Scientific Papers. Series D. Animal Science. Vol. LXIV, No. 2, 2021
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

ANALYSIS OF TRIGLYCERIDES AND CHOLESTEROL OF LAYING CHICKEN CARCASS ON REPLACEMENT OF FISH MEAL WITH DEGRADED MANURE FLOUR (MHD) LARVAE OF *HERMETIA ILLUCENS* L.

Merri ROTINSULU, Heidy MANANGKOT, Godlief REMBET, Delly RUMONDOR, Martha KAWATU

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

Corresponding author email: hmanangkot@gmail.com

Abstract

This study used an experimental method using a completely randomized design (CRD) consisting of 4 treatments, namely RA = 15% fish meal + 0% MHD meal, RB = 10% fish meal + 5% MHD meal, RC = 5% fish meal + 10% MHD meal and RD = 0% fish meal + 15% MHD flour. The results of the analysis of triglycerides and blood serum cholesterol in a sample of 20 laying hens, the analysis showed that MHD as a result of degradation of black fly larvae (Hermecia illucens L.) had a significant effect on triglycerides, LDL, HDL and total cholesterol but had no significant effect on VLDL (Very Low density). lipoprotein) blood serum of native chicken (p <0.05). RC treatment, namely administration with 10% MHD flour is a treatment with the best response to the content of the blood serum lipid profile, and the use of MHD in laying hens up to 15% is as good / effect as the use of MHD in domestic broilers.

Key words: *cholesterol, H. illucens L., laying free-range chicken, triglycerides.*

INTRODUCTION

Laying free-range chickens are part of local native chickens in Indonesia whose lives are closely related to the community. The appearance of laying free-range chickens is very diverse, as well as their genetic characteristics, their distribution is very wide because the domestic chicken population is found in cities and villages. The potential should be developed to improve community nutrition and increase family income, this can be seen from population growth and increasing demand for meat and eggs from year to year (Nyakeri et al., 2016).

H. illucens L. lives in organic matter, food waste disposal, and manure, produces enzymes such as: amylase, lipase, and protease to hydrate carbohydrates, fats, and proteins into smaller or individual parts such as maltose, fatty acids, glycerol, and amino acids respectively (Kim et al., 2011).

Thus, *H illucens* L. can break down waste disposal into quality feed ingredients and can also reduce pollution (Wang & Shelomi, 2017). *H. illucens* L. has been studied for its ability to convert organic waste into high quality protein, control certain harmful bacteria and insect pests, provide potential chemical precursors for producing biodiesel and for use as feed for various animals (Barbagan et al., 2017).

Considering this potential, it is necessary to seek a way out to increase the population and productivity. Laying free-range chickens have the advantage of high adaptability because they are able to adapt to various situations, environmental conditions and climate change and local weather. One of the contributing factors is the maintenance system which is still traditional in nature, the amount of feed given is insufficient and the feeding does not yet refer to the principles of nutrition, especially the feeding that has not taken into account the need for food substances for various levels of production (Wang & Shelomi, 2017).

In general, the nutritional requirements for chickens are highest during the first week (0-8 weeks) of life, therefore it is necessary to provide adequate rations containing energy, protein, minerals and vitamins in a balanced amount. Another factor is genetic improvement and increased management of native chicken

rearing must be supported by improved feed nutrition (Setioko and Iskandar, 2005).

Feed costs account for 70-75% of the total production cost of poultry farming, particularly native chickens (Teguia & Beynen, 2005; Mupeta et al., 2003).

The price of feed ingredients continues to increase due to an increase in the number of poultry farms (Hassan et al., 2014).

Poultry feed costs reach 70-80% of the total production. Fish meal is one of the ingredients of the ration and has a fairly good source of nutrition, especially as a source of animal protein. Fish meal as a raw material for poultry rations ranks first in the supply of animal protein sources because the very high crude protein based on its use in the composition of poultry ration/feed reaches 10% (Anggorodi, 1985).

One of the alternative feed ingredients that are easily available, cheap and can be used to replace fish meal is the degradation of manure flour (MHD) larva *H. illucens* L. The results of the study by Manangkot (2014) showed that MHD contains 51% protein which competes with fish meal.

