EAS Journal of Veterinary Medical Science
Abbreviated Key Title: EAS J Vet Med Sci
ISSN: 2663-1881 (Print) & ISSN: 2663-7316 (Online)
Published By East African Scholars Publisher, Kenya

OPEN ACCESS

Volume-2 | Issue-2 | Mar-Apr2020 | DOI: 10.36349/easjvms.2020.v02i02.001

Research Article

Substitution of rice bran and cocoa husk enzymes is in the feed on hybrid duck blood lipid profile

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Article History
Received: 04.04.2020
Accepted: 26.04.2020
Published: 27.04.2020

Journal homepage:
https://www.easpublisher.com/easjvms

Quick Response Code

Abstract: This study aims to determine the effect of feed substitution with the ingredients of bran and epidermis of Enzi matis cocoa on the hybrid duck blood profile. The method used was an experimental method with Complete Randomized Design (CRD) of 5 treatments and 5 replications. The treatment consists of P 0 = b ekatul 10% + 0% cocoa husk, P1 = bran husk 7.5% + 2.5% cocoa, P2 = bran 5% + 5% cocoa husk, P 3 = bran 2 , 5 % + cocoa shells 7.5 % and P4 = bran 0% + cocoa shells 10% . Feeding is done by self-mixing according to the treatment and feeding is done by dry feed. Cocoa epidermis used were from previous studies which gave the best results at an enzymatic 0.026%. The variables measured in this study included total blood cholesterol, triglycerides, LDL and HDL. Data were analyzed using ANCOV A. The results of this study showed that the enzymatic bran and cocoa substitution in the feed was able to give significantly different results ($P < 0.05\%$) on the total blood cholesterol value and the results were very significantly different ($P < 0.01$ %) on triglycerides, LDL and HDL. The conclusion of this study is the use of enzymatic cocoa shells as bran substitutes can give good results at a percentage of 10%.

Keywords: Enzymatic cocoa bean epidermis, total blood cholesterol, triglycerides, LDL, HDL.

PRELIMINARY

Increased body weight in livestock occurs along with increasing age of maintenance. Factors that affect body weight gain are differences in sex, feed consumption, environment, seed and feed quality. Gain weight cattle also followed by an increase in body fat deposition in the form of abdominal fat cattle and beef fat. This will affect the percentage of carcasses that will be produced in addition to the high fat content in the final product will have a negative impact on consumers such as health problems, especially related to body fat.

One way to reduce fat deposition in the body of cattle is to use active substances such as polyphenols. Polyphenols can be obtained from the epidermis of cocoa which will be used as a constituent in feed ingredients so that it will have a direct impact on the body of livestock. Polifenol can also stimulate the wall of the gallbladder to secrete bile fluid and stimulate the release of pancreatic sap that contains amylase, lipase, and protease enzymes that are useful for improving digestion of nutrients such as carbohydrates, fats, and proteins.

The main ingredient for forming bile salts is body cholesterol so that more bile salt is produced, the more cholesterol is remodeled in the body. The description above becomes interesting to know the impact of the use of bran substitution feed and enzymatic cocoa shells on the blood lipid profile of hybrid duck.

MATERIALS AND METHODS

Time and location of research

The research with treatment feed began on October 22, 2019 until December 10, 2019. Ducks that had entered the age of 14 days for 5 weeks and carried out in a cage owned by Mr. Eko Suhertanto addressed at Jalan Teratai Gang I, Karang Mloko Hamlet, Dadaprejo Sub-District, Junrejo District , Stone. Blood Profile Analysis was carried out at Kawi 31 Clinical Laboratory, Jln Kawi, no 31.

INGREDIENTS

Research using male hybrid ducks (*Peking* ♂ *Campbhell* ♀*)* aged 14 days as many as 125 tails. *Day Old Duck was* bought from Mr. Bayu (*supplier of* hybrid ducks) as many as 150 fish at a price of Rp. 6500 , 00 / fish.

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Feed for ages 1-14 days using commercial feed of Japfa duck and feed age> 14 weeks using self-made treatment feed . The cocoa husk used was obtained from the Coffee and Cocoa Research Center , Jember, which was purchased at a price of Rp. 800 / kg. In addition to cocoa epidermis, there are also other feed ingredients used in this study such as corn, rice bran, palm oil, soybean meal, meat bone meal and premix obtained from Sinar Abadi *Poultry Shop* (Karang Ploso Sub-District, Batu City, East Java).

Cocoa epidermis used is a material that has been carried out before cellulase treatment in previous studies to reduce raw fiber material.

