SUBSTITUSI TEPUNG IKAN DENGAN TEPUNG CACING TANAH (*Lumbricus rubellus*) PADA PAKAN TERHADAP KANDUNGAN ASAM LEMAK TIDAK JENUH, TRIGLISERIDA, LOW-DENSITY LIPO-PROTEIN DAN HIGH-DENSITY LIPOPROTEIN DAGING IKAN NILA (*Oreochromis niloticus*)

Substitution of Fish Meal with Earthworm Meal (*Lumbricus rubellus*) on Feed Toward Unsaturated Fatty Acids, Triglyceride, Low-Density Lipoprotein and High-Density Lipoprotein Content on Nile Tilapia's (*Oreochromis niloticus*) Meat

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Abstrak

Tepung cacing tanah dapat digunakan untuk substitusi tepung ikan karena tepung cacing tanah memiliki protein lebih tinggi dari tepung ikan. Tujuan dari penelitian ini adalah untuk menentukan pengaruh substitusi tepung ikan dengan tepung cacing tanah untuk meningkatkan kandungan asam lemak tak jenuh dan HDL dan menurunkan trigliserida dan LDL pada daging ikan nila. Penelitian ini dirancang menggunakan rancangan acak lengkap dengan 4 perlakuan dan 5 ulangan. Dosis yang diberikan pada setiap perlakuan adalah 0%, 30% 35%, 40%, 45%. Perlakuan diberikan selama 45 hari, dengan pemberian pakan 5% dari berat biomassa dengan frekuensi 3 kali sehari. Hasil penelitian ini mampu meningkatkan kandungan asam oleat, trigliserida dan HDL dan mengurangi LDL (P <0,05). Substitusi tepung cacing tanah tidak berpengaruh terhadap kandungan omega 3 dan omega 6 pada daging ikan nila (P> 0,05).

Kata kunci: Tepung cacing tanah, Asam lemak, HDL, LDL, Trigliserida

Abstract

Earthworm meal can be used to replaced fish meal because earthworm meal has higher protein than fish meal. The purpose of this study was to determine the effect of substitution of fish meal with earthworm meal to increase the content of unsaturated fatty acids and HDL and reduce triglycerides and LDL on tilapia's meat. This study was designed using a completely randomized design with 4 treatments and 5 replications. The dose given at each treatment is 0%, 30% 35%, 40%, 45%. Treatments were conducted for 45 days, by feeding 5% of the weight of biomass with a frequency of 3 times a day. The results of this study were able to increase the content of oleic acid, triglycerides and HDL and reduce LDL (P <0.05). Substitution of earthworm meal has no effect on the content of omega 3 and omega 6 in tilapia meat (P> 0.05).

Keywords **:** Earthworm Meal, Fatty Acids, HDL, LDL, Triglycerides

INTRODUCTION

Tilapia production from 2010 - 2014 increased significantly with an average increase of 19.03%. In 2014, tilapia production reached 82.96% (Directorate General of Fisheries Aquaculture, 2015). Increased aquaculture production also causes increased demand for feed. Generally, the feed quality can be measured from the higher protein content. The higher the protein contained in the feed, the more expensive the price of feed (Agustin et al. 2014). Protein is the main contributor to feed derived from fish meal.

Fish meal is a limited and expensive raw material. The availability of fish meal depends on imported component increasing the price of fish pellets, thus the

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production and marketing cost are also getting higher (Sullivan, 2008). Fish meal has good quality because it contains essential amino acids and non-essential amino acids that are good for fish growth (Sitompul, 2004).

Fish meal contains protein, fat, and crude fiber carbohydrates. Fish meal has a protein content of 31.55% 10.99% fat, crude fiber 6.32%, and carbohydrate 19.08% (Widaksi, 2014). Fish meal price is quite high, so the fish feed price is also high. Therefore, fish meal with appropriate nutrition is challenging to obtain. Consequently, a lot of fish meal coming from fish processing waste such as fish bones and thorns makes the fish meal nutrient content unsuitable. Therefore, we need protein raw materials that can replace fish meal.

Earthworms are the potential animal as the alternative feed raw material with high protein content, relatively equal to the protein content of fish meal. According to Haryati et al. (2011), the amino acid composition of earthworm meal is higher than fish meal. According to Fadaee (2012), earthworms contain 65.24% protein, 11% fat, 6% ash, 0.19% fiber. According to Pucher et al. (2012) earthworms can be used as an alternative feed for fish meal. Istiqomah et al. (2009) which stated earthworm meal could be the main protein for fish feed rations and become a substitute for fish meal which is increasingly difficult to find. In addition to protein, according to Resnawati (2003), earthworms contain fatty acids in the form of linolenic 1.64 - 2.07%, linoleic 2.34 - 2.88% and oleic 6.25 - 7.26%.

Besides being in the form of free (free fatty acids / FFA), fatty acids are also found to bind to glycerol. Acyl glycerol is an ester of fatty acids, trihydric alcohol, and glycerol which is usually called glyceride. Glyceride naming depends on the number of fatty acids if one fatty acid is called monoglyceride, two are called diglycerides, and three are called triglycerides (Holme et al. 1994). Accord-ing to Sartika (2008) triglycerides function as energy sources.

