Journal of Biology, Agriculture and Healthcare
ISSN 2224-3208 (Paper) ISSN 2225-093X (Online)
Vol.6, No.22, 2016

Catfish (*Clarias gariepinus*) Oil Intervention and its Effect on Lipid Profile and MDA Levels of Hypercholesterolemic Male Sprague-Dawley Rats

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Abstract

Catfish oil,which is rich in essential fatty acids,can be utilized as an alternative supplement solution. The objective of this study was to analyze the effect of catfish oil intervention on the lipid profile and malondialdehyde (MDA) levels of hypercholesterolemic male Sprague-Dawley rats. Four treatments were applied in this study: negative control (A1), refined fish oil (A2), fish oil with omega-3 concentrates (A3), and fish oil with omega-3 concentrates and vitamin E (A4). The results showed that catfish oil tended to reduce cholesterol levels. Negative control group had the highest value of lipid profile (58.3±9.93 mg/dL) among other groups. All intervention groups had very low MDA values (A1: 0.616±0.071, A2: 0.835±0.223, A3: 0.832±0.160, A4: 0.702±0.113 nmol/L), indicating that their MDA values were still normal (<4nmol/L).

Keywords: Catfish oil, Hypercholesterolemia, Lipid profile, Malondialdehyde

1. Introduction

Hypercholesterolemia is a cholesterol metabolism disorder caused by high cholesterol levels in the blood (Stapleton *et al,* 2010). This condition is also the main risk factor for coronary heart disease (CHD) and atherosclerosis (Stapleton *et al.* 2010; Onyeike *et al.* 2012; Aronow 2013; Rantung *et al.* 2014). In addition, previous study showed that hypercholesterolemia indicated a free-radical accumulation in the body. It might stimulate lipid peroxidation and lead to oxidative stress, which can be determined by measuring one of the parameters i.e. malondialdehyde (MDA). One of the causes of hypercholesterolemia is the low intake of essential fatty acids in the body due to unhealthy dietary pattern, obesity, and low physical activity (Malik *et al.* 2013).

Essential fatty acids play an important role in human physiological processes. They have been proven to have anti-inflammatory and anti-atherosclerosis effects, besides their effects on immunity and intelligence. Some studies suggest that essential fatty acid deficiency in the body may affect brain function and mental health (Mulder *et al,* 2014). Food sources containing high amount of essential fatty acids are the ones of marine origin (seafood) laut (Basmal, 2010). Seafood is known to be rich in omega-3 fatty acids, which is beneficial in reducing triglyceride levels and blood pressure (Inga-Britt, 2004).

Besides fresh fish flesh, fish oil as the by product of fish-flour processing can also be an alternative source of essential fatty acids. Fish oil is known for its omega-3 and omega-6 contents, which are beneficial for health if consumed as a supplement (Minis *et al,* 2006). In line with this, earlier study also suggested that catfish oil contained a high amount of polyunsaturated fatty acids (PUFAs) and monounsaturated fatty acids (MUFAs) (Srimiati, 2011). Several researchers stated that low content of omega-3 in the body had a significant association with hyperactivity, depression, aggression, and other cardiovascular diseases (Hibbeln *et al,* 2006**)**.

Therefore, it is necessary to provide food or alternative products as a solution for the availability of essential fatty acids. One of the products that can be used is fish oil. It is a derivative product of fish produced from the extraction of fish flour or canned fish, which can be produced either from seawater or freshwater fish. It may provide health and nutritional benefits (Irianto and Soesilo, 2007). Previous study stated that the global production of fish oil reached 1-1.25 million tons per year (Pike and Jackson, 2010)**.**

Potency development of freshwater fish oil increases along with the discovery of numerous studies, which prove that the oil's content has a great potential and it is quite competitive with seawater fish oil. The freshwater fish commonly used in fish oil production is catfish (*Clarias gariepinus*). It is easily found in Indonesia and is one of freshwater fish farming commodities, which is favored by people. Catfish production reached 758,455 tonsin 2013 with a mean increase of 47.21% from 2010. It is predicted to keep increasing with a target production of 840,000 tons in 2014 (Direktorat Jenderal Perikanan Budidaya, 2013).

