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IMPROVING MILK AND SOYBEAN FERMENTED WITH PROBIOTIC BACTERIA ON HDL AND LDL BROILER BLOOD

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Abstract

Probiotics can play an important role in immunological, digestive and respiratory functions and could have a significant effect in alleviating lipid. Therefore, a study was conducted to evaluate the effect of Milk and Soybean Fermented with Probiotic on cholesterol status i.e. High Density Lipoprotein (HDL) and Low Density Lipoprotein (LDL) indices of broilers. Materials and Methods: A total of 120, 5 weeks old broilers were used in this study in a Complete Randomized Design (CRD). The birds were randomly assigned into six treatment groups of P0, P1, P2, P3, P4 and P5 with 24 birds treatment G^1 replicated 4 times of 5 birds each. The broiler in first group (P0) basal feed, (P1) basal feed with cow's milk, (P2) basal feed with milk fermented, (P3)basal feed with soy milk fermented, (P4) basal feed with combination milk fermented and soy milk fermented, (P5) basal feed with combination milk fermented and soy milk fermented with different bacteria. Results: There were non-significant (p>0.05) increasing High Density Lipoprotein (HDL) level of broiler due to probiotic supplementation. Increasing blood HDL levels is (69.73 mg/dL) in group fed P4 (combination milk fermented and soy milk fermented) compared to control (45,16 mg/dL). A statistically significant (p<0.05) decrease in total number of Low Density Lipoprotein (LDL) level. Lowest LDL level (33.36 mg/dL) was found in group fed (P4) combination milk fermented and soy milk fermented. In conclusion, addition of probiotic milk fermented with soy milk fermented had beneficial effect increasing HDL levels and decreasing LDL levels broiler blood.

Key words: *probiotic, fermented milk, fermented soy milk, broiler, HDL, LDL.*

INTRODUCTION

Cholesterol in the blood circulation in lipoprotein particles. In lipoprotein, the most influence on cholesterol is High Density Lipoprotein (HDL) and Low Density Lipoprotein (LDL). The level of HDL and LDL cholesterol is needed to determine which is the total amount of LDL, HDL and 1/5 triglycerides in each deciliter of blood. Usually, the total and HDL cholesterol levels can describe the general conditions of cholesterol levels.

The goal of this study is expected to add science and knowledge, especially regarding meat quality of broilers. In addition, fermented milk, soy milk and the combinations with probiotics bacteria can increase levels of high density lipoprotein (HDL) and decrease levels of low density lipoprotein (LDL) blood broiler

HDL removes cholesterol from tissues and transports it to the liver. HDL is created mostly from components from other degraded lipoproteins. HDL converts cholesterol to cholesteryl esters by LCAT, an enzyme activated by apoA-I in HDL. HDL appears to get cholesterol to the liver 1) by transfer the cholesteryl ester to VLDL which after degradation IDL and LDL is taken to the liver and 2) by direct interactions between HDL and the liver via a specific HDL receptor. The liver disposes of cholesterol as bile acids. HDL is also called "good cholesterol" because it is associated with lowering cholesterol levels.

VLDLs are synthesized by the liver, like chylomicrons, are degraded by lipoprotein lipase. VLDL, IDL, and LDL are interrelated. IDL and LDL appear in the circulation as VLDL remnants. VLDL is converted to LDL by removal of all proteins except apo B-100 and esterification of most of the cholesterol by lecithin-cholesterol acyl transferase (LCAT) associated with HDLs. The esterification occurs by transfer of a fatty acid from lecithin to cholesterol (forming lysolecithin).

HDL can be classified into larger, less dense HDL_2 or smaller, denser HDL_3 which falls within the density ranges 1.063–1.125 and 1.125–1.210 g/mL, respectively. Although the major proportion of HDL is normally present in HDL_3, individual variability in HDL levels in human populations usually reflects different amounts of HDL_2.

Giving probiotics is one of the efforts to decrease LDL and increase HDL blood broiler. Probiotics are living organisms used as feed supplements when consumed can improve animal health by balancing the microflora in the digestive tract. One of the food products that contain probiotics are fermented milk and fermented soy milk are known to lower LDL and raise HDL blood. Based on previous studies it is known that fermented soy milk contains flavonoids. Flavonoids are one of the components which can reduce cholesterol by inhibiting the action of the enzyme system 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase. Based on this, the authors are interested in doing a study entitled "Improving Milk and Soybean Fermented with Probiotik Bacteria on HDL and LDL Broiler Blood".

