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Research Article

THE UTILIZATION OF PROBIOTIC LACTIC ACID BACTERIA FROM FISH WASTE ON STRAIN LOHMANN BROILER'S HDL LEVELS

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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 HDL broiler. Microbes are a source of research material LAB isolates isolated from fish waste faeces. And chicken used was strain Lohmann male broiler production 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 HDL blood of broiler chickens. So the administration of lactic acid bacteria does not affect blood levels of HDL broiler.

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INTRODUCTION

Lactic Acid Bacteria (BAL) is general term to refer bacteria that ferment lactose and produce lactic acid as its main product. (Widodo, 2003). BAL is one of bacteria group that have been used as a probiotic. Probiotic are active microbial as food supplements or feed which has beneficial effect on health thorugh microbial balance increased in digestive tract (Fuller, 1992). To be able as probiotics, several requirements must be fulfilled whiche are having high viability so that survive, grow, and active in digestive system, resistant to acids, bile salt, and anaerobic conditions, be able to grow rapidly and stick (colonizing) in the wall of digestive tract, and be able to inhibit or kill pathogenic bacteria. (Playne *et al*., 1999 dalam Widodo, 2003). One effect of LAB for health that many great interest of researcher is the ability to lower cholesterol level in both humans and animals. (Horison and Peat *et al*., 1975). In broiler rapid growth is often followed by high fatty particularly especially for final phase chicken. It is problem for consumer who wants a good quality meat (low fat) because high fat content is synonymous with high cholesterol anyway.

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This can lead to cotonary heart disease and clogged arteries. To improve the quality of broiler's ideal meat should have to be reduced to a minimum. In this study, will be tested *Streptococcus thermophilus* LAB in broiler's HDL levels. *Streptococcus thermophilus* is one of LAB which capable metabolism with more extreme temperatures (over summer). *Streptococcus thermophilus* have a temperature optimum growth temperature 20-53oC and 43-45oC. These bacteria are round / spiral, not berspora, gram-positive and are homofermentatif, the optimum pH for growth is 6.8, and anaerobic. These bacteria are resistant to acidity from 0.85 to 0.89%. The lactic acid produced is the result of the breakdown of glucose, fructose, galactose, sucrose and lactose (Whittier and Webb, 1970).

Problem Formulation

Based on the above problems can be formulated boundary probem as folloews:

Is the addition of Lactic acid bacteria *Streptococcus thermophilus* by *force feeding* can give effect of broiler's HDL level?

Research Purpose

The Purpose of this study is:

To know whether the addition of lactic acid bacteria *Streptococcus thermophilus* by *force feeding* can give effect of broiler's HDL level

Research Benefits

The theoretical benefit

- to know information about Lactic acid bacteria
- to know scientific information about *Streptococcus thermophilus* bacteria and its benefit
- to know mechanism of lactic acid bacteria action in influencing broiler's HDL level

The practical benefit

- to know the effect of lactic acid bacteria addition to broiler's HDL level

Hypothesis

Based on literature review and frame of mind, it can be made the hypothesis that the addition of lactic acid bacteria *Streptococcus thermophlius* by *force feeding* can give effect HDL level in broiler's blood.

RESEARCH METHODS

Research Design

This study is an experimental research. The study was designed using completely randomized design (CRD) with 4 variables are one control and three treatments lactic acid bacteria level variable, each treatment consisting of 10 repetitions.

Place and time research

Place of research

- Animal husbandary faculty of UGM, for maintenance of broiler
- Laboratory of biochemistry UNY, for manufacture of lactic acid bacteria biomass
- Laboratory of Biochemistry in the Faculty of Veterinary Medicine UGM

Time of research: Agustus – Desember, 2005

Research variable

Variables were observed in this study:

- **Independent Variable :** variations in dosing of lactic acid bacteria 1of 0^6 cfu/ml, 10^7 cfu/ml dan 10^8 cfu/ml, with the following conditions:

R0 : Group without addition of lactic acid bacteria (as a control)

R1 : Group was given dose of lactic acid bacteria by force feeding of 10^6 cfu/ml
R2 : Group was given dose of lactic acid bacteria by force feeding of 10^7 cfu/ml

R3: Group was given dose of lactic acid bacteria by force feeding of 10^8 cfu/ml

Dependent Variable: Broiler's HDL level

Population and Sample research

- **Research Population:** Strain Lohman broiler *Day Old Chick* (DOC)
- **Sample research:** 40 Strain Lohman broilers were divided into 4 treatments with each treatment consisting of 10 broilers and taken by random.

