EFFECT OF PROBIOTIC GIVING DURING MAINTENANCE ON HEMATOCRITES, ERITROSITES, AND BLOOD BIOCHEMISTRY POST TRANSPORTATION BROILER

Diding Latipudin[1*], Lovita Adriani[1], Roostita L. Balia[1]

[1]*Faculty Peternakan Universitas Padjadjaran, Indonesia*

Abstract

The research aimed to determine the effect of giving probiotics and which combination of probiotics that gave the best effect on hematocrit profile and broiler erythrocyte after transportation. Probiotics is an additional food or feed additive in the form of microorganisms that provide beneficial effects for health through increasing the balance of microbes in the digestive tract. Chicken broiler used is strain Cobb 398 start phase starter until finisher (1-35 days) as many as one hundred broiler chickens. The experimental study was conducted with Completely Randomized Design (RAL) with five treatments: P0 = (control), P1 = Probiotic (L. plantarum + L. acidophilus), P2 = Probiotics (L. plantarum + T. beigelii), P3 = Probiotics (L. acidophilus + C. humicolus), P4 = Probiotics (T. beigelii + C. humicolus) with four replications. The result of Duncan test analysis showed that P3 gave significant different effect ($P<0.05$) among all treatments on hematocrit and erythrocyte, and blood biochemistry after transportation. The conclusion is that giving probiotics with combination of bacteria and yeast (L. acidophilus + C. humicolus) can increase the hematocrit profile and broiler erythrocyte after transportation but still in normal range.

Key words: broiler, probiotics, hematocrit, erythrocytes, blood biochemistry, transportation

INTRODUCTION

Broiler chickens or better known as broiler chickens are a type of superior broiler chicken resulting from the crossbreeding of various chicken nations that have high productivity, especially in terms of meat production which has the potential as one of the food ingredients providing animal protein sources. Certain activities are carried out after the chickens are harvested, namely the transportation process to deliver livestock products from farmers to consumers.

One of the problems that is developing in the broiler chicken industry is the high level of stress before cutting caused by the transportation process which can result in shrinkage of body weight to the presence of cattle that DOA (Death on Arrival) [1]. The physiological response of post-transportation broilers can be known by doing a hematological study. Hematological analysis can be used to determine the health status of animals by observing changes in hematological parameters such as hematocrit profiles and erythrocytes. In normal animals PCV is proportional to the number of erythrocytes and hemoglobin levels [2]. If the number of red blood cells and hemoglobin levels changes, the percentage of hematocrit also changes. This can be affected by stress, one of them is when transporting [3]. The transport process in livestock can result in a decrease in hematocrit and hemoglobin values [4].

The performance of post-transportation broiler chickens can be maintained under normal physiological conditions with various treatments in the field of nutrition. Provision of probiotics in broiler chicken drinking water is one effort to minimize the onset of stress. Probiotics are feed additives that are beneficial and can increase growth and feed efficiency, so that a good and healthy physiological state can be maintained after transportation. This study used various types of probiotics with a mixture of *Lactobacillus plantarum*, *Lactobacillus acidophilus*, and *Cryptococcus humicolus* yeasts, Trichosporon beigelii with

*Corresponding author:
diding.latipudin@unpad.ac.id
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MATERIALS AND RESEARCH METHODS

1. Objects and Research Materials

Object of research

The animals used as the object of the experiment were 100 brob 398 strain broiler chickens starting from the starter phase to the finisher (1-35 days). The chickens were divided randomly into 20 cage units, each cage contained 5 tails.

Research Materials

The research material used was probiotics given through drinking water. The dosage of probiotics is 0.1-0.15% of daily drinking water needs. In the first week of starter used as much as 1 ml, second week 2 ml, third week 4 ml, fourth week 6 ml, and fifth week 8 ml. Each probiotic starter contains 107 CFU / ml

The ration used

The rations used during the study were mash-shaped rations. The ration used with PK content was 21.82% with EM of 3076 kcal / kg. The composition of the ration consisted of corn, soybean meal, fish meal, rice bran, coconut oil, bone meal, lysine, and methionine

Cages and Equipment

The cage used in the study is 20 units of litter cages and each unit is made of bamboo slats with a length × width × height of each unit 70 × 70 × 70 cm for a capacity of 5 head.