The existence of the cholesterol in the body is very important because it has a function as a component of the cell membrane, so that the body can not function without cholesterol (Laurencio, 2002). Blood cholesterol is influenced by the levels of HDL and LDL cholesterol is a major component of total blood cholesterol (Hendromartono, 2004). Low density lipoprotein (LDL) is a vehicle to bring a lot to the tissues. Normal levels of LDL in the blood of broilers is <130 mg/dL and HDL>22 mg/dL (Basmacioglu and Ergul, 2005).

Milk fermented containing lactic acid, bacteria that can lower total cholesterol, LDL cholesterol, and triglycerides and to increase HDL cholesterol. The processing of the soybean will hydrolyze isoflavone compounds into free aglycone isoflavones higher activity. Lactic acid bacteria in fermented soy milk has a very important role in improving the digestibility of soy isoflavones. This is due to the activity of β-glucosidase enzyme in the bacteria that can hydrolyze aglycon isoflavones into a compound that is easily absorbed (Larkin et al., 2009).

This research is very important for knowing combination milk and soy milk with probiotic bacteria that have result to increase HDL and decrease LDL.

MATERIALS AND METHODS

Methodology: 120 broilers were adapted period of 1 week was given to the birds, following which a trial was conducted that lasted 5 weeks. The broilers were offered the maintenance ration throughout the study. A total of 120, 5 weeks old were used in this study in a Complete Randomized Design (CRD). The birds were randomly assigned into six treatment groups of P0, P1, P2, P3, P4 and P5 with 24 broilers treatment G^1 group replicated 4 times of 5 broilers replicate G^1. The birds in the first group (P0) were given basal feed without milk, while as other groups were supplemented with milk (P1), milk fermented (P2), soy milk fermented (P3), combination milk and soy milk fermented (P4), and combination milk and soy fermented with different bacteria (P5). The feed and potable water were supplied *ad libitum* throughout the experimental period of 5 weeks, with strict adherence to all the conventional management practices.

Parameters recorded: Blood samples were randomly collected from 24 broilers per replicate at the end of study. The samples were analyzed at the Laboratory of Physiology and Biochemistry, Faculty of Animal Husbandry, University of Padjadjaran. The parameters recorded were HDL and LDL levels. The HDL and LDL was estimated using a HDL and LDL Direct method using Biolabo KIT.

Statistical analysis: Data collected were subjected to analysis of variance (ANOVA) as per Steel and Torrie[18] and where ever means difference existed, they were compared using Honestly Significant Difference (Tukey test) with 5% significant level.

The treatment consists of :

P_0 = Basal feed (Control)
P_1 = Basal feed with cow's milk (M)
P_2 = Basal feed with milk fermented (MF)
P_3 = Basal feed with soy milk fermented (SMF)
P_4 = Basal ration with milk fermented + soy milk fermented (MF+SMF)
P_5 =Basal ration with milk fermented + soy milk fermented, different bacteria (MF+SMF with different bacteria)

The measured variables were:

1. High Density Lipoprotein (HDL)
2. Low Density Lipoprotein (LDL)

RESULTS AND DISCUSSIONS

Based on data from Table 1. it can be seen that the average highest to the lowest HDL levels, respectivelly is P4 (MF+SMF)= 69.73 mg/dL, P3 (SMF) = 64.19 mg/dL, P5 (MF+SMF with different bacteria) = 62.95 mg/dL, P2 (MF) = 60.51 mg/dL, P0 (Control) = 45.16 mg/dL and P1 (M) = 45.11 mg/dL.

Table1. Average High Density Lipoprotein and Low Density Lipoprotein in Broilers Blood

PARAMETER	TREATMENT					
	P0	P1	P2	P3	P4	P5
	………………….mg/dL…………………..					
HDL	45.16 ^{a} ± 14.53	45.11 ^{a} ± 12.38	60.51 ^{a} ± 25.48	64.19 ^{a} ± 8.74	69.73 ^{a} ± 14.76	62.95 ^{a} ± 6.22
LDL	52.96^{a} ± 19.47	102.83^{b} ± 27.14	43.73 ^{a} ± 24.99	55.7 ^{a} ± 6.88	33.36 ^{a} ± 22.46	35.66 ^{a} ± 29.31

For more details can be seen in the graph in figure 1.

