Influence of liquid probiotic inclusion as feed additives on lipid profiles and meat cholesterol content of commercial broiler chickens

Imran¹, S Wajizah² and Samadi²*

¹ Magister Program of Animal Husbandry Department, The Faculty of Agriculture Universitas Syiah Kuala-Banda Aceh
² Animal Husbandry Department, The Faculty of Agriculture Universitas Syiah Kuala-Banda Aceh

*E-mail: samadi177@unsyiah.ac.id

Abstract. Due to consumer’s concern for food safety and the pathogenic bacterial resistance of antibiotics in humans, the usage of antibiotics as animal feed has been banned. The aims of this study was to investigate inclusion of liquid probiotic in drinking water on lipid profiles and meat cholesterol content of commercial chickens. Totally 100 chickens at the age of 28 d from growth study were selected for 20 chickens (5 birds per treatment) for further lipid profiles and meat cholesterol content study. Four treatment in this study was inclusion of liquid probiotics in the drinking water with different concentration (P1=control, P2=1.0 %, P3=1.5 % and P4=2.0 % of probiotic in drinking water). The result of the study indicated that inclusion of liquid probiotic up to 2% did not significantly reduce the weight and percentage of abdominal fat and cholesterol in the breast meat broiler. However, inclusion of probiotics at the level of 1.5% was the best level indicated low cholesterol concentration and high value of iodine concentration in meat broilers. Based on the study, it was concluded that probiotics can be used as alternative feed additive to replace antibiotics as growth promoters up to 1.5% at the best.

1. Introduction
Due to adverse effects of antibiotics as growth promoters in animal production (antibiotic resistance and residues in animal products and environment), the usage of antibiotics as animal feed has been banned in Europa since 2006 and Indonesian government released the regulation to prohibit antibiotics as growth promotes in animal production since January 2018. Nutritionists have investigated other alternative feed additives as growth promoters such as prebiotics, enzymes, organic acids, immunostimulants, phytogenic feed additives, bacteriocins, bacteriophages, nanoparticles and essential oils [1]. The purpose to inclusion of these alternative feed additive was to maintain a low mortality in animal production, improve animal performance and preserve consumer health without any adverse effects to animal and consumers.

Probiotics known as an alternative feed additive in the form of live micro-organisms function to improve feed digestion and host health when provided into adequate amount [2]. Furthermore, the roles of probiotics in animals are to maintain the balance of intestinal microflora in the digestive tract, to improve activity of digestible enzyme and feed consumption, to reduce ammonia production,
and to stimulate immune system of animals [3]. Administration of probiotics and other natural agents as in animal production has been reported [4]-[6].

The potential function of probiotics was not only for to improve animal performance but to reduce cholesterol and other unbeneﬁcial lipid as well. WHO predicted that cardiovascular diseases can be still leading causes of death in 2030 and hypercholesteolemia contributed the high risk of cardiovascular disease. It has been reported that probiotic was be able reduce three causes of coronary diseases namely high level of cholesterol, blood pressure and triglyceride [7]. The research conducted by [8] administration of lactobacillus bacteria reduced blood cholesterol 22% and triglyceride 33%. Low cholesterol concentration in the probiotic treatment due to the role of probiotic to inhibit hydroxymethyl-glutaryl-coenzyme A. This enzyme contributed in the pathway of cholesterol synthesis by reducing the synthesis of cholesterol [9]. Panda et al., [10] reported that poultry administrated probiotic _Lactobacillus sporogenes_ (6x10^8) signiﬁcantly reduced lipid proﬁles of boiler (serum total cholesterol, LDL, VLDL and triglycerides).

Probiotics “Nanggro” is liquid probiotics containing _Lactobacillus casei_ and _Saccharomyces cerevisiae_ by mixing commercial probiotics from bacteria and yeast. This probiotics produced by Animal Husbandry Department, Universitas Syiah Kuala. The study relating to the aﬀectivities of this probiotics in broiler production was still limited. Therefore, it is needed to study about administration this liquid probiotic in commercial broiler chickens with the measured parameters of proﬁles lipid.

2. **Materials and methods**

2.1. **Animal and feeding**

The growth study was carried out at private enterprise farm in Banda Aceh. Lipid proﬁles (abdominal fat and iodine values) was conducted at Animal Nutrition Laboratory, Animal Husbandry Department, the Faculty of Agriculture and Laboratory Analysis of Food and Agricultural Products, the Faculty of Agriculture Universitas Syiah Kuala Banda Aceh. Meat cholesterol content was analysed at Integrated Laboratory Feed Technology and Nutrition, Bogor Institute of Agriculture.