Fatty acids have an essential role in metabolic activities, membrane components, initial compounds of prostaglandin, thromboxane, prostacyclin, and leucotrin (Suwirya et al. 2002). Acid is divided into saturated and unsaturated fatty acids. Saturated fatty acids have a higher melting point than unsaturated fatty acids and are the basis for determining the physical properties of fats and oils. Unsaturated fatty acids are divided into two mono-unsaturated fatty acids which have one double bond (MUFA / Mono Unsaturated Fatty Acid) and monounsaturated fatty acids which have two or more double bonds (PUFA / Poly Unsaturated Fatty Acid) (Murray et al. 2009).

An example of palmitoleic acid MUFA derived from almost all fats, oleic acid which is often found in natural fats, elaidic acid is found in hydrogenated fats and ruminants. Erucic acid is found in rapeseed and mustard seed oil, nervonic acid found in cerebrosides. (Murray et al. 1996). The function of oleic acid accord-ing to Al Saghir et al. (2004) is lowering cholesterol levels, and solvent media for vitamins A, D, E, K.

PUFA is an unsaturated fatty acid which has two or more carbon bonds of C carbon at its chemical structure. PUFA consists of omega-3 (Alpha Linoleate, Eicosapentaenoate, and Docosahexa-enoate) and omega-6 (arachidonic and linoleic) (Wardlaw et al., 2004). Omega-3 plays a role in the development and growth of the brain, the formation of blood vessel and heart cells in the fetus and adults also function to nourish the blood and its veins and help the blood circulation mechanism (Titiek, 2007). Omega-6 performs as an anti-inflammatory besides that Omega-6 also functions against heart disease and depression and increases mass muscle growth (Diana, 2012).

Lipoprotein is a bond between protein and fat (cholesterol, triglycerides, and phospholipids). Lipoproteins divided ac-

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cording to their density consist of Very Low-Density Lipoprotein (VLDL), Intermediate Density Lipoprotein (IDL), Low-density Lipoprotein (LDL), and High - Density Lipoprotein (HDL) (Adam, 2009). Low-Density Lipoprotein (LDL) functions to carry cholesterol from the liver to the tissues while High-Density Lipoprotein (HDL) plays an important role in the destruction of triglycerides and cholesterol and for the transport and metabolism of cholesterol esters in plasma cells (Murray et al. 2009).

Based on the explanation above, the researchers are interested in conducting research on unsaturated fatty acids, triglycerides, and lipoproteins in tilapia (*Oreochromis niloticus*) which have been fed with earthworm meal substitution.

MATERIALS AND METHODS

Implementation

This research was conducted in January - July 2018 at the Education Laboratory, Faculty of Fisheries and Maritime, Universtas Airlangga, Surabaya. Whereas, the proximate test was conducted at the Animal Feed Laboratory, Faculty of Veterinary Medicine, Universtas Airlangga, Surabaya. Analysis of fatty acid content was carried out in the Testing Service Unit, Faculty of Pharmacy, Universtas Airlangga, and analysis of the content of triglycerides, HDL and LDL were carried out in the Laboratory of Physiology, Faculty of Medicine, Brawijaya University. Fish culture was carried out for 45 days. Measurement of fatty acid content, triglycerides, and lipoproteins was carried out at the end of maintenance by surgery and taking meat from test fish.

Test Fish

The test fish used were black tilapia fish (*Oreochromis niloticus*) with a size of 5-7 cm and weight of 1-3 g as many as 200 fish (10 fish per aquarium). Fish was obtained from the Umbulan Freshwater Aquaculture Technical Service Unit, Pasuruan, East Java. The earthworms used were obtained from Kepanjen Earthworm Farmers, Malang with 30 days of earthworm age

Feed ingredients

Feed ingredients used are fish meal, earthworm meal, corn meal, soybean meal, bran, vitamins, minerals, fish oil, and CMC.

Table 1. Nutrient Composition on dried ingredients (%) of tested diets

Ingredient	Raw Diet	Raw Protein	Raw Fat	Ash	Raw Fiber	NFED	ME
Fish meal	90.4156	36.5275	6.2823	25.5256	14.6046	7.4756	1979.6262
Earthworm Meal	92.7130	48.0587	18.6836	5.8823	5.0595	15.0289	3511.4553
Soybean meal	88.1071	44.0039	12.9401	6.6306	3.1098	21.4227	3193.4688
Corn meal	91.7156	9.6755	4.5771	1.4016	4.6321	71.4293	3290.4320
Bran	89.4807	12.5370	11.1344	7.3022	9.2592	49.2479	3052.8996

Notes:
NFED : Nitrogen Free Extracted Diet
EM : Energy Metabolism

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Table 2. Diet composition Nile Tilapia (*Oreochromis niloticus*) among the treatments

