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Research Article

Effect of Fermented Cow and Soybean Milk with Probiotic in Improving Blood Cholesterol and Triglyceride Levels on Broilers

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Abstract

Background and Objective: Probiotics have several positive benefits, such as reducing pathogenic bacteria population, providing enzymes to help food digestion and lowering blood lipid levels. This study aimed to determine the effect of fermented cow and soybean milk containing probiotic bacteria on the levels of blood cholesterol and triglyceride of broiler chickens. **Methodology:** The experiment applied the Completely Randomized Design with six levels ration and used 120 day-old broiler chicks. Six treatments were designed: T0, T1, T2, T3, T4 and T5 with 4 replications. The total experimental units were 24, which contained 5 chicken each. The treatments were; T0: Basal ration, T1: Basal ration+cow milk, T2: Basal ration+fermented cow milk, T3: Basal ration+fermented soybean milk, T4: Basal ration+fermented cow and Soybean milk and T5: Basal ration+fermented cow milk with *Lactobacillus acidophilus* and *Bifidobacteria* with ratio 1 : 1. **Results:** Result indicated that the addition of probiotic bacteria (T5) have a significant effect ($p<0.05$) to decrease the levels of cholesterol and triglyceride in the broiler chickens. T5 has the lowest blood cholesterol and triglyceride levels, i.e., 108.25 and 32.93 mg dL^{-1}, respectively. **Conclusion:** The fermented cow and soybean milk with *Lactobacillus acidophilus* and *Bifidobacteria* (T5) had the best result in decreasing blood cholesterol and triglyceride levels in broilers.

Key words: Blood lipid levels, broiler chicken, fermented cow milk, food digestion, soybean milk

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INTRODUCTION

Triglycerides and cholesterol are two separate types of lipids or fats circulating in the blood. Triglycerides are a form of stored fat as energy reserves in the adipose and liver tissue cells. When the cell requires energy, the lipase enzyme in the cell breaks the triglycerides into glycerol and fatty acids that are released into the blood vessels[1]. Cholesterol is formed by fatty acid oxidation (β-oxidation) in the mitochondria liver cells into Acetyl CoA[2].

The triglycerides and cholesterol can be synthesized by the liver (endogenous) and resulted from food (exogenous). The triglycerides and cholesterol esters derived from food will be absorbed by the intestinal mucosal cells. It will be re-synthesized and wrapped with the amount of protein and secreted to the lymph system (in kilomicron) and circulated throughout the body[3]. Consequently, triglycerides and cholesterol levels will be elevated in the blood because of the high synthesis occurred. This condition needs to be controlled as the normal blood levels, for example in broilers, are <150 mg dL^{-1} for triglycerides[4] and 52-148 mg dL^{-1} for cholesterol[5].

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[6]. It has been regarded as a way to regulate and decrease the blood triglycerides and cholesterol levels because of the enzymes or inhibitors produced by the probiotic bacterias. Bile Salt Hydrolase (BSH) enzymes play a role in decreasing cholesterol by hydrolyzing or breaking the C-24 N-Acyl amide bond formed between bile acids and amino acids in conjugated bile salts[7], while Acetyl CoA-carboxylase enzymes is associated with the ability to decrease the blood triglyceride levels as it plays a role in the rate of fatty acid synthesis[8]. Moreover, Statin or 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMG-CoA reductase) inhibitors produced by probiotic bacteria have a function as cholesterol biosynthesis regulators that can decrease Low-Density Lipoprotein (LDL), Very Low-Density Lipoprotein (VLDL) and blood triglyceride levels[9].

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

Products containing probiotics are fermented milk. It can be produced from various types of milk such as soybean and cow milk. These two products contain compounds or substances that can control blood cholesterol and triglyceride levels. However, there is no study on the combination of fermented soybean and cow milk in decreasing blood triglyceride and cholesterol levels in broilers.

The purpose of this study was therefore to determine the effect of fermented cow and soybean milks and the combination of both milk with probiotic bacteria on the blood cholesterol and triglyceride levels of broiler chickens.

MATERIALS AND METHODS

Materials: A total of 120 day-old broiler chicks were used in the experiment. The experiment was conducted for 35 days. The fermented milk as a probiotic was mixed in rations. The composition was based on the type of the fermented milk of each treatment. The dose of fermented milk is 1.25% of the broiler body weight. Cow and soybean milk were used as the basic ingredients to be fermented. The fermentation process used bacteria, namely *Streptococcus thermophilus*, *Lactobacillus bulgaricus*, *Lactobacillus acidophilus* and *Bifidobacteria* sp.