Tool and material

Tool and material used in this study are as follows:

Table 1. Feed composition and nutrient content

Feed	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 meal	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 for Indonesia. Hartadi et al(1994: 13).
Information:
BK: Dry weight
PK: Protein rough
ME: Metabolizable Energy
Pav : Phospor Available
Ca: Calcium
Met: Methionine
Lys: Lysine
Trp: Tryptofan

Table 2. Ration research nutrient content

Feed	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 meal	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

- **Tool**

  Test tube, hot plate, pH meter, analytical scale, autoclaves, incubators, Erlenmeyer tube, centrifuges, petri dish, mikrotip, coloni counter, water bath, spectrophotometer.

- **Material**

  Strain Lohman Broiler *Day Old Chick* (DOC), chicken feed consisting of corn flour, rice bran, fish meal, soybean meal and NaCl (an additional minerals), vitamins chicken (Vita chick), MRS broth, distilled water, 1 N HCl, 1 N NaOH, a solution of peptone, skim milk 10%, MRS agar, blood samples of chicken, chloroform, acetone, alcohol, acetic anhydride.

Research procedure

The research was conducted in several stages,

Preparation

This preparation involved cleaning of cage, disinfectant spraying of cage, and installation of light on the enclousure

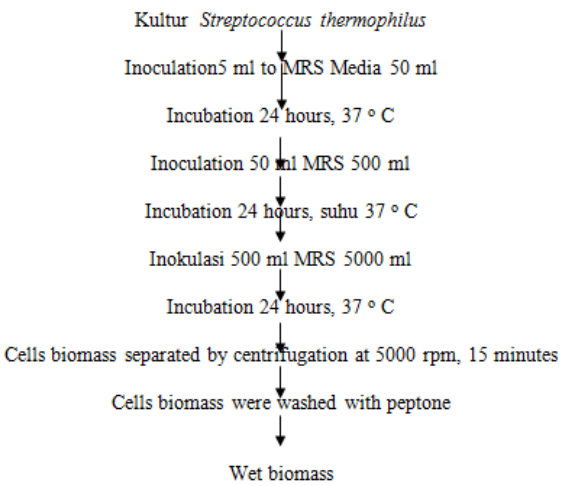
Ration preparation

The materials of ration preparation are corn flour, bran, soybean meal, fish flour, and mineral mix. Ration research is arranged with calculation result of feed composition table based on NRC (1994)

Lactic acid bacteria biomass production

Before lactic acid bacteria supplementation, necessary lactic acid bacteria production that will be given *Streptococcus thermophilus* Cell biomass production used MRS media. MRS media is made in the following manner: 3 Gram MRS Media is dissolved in 50ml water, then adjust pH 6,2. After boiling, media were sterilized by autoclaving at the temperature of 21^{o} C for 15 minutes. Media has been sterilized then gassed by CO_2. Furthermore inculating culture of *Streptococcus thermophilus* 10%/ v to the media then incubated at 37^{o} C for 24 hours.

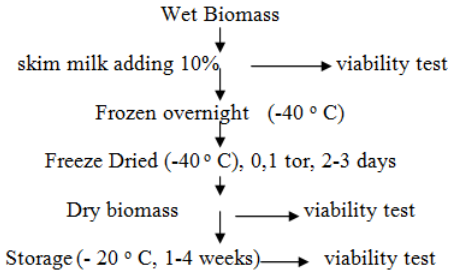
For the next procedure will be swon in the diagram:



3 gram Broth MRS media obtained by calculation:
50 X5,2 = 2,6 gram ~3 gram
100 ml
5,2 = size in Broth MRS recipe.
For a volume of 500 ,l and 5000 ml used MRS media as much as 26 grams and 260 grams

To maintain cell viability during the storage, necessary preservation cell biomass to be done. One alternative for cell biomass preservation is freeze drying. Freeze drying has advantage that small cell shrinkage, low chemical changes and stable during storage (Rudge, 1991).

The freeze drying procedures are indicated by the following diagram.



Lactic acid bacteria doses established

Before lactic acid bacteria are applied to the broiler, first determine the lactic acid bacteria dosage by counting the number of bacteria present in the dry biomass. The method is inculating dry biomass into MRS media by pour plate. 1 gram of dry biomass dissolved in 9 ml water (dilution 10^{-1}) then performed 10 times dilution in inoculated into media is dilution 10^{6}-10^{10}. After inoculated to MRS media, incubated at 37^{o} C for 24 hours then counting the number of colonies in cfu.

