THE INFLUENCE OF USING FISH FERMENTED BY LACTIC ACID BACTERIA AS FEED SUBSTITUTION ON SERUM LIPID PROFILE OF BROILERS

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ABSTRACT

The study was aimed to examine the effect of the use of fish fermented by lactic acid bacteria (LAB) as a subtitution for fish meal on serum lipid profile of broiler. One hundred and twenty five day-old broiler chicks of CP 707 strains were divided into five groups for five treatments. A Completely Randomized Design was used in this experiment. The treatments were 0 (control), 2, 4, 6 and 8% fish fermented by LAB substitution of basal diet containing 8% fish meal. The parameters were blood lipid profile including total cholesterol, Low Density Lipoprotein (LDL) and High Density Lipoprotein (HDL) cholesterol and triglycerides. Data was analyzed by one-way Analysis of Variance (ANOVA). When the F test indicated a significant effect, the differences between the mean values were analyzed by Duncan's Multiple Range test. Results showed that using fish silage fermented by LAB on broiler's feed did not affect total cholesterol and HDL cholesterol, but significantly affect ($p<0.05$) levels of LDL cholesterol and serum triglycerides of broilers. Based on serum lipid profile, it could be concluded that fish fermented by LAB can be used as a substitute for the 100% fish meal in broiler's feed.

Keywords : fish fermented, lactic acid bacteria, lipid profile

INTRODUCTION

There is a tendency towards increased consumption of fat in which parallel with the growing prosperity of the Indonesian people. Nationally, Indonesian people are still unable to reach the recommended number of nutrient adequacy. About 9% of the population consume more fat than standard safety considered. This fact is supported by the results of the household health survey which showed that death caused by vascular disease was very low in 1970, number seven position in 1986, first rank in 1992 and still in the same position right now.

One risk factor for vascular disease (cardiovascular, hypertension) is high blood cholesterol levels. Same efforts to prevent or reduce blood vessel disease are to lower fat intake or to consume food that is hipokolesterolemik.

Broilers produce meat that contains fat and cholesterol. Excessive fat deposition in meat and abdominal cavity is one of the main problems in the production of broilers (Leenstra, 1985). Fat and cholesterol content of broiler's meat consumed influenced by feedstuffs. Fish meal is one of broiler's feedstuff content as a source of crude protein.

Fulfillment of fish meal for feed ingredients is still dependent on imports, since domestic production can only provide about 5% of that required, while the use of fish meal tended to increase from year to year. One of the alternative products that can replace the role of fish meal as a source of animal protein is fermented fish.

The advantage of fermented fish compared to fish meal is 1) it is high quality and it can be used at any seasons throughout the year; 2) it can be given practically for cattle; 3) preparation method is relatively easy and does not require any special skills /high; 4) the products have a long usage time.

Fermented fish is product of microbiologically fish preservation process using lactic acid bacteria (Junianto, 2003). Lactic acid bacteria are bacteria that are able to produce lactic acid as a result of fermentation of carbohydrates. Lactic acid works to prevent the development of microbial spoilage. *Lactobacillus sp* is a lactic acid bacteria that have a potential as probiotics (Purwandhani and Rahayu, 2003) and its stability can be maintained during storage by dry cell preparation as a probiotics powder (Hartati and

Harmayani, 2006). Lactic acid bacteria are also known as the agent for hypercholesterolemia prevention, as reflected in the high content of High Density Lipoprotein cholesterol (HDL) and low content of Low Density Lipoprotein cholesterol (LDL) in the experimental animals (Harmayani, 2002).

This study was aimed to examine the effect of the use of fish fermented by lactic acid bacteria (LAB) as a substitution for fish meal on lipid profile (total cholesterol, LDL, HDL and triglycerides) of broilers serum. Results are expected to provide information on the use of fish meal fermented by lactic acid bacteria (LAB) as a substitute for fish meal in broiler. The hypothesis of this research was the using of fish meal fermented by LAB will decrease total cholesterol, LDL and triglycerides and increase HDL of broilers serum.

MATERIALS AND METHODS

Broilers, Diet and Samples Collection

One hundred and twenty five day-old broiler chicks of CP 707 strains were allocated in five cages that floor was made of litter and which was supported by a 40 watt lamp, drinker and feeder. The New Castle Disease vaccine strains Lasota was conducted when the broilers aged 5 days. The feed and water were provided *ad libitum.* BR-1 commercial was given at the age of 1-14 days. Feed's treatment was given starting from two-week-old chickens and terminated at the age of 4 weeks. Feed was made iso-protein and iso-energy (20% crude protein and 3000 kcals of ME / kg) containing 8% fish meal.

