analyzer method. Results, the addition of Pegagan powder to a treatment level of 2% in broiler feed and data analysis and ANOVA showed a very significant effect on hemoglobin and hematocrit levels but had no significant effect on RBC, MCV, MCH, and MCHC. As well as having a very significant effect on the number of Leukocytes, Monocytes, and Eosinophils but no significant effect on Heterophiles, Basophils, and Lymphocytes. So, administrating Pegagan powder up to 2% in broiler feed can improve hematological status.

Keywords: Broiler, Pegagan, Hemological Status, Mortality.

INTRODUCTION

Broiler chickens have the advantage of fast growth and efficiency in consuming feed to be converted into meat products. But behind this superiority, it also has weaknesses, that is to being susceptible to disease and easily stressed, so the speed of growth is inversely proportional to the endurance one has. This is exacerbated by environmental conditions that have high ambient temperature so that broilers will experience heat stress which will cause a decrease in performance (Ismail *et al*., 2022). To anticipate this, the farmer uses additional Antibiotics Growth Promoters (AGP) to increase the immune system, increasing growth and feed efficiency. This is as reported by Choi *et al*., (2022) that AGP can increase Average Daily Growth (ADG) and reduce broiler feed conversion ratio (FCR) in the starter to grower phases.

Good feed, prepared and formulated with ideal feed nutrition must contain sources of energy, protein, fat, minerals, and vitamins so that they can be used for living, production, and maintaining health. Furthermore, considering that broilers have fast growth with a relatively short rearing time (about 35 days), given of natural feed additives are needed in feeding to support this growth while being able to maintain health (Ismail *et al*., 2022), even though it is commonly done with vaccination (Ali *et al*., 2019).

An alternative solution to using safe feed additives is to use herbal plants (Ayalew *et al*., 2022; Ismail *et al*., 2022). This group of plants has been empirically commonly used by humans from the past time to now, as ingredients to maintain health, increase the immune system, increase feed digestibility, and even cure diseases and others. Because in these plants, it is proven to contain bioactive ingredients as

secondary compounds which are deposited in leaves, stems, roots, seeds, fruits, and flowers. Herbal plants contain phenolic group compounds (Ismail *et al.*, 2021a). This compound can function as an antioxidant (Ismail *et al*., 2021b) and can also be used to boost immunity (Ismail *et al*., 2022). On this basis, it is necessary to explore and carry out more and varied research related to the use of this herbal plant as an alternative to animal feed additives, both fresh and processed. In this case, Pegagan (*Centella asiatica* (L.) Urban.) was chosen as the research material.

Pegagan (Centella asiatica (L.) Urban.) is an annual herbaceous plant that grows in the tropics and flowers all year round. The part of the plant that is commonly used is the leaf. Centella Asiatica contains various active ingredients that can be utilized by the body to boost the immune system and increase appetite (Sutardi, 2016). Besides that Pegagan extract is also able to overcome heat stress, anti-forgetting, adaptogenic, antipyretic, antispasmodic, aphrodisiac, astringent, blood purifier due to metal poisoning, diuretic, nervine, and sedative.

The active compound of Pegagan includes triterpenoid saponins, triterpenoid genin, essential oils, flavonoids, phytosterols, and other components such as volatile oils, tannins, amino acids, and carbohydrates. It also contains salts including potassium, magnesium, calcium, iron, phosphorus, pectin, amino acids, B vitamins, and Bellarine substances. Where these materials are needed in the body's biochemical processes when forming enzymes and hormones which in turn increase appetite (palatability), improve metabolism, and can encourage the formation of body conformation. Therefore, it is necessary to evaluate the effect of giving Pegagan through feed on the hematological status of broilers reared without using vaccines.

MATERIAL AND METHODS

Material

This study used 100 Day Old Chicks (DOC) which were reared for 35 days in a closed-house cage system. The feed used is commercial feed and Pegagan (*Centella asiatica* (L.) Urban.) powder. Feed was given ad-libitum.

Methods

This study used a completely randomized design (CRD) which consisted of 5 treatments, namely the concentration of Pegagan powder in the feed (0, 0.5, 1, 1.5, and 2%) with 4 replicates and each replicate consisting of 5 chicks. The variables observed were Haematological Status including erythrocyte quality, hemoglobin, and blood sedimentation rate consisting of 3 parts, namely MCV (Mean Corpuscular Volume, MCHC (Mean Corpuscular Hemoglobin Concentration, and MCH (Mean Corpuscular Hemoglobin)) and Leukocyte Profile including Heterophiles, Eosinophils, Basophils, Lymphocytes, and monocytes were measured using the Automatic Hematology Analyzer method. Blood samples were taken using a 5 ml syringe through the pectoral vein as much as 3 ml, then put into the EDTA tube complete with anticoagulant Blood samples were taken proportionally in 2 maintenance phases, namely the starter and finisher respectively in 20 randomly selected samples.

