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The effect of non-AGP feed on blood cholesterol and profile of meat fat of broiler chickens reared in different regions

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ABSTRACT: This study aimed to determine the levels of blood cholesterol, meat fat and cholesterol, and fatty acids of broiler chickens fed non-AGP feed in three different regions. The material used consisted of 45 broilers from 3,000-10,000 broiler breeders and during maintenance they were not given any antibiotics at all. The research method used is a survey method. The data obtained were then analyzed by One Way ANOVA using the General Linear Model with regional differentiating factors, namely Banyumas, Cilacap, and Purbalingga. The variables observed included total blood cholesterol levels, blood low-density lipoprotein (LDL), blood high-density lipoprotein (HDL), blood triglycerides, meat cholesterol, and total fat of broiler chicken in Banyumas, Purbalingga, and Cilacap areas. The results showed that the use of non-AGP feed in broilers raised in three different areas resulted in total cholesterol, HDL, LDL, triglycerides, fats, and fatty acids which were not significantly different (P> 0.05), but produced meat cholesterol. different (P <0.05). It can be concluded that the non-AGP feed given to broilers in three different regions resulted in relatively the same blood cholesterol, fat, and meat fatty acid profile, but caused the meat cholesterol levels in the three regions to be different.

Keywords: Broiler chicken; Non-AGP feed; Cholesterol; Fat

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INTRODUCTION

The demand for animal-based food has been increasing as the human population grows. Meat is a popular livestock product among consumers, and it meets the average demand for animal protein, such as broilers. Broilers are chickens with fast growth, optimum meat production, relatively short meat growth, and relatively high body weight.

Central Java province is a region that produces the most broiler chickens after West Java and East Java. The populations of broiler chickens in Central Java was 180.634.329 spread across the regencies, such as Banyumas, Cilacap and Purbalingga (Ditjennak, 2018). There were 7.537.900 broiler chickens Banyumas (BPS Kabupaten Banyumas, 2018), 6.371.850 in Cilacap (BPS Kabupaten Cilacap, 2019), and 4.900.918 in Purbalingga (BPS Kabupaten Purbalingga, 2019).

Feed additives are used to increase feed efficiency for livestock. Farmers commonly use *Antibiotics Growth Promotor* (AGP) to increase livestock productivity, improve feed efficiency, and decrease the mortality rate. However, AGP also causes allergy, skin, and cardiovascular disorders, as well as the gastrointestinal tract, such as stomach ache, urticaria, and hypotension (Swastike, 2014). These factors lead to the prohibition of AGP for animal feed and the suggestion to replace it with other feed additives like probiotics, prebiotics, herbal plants, and others. Antibiotics in AGP are absorbed by nutrients in meat, milk, and egg; therefore, the small fraction of antibiotics is indirectly consumed by the consumers. Incorporating AGP into animal feed would not affect the germ-free animals and it also depends on the sanitation level of the farms (Kompiang, 2009).

Broilers' fast growth is always followed by rapid fat storage. Fat accumulation is synchronized with an increasing body weight because broilers have a high appetite but slow movement; therefore, the consumed energy is stored as body fat. Cholesterol is a product of fat metabolism which makes hormones and vitamin D as well as repairing damaged body cells. The type of transport lipoprotein which plays an important role in fat transportation and metabolism is plasma HDL, VDL, and LDL portomicrons. Yulianti *et al.* (2013) stated that cholesterol level differs in each livestock due to environmental factors, feed, and farming management. This study investigated the cholesterol level of broiler chickens farmed in three different regions and fed with non-AGP-fortified feed.

MATERIALS AND METHODS

This study used 45 broiler chickens aged 35-37 days obtained from three regions with different climatic conditions and altitudes: Banyumas, Cilacap, and Purbalingga. Banyumas has an average temperature of 23-29^{0}C, 93% humidity, 301-400-millimeter rainfall and 10 m.a.s.l (meter above sea level) altitude. Meanwhile, the average temperature of Cilacap and Purbalingga is 23-29,8 vs. 2-28,4^{0}C, the humidity is 90 vs. 95%, the rainfall is 201-300 vs. 401-500 millimeter, and the altitude is 6 vs. 40-3.000m m.a.s.l.

A survey was conducted on broiler chicken farmers who owned 3,000 – 10,000 chickens and the feed was antibiotic-free. From each regency was collected 15 farmers who incorporated feed additives (a probiotic and herbal supplement) into the feed and offered the feed and drinking water *ad libitum*.

