Effects Of Fermented Milk And Soybean With Probiotic On Blood Lipid In Rats

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Abstract. Probiotics have several positive benefits such as reducing pathogenic bacteria population to help food digestion and lowering blood lipid levels. Therefore, a study was conducted to evaluate the effect of fermented cow milk, soybean milk, and combination of both with probiotic bacteria on HDL, LDL, and blood cholesterol level of rats. A total of 30 rats, 8 weeks old were used in this study in a Complete Randomized Design. The rats were randomly assigned into 5 treatment and replicated 5 times of 1 rat each. The rats in first group (P0) were given basal feed without probiotic, P1 = basal feed + fermented cow milk; P2 = basal feed + 75% fermented milk + 25% soybean milk; P3 = basal feed + 50% fermented cow milk + 50% fermented soybean milk; P4 = basal feed + fermented cow milk with different bacteria. There was a non-significant reduction in LDL, and cholesterol level of rats, also non-significant addition in HDL due to probiotic supplementation and the highest reduction is in group fed P4. Addition of probiotic in diet of rats gave the same respond, but have beneficial effect in improving blood cholesterol although non-significant different

1. Introduction

Cholesterol plays a major role in human heart health. Cholesterol can be both good and bad. High-density lipoprotein (HDL) is good cholesterol and low-density lipoprotein (LDL) is bad cholesterol. High cholesterol in serum is a leading risk factor for human cardiovascular disease such as coronary heart disease and stroke America's number one killer [1]. Excess cholesterol in the bloodstream can form plaque (a thick, hard deposit) in artery walls. The cholesterol or plaque build-up causes arteries to become thicker, harder and less flexible, slowing down and sometimes blocking blood flow to the heart. When blood flow is restricted, angina (chest pain) can result. A heart attack will result when blood flow to the heart is severely impaired and a clot stops blood flow completely. When there is too much LDL cholesterol in the blood, it is deposited inside the blood vessels, where it can build up to hard deposits and cause atherosclerosis, the disease process that underlies heart attacks.

Cholesterol is formed by fatty acid oxidation (β-oxidation) in the mitochondria liver cells, into Acetyl CoA. The cholesterol is also synthesized by the liver (endogenous), and from food (exogenous). The triglycerides and cholesterol esters, which are derived from food, will be absorbed by the intestinal mucosal cells. Then, it will be re-synthesized and wrapped with the amount of protein and secreted in the kilo micron form to the lymph system and in the end, it circulated throughout the body [2]. The high synthesis of triglycerides and cholesterol will elevate the levels of triglycerides and cholesterol in the blood, the normal blood cholesterol levels have ranged from 52-148 mg/dL [3]. There are various ways to regulate and decrease the triglycerides and cholesterol blood levels. One of the ways is the use of probiotic. Probiotic is a live microbial as a dietary supplement that has beneficial effects for health by improving the balance of microbes in the digestive tract [4]. Probiotic bacteria such as lipolytic bacteria produces Bile Salt Hydrolase (BSH) enzyme. Lactic acid bacteria (LAB) grown in fermented milk as probiotic can also produce BSH enzymes. The BSH enzymes
The ability of probiotics to decrease the blood triglyceride levels is associated with the activity of acetyl CoA-carboxylase as an enzyme which plays a role in the rate of fatty acid synthesis [6]. [7] stated that the acetyl CoA-carboxylase produces malonyl CoA as the key of metabolism to regulate the synthesis of fatty acids and oxidation, but it can be affected by changes in diet and intestinal activity. Moreover, probiotics can produce statins. Statin is 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMG-CoA reductase) inhibitors, which are cholesterol biosynthesis regulators, Low Density Lowering (LDL)-lowering, Very Low Density Lipoprotein (VLDL), and blood triglyceride levels [8]. The mechanism of decreasing triglyceride levels by statins begins when the inhibitor reduces cholesterol concentrations in hepatocytes and improves the performance of LDL-receptors [9]. The receptors are also related to the VLDL components, which can reduce triglyceride level. Furthermore, [10] indicated that decreasing the synthesis of fatty acid in the liver is a primary factor to decrease the triglyceride synthesis in the liver, which resulting in low triglyceride concentration in the blood plasma.

