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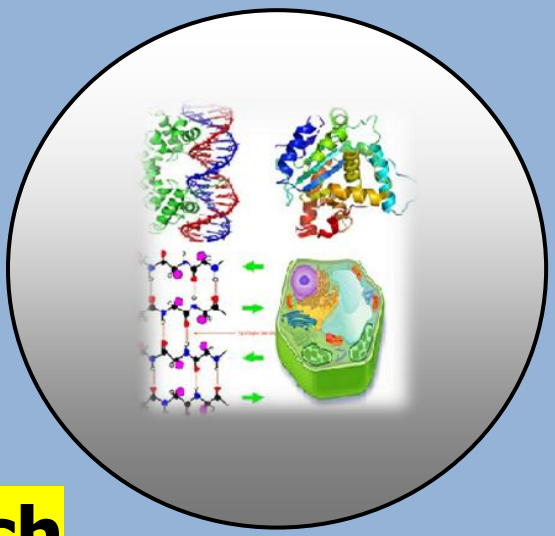

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The Use of Lactate Acid Bacterium, *Streptococcus thermophilus* and Fish Waste Feces to the Blood Cholesterol Level of Broiler Chicken Lohman Strain

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ABSTRACT

This research was conducted to examine whether giving Streptococcus thermophilus bacteria in a force feeding way could reduce the level of cholesterol in broiler chicken blood.The source of Microbe used as the research materials was isolate BAL isolated from fish waste in the fish digestion organ. 40 male broiler chickens strain Lohmannwith 4 week ages produced by PT Multi Breeder Adirama was used in this research. They were divided into five different treatments in which each treatment consisted of 8 chickens taken randomly and raised for 42 days.

Data collected was analyses with variance analysis used one way full random design, then, the data analyses by using Duncan's Multiple Range Test (DMRT).

Lactate Acid bacteria (BAL) used in this research were freeze drying Streptococcus thermopiles which were taken from Biochemistry Nutrition Laboratory of Faculty of Animal Breeding of Gajah Mada University. Treatment I was as a control (Without BAL). Treatment II used 10^6COLONY FORMING UNIT/ml BAL cell. Treatment III used 10^7COLONY FORMING UNIT/ml BAL cell. Treatment IV used 10^8COLONY FORMING UNIT/ ml BAL cell.

The result of the research showed that giving lactate acid bacteria, Streptococcus thermophiles, significantly reduced the cholesterol level in broiler chicken blood. The reduced of cholesterol level in the chicken blood seemed affected indirectly by the mechanism of gall saline de-conjugation.

Keywords: Lactate Acid Bacteria, Streptococcus thermophiles, Cholesterol and Freeze Drying.

INTRODUCTION

Lactate acid bacterium (BAL) is a general term for a bacterium which fermenting lactose and producing lactate acid as a main product. This bacterium has been consumed for a period of time since it gives a good effect for human body. BAL has important roles in the yoghurt, cheese, butter, and lactic acid fermentation industry. BAL is also a bacterium which is known as a natural preservative for food because its ability to produce anti bacteria compound such as lactate acid, hydrogen peroxide, *diacetyl*, and *bacteryosin*(Widodo, 2003).

BAL is one of bacteria which had been used as a prebiotic. Probiotic is a life microbe used as food supplement which is useful for health through increasing the balance of microbe in digestion organ (Fuller, 1992). For being a prebiotic, there are some requirements that must be met such as having high viability to keep it alive, growing, and active in the digestion organ, being able to endure acid, bile salt, and an-aerobic condition, being able to grow rapidly in digestion organ, and being able to diminish pathogen bacterium (Playne *et al*., 1999 in Widodo, 2003).

The use of BAL as probiotic for human body and animal health are decreasing the lactose intolerance, decreasing cholesterol level, decreasing diarrhea, stimulating human immunity, controlling infection caused by pathogen bacteria, changing the anti-biotic, and minimizing cancer possibility in the digestion organ by keeping the balance of microbe in the digestion organ (Sceinbech, 1998 in Widodo, 2003).

One of the BAL advantages that are very interesting for the researchers is its ability to decrease the level of cholesterol in the human or animal blood (Horison and Peat *et al*., 1975).

Cholesterol is the most sterol in human or animal body which is found almost in every part of human or animal body. Cholesterol can be found easily in the blood, gall, adrenal gland, and nerve system (Anna Poedjiadi, 1994).

