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EFFECTS OF SUPPLEMENTATION OF COW'S MILK AND SOYBEAN MILK FERMENTED WITH PROBIOTIC BACTERIA ON BLOOD LIPID LEVELS AND MEAT QUALITY OF BROILER CHICKEN

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Abstract

The aim of this study was to determine the supplementation of cow's milk-based probiotics and fermented soybean milk on blood lipids profile such as cholesterol levels, triglycerides, and meat cholesterol levels of broiler chicken. The study was conducted from January to February 2018. The method used was experimental with Completely Randomized Design (CRD) and to see the treatment effects. The data were analyzed using Analysis of Variance followed by Duncan's multiple range test. The treatment consisted of five treatments with four replications, namely P0 = control diet/ without giving probiotics, P1 = 100% fermented cow's milk, P2 = 50% fermented cow's milk + 50% soy milk fermentation, P3 = 75% fermented cow's milk + 25% soy milk fermentation. The results showed that the administration of probiotics had a significant effect (P <0.05) on a decrease in blood triglyceride levels and meat cholesterol compared with other treatments. There were no significant effect (P>0.05) on blood cholesterol levels among treatments. In conclusion, the administration of 100% fermented cow's milk and 75% fermented cow's milk + 25% fermented soy milk reduced blood triglyceride levels and meat cholesterol which is improved meat quality.

***Key words**: fermented milk, blood cholesterol, meat cholesterol, blood triglycerides, broiler chickens, probiotics.*

INTRODUCTION

Chicken meat is one of the main protein sources for humans. The price of chicken meat is lower compared with the price of beef or mutton. Therefore, the demand for chicken meat continues to increase as a result of the poultry industry in Indonesia is growing. Chicken meat, including poultry products which are foodstuffs, must be safe for consumption. People crave animal foods, especially poultry with low cholesterol and fat content.

High levels of cholesterol and triglycerides in diet can have a negative impact on health such as causing symptoms of pancreatitis and increasing the risk of arteriosclerosis (Wijaya et al., 2013).

Efforts to reduce cholesterol and triglyceride levels in livestock, especially in broiler chickens need attention, one of which is by providing probiotics.

Probiotics are living organisms that are commonly used as additional feed (feed additives), which when consumed can improve the health of livestock by balancing the microflora in the digestive tract.

Fermented products with milk-based probiotic bacteria and soy milk are known to reduce cholesterol and triglyceride levels. The consumption of probiotics is beneficial for the ecosystem balance in the intestinal tract by increasing the population of lactic acid bacteria and decreasing the population of pathogenic bacteria that have an impact on the process of absorption and improving animal health.

Some studies have been carried out to investigate the role of probiotics in lipid metabolism. Adriani et al. (2018) studied the use of fermented cow and soybean milk with *Lactobacillus acidophilus*, and *Bifidobacteria* in broiler's diet had to reduce the blood cholesterol and triglyceride levels.

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

MATERIALS AND METHODS

This study involved 100 broiler chickens. Probiotics are given at the age of 3 to 45 days. The type of probiotic bacteria consists of

Streptococcus thermophillus, Lactobacillus bulgaricus, Lactobacillus acidophilus, and *Bifidobacterium*. Probiotics used in the form of yogurt with the force-feeding method given 2% of the average body weight of chickens (Adriani et al., 2015).

Sampling was carried out at the end of the study, taken from blood and broiler meat. One bird per treatment and replication was taken for sample. Meat samples were taken from the chest and thighs.

Determination of blood and meat cholesterol levels was carried out by the CHOD-PAP (Cholesterol Oxidase Phenylperoxidase Amino Phenozonphenol) method (Richmond, 1983) and triglyceride

analysis using the GPO (Glycerol-3-Phosphate Oxidase) method.

Data obtained from the results of the analysis were tested using the one-way analysis of variance test (ANOVA) and significant results were then tested using the Duncan's multiple range test. The study was conducted by experimental method using a Completely Randomized Design (CRD) with 4 treatments 5 replications, 20 experimental units, bringing a total of 100 tails.