Diet	Treatments				
	P0 Fish meal 100% + Earthworms meal 0%	P1 Fish meal 70% + Earthworms meal 30%	P2 Fish meal 65% + Earthworms meal 35%	P3 Fish meal 60% + Earthworms meal 40%	P4 Fish meal 55% + Earthworms meal 45%
Fish Meal	40	28	26	24	22
Earthworm meal	0	12	14	16	18
Soybean meal	28,8	24,6	23,9	23,5	22,5
Corn	12,7	16,9	17,6	18,5	19
Bran	15	15	15	15	15
Fish oil	0,5	0,5	0,5	0,5	0,5
Mineral	1,5	1,5	1,5	1,5	1,5
Multivitamin	0,5	0,5	0,5	0,5	0,5
CMC	1	1	1	1	1
Total	100	100	100	100	100

Table 3. Nutrient component on on dried ingredients (%) of tested diets

Pellet	Ash	RP	RF	RFb	NFED	EM	Carbohydrate
P0	17,327	33,369	9,893	9,706	29,704	2940,123	39,410
P1	14,406	33,225	11,124	8,462	32,782	3134,158	41,243
P2	13,921	33,189	11,324	8,256	33,311	3166,317	41,567
P3	13,435	33,152	11,525	8,050	33,838	3198,436	41,888
P4	12,956	33,155	11,735	7,844	34,311	3230,501	42,155

Notes: Dried Substances (DS), Raw protein (RP), Raw fat (RF), Raw Fiber (RFb), Nitrogen Free Extracted Diet (NFED), Energy Metabolism (EM)

Dosage for substitution of fish meal with earthworm meal were 100% fish meal with 0% earthworm meal (P0); 70% fish meal with 30% earthworm meal (P1); 65% fish meal with 35% earthworm meal (P2); 60% fish meal with 40% earthworm meal (P3); and 55% fish meal with 45% earthworm meal (P4). The dosage was determined based on a research conducted by Asiah et al. (2015) which stated that the optimum dose of earthworm meal to support growth is 40%.

Tools

The tools used include: 20 aquaria sized 48x28x30 cm^3, aerator, aeration hose, aeration stone, pH meter, thermos-meter, gas mass spectrometry (gas chro-matography-mass spectrometry) (GCMS QP2010, Shimadzu Japan).

Enzychrome Triglycerides Assay Kit (ETGA-200, USA), DO meters (AZ-8403, Germany), LDL assay kits (E2HL-100, USA), HDL assay kits (E2HL-100, USA), digital scales, rulers, hammer mill machine, feed processing machine, tray, measuring cup, plastic bucket, scoop net, siphon hose, label paper, plastic, and stationery.

Earthworm Meal Production

The earthworm meal was processed into pellets using 25 kilograms of earth-worm that was cleaned, killed, dried, and shaped into pellets. Boiling worms in water did the process of executing the earthworms until the earth-worms died. The drying pro-cess was carried out twice, after the killing and during the pellets making process (Fahmi, 2010).

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Measurement of Fatty Acid Content

The fatty acid content was measured using GCMS. 1 gram of sample was weighed, then put in a bottle container. Then, 6 ml of petroleum ether was added then vortex for 3 minutes. It was set for 24 hours after it was densified for 15 minutes, then filtered and trans-ferred to the filtrate into a derivatization tube. Dried with a nitrogen spray; add 1ml of 2% NaOH in methanol, then close tightly and heated to 90 ^{o}C for 5 minutes. After cooled, add 1 ml BF3 in methanol, seal tightly, and reheat 30 minutes. Add 1 ml of hexane then vortex for 1 minute; let it cool until the two layers are separated, take the upper layer to be analyzed by GC.

Measurement of Triglyceride Content

Measurement of triglyceride content was carried out using ETGA-200 as the following: dilute the sample, take 10µl sample and add with 100 µl assay buffer, 2 µl enzyme mix, 5 µl lipase, 1 µl ATP and 1 µl dye reagent in a clean tube. Then transfer 100 µl of the reagent to put it in standard; incubate for 30 minutes, then read the optical density at 570 nm (550-585 nm).

Measurement of LDL Content

Measurement of LDL and HDL content was carried out using LDL and HDL brand assay kits (E2HL-100), measures of Low-density Lipoprotein content were carried out with LDL Assay kit testing method. The method of testing was by taking 100 µl of prepared solution and placing it in a centrifuge tube, then adding 1 ml of LDL assay kit (R1), then retrieve the supernatant, put 20 µl into a test tube and add 1 ml of assay kit LDL Cholesterol (R2). The solution was shaken until homogeneous and allowed to stand for 10 minutes, then absorbance was measured at λ 540 nm for the standard series.

A regression equation was used to determine the LDL cholesterol level of the sample, the LDL cholesterol level of the sample was determined with the help of the equation standard regression.

Measurement of HDL Content

Measurement of High-Density Lipoprotein content was carried out using the HDL Assay kit testing method. 100 µl of prepared solution was taken and put into a centrifuge tube, then add 1 ml of HDL assay kit (R1) solution, then take the supernatant, and put it in a test tube as much as 20 µl and add 1 ml of assay kit HDL Cholesterol (R2). The solution is then shaken until it is homogeneous and allowed to stand for 10 minutes, after that it measures the absorb-ance at λ 540 nm, the process is carried out on standard HDL cholesterol with a commitment of 0; 10; 20; 40; 80; 160 (mg / 100 ml), for the standard series determined regression equations used for the determination of sample HDL cholesterol levels. The sample HDL cholesterol levels were assessed with the help of a standard regression equation.