Blood samples were collected from chickens of each treatment. Blood was withdrawn from the wing vein and the serum samples were analyzed for lipid profile (total cholesterol, HDL cholesterol, LDL cholesterol and triglycerides) content. Analysis was carried out by spectrophotometry commercial kit.

Fish Silage

Fish silage was made by adding *Lactobacillus sp* isolates 2% (w/w) and rice bran as a source of easily digested carbohydrates as much as 4% (w/w) of the weight of fresh fish. Fish was fermented in anaerobic condition for two weeks. The treatment was using fish silage that was dried with oven at 40 ºC and milled into flour. Lactic acid bacteria as starter was isolated from *caecum's* broilers with MRS medium-1% CaCO3 plus 10 ppm sikloheksamat to suppress the growth of yeast and 10 ppm sodium azide to suppress micro aerobic (Rahayu, 2003). Identification of lactic acid bacteria was done by examining the phenotypic traits that appear based on morphological, biochemical and physiological based on Bergey's Manual of Determinative Bacteriology (Holt *et al*., 1999).

Experimental Design

One hundred and twenty five day-old broiler chicks of CP 707 strains were divided into five treatments. A Completely Randomized Design was used in this study. There were five replications for each treatment, and five chickens in each replication. The treatments were 0 (control), 2, 4, 6 and 8% fish silage substitution of basal diet containing 8% of fish meal. The parameters were blood lipid profile including total cholesterol, LDL and HDL cholesterol and triglycerides. Data was analyzed by one-way Analysis of Variance (ANOVA). When the F test indicated a significant effect, the differences between the mean values were analyzed by the procedures of Duncan's multiple range test (Steel and Torrie, 1981).

RESULTS AND DISCUSSION

Table 1 shows that the average of total cholesterol level of blood broiler serum receiving rations of fish fermented by lactic acid bacteria (LAB) as a substitution for fish meal were not significantly different from control. This indicated that there is no change in cholesterol metabolism in the body of broiler chickens. Ration nutrient content did not differ among the treatments, which cause the total cholesterol did not differ. Lactic Acid Bacterias can not change the nutrient but have a potential as probiotics so nutrient content did not differ among the treatments. Cholesterol levels are influenced by different nutritional components in foods (Linder, 1985).

Cholesterol metabolism is catalyzed by enzymes in the body Hydroxy Methyl Glutaril Co-A (HMG Co-A). The reaction controls rate of cholesterol synthesis. HMG Co-A disconnect the Co-A that is then reduced to form as early mevalonat acid formation in cholesterol. Enzyme HMG-Co-A is directly inhibited by dietary cholesterol intake. When cholesterol diet consumed is less, than more cholesterol is synthesized by the liver that causes a reduction of total cholesterol (Hermier, 1997)

Table 1. Total Serum Cholesterol Blood Broiler Chickens Fed Fish Fermented by Lactic Acid Bacteria (LAB)

Treatment	U1	U2	U3	U4	U5	Avg
	………………………………………		mg / dl.	………………………………………		
0	98.41	112.34	95.89	114.80	112.51	106.73
0.02	98.12	94.88	91.23	116.14	120.03	104.08
0.04	89.45	113.17	99.96	74.59	88.99	92.83
0.06	73.80	100.86	97.87	86.88	94.70	90.82
0.08	88.04	118.54	93.35	85.10	72.96	91.60

Total cholesterol in the blood can not describe the effect of cholesterol on health but the type of cholesterol provides a major influence on health conditions. There are two types of cholesterol, which gives a different impact on health, that is LDL cholesterol provide negative effects, while HDL cholesterol had a positive impact on health. Therefore, to know the effect of cholesterol is not only observe seen the changes of total cholesterol in the blood but also the types of cholesterol (Linder, 1985).

Mean blood serum levels of HDL cholesterol of broiler receiving rations of fish fermented by LAB as a substitution for fish meal were not significantly different from control (Table 2). This indicated that transport of cholesterol from tissues back to the heart was not different. High levels of HDL cholesterol in the blood serum will accelerate the transport of cholesterol from the cell edge, which means reducing the accumulation of cholesterol from the blood vessel wall (Linder, 1985). The HDL cholesterol plays a role in transporting cholesterol from body tissues to the liver to be converted into bile acids and then excreted in the bile as the main road into the mechanism of intestinal cholesterol removal from the body (Montgomery *et al.*, 1983).