Data Analysis

All the data were analyzed by one-way ANOVA of completely randomized design (CRD) using SPSS software version 21.0 (SPSS, 2012).

RESULTS AND DISCUSSION

Haematological Status

Hematological Status observed based on erythrocyte quality, hemoglobin, and blood sedimentation rate consisting of 3 parts, namely MCV (Mean Corpuscular Volume, MCHC (Mean Corpuscular Hemoglobin Concentration and MCH (Mean Corpuscular Hemoglobin)).

Erythrocyte quality

The results of research on the use of Pegagan powder on the number of erythrocytes are presented in Table 1, as follows:

Table 1: The average erythrocyte value

Erythrocyte Status	Treatments				
	0 %	**0,5%**	**1,0%**	**1,5%**	**2,0%**
Hemoglobin (g/dL)	4,74	4,68	5,28	5,55	5,50
RBC (10^6/µL)	2,51	2,71	2,75	2,94	2,81
Hematocrit (%)	34,75	38,50	43,25	47,50	48,25
MCV (10^{-15}L)	138,16	142,49	157,60	162,59	174,05
MCH (g/dL)	18,88	17,24	19,24	19,08	20,04
MCHC (g/dL)	13,92	12,27	12,22	11,71	11,43

Note: treatment was the administration of Pegagan powder with 5 different levels (0, 0.5, 1, 1.5, and 2%). RBC: Red Blood Cell. MCV: Mean Corpuscular Volume. MCH: Mean Corpuscular Hemoglobin. MCHC: Mean Corpuscular Hemoglobin Concentration).

Based on the results of these data indicate that the addition of Pegagan powder can increase erythrocyte status. This is by the opinion of Ahmed *et al*., (2020) blood hemoglobin levels are determined by body activity, the higher the body's activity the higher the hemoglobin level. Protein contained in feed influences the formation of hemoglobin, because protein is an important component in the formation of blood cells. Wulandari *et al*., (2014) explained that protein is a component that is closely related to blood formation. It is said so because protein intake is very important for the process of forming hemoglobin which has the form of amino acids.

According to Ulupi and Ihwantoro (2014), MCV which tends to decrease can be affected by a decrease in hematocrit and hemoglobin weight due to heat stress. Physiologically heat-stressed chickens will increase drinking water consumption and decrease feed consumption as a mechanism for heat dissipation. As a result, the percentage of blood hematocrit decreases which will also be followed by a decrease in the MCV value. MCV is the average size of erythrocytes which is divided into macrocytic (higher than normal erythrocytes), microcytic (lower than normal erythrocytes), and normocytic (not too big and not too small erythrocytes). To find out the effect of using Pegagan powder on the number of erythrocytes can be seen in Table 2.

Table 2: Recapitulation of the results of the analysis of variance in the number of erythrocytes

Erythrocytes Status	F-Value	F-Table	
		0,05	0,01
Hemoglobin	6**	3,06	4,89
RBC	1,28	3,06	4,89
Hematocrit	9,79**	3,06	4,89
MCV	2,51	3,06	4,89
MCH	0,76	3,06	4,89
MCHC	1,84	3,06	4,89

Note: ** mean very significant difference (P<0.01)

The results of the analysis of variance on the status of erythrocytes in the table above showed that administration of Pegagan powder up to 2% showed a very significant effect (P<0.01) on Hemoglobin and Hematocrit, but had no significant effect on the RBC, MCV, MCH, and MCHC variables.

Hematocrit is an indication of the proportion of cells and fluids in the blood. A low hematocrit can indicate several disorders including anemia, hemorrhage, damage to the spinal cord, damage to red blood cells, malnutrition, myeloma, rheumatoid, and arthritis. A high hematocrit value, on the other hand, would indicate dehydration, erythrocytosis, and venous polycythemia. In addition, hematocrit is also associated with changes in blood pressure. (Wahyuni, 2019). Virden *et al*., (2007) stated that the normal hematocrit value of broilers is in the range of 24% - 43%.