The research of proofing the BAL ability to reduce the cholesterol level has been conducted by many researchers. Grunewald (1982) did research about the effect of the fermented skim milk with *Lactobacillus acidophilus* to cholesterol level serum which was evaluated by rat as a research object. The research showed that after 4 weeks, the cholesterol level was decreased and it was believed that BAL was the factor causing the decreased of cholesterol level. Meanwhile, Alkalin*et al*., (1997) did a study of the effect of yoghurt and *Acidophilus yoghurt* to mouse cholesterol. From the study, he found that the average of mouse cholesterol serum and cholesterol LDL decreased significantly in the mouse that the research gave the Acidophilus yoghurt. From the study that was conducted byRao*et al*., (1981), it was found that consumed thermophiles milk can reduce mouse cholesterol level serum. Gilliland (1985) states that consume Acidophilus towards cholesterol in growth media can decrease the cholesterol level in pig that treating high cholesterol diet.

In this research, the researcher will examine the effect of BAL *Streptococcus thermophilus* towards broiler chicken with blood cholesterol level parameter. *Streptococcus thermophilus i*s a kind of BALs which is able to do metabolism in an extreme temperature. *Streptococcus thermophilus* has a growing temperature 20-53 degree Celsius and will be optimal at the temperature 43-45 degree Celsius. The shape of this bacterium is circle/ spiral, has no spore, gram positive, and homo-fermentative. It has optimal growing pH 6.8 and it has an-aerobic characteristic. This bacterium can endure acidity from 0.85 to 0.95%.

Lactate acid produced by this bacterium is a result of glucose, fructose, galactose, sucrose, and lactose splitting process (Whittier and Webb, 1970).

Broiler chicken was chosen because BAL has never been examined to this chicken. In addition this chicken is one of the most susceptible chicken towards any disease compare to the other chicken so that the given pro-biotic will also substitute the anti-biotic that commonly used for a chicken. This chicken is the fastest growing chicken compare to the other chicken (Akoso, 2004: 9). This chicken is also the most economical cattle and has the high rate production (BambangAgusMudjito, 2004: 8). In Indonesia, this chicken, even, is one of the most well-known poultry (Unknown, 2004).

BAL which was used in this study was the isolation result from freshwater fish (*Puntiusjavanicus*) digestion organ. Freshwater fish is one kind of fish that easily breed, cheap, and easily obtained. Isolate which was gained could be an pro-biotic because it had met the requirement as a pro-biotic like being able to endure high temperature, being able to endure low pH, and being able to endure gall saline.

The effect of pro-biotic bacterium towards the descent of cholesterol level was assumed by the ability of the bacterium in assimilating the cholesterol and de-conjugating gall saline (Gilliland, 1985). The descent of cholesterol level by BAL was assumed occurred directly by cholesterol assimilation mechanism or indirectly by gall saline de-conjugation mechanism. In the cholesterol assimilation mechanism, BAL would absorb cholesterol *micelle*in the intestine. Then, the taken cholesterol incorporated on the membrane cell of the bacterium. In the mechanism of gall saline de-conjugation, the descent occurred during enthero-hephatic. De-conjugation occurred because the bacterium has *bile salt hydrolase* enzyme (Noh *et al*., 1997).

Formulation of the Problem

Based on the limitation of the problem, the researcher formulate the problem whether giving lactate acid bacterium *Streptococcus thermophilus* in a *force feeding* way can reduce the cholesterol level towards broiler chicken blood.

The Objective of the research

The objective of this research is to examine whether giving *Streptococcus thermophilus* bacteria in a *force feeding* waycan reduce the level of broiler chicken blood cholesterol.

The Significances of the Research

1. Theoritical

a. To identify the information about BAL

b. To identify the scientific information about *Streptococcus thermophilus a*nd the advantage of this bacterium

c. To identify the work mechanism of BAL to decrease the blood cholesterol level of broiler chicken

2. Practical

Practically, this research is used to identify the effect of given BAL *Streptococcus thermophilus* towards blood cholesterol level of broiler chicken.

Hypothesis

Based on the literature review and theoretical framework, the researcher made a hypothesis that giving BAL *Streptococcus thermophilus* in *Force feeding* way can reduce the blood cholesterol level of broiler chicken.

MATERIAL AND METHODS

Research Design

This research is an experimental research. This research design with Complete Random Sampling Design (RAL) with 4 variables in which it was divided into two groups, they are, 1 control group and 3 treatment variables with different level of lactate acid. Each of treatment was repeated 5 times.