The study was the effect of fermented cow and soybean milks with probiotic bacteria on the meat cholesterol levels, cholesterol and blood triglyceride levels on broiler chickens so that the following experimental treatments were obtained:

P0= Basal ration (without fermented milk)

P1= Basal ration + 100% fermented cow's milk

P2= Basal ration + probiotic 50% fermented cow's milk + 50% fermented soy milk

P3= Basal ration + probiotics 75% fermented cow's milk + 25% fermented soy milk

RESULTS AND DISCUSSIONS

The results of the analysis of meat cholesterol levels, blood cholesterol, and triglyceride levels in broiler chickens are presented in Table 1.

Table 1. Effects of fermented milk and soybean using probiotic on blood cholesterol, meat cholesterol, and blood triglyceride in broiler

	P0	P1	P2	P3
Blood Cholesterol (mg/dL)	150.99 ± 6.73^{a}	135.49 ± 7.19^{a}	147.35 ± 19.92^{a}	141.34 ± 14.04^{a}
Meat Cholesterol (µg/mg)	13.93 ± 1.87^{b}	10.64 ± 1.97^{a}	12.19 ± 1.40^{ab}	10.65 ± 0.90^{a}
Blood Triglyceride (mg/dL)	93.00 ± 9.67^{b}	53.50 ± 7.30^{a}	72.67 ± 21.85^{ab}	65.17 ± 17.91^{a}

Blood Cholesterol Level

The average results show that blood cholesterol level treatments are in the normal range, except those without probiotics (P0) are above the normal range. According to Mitruka (1981) in Manoppo, et al. (2007) that the normal total blood cholesterol level in broiler chickens ranges from 52-148 mg/dL.

The results showed that the supplementation of probiotic-based fermented milk gave the same response to the average blood cholesterol level, but overall the average of each treatment given probiotic-based fermented milk tended to decrease the total blood cholesterol level.

The average blood cholesterol level which was given probiotic-based fermented milk treatment was relatively lower than P0 (without treatment) indicating that the supplementation of probiotics was able to balance the microflora in the digestive tract. According to Surono (2004) and Lengkey and Adriani (2013) states that the cholesterol reduction process is one of them because of the activity of lactic acid bacteria that produce enzymes that hydrolyze bile salt hydrolase or sever the bond of C-24, N-acyl amides formed between bile acids and amino acids in the conjugated bile salts. Lactic Acid Bacteria (LAB) produces the enzyme bile salt hydrolase (BSH), which is a deconjugated bile salt to release the glycine or taurine of steroids to produce bile salt-freeor cause to form colic acid which is poorly absorbed by the small intestine. This result shows that probiotics can increase the synthesis of bile salts which results in more cholesterol needed to synthesize bile salts so that it will reduce cholesterol levels in the blood, but this study has not had a significant effect. This is supported by the study by Sumardi et al. (2016), which confirms the supplementation of probiotics is not significantly different in reducing blood cholesterol levels.

Decrease in blood cholesterol levels, due to the presence of compounds produced such as short-chain fatty acids from the fermentation process by LAB which competes with Hydroxy Methyl Glutatyil-CoA reductase (HMG CoA reductase) which plays a role in mevalonate formation in the cholesterol synthesis process, so cholesterol synthesis will be hampered (Voet et al., 1999; Sudha et al., 2009). One of the LAB components is propionic acid. Propionate can inhibit the incorporation of acetate into plasma triacylglycerol. This element will result in decreased cholesterol synthesis because acetate is a precursor to cholesterol formation (Marie et al., 2000).

Meat Cholesterol Level

The results of statistical analysis of meat cholesterol levels ranging from the highest to the lowest P0 = 12.93 μg/mg, P2 = 12.19 μg / mg, P3 = 10.65 μg / mg, and P1 = 10.64 μg / mg. Statistical analysis showed that the results were significantly different ($P < 0.05$) to decrease meat cholesterol levels.

