BLOOD PROTEIN PROFILE DYNAMICS DUE TO PROBIOTIC YOGURT SUPPLEMENTATION IN BROILER CHICKENS

Lovita Adriani[1*], Muhammad Rifki[1], Tuti Widjastuti[1]

[1]*Faculty of Animal Husbandry, University Padjadjaran, West Java, Indonesia*

Abstract

This study aims to determine the effect of providing probiotics based on cow milk, its combination with soy milk and green beans on total protein and blood albumin levels of broiler chickens. The study used 100 DOC broiler chickens and reared for 28 days. The experimental design used was a completely randomized design (CRD) with 4 treatments and 5 replications, namely P0 (commercial ration), P1 (commercial ration + 2% probiotics (100% fermented cow milk)), P2 (commercial ration + 2% probiotics. (50% fermented cow milk + 50% fermented soy milk)), and P3 (commercial ration + 2% probiotics (50% fermented cow milk + 25% fermented soy milk + 25% fermented mung bean milk)). The results of statistical analysis showed that the treatment had no significant effect (P> 0.05) on total protein and albumin levels. The conclusion is that the provision of 2% probiotic based on cow milk, in combination with soy milk and green beans can maintain the total protein and blood albumin levels of broiler chickens in the normal range.

Key words: fermented milk, probiotics, total protein, albumin, broiler chicken

INTRODUCTION

Probiotics in feed have a positive effect because it can increase the content of lysine analog and aminoethyl cysteine in the digestive tract, most of them are hydrolyzed into amino acids lysine and cysteine and can increase protein retention which plays a role in meat formation [1]. The addition of probiotics increases the activity of the protease enzyme that needed in digestion to break peptide bonds in feed protein to free amino acids needed to the body [2]. Amino acids play a role in composing body tissues and in growth so the increased availability of amino acids will increase growth and body weight [3]. The accumulation of amino acids that can be absorbed by the small intestine, can increase the activity of the protease enzyme [1]. The increased secretion of protease enzymes associated with protein metabolism causes the rate of protein metabolism in the liver increases, so total plasma protein levels increases, with increasing of total plasma albumin [4].

Most of the blood protein comes from ration proteins that undergo digestion and absorption in the intestine in the form of free amino acids that are carried by the portal blood to the liver. Free amino acids are precursors for the synthesis of blood proteins, which are synthesized in the liver. Nearly 90% of blood protein is released into the blood circulation [5]. The liver releases protein into the blood circulation and then distributed to all tissues in the body. About 40% of the total protein from the blood flows to the tendon and the remaining 60% to the pancreas and epithelial cells [6].

The main blood proteins are albumin, globulin, and fibrinogen [4]. Blood proteins play a role as blood clotting, enzymes, hormones, immune defense, a role in the inflammatory response, and transport or binding proteins [7]. Blood protein functions are to maintain osmotic pressure, as a source of amino acids for tissues, transport nutrients to cells and waste products to secretory organs, the body's acid-base balance (buffer) [8] [4]. Albumin can bind various ligands and is responsible for 80% of the osmotic

[*]Corresponding author: lovita@unpad.ac.id; lovita_yoghurt@yahoo.co.id,
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pressure [4]. Normal levels of total blood protein in chickens are in the range of 3.27−4.40 g / dl [9]. Normal albumin levels in chickens are between 1.09−1.56 g / dl [10].

Research that has been carried out by giving probiotics derived from soy milk yogurt and fermented cow milk by 1.25% of the bodyweight of mice can increase the population of non-pathogenic bacteria and reduce the number of pathogenic bacteria in the colon [11]. The use of fermented cow milk and fermented soy milk with *Lactobacillus acidophilus* and *Bifidobacteria* in broiler feed can reduce blood cholesterol and triglyceride levels [12]. Also, the addition of 2% probiotics containing *Lactobacillus bulgaricus* and *Streptococcus thermophilus* in the ration of broiler chickens produces the highest body weight, reduces abdominal fat, and reduces meat cholesterol [13]. The use of fermented cow milk and fermented soy milk with *Lactobacillus acidophilus* and *Bifidobacteria* in broiler feed can reduce blood cholesterol and triglyceride levels [12]. Proteins play a very important role in organisms, namely in structure, function, and reproduction [14]. Protein is a polypeptide that has a very variable molecular weight and has different properties. Protein is divided into proteins that are easily soluble in water and proteins that are difficult to dissolve in water [15].