1. Setting of the Research

The research was conducted in

a. Faculty of Animal Husbandry Faculty of Gajah Mada University for breeding the chicken

b. Biochemistry laboratory of State University of Yogyakarta for making biomass of lactate acid bacteria and for measuring cholesterol level

2. Time

The research was conducted from August to December 2005

Research Variables

The variables which were observed in this research were:

1. Independent variables: the variation of giving BAL dosage about 10^6colony forming unit.ml, 10^7colony forming unit/ml, and 10^8 with following requirements:

R0: group without BAL (control group)

R1: group which was given lactate acid dosage in *Force feeding* about 10^6colony forming unit/ml

R2 : group which was given dosage of lactate acid in force feeding way about 10^7colony forming unit/ml

R3 : group which was given dosage of lactate acid in force feeding way about 10^8colony forming unit/ml

2. Dependent variable: blood cholesterol level of broiler chicken after the treatment (mg/ml)

Population and Sample of the Research

1. The research population were a day old chick (DOC) broiler chicken Lohmannstraine
2. The research sample was 20 broiler chicken Lohmann Strain which were divided into 4 treatments and each treatment consisted of 5 chickens as the repetition taken randomly.

Tools and Materials

This research used some tools and materials to support the process of this research.

a. Tools

Tools that were used in this research consisted of reaction vessel, hot plate, balance analytic, autoclave, incubator, Erlenmeyer tube, centrifuge,

b. Materials

The materials used in this research consisted of a Day Old Chick (DOC) Broiler chicken *Lohmann* Strain, chicken feed consisted of corn flour, brans, fish meal, soy bean, and NaCl (mineral addition), chicken vitamin (Vita Chick), MRS Broth, water, HCL 1N, NaOH 1 N, peptone liquid, skim milk 10 %, MRS Jelly, chicken blood sample, chloroform, acetone, alcohol, and acetate anhydrate.

The Procedure of the Research

This research was conducted in several stages as follows:

Preparation

The preparation stage consisted of henhouse cleaning, henhouse watering with disinfectant, and setting lamp in the henhouse.

1. **Ransom arrangement**

Ransom arrangement consisted of corn flour, bran, soybean, fish meal, and mix mineral. The ransom in the research made based on the measurement of ransom feeding composition according to NRC (1994) which is written on the table:

Tabel 1. The composition of feed and the nutrition.

Feed	WG %	RP %	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 meal	89,34	61,73	2.219	2,32	1,89	2,67	6,45	1,06	2,60	7,90

Source: the table of feed composition for Indonesia. Hartadi*et al*., (1994:13)

Description:

WG : Weight
RP : Raw Protein
ME : Metabolizable Energy
Pav : Phospor Available
Ca : Calcium
Met : Metionin
Lys : Lycin
Trp : Tryptofan

Tabel 2. Feed nutrition of research ransom.

Feed	Formulation %	RP %	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 meal	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
Total	100,00	21,08	3.000,64	0,28	0,58	0,50	1,32	0,27

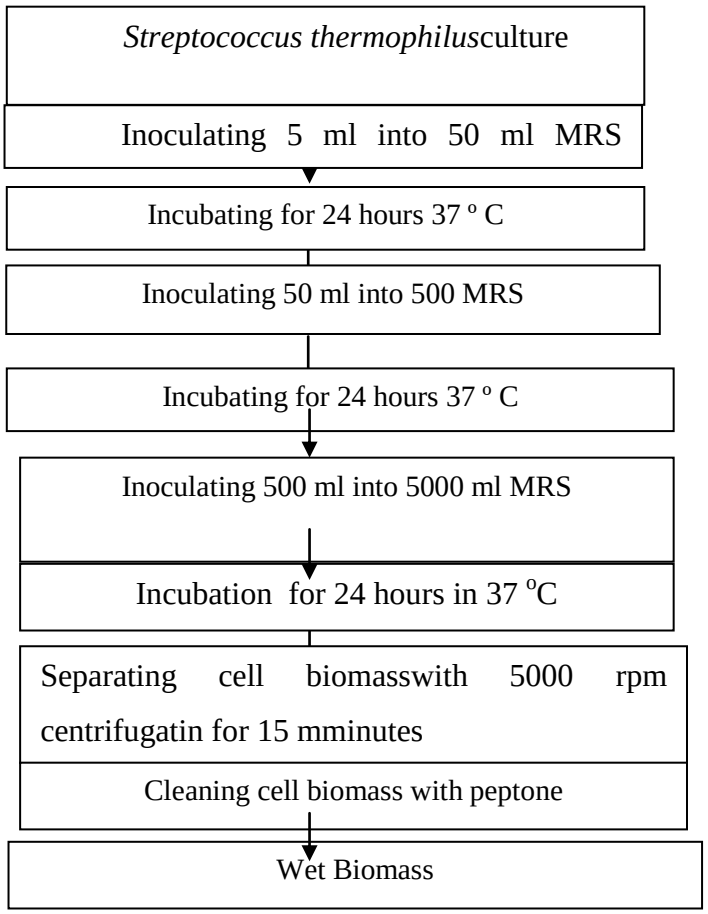
The making of lactate acid bacteria

Before doing the supplementation of lactate acid bacteria, the researcher produced the biomass of lactate acid bacteria which would be given to the chicken. The bacteria that would be produced were *Streptococcus thermophilus* To produce the cell biomass, the researcher used MRS as a medium.