METHOD

The method used is the method p ercobaan that t erdiri of 5 treatments and 5 replications. The treatment is as follows:

P 0: 10% bran and cocoa epidermis 0% cellulase
P 1: bran 5.5 % and cocoa epidermis 2.5 % cellulase
P 2: bran 5% and cocoa epidermis 5 % cellulase
P3: bran 2.5 % and cocoa epidermis 7.5 % cellulase
P4: 0% bran and cocoa epidermis 10% cellulase

Variables observed in the blood lipid profile in the hydride include total blood cholesterol, triglycerides, LDL and HDL.

DATA ANALYSIS

Analysis of the data used in the study was analysis of variance (ANCOVA) with initial body weight as a variation in the Completely Randomized Design (CRD) with 5 treatments and 5 replications / groups. If there is a difference in influence between treatments it will be continued with Duncan's Multiple Range Test. The purpose of this analysis is to find out the effect of the treatment of ult bran and cocoa cellulase husk on the performance of hybrid duck production.

DISCUSSION RESULT

The results of the study of substitution of form 1 and cocoa cellulase substitution for hybrid duck blood lipid perfilts including total blood cholesterol, triglycerides, LDL and HDL are shown in Table 1.

Table 1. Per ofil Blood Lipids

Treatment	Total Blood Cholesterol (mg / dl)	Teriglyceride (mg / dl)	LDL (mg / dl)	HDL (mg / dl)
P0	197.27 ^{C}	130.96 ^{C}	123.89 ^{B}	31,35 ^{A}
Q1	178.49 ^{BC}	84.07 ^{BC}	98.79 ^{B}	49,35 ^{B}
P2	158.39 ^{B}	75.07 ^{B}	58.97 ^{AB}	73.38 ^{C}
Q3	151,34 ^{AB}	69.38 ^{AB}	48.08 ^{AB}	86.98 ^{CD}
Q4	135.73 ^{A}	59.11 ^{A}	39,33 ^{A}	106.32 ^{D}

Description: The numbers on the row and column of the same followed by different letters indicate significantly different at 1% level test (Duncan's multiple range test)

Total Blood Cholesterol

The results showed that the use of the epidermis of cocoa cellulase in the feed was able to reduce the level of total blood cholesterol in P4 per treatment showed significantly different results (P <0 , 01) with the lowest percentage of 135.73 mg / dl. The use of cocoa shell cellulase by 10% has the most significant effect because the polyphenol content available in the material is also high in contrast to P0, which is feed with 0% cocoa shell cellulase which produces the highest percentage of 197.27 mg / dl.

This is caused by the control treatment or P0 does not experience a decrease in cholesterol levels even the resulting cholesterol level gives the highest results compared to other treatments, an increase because this treatment is not given feed ingredients from the cocoa cellulase. The positive treatment group by giving cocoa cellulase epidermis works due to polyphenols inhibiting 3-hydroxy-3-methyl-glutaryl (HMG) -CoA reductase in the early stages of sterol formation. The content of secondary metabolites in the epidermis of cocoa are suspected as lowering levels of total cholesterol is polyphenols. Polyphenols reduce cholesterol synthesis by inhibiting reductase 3-hydroxy-3-methyl-glutaryl (HMG) - CoA and inhibiting the secretion of triacylglycerol. Polyphenols are reported to be able to reduce total cholesterol levels and to be able to inhibit the formation of atherosclorosis through its antioxidant effects (Hartoyo, 2003). Antioxidant compounds such as polyphenols can "eradicate" free radicals such as peroxide, hydroperoxide or lipid peroxyl so that it inhibits the oxidation mechanism. K Adar total cholesterol can be scaled possibly through the mechanism of antioxidant compounds called polyphenols, cocoa husk owned, increases the metabolism of cholesterol to bile acids and increasing the excretion of bile acids in the feces. Low cholesterol in the liver will increase the uptake of cholesterol from the blood of the liver which then acts as a precursor of bile acids, thereby reducing total cholesterol in the blood (Septiana *et al.*, 2002). Reduction in total cholesterol in the blood will affect the percentage of constituents of cholesterol such as triglycerides, LDL

and HDL. So the high and low of total cholesterol will describe the condition of the blood lipid profile on the body of cattle Umarudin *et al.,* (2009).

