Scientific Papers. Series D. Animal Science. Vol. LXII, No. 1, 2019
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

THE INTERVENTION OF *Hermetia illucense* L. (DIPTERA: STRATIOMYIDEA) IN MANURE EMPOWERMENT ON BLOOD LIPID PROFILE OF NATIVE CHICKEN

Heidy MANANGKOT, Marie NAJOAN, Wisje TOAR, Merry ROTINSULU, Santi TURANGAN

Faculty of Animal Husbandry, Sam Ratulangi University, Jalan Kampus Unsrat. Manado 95115, Indonesia

Corresponding author email: hmanangkot@gmail.com

Abstract

The aims of this research were to evaluate the utilization of manure degraded by Hermetia illucens to be used as diets on blood lipid profile of native chicken. The treatments were R1 (control diet with 15% fish meal + 0% MBHI meal), R2 (diet with 10% fish meal + 5% MBHI meal), R3 (diet with 5% fish meal + 10%MBHI meal), and R4 (diet with 0% fish meal + 15% MBHI meal). The data were analyzed using analysis of variance according to Completely Randomized Design (CRD) and means separated by the application of Duncan's multiple range test. Variables measured were triglyceride, LDL, HDL, and cholesterol. The results shown that R3 gave a significant (P<0.05) lower in triglyceride (47,15 -59,66 mg/dl), LDL (45,68 – 81,06 mg/dl),cholesterol (124,98 -155,97 mg/dl) Whereas, R3 gave HDL (75,85 – 86,28 mg/dl) level significantly higher (P < 0.05). High blood HDL level at 10% MBHI meal in the diet not only maintains blood HDL at normal level but also increases native chicken meat quality, as was expected in the present study.

Key words: *manure, H. illucense, blood, native chickens.*

INTRODUCTION

The utilization of insect in animal husbandry become important (Rumokoy et al., 2017) especially as ingredients for feed has gained considerable interest recently, for example *H.illucens* or black soldier fly (BSF) can be used as feeds for animals and human food as well and further stated that BSF is rich in protein and fat (Wang and Shelomi, 2017); while Barragan-Fonseca et al. (2017) reported BSF has a protein content of 37-63%, fat 7-39%, ash 8-25% (DM basis), protein 40.8 ± 3.8%, fat 26.6 ± 8.6% and suited for poultry feed (Nyakeri et al., 2016). BSF lives in organic matter, food waste disposal, and manure, produces enzymes such as: amylase, lipase, and protease to hydolize karbohidrate, fat, and protein into smaller or individual parts as maltose, fatty acids, glycerol, and amino acids respectively (Kim et al., 2011). By doing this, BSF can break down waste disposal to become a high quality feedstuffs and can also reduce pollution (Wang and Shelomi, 2017). This BSF or *H. illucens* has been studied for its capability to convert organic waste to high quality protein, control certain harmful bacteria and insect pests, provide potential chemical precursors to produce biodiesel and for its use as feed for a variety of animals. BSF has been used as feedstuffs and can substitute parts or total proportion of soybean meal or fish meal in layer diets (Maruer et al., 2016; Marono et al., 2017), broiler (Schiavone et al., 2016), and quails (Widjastuti et al., 2014). Moula et al. (2017) reported that native chicken consumed horse manure biodegraded by *H. illucens* resulted in good performances.

Tohala (2010) reported that blood triglyceride, total cholesterol, HDL, LDL,VLDLP level of native chicken consumed high and low energy diets about 53.83 ± 2.24 mg/dL; 139.69±5.82 mg/dL; 57.50±4.39 mg/dL; 75.50±3.71; 10.57±0.46 mg/dL; and 50.17±1.40; 131.83±4.13 mg/dL; 53.33±1.7; 73.50±1.52; 10.03±0.29 mg/dL, respectively. The utilization of chicken manure biodegraded by *H. illucens* is an interesting choice as an inconvensional feed ingredient to replace conventional feed ingredients which are expensive and compete with human use. Yet little is known about the effect of utilization of chicken manure biodegraded by *H. illucens* in the diets on meat quality of native chicken. Therefore, the present study was arranged to elaborate the utilization of chicken manure biodegraded by *H. illucens* in the diets on blood lipid profiles of native chicken *(Bali chicken)*.