Water Quality Measurement

Water quality measurement was carried out in the form of temperature, DO, pH and ammonia measurements. Temperature measurements were carried out using a thermometer, the oxygen content was measured using DO meter. Ammonia was measured using the ammonia test kit and pH was measured using pH paper. Measurement of water quality temperature and pH was carried out during each day in the morning and evening, while ammonia and DO are carried out once a week in the morning and evening. This method followed the recommendations of SNI in 2009.

Experimental Design

This study used an experimental design in the form of 5 treatments with four replications. Data were obtained by using Analysis of Variance (ANOVA) to determine the effect of the treatment given. The treatment of substitution of earthworm meal showed significant results then continued with Duncan's multiple range test (Kusriningrum, 2012).

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RESULTS AND DISCUSSION

Omega-3, Omega-6, and Oleic Acid

Table 4. The average content of Omega-3, Omega-6, and Oleic Acid

Treatments	Average contents of omega-3 (%)±sd	Average contents of omega-6 (%)±sd	Average contents of oleic acid (%) ±sd
P0	3,63[a] ±1.93	11.94[a] ±6.73	28,463[a] ±3,045
P1	4,48[a]±1.60	9.20[a] ±6.99	30,945[a] ±8,884
P2	4,25[a] ±0.66	11.01[a] ±9.18	30,155[a] ±6,660
P3	2,84[a] ±1.85	2.50[a] ±0.95	40,908[b] ±4,560
P4	4,80[a] ±1.42	8.78[a] ±10.65	35,905[ab]±2,397

Notes: different a, b superscript in similar column showed the significant difference ($p<0.05$)

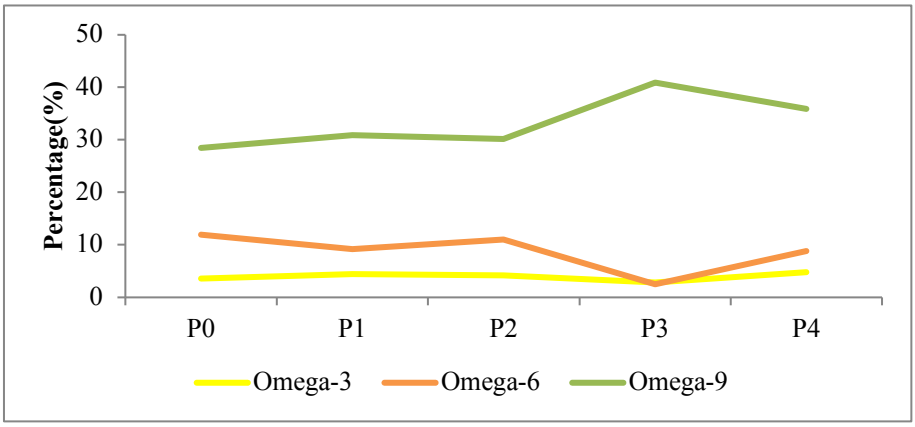

Figure 1. The graph of average contents of Omega-3, Omega-6, and Oleic Acid

The results of omega-3 (Alfa Linolenic Acid (ALA), Eicosapentaenoic Acid (EPA) and Docosahexaenoic Acid (DHA) content in tilapia (*Oreochromis niloticus*) in the calculation of Analysis of Variants (ANOVA) showed that substitution of fish meal with earthworm meal was not significantly different ($P> 0.05$). The content of omega-6 in tilapia ranged from 2.84% - 4.80%.

Huang et al. (2006) stated that the fatty acids contained in tilapia came from fatty acids consumed by the fish. The value of omega-3 (Alfa Linolenic Acid (ALA), Eicosapentaenoic Acid (EPA) and Docosahexaenoic Acid (DHA) in feed is 0.10% so that the omega-3 content is limited and only obtained from the feed given. Omega-3 content in the treatment P3 decreased by 2.84% but increased in P4 treatment by 4.80%.

Fatty acids consumed by fish caused decreasing omega-3 content in P3 treatment that will affects the formation of brain nerves, eye organs, blood vessels, and other body organs. The composition of omega-3 tilapia depends on feed, species, age, season and habitat level (Cedoloh et all, 2011).

Omega-3 is called essential fatty acid because it cannot be produced by the body and can only be obtained from food consumed daily (Rasyid, 2003). Omega-3 plays a role in the development and growth of the brain, the formation of blood vessel and heart cells in the fetus and adults. Besides, it also functions to nourish the blood and veins as well also help the blood circulation mechanism (Titiek, 2007). In addition, omega-3 unsaturated fatty acids play an essential role in morphological, biochemical and molecular development of the brain and other organs. Omega-3 deficiency can inhibit brain development, physical health (Diana, 2012).

Consuming Omega-3 as much as 4g/day can reduce plasma triglyceride con-

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tent by decreasing hepatic production and secretion of very low-density lipoprotein (VLDL). Also, omega-3 lowers triglycerides in the liver by increasing the work of lipoprotein lipase (Fitranti et al. 2017). The balance of consumption between omega-3 and omega-6 must be considered so that there is no competition for metabolism between omega-3 and omega-6. The content of fatty acids in tilapia can be influenced by several factors, one of which is through the food consumed containing fatty acids.