One hundred chickens from growth study were selected for 20 chickens at the age of 28 d for further measurement of lipid proﬁles (fat abdomen, meat cholesterol content and iodine values). In growth study, one hundred of mixed sex commercial broiler chickens (CP 707) with the beginning of body weight of 38.2 ±2.45 g, 38.4 ±2.38 g, 37.8 ±2.53 g and 37.2 ±2.45 g for P0, P1, P2 and P3 respectively was reared for 28 d. The treatment in the growth study was concentration of liquid probiotic in the water consisting of P1=control, P2= 1.0 % of liquid probiotic in the drinking water, P3= 1.5 % of liquid probiotic in the drinking water, and P4= 2.0 % of liquid probiotic in the drinking water. All animal provided free access of commercial feed and water. All broiler were reared under uniform environmental and management condition for the whole periods of study.

2.2. **Sample preparation for breast and drumsticks measurements and iod measurement**

Chickens from growth study (one per each unit) were selected based on closest weight to average weight for fat proﬁles and meat cholesterol measurements. The birds was fasted to complete evacuation of the gut. Broiler were slaughtered according to animal ethics and dressed for carcass and fat abdomen measurements. Meat cholesterol content of yields cut (breast and drumsticks chicken) was measured by using the method of Lieberman-Burchard. This method known as acetic anhydride test is applied to detect the cholesterol concentration. The colour of green or green-blue colour after a few second test indicating positive of cholesterol content. Further measurement of cholesterol content was by using spectrophotometry [11,12].

The principle to determine iodine values is by applying Hanus method. The procedure as follow: adding bromide iodine in the mixtures of acetic acid and tetra chloride carbon in the samples. After standard reaction, determination the excess of halogen is by adding potassium iodide solution which is exempted from titration by using the standard solution of sodium thiosulfate [13].
2.3. \textit{Statistical analysis}

All data were subjected to statistical analysis by employing analysis of variance for one way analysis and designed to completely randomized design (CRD) with the following equation: \( Y_{ij} = \mu + \alpha_i + \epsilon_{ij} \)

The statistical analysis were performed by using SPSS software and expressed as mean±SEM. The statistical significant was considered (\( P<0.05 \)). The means of treatment were compared with duncan multiple range test [14].

3. \textit{Results and discussion}

3.1. \textit{Fat profiles}

Abdominal fat is a very important profile fat in the poultry production since it deposits fat faster than other fat tissues. Abdominal fat can be used to evaluate total fat content on the poultry species [15]. The weight of abdominal fat consists of the whole of lipid from visceral fat to subcutaneous fat in the abdomen [16].

The result of the study indicated that inclusion of liquid probiotic in drinking water up to 28 d did not significantly influence (\( P>0.05 \)) on weight and percentage of abdominal fat (Table 1). However, the percentage of abdominal fat in this study was in the normal range from 1.24 to 1.59%. This is in accordance to [17] stated that abdominal fat in broiler carcass ranged from 0.73% to 3.78%.

Table 1. The Effect of liquid probiotics administration up to 28 d on weight and percentage of abdominal fat commercial broiler chickens (P1=control, P2= 1.0 % of liquid probiotic in the drinking water, P3= 1.5 % of liquid probiotic in the drinking water, and P4= 2.0 % of liquid probiotic in the drinking water).

| Parameters                          | Treatment       | P-value |
|-------------------------------------|-----------------|---------|
| Weight of abdominal fat (g)         | P0              | 26.20±4.14 |
|                                     | P1              | 20.72±1.00 |
|                                     | P2              | 22.30±4.37 |
|                                     | P3              | 27.42±5.56 |
| Percentage of abdominal fat (%)     | P0              | 1.45±0.20 |
|                                     | P1              | 1.24±0.11 |
|                                     | P2              | 1.30±0.24 |
|                                     | P3              | 1.59±0.35 |
| Meat Cholesterol Content (mg/100g)  | P0              | 90.51±6.15 |
|                                     | P1              | 79.47±3.94 |
|                                     | P2              | 66.63±4.46 |
|                                     | P3              | 78.96±7.00 |

Our result was similar to research conducted by Wulandari et al., [16] in which broiler administrated with \textit{Saccharomyces cerevisiae} at the level of 0.15% for 3 weeks did not reduce abdominal fat up to 36 d. Santoso et al., [18] also reported that inclusion various levels of probiotic \textit{immunobac} did not significantly decrease abdominal fat percentage of broiler up to 45 d.

No significantly decreased on weight and percentage of abdominal fat as a result of probiotic administration, it was assumed that protein content for all diet treatments has been formulated based on requirement (21%). Therefore, lipid accumulation was minimized. Suartika et al., [19] reported that reducing of protein in the diet increased of abdominal fat accumulation since it can increase nitrogen metabolism and deduce energy metabolism. Administration of \textit{Saccharomyces spp} 0.2% in the low protein diet (18%) significantly reduced abdominal fat compared to broiler fed low protein without probiotic supplementation. \textit{Saccharomyces spp} was able to improve the value of metabolic energy and feed digestibility.