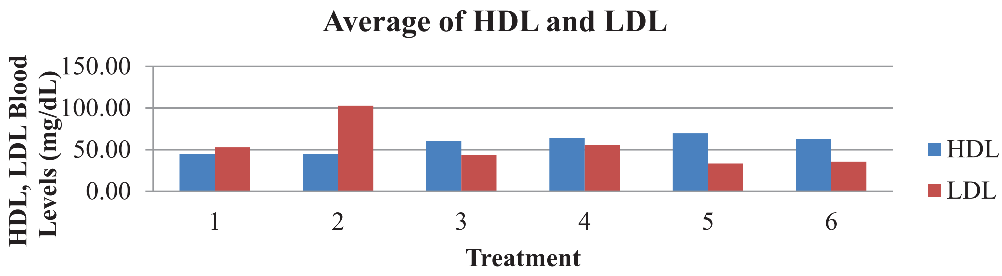

Figure 1. Effect of fermented milk, fermented soy milk and their combination on high density lipoprotein (HDL) and low density lipoprotein (LDL) broiler blood

According Basmacioglu and Ergul (2005), normal HDL levels in the blood of broilers is more than 22 mg/dL. All the treatments in the normal range, based on Table 1. and Figure 1., showed that P2 (MF), P5 (MF+SMF with different bacteria), P3 (SMF) and P4 (MF+SMF) is able to increase cholesterol levels, while levels of HDL P1 (M) lower than other treatments but there is in the normal range. Result of statistical analysis using analysis of variance showed that adding of milk fermented, soy milk fermented, and the combinations was not significantly different (P> 0.05) increase blood HDL levels. Although was not significant difference but the level tend increases. HDL is the smallest lipoprotein particles produced in the liver and small intestine, has the highest density because it contains more protein than cholesterol. The content is the most direct apolipoprotein Apo A-I and Apo A-II. The liver synthesizes lipoproteins as complexes of apolipoproteins and phospholipids, which form particles of cholesterol-free, the complex is capable of taking cholesterol carried internally of cells through the interaction with the ATP-binding cassette transporter AI (ABCA1), an enzyme plasma called lecithin-cholesterol acyl-transferase (LCAT) converts free cholesterol into cholesteryl ester (a more hydrophobic form of cholesterol), which is sequestered into the core of the lipoprotein particles, eventually causing the newly synthesized HDL. HDL particles increases because circulate through the bloodstream and incorporate more cholesterol and phospholipid molecules from cells and other lipoproteins, for example, by interaction with the transporter ABCG1 and Phospholipids Transport Protein (PLTP) (Murray, 2009).

The HDL binds cholesterol and phospholipids in the blood and transfer to other lipoproteins to be released into the bloodstream and flows throughout the body. The results of Moses et al. (2006) study showed that HDL can maintain a balance and not to accumulate inside the cell, the balance is managed by the removal of sterols from the membrane at a rate equal to the amount of cholesterol is synthesized to the liver (Diestchy, 2003).

Increased blood levels of HDL also due to the present of isoflavones from soy milk fermented. The fermentation process will hydrolyze soy isoflavones into compounds called free aglycone isoflavones. This is due to the activity of β-glucosidase enzyme in the bacteria that can hydrolyze isoflavone aglycone into a compound that easily absorbed (Larkin et al., 2009). Isoflavones activate the Peroxisome Proliferator activated receptor α (PPAR α), such as increasing the synthesis of lipoprotein lipase which can increase the catabolism of triglyceride-rich VLDL. HDL levels vary with plasma triacylglycerol and directly with the activity of lipoprotein lipase. This process is caused by byproducts that are release from hydrolysis chylomicrons and VLDL cholesterol, phospholipids, and Apo A-I to form a pre β-HDL. PPARα also can increase the expression of Apo A-I and Apo A-II directly from the cycle and establish praβ HDL-cholesterol after binding with phospholipids and cholesterol. The final result from isoflavones activated will increase HDL cholesterol (Medjakovic et al., 2010).