Eicosapentaenoic Acid (EPA) is one of the components of Omega-3 which functions in helping the formation of blood cells and heart, stabilizing the circulatory system by promoting blood circulation. EPA is beneficial for the growth of brain cells, visual organs and bones, as well as maintaining cells blood vessels and heart. EPA is needed in helping the growth and development of brain nerve cells to be optimal. The lack of this substance will make nerve cells in the brain lack energy for the process of brain development so that it can interfere with work and brain function drastically. Not only for the brain, but also for visual organs and bones (Razak, 2014).

EPA content in tilapia is also influenced by several factors, such as the content of fatty acids in feed and the amount of food consumed. According to Agustono (2014), the fatty acid content that can be absorbed by the fish's body is influenced by various things such as the quality of feed and the amount of food consumed. If the food eaten by fish has a good fatty acid content and is consumed in large quantities, the fatty acid will be well-absorbed by the fish.

EPA fatty acids (Eicosapentaenoic acid) in tilapia are affected by fatty acid content in the food that has been consumed by tilapia. Huang et al. (2006) states that the fatty acids contained in tilapia are derived from fatty acids consumed by the fish. However, the lower EPA content which does not match with the fatty acid on a diet consumed by the fish will be directly used for metabolic processes such as the formation of the brain's nerves, the eye organs, blood vessels and utilized by other fish organs.

Docosahexaenoic Acid (DHA) in the fish's body functions as a nerve wrapping network that plays a role in launching nerve commands and delivering nerve stimulation to the brain. DHA is one of the derivatives of Omega-3 that can help in healthy blood vessels and heart. Omega 3 that has been consumed by the body can reduce triglyceride and LDL levels in the blood, thereby decreasing blood fat accumulation that is not good in the blood vessels that trigger atherosclerosis (Razak, 2003). DHA has a role in the formation of retinal and neural networks (Pangkey, 2011). EPA and DHA play an essential role in physiological function (Watanabe, 1993).

Fatty acids with DHA type (Docosahexaenoic acid) in tilapia meat are affected by fatty acid content in the food that has been consumed by tilapia. This followed the statement of Huang et al. (2006), which states that the fatty acids contained in tilapia come from fatty acids consumed by the fish. However, the lower DHA content which does not match with the fatty acid on the diet consumed by the fish will be directly used for metabolic processes such as the formation of the brain's nerves, the eye organs, blood vessels and utilized by other fish organs.

Freshwater fish usually require more than *n*-3 fatty acids than n-6 fatty acids or a mixture of n-6 and n-3 fatty acids, while marine fish need n-6 fatty acids. The need for omega-3 for freshwater fish ranges between 0.5% -1.5% (Craig, 2002). Takeuchi (1983) states that the need for fish for essential fatty acids is at least 0.5%. Essential fatty acids can affect metabolism in cells so that the right fatty acid composition will produce the proper cell metabolism (Dedi et al., 2017).

The results of the content of omega-6 (Linoleic Acid (LA), Arachidonic Acid (AA) in tilapia meat (*Oreochromis*

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niloticus) in the calculation of Analysis of Variants (ANOVA) showed that the substitution of fish meal with earthworm meal was not significantly different ($P > 0.05$). The content of omega-6 in tilapia meat ranges from 2.50% - 11.94%.

The content of omega-6 (Linoleic Acid (LA), Arachidonic Acid (AA) in tilapia meat has decreased in several treatments including P1, P3, and P4. The omega-6 content obtained from P3 treatment is 2.50% with 40%earthworm meal substitution, lipid levels and fatty acid composition can vary depending on species, sex, age, season, food availability, salinity and water temperature (Awalludin, 2011).

Omega-6 functions as an anti-inflammatory. Besides, Omega-6 functions against heart disease and depression as well as increases muscle mass growth. Omega-6 deficiency in humans can cause hair loss, skin disorders such as eczema, behavioral changes, decreased immunity even to infertility in men. Intake of unequal omega-3 and omega-6 can cause chronic degenerative such as increasing the risk of cancer, heart disease and obesity. Omega-6 imbalances can also result in inflammation, coronary heart disease, asthma, depression and arthritis (Diana, 2012). Excessive intake of omega-6 will disrupt the action of omega-3.

Omega-3 and omega-6 will compete to metabolize Δ6 desaturase enzymes, so it will inhibit omega-3's work in reducing triglycerides (Fitranti et al., 2017). Tilapia (*Oreochromis niloticus*) requires higher omega-3 essential fatty acids compared to omega-6 to be converted into long hydrocarbon chains with the help of the Δ6 desaturase enzyme.

The results of oleic acid by analysis of variance (ANOVA) showed that the substitution of fish meal with earthworm meal (*Lumbricus rubellus*) had a significant effect ($P <0.05$) so further DMRT (Duncan multiple range tests) was needed. P3 is not significantly different from P4 but substantially different from P0, P1, P2 and P0, P1, P2, P4, each does not differ between treatments. The results of oleic acid content ranged from 28.463 to 40.908%.

