

FEEDING LOCAL FERMENTED WITH EM-4, AMMONIUM SULFATE, AND UREA ON THE BLOOD FAT LEVEL OF MALE DUCK

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ABSTRACT

The objective of this study was to determine the effect of commercial ration by the addition of ammonium sulfate and urea fermented feed ingredients on the blood fat levels of local male ducks. This study used a completely randomized design with 32 male ducks which were divided into four groups namely R0, R1, R2, R3, and R4. Each group was given the following treatment: R0 only given commercial ration; R1 was given 80% commercial ration + 20% fermentation of EM-4 + 1.5% urea; R2 was given 80% commercial ration + 20% EM-4 + 1.5% ammonium sulfate fermentation; and R3 was given 80% commercial ration + 20% EM-4 + 1.5% urea + 1.5% ammonium sulfate. Variables in this study were blood fat consisting of cholesterol, triglyceride, High-Density Lipoprotein (HDL), and Low-Density Lipoproteins (LDL). The data obtained were analyzed by using 5% real level and LSD. The results showed that the provision of commercial ration added with local feed fermented with ammonium sulfate and urea had a significant effect on cholesterol levels ($P < 0.5$), but not significant ($P > 0.05$) on triglyceride, HDL, and LDL levels. Furthermore, the ration that gave the best effect on the blood fat levels of male ducks blood was the ration given to R3 group.

Key words: ammonium sulfate, blood fat content, duck, urea

ABSTRAK

Penelitian ini bertujuan mengetahui kadar lemak darah itik jantan dengan pemberian ransum komersil yang ditambahkan bahan pakan lokal terfermentasi EM-4, amonium sulfat, dan urea. Dalam penelitian ini digunakan rancangan acak lengkap (RAL) dengan 32 sampel itik jantan. Perlakuan yang diberikan adalah ransum komersil (R0); 80% ransum komersil + 20% (Fermentasi EM-4 + Urea 1,5%, R1); 80% ransum komersil + 20% (Fermentasi EM-4 + Amonium sulfat 1,5%, R3); dan 80% ransum komersil + 20% (fermentasi EM-4 + Urea 1,5% + Amonium sulfat 1,5%, R3). Data yang diperoleh dianalisis dengan analisis varian dan dilanjutkan dengan uji beda nyata terkecil (BNT). Peubah dalam penelitian ini yaitu lemak darah yang meliputi kolesterol, trigliserida, high-density lipoprotein (HDL), dan low-density lipoproteins (LDL). Hasil penelitian menunjukkan bahwa pemberian ransum komersil dengan tambahan bahan pakan lokal terfermentasi amonium sulfat dan urea berpengaruh nyata terhadap kolesterol ($P < 0,5$) namun tidak berpengaruh nyata ($P > 0,05$) terhadap trigliserida, HDL, dan LDL darah itik jantan lokal. Disimpulkan bahwa ransum R3 memberikan pengaruh terbaik terhadap kadar lemak darah itik jantan lokal.

Kata kunci: amonium sulfat, kadar lemak, itik, urea

INTRODUCTION

The use of local feed ingredients such as palm kernel expeller, cassava pulp, and bran is an effort to improve feed efficiency and reduce production costs in duck farming, 70-80% of which is allocated for feed. However, the low quality of local ingredients makes it only a poultry feed. Palm kernel expeller contains 11.03% crude protein, 14.67% crude fiber, 13.67% crude fat and, 3.48% ash (Sukaryana *et al.*, 2013). Additionally, bran contains 8.50% crude protein, 8.50% crude fat, 12% crude fiber, and 1600 kcal/kg metabolic energy (Priabudiman and Sukaryana, 2011). While cassava pulp contain nutritional ingredients such as crude protein, crude fiber, crude fat, and ash with a content of 2.28%, 19.36%, 0.54%, and 3.66% respectively (Sukaryana *et al.*, 2013).

The fermentation process using microorganisms can improve nutritional quality and reduce or eliminate the negative influence on feed ingredients (Biyatmoko *et al.*, 2018). The EM-4 probiotic is a biotechnology product that can be used to improve the nutritional value of agricultural waste and ineffective materials that to be used as animal feed ingredients. This EM-4 is a probiotic solution containing mixed cultures of various microorganisms, namely Lactobacillus, photosynthetic bacteria, Actinomycetes, yeast, and fermented fungi.

