

## THE EFFECT OF GRANTING FERMENTED MILK, FERMENTED SOY MILK, AND THE COMBINATION AGAINST CREATININE AND BLOOD GLUCOSE LEVELS ON BROILER

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### Abstract

The research has been conducted from January 30 to March 7, 2017. The purpose of this research is to know the influence of addition of fermented milk, fermented soy milk, and the combination, as well as the influential composition to creatinine and blood glucose levels on broiler. The method used is experimental, with a complete random design. There are seven treatments,  $T_1$  = without treatment,  $T_2$  = milk granted,  $T_3$  = fermented milk granted,  $T_4$  = fermented soy milk granted,  $T_5$  = fermented milk and fermented soy milk granted with ratio 1:1,  $T_6$  = fermented milk granted LA + B 1.25%,  $T_7$  = fermented milk granted LA + B, 2% dose with four times repetition. Each unit has five farm animals, so the total is 140 broiler chicken, 1-35 days old. Based on the statistic analysis calculation, the effect of granting fermented milk, fermented soy milk, and the combination has a significant effect ( $P < 0.05$ ) to decrease creatinine levels and does not have a significant effect ( $P > 0.05$ ) to decrease blood glucose levels in broiler. The conclusion is by granting fermented milk combined with fermented soy milk with ratio 1:1 can decrease creatinine levels, but does not decrease blood glucose levels in broiler.

**Key words:** broiler, creatinine, blood glucose, fermented milk, fermented soy milk.

### INTRODUCTION

The research is focused on the role of probiotic, including Lactic Acid Bacteria (LAB) in the yoghurt for the enhancement of the gastrointestinal function through the kidney improvement, lactose intolerance (lactase deficiency) reduction, and *Lactobacillus acidophilus* and Bifidobacteria consumption (Vesa et al., 2000).

LAB enables the inhibition of the pathogenic infection through acetic acid, lactic acid, and bacteriocins production (Bianchi-Slvadori, 1986), as well as stabilizing the intestinal microflora after long-term antibiotic usage (Brown et al., 2005). Suppression of the harmful carcinogens is associated with colon cancer (Tavan et al., 2002; Wollowski et al., 1999) and increasing the immune response by producing secretory immunoglobulin (Perdigon, 1995, 2003).

This research aims to improve lipid profile and the health of farm animals, one of them is by increasing the probiotic. Beside that, it also

aims to see if probiotic can improve kidney function through creatinine levels. Probiotic function is to press pathogenic bacteria population, to supply the enzyme to help digesting food ingredients, to detox adverse food components and take it out from the digestive track, and to stimulate the intestinal peristaltic activity. Some microbacterias in probiotic can produce compounds that can inhibit fat synthesis, mobilize or reduce it to increase farm animals' health.

Creatinine is mass muscle production that comes from fosfoceratine. Generally, creatinine is produced in the body in the fixed amount and released into the blood. An increase in creatinine levels can be used as an indicator of kidney function disorder and the low ability of glomerulus filtration. On the other hand, blood glucose derived from the absorption of food digestion and the release of glucose from glycogen cell supplies. This research use fermented milk, using probiotic bacteria such as *Lactobacillus bulgaricus*, *Lactobacillus*, and *Bifidobacterium*. The advantages of probiotic is

to press pathogenic bacteria population, to supply the enzyme to help digesting food ingredients, to detox adverse food components and take it out from the digestive track, and to stimulate the intestinal peristaltic activity (Adriani, 2009). Creatinine is used to monitor kidney function in blood. An increase in creatinine levels can be caused by some conditions, such as heart failure, dehydration, pyelonephritis, and high urem levels in blood. Creatinine levels in blood can decrease where there is a reduction of the mass of muscle tissue.

Creatinine serum is an important indicator of renal health because it is an easily measured by product of muscle metabolism that is excreted unchanged by the kidneys. Creatinine itself is produced through a biological system involving creatine, phosphocreatine, and adenosine triphosphate (ATP, the body's immediate energy supply).

Creatine is synthesized primarily in the liver from the methylation of glycoamine by S-adenosyl methionine. It is then transported through blood to the other organs, muscle, and brain, where, through phosphorylation, it becomes the high-energy compound phosphocreatine. Creatine conversion to phosphocreatine is catalyzed by creatine kinase, a spontaneous formation of creatinine occurs during the reaction. High creatinine levels are rarely an independent problem. More often, this issue is a symptom of something more serious to decrease the levels permanently and improve human and animal vein. Kidney damage and chronic kidney disease are related to type II Diabetes, and also linked to high creatinine levels in human.

