International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2013): 6.14 | Impact Factor (2015): 6.391

The Use of Probiotic Lactate Acid Bacterium, *Streptococcus thermophylus* of Fish Feces Waste to the Meat Fat Level of Broiler Chicken *Lohmann* Strain

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Abstract: ***This research was conducted to study the effect of lactate acid bacterium Streptococcus thermophylus of fish feces waste to lower meat fat level of broiler chicken Lohmann strain. Forty a week old broiler chicken were divided into four probiotic treatments, they are: R-0 (without LAB), R-1 (LAB 10^6 CFU/ml), R-2 (LAB 10^7 CFU/ml), R-3 (LAB 10^8 CFU/ml) and each replication contains ten chicken broiler. Food and water are given twice a day in adlibitum way. This research was progressed in 28 days. Collected data are meat fat level. The data were analyzed by variance analysis using one way completely randomized design (CRD), followed by testing the significance means using Duncan's Multiple Range Test (DMRT). As the result, the meat fat level shows significant differences P<0.05%. The average of meat fat is 1,94; 1,84; 1,45 and 1,36%. It can be concluded that probiotic LAB Streptococcus thermophylus of fish waste feces can reduce the chicken broiler Lohmann strain meat fat level.***

Keywords: Broiler chicken, lactate acid bacterium, meat fat, probiotic

1. Introduction

Nowadays, people tend to choose healthy food which is free from pollution synthetic chemicals and antibiotic residues. Unhealthy food causes cancer, indigestion, respiratory disorder, and the other disease. Indonesia broiler export is rejected since there are antibiotic residues in carcass. Besides that, consumer also concerns with the meat fat level. There are many bad effect of using antibiotic, so that the use of probiotic lactate acid is needed to be developed.

The rapid growth of broiler is caused by giving too much fat particularly for the final phase broiler. As we know, high fat level is identically high cholesterol which causes coronary heart disease and clogged arteries. The meat fat should be reduced to produce healthy meat.

Since lactate acid bacterium (LAB) is able to balance microbes' population in the digestive tract and inhibit s the pathogenic bacterium growth, giving LAB as the probiotic can improve feed efficiency and livestock performance. Moreover, LAB is also able to produce *bile salt hydrolase*. It serves to de-conjugate bile salt which plays role in fat emulsifying to be absorbed by body. Giving probiotic lactate acid is expected to reduce the broiler meat fat level.

The Objective of the Research

This research aims to know the effect of using probiotic to the meat fat level of broilers.

The Significance of the research

The result of this research is expected as information related to the effect of using probiotic LAB to reduce fat level of broiler carcass.

2. Materials Dan Methods

Research Setting

This research was conducted in October-December 2005 at poultry cages Nutritional Biochemistry Laboratory, Nutrition and Animal Feeding Department, Animal Husbandry Faculty, Gajah Mada University, Yogyakarta. While fat analysis was conducted at Nutritional Biochemistry Laboratory, Nutrition and Animal Feeding Department, Animal Husbandry Faculty, Gajah Mada University, Yogyakarta.

Materials

Chicken

The chicken used were forty a week old male broilers *Lohmann* strain produced by PT. Multi Breeder Adirama.

Cages

The cages used were 40 individual battery cages which sized 30 x 50 x 25 cm. Each cage was equipped plastic feed and drinking place. The other equipments were heating lamps, blending place, scales from Daema brand with 5 kg capacity and 20 gram sensitivity and Airlux brand 3 kg capacity and 10 gram sensitivity.

Probiotic Treatments

Isolates Lactate Acid Bacterium (LAB) used was *Streptococcus thermopillus* bacterium in *freeze drying* form from Nutritional Biochemistry Laboratory, Animal Husbandry Faculty, Gajah Mada University. The feeding treatments were R-0 (without LAB), R-1 (LAB 10^6 CFU/ml), R-2 (LAB10^7 CFU/ml), R-3 (LAB 10^8 CFU/ml).