The increasing oleic acid in P3 occurred because the amount of oleic acid in earthworms is higher than fish meal. The content of oleic acid in earthworm meal was 13.05% while in fish meal was 11.51%. According to Vucinic and Katarina (2015), the oleic acid content of earthworm meal was 12,14-13,14%. Whereas in the P4 treatment oleic acid content occurs, this is also possible because the source of oleic acid from the feed is not only from earthworm meal but also from other feed ingredients.

According to Handayani and Joko (2005), the content of oleic acid is influenced by various things such as the quality of feed and the amount of food consumed. if the food eaten by fish con-tains a lot of oleic acid and is consumed in large quantities, the fish body will absorb it maximally. Except feed, the internal factor on the fish such as desaturase enzyme performance affects oleic acid content in fish meat.

Essential fatty acids consist of omega-3 and omega 6 which cannot be synthesized by the body so they must be obtained from food sources (Wardlaw and Hampl, 2007). The need for fatty acids is very dependent on the natural ability of the fish in deciphering essential fatty acids both anabolically and catabolically (Sargent et al., 2002).

Oleic acid is a group of MUFA (mono unsaturated fatty acids) fatty acids. This fatty acid has a structure of 18: 1 D9 with the molecular formula CH_3 (CH_2) 7C = C (CH_2) 7COOH, and is a class of omega-9 because it has a double bond at position nine from the end of the chain (Murray et al., 1999).

Oleic acid functions as a source of energy and anti-inflammatory substances, lowers cholesterol levels, improves glucose control and insulin sensitivity, and solvent media for vitamins A, D, E, K. Oleic acid deficiency can cause vision disturbances, and growth disorders (Calder, 2015).

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Triglyceride

Table 5. Average contents of triglyceride

Treatments	Average content of Triglyceride (mmol/L) ± SD
P0	0,898[a]±0,180
P1	1,041[a]±0,094
P2	1,076[a]±0,087
P3	1,162[a]±0,366
P4	1,982[b]±0,147

Notes: different a, b superscript in similar column showed the significant difference ($p<0.05$).

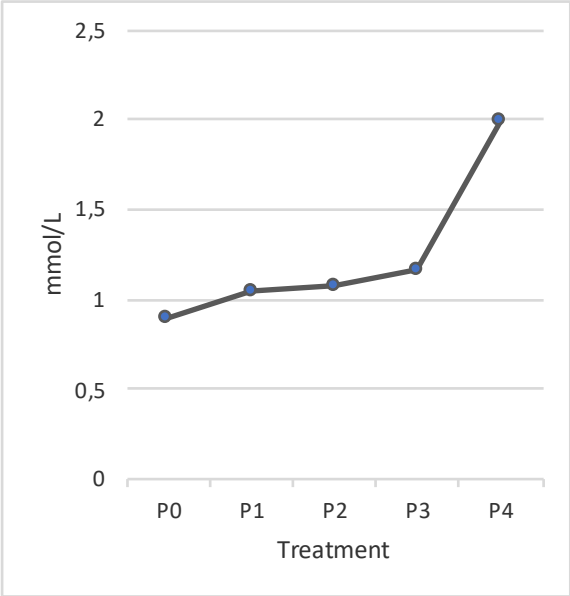

Figure 2. The graph of average contents of Triglycerides

Based on Table 5 triglyceride content in tilapia meat ranged from 0.898 to 1.982 mmol/L. The lowest triglyceride content in tilapia meat was found in P0 (control) at 0.898 with 0% earthworm meal (*Lumbricus rubellus*). On the other hand, the highest triglyceride content was 1.982 mmol/L found in treatment P4 with a 45% earthworm meal content (*Lumbricus rubellus*). Triglyceride content in P1 treatment was 1,041 mmol/L, P2 was 1,076 mmol/L and P3 was 1,162 mmol/L.

The amount of triglycerides in earthworm meal (*Lumbricus rubellus*) was 0.824 mmol/L, lower than the amount of fish meal triglycerides which amounted to 0.980 mmol/L. According to Iriyanti et al. (2005), triglyceride content in feed ingredients will reduce triglyceride content, but the triglyceride levels did not decrease. This is because the amount of carbohydrates from P4 feed is 42,567 greater than the amount of carbohydrate feed formulation.

The highest content of triglycerides in fish meat is at P4 at 1.982 mmol/L and the smallest at P0 is 0.898 mmol/L. This is because the feed formulation in P4 contai 55% fish meal, 45% worm meal, and 38.184% carbohydrate. Feed containing high carbohydrate will increase fructose 2,6 bisphosphate levels so that phosphorfructinase-1 becomes more active and stimulate the glycolysis reaction. The in-creased glycolysis will increase the glucose converted into fat. Then, this free fatty acid is combined with glycerol to form triglycerides (Tsalissavrina et al., 2006).

The distribution of high carbohydrate feed causes a faster increase in blood glucose levels; an increase in blood glucose levels commonly called hyperglycemia causes hyperinsulinemia. This condi-

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tion causes insulin resistance over time. Insulin resistance can cause a decrease in the activity of the enzyme lipoprotein lipase (LPL). The lower the LPL enzyme activity, the more triglyceride levels will be (Tsalissavrina et al, 2006).

