The Use of Lactate Acid Bacterium, *Streptococcus thermophillus* from Fish Digestion Organ to Growth and Cholesterol Level of Broiler Chicken *Hubbart* Strain

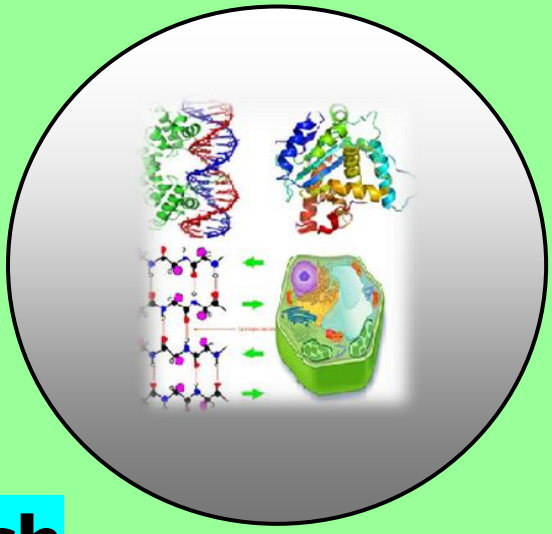
By

Astuti, Zaenal Bachrudin, Supadmo and Eni Harmayani

ISSN 2319-3077 Online/Electronic
ISSN 0970-4973 Print

Journal Impact Factor: 4.275
Global Impact factor of Journal: 0.876
Scientific Journals Impact Factor: 3.285
InfoBase Impact Factor: 3.66
Index Copernicus International Value
IC Value of Journal 47.86 Poland, Europe

J. Biol. Chem. Research
Volume 33 (1) 2016 Pages No. 537-546



Journal of Biological and Chemical Research

An International Peer Reviewed / Referred Journal of Life Sciences and Chemistry

Indexed, Abstracted and Cited in various International and National Scientific Databases

Published by Society for Advancement of Sciences®

J. Biol. Chem. Research. Vol. 33, No. 1: 537-546, 2016
(An International Peer Reviewed / Refereed Journal of Life Sciences and Chemistry)
Ms 33/2/32/2016

ISSN 0970-4973 (Print)
ISSN 2319-3077 (Online/Electronic)

Dr. Astuti
http:// www.jbcr.co.in
http:// www.sasjournals.com
http:// www.jbcr.in
jbiolchemres@gmail.com

RESEARCH PAPER

Received: 12/05/2016 Revised: 24/05/2016 Accepted: 28/05/2016

The Use of Lactate Acid Bacterium, *Streptococcus thermophillus from* Fish Digestion Organ to Growth and Cholesterol Level of Broiler Chicken *Hubbart* Strain

Astuti, *Zaenal Bachrudin, *Supadmo andEni Harmayani**

Faculty Mathematics and Science Yogyakarta State University, Yogyakarta, Indonesia
***Animal Husbandry Faculty Gadjah Mada University, Yogyakarta, Indonesia**
****Faculty of Agricultural Technology, Gadjah Mada University, Yogyakarta, Indonesia**

The purpose of this research was to determine the effect of probiotic LAB isolates of Streptococcus thermophillus of fish waste on the growth of broiler chickens that weight gain and cholesterol levels of broiler chicken meat. This research material is Hubart Strain male broiler production PT Multi Breeder Adirama much as 40 chicken attain the age of 1 day. Lactate Acid bacteria (LAB) 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 LAB). Treatment II used 10^6COLONY FORMING UNIT/ml LAB cell. Treatment III used 10^7COLONY FORMING UNIT/ml LAB cell. Treatment IV used 10^8COLONY FORMING UNIT/ ml LAB cell. Data was collected every week include weight gain and data for cholesterol levels at the end of the research. Data taken include: weight gain and cholesterol levels of broiler chicken meat. The result of the research showed that giving lactate acid bacteria, Streptococcus thermophiles, significantly reduced the cholesterol level in broiler chicken meat. LAB probiotic effect on weight gain and the best level is the level LAB 10^8 CFU / ml (R3).
Keywords: Lactate Acid Bacteria, Growth, Cholesterol, and Broiler Chicken.