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:

Table 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 : Calsium
Met : Metionin
Lys : Lysin
Trp : Tryptofan

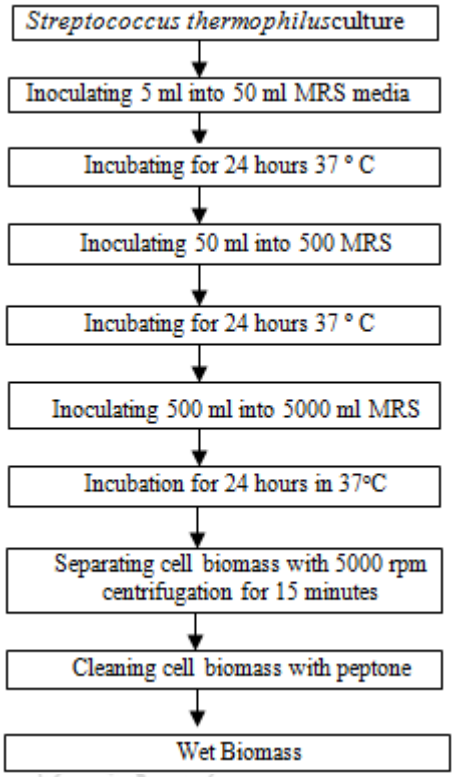
Table 2: Feed nutrition of research ransom

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

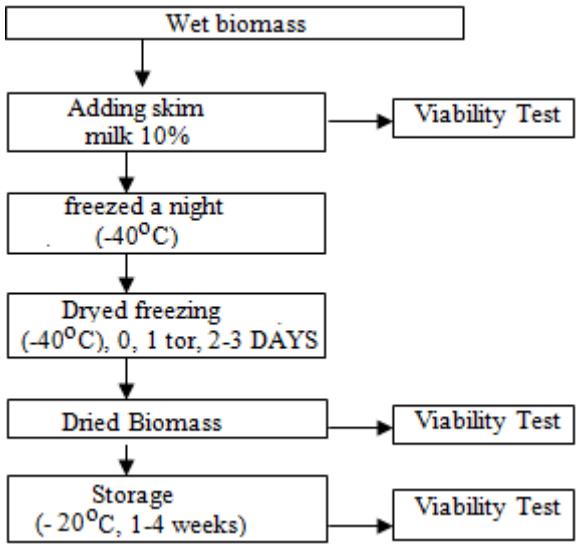
For volume 500 ml and 5000 ml, MRS medium used was 26 gram and 260 gram.

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 (dilution 10^{-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 the 10^{-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

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

The average of colony forming unit dilution

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

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

3. Research Methods

Preparation

The cages and the tools were disinfected using biochid first. Vaccination was done two times: ND - 1 vaccination at 3 days age and ND – 2 vaccinations at 20 days age. Based on the calculation result of NRC (1994) feed composition tables, feeding and drinking water were given twice a day at 07:00 AM and 15:00 PM. Probiotic were given in the afternoon through drinking water by using spet 1.5 ml per oral.

Research Plan

The model design used was one way completely randomized design. Forty boilers are divided into 4 categories (10 boilers in each category). Each treatment was repeated 10 times using 10 boilers for each test.

Collected Data

Fat boilers meat level.

Meat fat sample. The sample was obtained by blending the right chicken breast meat, and then took 1 g to be analyzed ether extract by Soxhlet Extraction methods (Sudarmaji *et al*., 1989).

Meat fat percentage. Meat fat percentage was obtained by using Soxhlet Extraction Methods (Sudarmaji *et al*., 1989).