Energy levels also affect the increase in triglyceride levels. The energy level in earthworm meal of 3511.4553 kcal/kg is greater than the fish meal which amounted to 1979.6262 kcal/kg. Energy levels in P4 feed amounted to 3230,501 is greater than other treatment feeds. According to Wulandari and Pramono (2014), if the energy in food is excessive, then the unused energy will be converted into triglycerides.

When Omega 3 fatty acids in the form of EPA and DHA are used at 3-4 grams/day, they can reduce triglyceride levels. Triglycerides go down with the mechanism of omega 3 inhibiting triglyceride synthesis by inhibiting the enzyme Diacil Glycerol Tranferase (DGAT) or the enzyme Phospatic Acid Phospohydrolase (PAP). PPARα (Peroxi-some poliferator-activated receptor α) stimulates β oxidation of fatty acids. Omega 3 decreases the availability of fatty acids for triglyceride synthesis (Purnomo, 2014).

Triglycerides are the result of esterification between glycerol and three fatty acids. Most lipids are difficult to dissolve in water. Thus, to be digested and absorbed, they must be emulsified first. Triglycerides in the diet are emulsified in the duodenum by the bile.

Digestion takes place by hydrolysis catalyzed by the enzyme lipoprotein lipase, which hydrolyzes triglycerides into a mixture of monoglycerides and fatty acids. Freed fatty acids and mono-glycerides diffusely enter the intestinal mucosa. Then, triglyceride resynthesis takes place in it. This process is followed by its release into lymph particles called chylomicrons which contain lipids and proteins, which then enter the venous zone and eventually are stored in tissues. Fatty acids that are shorter and more soluble in water are absorbed directly into the portal blood and inserted into the liver as fatty acids (Montgomer et al. 1993).

***Low-density Lipoprotein* (LDL) and *High-Density Lipoprotein* (HDL)**

Table 6. Average Contents of LDL and HDL

Treatments	Average Contents of LDL (mg/dL) ± SD	Average Contents of HDL (mg/dL) ± SD
P0	$70,2600^{a} \pm 12,401$	$54,7345^{a} \pm 5,608$
P1	$72,3167^{a} \pm 7,910$	$51,5482^{a} \pm 1,462$
P2	$66,1465^{a} \pm 4,302$	$135,0442^{c} \pm 7,645$
P3	$33,0970^{a} \pm 22,860$	$75,4770^{b} \pm 10,942$
P4	$172,6712^{b} \pm 68,800$	$138,4955^{c} \pm 22,995$

Notes: different a, b, c superscript in similar column showed the significant difference ($p<0.05$)

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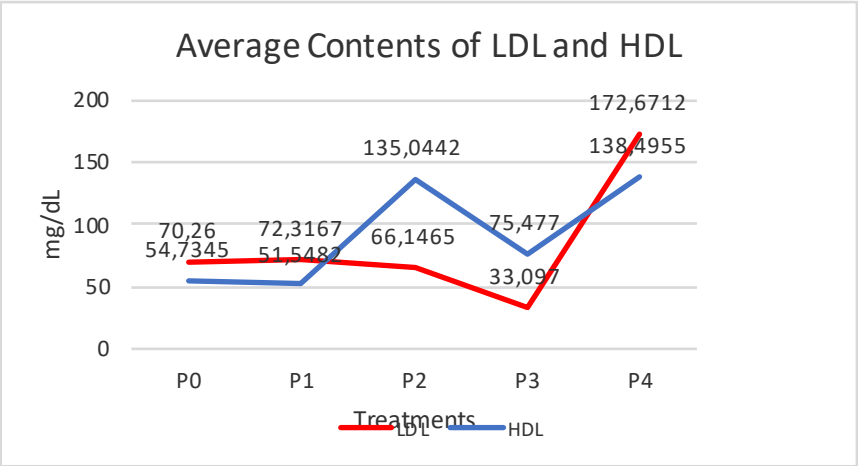

Figure 3. Graph of Average LDL and HDL content

The results in table 6 showed that P4 treatment is the highest content with 172.6712 mg/dL, while P3 treatment is the lowest with 33.0970 mg/dL. Increased LDL content is influenced by several factors, one of which is the consumption of trans fats (Sun et al. 2015). The fat consumed by tilapia from the formulation feed in P4 treatment is 11,735%, this content exceeds the maximum limit given to tilapia according to BBAT Sukabumi (Center of Freshwater Aquaculture Sukabumi) in 2005, which is 6-10%.

According to Sun et al., (2015) an increase in LDL content can be caused by excessive consumption of fat. Excess fat in feed will cause an increase in the amount of fat deposited in adipose tissue. Excess fat in adipose tissue will cause hepatocytes to change cholesterol secretion, lipoprotein composition, and catabolic reactions in apolipoprotein. Hepatocytes will work with apolipoprotein-E (apoE) to form VLDL in which the VLDL will change to LDL (Phillips, 2014).