INTRODUCTION

Background

Nowadays people awareness on health is increasing. Along with the increase of Science and Technology causes the awareness to maintain a healthy increase. The cost of expensive

medical treatment causes people prefers preventive measures to prevent the illness. One of the dreaded diseases is coronary heart disease. This disease is the number one disease in developed countries and in a group of society in developing countries.

According to health experts there is a positive correlation between blood cholesterol levels and the risk of coronary heart disease. The accumulation of cholesterol in the walls of blood vessels can cause narrowing of the arteries and cause atherosclerosis risk. Atherosclerosis is what causes coronary heart disease. Prevention of coronary heart disease can be done by reducing the consumption of food high in cholesterol or consuming foods with low cholesterol. Animal foods such as milk, eggs, meat and processed products generally have high cholesterol levels. High cholesterol content in foods has been associated by consumers as one of the causes of coronary heart disease; therefore the majority of consumers tend to limit the consumption of cholesterol, among others, by consuming animal products that lower cholesterol. If the cholesterol content in animal food can be reduced, then the formulation and use of animal products are highly nutritious diet can be overcome.

Cholesterol is the principal sterol in the human body. Cholesterol is a compound of the metabolism of the animals and more stored in meat, liver, brain and eggs. Although cholesterol negative effects if over in consumed, cholesterol has also an important role for the body. Mayes [**1987.**] said that cholesterol has a functional role for the body as a precursor compound that steroids (corticosteroids, sex hormones, bile acids, and vitamin D), and forming a structural component of cell membranes as well as the external layer of plasma lipoprotein.

As discussed by Gilliland [**1999**] the effect of probiotic bacteria to decrease cholesterol levels is suspected because of its ability to assimilated cholesterol and conjugated bile salts. Lactic acid bacteria that have specific capabilities will be effective if it can hold out in the digestive tract conditions. Therefore strains of Lactic Acid Bacteria should be resistant to bile salts and gastric pH conditions (pH 1-2) when consumed. LAB strains potential to be commercialized as a probiotic product must have high viability and stable during processing. Some production processes using freeze drying or spray drying often causes the decrease cell viability so that it can affect the resulting product (bio mass LAB cells).

Giving probiotics in chicken will have a positive impact, which can improve the health or productivity of chickens, changing components and the balance of microflora in the digestive tract of chicken. Hanson [**1999**] reported a probiotic (probiolac at the level of 100 mg / kg feed) can improve egg production, eggshell weight and eggshell thickness as well as lowering cholesterol in egg yolk. Grunewald [**1982**] observed that the giving of skim milk fermented with *Lactobacillus acidophilus* can decrease serum cholesterol levels.

This research was to determine the effect of LAB isolates from fish waste as probiotics given to broiler chickens through drinking water by means of drinking use spet with amount of 1.5 ml per oral to decrease cholesterol levels of broiler chicken meat. Besides, the presence of this research are expected later will realize a broiler chicken farm that is healthier because it is not very high cholesterol content. This research is also expected to be useful for the development of Science in the field of animal husbandry.

Benefits of research

Research purposes

The research was conducted to determine the effect of probiotics in broiler chickens on performance of broiler chickens which include weight gain and cholesterol levels of broiler chicken meat.

The benefit from this research is obtaining selected strains of lactic acid bacteria are able to act as a probiotic and cholesterol can lower broiler meat.

MATERIALS AND METHODS

Implementation of the maintenance

Before use to research, stable and equipment was disinfected beforehand using brochid. Vaccination is done two times, that vaccination ND-1 at the age of 3 days and ND-2 at the age of 20 days. Feed compiled based on the results of the consideration of the table according to the material composition NRC, as can be seen in Table 1. Feed and water were given two times a day, it is at 07.00 am and at 03.30 pm. Probiotics are given every afternoon through the water by means of drinking from use spet the amount of 1.5 ml per oral.

The location and time of the research

The research was conducted for 35 days in a stable poultry Biochemistry Nutrition Laboratory of Faculty of Animal Breeding of Gajah Mada University, Yogyakarta from 1 October until 15 October 2005. Analysis cholesterol levels of meat held in Nutritional Biochemistry Laboratory, Department of Nutrition and Feed, Faculty of Animal Breeding, Gadjah Mada University, Yogyakarta.