MATERIALS AND METHODS

The wild adult *H. illucens* or BSF were captured from the chicken farm environment and then thirty pairs flies were placed into each manure box. The manure box was designed with a dimension 100 x 100 x 70cm, each side is made of gauze. Thirty kg of broilers manure, as the BSF larva media, were placed in this manure box. The flies laid down their eggs until day fourth. The BSF larvae were reared in this media to take place the biodegradation of the manure during eight days of their life cycle. The study was conducted for two months. A total of 100 four month-old native chicken *(Nunukan X Kedu)* known as *Bali chicken*, were allocated in twenty 0.7 x 0.5 x 0.5 m battery cages. Birds were randomly divided into 20 experimental units of 5 chicks each and each diet was offered at random. Each pen was equipped with three separate tube feeders and waterers. Fresh water and feed were provided *ad libitum* throughout the experimental period. The dietary treatments were in a completely randomized design with 4 treatments and 5 replications according to Steel and Torrie (1980). Each treatment was formulated in iso-nutrient and iso-caloric arrangement. Treatments were formulated as follow: R1 as control diet with 15% fish meal + 0% MBHI meal; R2 was a diet with 10% fish meal + 5% MBHI meal; R3 was a diet with 5% fish meal + 10% MBHI meal; and R4 was a diet consisted with 0% fish meal + 15% MBHI meal. Composition and nutrient content of treatment diets is presented in Table 1.

Data collection

At day 60, 2 chicks from each pen were selected and blood samples collected. The triglyceride (TG), cholesterol (CHOL), high density lipoprotein (HDL) levels were measured using Cholesterol Oxidase-peroxidase amino antipyrine phenol (CHOD-PAP) method (Enzymatic Calorimetric/Method NS), using commercially available kits. (Kit/Spectrophotometry Analysis (Biotech England, 2011).

Table. 1. Composition and nutrient content of experimental diets (%)

Ingredients (%)	Treatments			
	R1	R2	R3	R4
Yellow corn	55	55	55	55
Rice bran	11.5	11.5	11.5	11.5
Copra meal	7	7	7	7
Soybean meal	10	10	10	10
Fish meal	15	10	5	0
MBHI meal	0	5	10	15
Bone meal	1	1	1	1
Vitamin Premix*)	0.5	0.5	0.5	0.5
Nutrient assayed				
Crude protein (%)	20.30	20.17	19.83	19.55
Crude fiber (%)	4.56	5.02	5.49	5.95
Ether extract (%)	5.27	5.23	5.20	5.03
Ca (%)	1.00	0.92	0.83	0.67
P (%)	0.74	0.61	0.55	0.42
ME (kcal/kg)	2951.40	2934.60	2917.25	2905.75

*)Vitamin premix is a mixture of vitamins and mineral premix supplied the following per kilogram of feed: vitamin A = 6000 IU, vitamin D3 = 1000 IU, vitamin E = 12.50 mg, vitamin K = 1.450 mg, vitamin B1 = 4.50 mg, vitamin B2 = 2.250 mg, vitamin B6 = 0.450 mg, vitamin B_{12} = 2.250 mg, niacin = 5.500 mg, pantothenic acid = 0.150 mg, iron = 18.50 mg, copper = 2.5 mg, manganese = 50.0 mg, zinc = 27.50 mg, iodine = 4.0 mg, and selenium = 0.0500 mg.

Statistical analysis. Data were subjected to analysis of variance and significant differences observed in means subjected to Duncan's multiple range test. All data were analyzed for variance analysis using the general linear model (GLM) procedures of the SAS Institute.

RESULTS AND DISCUSSIONS

The results of the blood lipid indices of the experimental birds are presented in Table 2 and Diagram 1.

Triglyceride as a lipid component in the blood normally increases drastically soon after meals, especially with simple carbohydrate such as sugars and saturated fat. Since simple carbohydrate cannot be used directly as an energy sources, instead of being transformed into triglycerides and stored in a form of energy. At the time when body needs energy, triglyceride will be mobilized by hormones.