2. Research Methods

Research procedure

Preparation phase

The preparation phase begins with the preparation of the cage including cage sanitation, and calculation of probiotic requirements per week. After everything is ready, then livestock maintenance begins with the chick in process.

Maintenance stage

Feeding is done twice a day (morning and evening). Drinking water is given adlibitum. Probiotics are given by drinking water in the afternoon. Probiotics are added to the skim milk solution according to treatment, after which it is divided into 4 according to the replication, each test is given probiotics which have been dissolved with 50 mL of skim milk. Probiotics are put into the drinking area and wait until they run out. After the probiotics run out, the drinking water is replenished with clean water to give to the chicken adlibitum.

Transportation Phase

Broiler transportation or transportation is carried out at the end of maintenance, which is 5 weeks. Broilers are transported using pick-up cars from cages in Sukasari from 11.00 PM to Subang, and return to Sukasari at 17.00 PM.

Sampling Phase

Data collection at the end of the study was carried out by taking blood from the sample using a syringe from the pectroralis vein as much as 3 ml. Blood samples are collected in vakutainer containing EDTA with a purple lid.

Sample Analysis Phase

Hematocrit and erythrocyte analysis was performed at the Laboratory of Animal Physiology and Biochemistry, Faculty of Animal Husbandry, Padjadjaran University using a hematology analyzer

Variables observed

1.Hematocrit
2.Erythrocytes
3.Blood Biochemistry

Experimental design

The study was carried out using experimental methods using a completely randomized design (CRD) with 5 treatments with 4 repetitions, so that 20 experimental units were obtained. Treatment consists of:

RESULTS AND DISCUSSIONS

Table 1 Average of Hematocrites, Eritrosites, And Blood Biochemistry Post-Transportation Broiler Chicken given Probiotics During Maintenance

Variable	Treatment				
	P0	P1	P2	P3	P4
Hematocrites(%)	29.2±2.03	27.4±2.28	29.0±2.00	30.7±1.10	27.7±2.62
Eritrosites (10^6/ml)	2.56±0.17	2.34±0.16	2.51±0.10	2.61±0.07	2.52±0.32
Glucose Levels(mg/dl)	232.33±11.15	247.27±20.62	234.31±7.16	242.81±25.30	243.84±20.37
Protein Level (mg/dl)	2.629±1.73	5.748±2.42	2.576±0.96	1.962±0.44	2.318±0.86

***Note**:*
P0 = Without giving probiotics (controls)
P1 = Probiotics (*Lactobacillus plantarum* + *Lactobacillus acidophilus*)
P2 = Probiotics (*Lactobacillus plantarum* + *Trichosporon beigelii*)
P3 = Probiotcs (*Lactobacillus acidophilus* + *Cryptococcus humicolus*)
P4 = Probiotics (*Trichosporon beigelii* + *Cryptococcus humicolus*)

Table 1 shows the average hematocrit values for each treatment in sequence, P0 (29.2%), P1 (27.4%), P2 (29.0%), P3 (30.7%), and P4 (27.7%), the hematocrit value of post-transport broiler chickens is still in the normal range of 27-30% in accordance with other opinions that the normal value of chicken hematocrit is between 22-35% with an average of 30%. If there is a deviation from the normal hematocrit value, it has an important effect on the ability of the blood to carry oxygen and affect the health status of the chicken. The results showed the hematocrit value of post-broiler broilers for all treatments were still in the normal range, this indicates that the administration of various combinations of probiotics does not give a negative response or interfere with the amount of hematocrit so that the condition of the chicken remains healthy.