On the other hand, reviewed articles by Pourakbari et al., [20] indicated that some previous studies showed that abdominal fat reduced by inclusion of probiotics in the diet compared to control. Santoso et al., [21] stated that administration of probiotic (\textit{Bacillus subtilis}) to female boilers reduced abdominal fat due to reduction of acetyl-CoA carboxylase activity. This enzyme function to synthesis lipid after supplementation of \textit{Bacillus subtilis}. Probiotics has a role to manage lipid metabolism in the body. In addition, inclusion of 12 mixed strain \textit{Lactobacillus} at 0.1% to broiler up to 4 weeks
decreased concentration of triglyceride serum followed by decreasing of abdominal fat up to 42 d [22].

3.2. Meat cholesterol content
Cholesterol is metabolism product from animals and can be obtained from animal origin food such as yolk, meat, liver and brain. In the body, cholesterol was produced from the liver synthesis [23]. From previous studies indicated that administration of probiotics in broilers was able to reduce cholesterol either in blood or meat [24]. In this study, even though probiotics was not able statistically reduce concentration of cholesterol in meat, administration of probiotics 1.5% in drinking water tended to reduce cholesterol content in meat (P=0.056) as shown in the Table 1. Compared to control, administration of probiotic 1.5% reduced cholesterol content in breast meat 26.4% (90.51 vs 66.63 mg/100 g).

Andriani et al., [25] reported that inclusion of L. casei dan L. rhamnosus 0.05g/kg, 0.01g/kg in feed and 0.025g/liter, 0.05 g/liter in drinking water as AGP reduced cholesterol total of broilers. Reduction of cholesterol due to probiotics administration was also reported by Anjarawati et al., [26] inclusion of Saccharomyces spp G-7 to broilers at the age of 2-6 weeks. Wulandari et al., [16] also reported that inclusion of probiotics S. Cerevisiae reduced breast meat with the best of 0.1% in the diet.

The content of L. casei in the probiotics was able to reduce cholesterol content by the mechanism involved deconjugation of bile acids by bile-salt hydrolase (BSH) produced by probiotics. As a result of deconjugation, bile acids solubility will reduce and absorption in the intestine was hampered and excreted to feces. Therefore, it required the other amount of cholesterol as precursor to synthesis new bile acids as homeostatic respond and reduced cholesterol content in the blood [27]. Furthermore, application of S. cerevisiae as yeast group to broilers increased bile salt in the intestine and depressed hydroxyl3-methylglutaryl-coenzyme reductase as precursor for cholesterol synthesis, therefore concentration cholesterol reduced [28].

In addition, low pH condition as result of lactic acid produced by probiotics in the digestive tracts will stimulate bile salt secretion to neutralize pH by using cholesterol in the blood as materials to synthesis salt solution. The use of cholesterol in the blood caused deposition of cholesterol in meat to be low [29]. This is the reason why appliation of probiotocs in the diet was able to reduce cholesterol content in the meat.

3.3. Relationship of abdominal fat weight with cholesterol of broiler breast meat linier
Regression analysis in this study indicated that there was weak correlation between cholesterol concentration and abdominal fat due to probiotic administration in drinking water as presented in Figure 1.

The result indicated that there was weak correlation between concentration of cholesterol in meat and abdominal fat with the regression equation was $y = -4.749x + 90.765$. Determination value of this regression was $R^2= 0.394$ meaning that 39.4% of cholesterol reduction was influenced by the percentage of abdominal fat administrated various level of probiotics in drinking water. Meanwhile, 60.6% was affected by other factors. The value of coefficient correlation (r) based on the regression equation was 0.48 or 48.2%. It was indicated that reduction of cholesterol in meat broiler had weak correlation with reduction of lipid percentage in abdominal fat.

Deposition of abdominal fat was more influenced the composition of feed nutrients such as energy, protein and the availability of lipid in the blood either from ration or lipogenesis prose de novo in the liver [15]. Some studies report that abdominal fat decreased by probiotics administration, however the other reports informed that administration of probiotics did not influence abdominal fat reduction. On the other hand, probiotics has more convincing role in reduction cholesterol content both in blood and meat. One of the mechanism was by enzymatic deconjugation from bile acids by bile-salt hydrolase (BSH) produced by probiotics [27].
3.4. Iodine values

Iodine value expressed the level of oil and fat saturation. Unsaturated fatty acids indicated double bonds from fats and reacted with iodine compounds. The higher iodine values in the oil or fat indicated that the higher double bonds in those substances [30, 31]. The iodine values of meat boiler administrated probiotics in drinking water is presented in Figure 2.

![Graph showing correlation between weight of abdominal fat and cholesterol concentration](image_url)

**Figure 1.** Correlation between the weight of abdominal fat and cholesterol concentration in meat of broiler administrated liquid probiotics in drinking water up to 28 d.