Based on the above thinking, research has been carried out to determine the effect of replacing fish meal with manure meal resulting from degradation of *H. illucens* L. larvae on triglycerides and blood serum cholesterol of laying hens. Does the replacement of manure flour degraded by *H. illucens* L. larvae with fish meal affect the quality of carcass on triglycerides and blood serum cholesterol of laying hens? This study aims to determine the effect of replacing fish meal with manure flour from degradation of *H. illucens* L. larvae on blood serum cholesterol and triglycerides of laying native chicken meat.

Previous research has been carried out by Rotinsulu (2020), Exploration of Degraded Manure Flour (MHD) of Black Fly Larvae (*Hermetia illucens* L.) Against Carcass of Layed Buras Chicken with Soaking Sweet Orange (Citrus sinensis). This study aims to determine the effect of replacing fish meal with manure meal resulting from degradation of *H. illucens* L. larvae on triglycerides and blood serum cholesterol of laying native chicken carcasses and testing the carcass quality.

MATERIALS AND METHODS

H. illucens L. was obtained from the chicken farm environment and then thirty pairs of flies were placed in each litter box. The manure box is designed with a size of 100 x 100 x 70 cm, each side made of gauze. Thirty kg of broiler manure as a medium for *H. illucens* L. larvae were placed in this litter box. The flies lay eggs until the fourth day. *H. illucens* L. larvae were reared in this medium for biodegradation of manure for eight days of its life cycle.

MHD was obtained from biodegradation of 8 day old larvae through rearing results of *H. illucens* L. flies with 2 week old broiler chicken manure.

Maintenance was carried out for 6 months. A total of 60 6-month-old laying hens, known as Balinese chickens, are housed in 20 cages, each measuring 50 x 50 x 70 cm. The laying hens used were divided randomly into 20 experimental units, each consisting of 3 chickens and each food was given randomly. Each cage is equipped with separate eating and drinking areas. Drinking water and feed were given ad libitum during the experimental period.

The treatment used a completely randomized design with 4 treatments and 5 replications according to Steel and Torrie (1991).

Table 1. Composition and content of food substances in the experiment

Ingredients	Treatments (%)			
	R1	R2	R3	R4
MHD	0	5	10	15
Fish meal	15	10	5	0
Copra meal	7	7	7	7
Soybean meal	10	10	10	10
Rice bran	11.5	11.5	11.5	11.5
Yellow corn	55	55	55	55
Bone meal	1	1	1	1
Vitamin Premix*)	0.5	0.5	0.5	0.5
The content of food substances				
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

Each treatment is formulated in terms of iso-nutrient and iso-calorie compositions. The treatments were formulated as follows: RA as control feed with 15% fish meal + 0% MHD flour; RB, 10% fish meal + 5% MHD flour; RC is a diet with 5% fish meal + 10% MHD flour; and RD is feed consisting of 0% fish meal + 15% MHD flour (Table 1).

Data collection for 4 months and age 12 months after harvest for analysis of triglycerides and blood serum cholesterol in laying hens.

Data analysis. Analysis of variants was performed using the SPSS procedure. The research data was tabulated, then tested according to analysis of diversity to see the effect of treatment. The level of difference for each treatment ration was tested according to Duncan's Multiple Range Test (Steel and Torrie, 1991). TAG and Cholesterol Test using the CHOD-PAP Method. The independent variable is feeding/ration of manure flour as a result of degradation (MHD) of *H. illucens* L. larvae, while the dependent variable observed in this study is the content of triglycerides and cholesterol (VLDL, LDL, and HDL) in mg/dL in chicken blood serum.

CHOD-PAP Method (Enzymatid Calorimetric Method/NS Method), with using Kit/Spectrophotometry Analysis (Biotec England, 2011), as the following:

Triglycerides (TAG)

Blank tubes containing 10 µl of distilled water and 1,000 µl of kit reagent were prepared, standard tubes containing 10 µl of standard triglycerides and 1,000 µl of kit reagents, sample tubes containing 10 µl of serum and 1,000 µl of kit reagents. The mixture was then homogenized, incubated at a temperature of 20-25ºC for 10 minutes. The absorbance is read at the Hg 546 nm wavelength within one hour.