Table 2. Average blood lipid profile native chicken fed chickens manure mealbiodegraded by *Hermetia illucens* (*Diptera:* L *stationyidea)* (MBHI) (mg/dL)

Blood Lipid Profiles	Treatments			
	R1	R2	R3	R4
Triglycerid	59.66^{c}	52.77^{b}	47.15^{a}	49.69^{a}
LDL	81.06^{d}	62.48^{c}	45.68^{a}	51.96^{b}
HDL	75.85^{a}	81.20^{b}	86.28^{c}	78.58^{a}
Total Cholesterol	155.97^{c}	131.73^{b}	124.98^{a}	129.77^{a}

^{a,b,c} Means on the same row with different superscripts differ significantly (P < 0.05)

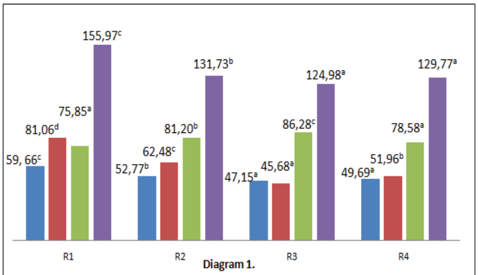

Diagram 1.
Avarage blood lipid profile native chicken fed chickens manure biodegraded by *Hermetia llucens* larvae meal (mg/dL)

Research results showed that blood triglyceride levels in the present study are about 47.15 – 59.66 mg/dL. The highest blood triglyceride level found at R1 (control diet without MBHI in the diet) (59.66 mg/dL; whereas the lowest level of triglyceride found at R 3 (10% MBHI and 5% fish meal in the diet)(47.15 mg/dL. The average level of triglyceride in the present study was in agreement with Tohala (2010) who reported that blood triglyceride level in broiler chicken is about 50.17±1.4 to 52.83±2.44 mg/dL. Blood triglyceride level is determined by feed consumption especially simple carbohydrate such as sugar, saturated fat, high level of circulated free fatty acids, high insulin level, and low glucagon level. Carbohydrate in the liver is broken down to fatty acids and transformed back to triglyceride (Murray, 2012). Chicken manure biodegraded by *H.illucens* (MBHI) in the present study is assumed to have high enzymes (amilase, lipase, and protease) activity specifically lipase. Initial study (Manangkot, 2014) revealed that at day 7 of manure after being biodegraded by *H. illucens* and day 8 of larvae growth in the manure, gave a highest amylase, protease, and lipase enzymes activity. Lipase showed a value of 44.84±17.39 mg/dL. It is assumed that the higher the level of MBHI in the diets, the higher the lipase activity. Blood LDL level in the present study ranged from 45.68 to 81.06 ml/dL. R1 (control diet without MBHI meal in the diet) gave a highest blood LDL level of about 81.06 ml/dL; whereas R3 (10% MBHI meal in the diet) gave a lowest blood LDL level of about 45.68 ml/dL. Statistical analysis revealed that treatments gave a significant difference (P < 0.05) on blood LDL level of native chicken in the present study. It can be said that MBHI has an ability in lowering blood LDL level of native chicken in the present study. Lower blood LDL level in the present study compared with a normal level (46.68 – 81.06 ml/dL vs 95 – 125 ml/dL) is a result of high MBHI meal level in the diets, so that the diets have more active enzyme to mobilize cholesterol and LDL itself. The average blood HDL level in the present study is 75.85 – 86.28 mg/dL. R1 (control diet without MBHI meal in the diet) has a lower (P < 0.05) blood HDL level compared with R2, R3, and R4. Blood HDL level of R2 (5% MBHI meal in the diet) was significantly lower (P < 0.05) than R3 (10% MBHI meal in the diet). Blood HDL level of R4 (15% MBHI meal in the diet) was significantly lower (P < 0.05) than R2 and R3; while blood HDL level of R3 (10% MBHI meal in the diet) was significantly higher (P < 0.05) than the rest of treatments. Hariyanto (2017) also reported that blood triglyceride level in broiler chicken ranged from 54.20±20.9 to 84.78±23.6 mg/dL. R1 gave a significantly higher (P < 0.05) compared with R2, R3, and R4 treatments. R2 gave a significantly (P < 0.05) higher blood triglyceride level than R3; while there was no significant different (P > 0.05) was observed on blood triglyceride level between R3 and R4. The results indicated that the higher the MBHI meal level in the diets, the higher the blood HDL level of native chicken in the present study. It can be said that MBHI was able to increase blood HDL level of native chicken in the present study. High blood HDL level can prevent the incidence of atherosclerosis. HDL is a lipoprotein that function as a carrier for cholesterol from body tissues to liver. The excess of cholesterol is transported by HDL to the liver (Murray et al*.,* 2003) degraded and excreted as bile acids.