The results showed that P2 treatment had no significant effect, but treatment P1 and P3 significantly affected the treatment of P0 in reducing cholesterol levels in broiler chicken meat. This finding might indicates that probiotics are capable of producing cholesterol reductase enzymes. The cholesterol reductase enzyme can convert cholesterol to coprostanol, a type of sterol that cannot be absorbed by the intestine. Coprostanol is a natural steroid that can be produced by bacteria in the lower intestine of humans or animals and released through feces (Andi et al., 2015).

The treatment of P1 (100% fermented cow's milk) and P3 (75% of fermented cow's milk and 25% of fermented soy milk) reduced broiler chicken meat cholesterol level. Previous study showed that the higher percentage of cow's milk the higher value of lactic acid levels study (Abu Bakar and Syalawudin, 1999). The difference in basic materials will affect microbial activity in culture, because the basic ingredients affect the growth of lactic acid formation (Tamime and Deeth, 1980).

Flavonoid compounds contained in soy milk are also able to reduce cholesterol levels in meat. Flavonoid is one of the phytochemical groups that have the same structure, namely polyphenols, whose mechanism can reduce cholesterol levels due to HMG-CoA (Hydroxy Methyl Glutatyil-CoA)reductase activity, reduce the activity of the enzyme acyl-CoA cholesterol acyltransferase (ACAT), and reduce cholesterol absorption in the digestive tract (Choi et al., 2008).

Isoflavone compounds in soy milk are also reported to reduce cholesterol levels in addition to flavonoids. Isoflavones are included in the class of flavonoids which are polyphenolic compounds in soy milk. The mechanism for reducing cholesterol by isoflavones is explained by the effect of increasing fat cell catabolism for energy formation which results in a decrease in cholesterol content (Sekiya 2000; Pawiroharsono, 2007).

The conclusion shows that blood cholesterol levels decrease while meat cholesterol levels decrease and are significant. This finding occurs in P1 and P3 treatments, which is 100% fermented cow's milk, and 75% fermented cow's milk + 25% fermented soy milk, respectively (Figure 1).

Blood Triglyceride

The average blood triglyceride level of broiler chickens <150 mg/dL (Basmacioglu and Ergul, 2005), Other previous study of Melluzi et al. (1992) reported that normal averages of triglyceride levels were 47.2-162 mg/dL. This shows that blood triglyceride levels in each treatment are in the normal range.

The decrease of triglyceride levels blood in broiler chicken the supplementation of 100% fermented cow's milk (P1) in the diet in the present study maybe due to the ability of probiotic bacteria to produce statins that play a role in the biosynthesis of triglycerides. In line with the statement of Cavallini et al. (2009) that probiotics can produce statins, namely 3-hydroxy-3-methyl-glutaryl-CoA reductase inhibitors (HMG-CoA reductase) which are cholesterol biosynthesis regulating enzymes, lowering LDL, VLDL, and blood triglyceride levels.