Based on the results of the analysis of variance obtained results of F hit <F table, which means that the probiotic treatment was not significantly different (P> 0.05) against the value of broiler hematocrit after transportation. Although the results of the analysis of variance showed that the administration of probiotics during maintenance did not significantly influence the hematocrit value of broiler chickens after transportation, but to test the differences between all pairs of treatments Duncan test was carried out. Based on Duncan's test analysis showed that (P1 is significantly different from P2, P0 and P3), (P4 is significantly different from P0 and P3), and (P2 is significantly different from P3). The treatment of P3 which combines *Lactobacillus acidophilus* and *Cryptococcus humicolus* results in the highest hematocrit value but still in the normal range.

Yeast as a source of probiotics can increase the amount of BAL that will affect the performance of BAL. This increase in BAL will benefit the host cell. [5] stated that microbes belonging to BAL have high microbial activity because the products they produce such as acidophilin produced by *Lactobacillus acidophilus* will inhibit the growth of pathogenic bacteria, which can damage the permeability of blood cell membranes and will end with leaking or damage to blood cell walls. When BAL increases, blood cells will be protected from damage so that the hematocrit value of post-transport broilers in P3 is highest among all treatments.

During the transportation process, an increase in free radicals both from metabolic activities in the body and from outside the body such as from cigarette smoke, haze, and vehicle emissions will trigger oxidative stress. During the process, there is an increase in heat entering the body and potentially increasing the radicals formed. Increased production of free radicals that are not comparable to the body's antioxidants triggers oxidative stress [6].

[7] stated that lactic acid bacteria (BAL) can produce organic acids and phenol

compounds. Lactic acid levels that continue to increase in the fermentation process can increase antioxidant activity. Lactic acid contains α-hidroxyacids (AHA) which function as antioxidants. Increased antioxidant activity other than lactic acid can be caused by metabolites secondary to bacterial metabolism. Probiotic bacteria produce antioxidant compounds in the form of vitamin C and vitamin E. According to [8] vitamin E can protect the membrane from damage by preventing the oxidation (peroxide) of unsaturated fatty acids that are in the phospholipids of the membrane while vitamin C acts as a reducing agent (antioxidant) in a liquid solution such as blood and in cells.

The hematocrit value is closely related to the number of erythrocytes / red blood cells in the body. Hematocrit values in general are also an indicator of determining the ability of blood to carry oxygen (O_2) commonly known as Oxygen Carrying Capacity. Hematocrit values in the body of livestock can experience a decrease and increase caused by the condition of the chicken's body itself or commonly called homeostasis [9]

This study although analysis of variance was not significantly different, but there was one positive value that was significant in the administration of probiotics during maintenance of the hematocrit value. When viewed from the hematocrit value before transportation and post-transportation, the hematocrit value of broiler chickens treated with tended to not decrease compared to those who were not treated. This decrease in hematocrit values can be an indicator that these animals experience stress. This is consistent with the opinion [10] which states that a decrease in hematocrit values can be caused by several factors, namely the level of stress that is influenced by nutritional and temperature factors, dehydration, and parasites in the blood. Provision of probiotics during maintenance can increase the resistance of the livestock so that it can reduce stress levels in livestock when transport is carried out, seen from the ability to maintain the value of post-transportation hematocrit.

2. Effect of Treatment on Erythrocyte Amounts

Table 1. shows the average number of erythrocytes in sequence, P0 (2.56 million / mm^3), P1 (2.34 million / mm^3), P2 (2.51 million / mm^3), P3 (2.61 million / mm^3), and P4 (2.52 million / mm^3). The results showed that the number of post transport erythrocytes from each treatment was still within the normal range. This indicates that metabolic processes in the body are normal and there is no disturbance in the formation of erythrocytes during transportation.

The results of the analysis of variance in blood profiles of post-transportation broiler chickens that were given probiotics with various combinations of probiotics during maintenance gave no significant different effect ($P> 0.05$) on the number of erythrocytes. The number of erythrocytes in post-transport broiler obtained in this study was in the normal range of 2.34 million / mm^3 to 2.61 million / mm^3. This amount is in accordance with the opinion [11], namely the number of normal erythrocytes in chickens is about 2.0 - 3.2 million / mm^3. The main function of erythrocytes is to transport hemoglobin to carry oxygen from the lungs to the tissues and carry carbon dioxide from the tissues to the lungs. [12] Added to that the function of other erythrocytes is to maintain the osmotic balance of the body and form ATP.