Blood cholesterol level in the present study is about 124.98-155.97 ml/dL. R1 gave the highest blood cholesterol level and R3 gave the lowest blood cholesterol level. The average blood cholesterol level found in the present study was in agreement with a finding by Iriyanti et al., (2014) who reported that blood cholesterol level in native chicken ranged from 123.04±7.07 to 170.27±9.68 ml/dL. Statistical analysis revealed that treatments gave a significant difference (P< 0.05) on blood cholesterol level. R1 (control diet without MBHI meal in the diet) had a higher (P<0.05) blood cholesterol level compared with R2, R3, and R4 treatments. R2 (5% MBHI meal in the diet) also gave a higher (P < 0.05) blood cholesterol level compared with R3 and R4 treatments. As mentioned above that MBHI used in the present study is high in enzymes activity. So that the higher the MBHI level in the diets, the higher the enzymes activity (more specifically lipase), in turn the higher the production of fatty acids and glycerol.

Blood triglyceride level is determined by feed consumption especially simple carbohydrate such as sugar, saturated fat, high level of circulated free fatty acids, high insulin level, and low glucagon level. Carbohydrate in the liver is broken down to fatty acids and transformed back to triglyceride (Murray, 2012). Chicken manure biodegraded by *Hermetia illucens* L (*Diptera: statiomyidea)* (MBHI) in the present study is assumed to have high enzymes (amilase, lipase, and protease) activity specifically lipase. Initial study (Manangkot, 2014) revealed that at day 7 of manure after being biodegraded by *Hermetia illucens* L (*Diptera: statiomyidea)*and day 8 of larvae growth in the manure, gave a highest amylase, protease, and lipase enzymes activity. Lipase showed a value of 44.84±17.39 mg/dL. It is assumed that the higher the level of MBHIL in the diets, the higher the lipase activity. Lipase is a group of enzyme that normally hydrolyzes triglyceride to free fatty acids and glycerol. This what was expected to be a key factor in decreasing blood triglyceride level in chicken (Mingrui Yu et al., 2007 in Supriyatna et al., 2015).

Low Density Lipoprotein (LDL). Blood LDL level in the present study ranged from 45.68 to 81.06 ml/dL. R1 (control diet without MBHIL meal in the diet) gave a highest blood LDL level of about 81.06 ml/dL; whereas R3 (10% MBHIL meal in the diet) gave a lowest blood LDL level of about 45.68 ml/dL. The normal level of LDL for broiler chicken is about 95 – 125 ml/dL. Statistical analysis revealed that treatments gave a significant difference (P < 0.05) on blood LDL level of native chicken in the present study. The result indicated that the higher the MBHI meal level in the diets, the lower the blood LDL level. It can be said that MBHIL has an ability in lowering blood LDL level of native chicken in the present study. Lower blood LDL level in the present study compared with a normal level (46.68-81.06 vs 95 – 125 ml/dL) is a result of high MBHI meal level in the diets, so that the diets have more active enzyme to mobilize cholesterol and LDL itself. LDL is a lipoprotein that acts as a main carrier for cholesterol to be transported to the tissues. The blood level of LDL is highly influenced by cholesterol itself. Montgomery et al., (1993) expalined that blood LDL level is simultaneously produced with triglyceride and cholesterol.