Triglycerides

The results of the study in Table 16 obtained very significant results (P <0.01) in feed with the addition of cocoa shells to triglyceride values, obtained the highest value at P0 130.96 mg / dl which is a feed with 10% bekatu and cocoa shells 0%, the lowest triglyceride value was P4 59.11 mg / dl with 10% cocoa shell and 0% bran, followed by P3 69.38 mg / dl, P2 75.07 mg / dl, and P1 84.07 mg / dl. Triglycerides are the main fat that will be stored in the body and high triglycerides affect the deposition of abdominal fat which will reduce the percentage of meat in cattle (Murray , 2003). The main factor increasing body fat storage is dietary fat in the form of triglycerides and fatty acids which then enter the body and metabolize. Excessive dietary fat will be stored in the body's tissue and abdominal. The percentage of abdominal fat decreases with decreasing triglyceride content in the blood and vice versa. High abdominal fat depends on triglyceride synthesis activity which can be seen from the blood lipid profile Yadnya *et al.,* (2010). This is in accordance with George *et al.,* (2015). Which states that high concentrations of triglycerides in the blood stem from high absorption of fat in the intestine and synthesis of fatty acids in the liver that increase levels of tissue fat and abdominal fat. A low percentage of triglycerides results in reduced abdominal fat storage and can increase the percentage of meat.

Cocoa epidermis used as bran substitutes contains polyphenols which can increase the production of bile salts and can reduce the process of lipogenesis. This is in accordance with (Wijaya *et al.,* 2013) administration of materials containing polyphenols in the ration can reduce abdominal fat and blood serum cholesterol levels. The content of polyphenols in a suspected material can improve the lipid profile may improve lipid profiles by way of forming bile through extra hepatic cholesterol excretion Yadnya *et al.,* (2016). Polyphenols have an antidislipidemia effect and in large doses polyphenols can reduce cholesterol and free fatty acids in the blood. Polyphenols will inhibit fat transport to the liver so that it will reduce triglyceride synthesis Perez *et al.,* (2006). This was also revealed by Sukmawati (2014) that polyphenols contribute to pancreatic lipase inhibition which inhibits the action of lipases in remodeling lipids / triglycerides so as to make absorption of triglycerides in the body inhibited and reduce triglyceride levels in the body. The process of polyphenols in inhibiting fat absorption and reducing triglyceride content begins by lowering the pH of digestion by creating acidic conditions in the digestive tract which can reduce the activity of enzymes that digest fats that are active at neutral pH, consequently the absorption of fat in the intestine is reduced (Natsir, 2005). Polyphenols can also bully the secretion of bile salts thereby forcing the use of cholesterol in the body to be converted into bile salts thereby reducing the percentage of triglycerides, LDL and total cholesterol in the blood. This is consistent with research conducted by (Dietschy, 2003) that polyphenols can improve lipid profiles by several mechanisms including inhibiting the absorption of cholesterol in the small intestine, inhibiting cholesterol synthesis in the liver, inhibiting fatty acid transport, and increasing the excretion of cholesterol in the form of salt bile. Percentage value of Teriglyceride in hybrid ducks that use P0 feed is still normal value which is 130.96 mg / dl compared to the standard value of duck triglyceride value proposed by Sukmawati (2014) which is 230 mg / dl and lower than the percentage stated by Tugiyanti (2016) which is 232 mg / dl.

Low Density Lipoprotein (LDL)

The results of the study shown in Table 16 show an increase in LDL levels from feeding P0 123,89 mg / dl in line with the increased content of Triglycerides 130.96 mg / dl in the blood.

Increased triglycerides are caused by the re-formation of cholesterol to synthesize bile salts which are used to absorb nutrients, especially triglycerides that are in the body cells, which will later be used as livestock energy Abdel *et al.,* (2008). Increasing the percentage of triglycerides from P0 administration can increase the formation of VLDL (Very Low Density Lipoprotein) which is the parent of LDL formation. The teriglycerides present in VLDL are broken down into LDL by lipoprotein lipase. LDL will then be carried to the liver and other tissues that have the LDL receptor Al-Waili (2004). This is in accordance with (Hawab, 2003) triglycerides have an influence on the increase in LDL in the blood where cholesterol, triglycerides, and various other lipids obtained from food are absorbed from salt micelles into intestinal epithelial cells, this cholesterol along with cholesterol synthesized by intestinal cells then packed in the form of chylomicrons, then enter the blood through lymph vessels. The chylomicrons in the blood are hydrolyzed by the enzyme lipoprotein lipase to triacylglycerol and the rest of the kilmicrons. Triacylglycerol enters the cell and is hydrolyzed by lipase to fatty acids and glycerol. Fatty acids and glycerol in cells undergo further metabolism to produce energy, then the rest of the chylomicrons will bind to specific receptors in liver cells and internalize endositosis. The remaining chylomicrons are rich in cholesterol and the cholesterol esters are digested by lysosomes to form fatty acids and free cholesterol, the increased free cholesterol content then causes inhibition of cholesterol synthesis and LDL receptor synthesis by the liver decreases, this causes an increase in triglycerides in the blood affecting high levels of LDL in the blood due to the decreasing amount of LDL in the liver.