Increased LDL content is also influenced by consumption of carbohydrates from excess formulation feed (Anand et al. 2015). Carbohydrates con-tained in P4 treatment feed were 42,154%. These carbohydrates exceed the maximum amount of carbohydrates intended for tilapia. The need for Tilapia carbohydrate, according to BBAT Sukabumi (Center of Freshwater Aquaculture Sukabumi) in 2005 was 25%. According to Sacks et al., (2014) high carbohydrate content will reduce insulin and will increase LDL content. Insulin will stimulate LDL receptors to capture LDL from fibroblasts so that LDL levels in tilapia meat will increase (Haas et al., 2013).

High protein content in feed can cause LDL to increase. This situation happens because one of the many types of proteins will bind LDL. Apoprotein B-100 will recognize and bind LDL receptors (Shelness and Ledford, 2005). Apoprotein B-100 (APO-B100) is an apoprotein or one of the protein groups in the main lipoprotein in low-density lipoprotein (LDL). Apoprotein B-100 (APO-B100) is also present in very low-density lipoprotein (VLDL) and chylomicrons (Setiawan et al. 2017).

The lowest LDL content was found in P3 treatment, which was 33.0970 mg / dL. LDL content is related to the ability of omega-3 fatty acids to inhibit the production of apolipoprotein-B. According to Scorletti and Byrne (2013) the inhibited production of apolipoprotein-B will also inhibit the production of VLDL to turn into LDL, so LDL content in tilapia decreases.

The results in table 3 showed that P1 treatment has the lowest content with 51.5482 mg / dL, while P4 treatment is the highest content with 138.4955 mg / dL. The low HDL content can be caused by high levels of LDL and triglycerides in the blood, thus making HDL high in triglyceride content. Increased levels of trigly-

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cerides will increase catabolism from HDL, so that cholesterol levels in the blood become higher (Tsalissavirna et al. 2006).

Decreased HDL content can also be caused by dysfunction of ApoA-I (Apoprotein A-I). Apoprotein A-I (ApoA-I) is the main protein that composes HDL. ApoA-I dysfunction occurs because ApoA-I binds to various proteins from feed that has been digested, resulting in disformation and dysfunction of HDL (Nayoung and Kijin, 2016). HDL dysfunction can result in the failure of HDL to transport LDL which is rich in tri-glycerides to the liver for the esterification process so that the LDL content which is rich in triglycerides becomes high.

The highest HDL content was found in the results of P4 treatment, which was 138.4955 mg / dL. Increased HDL content is closely related to omega-3. Omega-3 can provide a hypocholesterolemic effect by lowering LDL levels, so that HDL levels in the body increase (Yulianti et al. 2015). Hypocholesterolemia is an effect when omega-3 and decreasing activity of CETP so that HDL cholesterol levels increase (Chadli et al. 2013).

Cholesterol Esther Transfer Protein (CETP) is a plasma protein that mediates the exchange of cholesteryl ester from HDL with triglyceride molecules from LDL, VLDL, and chylomicrons, so VLDL is rich in cholesterol, while HDL becomes rich in triglycerides or triglyceride-rich lipoproteins (TGrL). Inhibition of CETP resulted in a decrease in LDL and an increase in HDL (Chadli et al. 2013).

Water Quality

Table 7. Water Quality

Parameter	Unit	Range
DO	mg/L	3,36-5,84
pH	-	7
Temperature	^{0}C	26-27
Ammonia	mg/L	0

A critical factor in culture is water quality. Oxygen is an important parameter in fish farming. Oxygen is needed by fish to metabolize in the body. Dissolved Oxygen (DO) in the water medium during the study ranged from 3.36 to 5.84 mg / L. BSN (2009) states that the dissolved oxygen needed by fish is at least 3 mg / L. Observation of water quality during the study found that the temperature ranged from 26-27 ^{o}C that was optimal for fish growth.

The pH value is an indicator of the acidity of water. Several factors that affect the pH of waters include photosynthesis activity, temperature, and the presence of anions and cations. During the cultivation of tilapia, the pH value is 7. The pH is ideal for the survival of tilapia. It is based on the standard made by BSN (2009) which states that the pH range of water for tilapia is 6.5 - 8.5.

Measurement of ammo-nia levels in this study showed that tilapia ranged between 0 mg / L. According to Ogbonna and Amajuoyo (2010), ammonia compounds that are harmful to aquatic organisms are equal to 0.6 mg / L. Thus, the quality of water in the research media as a whole has fulfilled the requirements for fish life and is safe for fish life.

CONCLUSION

Based on research on the substitution of fish meal with earthworm meal (*Lumbricus rubellus*), it can be concluded that it can increase the content of oleic acid, triglycerides and HDL and reduce LDL while the substitution of fish meal with earthworm meal (*Lumbricus rubellus*) does not affect on the content of omega 3 and omega 6 in tilapia meat (*Oreochromis niloticus*). The best dose substitution of fish meal with earthworm meal (*Lumbricus rubellus*) in fish feed toward the content of oleic acid and triglycerides on P3 treatment (40% earth-worm meal and 60% fish meal) because it increases the oleic acid, yet not increasing triglycerides. The best dose to increase HDL and reduce LDL is in P2 treatment feed (35% earthworm meal and 65% fish meal).

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