Total cholesterol

Blank tubes containing 10 µl of distilled water and 1,000 µl of kit reagent were prepared, standard tubes containing 10 µl of standard cholesterol and 1,000 µl of kit reagents, and the sample containing 10 µl of serum reagent kit and 1,000 µl of kit reagent. The mixture was then homogenized, incubated at a temperature of 20-25ºC for 10 minutes. The absorbance is read at the Hg 546 nm wavelength within one hour.

HDL (High Density Lipoprotein)

A total of 500 µl of serum was added with 1,000 µl of precipitation, mixed until it was homogeneous, then left to stand for 10 minutes at room temperature. Centrifugate for 10 minutes with 3,500 revolutions per minute. The supernatant was prepared from the precipitate within two hours after centrifugate. A total of 100 µl of supernatant plus 100 µl of CHOD-PAP reagent were mixed, incubated for 10 minutes at 20-25ºC. The absorbance was read within one hour at a wavelength of Hg 546 nm.

LDL (Low Density Lipoprotein) and **VLDL** (Verry Low Density Lipoprotein)

A total of 100 µl of serum was added with 1,000 µl of precipitation, mixed until it was homogeneous, then left to stand for 10 minutes at a temperature of 15-25ºC. Centrifugate for 15 minutes with 3,500 turns per minute. The supernatant was prepared from the precipitate within two hours after centrifugate. A total of 50 µl of supernatant plus 100 µl of reagent kit were mixed, incubated for 10 minutes at 20-25ºC.

RESULTS AND DISCUSSIONS

The response of laying free hens after being given manure flour from degradation results (MHD) of *H. illucens* L. larvae for 6 months, showed different variations in the lipid profile (Triglyceride and Cholesterol content) between treatments. The lipid profile in this study, namely cholesterol (Triglycerides/TAG, LDL, VLDL, HDL and total cholesterol) VLDL, LDL and HDL) was obtained through spectrophotometric analysis of blood serum in laying hens. The results showed that blood triglyceride levels in this study ranged from 49.20-74.86 mg/dL. The highest triglyceride content was in RA (control ration without MHD on the ration) (74.86 mg/dL; while the lowest triglyceride content was found in RC (10% MHD and 5% fish meal on the ration) (49.20 mg/dl) The average triglyceride levels in this study are in line with Tohala (2010) who reported that blood triglyceride levels in broiler chickens ranged from 50.17 ± 1.4 to 52.83 ± 2.44 mg/dL. consumption of feed, especially carbohydrates such as sugar, saturated fat, high levels of free fatty acids, high insulin levels, and low levels of glucagon carbohydrates in the

liver are broken down into fatty acids and converted back into triglycerides. Manure results of degradation (MHD) with *H. illucens* L. larvae in this study were assumed to have high enzyme activity (amylase, lipase, and protease), especially lipase. Early studies (Manangkot, 2014) revealed that on the 7th day of manure after being broken down *by H. Illucens* L. and the 8th day of larval growth in manure, gave the highest activity of amylase, protease, and lipase enzymes.

Blood LDL content in this study ranged from 47.58-82.26 ml/dL. RA (control ration without MHD administration) was the highest blood LDL content around 81.06 ml / dL; whereas RC (10% MHD in the ration) gave the lowest blood LDL level of around 47.58 ml / dL. Statistical analysis showed that the treatment provided a significant difference (P <0.05) to the LDL level of laying native chicken blood in this study. It appears that MHD has the ability to reduce the LDL content of laying hens' blood in this study.

The results of the VLDL experiment showed that the manure treatment resulting from degradation (MHD) of H. illucens larvae had no effect on the VLDL content of chicken blood serum (P> 0.05) RA=9.30, RB=9.24, RC=9.20, RD=9.29 like the TAG content, the RC treatment also showed the smallest average VLDL content compared to all treatments, namely 9.20 mg/dL. RA treatment showed the largest average VLDL, namely 9.30 mg/dL. Very Low density lipoprotein (VLDL) is synthesized in the liver and is rich in endogenous triglycerides. In the blood will be degraded into LDL. The main function is as a carrier of triglycerides that are carried from the liver to other tissues in the body, especially to stored adipose tissue. VLDL contains high concentrations of triglycerides and moderate concentrations of cholesterol and phospholipids (Mokosuli, 2012). The TAG content is related to the VLDL content because the largest charge in this lipoprotein is TAG. The average HDL content in this study was 77.00-87.10 mg/dL. RA (control ration without MHD administration) had a lower blood HDL content (P<0.05) (77.00 mg/dL compared to RB (83.15 mg/dL), RC (87.10 mg/dL), and RD (79.05 mg/dl).

