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THE EFFECT OF FERMENTED MILK, SOY MILK AND THE COMBINATION OF IT ON MEAT CHOLESTEROL AND INTESTINE PH OF BROILER

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Abstract

This research is aimed to determine the effect of giving fermented milk, soymilk, and thecombinationof it on meat cholesterol and intestine pH of broiler. The research was conducted on December, 19th2016 to February, 16th 2017 at Cipacing Village, Jatinangor, Sumedang, West Java, Indonesia. Thisexperiment uses a Completelty Randomized Design (CRD) in five kinds of treatment. Those are T0 (Control), T1 (basal ration with cow's milk), T2 (basal ration with fermented milk), T3 (basal ration with fermented soymilk), T4 (basal ration with fermented milk and fermented soy milk combination in comparison of 1:1) done with four replications. There are 20 experimental units. In each unit, there are five heads with 100 broilers for the 35-day maintenance. Based on the statistical analysis of ANOVA, the result has no significant effect (P>0,05) on cholesterol content of meat. Besides, the result shows that the total of cholesterol content of meat and intestine pH of broiler tend to decrease equal to T0=0.00% (Control), T1= 2.24% (102.55±3,56), T2= 5.42% (99.22±3,06), T3= 2.29% (102.50±3,00), T4= 8.65% (95.83±17,88). The conclusion of this research is that giving of fermented milk, fermented soy milk, and combination of it can decrease meat cholesterol up to 8.65% and intestine pH of broiler.

Key words*: Fermented milk, Fermented soy milk, broiler, Meat cholesterol, Intestine pH.*

INTRODUCTION

One of the animal protein sources that has high nutritional value is meat. Meat got a top rank as one of the most animal protein sources consumed by enormous number of people due to the fact of its delicious taste and high nutritional value. One of common sources of meat is broiler.

Based on the General Directorate of Livestock and Animal Health, data processed by the Agriculture Data and Information Services Center in 2015, the average daily feed consumption of broiler meat in Indonesia is 3.9733 kg per capita per year. Chicken meat production in Indonesia reached 1.62711 million tons with a total population of 1,497,625,658 chickens.

The average daily growth of broiler meat demand in the period time of 2015-2019 gainedto 1.90% per year (Direktorat Jenderal Peternakan, 2015).

Broiler as one of meat sources that has high nutritional value is the largest contributor of animal protein from livestock production a leading commodity. The growth of broiler gain peaked due to a meat producing in a relatively short time of five to six weeks.

Cholesterol content of broiler is relatively high compared to native chicken. Part of the broilercarcass that contains cholesterol are chest and thigh. This is because they contain lipids especially on oily skin (Setiawan, 2009).

Cholesterol is the main sterol in animal tissues. It is a typical product of the metabolism of animals. In result, all animal-based production foods such as yolk, meat, liver, and brain clearly contain cholesterol (Murray et. al., 1996 : 248).

Cholesterol in meat can be lowered by probiotic microbes used as feed additive. It can be profitable the host by improving the ecosystem in the digestive tract. One of probiotic products is a fermented milk that can be made from cows and soy milk.

In fermented milk product, there are groups of lactic acid bacteria that can lower cholesterol content. Lactic acid bacteria is found in

probiotics produce Bile Salt Hydrolise (BSH) enzymes through feces together with the cholesterol that causes reducing cholesterol levels (Sunarlim, 2009).

The use of cow's milk and fermented soy milk can lower the pH of the digestive tract of broiler. Increasing the use of probiotics can improve non pathogenic bacteria and reduce bacterial pathogen so that the balance of microflora in the digestive tract of broiler maintained.

When the lactic acid bacteria come into the system, it can reduce the bile acids and lower the pH in the digestive tract. In the acidic pH conditions, most of pathogenic bacteria will come out of the colon

MATERIALS AND METHODS

MATERIALS

The research used 100 broilers with 35-days treatment. The samples of meat are taken at the end of the research. This research has been carried out in 35-days. The broilers are randomly divided in to 20 units with 5 treatment rations, 4 repetitions for each containing 5 broilers.