MRS medium was made as follows:

3 gram of MRS medium dissolved in 50 ml water, set pH6.2. After boiled, sterilize the medium with autoclave in121ºC temperature for about 15 minutes. Give CO2 to the medium which has been sterilized. The next step is inoculating culture of *Streptococcus thermophilus*10%/ v into the medium. After that, incubate the culture in 37 º C for 24 hours.

The diagram below shows the later procedure of making lactate acid bacteria



3 gram of Broth MRS Medium was gained from the measurement as follows:

$$\frac{50}{100\ ml} \times 5{,}2\ = 2{,}6\ gram \sim 3\ gram$$

5,2 = standard in Broth MRS recipe

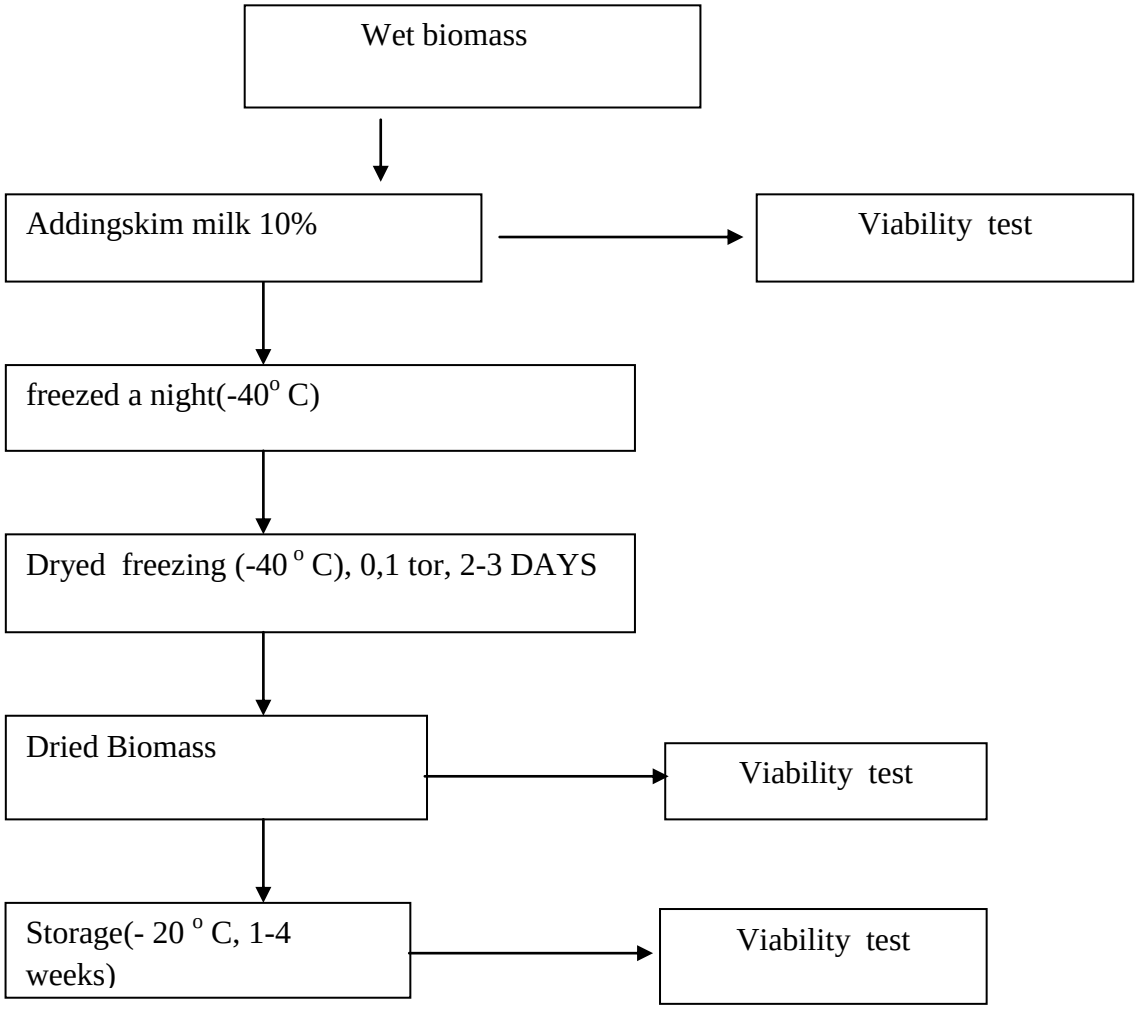
For volume 500 ml and 5000 ml, MRS medium used was 26 gram and 260 gram.