A number of studies have been carried out to investigate the role of probiotics in lipid metabolism [10, 11]. For example, [12] have studied the combination of chocolate and probiotic yoghurt that can improve blood lipid, and reduce LDL level in the blood of rat. [11] have investigated the role of probiotics on lipid profiles and reported the effect of probiotics on total cholesterol and LDL. However, there is a little study on the role of probiotics in reducing blood lipid concentration particularly improving HDL blood in broilers.

The product that contains probiotic is fermented milk. Fermented milk can be made from various types of milk such as soybean milk and cow milk. These two products contain compounds or substances that can control the blood cholesterol, HDL and LDL levels.

The combination of fermented soybean milk and cow milk in decreasing blood triglyceride and cholesterol levels in broilers have done, however, there is no study on the combination of fermented soybean milk and cow milk in decreasing others blood lipid levels in rats, so the research is very important done.

The purpose of this study is therefore to determine the effect of fermented cow milk and soybean milk, and the combination of both with probiotic bacteria on blood cholesterol, HDL and LDL of blood of rats.

2. Materials And Methods
A total of 30 female rat, age 2 month were used in the experiment. The experiment was conducted for 30 days. The fermented milk as a probiotic was given by force feeding. The composition is based on the variant fermented milk of each treatment. The dose of fermented milk is 1.25% of the body weight [13]. Cow milk and soybean milk were used as the basic ingredient for fermentation milk. The fermentation process used bacteria, namely Streptococcus thermophilus, Lactobacillus bulgaricus, Lactobacillus acidophilus and Bifidobacteria sp. The balance of probiotic bacteria used is 1:1 [14]. Rat’s standard food (pellet) in this research, and 5 treatment was used. Probiotic yoghurt is taken from product of Lovita yoghurt. The concentration of probiotic yoghurt was given to the rats was calculated following the research of [14]. The experiment design used Completely Randomized Design with 5 treatments and 6 replications. The experiment units were 30, and each unit contained 1 female rats.

The treatments consisted of: T0 = Basal ration (without fermented milk), T1 = Basal ration + fermented cow milk, T2 = Basal ration + probiotic 50% fermented cow milk: 50% fermented soybean milk, T3 = Basal ration + probiotic 75% fermented cow milk: 25% fermented soybean milk, T4 = Basal ration mixed with combination of fermented cow milk with different probiotic, Bifidobacteria sp and Lactobacillus acidophilus.

Cholesterol, HDL and LDL were analyzed using Biolabo products. Cholesterol was analyzed by CHOD-PAP (Cholesterol Oxidase Phenylperoxidase Amino Phenoazonphenol) methods.
Data measurements obtained using spectrophotometers (Shandong, China) were analyzed statistically using the One way Analyzed of Variance (ANOVA) and followed by Tukey-test (Honestly Significant Difference / HSD) to test the average value between treatments. The hypothesis applied in this study is the level of giving probiotic with ratio 50% cow milk: 50% soybean milk with dose 1.25% of body weight (T4) can give the most significant influence. In this study, three parameters were measured: blood cholesterol, HDL and LDL levels.

3. Results And Discussion

3.1. Blood Cholesterol Level
Table 1. showed that the average blood cholesterol levels ranging from 80.27-113.03 mg/dl. According to [4] total normal blood cholesterol levels in broilers ranged from 52-148 mg/dL. Hence, most cholesterol levels in each treatment were at normal levels, except in T1 and T5 can decrease the blood cholesterol until 27-29%, compare to control. Nevertheless, the result of the statistical analysis showed that the fermented milk as probiotic supplementation have non significant effect (P >0.05) in decreasing blood cholesterol level.