According to Septinova *et al.* (2017), fermentation with EM-4 was not optimal to improve the nutrition of expeller palm kernel, cassava pulp, and bran mixture. Giving a mixture of local feed ingredients in the ration has not been able to increase the production of local male ducks either. The production of ducks as control was not significantly different from the production of ducks that received a fermented mixture of expeller palm kernel, cassava pulp, and bran. Therefore, other efforts need to be made to improve the quality by adding urea and ammonium sulfate in the fermentation process of the feed mixture. However, administration of urea and ammonium sulfate in poultry rations must be done with caution because it can interfere with liver function. The liver is strongly associated with the levels of total blood plasma cholesterol because cholesterol metabolism mostly occurs in the liver. Therefore, damage to liver function will reduce blood cholesterol levels (Surasa *et al.*, 2014).

Ducks are a type of poultry whose meat contains high fat and cholesterol. According to Rahmat and Wiradimadja (2011), cholesterol in the meat and eggs will increase in line with increasing blood cholesterol levels. According to Bidura *et al.* (2008), the use of fermented products in rations can significantly increase the quantity and quality of the carcasses and reduce the amount of abdominal fat and cholesterol levels in blood plasma of poultry.

MATERIALS AND METHODS

This study used 32 male hybrid ducks, aged 3 weeks that were kept for 4 weeks in a litter cage. The litter cage was divided into 16 plots measuring 80 cm long, 60 cm wide, and 60 cm high. The ration used was a commercial broiler II (BR2) added with a mixture of expeller palm kernel, cassava pulp, and bran that was fermented with EM-4, molasses, ammonium sulfate, urea, and water. The nutritional content of ration is listed in Table 1.

This research was conducted experimentally using a Completely Randomized Design (CRD). All ducks were divided into four groups: R0, R1, R2, and R3 where each group consists of four ducks. The groups were given treatments as follows: R0 group was given commercial rations (BR2), whereas R1, R2, and R3 groups were given 80% of commercial rations that added 20% of fermented feed ingredients I (FF1) for R1, 20% fermented feed ingredients II (FF2) for R2, and 20% fermented feed ingredient III (FF3) for R3. The composition of fermented feed is 80% palm kernel expeller, 10% cassava pulp, 10% bran, 10% EM4, 5% molasses, and 40% water. Furthermore, those feed was fermented with 1.5% Urea (FF1), 1.5% ammonium sulfate (FF2), and 1.5% urea and 1.5% ammonium sulfate (FF3). Fermentation of feed process was carried out according to Muhammad *et al.* (2014). The fermented feed material had a smell nice, was overgrown with white or red fungus, and felt warm. The ration treatments were carried out for 4 weeks.

At the end of the 8th week, a blood sample was taken from the brachial vein. The skin was cleaned using a cotton swab moistened with alcohol. The blood taken was about 3 ml and put into a vacutainer tube and stored in a cool box before examined at the laboratory. The observed variables were blood lipid levels of cholesterol, triglycerides, High-Density Lipoprotein

(HDL), and High-Density Lipoprotein (HDL), dan low-density lipoproteins LDL. The data obtained were analyzed by Analysis of Variance (ANOVA) at 5% level. To determine the best treatment, the Least Significant Difference (LSD) test was performed.

RESULTS AND DISCUSSION

Effect of Treatments on Duck Blood Cholesterol

The average level of blood cholesterol in duck is presented in Table 2. The results showed that the treatments had a significant effect on duck blood cholesterol ($P < 0.05$). According to LSD test results showed that blood cholesterol level at R0 group was not significantly different ($P > 0.05$) than R1 and R3 groups but that level was significantly lower ($P < 0.05$) than R2.

The level of blood cholesterol at R0, R1, and R3 was significantly lower ($P < 0.05$) than R2. This is presumably because the average energy content in rations of R0, R1 and R3 groups are higher and the crude fiber content is lower. The high energy content in rations of R0, R1 and R3, causes high fat deposits in the liver so that it can increase fat absorption and decrease cholesterol formation through *de novo* biosynthesis.