The blood HDL level of the RB (5% MHD) was significantly lower (P<0.05) than the RC (10% MHD in the ration). The blood HDL level of RD (15% MHD in the ration) was significantly lower (P <0.05) than RB and RC while the blood HDL level of RC (10% MHD in the ration) was significantly higher (P<0.05) compared to other treatments. Hariyanto (2017), also reported that blood HDL levels in broilers ranged from 54.20 ± 20.9 to 84.78 ± 23.6 mg/dL. RA gave significantly higher results (P<0.05) compared to the RB treatment. RC, and RD.RB provided significantly higher blood triglyceride content (P <0.05) than RC.

Total blood cholesterol in this study ranged from 128.05 to 163.05 ml/dL. The highest total cholesterol in RA (163.05 mg/dL) of RB, RC, RD because RA is a control diet without MHD and RC gives the lowest total blood cholesterol level (12.05 mg/dL = MHD 10%). The average total blood cholesterol found in this study is in accordance with the findings of Iriyanti et al., (2014) who reported that blood cholesterol levels in native chickens ranged from 123.04 ± 7.07 to 170.27 ± 9.68 ml/dL. Statistical analysis showed that the treatment gave a significant difference (P<0.05) to total blood cholesterol. RA (control ration without giving MHD on the ration) had higher total blood cholesterol (P <0.05) compared to RB, RC, and RD treatments. RB (5% MHD in the ration) also gave higher total blood cholesterol (P <0.05) compared to RC and RD treatments. As mentioned above, the MHD used in this study had high enzyme activity. So that the higher the MHD level in the ration larva growth in manure gave the highest amylase, protease, and lipase enzymes activity. The production of these bile salts requires cholesterol. So, when cholesterol levels in the liver are low, high density lipoprotein (HDL) will mobilize cholesterol from body tissues to meet their needs for cholesterol (Mayes, 1997). The results of the blood lipid indices of the experimental birds are presented in Table 2 and Figure 1.

Table 2. Average content of the blood fat profile of laying hens given manure flour degraded (MHD) larvae of *Hermetia illucens* L.

Treat-ments	Blood Lipid Profiles (mg/dL)			
	Triglycer id (TAG)	LDL	HDL	Tot Cholesterol
RA	74.86	82.26	77.00	163.05
RB	57.87	63.40	83.10	134.00
RC	49.20	47.58	87.10	128.05
RD	50.20	54.00	79.05	130.07

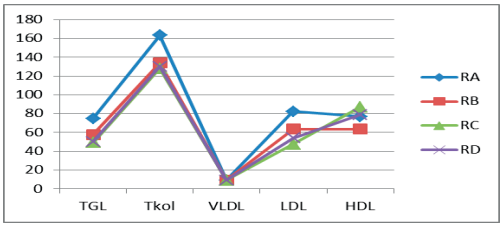

Figure 1. Comparison of Triglyceride Content, Total Cholesterol, VLDL, LDL and HDL between treatment of MHD rations of *H. illucens* L. larvae in laying native chickens

CONCLUSIONS

Analysis of triglycerides and carcass cholesterol of laying hens on replacement of fish meal with Degradation Product Manure (MHD) of *Hermetia illucens* L. larvae can replace fish meal up to 15% in the ration of laying free hens and degraded manure (MHD) of *H. illucens* L. larvae. significant effect on triglycerides, LDL, HDL and total cholesterol but not significant effect on blood serum VLDL of laying hens. MHD rations resulting from degradation of *H. illucens* L. larvae can maintain the triglyceride and total cholesterol content of laying hens in the normal range (MHD RC = 10% treatment), capable of producing low-cholesterol carcasses.