Glomerulus is an indication of increasing blood glucose with metabolism changing on poultry. Blood glucose is sugar in blood, which formed from carbohydrate in food and stored as glucose in liver and skeletal muscle. Blood glucose will decrease if the rate absorption by metabolism or stored higher than additional rate. Glucose absorption by cells is stimulated by insulin, which secreted by  $\beta$  cell from Langerhans Islands. Glucose is spreading from plasma to cells because the concentration of glucose in plasma is higher than in cells. The consumption of fermented soy milk is beneficial to the balance of the ecosystem in

the intestinal tract by increasing probiotic population and decreasing pathogenic bacteria population (Lengkey and Adriani, 2009). One of the component of soy milk that has lots on benefits is isoflavones, which is the instrumental in the improvement in decreasing creatinine and blood glucose. Other components such as saponins and soy protein also have antioxidant effect. Fermented soy milk can decrease total cholesterol and the accumulation liver triglycerides on oxidative process. Generally, soy milk has vitamin B<sub>2</sub>, B<sub>1</sub>, niacin pyridoxine, and vitamin B, E, and K. Soy contains lots of Ca, F, but has less Fe. Soy also contains lots of nutrients, such as isoflavones, saponins, phytosterols, pitat acid, and felonic acid (Anderson and Wolf, 1995).

Blood glucose profile is arranged to always be in stable condition in the body through homeostatic process, which involved other glucose resources such as glycogen, fat acid, and amino acid (Lovita et al., 2010). According to Piliang (1996), the constant blood glucose profile is maintained at all times. Homeostatic sugar in blood is reached through some mechanisms that set the speed of glucose conversion to glycogen or fat to be stored and released, then converted to glucose that enter blood circulatory system (Asril, 2002).

Probiotic is needed to decrease creatinine and blood glucose levels. Based on the explanation, it is important to do research about giving probiotic that affected creatinine and blood glucose levels on broiler chicken.

## MATERIALS AND METHODS

Farm animals observed in this research are 140 strain Cobb broiler chicken, from starter to finisher phase (1 – 35 days). The research use experimental method with Complete Random Design with seven treatments and four times repetition, so 28 experimental units are retrieved. Drinking water *ad libitum* is provided during experimental period and feed is given according to needs.

It were taken random blood sample from 56 broiler chicken, two from each cage, which analyzed at Physiology and Biochemistry Laboratory, Faculty of Animal Husbandry, Padjadjaran University. The parameter measured is creatinine and blood glucose

levels. The analysis of creatinine levels is measured with kolorimetric method, and the analysis of blood glucose levels is measured with Folin-Wu method.

Data collected from the analysis is tested using Anova and the significant result is tested using Tukey test. Probiotic used consists of T<sub>0</sub>, T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub>, and T<sub>4</sub> is using mixture of three bacteria; *L. bulgaricus*, *L. acidophilus*, and Bifidobacteria. T<sub>5</sub> and T<sub>6</sub> is using mixture of two bacteria: *L. acidophilus* (LA), and Bifidobacteria (B).

T<sub>1</sub> = The rations is given *ad libitum* without fermented milk granting.

T<sub>2</sub> = The rations is given *ad libitum* with milk by force feeding with 1.25% from body weight.

T<sub>3</sub> = The rations is given *ad libitum* with fermented milk by force feeding with 1.25% from body weight.

T<sub>4</sub> = The rations is given *ad libitum* with fermented soy milk by force feeding with 1.25% from body weight.

T<sub>5</sub> = The rations is given *ad libitum* with fermented milk and fermented soy milk with ratio 1:1 by force feeding with 1.25% from body weight.

T<sub>6</sub> = The rations is given *ad libitum* with fermented milk with LA + B by force feeding with 1.25% from body weight.

T<sub>7</sub> = The rations is given *ad libitum* with fermented milk with LA + B by force feeding with 2% from body weight.

## RESULTS AND DISCUSSIONS

The result shows that treatments of fermented milk, fermented soy milk, and the combination of it has significant effect ( $P < 0.05$ ) to decrease creatinine levels (Table 1 and Figure 1). Creatinine levels are significantly different in T<sub>4</sub> and T<sub>5</sub> compared to other treatments. Normal creatinine levels in broiler blood is ranged from 0.90 – 1.85 mg/dl (Girindra, 1989).

An increase in creatinine levels in blood and the amount of creatinine in urine can be used to estimate glomerular filtration rate (Kramer et al., 2004). If the glomerulus filtration is decreasing, the kidney function is also decreasing and the glomerular function in the filtration is reducing. This will cause an increase of creatinine levels in blood.