Methods: The experiment design used Completely Randomized Design with 6 treatments and 4 replications. The total experimental units were 24 and each unit contained 5 day-old chicks. The treatments consisted of:

- T0 = Basal ration (without fermented milk)
- T1 = Basal ration mixed with the cow milk at a dose of 1.25% of body weight
- T2 = Basal ration mixed with the fermented cow milk at a dose of 1.25% of body weight
- T3 = Basal ration mixed with the fermented soybean milk at a dose of 1.25% of body weight
- T4 = Basal ration mixed with the combination of fermented cow and soybean milk (ratio 1:1) at a dose of 1.25% of body weight
- T5 = Basal ration mixed with the combination of fermented cow and soybean milk (ratio 1:1) using different probiotics, i.e., *Bifidobacteria* sp. and *Lactobacillus acidophilus*

Cholesterol and triglycerides were analyzed using Biolabo product. CHOD-PAP (Cholesterol Oxidase Phenylperoxidase Amino Phenozonphenol) method was used to analyze the cholesterol[12]. GPO (Glycerol-3-Phosphate Oxidase) method was used to analyze the triglycerides[13].

Measured data obtained by using spectrophotometers (Shandong, China) were analyzed statistically using the one-way analysis of variance (ANOVA). It was followed by Tukey-test (Honestly Significant Difference/HSD) for comparisons among means. The hypothesis applied in this study is that the level of probiotics with the ratio of fermented cow and soybean (ration 1 : 1) at a dose of 1.25% of body weight can have the most significant influence to reduce the levels of blood cholesterol and triglycerides.

RESULTS AND DISCUSSION

Blood cholesterol level: Table 1 shows the average blood cholesterol levels per treatment. The blood cholesterol levels in T0: 137,47 mg dL^{-1}, T2: 129.08 mg dL^{-1}, T4: 118.20 mg dL^{-1} and T5: 108.25 mg dL^{-1} were at normal levels as the values were in the range of 52-148 mg dL^{-1} [5]. These treatments have better results in decreasing the blood cholesterol levels compared to; T1: 171,86 mg dL^{-1} and T3: 155,37 mg dL^{-1} that had high blood cholesterol levels in broilers. However, based on the result of the statistical analysis, only the treatments using the fermented milk (T4 and T5) had a significant effect ($p<0.05$) in decreasing blood cholesterol levels.

The decreases of the blood cholesterol levels in T4 and T5 were primarily due to the combination of the fermented cow and soybean milk. The presence of Lactic Acid Bacteria (LAB) and active substances contained in the fermented soybean milk can decrease the blood cholesterol levels[14]. Although, soybean milk consists of an oligosaccharide group of carbohydrates, it cannot be used by the LAB as energy or carbon sources. This is supplied by the fermented cow milk as it contains lactose that can be used as a food source for the LAB.

Biliary salt deconjugation and cholesterol assimilation processes in T4 and T5 decreased the blood cholesterol levels. These processes are related to the LAB used in the treatments. In fact, from the results, it can be seen that the LAB in T5 (i.e, *Bifidobacteria* sp and *Lactobacillus acidophilus*) had lower blood cholesterol level than T4 (i.e, *Streptococcus thermophillus* and *Lactobacillus bulgaricus*). This LAB produce BSH enzymes that break 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[7,15,16]. Unconjugated bile salts have a lower solubility rate at physiological pH when produced. If the production is more hydrophobic, 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 faeces[17,18]. Similarly, 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 coprostanol, which is a sterol that cannot be absorbed by the intestine. Therefore, it will not be absorbed and released with the faeces[19,20].

The content of isoflavones in soybeans also plays a role in reducing blood cholesterol levels[21]. The fermentation process (in T4 and T5) can activate isoflavone and change it into aglycone by a β-glucosidase enzyme. Aglycone in genistein and daidzein soybean can increase bile acid excretion and regulate LDL receptor activity[22]. 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[23]. Hence, the synthesis of cholesterol and ACAT (acyl-CoA) were inhibited resulting in the lower values of the blood cholesterol levels. The soybean also contains a phytosterol, which acts to bind and inhibit the absorption of cholesterol from food in the intestine. The cholesterol-binding phytosterol enters the intestinal lumen by the ABCG5 enzyme transporter that will be released through the faeces[24].