In this study, feed fermentation with EM-4 and ammonium sulfate did not reduce the crude fiber content of the feed ingredient mixture. As a result, the crude fiber content in R2 was higher than R0. The high content of crude fiber in R2 cause a decrease in absorption of fat in the body. The impact of an increase in the *de novo* biosynthesis process in the body makes the cholesterol in the blood significantly higher in R2 ducks than R0 ($P < 0.05$). According to Hartoyo *et al.* (2015), the fat, protein, crude fiber, and energy contents in the ration will affect the body fat level which subsequently will affects the blood fat level. According to Joseph *et al.*

Table 1. Nutrient content of the ration treatments

| Treatment name | Analysis results | | | | | | |
|----------------|------------------|-----------------|-------------|---------------|-------|--------|-------------|
| | Dry matter % | Crude protein % | Crude fat % | Crude fiber % | Ash % | BETN % | EM (kkl/kg) |
| R0 | 91.65 | 17.96 | 0.20 | 4.07 | 4.81 | 72.20 | 3319.11 |
| R1 | 90.29 | 17.43 | 0.48 | 7.62 | 4.97 | 67.95 | 3171.99 |
| R2 | 89.71 | 17.17 | 0.24 | 10.00 | 5.09 | 65.85 | 3068.35 |
| R3 | 90.51 | 17.71 | 0.18 | 8.51 | 6.08 | 66.24 | 3097.41 |

* Laboratory of Animal Nutrition and Food, Department of Animal Husbandry, Faculty of Agriculture, Lampung University (2018); ** Calculation results based on the Carpenter and Clegg formula (Anggorodi, 1994) $EM = 40.81 \{0.87 [Crude\ protein + 2.25\ Crude\ fat + BETN] + 2.5\}$. R0= commercial ration (BR2); R1= 80% commercial ration + 20% FF1; R2: 80% commercial ration + 20% FF2; R3= 80% commercial ration + 20% FF3

Table 2. The level of blood cholesterol, triglycerides, HDL, and LDL duck

| Replication | Treatment | | | |
|---------------|---------------------------|---------------------------|--------------------------|--------------------------|
| | R0 | R1 | R2 | R3 |
| | mg/dL | | | |
| Cholesterol | 160.75±16.11 ^a | 180.75±15.56 ^a | 200.5±24.52 ^b | 168.5±16.03 ^a |
| Triglycerides | 68.50±28.90 | 85.50±21.32 | 91.25±20.71 | 58.00±11.81 |
| HDL | 87±14.47 | 89.5±5.74 | 101±8.91 | 94±8.83 |
| LDL | 70.50±7.94 | 84.00±30.92 | 78.75±18.41 | 60.00±10.55 |

Values with different superscripts showed a significantly different ($P < 0.05$) based on the LSD test (HDL= High Density Lipoprotein; LDL= Low Density Lipoprotein)

R0= commercial ration (BR2); R1= 80% commercial ration + 20% FF1; R2: 80% commercial ration + 20% FF2; R3= 80% commercial ration + 20% FF3

(2002), 25% of cholesterol comes from the treatment of feed given, while 75% comes from de novo synthesis that occurs in the body, which is concentrated in the liver. Muliani (2014) states that when cholesterol intake from the food is low, cholesterol production through de novo biosynthesis will increase.

In this study, the provision of local feed ingredients fermented with EM-4 with the addition of ammonium sulfate has not had a positive effect on reducing cholesterol levels, but the provision of local feed ingredients fermented with EM-4 with the addition of ammonium sulfate and urea can reduce cholesterol levels. Blood fat levels depend on the synthesis of proteins, fats, and carbohydrates in the body and the content of this nutrition in feed ingredients. Fermentation of local feed ingredients with EM-4, urea, and ammonium sulfate can increase protein and ash content and reduce the crude fat of feed ingredients. The use of 20% fermented feed in commercial rations (R3) produces relatively high protein content, lowest fat content, and crude fiber content that is still within the limits and can be tolerated by the body of ducks, while cholesterol, triglyceride, LDL, and HDL levels are all within normal limits.

In this present study, the amylase, protease, and lipase enzymes contained in EM-4 function to break down the components of carbohydrates, proteins, and fats within the feed. However, during drying and storage, the bacteria present in the fermented feed are inactive for a long time even the bacteria die so that the production of enzymes that are expected to help the digestive process of the feed becomes disrupted in the duck's body. Cavallini *et al.* (2009) stated that probiotics can produce statins, namely inhibitors of 3-hydroxy-3-methyl-glutaril-CoA reductase (HMG-CoA reductase), which are enzymes that regulate cholesterol biosynthesis and reduce LDL, Very Low Density Lipoprotein (VLDL), and blood triglyceride levels.