Based on data from Table 1, the average highest to the lowest levels of LDL, respectively is in P1 (M) = 102.83 mg/dL, P3 (SMF) = 55.70 mg/dL, P0 (Control) = 52.96 mg/dL, P2 (MF) = 43.73 mg/dL, P5 (MF+SMF with different bacteria) = 35.66 mg/dL and P4 (MF+SMF) = 33.36 mg/dL.

According Basmacioglu and Ergul (2005) that normal LDL levels in the blood is <130 mg/dL. Blood levels of LDL in each treatment are in the normal range. Based on Table 1 and Figure 1 showed that P2 (MF) and P4 (MF+SMF) can decrease blood LDL levels, while LDL levels P1 (M) and P3 (SMF) increased compared to the other treatments, but the number is still in the normal range. Results indicate that adding of milk fermented, soy milk fermented and the combinations have significant effect ($P<0.05$) decrease LDL levels.

Decreased levels of blood LDL broiler chicken in milk fermentation occurs 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. Activities LAB produces the enzyme bile salt hydrolase (BSH) deconyugated bile salts by separating the glycine or taurine of steroids to produce bile salt-free or cause to form cholic acid-free which is poorly absorbed by the small intestine (Surono, 2004; Lengkey and Lovita, 2013) ,

A decrease in blood LDL with combination fermented cow's milk and soy milk is the best result, due to the presence source of food for lactic acid bacteria. Cow's milk contents lactose, sources of food for Lactic acid bacteria, while the type of carbohydrate in soy milk fermented can not be used as a food source.

The nutritional content of cow's milk and soy milk are high in carbohydrates and fat causes an increase in the amount of cholesterol that is hydrolyzed by the help of bile salts in the intestine. Carbohydrates in soy milk oligosaccharides consist of classes that can not be used as an energy source and a carbon source by lactic acid bacteria. In addition, the soy milk is not lactose which is a food source for lactic acid bacteria, so that the fermentation process is not perfect. This causes the population of BAL in the digestive tract of broilers was not optimum, so it is not capable of inhibiting the absorption of cholesterol that cause increase LDL in blood. Thus, dietary inclusion of milk, soy milk and the combination fermented is recommended for the welfare of broilers.

Flavonoids contain in probiotic may release one hydrogen atom from one cluster reducing associated with one free radical synthesis of forming 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) to be blocked that serves as a precursor forming cholesterol and subsequent oxidation of LDL cholesterol is inhibited (Reynertson, 2007).

High LDL levels will cause more cholesterol attached to the walls blood vessels at the time of transport carried out and slowly it will to form stacks which precipitate, such as plaque (Graha, 2010)

Comparison of HDL and LDL levels

HDL levels of broiler chickens in this study did not experience a significant increase and LDL levels did not decrease significantly, but can be seen changes in the balance between the levels of HDL/LDL each treatment, shown in Table 2.

Table 2. Comparison of Blood levels of HDL and LDL Broiler

Treatment	HDL (mg/dL)	LDL (mg/dL)	Comparation
P0	45.16	52.96	0.85 : 1
P1	45.11	102.83	0.44 : 1
P2	60.51	43.73	1.38 : 1
P3	64.19	55.70	1.15 : 1
P4	69.73	33.36	2.09 : 1
P5	62.95	35.66	1.76 : 1

The best ratio of HDL and LDL are 2.09:1 (P4). Levels of HDL have an inverse relationship to each other with various illnesses, so that the ratio of HDL/LDL is an important predictive parameter (Murray et al., 2009).

Low levels of blood LDL are good for health, where the risk of blood vessel will be low, because cholesterol is transported throughout the body slightly.

This research can improve the ratio of HDL/LDL in the blood of 2.09:1 in P4. According Laihad study (2000) that rasio HDL/LDL blood of broiler chickens was 1.6:1.

CONCLUSIONS

Based on the results of this study concluded that:

Fermented cow's milk, soy milk fermentation and the combination of fermented cow's milk with soy milk fermented able to increase blood HDL levels despite statistical improvement was no significant difference in the numbers HDL levels, but using fermented milk and soy milk cause the number of HDL tend increase, while cow's milk causes a decrease in blood HDL levels.

The best treatment for decreasing blood LDL levels is combination fermented cow's milk and soy milk in (P4) = 33.36 mg/dL.

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