![Graph showing iodine values](image_url)

**Figure 2.** The iodine value of meat boiler administrated liquid probiotics in drinking water up to 28 d (P1=control, P2= 1.0 % of liquid probiotic in the drinking water, P3= 1.5 % of liquid probiotic in the drinking water, and P4= 2.0 % of liquid probiotic in the drinking water).
Figure 2 shows that the most optimal of reduction saturated fat acids in the breast meat broilers was by inclusion of 1.5% probiotic in drinking water. Saturated fat acids reduction was directly proportional to reduction of cholesterol in meat. Santoso et al., [21] reported that inclusion of probiotics reduced triglyceride since probiotics effectively reduced the activity of acetyl CoA carboxylase. This enzyme functions to accelerate lipid synthesis mainly saturated fat. Reduction of saturated fat improved meat quality and meat tenderness [24].

4. Conclusions
Inclusion of liquid probiotic in drinking water up to 2% did not significantly reduce the weight and percentage of abdominal fat and cholesterol in the breast meat broiler. However, inclusion of probiotics at the level of 1.5% in drinking water was the best level indicated by the trend of cholesterol concentration reduction in this level compared to control. In addition, the level of 1.5% probiotics in drinking water had high level of iodine concentration meaning that concentration of unsaturated fatty acids in this meat was higher than other treatments.

References
[1] Mehdi Y, Letourneau-Montminy M, Gaucher M, Chorfi Y, Suresh G, Rouissi T, Brar S T, Cote C, Ramirez A A and Godbout S 2018 Anim. Nutri. 4 170-178
[2] FAO/WHO 2002 FAO/WHO, London, UK
[3] Jin L Z, Ho Y W, Abdullah N and Jalaludin S 1997 Journal World's Poult. Sci. J 53 4
[4] Samadi, Delima M and Latief H 2019 IOP Conference Series: Earth and Environmental Science, 2019 260
[5] Samadi, Wajizah S and Tarman A 2020 IOP Conference Series: Earth and Environmental Science, 2020. 497
[6] Samadi, Wajizah S and Tarman A 2020 IOP Conference Series: Earth and Environmental Science, 2020. 425
[7] Safaloo A C L 2006 Afric. J. of Food Agric. Nutri And Devl. 6 1-10
[8] Taranto M 1999 J. of Dairy Sci. 83 401-403
[9] Fukushima M and Nakano M 1995 Brit. J. of Nut. 73 701-710
[10] Panda A K, Rama Rao S V, Raju M V L N and Sharma S R 2006 J. of Poult. Sci. 43 235-240
[11] Campbell, Mary K and Shawn O F 2005 Singapore: Thomson Asia Pte Ltd
[12] Witantra 2011 Artikel Ilmiah. Universitas Airlangga. Surabaya
[13] Paqout C and Hautfenne A 1987 Blackwell Sci. Pub, California
[14] Steel R G D dan Torrie J H 1995 Gramedia Pustaka Utama. Jakarta
[15] Fouad A M and El-Senousey H K 2014 Asian Australas J. Anim Sci. 2 1057-1068
[16] Wulandari S, Syahniar T M and Pantaya D 2020 Bulletin of Anim. Sci. 44 27-33
[17] Salam S, Fatahilah A, Sunarti D dan Isroli 2013 Jurnal Sains Peternakan. 11 84-89
[18] Kafilzadeh F, Safari, Parvar M R 2003 J. Agric. Sci. Nat Resour. 9 173-184
[19] Suartika I G B, Sumadi I K, and Bidura I G N G 2014 E-Journal Anim. Sci. Udayana University. 3 1-10
[20] Pourakbari M, Seidavi A, Asadpour L and Martinez A 2016 Annals of the Brazilian Academy of Sci. 88 1011-1021
[21] Santoso U, Tanaka K and Ohtania S 1995 Br. J. Nutr. 74 523-529
[22] Kalavathy R, Abdullah N, Jalaludin S and Ho Y W 2003 Br. Poult. Sci. 44 139-144
[23] Murray R K, Bender D A, Bothan K M, Kennelly P J, Weil P A and Rodwell V W 2012 The Mc Graw-Hill Companies Inc. USA
[24] JadHAV K, Sharma K S, Katoch S, Sharma V K and Mane B G 2015 J. of Anim. and Vet. Sci. 2 04-16
[25] Andriani A D, Lokapirnasari W P, Karimah B, Hidanah S, Al-Arif M A, Soeharsono and Harijani N 2020 J. Medik Vet. 3 114-122
Acknowledgments
Thanks to FA Pronak as small industry providing probiotics sources for this study. We would like also thanks to Universitas Syiah Kuala for community service grand under Research and Community Service Institution to support FA Pronak to produce various kinds of feed additives.