Based on Duncan's test analysis showed that (P1 was significantly different from P4, P0 and P3), (P2 was significantly different from P0, and P3), and (P4 was significantly different from P3). In illustration 2 shows that the average number of erythrocytes / red blood cells of broiler chickens after transportation in P3 giving probiotics combination of BAL (*Lactobacillus acidophilus*) and yeast (*Cryptococcus humicolus*) was higher than other treatments. It is suspected that the combination probiotics between BAL and yeast have better interaction, so that it can increase the elasticity or flexibility of blood cell membranes and will lead to better blood cell membranes which will ultimately improve the ability of the erythrocyte membrane to maintain its integrity [13]. [5] stated that the administration of yogurt containing additional probiotics *Lactobacillus acidophilus* could improve the state of hematology of mice, especially an increase in the number of erythrocytes but still within the normal range. But overall, the addition of all four types of probiotics with various combinations is still within the criteria of normal erythrocytes

The results of the analysis of variance in this study did not give a significantly different effect, but by administering probiotics during maintenance, the profile of erythrocyte post-transport broilers was maintained in the normal range without a decrease in the number of erythrocytes. When viewed from the number of erythrocytes before transportation and post-transportation, the number of broiler erythrocytes treated were less likely to decline compared to those not treated. This is because for those who are not treated, the blood cell membrane will be more easily damaged due to pathogenic bacteria and free radicals. Whereas for those treated, blood cell membranes will be protected by microbial activity such as the presence of antioxidants from microbes that are used to prevent damage or leakage to the blood cells.

The transportation process carried out during the day in hot conditions will cause heat stress on the chicken. [14] reported that heat stress caused an increase in ROS levels in mitochondria. Free radicals (ROS: Reactive Oxygen Species) are natural products of oxygen metabolism in cells whose formation is strongly influenced by environmental conditions. [15] When the amount of free radicals increases, it will cause changes in blood profile, especially in erythrocytes. Red blood cells (erythrocytes) are one of the cells that are very susceptible to free radicals. Free radicals can cause changes in blood chemical bonds or known as oxidative stress so that blood cannot carry oxygen to the maximum.

BAL can produce antioxidants that make damage to erythrocyte membranes due to free radicals can be prevented or reduced. Superoxide is one of the free radicals that can cause peroxidative damage to the phospholipid component of the membrane. Oxidative damage that accumulates in the membrane component will affect aging and destruction of erythrocytes, which is an erythrocyte life span [16].

High and low erythrocytes can be affected by environmental temperature factors. Environmental temperatures that are too high will cause livestock to experience heat stress so that the adequacy of oxygen in the body decreases, then affects the formation of red blood cells. Heat stress will increase corticosterone hormone levels in poultry that will trigger cell damage including blood cells due to reduced body oxygen intake. The resulting corticosterone hormone plays a role in breaking down proteins into glucose so that the energy produced is widely used for homeostasis, resulting in reduced availability of protein for erythrocyte formation.

The addition of probiotics into drinking water also serves to maintain the balance of the microflora ecosystem in the digestive tract and provides enzymes that can digest crude fiber, protein, fat and detoxify toxins or metabolites. [3] Food substances that are digestible and absorbed through the intestine are precursors for cell formation blood.