The blood sample was collected from the vena brachialis of harvested chickens to observe the cholesterol level. The chickens were slaughtered and the breast meat was removed to analyze the cholesterol and fat content. The obtained data were subjected to One Way Anova analysis using *General Linear Model* and the independent variables were three regencies in Central Java, namely Banyumas, Cilacap, and Purbalingga. The observed variables were total blood cholesterol, *Low-density Lipoprotein* (LDL), *High-density Lipoprotein* (HDL), triglyceride, meat cholesterol dan total meat fat of broiler chickens.

One broiler chicken aged 35 days was randomly selected from each farmer for blood sampling. The chickens were deprived of food two hours before the blood sampling. The blood was drawn from vena brachialis using a 3ml-syringe then stored in a label 3ml-tube.

This study used CHOD-PAP (cholesterol oxidase p-aminophenazone) enzymatic colorimetric method to measure the broilers' blood cholesterol (mg/dl), GPO-PAP (glycerol phosphate oxidase p-aminophenazone) for triglyceride serum (mg/dl), and enzymatic colorimetric to determine HDL and LDL (mg/dl) after precipitating β-lipoprotein with phosphotungstate acid and magnesium chloride (MgCl2). The LDL (mg/dl) level was obtained using the Friedewald formula (1972), namely LDL = total cholesterol – HDL – 1/5 triglyceride.

Meat fat was measured using the Soxhlet method and ethanol dissolvent while meat cholesterol was analyzed using the Lieberman-Burchard method. Samples were measured (0.2g) and put into 15-ml centrifuge tubes, incorporated with 12ml alcohol ether solution (3:1), and stirred to obtain a homogenous suspension. The suspension was let sit and shaken occasionally for 30 minutes. After that, the stirrer was cleansed using 3:1 ether, the suspension was made equal to 15ml and centrifuged at 3000rpm speed for 15 minutes. The supernatant was removed to a 50-ml beaker glass and heated on a water bath to dry.

The residual extract was dissolved with 25ml chloroform (poured gradually) or cleansed twice and put into a 10-ml reaction tube to have an equal 5ml volume. In other tubes, 5ml standard cholesterol (0.4mg cholesterol into 5 ml chloroform) was incorporated and added with 2ml acetic anhydride and thick 1000 µl H2SO4. At last, reading was carried out using a spectrophotometer at 420nm wavelength (Fenita *et al*., 2012). Analyzed meat fatty acids using gas chromatography. The derivative fat sample was analyzed using GCMS QP 2010 (Shimadzu) with SP ™ 2560 column, 100m x 0.25 mm, 0.2 µm. Nitrogen is used as a carrier gas at a rate of an average of 20cm3 / s. The column temperature is set at temperature 100ºC for 5 minutes. Increased to 240ºC at temperature 4ºC / min. Hold at 240ºC for 30 minutes. Volume injection sample of 1.00 µl.

RESULT AND DISCUSSION

1. Blood cholesterol

The result showed that the level of blood cholesterol, HDL, LDL, and triglyceride broiler chickens fed with non-AGP feed were not significantly different (P>0.05) across three regencies. Table 1 presents the blood cholesterol, meat cholesterol, and fat of broiler chickens fed with non-AGP feed farmed in different regions.

Table 1. The mean value and standard deviation of blood cholesterol of broiler chickens

Parameter	Effect of Non-AGP Feed in Region		
	Banyumas	Cilacap	Purbalingga
Total Blood cholesterol (mg/dl)^{ns}	186.67 ± 25.34	195.56 ± 39.75	231.11 ± 25.34
Low-density Lipoprotein (LDL) darah (mg/dl)^{ns}	113. 04 ± 27.19	123.00 ± 52.39	160.53 ± 22.87
High-density Lipoprotein (HDL) darah (mg/dl)^{ns}	22.2 ± 6.08	23.97 ± 5.06	11.72 ±10.08
Triglyceride (mg/dl)^{ns}	157.14 ± 28.97	142.86 ± 21.43	174.28 ± 22.72

Note: ^{ns}= the mean values sow non-significant effect (P>0.05)

Total blood cholesterol of broiler chickens

Table 1 shows that the average total blood cholesterol of broiler chickens in Purbalingga (231.11 ± 25.34 mg/dl) is higher than Cilacap (195.56 ± 39.75 mg/dl) and Banyumas (186.67 ± 25.34 mg/dl). Citrawidi *et al.* (2012) stated that the normal total cholesterol was 125-200 mg/dl. Therefore, blood cholesterol in this study was within the normal range, except for those in Purbalingga which were above 200 mg/dl.