Blood triglyceride levels: The average blood triglyceride levels of all treatments (Table 1) were in the normal ranges (<150 mg dL^{-1} [4]). T5 has the lowest value of blood triglyceride level (32.93 mg dL^{-1}) compared to the other treatments; (T0: 111.67 mg dL^{-1}, T1: 106.14 mg dL^{-1}, T3: 103.53 mg dL^{-1}, T2: 101.15 mg dL^{-1} and T4: 97.39 mg dL^{-1}).

The result of the statistical analysis showed that the supplementation of fermented cow and soybean milk as probiotic using *Bifidobacteria* sp and *Lactobacillus acidophilus* (T5) had a significant effect ($p<0.05$) in lowering blood triglyceride level. This might be related to the type of bacterias and the use of fermented milk[14].

Table 1: The average levels of blood cholesterol and triglycerides in broilers

	Treatments (mg dL^{-1})					
Parameters	T0	T1	T2	T3	T4	T5
Triglyceride	111.67±23.13[b]	106.14±27.79[b]	101.15±37.12[b]	103.53±22.96[b]	97.39±23.17[b]	32.93±5.56[a]
Cholesterol	137.47±24.19[ab]	171.86±16.23[c]	129.08±26.83[ab]	155.37±19.70[bc]	118.20±21.16[a]	108.25±17.88[a]

[a,b,c]Means with different superscript are significantly different ($p<0.05$)

Several studies have indicated that specific bacterias have an effect on blood lipid concentrations[25,26]. These are associated with differences in specific metabolite production among the bacterias[27] or with survival ability in acid and bile environment[28]. Apparently, the bacterias applied in T5 (*Bifidobacteria* sp and *Lactobacillus acidophilus*) were able to survive in acid and bile environments and easily colonize the human intestinal tract[28]. Therefore, the blood triglyceride levels in broilers were low.

The LAB in all treatments in principle can influence the process of fatty acid synthesis in the broilers, which can also be useful to lower blood triglyceride levels. LAB can effectively decrease the activity of acetyl CoA carboxylase as an enzyme that plays a role in the rate of fatty acid synthesis. It is a positive correlation that fewer secretion of acetyl CoA carboxylase will form less fatty acids. Decreasing fatty acids forms was associated with the decrease of triglycerides levels[9,29].

The mechanism of cholesterol assimilation by LAB will also help to decrease the blood triglyceride levels. The assimilation of cholesterol leads for disturbing micelle formation. The lower micelle form will decline lipid absorption in the intestinal lumen and eventually reduce the number of triglycerides in the bloodstream and lower the blood triglyceride levels[30].

The use of the fermented soybean milk in the treatments (T3, T4, and T5) also helped to decrease blood triglyceride levels in broilers. This is because the isoflavones contained in soybean plays an important role in activating Peroxisome Proliferator-activated Receptor-α (PPAR-α). PPAR-α can decrease gene activity to produce triglycerides. This PPAR-α can also increase lipoprotein lipase activity that has a role as lipolysis triglyceride in kilomicron and VLDL[31]. Moreover, the content of flavonoids in soybeans can inhibit the early stages of the triglyceride synthesis reaction. Flavonoids will release one of H atom from hydroxy acetone phosphate group then bind free-radical. Thus, the activation energy is reduced resulting in the limitation of the triglyceride oxidation reaction[32].

CONCLUSION

In the present study, supplementing basal rations with the fermented cow and soybean milk using *Lactobacillus acidophilus and Bifidobacteria* microbes (T5) was the best treatment to decrease the blood cholesterol and triglyceride levels in broilers. The levels of the blood cholesterol and triglycerides of broilers could be decreased to 108.25 ± 17.88 and 32.93 ± 5.56 mg dL^{-1}, respectively.

SIGNIFICANT STATEMENT

This study discovered that the use of fermented cow and soybean milk with *Lactobacillus acidophilus* and *Bifidobacteria* in broiler's diet had the potential to reduce the blood cholesterol and triglyceride levels. This study will help the researchers to explore new ways of using fermented milk in the diet of broilers to produce healthy chicken. Thus a new theory on using the combination of cow and soybean fermented milk in the ration of broilers may be arrived at.

RECOMMENDATION

The results of the study provide an important information for the use of probiotics in broilers, particularly for decreasing blood cholesterol and triglyceride levels.

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