LDL particles contain triglycerides as much as 10% and cholesterol as much as 50%, the main pathway of LDL catabolism takes place through receptor mediated endocytosis in the liver and other cells. Cholesterol esters from the LDL core are hydrolyzed to produce free cholesterol, for the synthesis of cell membranes and steroid hormones. Aside from the endocytosis process, cells also get cholesterol from de novo synthesis through HMG-CoA enzymes, Basmacioglu and Ergul (2005). The increase in LDL was also related to the P0 treatment feed that was not given cocoa epidermis containing polyphenols as antioxidants. This was also explained by Susmiati (2010) who stated that the lack of antioxidants given to feed can cause *Reactive Oxygen Species* (ROS) which is a by-product of biochemical processes causing cells to experience oxidative stress. The reaction of ROS to lipids that are not saturated with cell membranes and plasma lipoproteins causes the formation of lipid peroxide (malondialdehyde) which can chemically modify proteins and nucleic acid bases. Chemical modification in protein and fat in lipoprotein (LDL) causes LDL to no longer be known by the LDL receptor of the liver, consequently LDL cannot be cleansed by the liver.

Polyphenols are bioactive compounds that can affect lipid metabolism, polyphenols are antioxidant compounds that function in reducing cholesterol and fat. Antioxidants can inhibit the activity of the HMG-CoA reductase enzyme which converts 3-Hydroxyl, 3-Methyl Gluteryl-CoA to mevalonate. Mevalonic acid is a cholesterol-forming compound with reduced mevalonic acid, cholesterol that is synthesized by the liver will be reduced, as well as in blood circulation (Agarwal and Rao, 2000). LDL and HDL are two types of lipoproteins that function to circulate cholesterol in the blood so that their concentration in the blood is strongly influenced by the amount of cholesterol synthesized by Musa *et al.,* (2006). It was also stated by Montgomery *et al.,* (2003) that LDL plays a role in providing cholesterol in body tissues because it is the main career for cholesterol from the liver to body tissues, so LDL levels in the blood are influenced by cholesterol concentrations. This was also reported by Naufalina (2014) the content of polyphenols in cocoa powder can reduce LDL cholesterol levels by 0 , 4 -3.9 mg / dl and increase HDL cholesterol levels by 1.95.4 mg / dl. Polyphenols reduce LDL cholesterol in various ways such as preventing absorption of cholesterol in the small intestine, then preventing LDL biosynthesis by decreasing the activity or amount of hydroxymethylglutaryl - CoA synthase, hydroxymethylglutaryl - CoA reductase, acyl CoA: cholesterol acyltransferase and transfer of microsomal proteins in the liver, decreasing hepatic apoliprotein secretion B-100, and can increase the number of LDL receptors in the liver thereby increasing LDL clearance in the bloodstream which causes a reduction in LDL in the blood.

Polyphenols also play a role in stimulating bile salt secretion due to the nature of the polyphenols which are acidic and can affect the acidity of a substance (Trissanthi and Wahono 2016). The content of phenol compounds (ph 4) contained in polyphenols can increase the acidity of the material so that it can stimulate the excretion of bile salts to balance the pH of the digestive tract (Sumardika and Jawi 2010). This is consistent with studies using lime (Citrus aurantifolia) containing citric acid in Magelang duck cattle. Citric acid can lower the pH of the digestive tract so that the acidic conditions stimulate the increase in collection of blood cholesterol as bile salt-forming material to normalize the pH of the gastrointestinal tract, resulting in a decrease in the levels of cholesterol in the blood (Yulianti *et al.,* 2013). In that study, cholesterol levels fell from 164.71 mg / dl to 143.53 mg / dl, triglyceride levels dropped from 148.67 mg / dl to 117.14 mg / dl and LDL levels dropped from 84.59 mg / dl to 61.02 mg / dl, while HDL levels rose from 50.4 mg / dl to 143.53 mg / dl. Decrease in pH in the digestive tract can increase the digestibility of feed protein, this is because the acidic pH of the digestive tract can support the performance of digestive enzymes, especially pepsin which plays a role in digesting protein so that protein digestion is much better and impact on increasing the body's meat deposition (Ati *et al* ., 2019). Protein has an important role in the process of transporting cholesterol in the blood through the formation of lipoproteins formed from a combination of protein and fat. According to Daniels *et al* . , (2009) Lipoprotein functions to mediate the transportation of lipids from the liver to the tissue and from the tissue to the liver, so that lipoprotein has a very important role in maintaining homeostasis (balance) of cholesterol in the blood. Cholesterol homeostasis aims to meet the needs of cholesterol in the blood when the intake of cholesterol in the diet is insufficient, then cholesterol will be synthesized from both the institutional and extrahepatic tissue to meet the needs of cholesterol in the blood Trapani (2012).

