Life Science Informatics Publications

Research Journal of Life Sciences, Bioinformatics, Pharmaceutical and Chemical Sciences

Journal Home page http://www.rjlbpcs.com/

RJLBPCS

ISSN 2454-6348

Original Research Article **DOI - 10.26479/2017.0205.14**

THE UTILIZATION OF LACTIC ACID BACTERIA PROBIOTIC FROM FISH WASTE ON STRAIN LOHMANN BROILER'S LDL (LOW DENCITY LIPOPROTEIN) LEVEL

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ABSTRACT: This study aims to determine whether additional of lactic acid bacteria from sewage force feeding fish can lower blood levels of LDL broiler. Microbes are a source of research material LAB isolates isolated from fish waste phase. And chicken used was *Strain Lohmann* male broiler production by PT. Multi Breeder Adirama as many as 40 fish age 1 day. Which is divided into 4 treatments with each treatment consisted of 10 individuals as replicates, were taken randomly and maintained for 42 days. Data were analyzed by analysis of variance using a completely randomized design unidirectional pattern, followed by a test of Duncan's Multiple Range Test (DMRT).Probiotic Lactic Acid Bacteria Isolates treatment (BAL) were used in this study is the bacterium Streptococcus thermopillus in the form of freeze drying from the Laboratory of Nutritional Biochemistry, Faculty of Animal Husbandry, UGM. I as a control treatment (without BAL) Treatment II BAL cell count was 106 CFU / ml., The third treatment is the number of BAL Cells are 10 7CFU / ml. , IV treatment BAL cell count was 10 8 CFU is / ml. RESULTS: Treatment administration of lactic acid bacteria are not significantly different ($P < 0.05$) on LDL blood of broiler chickens. So the adding of lactic acid bacteria does not affect blood levels of LDL broiler.

KEYWORDS: Freeze drying, lactic acid bacteria, LDL.

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Peer review under responsibility of Life Science Informatics Publications

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1. INTRODUCTION

BAL has important role in industry of yogurt fermentation, cheese, butter, yakult and milk. Research related BAL ability in cholesterol decrease have been done. Grunewald (1982) researched about the effects of skim milk fermented with Lactobacillus acid serum cholesterol level, evaluated using a mouse as experimental animal. This research show that after 4 weeks, cholesterol decreased and suspected factors that affect cholesterol is BAL. this research would be tasted BAL Streptococcus thermophilus in broilers with LDL level of broilers blood parameter. Streptococcus thermophilus is one of BAL which able to metabolize the extreme temperature (hotter). Streptococcus thermophilus has growth temperature 20-53^{o}C and optimum temperature 43-45^{o}C. These bacteria are round/spiral, does not spore, gram positive and homo fermentative, optimum pH for growth is 6,8 and anaerobic. These bacteria are resistant to acidity from 0,85-0,89%. The lactic acid produced is the result of glucose breakdown, fructose, galactose, sucrose and lactose. (Whittier and Webb, 1970). Broilers were chosen because BAL has never been attempted before on this cock. Moreover, broilers are also susceptible to disease, so that probiotics provided can also have function as a replacement antibiotic given to chicken. Broiler is chicken which is the fastest can be cut compared the others. Broiler is most economical livestock, and has high production speed (Bambang Agus Mutidjo, 2004: 8). In Indonesia, broiler is one of fowl which produced of meat widely known in community.

Formulation of Problems: Based on limitation of the problem which talked before, it can be formulated as follows:

1. Does adding of lactic acid bacteria Streptococcus thermophilus in force feeding give affecting the LDL level in broiler's blood?

Research Purpose

The purpose of this research is: To know does adding of lactic acid bacteria Streptococcus thermophilus in force feeding give affecting the LDL level in broiler's blood.

Benefits of Research

1. Theoretical benefit
a. to know information about BAL
b. to know information scientific about *Streptococcus Thermophiles* bacteria and benefit
c. to know BAL work mechanism in affecting the LDL level in broiler's blood
2. Practical benefit
a. To know the effect of BAL giving *Streptococcus Thermophiles* to LDL level in broiler's blood.

Research Design

This research is experimental research. This research was designed using completely randomized with 4 variables: one as control variable and three as treatment variable level of lactic acid bacteria, each treatment consist of 10 times repetition.