The percentage of hematocrit values in the blood is also affected by the presence of erythrocyte damage. According to Wardhana *et al*., (2001), a high or low percentage of hematocrit can be affected by erythrocytosis (erythrocyte damage). The factors that affect the hematocrit value include age, gender, nutritional status, hypoxic state, number, and size of erythrocytes. According to Samour (2015), the normal MCH value for broilers is around 32.0-43.9 pg. According to Rais *et al*., (2017), MCH is the average amount of hemoglobin in the blood, which is calculated by dividing hemoglobin by erythrocytes, so that these two components will affect the resulting MCH value. Heat stress in broilers will cause a decrease in the resulting MCH value. This is the opinion of Ulupi and Ihwantoro (2017) who states that a decrease in hemoglobin weight (MCH) and a decrease in hematocrit due to heat stress will cause the MCV to also decrease. The MCH value is directly proportional to the number of erythrocytes, meaning that large (macrocytic) erythrocytes usually have a high MCH value and vice versa.

MCHC (Mean Corpuscular Hemoglobin Concentration) is the average hemoglobin concentration for red blood cells. The MCHC value is calculated by dividing hemoglobin by the mass of red blood cells (Hematocrit) so that the results are obtained in units (%) or grams/deciliter (g/dL). Samour (2015) explained that normal values are in the range of 30.2 – 36.2 g/dL. The MCHC value can be affected by hematocrit and hemoglobin and the factors that affect these two components. The absence of a significant difference in the MCHC value of broiler chickens indicates that the chickens are kept in healthy conditions and there is no disturbance in the red blood profile of the chickens.

Leukocyte Status

White blood cells are cells of several blood components that act as the body's defense by providing an immune response, destroying antigens, and forming antibodies. Sulaiman et al (2017) explained that the increased total leukocytes indicated that there was an increase in immunity against antigens that were in the

livestock body. The results of the study using Pegagan powder on leukocyte status are presented in Table 3.

Table 3: Leukocyte Status Mean Data

Leukocyte Status	Treatments				
	0 %	**0,5%**	**1,0%**	**1,5%**	**2,0%**
Leukocyte Total (x 10^3)	12,38	12,53	14,78	17,03	22,75
Heterophile	2,15	2,38	2,00	1,80	1,63
Eosinophils	4,3	0,00	0,00	0,00	0,00
Basophils	0,4	0	0	0	0
Lymphocytes	43,1	46,9	43,78	44,15	44,08
Monocytes	2,25	1,73	1,50	1,50	1,53

Note: treatment was the administration of Pegagan powder with 5 different levels (0, 0.5, 1, 1.5, and 2%). RBC: Red Blood Cell. MCV: Mean Corpuscular Volume. MCH: Mean Corpuscular Hemoglobin. MCHC: Mean Corpuscular Hemoglobin Concentration).

The percentage of normal leukocytes in broilers ranges from 12 – 30 × 10^3/µl (Jannah *et al.*, 2017). The number of leukocytes is a parameter to determine the health status of livestock. To determine the effect of the addition of Pegagan powder on the number of broiler leukocytes, an analysis of variance was performed which is presented in the following Table 4:

Table 4: Analysis of Variance of Leukocyte Status

Leukocyte Status	F-Value	F-Table	
		0,05	**0,01**
Leukocyte Total	19,69 **	3,06	4,89
Heterophile	**1,17**	3,06	4,89
Eosinophils	**3,02 ***	3,06	4,89
Basophils	**1,02**	3,06	4,89
Lymphocytes	**0,50**	3,06	4,89
Monocytes	**7,0 ****	3,06	4,89

Note: ** mean very significant difference (P<0.01)

The results of the analysis of variance on leukocyte status presented in the table showed that the feed given Pegagan powder had a very significant effect (P<0.01) on the number of leukocytes and the Monocyte component and had a significant effect on Eosinophils but had no significant effect on the heterophil, basophil, and lymphocyte components.

This means that the addition of Pegagan powder to the number of leukocytes in all treatments was an increasing condition. Described by Purnomo *et al*., (2015) states that the number of leukocytes produced can determine the health condition of livestock, whereas an increased number of leukocytes indicates an increase in the body's defenses. These conditions indicate that the addition of Pegagan powder also contributes to its function of increasing the immune system of broilers. Several factors affect the number of leukocytes, namely feed and the percentage of addition of Pegagan powder.

The different levels of addition of Pegagan powder affect the number of leukocyte cells. This is presumably because the high or low consumption of Pegagan affects the intake of active compounds that act as immunomodulators. In addition to protein as a raw material for producing leukocytes, other compounds also contribute to blood performance which ends in an increase in endurance. Asri *et al*., (2019) stated that medicinal plants contain secondary metabolites for disease prevention. Compounds with antibacterial properties are triterpenoids, flavonoids, saponins, and tannins with their ability to suppress bacterial growth (Ngajow *et al*., 2013).