However, further analysis demonstrated a non-significant difference across the three regencies (P<0.05). Meliandasari *et al.* (2015) reported that the cholesterol feedback system was conducted to maintain homeostatic conditions, so in case of small cholesterol intake, the body will synthesize endogenous cholesterol until it reached a normal cholesterol level.

Furthermore, a previous study suggested that cholesterol in the blood is derived from de novo biosynthesis (Setyadi *et al.,* 2013). Feed plays a critical role in absorbing nutrition and producing cholesterol. This study found that feed intake of broiler chickens farmed in Banyumas, Burbalingga, and Cilacap regencies were near equal, namely 2678 ± 167.24 g; 2694.72 ± 183.51g and 2645±178.33 g, respectively.

The contributing factors to total blood cholesterol levels in broiler chickens include genetic factors, feed, medicines, and environmental factors. The high total cholesterol of broiler chickens in Purbalingga may be due to a lower temperature and higher rainfall than the other regencies. Consequently, the chickens would consume more feed, hence increasing blood cholesterol. Meliandasari *et al.* (2016) found that 25-40% of the cholesterol in the body is derived from the food consumed. It demonstrated that broiler chickens in Purbalingga consumed more feed, so their cholesterol level was higher than that of broiler chickens in Cilacap and Banyumas.

***Low-density lipoprotein* (LDL)**

The average LDL of broiler chickens fed with non-AGP feed-in Cilacap, Purbalingga, and Banyumas was 123.00 ± 52.39 mg/dl; 160.53 ± 22.87 mg/dl; and 113.04 ± 27.19 mg/dl, respectively. It shows that the LDL was relatively low among broiler chickens in Cilacap and Banyumas, but higher in Purbalingga. It was in line with Tuli *et al.* (2014) that the normal LDL of broiler chickens was 95-125 mg/dl, hence higher than the other regencies. It is evident that the higher the blood cholesterol, the higher the *Low-density Lipoprotein* (LDL). Yulianti *et al.* (2013) stated that LDL level is affected by cholesterol concentration and LDL is the main carrier of cholesterol from the liver to body tissues.

The analysis showed that the average LDL of broiler chickens in three regencies was not different (P<0.05). The contributing factors to LDL level include genetic factors, environmental factors, and feed (ingredients and feed additives). Tuli *et al.* (2014) reported that *feed additive*s and their dosage did not affect LDL levels in broilers. It is because feed additives offered by the farmers in form of probiotics and natural herbs like turmeric and curcumin may affect LDL levels. Swastike (2012) stated that turmeric contains an active antibacterial curcumin compound while Curcuma contains an active xanthohumol that inhibits fungal growth. Furthermore, the chemical substances in turmeric could reduce body fat during bile and pancreatic secretion and excrete it through feces. It shows that incorporating turmeric or Curcuma in feed could lower blood LDL. The different level of LDL is affected by varying doses of feed additive given by the farmers in each regency.

High-density lipoprotein (HDL)

The average High-density Lipoprotein (HDL) of broilers in this study was relatively similar in Cilacap, Purbalingga, and Banyumas, namely 23.97 ± 5.06 mg/dl; 21.72 ±10.08 mg/dl; and 22.2 ± 6.08 mg/ dl. Basmacioglu and Ergul (2005) stated that the minimum HDL of broiler chickens was

22 mg/dl. Therefore, the HDL level of broiler chickens in this study was within the normal range. Broiler chickens in these regencies can tolerate the temperature, humidity, and rainfall so their HDL level remains unaffected despite different feed intakes.

The analysis showed that the HDL of broiler chickens in three regions is not significantly different (P>0.05) because all farmers used feed additives in form of probiotics, acidifiers, or herbs (mixed garlic, ginger, and turmeric) to substitute antibiotics. Tuli *et al.* (2014) reported that HDL levels can be regulated through the flow of cholesterol from lipoprotein to liver cells and the use of HDL to synthesize steroids like hormones or bile salt in the liver. Probiotics supplement would decrease cholesterol absorption. Sumardi *et al.* (2016) stated that probiotics tend to increase blood HDL by limiting the activity of acetyl CoA carboxylase – an enzyme that regulates the synthesis rate of fatty acid. HDL molecules are relatively smaller than other lipoproteins so they could pass the endothelial vascular cells and into the intima to re-transport the cholesterol accumulated in the macrophage. According to Sutama *et al.* (2010), the faster the cholesterol was excreted through feces, the lower cholesterol accumulation in the liver, hence cholesterol binds salt to produce bile acid. Furthermore, it stimulates HDL synthesis in the liver.