Research design

Design model used in this research is completely randomized design direction. 40 broiler chickens were divided into 4 groups treat each treatment was repeated 10 times each repetition using 1 chickens.

Data collected

Data recording is done every week for the performance include weight gain. Collecting data for cholesterol meat is done at the end of the research.

Weight gain (g / tails / day). Chicken weighing every 1 week of age 0 weeks until the age of 5 weeks. The weighing is done in the morning before the feed given.

Cholesterol percentage of meat. The percentage of meat cholesterol obtained by the method Bunchad Lieberman.

Meat Intake

Meat intake of Lohman strain broiler after slaughtered. Chicken meat is taken on the chest, precisely in the area of the sternum. Rasyaf [**2001**] said that meat is this body are mostly broiler meat, especially meat section of the best chest.

Data analysis technique

Data were analyzed variants: Completely Randomized Design (CRD). If there are differences tested by Duncan's Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

Weight gain

Weight gain in broiler chickens were given the probiotic LAB significantly different (P <0.05) with the control diet (not given probiotic LAB) as shown in table 1.

Table 1. Effect of LAB against weight gain.

	Treatment				Significa nly
	R-0	R-1	R-2	R-3	
Weight gain (g/tails/day)	44,07[ab]	47,14[a]	45,03[ab]	43,28[b]	*

1) Treatment

R 0: The group without administration of lactic acid bacteria (as a control)
R 1: The group that was given doses of lactic acid bacteria of 106 CFU / ml
R 2: The group that was given doses of lactic acid bacteria of 107 CFU / ml
R 3: The group that was given doses of lactic acid bacteria of 108 CFU / ml

2) Description

[ab] Different superscripts indicate significant differences (p <0.05)

Weight gain

From Table 1 there are significant differences (P <0.05) for weight gain. The cumulative weight gain that shows the highest number is the treatment of R 1 (106 CFU / ml) that is equal to 46.90. While the lowest is the treatment of R 0 (control) and treatment of R 3 (108 CFU / ml).

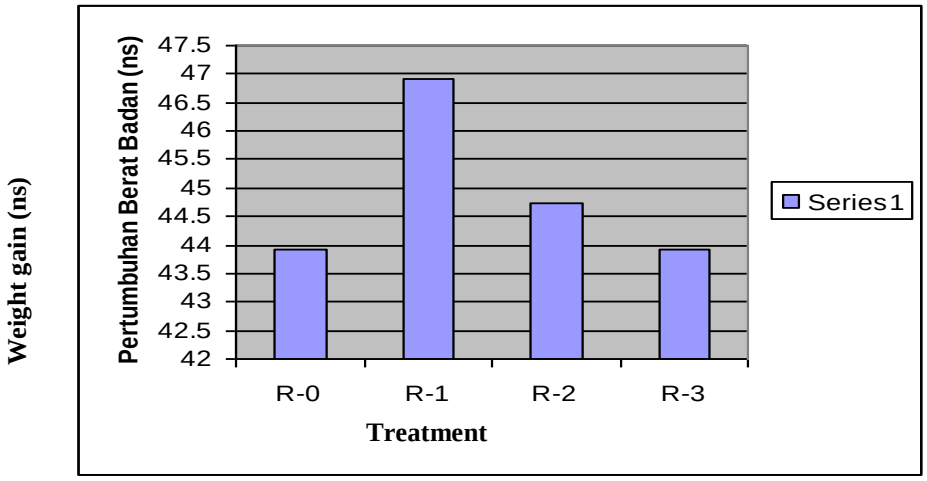


Figure 1. Graph of weight gain.

Based on growth data there are differences in body weight significantly (P <0.05) and broiler chickens were given probiotics increased weight gain. This is in accordance with the opinion of Havenwar et al., [**1992**].

There are several advantages of the use of probiotics in animals / livestock is able to spur growth and improve feed conversion and in accordance with the opinion of Barrow [**1992**], Yeo and Kim [**1997**]. Giving probiotics in broiler chickens reported to improve growth and feed conversion and probiotics in broiler chickens should be carried out for 3 weeks maintenance. It is also supported by Soeharsono [**2002**] which states that EM4 probiotics can increase weight gain.