To protect the cell viability during the storage, cell biomass needed to be dried. One of the alternatives that can be done was freeze drying. Freeze drying had several advantages such as lowering the cell reduction, lowering the chemistry process changing and stable during the process of the storage (Rudge, 1991)

Determine the dosage of lactate acid bacteria

Before applying the lactate acid bacteria to the chickens, the first step was determining the dosage of lactate acid bacteria that would be applied to the chicken by measuring the number of bacteria in the dried biomass. This step could be done by inoculating dried biomass into jelly MRS media in pour plate way. 1 gram dried biomass needed to be dissolved in 9 ml sterile water (dilution10^{-1}). After that, the series of 10 times dilution needed to be done. From the dilution, inoculation which could be done was the result of 10^{6} - 10^{10} dilution. After doing inoculation to MRS media, it should be incubated in the 37ºC temperature for 24 hours. Finally, calculate the colony in colony forming unit.

The procedure of freeze drying is shown in the diagram below



The result of the measurement is shown as follows

10^{-6} = spreader	10^{-7} = 112	10^{-8}= 39	10^{-9}= 10	10^{-10}= not growing
10^{-6} = spreader	10^{-7} = 94	10^{-8}= 107	10^{-9}= 27	10^{-10}= not growing
10^{-6} = spreader	10^{-7} = 128	10^{-8}= 44	10^{-9}= 17	10^{-10}= not growing

Since the10^{-6} , 10^{-9}, and 10^{-10}dilution did not meet the requirement in the colony measurement, dilution which was used as measurements were 10^{-7} and 10^{-8}.

Dilution	1st repetition	2nd repetition	3rd repetition
10^{-7}	112	94	128
10^{-8}	39	107	44
Kontrol	0	0	0

The average of colony forming unit dilution 10^{-7}= $\frac{112+94+128}{3}$ = 111,3 ×10^{7}

The average of colony forming unit dilution 10^{-8}= $\frac{107+39+44}{3}$ = 63,3 ×10^{8}

Comparison = $\frac{63,3x108}{11,13x108}$ > 2, therefore smaller dilution was used, 10^{-7}.

Therefore the number of cell = 11,03 x 10^{8} colony forming unit/gr= 1,103 x 10^{9}colony forming unit/gr

Therefore it was converted 10^{9} = 1 gr

For treatment of R1 = 10^{6} ~ 0,001 gram/ml

R2 = 10^{7} ~ 0,01 gram/ml

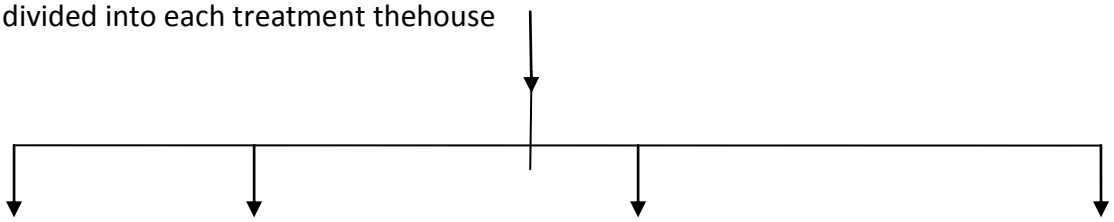
R3 = 10^{8} ~ 0,1 gram/ml

The application of probiotic to the Chicken

The applications of probiotic lactate aid bacteria to the chicken were done in force feeding way. A number of 10^{6} colony forming unit/ ml or equal 0,001 gram/ml was given to the group R1, 10^{7} colony forming unit/ml or equal 0,01 gram/ml was given to group R2, 10^{8} colony forming unit/ml or equal 0,1 gram.ml was given to R3. The complete scheme is shown as follows:

Chickens for conducting research

20 chicken of 7 days old chicken were placed in the henhouse treatment. They were divided into each treatment thehouse



5 chickens as control 5 chicken as 1st treatment5 chicken as 2nd treatment 5 chicken as 3rd trt10^{6} ~ 0,001 gram/ml 10^{7} ~ 0,01 gram/ml 10^{8} ~ 0,1 gram/ml

The Measurement of Blood Cholesterol Level

Cholesterol level was determined by Liebermann-Burchard method (Plummer, 1977: 256).

1 ml blood/ 1 gram digest added with 10 ml acetone liquid: alcohol (1:1) boiled in the boiling water by shaking the alcohol until it boiled. Then, it needed to be refrigerated in the room temperature. Filter the liquid. Then centrifuge the filtrate for 15 minutes 2750 rpm. Evaporate Supernatant which was resulted from the filtrate in the water bath at 100 º C until it dried. Refrigerate it then, dissolve it with the chloroform 3 ml. add 3ml acetate anhydride: thick sulphate acid (30:1).