A decrease in creatinine levels occurs because of lactic acid bacteria contained in probiotic. Because of the lactate production of glucose correlates with glomerular filtration rate, lactate production is also correlates with urine flow rate and sodium resorption (Hohmann et al., 1974).

Creatinine serum levels in probiotic addition due to the combination of fermented milk and fermented soy milk ca suppress free radical compounds and fibrosis tissue formation by using antioxidant compounds in the form of vitamin E ( $\alpha$ -Tocopherol). Vitamin E is able to inhibit the formation of oxidative stress and protect polyunsaturated fatty acids, such as linoleic, linolenic, and arachidonic acids (Pryor, 2001). Antioxidant in vitamin E can be used as a therapy in the process of curing renal fibrosis disease, by inhibiting the formation of factors that stimulate the production of excess ECM (Extracellular Matrix) components. Free radicals trigger lipid peroxidation that will damage the glomerulus and tubules, and cause inflammation (Peter, 2007). Inflammation leads to activation of fibroblast proliferation. Fibroblasts produce excess ECM and are deposited on the glomerulus and tubules, which in turn, ECM deposition forms a fibrous tissue in the kidneys (Eddy, 2000). Kidney fibrosis results in decreased renal function. The decreased kidney function causes the glomerular filtration rate to fall, resulting in the filtration of some substances, such as damaged creatinine. This will lead to creatinine and non-protein nitrogen substances retention in the kidneys so that the level of creatinine in the blood increases (Razzaque, 2004).

Blood glucose in the body serves as a source of energy in order to stay in normal homeostasis conditions. Glucose is the fuel for important tissues such as the brain and red blood cells. The source of blood glucose is feed after meal, the liver oxidizes glucose, and stores its excess as glycogen (Tan et al., 2010).

Microbes that live in the chicken intestine have a big effect on regulating blood glucose levels. That is why the addition of probiotics is also beneficial to lower blood glucose levels (Table 2 and Figure 2).

Table 1. Levels of broiler chicken creatinine (mg/dL) in various treatments

Repeat	Treatment						
	T1	T2	T3	T4	T5	T6	T7
1	2.23	2.05	1.67	1.49	1.12	1.86	1.49
2	1.86	1.67	1.58	1.67	1.49	1.49	1.67
3	1.67	1.86	1.67	1.49	1.30	1.67	1.86
4	1.49	1.49	1.86	1.86	1.12	1.86	1.67
Average	1.81±0.32	1.77±0.24	1.70±0.12	1.63±0.18	1.26±0.18	1.72±0.18	1.67±0.15

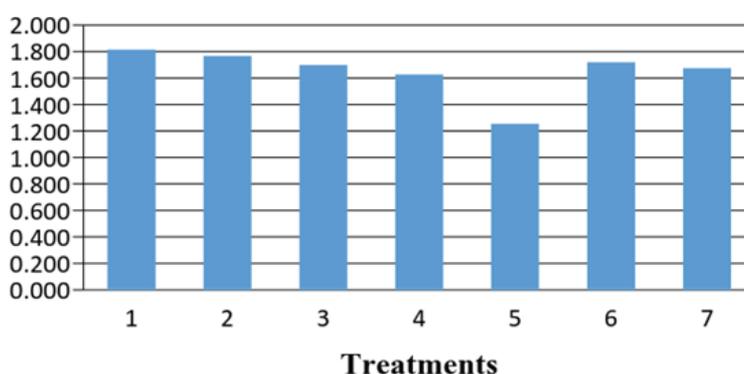


Figure 1. Average levels of broiler chicken creatinine in various treatments

Table 2. Levels of blood glucose (mg/dL) of broiler chicken in various treatments

Repeat	Treatment						
	T1	T2	T3	T4	T5	T6	T7
1	84.42	85.48	84.81	82.98	86.63	86.25	85.00
2	89.71	79.52	85.58	89.62	87.60	83.85	82.69
3	89.81	93.75	82.50	90.10	84.04	82.12	84.42
4	83.75	89.13	86.06	85.48	80.00	88.65	87.79
Average	86.92±3.29	86.97±6.01	84.74±1.58	87.05±3.41	84.57±3.40	85.22±2.85	84.98±2.12