The research was carried out on blood protein because it is one of the blood components that can be used to determine the health of livestock [16]. Proteins from blood plasma consist of albumin and globulin, where blood proteins function to maintain blood volume through osmotic pressure, as a buffer for blood pH, as transport of hormones and drugs, participate in cell coagulation, catalyze enzyme reactions, regulate hormone metabolism and participate in defense body [17]. Albumin functions as a transport substance for various small molecules such as fatty acids and bile pigments, as well as a precursor for white blood cells for the formation of immune components [18]. Albumin also plays a role in the binding and transport of various substances in the blood. Albumin is the most protein component in the blood produced by the liver [8]. Albumin is synthesized in liver cells which are carried out in two places, namely the free polysome where albumin will be used for intravascular purposes and in the polyribosomes associated with the endoplasmic reticulum, where albumin will be distributed and utilized throughout the body. In the endoplasmic reticulum, protein is converted into pro albumin which will then be broken down in the Golgi body into plasma albumin.

MATERIAL AND METHODS

The broilers used in this study were 100 tail. The research ration used in this study used a commercial ration with the addition of probiotics as much as 2% of the ration.

Table 1 Nutrient content and Energi Metabolism (EM) of research rations

Content	Units	Amount	
		starter	finisher
Water (Max)	%	13.0	13.0
Protein	%	22.0 -24.0	19.5-21.5
Fat (Min)	%	5.0	5.0
Ash (Max)	%	7.0	7.0
Calcium (Min)	%	0.9	0.9
Phosphorous (Min)	%	0.6	0.6
EM	Kcal/kg	3070-3170	3125-3225

Source: PT. Charoen Pokphand Indonesia

The probiotics in this study were *Lactobacillus bulgaricus, Streptococcus thermophilus, Lactobacillus acidophilus, and Bifidobacterium*, fermented in mixed cow milk, soy milk, and green bean milk. The combined use of the four probiotic bacteria refers to the research of Adriani and Hendrotono [11]. The total lactic acid bacteria after the drying process ranged from 7.09×10^2 to 4.5×10^3 [19]. The fermented milk is then processed into powder and prepared according to the predetermined amount as needed. The procedure for making probiotic powder is very simple, that is, just coat it with maltodextrin as much as 5 percent of the total yogurt and dry it with an over-drying equipped with an air fan at 39°C. The dose of probiotic is 2% of the total ration. The use of probiotics at a dose of 2% of this ration refers to the research of Lengkey and Lovita [13]. Mixing feed with probiotics is done by self-mixing.

RESULTS AND DISCUSSION

Effect of Treatment on Total Protein Levels

Based on the results of the study, the effect of giving probiotics based on cow milk, its combination with soy milk and green beans by 2% of the ration on the total protein content of broiler chicken blood is presented in Table 2.

Table 2 Total Protein and Blood albumin levels

Parameter	Treatment			
	P_0	P_1	P_2	P_3
	……g/dl……			
Kadar Protein Darah	3.39 ± 0.43	3.55 ± 0.11	3.71 ± 0.34	4.00 ± 0.34
Kadar Albumin Darah	1.37 ± 0.06	1.39 ± 0.15	1.26 ± 0.08	1.38 ± 0.12

Note :P0 = commercial ration without probiotics
P1 = commercial ration + 2% Probiotics (100% fermented cow milk)
P2 = commercial ration + 2% Probiotics (50% fermented cow milk + 50% fermented soy milk)
P3 = commercial ration + 2% Probiotics (50% fermented cow milk + 25% fermented soy milk + 25% fermented mung bean milk)

The total protein level of blood broiler chicken after given probiotic treatment was in the range 3.27-4.40 g / dl [9]. The total protein content of the treated chickens was still in the normal range. Based on variance, giving probiotics based on cow milk, combination with soy milk, and green beans as much as 2% of the feed had no significant effect (P> 0.05) on total blood protein levels. These results indicate that the administration of cow milk-based probiotics, in combination with soy milk and green beans, gives the same response to the average total blood protein levels.