Homogenize and put it in the dark room for 5 minutes so that the liquid mixture colour will become green. Make a Blanco in the same way. Measure the absorbency in the 680nm wave length. Put the absorbance in the regression equality.
The regression equality is Y = 0,000697 + 42,515 X

The Research Design and Data Analysis
This research used one way complete random sampling. The gained data which was resulted were analysed by one way Variant Analysis (ANAVA). If the treatment significantly affected the chicken, Duncan's Multiple Range would be applied (DRMT) (Gaspers, 1991).

RESULTS AND DISCUSSION
Based on the research about the effect of applying lactate acid bacteria to the level of blood chicken cholesterol, the researchers found several data as follows:

Table 3. The level of blood cholesterol (mg/ml) after applying lactate acid bacteria during the research study.

Repetition	The level of blood cholesterol			
	R0	R1	R2	R3
1	51,8	48,1	34,2	17,7
2	54,2	41,8	43,7	23
3	53	49,5	34,2	18,9
4	50,9	36,5	39,6	26,9
5	49,1	50,3	23,8	35,3
Mean	51,8a	45,24a	35,1b	24,3c

[abc] the different Superscript on the same row shows the significance difference (P<0.01)
table 3 above shows that the average of the level of broiler blood cholesterol after applying the treatment of lactate acid bacteria from 51,8 mg/ml to 24,3 mg/ml. The highest mean is 51,8 mg/ml on the control group or on the group (R0) without being applied the lactate acid. Meanwhile the mean of the level of blood cholesterol on the treatment group (R3) which was applied the lactate aid bacteria 10^8colony forming unit/ml is low, 24,3 mg/ml.

Table 4 collate acid produced from the gall saline de-conjugation by lactate acid bacteria (micromole/ml).

Repetition	The amount of Gall saline					
	0%	0,1%	0,2%	0,3%	0,4%	0,5%
1	-0,005	0,078	0,170	0,206	0,184	0,112
2	-0,004	0,096	0,129	0,232	0,227	0,142
3	-0,005	0,065	0,196	0,196	0,167	0,144
4	-0,005	0,089	0,172	0,201	0,206	0,117
5	-0,008	0,105	0,210	0,220	0,226	0,233
Mean	-0,005a	0,087b	0,175cd	0,211d	0,202d	0,150c

a bcd The difference superscript on the same rows shows the significant difference (P<0.01)
table 4 above shows that the mean of collate acid produced from the gall saline de-conjugation by lactate acid bacteria for a control group or group without applying gal saline is -0,005 micromoles/ml.

Meanwhile, the mean of collate acid produced by applying 0,1% gall saline is 0,087 micromoles/ml. whereas, the mean of collate acid produced by applying 0,2%, 0,3%, 0,4%, 0,5% are 0,175 micromoles/ml, 0,211% micromoles/ml, 0,202 micromoles/ml, 0,150 micromoles/ml.

DISCUSSION

The use of lactate acid bacteria as a pro-biotic is one of the potential approaches for reducing cholesterols. Based on the previous research findings, it was known that consuming fermented products which contain lactate acid bacteria could reduce the level of blood cholesterol not only for animals but also for human (Alkalin *et al*, 1997: 67).

From the research findings that had been conducted, it was found that giving lactate acid bacteria could reduce the level of chicken blood cholesterol. Table f shows that the mean of the highest cholesterol level found in the control group in which the researcher did not give them lactate acid bacteria. The mean of the level of blood cholesterol was 51.8 mg/ml. the mean of the treatment group R1 was 45.24 mg/ml, R2 was 35.1 mg/ml, and R3 was 24,3 mg/ml. it means that the level of the blood cholesterol was significantly reduced. The level of blood cholesterol can be seen from the diagram below:

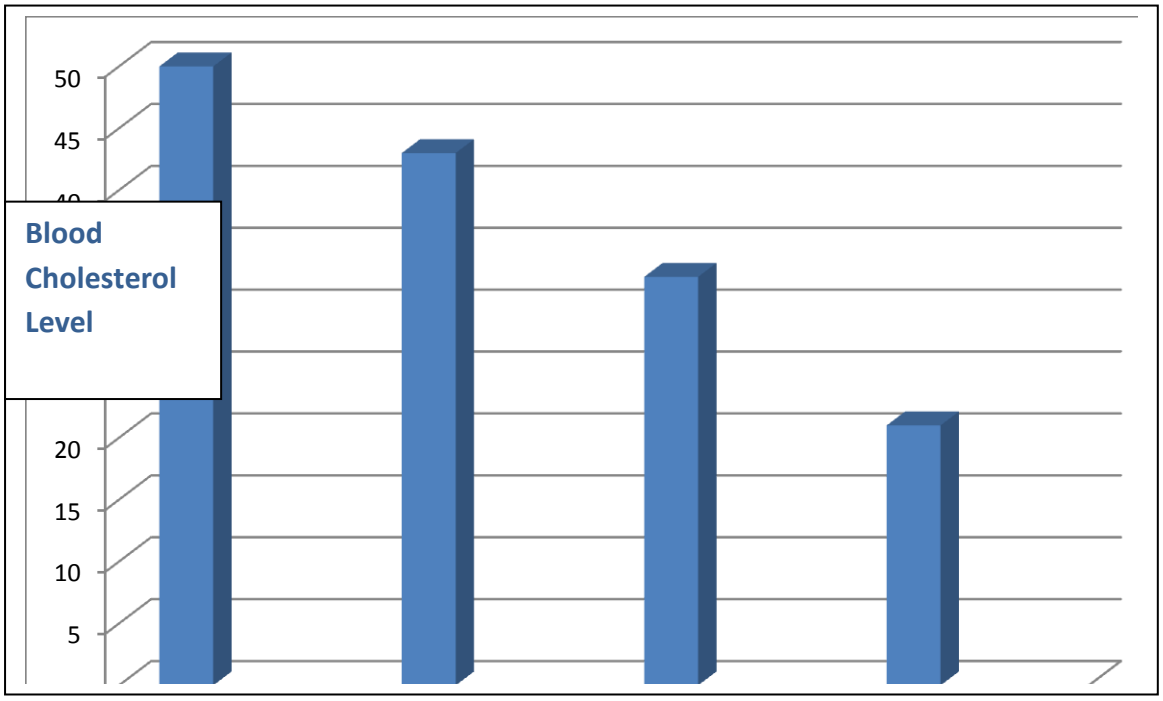


Chart 1. The Diagram of broiler blood cholesterol level with lactate acid bacteria treatment from the diagram above, it is known that the cholesterol level was reduced. The cholesterol level of treatment groups, R1, R2,R3 were lower that R0 or control group.

The result of this research showed that giving lactate acid bacteria could reduce the level of blood cholesterol level to the broiler chicken. It happened since lactate acid bacteria have an ability to de-conjugate the gall saline.

On the mechanism of gall saline de-conjugation, the reduction of cholesterol level occurred indirectly during anther hepatic cycle. On this mechanism, it was explained that cholesterol was the component of gall saline so that the catabolism and the secretion of gall saline in the feces would reduce the level off cholesterol level.

The main gall saline that is synthesized from the cholesterol in the liver is collate acid and Cenodeoxy-collate. Both of gall saline can conjugate and can also de-conjugate. This primer gall saline conjugate with glicyn and taurin and it is stored as a conjugated gall saline in the gall to be secreted gradually in the digestion organ. The conjugated gall saline is secreted to the small intestine to help absorbing the fat, cholesterol, and vitamins which can be dissolved by fat. In the ileum and caecum the conjugated gall saline will be de-conjugated by bacteria and create lithocollate and deoxlate. About 97% conjugated gall saline is absorbed from small intestines and transferred back to the liver by portal hepatic circulation. The rest of gall saline which is not absorbed (250-400) in this process will be dissolved and secreted with fesses as a free gall. The character of free gall saline are difficult to dissolve and can be absorbed by lumen intestines compared to conjugated gall saline.

De-conjugation of gall saline can stimulate the reduction of cholesterol serum by raising the formation of new gall saline which is needed to change the lost gall saline during the anthero-hepatic circulation in which it needs a cholesterol as a precursor. Therefore, this cycle will be keep going so that the cholesterol catabolism is getting faster and finally reducing the accumulation of cholesterol.

De-conjugation of gall saline occurs because lactate acid bacteria have *bile salt hydrolase* enzyme. This enzyme catalyzes the hydrolyze reaction of conjugated gall salineand produces free gall saline and amino acid. This enzyme attracts much attention because it has potention as a cholesterol reduction (Gilliland, 1999).

In this research, the result showed that giving lactate acid bacteria 10^7 colony forming unit/ ml (R2) and 10^8 colony forming unit/ml were able to reduce the cholesterol level significantly. Meanwhile, the treatment of giving lactate acid bacteria 10^6 colony forming unit/ml did not significantly reduce the cholesterol level significant compare to the control group. This case was probably caused by the amount of cell which was given to the treatment group R2 and R3 was more than R1. The amount of the cell given to the treatment group will determine the reduction of the cholesterol level. It can be concluded that based on this research, the most effective treatment occurred in R3 which was given the most amount of lactate acid bacteria (10^8 colony forming unit/ml). The more probiotic cell intake, the more cell could pass the digestion organ to enlarge intestines because in the large intestines the de-conjugation process of gall saline occurred.