|          | P0           | P1           | P2           | P3           | P4           |
|----------|--------------|--------------|--------------|--------------|--------------|
| Cholesterol (mg/dl) | 113.03 ± 50.03 | 80.27 ± 43.18 | 93.02 ± 21.49 | 96.9 ±17.28 | 81.52 ± 9.62 |
| HDL      | 45.27 ± 22.52 | 41.90 ± 18.67 | 45.33 ± 23.42 | 49.46 ± 13.83 | 41.54 ± 14.84 |
| LDL      | 36.17 ± 29.70 | 23.97 ± 51.57 | 23.65 ± 8.07  | 30.53 ± 31.56 | 29.27 ± 15.61 |

Carbohydrate type in soybean milk consists of oligosaccharide group, it cannot be used by LAB as energy sources or carbon sources. This is supplied by cow milk as it contains lactose that can be used as a food source for LAB. The lactose helped LAB to maintain them to keep alive and to be able to remodel and ferment carbohydrates, which are contained in fermented cow milk and also the combination of fermented cow milk and soybean milk.

Biliary salt deconjugation and cholesterol assimilation process in T1 and T5 have better performances compared to other treatments. However, the LAB in T5 has better performances than others. The LAB produced BSH enzymes that hydrolyze or break the C-24 N-Acyl amide bond formed between bile acids and amino acids in conjugated bile salts. The bile salts are deconjugated by separating the glycine or taurine from steroids to produce free or conjugated bile salts in the less absorbed free cholesterol acids formed by the small intestine [15, 16]. Unconjugated bile salts have a lower solubility rate at physiological pH when produced. If the production makes it more hydrophobic, it will be less ionic. It will have an impact on low intestinal absorption rates. As a result, secondary bile acids will be settled in the intestine and released through the feces [17]. The release of bile acids causes a decrease in the number of bile acids absorbed in the enterohepatic cycle. In that cycle, bacteria released bile salt hydrolase enzymes, to break the glycine or taurine from steroids, and resulting in free or deconjugated bile salts [18]. Thus, it has an effect in increasing de novo bile synthesis [19]. The formation of bile acids requires cholesterol as a precursor, so then biliary salt deconjugated indirectly. It can decrease blood cholesterol levels because the existing cholesterol is used to form new bile acids. In the mechanism of cholesterol assimilation, LAB will absorb the micelle cholesterol in the lumen intestine and will enter on the cellular membrane of bacteria and converted into carotenol, which is a sterol that cannot be absorbed by the intestine; so it will not be absorbed and tends to be released with faces [20, 21].

The content of isoflavones in soybeans also plays a role in reducing blood cholesterol levels. It can be seen in T2 and T3. The fermentation process can activate isoflavone in the soybean milk and changed into aglycone by a β-glucosidase enzyme. The aglycone acts to increase soybean milk activity.
in order to lower blood cholesterol level [22]. Aglycone in genistein and daidzein soybean can increase bile acid excretion and regulate LDL receptor activity [23]. Moreover, it was also reported that the flavonoids in the soybeans were able to inhibit the activity of 3-hydroxy-3-methyl-glutaryl CoA (HMG CoA) enzyme, which inhibits synthesis of cholesterol and ACAT (acyl-CoA). The compound will compete with HMG CoA to bind the HMG CoA reductase enzyme, then to inhibit the cholesterol synthesis [24]. Cholesterol acyltransferase acts in lowering cholesterol esterification in the intestine and liver [25]. Hence, the blood cholesterol levels will decrease.

The soybean also contains a phytosterol, which acts to bind and inhibit the absorption of cholesterol from food in the intestine. It means that the Niemann-Pick C1 Like 1 (NPC1L1) enzyme transporter cannot promote cholesterol absorption into the lymph system. The cholesterol-binding phytosterol enters the intestinal lumen by the ABCG5 enzyme transporter. Then, it will leave through the feces. However, another study indicated that the succeeding cholesterol moves to the endoplasmic reticulum by Acyl-CoA Cholesterol Acetyl Transferase 2 (ACAT 2) enzyme, which is further esterified into Kilo micron. That action will inhibit the activity of HMG CoA reductase to stimulate the formation of cholesterol, which will reduce cholesterol synthesis, and thereby can lower blood cholesterol [26].