Research Variable: Variables were observed in this study is:

1. Independent variable : variations in dosing of lactic acid bacteria is 106cfu/ml, 107cfu/ml and 108cfu/ml, with the following conditions :

R0 : the group without dose of lactic acid bacteria (as a control)

R1 : The group was given dose of lactic acid bacteria in force feeding is 106cfu/ml

R2 : the group was given dose of lactic acid bacteria in force feeding is 107 cfu/ml

R3 : the group was given dose of lactic acid bacteria in force feeding is 108 cfu/ml

2. Dependent variable: LDL level in broiler's blood.

Population and Sample Research

1. The research population: strain broiler age of a day or Day old Chick (DOC)

2. The research sample: 40 broilers *Strain Lohmann* and broilers were divided into four treatments with each treatment consist of 10 as repetition and were taken by random.

Tools and materials

Tools and materials used in this study are as follows:

a. Tool: Test tube, hot plate, pH meter, analytical scale, autoclaves, incubators, Erlenmeyer, centrifuge, petri dish, microtip, colony counter, water bath, spectrophotometer.

b. Material : *Strain Lohmann* broilers are a day old / Day Old Chick (DOC), the ration of chicken feed which is corn flour consisted, rice bran, fish flour, soybean and NaCl (an additional minerals), chicken vitamin (Vita chick), MRS broth, distilled water, HC1 1 N, NaOH, peptone solution, skim milk 10%, MRS agar, chicken's blood sample, chloroform, acetone, alcohol, acetic anhydride.

Research Procedure

This study conducted in several steps. They are:

1. Preparation

This step involves cleaning cages, spraying cage with disinfectant and installation light on the cage.

2. Ration arrangement

Ration arrangement material on this study is corn flour, bran, soybean, fish flour and mix mineral. Ration research is based on calculation of feed ration composition by the NRC (1994). Listed in this table:

Table 1. Composition of feed ingredients and nutrient content

feedstuff	BK %	PK %	ME Kkal/kg	Ca %	Pav %	Met %	Lys %	Trp %	SK (%)	EE (%)
Corn	88,70	8,74	3.350	0,04	0,26	0,21	0,34	0,09	2,50	4,20
Bran	90,59	11,44	3.020	0,05	1,48	0,22	0,58	0,11	11,50	14,10
Soybean	90,00	49,83	2.230	0,28	0,20	0,60	2,67	0,58	6,20	5,70
Fish flour	89,34	61,73	2.219	2,32	1,89	2,67	6,45	1,06	2,60	7,90

Source: feed composition table for Indonesia. Hartadidkk (1994:13).

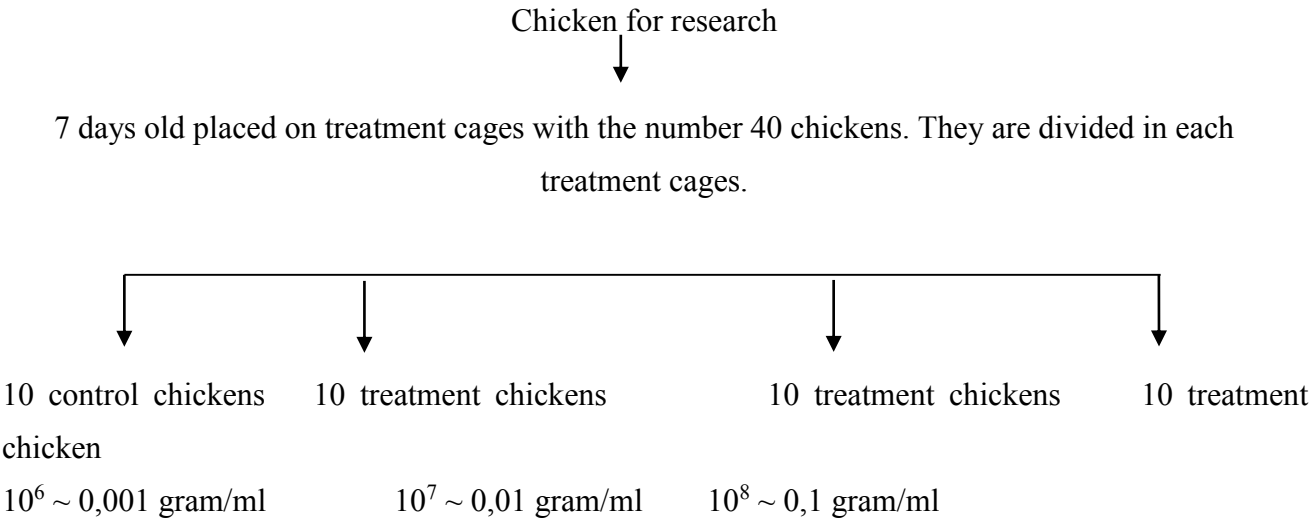
Table 2. Nutrient content of ration research