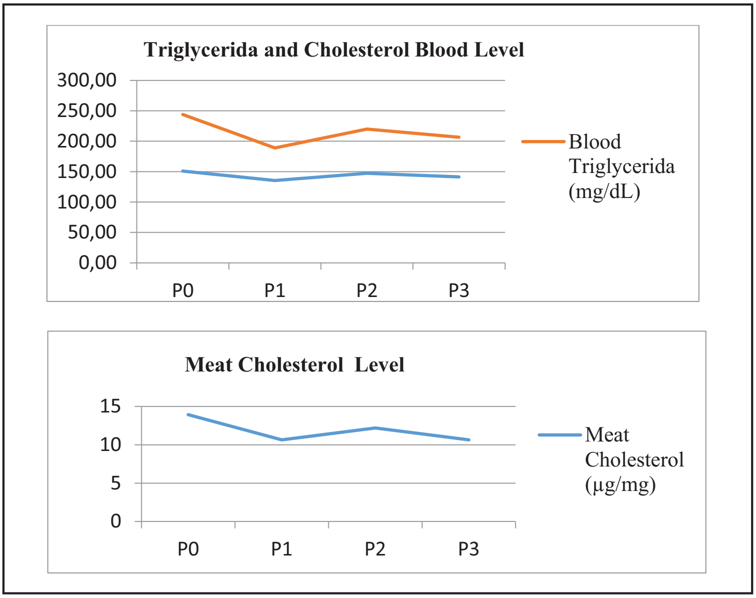

Figure 1. Mean blood cholesterol, blood triglycerides, and meat cholesterol

The mechanism of decreasing triglyceride levels by statins begins when the inhibitor reduces cholesterol concentration in hepatocytes and increases the performance of LDL-receptors that are closely related to VLDL components so that triglycerides will be reduced (Grundy, 1988). The occurrence of inhibition of triglyceride synthesis in the liver and small intestine will result in a decrease in blood triglyceride levels. Scorve et al. (1993) reported that the reduction of fatty acid synthesis in the liver is the main factor causing the decline in triglyceride synthesis in the liver which results in a reduction of the concentration of triglycerides in plasma.

Supplementation of 75% fermented cow's milk + 25% fermented soy milk (P2) decreased triglyceride in plasma due to the closely related decrease in the number of pathogenic bacteria in the intestine. Adriani, et al. (2008) reported that probiotic bacteria such as *Lactobacillus acidophilus* and *Bifidobacterium* can inhibit the growth of pathogenic bacteria. Moreover, Purwati et al. (2005) showed that probiotic administration created a balance of intestinal microflora because the presence of BAL in the intestine which creates an acidic atmosphere that suppresses the growth of pathogenic bacteria in the small intestine. The acidic environment inhibit the secretion of the lipase enzyme, therefore the synthesis of fatty acids in the digestive tract decreases and causes fat to be brought to the liver to decrease which results in a reduction in blood triglyceride levels.

Abu Bakar and Syawaludin (1999) reported that the manufacture of fermented milk with the addition of soy milk. That study recommended that the addition be done at a maximum of 20% of the amo„unt of cow milk that will be made yogurt, so that the lactic acid level and pH of the fermented milk are optimal. Therefore, supplementation to 50% fermented cow milk: 50% fermented soy milk (P2) does not have a significant effect in reducing blood triglyceride levels. It assumed that carbohydrates contained in soybeans are a group of oligosaccharides that are underutilized as an energy source or as a carbon source for LAB so that the fermentation process is not perfect and causes the LAB population in broiler digestive tract lesser than normal condition, so it cannot inhibit the absorption of lipids correctly.

Flavonoid in soybean is another factor to reduce triglyceride levels in blood. Flavonoid in soybeans can reduce the activity of Glycerol-3-Phosphate Dehydrogenase (GPDH), which is an enzyme that plays a role in the synthesis of triglycerides. Ta'inindari and Sopandi (2013) reported that flavonoids could inhibit the activity of the GPDH enzyme in adipocytes. Other active substances in soybeans are isoflavones (genistein and daidzein). Isoflavones belong to the flavonoid group. The amount of free isoflavones (aglycones) contained in fermented soy milk is high so that it can activate Peroxisome Proliferator-activated α receptors (PPAR α) (Medjakovic et al., 2010; Kersten, 2001; Kartika and Siti., 2016). PPAR α plays a role in decreasing gene activity which produces triglyceride availability for Very Low-Density Lipoprotein (VLDL) and increases lipoprotein lipase activity. Lipoprotein lipase has a role in lipolysis of triglycerides in chylomicrons and VLDL. The increase in these activities occurs the breakdown of triglycerides into fatty acids and glycerol. This finding is consistent with research by Rayalam and Mary Anne (2007) reporting that injection of genistein and daidzenin can stimulate the occurrence of lipolysis in mice.

CONCLUSIONS

Supplementation of basal ratio with cow's milk and fermented milk can reduce blood cholesterol levels, blood triglycerides, and cholesterol in broiler chicken meat.

Supplementation of probiotics-based fermented milk with 100% fermented cow's milk and 75% fermented cow's milk + 25% fermented soy milk can increase blood triglyceride levels and broiler chicken fat optimally.

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