All types of animals will have the same growth process; it is at the beginning of their growth so very quickly. But the subsequent growth has decrease; even at a certain age it was a standstill. All of which was caused by a growth rate form the body's tissues. Growth rate (growth rate) in poultry is usually measured by weight gain by weighing chickens that have research based unit of time. Measurement of growth turned out to vary, however it is same on based weighing.

Once treated, it appears that the chickens were given the treatment had a greater weight than the chickens are not given treatment. It can be said that probiotics can increase the weight of chicken. Digestive tract in both humans and animals consist of various types of bacteria. The existence of these bacteria due to the interaction of bacteria from contaminating the surrounding environment through feed chickens. A normal environment, the intestinal tract in chicks colonized by microorganisms. LAB which in the digestive tract is the most dominant microbes. This balance will shift when the animal is stress conditions such as temperature and high humidity, changes in feed, due to transportation and others. Giving probiotics regularly and continuously proven to maintain the existing balance of the natural microflora in the digestive tract through two ways, namely by means of competition and in a manner antagonistic activity against pathogenic bacteria. Natural microflora in the gastrointestinal tract has an important role in digestion and absorption of food. This microflora plays a role in the metabolism of nutrients such as carbohydrates, proteins, lipids and minerals, as well as in the synthesis of vitamins.

Probiotics can inhibit pathogenic bacteria and destroyer because the production of organic acids, hydrogen peroxide and bacteriocins. In Ouwehand [**1998**] states that the natural form of organic acids (lactic acid, acetic acid) can diffuse across cell membranes because it is soluble in fat.

Meat Cholesterol Levels

After 35 days the whole chicken for meat. The chicken meat was taken on the chest. Results of statistical analysis known the effect of probiotic LAB against cholesterol meat can be seen in table 2.

Table 2. Cholesterol Levels

	Treatment				Signific anly
	R-0	R-1	R-2	R-3	
Level of Chollesterol Meat (mg/100 g)	173,8^{b}	153,5^{ab}	143.02 ^{ab}	127,9^{a}	*

[ab]Different superscripts in the same row indicate significant differences (P <0.05)
[ns] non significant

R0: group without administration of lactic acid bacteria (as a control)
R1: The group that was given doses of lactic acid bacteria of 10^6 CFU / ml
R2: The group that was given doses of lactic acid bacteria of 10^7 CFU / ml
R3: The group that was given doses of lactic acid bacteria of 10^8 CFU / ml

Cholesterol levels of meat

It can be seen that the cholesterol levels of broiler chicken meat results are significantly different (P <0.05). Cholesterol levels in the R-1, R-2, R-3 dropped significantly (P <0.05) when compared with controls.

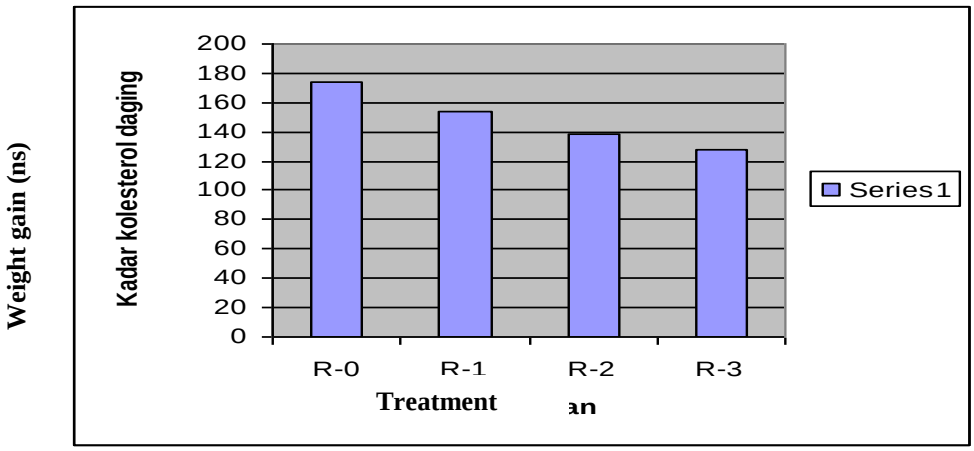


Figure 2. Graph of Meat Cholesterol Levels.