Feed stuff	Formulation %	PK %	ME Kkal/kg	Ca %	Pav %	Met %	Lys %	Trp %
Grits	60,75	5,31	2.035,13	0,02	0,16	0,13	0,21	0,05
bran	12	1,40	369,95	0,01	1,18	0,03	0,07	0,01
soybean	18	8,97	401,40	0,05	0,04	0,11	0,48	0,10
Fish flour	9	5,40	194,16	0,20	0,17	0,23	0,56	0,09
Top mix	0,25	0,00	0,00	0,00	0,00	0,00	0,00	0,00
amount	100,00	21,08	3.000,64	0,28	0,58	0,50	1,32	0,27

1. Chicken Probiotics application

Application or probiotics adding lactic acid bacteria to chicken was given by force feeding. For R1 group is 10^6cfu/ml or 0,001 gram/ml, for R2 group is 10^7 cfu/ml or 0,01 gram/ml and R3 group is 10^8 or 0,1 gram/ml.

The scheme is as follow:



Measurement of LDL level in broiler's blood

a. Measurement of LDL in broiler's blood

This study used CHOD-PAP method which is an enzymatic colorimetric test procedure is as follows

Reagents used are follows:

1. Reagent – precipitating, 250 ml

(0,68 g/l heparin, about 100.000 IU/1; 0.064 mol/l sodiumsitrat, stabilizer).

2. Reagent solution for cholesterol deterination

The working procedure of LDL-Cholesterol analysis is precipitator reagent was warmed in temperature between 15ºC to 25ºC then entered into centrifuged tube to get supernatant. Supernatant amount100 µl and second reagent solution amount 1000 µ entered into tube then mix carefully. The solution was centrifuged for 15 minutes and approximately than 4000 rpm. Cholesterol concentration in supernatant are determined within 1 hour after centrifugation. Supernatant amount 100 mL and 1000 mL are mixed carefully then incubated for 10 minutes in

temperature 15°C to 25°C or for 5 minutes in temperature 37°C and measured its absorbance (A) to the reagent solution.

The calculations are as follows:

The cholesterol concentration in the supernatant: A.F

	546 nm	500 nm
	1000	700 mg/dl
F	25.9	18.1 mmol/l

The calculation of LDL-cholesterol:

LDL cholesterol = cholesterol amount – Cholesterol in supernatant

Research Design and Data Analysis

This study used a completely randomized design (CRD) pattern in a direction and the data have been obtained from the measurement results were analyzed by Variant Analysis (ANAVA). When the treatment effect significantly, then followed by a test of Duncan's Multiple Range Test (DMRT) (Gaspers, 1991).