This research is conducted using the experimental method of Completely Randomized Design (CRD) with 5 kinds of treatments, each treatment is repeated 4 times. Each experimental unit consists of five broilers. In fermented cow milk and soy milk using three kinds of lactic acid bacteria (*Streptococcus thermophillus, Lactobacillus bulgaricus* and *Lactobacillus acidophillus)*

The treatment consists of :

T_0 = Basal Ration (Control)
T_1 = Basal ration with cow milk (CM)
T_2 = Basal raton with fermented milk (FM)
T_3 = Basal ration with fermented soymilk (FSM)
T_4 = Basal ration with fermented milk + fermented soy milk (FCM)

METHODS

The measured variables are:

1. **Meat cholesterol broiler**
   Cholesterol Test CHOD-PAP method (Cholesterol Amino OxidasePHenylperoxidase PHenozonpHenol) (Richmond, 1973).
   Setting up the tube. Filling the first tube with 10 mL of plasma plus 1 mL reagent, a second tube filled 10 mL standard cholesterol, and a third tube is a reagent blank containing 1 mL of color reagent, a standard 1 mL and 1 mL plasma. Incubate for 20 minutes at a temperature of 20-25^{o}C. Measuring the absorbance of the sample and standard absorbance against reagent blank daam 60 minutes. The measurements is using a spectrophotometer with a wavelength of 500 nm, with a calculation:

$$\text{Cholesterol(mg/dL)} = = \frac{AbsorbanSampel}{AbsorbanStandar} x standarkolesterol$$

2. **Intestine pH broiler**
   PH measurements performed with pH instructions (Bloom,1988).
   PH measurement principles that determine the condition of acids and bases. PH testing uses an electronic pH meter. before cleaning the cathode indicator with distilled water to neutral (pH 7 listed). Then clean with a tissue and then placing the cathode put the indicator on broiler intestine and colon.

Probiotics and meat samples are taken and analyzed at the Laboratory of Physiology and Biochemistry, Faculty of Animal Husbandry, Universitas Padjadjaran.

RESULTS AND DISCUSSION

1. The Effect of Fermented Milk, Soy Milk and The Combination of it On Meat Cholesterol Broiler

Table 1. Average Meat Cholesterol in Broiler

Repeat	Treatment				
	T0	T1	T2	T3	T4
			.....mg/dl.....		
1	103.02	98.44	97.81	103.65	101.98
2	103.86	101.56	95.93	105.53	111.37
3	110.32	106.99	103.02	102.40	99.90
4	102.40	103.23	100.10	98.44	70.07
Average	104.90	102.55	99.22	102.50	95.83

Based on the statistical analysis, the research has no significant($P>0.05$) result, but it shows that there is a tendency of cholesterol content of meat of broiler decreased. Based on Table 1, the average daily of the highest cholesterol content of meat to the lowest one are as follows; T0(Control) = 0, 00% (104.90 mg/dl), T1 (CM) = 2.24% (102.55 mg/dl), T3 (FSM) = 2.29% (102.50 mg/dl), T2 (FM) = 5.42% (99.22 mg/dl), T4 (FM+FSM) 8.65 % (95.83 mg/dl), below is the graph for giving informations more detail.

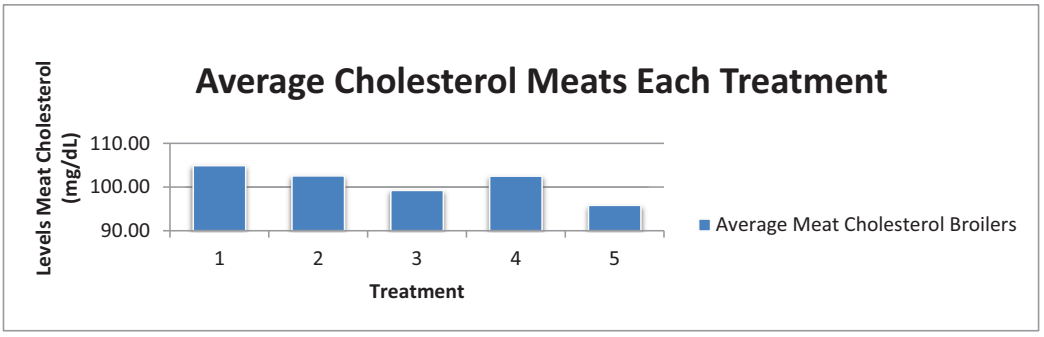

Figure 1. The effect of fermented milk, fermented soy milk and their combinations on meat cholesterol broiler