Asiaticoside is a part of the triterpenoids and dominates the active compounds in Pagagan. Sutardi (2016) explained that triterpenoid compounds function to facilitate blood circulation, stimulate blood cells and the immune system, and as natural antibiotics. Flavonoid compounds are capable of producing and releasing cytokines and interleukins IL-1; IL-6; IL-12 (Ulfa *et al*., 2020). The research results of Arjuna *et al*., (2019) explained that medicinal plants containing tannins and saponins can function as bactericidal so that the performance of heterophils is reduced. These compounds are contained in Pagagan and if they are in the body, they optimize the leukocyte work system.

As for the status of the monocyte component which is the second line of defense after heterophile. When these components leave the blood vessels, they develop into macrophages and enter the infected tissue. Like heterophils, monocytes are directed to rush towards factors that cause inflammation and damaged

tissue or tissues that are subject to the action of pathogenic bacteria.

Besides that, the contribution of active compounds in monocyte components is due to the intake of important compounds that act as antibacterial, namely asiaticoside, flavonoids, saponins, and tannins. Noor *et al*., (2019) explained that when asiaticoside compounds enter the body, granulocytes, and macrophages become more active. Sami'Anwari *et al*., (2012) added that flavonoid compounds are compounds with the ability to bind to bacterial proteins which have implications for suppressing enzyme activity so that the bacterial metabolic process is disrupted. Saponins are active compounds that, when interacting with bacteria, increase membrane permeability which causes hemolysis of bacterial cells (Jumadin, 2020). Asrtiyani *et al*., (2017) continued tannins function as antibacterial by interfering with the permeability of cell membranes so that the process of exchanging needed substances is disrupted which has an impact on inhibiting bacterial growth and death.

The cause of the number of monocytes tends to be lower than normal limits and is thought to be due to the content of tannin compounds in Pagagan. The excessive addition of Pagagan powder causes the content of tannin compounds mixed in the feed is also high, causing a negative impact. Tannins given in excessive concentrations can bind to protein components which have implications for low protein metabolism results. Most of the protein components are used for growth, this causes the protein used for the immune system to decrease. Jannah *et al*., (2017) explained that a low monocyte value indicates that there are no bacteria that enter the body so there is no trigger for the spinal cord to produce monocytes.

Conversely, an increase in the number of monocytes indicates that there has been an attack by microorganisms. According to Siskia *et al*., (2018), an increase in monocytes or monocytosis indicates the active spread of microorganisms. This condition is caused by the absence of the contribution of active compounds in the blood. It is suspected that the responsiveness of other white blood cell components to microorganisms is lower so that the spinal cord is not triggered to produce monocytes. Risa *et al*., (2014) stated that the bioactive compounds contained in herbal medicines will have a positive and complementary effect (sparing effect) if given at the right dose. For this reason, a sufficient dose is needed to have an effect.

The results of the study of adding Pegagan powder to eosinophils were significant. This means that pagan powder contributes to increasing the body power of broilers. Factors that cause changes in the number of eosinophil components are overreactions in the body (hypersensitivity), types of parasites, immune responses, and levels of inflammation. According to Jannah *et al*., (2017) explained that the function of eosinophils is to neutralize the presence of toxic materials which then stimulate an increase in the number of eosinophils to perform their function as penetration of foreign bodies in the body.

Eosinophils are leukocyte cells produced from the spinal cord and can recognize antigens, capturing and consuming antigens. Marlinda *et al*., (2016) explained that eosinophils are part of the leukocytes with the main function of responding to antigens or foreign bodies in the body. Explained by Jatmiko (2015) states that when the cell is mature, it will enter and follow the blood circulation. Then it migrates to where the tissue needs it, especially parts close to the outside world such as the digestive tract and respiratory tract. The percentage of eosinophils in broilers is 0 – 7% (Sulaiman *et al.,* 2017) The number of eosinophils in livestock is a parameter to determine livestock health status.

This real effect is suspected because there is nothing that causes hypersensitivity, so there is also nothing that needs to be neutralized by eosinophils so that the number of cells is 0%. Followed by Adrial *et al*., (2018) stated that the low number of eosinophils is caused because the body does not need these cells and many of their roles have been taken over by the specific defense system. This condition is thought to be caused by the contribution of asiaticoside compounds in the blood. Noor *et al*., (2019) explained that asiaticoside can activate macrophages and granulocytes. These conditions encourage the specific defense system to become more active toward its function so that the role of eosinophils tends to be less needed.

CONCLUSION

The results of the study can be concluded that the addition of Pegagan powder to a treatment level of 2% in broiler feed for Erythrocyte status influenced Hemoglobin and Hematocrit levels but had no effect on RBC, MCV, MCH, and MCHC. Meanwhile, Leukocyte Status influenced the number of Leukocytes, Monocytes, and Eosinophils but did not affect Heterophiles, Basophils, and Lymphocytes.

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