In this research, the reduction of cholesterol level was caused by de-conjugation of gall saline because of bile salt hydrolysis in *Streptococcus thermophiles*. The ability of probiotic cell in conjugating gall saline is related to the cholesterol in the blood and digestion process. If the cell has bile salt hydrolysis activity, the gall saline will be de-conjugated to be free gall acid which cannot be absorbed and secreted with feces. The more bile salt hydrolysis activity in conjugating gall acid, the more gall acid will be released or secreted. The body will use cholesterol in the blood for precursor of the new gall saline synthesis so that the blood cholesterol level will be reduced. The mechanism of gall saline de-conjugation is still needed to be proved in the later research.

Streptococcus thermophilus which was used in this research was able to de-conjugate gall saline. This case was known because the previous research had examined the bacteria in terms of de-conjugating gall saline (Burhani, 2006).

To identify whether *Streptococcus thermophilus* bacteria were able to de-conjugating gall saline, the production of collate acid by bacteria during the process of gall saline de-conjugation was tested. The bacteria which were able to de-conjugate gall saline were bacteria which could produce bile salt hydrolysis enzyme. This enzyme would hydrolyze glyooxyolate acid and taurocolate acid from gall saline and create collate acid and cenodeoxyllate acid. Collate acid was not fat emulsion and was not absorbed in the intestine in the anthero-hepatic cycle. Collate acid will be secreted together with the feces (Drassar and Barrow, 1995).

The result of statistica analysis showed that collate acid which was produced increased significantly ($P \leq 0.01$) on the groups which were given gall saline 0.1%-0.5%. This result showed that lactate acid bacteria which were studied had an ability to de-conjugate gall saline. The released collate acid increase significantly ($P \leq 0.01$) after giving gall saline up to 0.2%. However there was no significant addition by giving 0.2%-0.4% gall saline. On the addition of 0.5 gall saline, the released collate acid decrease significantly ($P \leq 0.01$) which was lower than adding 0.3% gall saline.

This result showed that lactate acid bacteria which were studied had an ability to de-conjugate gall saline well in level 0.2%. and level 0,4%, the de-conjugation activity still run well. In level 0.5% gall saline, the ability of lactate acid bacteria to de-conjugate was getting lower. The reduction was related to the growing reduction of lactate acid bacteria because of the obstacle by gall saline. The data can be seen in the attachment.

The ability of lactate acid bacteria to de-conjugate gall saline showed that the bacteria which were used in this research had a potential as a probiotic which could reduce the cholesterol level. As explained by Tanaka (1994: 34) the ability to de-conjugate gall saline was a main mechanism of the cholesterol level reduction. The reduction of cholesterol level as a result of gall saline de-conjugation occurred in the digestion organ and in the body.

In this research, the endurance of *Streptococcus thermophiles* to the gall saline and the temperature which was one of the requirements for the bacteria to be probiotic was examined (Burhani, 2006).

To test the endurance of the bacteria to the gall saline, the bacteria growth pattern was measured in the liquid medium which contained gall saline. It was done by observing the changing of liquid medium density every hour. From the test result, the density was raising during the fermentation process on every treatment. This case showed that lactate acid bacteria were able to grow in the medium which contained gall saline. The growth was shown by the raising of the density to every treatment. The bacteria was still able to grow in the medium with 0.5% gall saline level. The data can be seen in the attachment.

The endurance of the bacteria toward the temperature is also needed to be tested to adapt the chicken condition and environment. From the test result, the *Streptococcus thermophilus* had optimal growth on 40º C. it shows that the bacteria is suitable with the chicken environment which also has 40º C body temperature.

CONCLUSION AND SUGGESTION

A. Conclusion

Based on the research result and discussion, it can be concluded that giving lactate acid bacterium, *Streptococcus thermophilus* caused the reduction of the blood cholesterol of broiler chicken significantly. It is assumed that the mechanism of the blood cholesterol level reduction occurred indirectly with the mechanism of gall saline de-conjugation.

B. Suggestion

Based on the conclusion the research team creates several suggestions as follows:

1. Developing the research about lactate acid bacteria with higher dosage so that it can be identified the effectiveness of Lactate acid bacteria to reduce cholesterol level.
2. Developing the research about lactate acid bacteria together with observing the amount of gall saline in the chicken feces so that it can be identified exactly the mechanism of the cholesterol level reduction.
3. Examining the adhesion ability of the bacteria in the digestion organ to ensure that lactate acid bacteria which is used can be a probiotic.

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