Based on Figure 2, the highest decrease cholesterol levels of meat that is treated R-3, namely the giving of LAB 10^8 CFU / ml in the amount of 127.9 followed by treatment R-2 giving LAB 10^7 CFU / ml at 143.02 then the treatment R-1 giving LAB 10^6 CFU of 153.5. This is consistent with research from Arsanti Lestari [**2004**], namely research on hypocholesterolemic effects of probiotic yogurt that supplemented Indigenous probiotic in mice results can decrease blood cholesterol levels up to 36.14%.

Some researchers propose mechanisms cholesterol reduction by probiotic bacteria, such as: the assimilation of cholesterol, bile acids deconjugated. Cholesterol reduction by Streptococcus thermopillus cell biomass in this research suspected of indirectly because of the occurrence deconjugated bile salts.

From table 2 it can be seen that the cholesterol levels of broiler chicken meat results are significantly different (P <0.05). Cholesterol levels in the R-1, R-2, R-3 dropped significantly (P <0.05) when compared with controls. The highest decrease cholesterol levels of meat that is treated R-3, namely the provision of lactic acid 10^8 CFU / ml in the amount of 127.9 followed by treatment R-2 administration of lactic acid is 10^7 CFU / ml at 143.02 then the new treatment R-1 administration of lactic acid 10^6 CFU amounted to 153.5.

Some researchers propose mechanisms cholesterol reduction by probiotic bacteria, such as: the assimilation of cholesterol, bile acids deconjugated.

Cholesterol reduction by cell biomass *Streptococcus thermopillus* in this research suspected of indirectly because of the occurrence deconjugated bile salts. In the indirect mechanism, bile reaching the ileum and cecum will dideconjugated by *Streptococcus thermopillus* and form a primary bile acids. Deconjugated occurs because bile salt hydrolase enzymes produced by these bacteria. The primary bile acid will undergo dehydroxylation into secondary bile acids and excreted with feces. The higher the bile salt hydrolase activity of the enzyme in the bile acid deconjugation, the more bile acids to be issued. The body will form a new bile acids to replace the bile acids are released. This new bile acid formation requires cholesterol as a precursor to serum cholesterol levels will decrease.

These results are supported by the NII research conducted Rodas et al [**1996**] in pigs hypercholesterolemia. Research results indicate that administration of *Lactobacillus acidophillus* can decrease serum cholesterol greater than that without giving these bacteria. Taranto et al [**1998**] suggested that the giving of *Lactobacillus reuteri* CRL 1098 in mice hypercholesterolemia can reduce serum cholesterol by 38%. This discovery probably caused deconjugated bile acids. Usman and Hasono [**1999**] also reported a decrease in serum cholesterol in rats fed milk supplemented with *Lactobacillus gasseri* SBT 0270 (non-fermented) due to the occurrence deconjugated bile salts.

Results of a research of chickens fed the probiotic S. thermopillus of 10^8 CFU / ml showed cholesterol levels of meat and lowest levels of blood cholesterol compared with R 0 and R 1, R 2., This is presumably because of the greater number of cells in this group (10^8 CFU / ml). The more intake of probiotic cells, the more cells that can survive the digestive tract to the colon so the level of cholesterol meat was decrease.

Use of lactic acid bacteria as probiotics is one potential approach for decrease cholesterol. Based on the results of previous research in Akalin et al [**1997**], it is known that eating fermented products containing lactic acid bacteria can decrease cholesterol levels in both animals and humans.

Results of this research indicate that the administration of lactic acid bacteria can lower blood cholesterol levels of broiler chickens. This can occur because the lactic acid bacteria have the ability to perform deconjugated bile salts.

At deconjugated mechanism of bile salts, the decrease of cholesterol occurs indirectly and occurs during enterohepatic cycle. At this mechanism explained that cholesterol is a constituent component of bile acids so that bile acid catabolism and expenditures with feces will affect in a reduction in cholesterol levels.

Deconjugated bile acids can stimulate decrease cholesterol levels of meat by increasing the formation of new bile acids needed to replace lost during enterohepatic circulation, which require the formation of cholesterol as a precursor. Thus this cycle will continue, so that catabolism of cholesterol more quickly and ultimately can reduce cholesterol buildup.