High Density Lipoprotein (HDL). The average blood HDL level in the present study is 75,85 – 86,28 mg/dL. The highest blood HDL level is showed by R3 (10% MBHI meal in the diet), whereas the lowest blood HDL level is showed by R1 (control diet without MBHIL meal in the diet). The average blood HDL level in the present study is in agreement with a research results by Hariyanto et al., (2016) who reported that average blood HDL level in chicken is about 87.26 ±5.16 mg/dL. Statistical analysis revealed that blood HDL level among treatment showed a significant defferences (P< 0.05). R1 (control diet without MBHI meal in the diet) has a lower (P < 0.05) blood HDL level compared with R2, R3, and R4. Blood HDL level of R2 (5% MBHI meal in the diet) was significantly lower (P < 0.05) than R3 (10% MBHI meal in the diet). Blood HDL level of R4 (15% MBHI meal in the diet) was significantly lower (P < 0.05) than R2 and R3; while blood HDL level of R3(10% MBHI meal in the diet) was significantly high(P< 0.05) than the rest of treatments. The results indicated that the higher the MBHI meal level in the diets, the higher the blood HDL.level of native chicken in the present study. It can be said that MBHI was able to increase blood HDL level of native chicken in the present study. High blood HDL level can prevent the incidence of atherosklerosis. HDL is a lipoprotein that function as a carrier for cholesterol from body tissues to liver. The excess of cholesterol is transported by HDL to the liver ((Murray et al., 2012) degraded and excreted as bile acids. Approximately 75-80% of cholesterol in HDL particles will be converted to HDL particles (Mayes et al. 1997).

Cholesterol. Cholesterol is a main lipid component that functions as a precursor of all steroid hormones and bile acids. Cholesterol is synthesized in the liver. Blood cholesterol level in the present study is about 124.98-155.97 ml/dL. R1 gave the highest blood cholesterol level and R3 gave the lowest blood cholesterol level. The average blood cholesterol level found in the present study was in agreement with a findings by Iriyanti et al. (2014), who reported that blood cholesterol level in native chicken ranged from 123.04±7.07 to 170.27±9.68 ml/dL. Statistical analysis revealed that treatments gave a significant difference (P < 0.05) on blood cholesterol level. R1 (control diet without MBHI meal in the diet) had a higher (P < 0.05) blood cholesterol level compared with R2, R3, and R4 treatments. R2 (5% MBHI meal in the diet) also gave a higher (P < 0.05) blood cholesterol level compared with R3 and R4 treatments. It can be said that the higher the MBHIL meal level in the diets, the lower the blood cholesterol level in native chicken in the present study. It is indicated that MBHIL meal in the diets could reduce blood cholesterol level of native chicken in the present study. The explanation is that changes in blood cholesterol level is a direct response related to the rate of free fatty acids change in the diets as a result of increased MBHIL meal level in the diets. In this case, free fatty acids is converted to acetyl co-A as a main precursor for cholesterol formation (Lovita, 2005). In the body, cholesterol is a precursor for bile acid in producing bile salts to emulsify fats. The production of these bile salts requires cholesterol. So, when level of cholesterol in the liver is low, high density lipoprotein (HDL) will mobilize cholesterol from body tissues to accomplish its requirement for cholesterol (Mayes, 1997). As mentioned above that MBHIL used in the present study is high in enzymes activity. So that the higher the MBHIL level in the diets, the higher the enzymez activity (more specifically lipase), in turn the higher the production of fatty acids and glycerol. As a consequence it trigger the production of bile salt to emulsify fatty acids, then it will increase the use of cholesterol to balance the high secretion of bile salt and the end results is lower blood cholesterol level.

CONCLUSIONS

Chicken manure biodegraded by *Hermetia illucense larvae* (MBHI)can be used up to 10% in the diets to maintain blood triglyceride, cholesterol, LDL, and HDL in a normal level. High blood HDL level at 10% MBHI meal in the diet not only maintains blood HDL at normal level but also increases native chicken meat quality, as was expected in the present study.

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