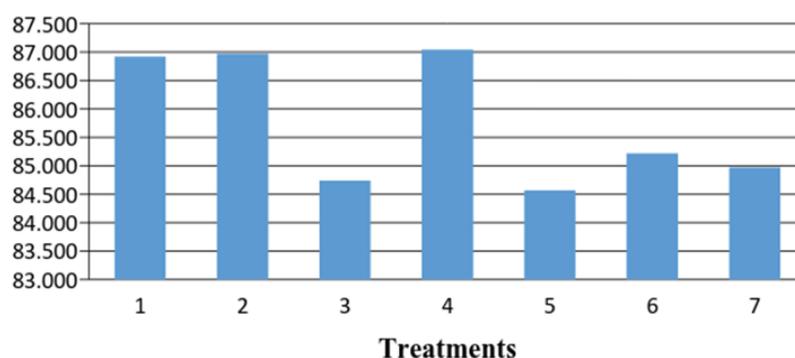


Figure 2. Average levels of broiler chicken blood glucose in various treatments

Probiotics, alias good bacteria, are included in functional food because of its benefits to health. A healthy digestive organ is trusted by experts as the foundation for excellent physical health. As glucose levels increase, glucose output stops, and at high levels occurs as uptake (Martin et al., 1984).

These data show that according to Ejtahed et al. (2012), the function of probiotics in lowering glucose is not clear. This is associated with

decreased oxidative stress, which is shown to be present in hyperglycemia (Ferreira et al., 2010). Specific strains of lactic acid bacteria have antioxidant properties (Amaretti et al., 2013). Yadav et al. (2006) reported that fermented milk containing *Lactobacillus acidophilus* and *L. casei* delay the development of glucose intolerance, hyperglycemia, and hyperinsulinaemia through decreased oxidative stress, thus, probiotics may modulate the

systemic immune response and low-level inflammation, in particular by reducing cytokines (de Moreno De Leblanc and Perdigon, 2010). And suppress the NF- $\kappa$ B pathway, which mediates microbial activation of the immune system via receptors such as tolls (Shi et al., 2006).

Consumption of probiotics can increase plasma antioxidant levels and neutralize the effects of oxygen-reactive species. Furthermore, the stimulation of the immune system decreases the inflammatory state and prevents oxidative stress induced by cytokines (Martarelli et al., 2011). Anti-oxidative and anti-inflammatory properties of probiotics, benefits can be given to the immune system, consequently neutralizing the production of oxygen reactive species (Martarelli et al., 2011). This is consistent with previous studies of the effects of probiotics on renal function by short-term randomized trials and the results show a decrease in blood urea nitrogen (BUN) (Ranganathan et al., 2009).

Probiotics contain flavonoid compounds, which are important antioxidants in neutralizing and destroying free radicals that can cause cell damage and also damage biomolecules in the body that can eventually lead to degenerative diseases (Silalahi, 2002).

When the amount of free radicals in the body is excessive, creatinine levels also increase, it takes antioxidant that come from outside the body, such as flavonoids, vitamin A, vitamin C, and vitamin E (Pratiwi et al., 2006,).

The mechanisms of antioxidants are: 1) interact directly with oxidants, free radicals; 2) prevent the formation of reactive oxygen species; 3) fixed the damage that arises (Ong et al., 1995).

Provision of probiotics is the reason for the blood creatinine profile does not increase in a state of stress.

The results of Wyss and Kaddurrah (2000) and Adriani et al. (2017) show adequate availability of glucose in muscle cells inhibiting the creatine kinase enzyme to catalyze creatine phosphate to creatinine.

Associated with the results of these studies of Wyss and Kaddurrah (2000), it can be explained that probiotics contain glucosamine, a source of glucose as a precursor of energy. So the phosphate creatine decomposition becomes less creatinine. With the provision of fermented

milk as a probiotic in broiler chicken, it will improve kidney function so that kidney function and filtration by glomerulus can increase which is the creatinine levels will decrease at the end.

High dose probiotics have been hypothesized to metabolize the nitrogenous waste products. Parameters of blood glucose result of variance analysis shows that the provision of fermented milk has no significant effect ( $P>0.05$ ) to decrease blood glucose level on broiler chicken. Blood glucose is derived from several sources, such as from food carbohydrates, glycogenic compounds through glycogenogenesis, as well as from liver glycogen by glycogenesis. The guard system of glucose profiles in the blood of livestock through the process of glycolysis, gluconeogenesis, etc. so that the blood glucose concentration will be relatively constant (Poedjiadi, 1994; Adriani et al., 2015).

Consumption of probiotics can simply improve glycemic control. Modification of intestinal microbiota by probiotic supplementation may be a method for preventing and controlling hyperglycemia in clinical practice (Yuting et al., 2015).

## CONCLUSIONS

The given of fermented milk and fermented soy milk has a significant effect in decreasing creatinine levels, but had no significant effect in decreasing blood glucose in broiler chicken.

The given of fermented milk with mixture of two bacteria – *Lactobacillus acidophilus*, and Bifidobacteria can decrease creatinine in the highest levels compared to other treatments.

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