REFERENCES

Anggorodi (1985). *Ultimate Ability in Poultry Feed Science*. University of Indonesia Press. Jakarta.

Barbagan-Fonseca, K.B., Dicke, M., & van Loon, J.J.A. (2017). Nutritional value of the black soldier fly *(Hermetia illucens L.)* and suitability as animal feed-a review. *J. Incects as Food and Feed*, 3 (2), 105–120.

Biotec England (2011). *Metode CHOD-PAP* (Metode Enzimatid Kalorimetrik/Metode NS).

Haryanto, A., Purwaningrum, M., Adityas, M., & Wijayanti, N. (2017). Effect of chicken feather meal on the feed conversion ratio and blood lipid profiles broiler chickens. *Asian Journal of Poultry Science*, 11, 64–69.

Hassan, A.A., Sani, I., Maiangwa, M.W., & Rahman, S.A. (2014). The effect of replacing graded levels of fishmeal with grasshopper meal in broiler starter diet. *PAT*, 5(1), 30–38.

Iriyanti, N., Santosa, R.S.S., & Rachmawati, W.S. (2014). Blood profile and performance of native chicken with functional feed. *International Journal of Poultry Science*, 13 (11), 645–651.

Kim, W., Bae, S., Park, K., & al. (2011) Biochemical Characterization of Digestive Enzymes in the Black Soldier Fly, *Hermetia illucens* (Diptera: Stratiomyidae). *J. Asia Pac. Entomology*, 14 (1), 11–14.

Manangkot, H.J., Rondonuwu, LS.J., Pinontoan, O.R., & al. (2014). Black soldier fly larvae manure degradation as fish meal replacer in native chicken ration. *Lucrări Ştiinţifice - Seria Zootehnie,* 62 (2014), 139–142.

Mayes, P.A, Daryl, K.G., Rodwelt V.W., & Martin, W.D. (1997). *Harper's Biochemistry*, Issue 20. Indonesian Translation, EGC, Jakarta.

Mokosuli, Y.S. (2012). *Digest of Biochemistry*. LP2AI, State University of Manado.

Mupeta, B., Coker, R., & Zaranyika, E. (2003). The added value of sunflower performance of indigenous chickens fed a reduce-fibre sunflower cake diet in pens and on free-range. *www.dfid.gov.uk/r4d/PDF/outputs/R7524e.pdf Juli 2014.*

Nyakeri, E.M., Ogola, H.J., Ayieko, M.A., & Amimo, F.A. (2016). An open system for farming black soldier fly larva as a source of protein for smallscale poultry and fish production. *J. Insects as Food and Feed*, 3 (1), 51–56.

Rotinsulu, M.D., & Manangkot, H.J (2020). Exploration of Degraded Manure Flour (MHD) of Black Fly Larvae (*Hermetia illucens* L.) Against Carcass of Laying Buras Chicken by Soaking Sweet Orange (*Citrus sinensis). Lucrări Ştiinţifice – Seria Zootehnie*, 62 (2014), 139-142.

Setioko, A.R., & Iskandar, S. (2005). Review of Research Results and Technology Support in Local Chicken Development. *Proceedings of the National Workshop on Local Chicken Development Technology Innovation. Semarang, Center for Animal Husbandry Research and Development*, Bogor, Thing, 10–19.

Steel, R.G.D., & Torrie, J.H. (1991). *Principles and Procedures of Statistics*, 2nd Ed. Mc Graw-Hill Book Co.Inc. Pub. Ltd. London.

Teguia, A, & Beynen, A.C. (2014). Alternative feedstuffs for broilers in Cameroon. *Livestock Research for Rural Development*, 17 (3), Retrieved 21 July 2012, from http://lrrd.cipav.org.co/lrrd17/3/tegu17034.htm Juli 2014

Tohala, S.H. (2010). The relationship between blood lipid profile and performance of broiler fed two type of finisher diet. *Iraqi J. Vet. Sci*., 24(2), 87–91.

Wang, Y.S., & Shelomi, M. (2017). Review of Black Soldier Fly *(Hermetia illucens* L.*)* as animal feed and human food. *Foods,* 6 (10), 91, 1–23.