Determination of meat fat level using soxhlet extraction

First, wrap one gram of meat sample in fat-free filter paper. Second, bake the sample at a temperature 105 ^{0} C for 12 hours and then weighed in hot condition. Third, put the sample into soxhlet extraction and add methanol and chloroform in the ratio 1:2. The extraction was conducted for 8 hours till methanol and chloroform in the extraction change into clear colored. Then, bake the sample which has been fat extracted at a temperature 105 ^{0} C for 12 hours then weighed in hot condition.

% fat level = X 100%

X= sample weight

Y= baked sample weight

Z= baked and fat extracted sample weight

Data Analysis

Collected data were analyzed one way using completely randomized design and if there are differences between the average, it will be continued using *Duncan's Multiple Range Test* (DMRT) (Gomez dan Gomez ,1984).

4. Results and Discussion

Meat fat

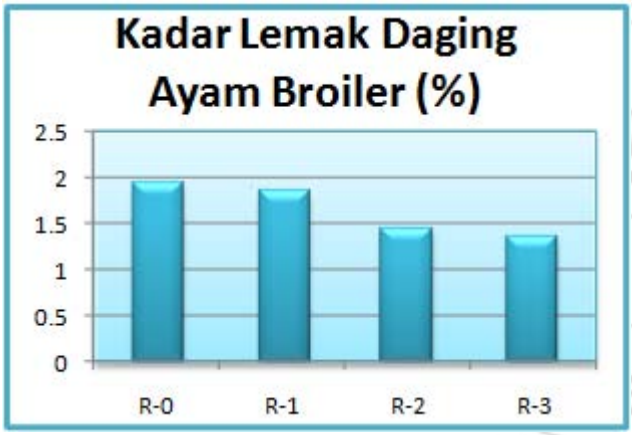
The significant impact (P<0, 05) of giving probiotic to the meat fat content of broilers can be seen in Table 1. The meat fat content decreased with the increase use of LAB. De-conjugation of bile salt by LAB inhibited fat absorption in small intestine so that fat production is slightly.

Table 1: The average of chicken broiler meat fat (%)

Repetition	R-0	R-1	R-2	R-3
1	1.87	2.26	1.26	1.29
2	1.56	2.18	1.56	1.04
3	1.62	2.28	1.37	1.15
4	2.14	0.98	1.42	1.81
5	1.82	2.56	1.37	1.96
6	1.58	1.15	1.69	1.17
7	2.52	2.29	1.14	1.95
8	1.70	1.06	1.76	0.87
9	2.04	1.87	1.75	0.98
10	2.62	2.01	1.22	1.45
Average	1.95^{c}	1.85^{ab}	1.45^{b}	1.37^{a}

^{ab} superscript on the same average shows significance differences (P<0,05)

Data in the Table 1 shows that the more bacterium amount, the fat content is decreased. R3 treatment shows the lowest fat content (1.37) since the bacterium amount at most (10^{8} CFU/ml). It is in line with Safalaoh (2005), probiotic lactate acid bacterium is able to reduce fat content up to 0, 06 %.



The average graphic of meat fat content. Based on graphic 2 above, there is reduction of broiler meat fat level. The lowest reduction can be seen in the R-3 i.e 1.37. Using probiotic LAB gives significant impact (P<0, 05) to the broiler meat fat content. It shows that LAB is able to de-conjugate bile salt to inhibit the fat absorption in the small intestine, so fat production will be slightly. The more of LAB amount, the fat content will be decreased. It is proved in R3 which contains LAB at most (10^{8} CFU/ml) and fat content at lowest (1,37 %).

5. Conclusion and Suggestion

5.1 Conclusion

Based on the research, it can be concluded that:

1) Giving probiotic LAB causes the reduction of meat fat level of chicken broiler strain lochman significantly.
2) The best LAB level is in the 10^{8} CFU/ml (R3) level i.e. 1.37.

5.2 Suggestion

Further research is needed for the use of LAB as a probiotics for chicken in order to reducing the fat content of meat fat level and cholesterol content with different level and more practical and efficient methods.

6. Acknowledgements

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) Prof. Eni Harmayani

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