Treatments (P1, P2, and P3) had a higher total protein content when compared to treatments without probiotics (P0). This is due to the higher activity of the protease enzyme in the intestine compared to treatment without probiotics (P0). The addition of probiotics can increase the activity of the protease enzyme needed in digestion to break the peptide bonds in feed protein and free the amino acids needed by the body [2]. Furthermore, Wang and Qing (2010) stated that broiler chickens that were given probiotics in the ration could significantly increase the protease enzyme activity, thus, the utilization of feed protein indigestion would increase.

The increase in protease enzyme activity can occur due to the use of fermented milk as a growing medium for probiotics where milk contains several enzymes, one of which is a protease. [21] stated that starter bacteria added to milk fermented can produce a protease enzyme that causes the protein hydrolyzed into peptides and amino acids which are dissolved proteins. [22] states that *Lactobacillus bulgaricus* and *Lactobacillus acidophilus* are lactic acid bacteria with high proteolytic activity. [23] stated that the addition of *Lactobacillus acidophilus* and *Bifidobacterium* to milk fermentate increase

the protease activity twofold so that more protein would be broken down into simple compounds such as amino acids, both essential and non-essential.

The increase in amino acid availability will also be followed by an increase in plasma protein because at the time of protein synthesis amino acids are needed [4]. The protein synthesis process requires 20 kinds of amino acids, mRNA, and tRNA as the executor, ATP as an energy source, and the RNA polymerase enzyme. Broadly speaking, protein synthesis takes place in two stages, that is the transcription and translation stages. Amino acids are chemical molecules that are present in proteins and form proteins. Proteins are biologically active polymers consisting of amino acids linked by peptide covalent bonds. One protein molecule consists of 12 to 18 kinds of amino acids and can amount to hundreds of amino acids. About 75% of amino acids are used for protein synthesis [24].

The activity of lactic acid bacteria produces short-chain fatty acids that function to repair the microvilli, namely increasing the villi height, width of the villi, and goblet cells in the small intestine, so that the area of absorption of food substances increases. This is by the statement of [25] that giving probiotics can improve microvilli by increasing the width of the jejunal villi by 20.52% and the number of jejunum villi by 13.70% of the treatment that is not given probiotics, thereby increasing the area of absorption of nutrients. [26] adds that increasing the number of microbial populations that are beneficial for livestock will prevent the development of pathogenic microbes in the digestive tract which leads to increased digestion of food. Increased digestibility and absorption of food substances will have an impact on increasing total blood protein.

P3 treatment showed that the highest total protein content compared to other treatments. This can occur because the fermentation results of legumes at pH 5 can hydrolyze isoflavones in the form of glycosides (glycons) to aglycones by the β-glucosidase enzyme by releasing the glycoside bonds [27]. Glycosides are inactive, whereas in the form of aglycones they become more active because of an increase in the hydrosil group in their molecular structure. Isoflavones in the form of aglycones are more easily absorbed by the small intestine and have better antioxidant activity when compared to the form of glycones. Fermentation causes isoflavone compounds in the form of aglycones to have higher antioxidant activity than the bound form [28]. In addition to the isoflavone content, green beans also contain beta-carotenes and vitamin E which have antioxidant functions.

Both livestock and human cells under normal metabolic conditions produce low numbers or low levels of high-energy particles, known as free radicals. At high concentrations, free radicals are harmful to living things because they can damage all the basic parts of the cell. Apart from endogenous free radicals can also be caused by exogenous, one of which is high environmental temperature, which can cause oxidative stress (oxidative stress) [29]. Oxidative stress, indicated by the cellular increase in Reactive Oxygen Species (ROS), including superoxides, hydrogen peroxides, hydroxyl radicals. Excessive production of ROS as a result of heat stress causes oxidative damage, namely disruption and reduction of protein, lipid, and DNA synthesis, as well as decreased ATPase activity. This means that oxidative damage will cause a decrease or even cessation of livestock production [34]. In other words, it can be concluded that free radicals inhibit protein synthesis, therefore reducing or even stopping livestock production.