Effect of Treatments on Duck Blood Triglycerides

Consumers do not prefer high levels of triglycerides in duck meat because it can be a cause of coronary heart disease. The results of the analysis of variance showed that treatments had no significant effect ($P>0.05$) on duck blood triglycerides (Table 2). The results of this study are similar to those of Tugiyanti *et al.* (2016). The levels of blood triglycerides that are not significantly different are caused by the low influence of fermented feed ingredients on the nutritional content of rations and consumption of rations. Table 1 shows that the nutritional content of the R0, R1, R2, and R3 treatment rations ranged from 65.85 to 72.20% carbohydrates, 17.17-17.76% protein, and 0.18-0.48% fat. Triglycerides in blood plasma are formed from fats, carbohydrates, and proteins in feed. Carbohydrate content in the ration is the most influential on triglyceride levels. The organ that plays a role in the formation of triglycerides is the liver. The liver converts carbohydrates to free fatty acids and turns them back into triglycerides (Citrawidi, 2012).

Effect of Treatments on Duck Blood HDL

HDL is a lipoprotein compound known as good cholesterol because it carries cholesterol in the blood vessels to the liver, which is then converted into bile. The results of the analysis of the variance showed that treatments did not significantly affect the HDL level ($P>0.05$) in duck blood (Table 2). This is because the nutritional content in the treatment rations is relatively the same in R0, R1, R2, and R3. According to Setyadi *et al.* (2013), the ration is a very influences factor on blood HDL and LDL levels. Other factors that can affect HDL and LDL levels are genes, environment, and condition of livestock.

The addition of fermented products in the duck ration tended to increase the blood HDL of duck. The mean HDL in R0, R1, R2, and R3 was 87 mg/dl, 89.5 mg/dL, 101 mg/dl, and 94 mg/dl respectively. An increased HDL levels is associated with an increased cholesterol levels in serum (Hasanudin *et al.*, 2013).

Effect of Treatments on Duck Blood LDL

LDL plays an important role in providing cholesterol needs in the body. The results of the analysis of the variance showed that the treatments had no significant effect on duck blood LDL ($P>0.05$) (Table 2). Nutrient content in rations, especially fats and proteins that are almost the same in four groups (R0, R1, R2, and R3) is suspected as a factor in the absence of significant differences. Low-density lipoprotein is a class of lipoproteins and transport agents containing 25% proteins, 45% cholesterol, and phospholipids and triglycerides that function in cholesterol transport from liver cells to peripheral cells. Additionally, LDL and cholesterol is composed of protein, triglycerides, cholesterol, and phospholipids with cholesterol as the major constituent (Rosadi *et al.*, 2013).

The relatively similar levels of LDL are also thought to be because the triglyceride and HDL levels in duck blood are not significantly different. Even though blood cholesterol in R0, R1, and R3 was significantly different ($P<0.05$), but this difference could not produce differences in blood LDL levels. That condition caused by HDL levels in R2 is higher than HDL levels in R0 group. The average LDL level in R0, R1, R2 and R3 was 70.5 mg/dL, 84 mg/dL, 78.75 mg/dL, and 60 mg/dL for, respectively. Normal LDL level in blood according to Basmacioglu and Ergul (2005) is <130 mg/dL. In addition, Fita (2007) stated that blood LDL levels in poultry are 31.6-62.07 mg/dL whereas LDL levels of this study range from 60.00-84.00 mg/dL.

The addition of urea and ammonium sulfate helps the process of overhauling organic material in feed fermented with EM-4. In poultry, giving urea and ammonium sulfate must be done carefully because it can disturb the body's acid-base balance and metabolism, especially the work of the liver and kidneys, which may result in poisoning and even death. Administration of urea and ammonia in high levels forces the liver to overwork, making liver cells

damaged, which in turn causes low blood plasma total cholesterol levels. In this study, giving feed fermented with EM-4, urea, and aluminum sulfate up to the level of 1.5% did not have a negative impact on the function of the liver of ducks. Cholesterol, triglycerides, HDL, and LDL levels in blood ducks are all within normal limits. Therefore, this feed is safe to use.

CONCLUSION

The ration that gave the best effect on the blood fat levels of male ducks blood was the ration given to R3 group (80% commercial ration + 20% FF3).

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