Probiotic treatment during maintenance by analysis of variance gave results that were not significantly different ($P > 0.05$) on the number of erythrocytes of broiler chickens after transportation. This is possible because chickens can still tolerate travel conditions when transported so that the number of erythrocytes can still be maintained in the normal range. The response of livestock was not significantly different from the number of erythrocytes treated and untreated showed that the microorganisms contained in probiotics used could adapt in the digestive tract of livestock, so by giving various combinations of treatments did not show any significant increase or decrease in the number of erythrocytes

3. Effect of Treatment on Blood Glucose Levels

Based on data Table 1. shows that the highest average glucose level is up to the lowest was P1 = 247.273 mg / dl, P4 = 243.835, P3 = 242.813 mg / dl, and P0 = 232.330 mg / dl. The effect of each treatment can be known through analysis of variance. Based on the results of analysis of variance obtained Fhit results <Ftable, which means that probiotic treatment was not significantly different ($P > 0.05$) in maintaining broiler blood glucose levels after transportation, but overall glucose level post transport broiler blood is still within the normal range, according to the study

[17] the normal range of blood glucose levels in broiler chickens is 230-370 mg / dl. In monogastric animals, the highest blood glucose concentration is reached within 2 hours after absorption of carbohydrates after eating, the function of gluconeogenesis of the liver provides the glucose needed to maintain fasting blood glucose levels [12].

The high blood glucose found due to the length of transportation for 6 hours causes the decomposition reaction of glycogenolysis or glycogen to produce glucose 6-phosphate, 1-4 termination of bonds or glycogen phosphorylase produces glucose 1-phosphate. Catalyzed with the enzyme phospho-glucomutase, glucose 6-phosphate can be formed from glucose 1-phosphate. Glucose 6-phosphate is converted to glucose by the phosphate enzyme alkalisation, thus facilitating the diffusion of glucose from the blood into the cell causing a stable blood glucose level [18].

The tendency to give the best results in maintaining blood glucose in P1 is because probiotics are able to produce vitamin K produced by *Lactobacillus plantarum*. This is according to [19] that probiotics containing lactic acid bacteria in the form of Lactobacillus can produce vitamin K. Vitamin K is the pancreas and insulin sensitivity in health by regulating glucose metabolism by modulation of osteocalcin [20].

In accordance with the results of research conducted [21] showed that consumption of probiotic fermented milk for 6 weeks could significantly improve glucose. According to him the consumption of probiotic yogurt increases blood glucose in a state of fasting. Blood glucose can come from several sources including food carbohydrates, glycogenic compounds through glyconeogenesis, and from glycogen tissue cells, especially liver cells through the pathway of glycogenolysis. The system guards the level of glucose in the blood in livestock through the process of glycolysis, gluconeogenesis, and so on so.

Blood glucose concentration will be relatively constant [22] glucose is a micromolecule of carbohydrate catabolism or non carbohydrate anabolism. Blood glucose serves as a provider of energy to all cells and tissues, including when livestock experience transportation stress. Some of the roles of glucose are known not only as energy precursors, but some of the results of previous studies reported the function of glucose as a buffer molecule for osmotic pressure so that blood pressure canmaintained even in a state of dehydration and heat stress [23].

Glucose levels are physiologically maintained at a fixed amount, through the role of the insulin hormone supported by Cortictropin Realeasing Hormone (CRH) secreted by the hypotalamus which then induces the anterior pituary to secrete adreno corticotropic hormone (ACTH), then ACTH stimulates the adrenal cortex to produce hormones glucocorticoids and epinephrine by the adrenal cortex and medulla [24]; [25]; [26].

This mechanism is very important to maintain because glucose directly affects the osmotic pressure of extracellular fluid. Changes in osmotic pressure negatively affect many metabolic pathways in the cell. Glucose levels in a stressful or increased state that can still be tolerated are always maintained to prevent changes in osmotic pressure. [27]. Changes in osmotic pressure cause the permeability of the cell membrane to decrease, which can cause abnormalities. Based on the results of the analysis, with or without the provision of probiotics is not different in maintaining blood glucose levels in broiler chickens

4. Influence of Treatment of Blood Protein Levels

Based on Table 1. average blood protein levels ranged from 2.00 to 5.75 g / dL..The lowest blood protein levels were found in P3 treatment, which was 1.962 g / dL and highest in P1 treatment was 5.748 g / dL.