The mean levels of LDL cholesterol in the control treatment was significantly higher ($P < 0.05$) than those in other treatments (Table 3). This means that diet containing fish fermented by LAB capable to lower LDL cholesterol and triglycerides. Research by Harmayani (2002) showed that LAB are also known as the agent for hypercholesterolemia prevention, as reflected in the high content of High Density Lipoprotein cholesterol (HDL) and low content of Low Density Lipoprotein cholesterol (LDL) in the experimental animals. LDL cholesterol is the end product of VLDL (very low density lipoproteins) with high cholesterol ester content (Linder,1985).

Table 4 shows that blood serum triglyceride levels of broiler chickens receiving the control diet was significantly higher ($P<0.05$) than those of broilers receiving diets containing fish meal fermented by LAB as a substitution for fish meal. The high level of LDL cholesterol is usually followed by high level of triglycerides. Lipids from the diet, mainly the triglyceride hydrolysis, is absorbed and reprocessed in the intestinal mucosa cells into several lipoprotein particles are transported in the form of VLDL and then converted to LDL by the liver (Hermier, 1997). VLDL is the form of triglycerides that is transported from the liver to the tissue outside the liver. The VLDL triglyceride that is reduced the density of particles is thus gradually transformed into LDL cholesterol (Montgomery *et al.*, 1983).

Triglycerides are lipids that are stored as energy reserves in the form of fat tissue (Frandson, 1992). It was stated by Montgomery *et al.* (1993) that triglycerides from digested food after 2-monoasilgliserol forming compounds and fatty acids are absorbed into the mucosal cells. Enzymes within the cell by catalyzing monoasilgliserol asilKoA forming 1,2-diacylglycerol can be asilazed into triglycerides and transported back into the porta as portomikron (Annison, 1983). Portomikron is hydrolyzed by lipoprotein lipase in the capillary walls into fatty acids and glycerol. Fatty acids in the blood as free fatty acid (FFA) and is bound by serum albumin and glycerol back to the liver to be converted into dihydroxy acetone phosphate and enter the glycolytic pathway.

Results of this study showed that the use of fish meal fermented by LAB can be used as a substitution for the 100% fish meal in broiler. LAB has a positive influence on serum lipid profile of broiler as indicated by the low levels of LDL cholesterol and triglycerides blood serum of broilers.

Table 2. Serum of HDL Cholesterol in Blood of Broiler Chickens Fed Fish Fermented by Lactic Acid Bacteria (BAL) as Fish Meal Substitution

Treatment	U1	U2	U3	U4	U5	Avg
	.... mg / dl					
0	1.13	1.88	2.57	1.01	4.11	2.14
0.02	0.50	1.51	4.27	3.14	3.14	2.76
0.04	3.14	1.38	1.63	2.70	2.70	2.03
0.06	2.37	1.63	1.50	3.28	3.28	2.30
0.08	1.64	2.01	2.01	2.39	2.39	2.14

Table 3. Serum of LDL Cholesterol in Blood of Broiler Chickens Fed Fish Fermented by LacticAcid Bacteria (LAB) as Fish Meal Substitution

Treatment	U1	U2	U3	U4	U5	Avg
	....mg / dl					
0	77.43	82.75	72.56	96.10	92.43	84.25[a]
0.02	71.41	67.08	64.52	78.81	84.38	73.24[b]
0.04	76.01	88.31	76.51	62.89	67.77	74.30[b]
0.06	55.03	69.71	76.55	69.44	74.44	69.03[b]
0.08	58.08	89.69	62.18	66.45	57.89	66.86[b]

a-b: The different superscript in the same column indicate the differ significantly ($P<0.05$)

Table 4. Tligliserida Level in Blood of Broiler Chickens Fed Fish Fermented by Lactic Acid Bacteria (LAB) as Fish Meal Substitution

Treatment	U1	U2	U3	U4	U5	Avg
	.... mg / dl..					
0	127.90	129.60	120.70	181.60	157.70	143.50[a]
0.02	102.40	140.40	95.30	71.50	83.20	98.56[b]
0.04	51.50	117.40	109.10	52.00	82.60	82.60[b]
0.06	92.00	147.60	104.10	73.50	84.90	100.42[b]
0.08	141.60	139.20	145.80	85.10	73.40	117.02[b]

a-b: The different superscript in the same column indicate the differ significantly ($P<0.05$)

CONCLUSION

Fish fermented by LAB can be used as a substitution for the 100% fish meal in broiler. Using fish fermented by LAB on broiler's feed did not have a significant effect on the total cholesterol and HDL cholesterol, but significantly lower the LDL cholesterol and serum triglycerides content in broilers.

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