The result of the calculation is:

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

Because dilution 10^{-6}, 10^{-9}, and 10^{-10} did not qualified in the colonies calculation, so the dilution is used as calculation are 10^{-7} dan 10^{-8} .

Dilution	Repeat 1	Repeat 2	Repeat 3
10^{-7}	112	94	128
10^{-8}	39	107	44
Control	0	0	0

Cfu dilution average 10^{-7}= $\frac{112+94+128}{3}$ = 111,3 $\times 10^{7}$

Cfu dilution average 10^{-8}= $\frac{107+39+44}{3}$ = 63,3 $\times 10^{8}$

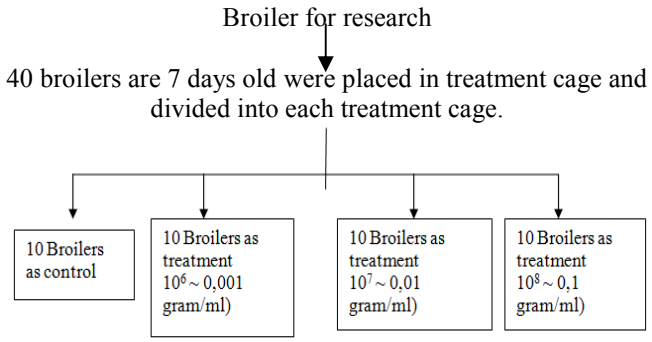
Comparation = $\frac{63{,}3x108}{11{,}13x108}$ > 2, using the smaller dilution 10^{-7} .

Cell amount = 11,03 x 10^{8} cfu/gr= 1,103 x 10^{9} cfu/gr
Converted 10^{9} = 1 gr

Treatment R1 = 10^{6} ~ 0,001 gram/ml
R2 = 10^{7} ~ 0,01 gram/ml
R3 = 10^{8} ~ 0,1 gram/ml

Probiotic Application to broiler

Application or probiotic lactic acid bacteria give to broiler by force feeding. R1 Group 10^6 cfu/ml equal 0,001 gram/ml, R2 10^7 cfu/ml equal 0,01 gram/ml and R3 = 10^8 equal 0,1 gram/ml. The scheme is as follow:



Broiler's HDL Level Measurement

CHOD-PAP method which is an enzymatic test Full Kolometrik whose sequence is as follows:

Reagents

- Reagent – precipitator 250 ml
- (1.4 mmol phosphotungstic acid; 8.6 mmol/1 magnesium chloride; stabilizer).
- Reagents solution for cholesterol determination

Procedure

Maximal Absorbance: 500 nm. Filter: 546 nm
Diameter in cuvvete: 1 cm
Put into centrifuge tube L

Serum : 200 µl
Precipitating reagent : 500µl

Mix carefully, let for 10 minutes at temperature +15 up to 25°C and then centrifugated for 15 minutes at 4000 rpm. In two hours after centrifugation, use the clear supernatant (see note) for cholesterol concentration determining by CHOD-PAP method.

	Sample	Reagent blanko
Put into test tube.		
Supernatant	100 µl	-
Aquabidest	-	100 µl
Reagent solution	1000 µl	1000 µl

Mix carefully, incubated for 10 minutes at the temperature +15 up to +25°C or for 5 minutes at the temperature +37°C and then measured the sample absorbance (A) of the reagent blank.

Calculation

HDL concentration -cholesterol = A.F

	546 nm		500 nm	
	mg/dl	mmol/l	mg/dl	mmol/l
F	222	5,74	318	8.22

Note:

Supernatant formed after centrifugation should be clear. Serum triglycerides contained more than 1000 mg.dl tend to produce of supernatant turbid or sediment that floats. If this is the case happen, dilute sample 1+1 with isotonic saline (9 g/l Δ 154 mmol/NaCl) and then do it again the deposition. Multiply the result by 2.

G. Research Design and Data Analysis

This research used a completely randomized design (CRD) one direction pattern and data has been obtained from the measurement result were analyzed by one way of Analisis Varian (ANAVA). When the treatment effect significantly, then followed by Duncan's Multiple Range Test (DMRT) test (Gaspers, 1991).