Based on Table 1 it can be seen that the highest average liver protein content was aimed at P1 *(L. plantarum + L. acidhopilus*) of 5.748 g / dL and P0 (treatment without probiotic administration) of 2.629 g / dL, then followed by P2 (*L. plantarum + T.beigelii*) of 2.576 g / dL, P4 (*C. humicolus + T. beigelii*) of 2.318 g / dL, and the lowest average was obtained for P3 (C. *humicolus + L. acidhopilus*) of 1.962 g / dL

The results of the analysis in all treatment groups showed that the administration of probiotics had a significant effect (P <0.05) on blood protein levels. The average level of P1 blood protein is in the normal range of 5.74 g / dl. According to the opinion [28] that normal levels of blood protein in broiler chickens are between 4.0 to 5.2 g / dl. [29] say that protein levels are influenced by age, hormonal, nutritional, stress and fluid loss.

Provision of probiotics during maintenance will make livestock have a more extra body defense, because the absorption of nutrients from feed is more optimal so that when experiencing stress transport livestock perform a low gluconeogenesis process, so that blood protein levels do not decrease. The probiotic tendency that gives the best results among all treatments is P1 combination of lactic acid bacteria (BAL)*Lactobacillus plantarum* and Lactobacillus acidophillus can minimize the gluconeogenesis process. This is in accordance with the opinion [29] that the probiotic microbes of Lactobacillus acidophillus are bacteriostatic whose targets protect the plasma membrane which acts as the entry of nutrients and the release of metabolic waste. According to [30] Lactobacillus sp. can have a positive effect as an antidepressant and provide a good state after consuming it.

Increased blood protein levels, presumably because the administration of probiotics can synthesize protease enzymes needed in the process of digestion of body nutrients so that the digestion of broiler chicken protein increases, when compared with treatment without probiotic administration because the protease enzyme produced only comes from the pancreas. This is consistent with the statement [31] stating that probiotics produce various protease digestive enzymes, so that nutrient digestibility increases, increasing nutrient absorption by thickening intestinal villi and expanding the surface of intestinal villi. [32] states that feed fermented by microorganisms undergoes a more simple change in particles so that the organic feed ingredients contained in it will be more easily absorbed by the body. This is caused by fermentation which produces certain enzymes which can break down proteins into amino acids so that they are more easily absorbed by the body [33].

Fermentation of organic matter will release amino acids and saccharides in the form of compounds that are dissolved and easily absorbed by the digestive tract of the chicken, thus causing absorption and utilization of nutrients for better growth. [34] states that in general the protein in the ration will be digested in the small intestine with the help of protease enzymes that hydrolyze proteins then flow through the intestinal wall into the blood vessels. Automatic protein that absorbs into the blood will be more or increased.

Probiotics in the form of yeast contain amino acids, proteins and complex B vitamins as well as minerals phosphorus, potassium, magnesium and calcium. Minerals in the yeast act as a metabolic modulator, which modulates the process of protein metabolism, so that blood protein levels do not undergo rapid changes because the absorption of the protein component is relatively the same and temporarily stored in the blood [35]. This shows that the administration of probiotics during maintenance is able to make the immune system of livestock increase so that it can reduce stress levels in livestock when transported and able to maintain levels of broiler blood protein.

CONCLUSION

1. Probiotic administration of P3 (*L. acidophilus* + *C. humicolus*) mapu maintains the physiological status of post-transport broilers, seen from hematocrit values and the number of erythrocytes, and blood biochemistry shows the highest value as well as the absence of a decrease in hematocrit values and the number of erythrocytes of post-transport broiler chickens

2. Probiotic treatment of P3 with a combination of bacteria and yeast (*L. acidophilus* + *C. humicolus*) is able to provide an increase in hematocrit profile and erythrocyte broiler chickens after transportation but still within the normal range.

3. Probiotic administration during maintenance showed significantly different results ($P < 0.05$) on blood protein levels of broiler chickens but not significantly different

(P> 0.05) against post-transport broiler blood glucose levels.

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