The lowest reductionis in the treatment of T4 (FM+FSM) (95.83 mg/dl) and the highest one is in the treatment ofT0 (Control) (104.90 mg/dl). The result of this research indicates that giving fermented cow's milk, fermentedsoy milk and their combinations led to a decreased cholesterol content of meat instead of having the untreated meat and cow's milk treatment. Cholesterol content of broiler meat in the treatment of T2(FM), T3 (FSM), and T4 (FM+FSM) produce lower cholesterol content of chicken meat than T0's (Control) and T1's (CM). The resulting of T4 (FM+FSM) treatment is the lowest cholesterol content which amount is 95.83 mg/dl decreased for 8.65% due to the fact that fermented milk can improve the balance of microorganisms in the digestive tract (Daud *et al*, 2007). Fermented milk can reduce the bile acids so that it can lower the pH of intestines in which the beneficial microbes will increase and suppress the growth of harmful microbes' mostly disease-causing microbes (pathogens). The use of probiotics has no negative effect to both livestock and humans who consume livestock (Budiansyah, 2004). In acidic conditions, the

pathogenic bacteria will be reduced so that the nutrients can be absorbed in the intestine optimally (Fuller, 1992).

Probiotics produce Bile Salt Hydrolise (BSH) enzymes to conjugate bile salts. This enzymes result conjugated bile salts and is excreted through feces together with the cholesterol leading to reduce cholesterol content (Sunarlim, 2009). Based on Lee, bile salts will be disposed of through the feces, where the conjugated bile saltthat is unabsorbed by the intestine is more easily removed from the digestive tract compared to the conjugated one. This explain that the more cholesterol is needed to synthesize, the more bile salt will lower cholesterol content as long as lactid acid bacteria binds cholesterol so that it prevents the absorption of cholesterol back to the liver (Lee et al., 2009). Cholesterol assimilation occurs through the mechanisms of cholesterol by lactic acid bacteria cell walls which then it will corporate the cholesterol with bacterial cell membranes, causing reduction in the number of free cholesterol in the body (Surono, 2004).

Maximally lipid absorption occurs in the distal and proximal ileum jejenum, also dekonjugasi bile salts in the ileum by *Lactobacillus* that can affect the efficiency of feed conversion because it has an important role in emulsify and absorption of lipids (Adriani et al., 2015). In addition, the giving of fermented milk products which contains a mixture of three bacterial interactions is better than the second bacterial mixture for fermentation since more bacteria will result more metabolites.

Fermented soy milk also contains lactic acid bacteria that has a very important role in improving the digestibility of isoflavones soy. The effects of isoflavones in decreasing cholesterol have been proven not only in animal testing such as mice and rabbits, but also in broiler and humans. Wider effects evident also are found in the treatment of soyflour; there is not only downing cholesterol content, but also triglycerides VLDL (very low density lipoprotein) and LDL (low density lipoprotein). On the other hand, soy flour can increase HDL (high density lipoprotein) (Amirthaveni and Vijayalakshmi, 2000). Zilliken (1987) elaborates that factor-II (6,7,4' tri-hidroksiisoflavon), isoflavone compounds the greatest effect. Another isoflavones-decreasead mechanism which is explained by its influence to the increasing catabolism of fat cells for energy production resulting in a decrease of cholesterol content.

This research shows that isoflavones from soy is an active substance which has a variety of useful biological activities. Therefore, the increasing of the fermentation of soy isoflavone content is due to the activity of β-glucosidase enzyme in the bacteria that can hydrolyzes isoflavone set to be free isoflavone compounds which is called aglycone (Larkin et al., 2009).Aglycone has higher activity in lowering total cholesterol. Ralston (2005) conductes a researchwhich shows that the enzymes produced by lactid acid bacteria can change flavanones into isoflavone compounds during the fermentation process.