Triglyceride

This study reported the average triglyceride of broiler chickens in Cilacap (142.86 ± 21.43 mg/dl), Purbalingga (174.28 ± 22.72 mg/dl), and Banyumas (157.14 ± 28.97 mg/dl). This result was higher than 38.97 – 54.18 mg/dl by Meliandasari *et al.* (2016) on broiler chickens that received *Salvinia molesta* leaf powder in their feed. Furthermore, Adityo *et al.* (2013) stated that the normal triglyceride of broiler chickens was 43,4 – 168 mg/dl.

This study found a non-significantly different (P<0.05) triglyceride level in broiler chickens across three regencies. It shows that the temperature, humidity, and rainfall are tolerable, so the triglyceride level was unaffected despite different quantities of feed intake. The contributing factors to triglyceride level include feed. Citrawidi *et al.* (2012) stated that the level of triglyceride is affected by carbohydrate and crude fiber in feed as well as the circulation of free fatty acid. Furthermore, Bariyah (2008) reported that the increased triglyceride level in the liver was due to excessive carbohydrate in feed. Meanwhile, a high crude fiber could affect fat absorption and lower triglyceride. This study used commercial feed that farmers obtained from a partnership venture and the feed contained relatively high carbohydrate and crude fiber. According to Pratama *et al.* (2012), high triglyceride has been attributed to the hydrolysis of abdominal fat by essential oil contained in ginger, turmeric, and garlic which is incorporated in the feed to substitute antibiotics. On the other hand, high triglyceride may be due to high fatty acid synthesis in broiler chickens aged 35-37 days.

2. Profile of meat fat

The result showed that the fat and fatty acid of meat broiler chickens fed with non-AGP feed were not significantly different (P>0.05) across three regencies, but the meat cholesterol levels were significantly different (P<0.05) (Table 2).

Meat fat

The average total fat of broiler chickens in three regions was near equal, namely 3.18 ± 0.47 % (Cilacap), 3.19 ± 0.28 % (Purbalingga), and 3.58 ± 0.53 % (Banyumas) regencies. This result is higher than 1.15–1.66 % by Adityo *et al.* (2013); therefore, the meat fat produced in this study is relatively high. One contributing factor to body fat level is feed. According to Dorisandi *et al.* (2017), the feed contains high energy which significantly increases body fat. The excessive energy unused in the body will be stored as fat for energy reserve.

This study has demonstrated non-significantly (P>0.05) different levels of meat fat in broiler chickens across three regencies. Concerning physiology, the broilers could tolerate local temperature, humidity, and rainfall. Additionally, feed additives in feed did not significantly affect total fat content in broilers because either probiotics, acidifiers, or herbs play an equal role in synthesizing fat. Probiotics and acidifiers have effectively reduced the activity of acetyl CoA carboxylase – the enzyme which regulates the rate of fatty acid synthesis (Huda *et al.,* 2019). Pratama *et al.*, (2012) reported that the active compounds in turmeric and Curcuma could increase the production and secretion of bile, thus supporting fat disruption. Besides herbal ingredients, farmers also used probiotics as feed additives to substitute AGP. Daud (2004) stated that probiotics provide enzymes to digest crude fiber, protein, and fat, as well as maintain balance in the ecosystem. It is evident that the less abdominal fat, the higher the meat production.

Table 2. The mean value and standard deviation of fat, cholesterol and fatty acid of meat broiler chickens

Parameter	Effect of Non-AGP Feed-in Region		
	Banyumas	Cilacap	Purbalingga
Meat fat^{s}	3.58 ± 0.53	3.18 ± 0.47	3.19 ± 0.28
Meat cholesterol (mg/100g)*	140. 84 ± 2.17^{b}	132.92 ± 2.34^{b}	124.58 ± 0.87^{a}
Saturated fatty acid (%)^{ns}	52.43±0.35	51,96±1,44	52.18±0.57
Unsaturated fatty acid (%)^{ns}	41.71±2.10	43.22±1.58	39.88±1.57

Note: * = the mean values show significant difference (P<0.05)
^{ns}= the mean values sow non-significant effect (P>0.05)

Meat cholesterol

The average meat cholesterol of broiler chickens in Cilacap, Purbalingga and Banyumas was 132.92 ± 2.34 mg/100g; 124. 58 ± 0.87 mg/100g; and 140.84 ± 2.17 mg/100g, respectively. This result was lower than 142-152 mg/100g by Ibrahim *et al.* (2015). The different levels of meat cholesterol across regencies in the present study may be due to feeding additives offered to the broiler feeds.