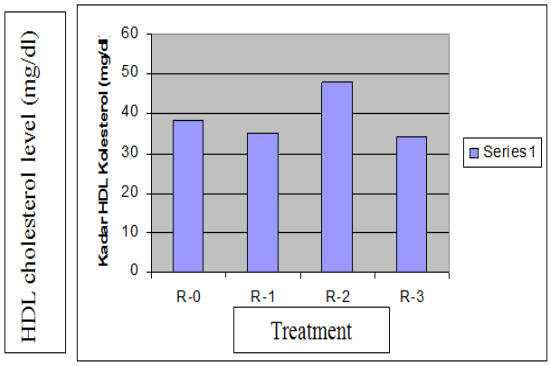
RESEARCH RESULTS AND DISCUSSION

HDL Level (*High Density Lipoprotein*)

Result of HDL research

Repeat	Treatment			
	R-0	R-1	R-2	R-3
1	26,64	19,98	35,52	35,52
2	35,52	53,28	51,06	38,85
3	31,08	14,41	51,06	28,86
4	56,61	52,17	48,84	44,40
5	41,07	35,52	53,52	23,53
Average^{ns}	38,18	35,07	48,00	34,23

High Density Lipoprotein (HDL) is a lipoprotein that helps carry cholesterol from the tissues to the liver and is then converted into bile acids. High HDL levels in the blood can reduce the risk of cholesterol piles, thus preventing atherosclerosis. Research was conducted by Usman and Hasono (2000) showed there is reduction of HDL cholesterol in hypercholesterolemia rats by skim milk adding and gived non fermentated milk of *Lactobacillus gasseri*. Rossouw et al (1981 in Usman and Hasono, 2000) also reported there is reduction in HDL cholesterol in humans who consume yogurt or milk.



Picture 1. HDL cholesterol level graphic

For HDL results showed no significant difference (P <0.05) each treatments. The highest HDL achieved by treatment of the R-2 is the provision of lactic acid bacteria 107 CFU / ml at 48.00.

While the lowest achieved by treatment of the R-3, namely the provision of lactic acid bacteria 108 CFU / ml at 34.23.

CONCLUSION AND SUGGESTION

Conclusion

Based on the result of this research and discussion can be concluded:

Lactic acid bacteria *Streptococcus thermophillus* treatment is not significantly different (P<0.05) to broiler's HDL level. Thus, lactic acid bacteria does not effect to broiler's HDL level.

Suggestion

Based on the conclusion, so that can be suggested:

- Research development about lactic acid bacteria (LAB) with the greater dose so it can be known LAB effectiveness in lower cholesterol level.
- To ensure that lactic acid bacteria using as probiotic also necessary to attachment test or bacteria adhesion in digestive tract.

REFERENCES

Akalin, A.S., S. Gone and S. Duzell. 1997. *Influence of Yogurt And Acidophilus Yogurt On Serum Cholesterol Levels In Mice.* J. Dairy Sci.

Akoso, Budi Tri. 2004. *Manual Kesehatan Unggas*. Yogyakarta: Penerbit Kanisius.

Altmasier, Sunita. 2002. *Prinsip Dasar Ilmu Gizi.* Jakarta: PT. Gramedia Pustaka Utama.

Axelsson, L. 1998. *Lactic Acid Bacteria: Classification And Physiologi, In:* Salminem, S. and A. Wright (eds). *Lactic Acid Bacteria, Microbiology and Functional Aspect 2nd ed.* New York: Marcel Dekker Inc.

Brock, T. and Madigan, M. T. 1988. *Biology Of Microorganism 8th (eds).* New Jersey: Prentice Hall Inc, Englewood Cliffs.

Drasar, B.S. and P.A. Barrow. 1985. *Intestinal Microbiology Am Soc for Microbial.* Washington, AS.

Fardias, S. 1988. *Fisiologi Fermentasi.* Bogor: PAU Bogor.

Fardias, S. 1989. *Mikrobiologi Pangan.* Yogyakarta: PAU Pangan dan Gizi UGM.

Frost, G.M. and Most, D. A. 1987. *Production of Enzyme By Fermentation.* Dalam: *Biotechnology* Rehm, M. J. and Reed, G. (eds). Vol. 7A. Weinheim: Verlag Chemic.

Fuller, R. 1992. *History and Development of Probiotics* . Edited by Fuller. *Probiotic: The Scientifis Basis.* London: Chapman And Hall.

Gilliland, S.E., Nelson, C. R. and Maxwel, C. 1985. *Assimilation of Cholesterol by Lactobacillus acidophilus.* J. App Environ Microbiology.

Gilliland, S.E., and Walker, D.K. 1990. *Factor To Consider When Selecting a Culture of Lactobacillus acidophilus As Dietary Adjunt To Produce a Hypocholesterolemic Effect In Humans.* J. Dairy Sci.

Gilliland, S.E., and Corzo, G. 1999. *Bile Salt Hydrolase Activity of Three Strains of Lactobacillus acidophilus.* J. Dairy Sci.