Total protein content in P3 treatment could increase total protein levels in broiler chicken blood by 18% compared to treatment without probiotic treatment although it was not statistically significant. The total protein content of broiler chicken blood at P3 is still at normal levels, indicating that protein is not only accumulated in the blood but is also distributed into enzymes, hemoglobin, muscle, and collagen. Normal total protein levels indicate healthy chickens because they get the adequate intake and can metabolize well.

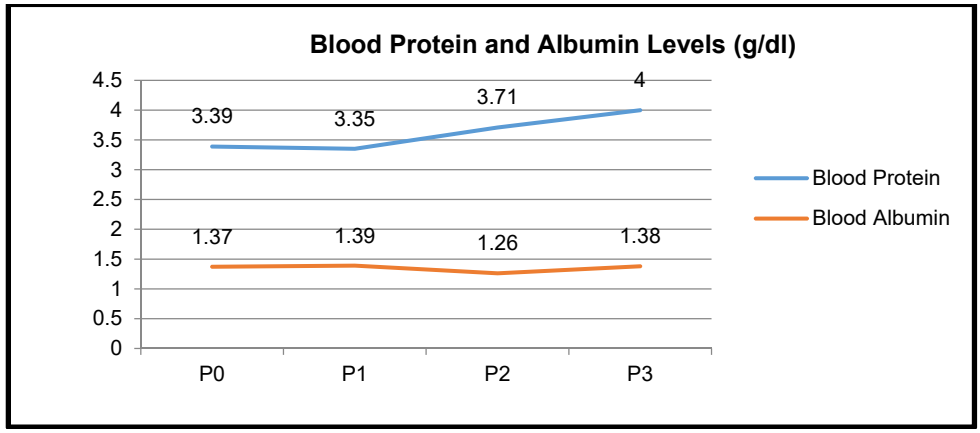

Figure 1. Average blood Total Protein and Albumin levels

Effect of Treatment on Albumin Levels

Blood albumin plays a role in regulating blood osmotic pressure and keeping fluids in the blood from leaking into the surrounding body tissues. The level of albumin in the blood can describe the health condition of the liver and kidneys. The range of albumin levels in this study in the range of 1.26−1.38 g / dl. Based on the research by [10] the blood albumin levels of broiler chickens were in the range of 1.09− 1.56 g/dl. The blood albumin levels of broiler chickens in this study still in the normal range [10]

Based on variance, giving probiotic based on cow milk, the combination with soy milk and green beans as much as 2% of the feed had no significant effect (P> 0.05) on blood albumin levels. These results indicate that the administration of cow milk-based probiotics, in combination with soy milk and green beans, gave the same response to the mean blood albumin. In this study, the addition of probiotics in feed to albumin levels did not show any significant difference. The results of this study are consistent with the research of [30] [31] which stated that the provision of probiotics in feed did not affect the blood albumin levels of broiler chickens.

Broiler chickens' blood albumin levels are still in the normal range, indicating that the protein deposition process into meat is also in the normal range. Low albumin will affect the total protein. Reduced protein intake, the albumin levels will also decrease so that protein deposition will also be reduced because albumin and total protein play a major role in protein deposition into the meat. This is by the statement by Liu, et al, (2015) in [16] which states that albumin affects the growth rate. Albumin in the blood, apart from being used for growth, is also used for protein deposition and is also used as an antibody in the body. [32] stated that an increase in albumin in the blood indicates that there is an infection in the body.

Albumin is the main protein found in the blood produced by the liver, the amount of blood albumin is about 50-60% of the total plasma protein. As previously explained, giving probiotics to broiler chickens can increase the activity of the protease enzyme, followed by an increase in the availability of amino acids, which are precursors for protein synthesis that occurs in the liver. The increased activity of the protease enzyme which maximizes the breakdown of feed protein into a simple form, namely amino acids, does not mean that all of these amino acids will be synthesized into albumin or all of the plasma proteins. Because the protein in the body is continuously replaced (protein turnover). Examples of turnover proteins are enzymes, hemoglobin, muscle, plasma protein, and collagen. In other words, the increased availability of amino acids does not mean an increase in albumin, because the body also needs amino acids for protein synthesis in addition to plasma proteins.

CONCLUSION

The conclusion is the provision of 2% probiotic based on cow milk, in combination with soy milk and green beans can maintain the total protein and blood albumin levels of broiler chickens in the normal range.

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