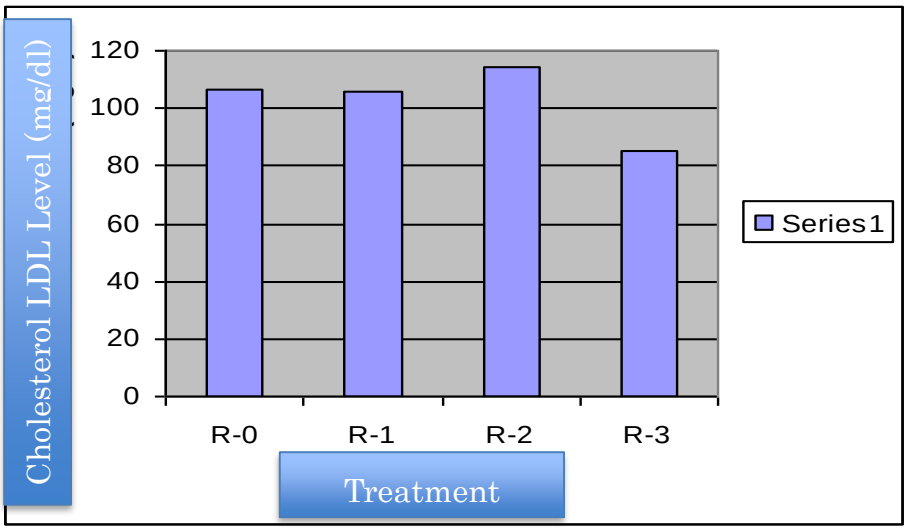
3. RESULTS AND DISCUSSION

A. Low Density Lipoprotein (LDL) Level

Result of LDL Research

Repetition	Treatment			
	R-0	R-1	R-2	R-3
1	109,69	89,11	167,12	61,44
2	96,81	128,53	140,75	124,78
3	78,01	118,92	120,85	71,43
4	149,45	117,52	108,73	68,10
5	116,50	73,57	43,68	101,05
Average^{ns}	110,29	105,53	114,23	85,36

LDL cholesterol has function to transfer cholesterol from liver to the body. High level of LDL cholesterol in blood has risk coronary heart disease. In the other hand, LDL cholesterol level which is not making down through normal way, will give effect cholesterol deposited in blood vessel and trigger atherosclerosis. Rodas et al (1996) said that giving of *Lactobacillus acidophillus* can lower LDL cholesterol greater than without giving these bacteria. This case is supported by research conducted by Sibel et al (1997) which told that yogurt supplementation used L. *Acidophillus* in rat diet can lower serum cholesterol and LDL cholesterol. LDL cholesterol is known as bad cholesterol because stack of LDL cholesterol in the blood vessel can give atherosclerosis. In the other word, if LDL cholesterol is low, it is also low risk of atherosclerosis. Jaspers et al (1984) observed hypocholesterolemic effect in fourteenth day to twenty first after giving the tablet consisting *Lactobacillus acidophilus* dan *L. Delbrueckii subsp. Bulgarius.* Jaspers *et al* (1984) mengamati efek hipokolesterolemik pada hari ke-14 sampai 21 setelah pemberian tablet yang berisi *Lactobacillus acidophilus* dan *L. Delbrueckii subsp. Bulgarius* on volunteers. Research of the subject of rat has been done by Rao et l (1981), Grunewald (1982), Pulusani and Rao (1983) and Akalin et al (1997). Rao et al (1981) reported a decrease of plasma cholesterol in the rats that consuming fermented milk S, *Thermophilus* Grunewald (1982) got the same effect at fermented milk by L. *Acidophilus.* Pulusani and Rao (1982) found decrease of plasma cholesterol and liver in rat was given milk fermented by S. *Thermophilus, L.Bulgaricus,* and *L.acidophilus.* Akalin *et al* (1997) studied the effect of yogurt and *acidophilus* yogurt on cholesterol level of mice. It was found that the average total cholesterol level and LDL level are decrease in *acidophilus* yogurt giving. The increase of the number of *Lactobacillus* and decrease of fecal coliform occur after *acidophilus* yogurt consumed. Based on this study, LDL level were not significantly different ($P \leq 0,05$) every treatment. Treatment of probiotic lactic acid bacteria in LDL broiler is not significantly different ($P < 0,05$). The highest LDL is R2 treatment in lactic acid bacteria 10^7CFU/ml with amount 114,23. The lowest LDL is R3 treatment in lactic acid bacteria 10^8 CFU/ml with amount 85,36. Picture 2.



Picture 2. Graphic of LDL cholesterol level

4. CONCLUSION AND SUGGESTION

A. Conclusion

Based on the result of the research and discussion can be concluded: treatment of lactic acid bacteria *Streptococcus Thermophiles* is not significantly different ($P<0.05$) to LDL level in broiler's blood. Thus, the supply of lactic acid bacteria does not LDL level in broiler's blood.

B. Suggestion

Based on the conclusion, it can be suggested as follows:

1. Research developing about lactic acid bacteria (BAL) with a larger dose so that can be known BAL effectiveness in lowering cholesterol levels

2. To make sure that the used of lactic acid bacteria can act as a probiotic and also need doing attachment test or adhesion of bacteria in digestive tract.

CONFLICT OF INTEREST

The authors have no conflict of interest.

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