The percentage of LDL P0 levels in Table 16 shows a value of 123.89 (mg / dl) higher than the LDL standard in broiler ducks proposed by Sukmawati (2014) which is 88 (mg / dl) and reported by Tugiyanti (2016) which is 120 (mg / dl).

High Density Lipoprotein (HDL)

The results of the study in Table 16 which showed the highest HDL blood lipid profile in feeding P4 (rice bran 0% and cocoa 10%) of 106 , 32 (mg / dl) showed very significant results (P <0.01) and the lowest in feeding P0 (10% bran and 0% cocoa) which showed a decrease in HDL content by 31.35 (mg / dl).

The increased value of HDL in the blood is in line with the decrease in LDL and triglyceride levels in the blood. HDL is a lipoprotein in charge of delivering cholesterol to the liver to be converted into bile salts because the percentage of HDL in the blood is inversely

proportional to LDL. A high percentage of triglycerides in the blood will also reduce the HDL content in the blood because triglycerides are one of the constituents of LDL formation. This is in accordance with Murray *et al.,* (2003) states that the process of releasing cholesterol ester content into the liver is carried out by hepatic lipase that hydrolyzes HDL and triglycerides to provide cholesterol for the production of bile acids, blood HDL concentrations have varied levels and have a reciprocal relationship with blood triglyceride concentration. The level of fat in the blood can be reduced by accelerating the removal of lipoproteins from the blood and inhibiting the entry of lipoproteins into the blood vessels. The acceleration of the removal of lipoproteins from the blood occurs with the help of HDL which can transport lipoproteins back to the liver. Supplying cocoa husk with polyphenols can increase HDL in the blood by stimulating the production and secretion of bile in the liver. This is in accordance with Hasanuddin *et al.,* (2014) the mechanism of increasing HDL cholesterol levels by polyphenols by increasing the process of Reverse Cholesterol Transport (RCT) by macrophage. HDL in the blood increases due to the need for cholesterol synthesis in the liver, thus requiring the removal of fat in adipose cells, the increased HDL flow brings catabolic fatty acids to be synthesized into cholesterol and the increase in HDL is inversely proportional to the LDL used for synthesis of steroids and bile salts. This was also revealed by (Lehninger, 2004) that cholesterol transported to the liver is mainly in the form of cholesterol which will be used as raw material for making bile salts and hormones. HDL with high enough levels in the blood will inhibit the process of deposition of fat in the walls of blood vessels.

The formation of HDL itself is synthesized and secreted from both the liver and the intestine as small cholesterol-poor particles containing apolipoprotein (apo) A, C, and E; and is called as nascent HDL. Nascent HDL comes from the small intestine and liver, has a flat shape and contains apolipoprotein A1. Nascent HDL will approach the macrophage to take cholesterol stored in the macrophage, then the nascent HDL changes into a rounded adult HDL. (Rosadi et al, 2013). This is also explained by Murray *et al.,* (2003) HDL nascent (recently excreted) from the intestine does not contain apolipoprotein C and E, but only contains apoprotein A, whereas HDL from the liver contains apo A, C and E. The function of HDL is as a storage place for apolipoprotein C and E needed in chylomicron metabolism and VLDL. VLDL, chylomicron, and cholesterol metabolism also transport HDL. HDL has 2 subclass molecules, namely HDL2 and HDL3, HDL3 molecules are synthesized in the liver and enter the blood vessels to take cholesterol, when HDL3 molecules cholesterol content increases its density decreases and becomes HDL2, then HDL2 re-enters the liver to be disassembled again and then HDL3 is re-flowed to blood circulation (Madani *et al.,* 2003). The percentage of HDL levels at P4 106 , 32 mg / dl is higher than the standard HDL levels of ducks expressed by Sukmawati, (2014) is 80 (mg / dl) and Tugiyanti (2016) 98 (mg / dl).

CONCLUSION

Pen gunaan epidermis cocoa cellulase in substitution of bran in the feed may give effect had highly significant (P < 0,01 %) in the blood lipid profile of duck hybrid P en gunaan epidermis cocoa cellulase in substitution of bran can give good results in the percentage of 10% .

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