The deconjugated occurs because bile acids lactic acid bacteria have bile salt hydrolase enzymes. These enzymes catalyze the hydrolysis reaction of conjugated bile acids and generate free bile acids and amino acids. This enzyme gets a lot of attention due to its potential as a cholesterol-decreasing as discussed by Gilliland [**1999**].

In this research, it appear giving of lactic acid bacteria treatment of 10^7 CFU / ml (R 2) and the giving of lactic acid bacteria 10^8 CFU / ml (R3) capable of decrease cholesterol significantly.

This might be due to the number of cells in the treatment given more R2 and R3 is 10^7 CFU / ml and 10^8 CFU / ml compared to treatment that only R1 10^6 CFU / ml. The number of cells of more highly determines the occurrence of cholesterol reduction. It can be said that in this research the most effective for lowering cholesterol is in treatment R3 is the number of cells at most 10^8 CFU / ml. The more intake of probiotic cells, so the more that can survive the digestive tract and reach the large intestine in the colon because it is a process deconjugated bile salts.

The ability of lactic acid bacteria to carry bile salts deconjugated showed that the bacteria which research, potential as probiotics that can decrease cholesterol levels. As described by Tanaka [16] that the ability to perform deconjugated bile salt is the main mechanism reduction in cholesterol levels. Decrease in cholesterol levels that occur as a result of this happening deconjugated bile salts in the digestive tract and in the body of the chicken.

Results of this research indicate that the giving of lactic acid bacteria can decrease cholesterol broiler meat. This can occur because the lactic acid bacteria have the ability assimilated of cholesterol and conjujated bile salts. Both phenomenon is what makes the lactic acid bacteria are able to decrease cholesterol levels of meat.

In the assimilation mechanisms of cholesterol, this process occurs directly. As discussed by Buck et al [**1994**] in this process occurs binding cholesterol by bacteria, so the amount of cholesterol in the intestine will not be available for absorption into the blood. Most cholesterol is assimilated to join the bacterial cell membrane. Meanwhile Gilliland [2] adds that cholesterol is not only joined the cell membrane but also adsorbed to the surface of the cell. Assimilation of cholesterol into the cell membranes increases the resistance of cells to lysis. Cholesterol assimilated by bacteria causes cholesterol cannot be absorbed by the body so that it will be issued with feces.

Meanwhile on deconjugated mechanism of bile salts, cholesterol reduction occurs indirectly and occurs during enterohepatic cycle. At this mechanism explained that cholesterol is a component of bile acids so that bile acid catabolism and release with feces will result in a reduction of cholesterol levels. Primary bile acids are synthesized from cholesterol in the liver; these are cholic acid and chenodeoxycholic acid. Both of these can be conjugated and deconjugated. The primary bile acids conjugated with glycine and taurine, and stored in the form of conjugated bile acids in the bile to gradually secrete in the gastrointestinal tract. Conjugated bile acids are secreted into the small intestine to help the absorption of fat, cholesterol and fat-soluble vitamins. In the ileum and cecum, bile acids conjugated will be deconjugated by bacteria forming lithokolat and deoksikolat. As much as ± 97% in absorption of conjugated bile acids from the intestine and returned to the liver by the hepatic portal circulation. A small amount of bile salts (250-400 mg) that are not absorbed in this process will be lost and out with feces as free bile acids. Bile acid-free nature of which is less soluble and less able to be absorbed by the intestinal lumen than conjugated bile acids. Deconjugated bile acids can spur a decrease in serum cholesterol by increasing formation of new bile acids needed to replace bill acid which lost during enterohepatic circulation. This formation was needed cholesterol as a precursor. Thus this cycle will continue, so that catabolism of cholesterol more quickly and finally can reduce accumulation of cholesterol.