Grunewald, K.K. 1982. *Serum Cholesterol Levels in Rats Fed Skim Milk Fermented By Lactobacillus acidophilus.* J. Dairy Sci.

Haris, Abdul. 1997. *Beternak Ayam Pedaging Super.* Pekalongan: CV. Gunung Mas.

Irawan, Agus. 1995. *Penanggulangan Berbagai Penyakit Ayam.* Solo: CV. Aneka.

King, M.W., 2002. *Cholesteroland Bile Metabolism.* http: indstate.edu/ thcme/ mwking/cholesterol. html.

Linder, M.C.1992. *Biokimia Nutrisi dan Metabolisme dengan Pemakaian Secara Klinis* (Aminuddin Parakkasi. Terjemahan). Jakarta: UI Press.

Liong, M.T. and N. P. Shah. 2004. *Bile Salt Deconjugation Ability, Bile Salt Hydrolase Activity and Cholesterol Content of Fresh Lambs With Lactobacillus acidohlius.* Meat Sci.

Mc. Donald, P.R.A. et al. 1995. *Animal Nutrition Fifth Editian*. Singapore: Longman Singapore Published (Pte). Ltd.

Montgomery, R. et al. 1993. *Biokimia Suatu Pendekatan Berorientasi Jilid 2*. Yogyakarta: Gadjah Mada University Press.

Muchtadi, Deddy, dkk. 1993. *Metabolisme Zat Gizi*. Jakarta: Pustaka Sinar Harapan.

Murray, R. K. et al. 1985. *Harper's Biochemistry* (Andry Hartono. Terjemahan). Jakarta: EGC.

Murtidjo, Bambang Agus. 2004. *Pedoman Beternak Ayam Broiler*. Yogyakarta: Penerbit Kanisius.

Noh, D.O., S.H. Kim and S.E. Gilliland. 1997. *Incorporation of Cholesterol into The Celluler Membrane of Lactobacillus acidophilus ATCC 4321.* J. Dairy Sci.

Playne. M.J.1999. *Classification and Identificationof Probiotic Bacteria Strains.* Probiotica.

Plummer, D.T.1977. *An Introduction to Practical Biochemistry.* New Delhi: Tata Mc. Graw Hill Pub. Co. Ltd.

Poedjiadi, Ana. 1994. *Dasar- Dasar Biokimia*. Jakarta: Penerbit UI Press.

Rao, D.R., C.B. Chawan and S.R. Pulusani. 1981. *Influence of Milk and Thermophilus Milk on Plasma Cholesterol Levels and Hepatic Cholesterolgenesis in Rats.* J. Food Sci.

Rasyaf, Muhammad. 1991. *Produksi dan Pemberian Ransum Unggas.* Yogyakarta: Penerbit Kanisius.

Rasyaf, Muhammad. 1994. *Makanan Ayam Broiler.* Yogyakarta: Penerbit Kanisius.

Rasyaf, Muhammad. 2003. *Beternak Ayam Pedaging.* Yogyakarta: Penerbit Kanisius.

Scheinbach, S. 1998. *Probiotics: Functionally and Commercial status.* Biotechnology Advances.

Sudaro, Yani dan Siriwa, Anita. 1997. *Ransum Ayam dan Itik* . Jakarta: Penerbit Penebar Swadaya.

Sudaryani, Titik dan Santosa Hari. 1994. *Kualitas Telur.* Jakarta: Penerbit Penebar Swadaya.

Wahju, Juju. 2004. *Ilmu Nutrisi Unggas*. Yogyakarta: UGM.

Whittier, E.O and B.H. Webb.1970. *By Product Milk.* Connecticut: The AVI Publishing Company.

Wibowo, J. 1993. *Produksi Antibiotik dengan Proses Fermentasi.* Kumpulan Handout Lokakarya. 11-26 Januari 1993. Yogyakarta: PAU Bioteknologi UGM.

Widodo, Wahyu. 2002. *Nutrisi dan Pakan Unggas Kontekstual.* Jakarta: Direktorat Jenderal Pendidikan Tinggi Departemen Pendidikan Nasional.

Widodo. 2003. *Bioteknologi Industri Susu.*Yogyakarta: Lacticia Press.

Winarno, F.G. 1992. *Kimia Pangan dan Gizi.* Jakarta: PT. Gramedia Pustaka Utama.

Wirahadikusumah, M. 1985. *Biokimia Metabolisme Energi, Karbohidrat dan Lipid.* Bandung: ITB.

Yuwanta, Tri. 2000. *Beberapa Metode Praktis Penetasan telur.* Yogyakarta: UGM Press

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