Fermentation process can also hydrolyze aglycone flavone compounds into its glycoside which shows a higher antioxidant activity. Isoflavone compound is one component that is also metabolized. Another compound found in fermented soy milk that can inhibit the absorption of cholesterol is a flavonoid. Flavonoids are also capable of inhibiting the activity of the enzyme 3-hydroxy-3-methylglutaryl CoA that plays a role of the inhibition of cholesterol synthesis and enzyme actylCoA: cholesteryl aciltransferase takes a role in the decline of esterification of cholesterol in the intestine and liver (Fuhrman and Aviram., 2001).

The T4 (FM+FSM) treatment shows thatits cholesterol content is lower than T3 due to the combination of fermented milk and fermented soy milk. This is because of the type of carbohydrate contained in soy milk is not in the form of lactose found in cow's milk so thatlactic acid bacteria could not be taken as an advantage. Lactic acid bacteria does not grow in the fermented soy milk in resulting flavonoid compounds contained in soy cannot be converted into free isoflavone which is called aglycone.Flavonoids in the form of glycosides cannot be absorbed. So in order to make fermented milk requires a mixture of soy milk with cow's milk to get more benefits.Fermented soy milk can lower cholesterol content because of the presence of compounds such as an fatty acid-generated short chain from either fermentation of soy or milk products as a result

of the activity of probiotics in the digestive tract. Such compounds will compete with HMG CoA binding to the *reductase* enzyme of HMG CoA, so that cholesterol synthesis is inhibited (Hardiningsih and Nurhidayat, 2006).

2. The Effect of Fermented Milk, Soy Milk and The Combination of it On pH Intestine Broiler

Table 2. The average of Ileum pH in Broiler

Repetition	Treatments				
	T0	T1	T2	T3	T4
	.....pH of ileum.....				
1	5.97	6.06	5.9	6.03	5.83
2	6.03	5.98	6.2	5.97	6.16
3	6	6.43	6.11	6.01	6.21
4	5.96	5.86	6.23	6.38	6.44
Average	5.99	6.08	6.11	6.10	6.16

Based on the result of statistical, there is a treatment which does not give a real effect (P> 0,55) to the intestine pH of broiler and pH of ileum. From above table, the lowest average pH on ileum can be seen as follows: T0 (5.99), T1 (6.08), T3 (6,10), T2 (6,11), T4 (6, 16). These results indicate that the treatment has no significant effect on pH of ileum. pH of the broiler's digestive tract ranged from 3.47 (gizzard) to 6.43 (small intestine) (Mabelebele et al., 2013). The broiler's digestive system in the small intestine is divided into 3 parts namely dudenum, jejenum and ileum. Duodenum is the small intestine that is grooved and united by the pancreas gland. The pancreas gland produces enzymes and bicarbonates that are channeled into the duodenum. Bicarbonate serves to neutralize the acidity or pH of the intestinal contents.

The non-acidic conditions of the broiler ileum can also be caused by the temperature in the broiler intestine which does not support the growth of lactic acid bacteria. This is in accordance with (Fardias, 1992) who elaborates that the environment which is suitable for living of lactic acid bacteria is temperature, hydrogen potential and nutritional content. The temperatures that are too high will damage the proteins which support the life of bacteria. This damage will result the bacteria being die. Temperatures that are too low will result BAL dormant and do not grow (Fardias, 1992). Lactic acid bacteria has an optimum temperature range of 37^{o}C – 42^{o}C (Husmaini et al., 2011) and can live at pH of 2 - 6.5 (Hardiningsih et al., 2006).

The giving of 1,25% Probiotics dose from broiler body weight is considered ineffective because it does not provide a meaningful effect on the pH conditions of broiler's intestine. Based on many studies, intestine ph will cause the decrease in broiler's colon because only the probiotic bacteria which can enter until the broiler's colon so the pathogenic bacteria in the broiler's digestive track can be reduced and come out with feces. Yet, this study does not analyze the colon ph of broiler.

CONCLUSIONS

The conclusion of this research is giving fermented milk, fermented soymilk, and the combination of it can decrease cholesterol content of broiler meat up to 8.65% (95.83±17.88) at T4 containing fermented milk + fermented soy milk, T2=5.42% (99.22±3.06) fermented cow's milk, T3=2.29% (102.50±3.00) fermented soy milk, and T1=2.24% (102.55±3,56) cow's milk.

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