This study reported significantly (P<0.05) different levels of meat cholesterol in broiler chickens across three regencies. Broiler chickens in Purbalingga had the highest meat cholesterol because of the highest rate of rainfall which encourages the farmers to give different feed additives, such as probiotics, acidifiers, and mixed herbs of ginger, turmeric, and garlic. Meanwhile, chicken farming in Cilacap and Banyumas mostly offered probiotics or acidifiers. It shows that although the broilers in three regencies could tolerate the environmental factors (temperature, humidity, and rainfall) which did not affect their feed intake, feed additives may affect differently to cholesterol synthesis in their meat. Sujana *et al.* (2007) reported that different meat cholesterol levels are affected by total blood cholesterol for bile synthesis. Furthermore, blood cholesterol transport to body cells involves the interaction between cholesterol from feed and cholesterol synthesis in the liver (Suyatna, 2007). When the total cholesterol from food increases, the liver would stop cholesterol synthesis because blood cholesterol automatically inhibits an important liver enzyme to perform cholesterol synthesis. Furthermore, Mark (2000) stated that food cholesterol is absorbed from the bile salt micelles to the intestinal epithelial cells then together with cholesterol (synthesized by the cells) would enter the bloodstream through the lymph. In the lymph and blood, chylomicrons obtain apoC11 apoE from HDL. After triacylglycerol chylomicrons are digested by lipoprotein lipase in the blood, the remaining chylomicrons would bind with receptors in the liver cells and undergo internalization through endocytosis. In this way, digestion occurs in the lysosome where protein and fat are degraded, a fatty acid is detached from esterified cholesterol, and the cholesterol, as well as other digestive products after chylomicrons, would form a depot in the liver cells.

After being formed in the liver, triacylglycerol is transported with cholesterol from the depot of cholesterol, phospholipid, and apoB-100 into VLDL which is secreted into the bloodstream. VLDL carries liver triglyceride to the peripheral cells. VLDL contains 8% protein, 90% fat (50% triglyceride, 20% cholesterol, 90% phospholipid) and 2% free fat. Zahra *et al.* (2014) reported that the absorption of meat cholesterol is affected by nutrition, genetic factors, and medicine. Some methods to decrease meat cholesterols include cutting down consumption, intestinal absorption, and endogen synthesis as well as increasing bile excretion and fecal excretion.

The decreased meat cholesterol indicates a mobilization by bile acids which are synthesized by bail cells using cholesterol as the precursor.

Percentage of saturated and unsaturated fatty acid of meat

The average saturated fatty acid of broilers meat in this study was relatively similar in Banyumas, Cilacap, and Purbalingga, namely 52.43±0.35%, 51.96±1.44%; and 52.18±0.57%. Likewise, the unsaturated acid content of broiler chicken meat in Banyumas, Cilacap, and Purbalingga, namely 41.71±2.10%, 43.22±1.58%, and 39.88±1.57%. The results are relatively the same as the research of Setyawati *et al.* (2016) which reported saturated fatty acids of broiler chicken meat ranged from 24.57-57.97%, while unsaturated fatty acids ranged from 35.34-68.79%.

The results of the analysis of variance showed that the levels of saturated and unsaturated fatty acids of broiler chicken meat in three different regions were relatively the same (P>0.05). This is because chickens consume commercial feed (factory-made) whose nutrient content is by the standard needs. In addition, Milicevic *et al.* (2014) stated that the fatty acid profile of tissue depends on the role of fatty acids and the content of phospholipids in the tissue.PUFA are preferentially incorporated into phospholipids [36] and phospholipids are in higher proportion in muscle fat.

CONCLUSIONS

The non-AGP feed given to broilers in three different regions resulted in relatively the same blood cholesterol, fat, and meat fatty acid profile, but caused the meat cholesterol levels in the three regions to be different.

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