3.2. Low Density Lipoprotein (LDL)

The Duncan test results in Table 1. Showed that LDL levels between treatments were not significantly different. All treatments showed a positive effect of lowering LDL levels although statistically not significantly different (p> 0.05) compare to control. Adding probiotic bacteria can decrease up to 33.7%. Reduced levels of LDL in rats on fermented milk, fermented soy milk and the combination also occur due to the activity of lactic acid bacteria producing bile salt hydrolyze enzymes that hydrolyze or break the C-24 N-Acyl amide bond formed between bile acids and acids amino in conjugated bile salts. BAL activity. Bile salt hydrolyase (BSH) enzyme deconjugates bile salts by separating glycine or taurine from steroids, produce free bile salts or deconjugate in the form of free cholic acid less absorbed by the small intestine [5].

Aside from lactic acid bacteria the decrease in blood LDL levels is also caused by the presence of isoflavones contained in soybeans. The fermentation process in soybeans will hydrolyze isoflavones into free isoflavone compounds called aglycons. Isoflavones of flavonoids that are polyphenolic compounds. Isoflavones can liberate one hydrogen atom from one reducing group associated with one free radical, synthesizing the 3-hydroxy-3-methylglutaril-CoA (HMG-CoA) synthesizer becomes inhibited which acts as a cholesterol-forming precursor and then the oxidation reaction of LDL cholesterol is inhibited.

Isoflavones in the treatment can dilate blood vessels and lower LDL oxidation levels, also inhibit the activity of HMG-CoA enzyme that plays a role in inhibition of cholesterol synthesis and acylCoA enzyme: Cholesteryl acyltransferase which plays a role in decreasing esterification of cholesterol in the intestine and liver, and able to bind LDL lipophilic flavonoid properties. The role of isoflavones here as antioxidants is a compound capable of inhibiting the oxidation of other molecules, which demonstrate the ability to prevent oxidation of Low Density Lipoprotein (LDL) by capturing free radicals.

The next factor, the HDL present in the blood plasma that binds to cholesterol or its ester and carries it along the bloodstream from the peripheral cells to the liver cells. The cholesterol that is bound to undergo the process of reshuffle produce liver cholesterol reserves, cholesterol reserves required for the synthesis of VLDL and biosynthesis of other compounds. VLDL binds LDL by receptor molecules on the peripheral tissue membranes inhibition by HDL, so that high levels of HDL will prevent LDL accumulation in blood vessel walls.

Based on the results of this study showed that LDL level of blood rats was lower than control of either fermented milk treatment or the combination of fermented milk and fermented soy milk. HDL levels have an inverse relationship with each other with various diseases, so the ratio of LDL / HDL cholesterol is an important predictive parameter [27]. Low blood levels of LDL are good for
health, where the risk of constricts of blood vessels will be low because cholesterol is transported throughout the body slightly.

A product can know the level of safety and health of the ratio of LDL / HDL. This research can increase the ratio of LDL / HDL of blood that is on control, is 1: 1.2 become respectively 1: 1.69 ; 1: 1.75; 1: 2.8; 1: 1.63, and 1: 1.42 / Based on these results, in this study obtained the ratio of LDL / HDL above the ideal number, the ratio of LDL/HDL of monogastric blood is 1: 1.6, therefore obtained security and health is higher than control.

4. Conclusion
Supplementation basal rations with fermented cow’s milk (T1) and the combination of fermented cow milk and soybean milk with Lactobacillus acidophilus and Bifidobacteria microbes (T5) showed the highest decreases in blood cholesterol compare to others. High Density Lipoprotein and Low Density in all treatments gave the same results. However the balance of LDL/HDL have improved. Using fermented cow milk, fermented soybeans or the combination of both with Lactobacillus acidophilus, Lactobacillus bulgaricus, and Streptococcus thermophilus, therefore it is recommended compare to without adding probiotic.

Acknowledgements
We gratefully would like to thank a group of students in conducting the study of the utilization of probiotic. Furthermore, we would also like to thank the PUPIT Project in 2016 of The Ministry of Research, Technology and Higher Education and also Padjadjaran University.

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