CONCLUSION

Based on the results of this research concluded that giving of probiotic lactic acid bacteria from fish waste in broiler chickens:

1. Probiotic lactic acid bacteria *Streptococcus thermophillus* of fish digestive tract can increase weight, that is treated with a dose of R-1which giving LAB 10^6 CFU / ml.
2. Giving probiotic lactic acid bacteria *Streptococcus thermophillus* of fish digestive tract can lower cholesterol levels in meat that is treated with a dose of R-3 LAB 10^8 CFU / ml

ACKNOWLEDGEMENTS

Authors are thankful to the following for their help during the tenure of the Research work presented in this paper :

1. Prof. Dr. Tri Yuwanta, S.U. DEA

2. Prof. Dr. Lies Mira Yusiati

3. Prof. Dr. Zaenal Bachrudin M.Sc

4. Dr. Supadmo, M.S.

5. Ir Edy Suryanto M.Sc, Ph.D.

REFERENCES

Mayes, P. A. (1987). Metabolisme Lipid II : Peranan jaringan. Pada: Biokimia Harper. 20th ed. David W. Martin, ed. Terjemahan Iyan Darnawan. EGC. Jakarta.

Corzo, G. and S.E. Gilliland (1999). Bile salt hydrolase activity of three strains of Lactobacillus acidophilus. *J. Dairy Sci.* 82: 472-480.

Hanson, D., Calkins, C., Gwartney, B., Forrest, J. and Lemenager. R. (1999). The relationship of beef primal cut composition to overall carcass composition. http.//ianrpubs.unl.edu/beef/report/mp71-30.htm.

Grunewald, K. K. (1982). Serum Cholesterol levels in rats fed skim milk fermented *Byobacillus acdopilus. J. of Food Sci.* 47: 2078-2079.

Rasyaf, M. (2001). *Beternak Ayam Pedaging*. Jakarta: Penebar Swadaya.

Havenwar, R. and J. H. J. Hulis in'Veld (1992). Probiotic: A general Viev, In: B. J. B. Wood (ed) The Lactic Acid Bacteria. Elsevier Applied Science. London.

Barrow, P. A. (1992). Probiotic for Chicken. Edited by Fuller, R. Probiotic: The Scientific basis. Chapman & Hall. London.

Yeo, J., and K. Kim (1997). Effect of feeding diets containing an antibiotik, a probiotik, or yucca extract on growth and intestinal urease activity in broiler chicks. *Poult. Sci.* 76:381–385.

Soeharsono (2002). Penyakit Menular dari Hewan ke Manusia. Yogyakarta: Kanisius.

Ouwehand A. C. (1998). Antimicrobial Components from Lactic Acid Bacteria. In S. Salminen & AV Wright, eds. Lactic Acid Bacteria: Microbiology and Functional Aspects. Second Edition. Marcel Dekker, Inc. New York.

Lestari, L.A., E. Harmayani and Y. Marsono (2004). Efek Hipokolesterolemik Yogurt yang Disuplementasi Probiotik *Indegenous* pada tikus *Sparague Dawley*. Berkala Penelitian Pascasarjana Ilmu-ilmu Penelitian UGM. Agrosains. Vol. 17 No. 1., p51-64.

Rodas, B.Z., S.E. Gilliland and C.V. Maxwell (1996). Hypocholesterolemic action Lactobacillus acidophilus ATCC 43121 and Calcium in Swine with Hypercholesterolemic Induced by diets. *J. Dairy Sci.*

Taranto, M.P., M. Medici, G. Perdigon, A.P. Ruiz Holdago and G.F. Valdez (1998). Evidence for hypocholesterolemic effect of Lactobacillus reuteri in hypercholesterolemic mice. *J. Dairy Sci.*

Usman and dan A. Harsono (1999). Bile Tolerance, Taurocholate Deconjugation, and Binding of Colesterol by *Lactobacillus Gasseri* Strains. *J. Dairy Sci*. 82: 243-248.

Akalin, A. S., S. Gone, and S. Duzze (1997). Influence of Yogurt and Acidophiles Yogurt on Serum Cholesterol Levels in Mice. *J. Dairy Sci.* 80:2721-2725.

Tanaka, H., K. Doesburg, T. Iwasaki, and I. Mierau (1999). Screening of lactic acid bacteria for bile salt hydrolase activity. *J. Dairy Sci*. 82:2530-2535.

Buck, M. L. and S.E. Gilliland (1994). Comparisons of Freshly Isolated Strains of *Lactobacillus acidophilus* of Human Intestinal Origin for Ability to Assimilate Cholesterol during Growth. *J. Food Sci.* 77:2925-2933.

Corresponding author: Dr. Astuti, Faculty Mathematics and Science Yogyakarta State University, Yogyakarta, Indonesia
Email: mp_astuti@yahoo.com