The use of catfish as a raw material has a great potential to be developed in the future. Catfish oil is known for its high content of essential fatty acids, i.e. 17.7% linoleic fatty acids (omega-6) and 1% linolenic fatty acids (omega-3). In line with this, earlier study suggested that fish oil had been proven to be beneficial for health due to its omega-3, EPA and DHA contents (Minis *et al*, 2006). Catfish oil, a freshwater fish oil, is predicted to be one of alternative supplements that competes with seawater fish oil, which has been widely known and utilized.

There has not been any scientific information so far regarding the effect of catfish oil consumption on the decreased blood cholesterol levels. Therefore, a research on that issue needs to be conducted to analyze the effect of catfish oil on lipid profile of people with hypercholesterolemia. The information is obtained by using experimental animals, i.e. male rats of Sprague-Dawley strain. This study aimed to analyze the effect of catfish oil intervention as an alternative source of essential fatty acids, and to observe its effect on lipid profile and malondialdehyde (MDA) levels of male Sprague-Dawley rats.

2. Material and Methods

2.1. Design, Location, and Time of Study

This study was part of the major study entitled "Catfish (*Clarias gariepinus*) as An Alternative Supplement for Alzheimer Prevention in The Elderly". It was an experimental study conducted on male Sprague-Dawley rats using randomized controlled trial (RCT) design. Catfish oil was obtained from PT. Carmelita Lestari, Bogor. Male rats aged 6-7 months were acquired from Faculty of Veterinary Medicine, Bogor Agricultural University. This study was conducted from March to September 2015.

Oil refining process was conducted in the Laboratory of Biochemistry, Department of Community Nutrition, Faculty of Human Ecology. Meanwhile, the nurturing of experimental animal and the intervention were carried out in the Management Unit of Experimental Animal (UPHL), Faculty of Veterinary Medicine. Lipid profile analysis was performed in Regional Health Laboratory, Bogor; while serum MDA was analyzed in the Laboratory of Biochemistry, Cipto Mangunkusumo Hospital, Jakarta.

2.2. Materials

The materials used in this study were refined catfish oil, NaOH, magnesol, omega-3 concentrate (K-Link Omega Squa), vitamin E (α-tocopherol), corn starch, fish flour, soybean meal, coconut oil, premix, NaCl, and $CaCO_3$.

2.3. Research Methods

This study was conducted in two phases. The first phase was the preparation of intervention materials (catfish oil) and experimental animals that were fed with high-cholesterol diet. Catfish oil refining process consisted of two phases, i.e. neutralization and bleaching. The bleaching was conducted in a temperature of 50^0C (Estiasih, 2009). Vitamin E was then added into the refined oil to protect it from oxidative damage during storage (Ngadiarti and Darmawan, 2014). Meanwhile, cholesterol levels of rats as the experimental animals were increased by feeding them with high-cholesterol diet. The diet formula was a mixture of corn starch, fish flour, soybean meal, egg yolks, coconut oil, premix, NaCl, and $CaCO_3$ (Hernawati *et al*, 2013; Astuti, 2015). High-cholesterol diet was given for two weeks (Gani *et al*, 2013).

The second phase was catfish oil intervention on male Sprague-Dawley rats, and the analyses of lipid profile and MDA levels that were conducted after the intervention period ended. The 2-week intervention was conducted by giving the catfish oil to the hypercholesterolemic Sprague-Dawley rats. Here is the order of interventions conducted in this study:

A1: Negative control;

A2: Catfish oil intervention;

A3: Intervention using catfish oil with concentrate addition (omega-3 oil);

A4: Intervention using catfish oil, concentrate (omega-3 oil), and vitamin E.

In this study, the experimental animals were weighed six times in each intervention phase. It was performed because body weight might affect the increase and decrease in lipid profile. It was stated in earlier study that there was an association between body weight, triglyceride concentrations and high-density lipoprotein cholesterol (HDL-c) concentrations (Hernawati *et al*, 2013) The first weighing was performed in acclimatization phase, before the high-cholesterol diet was given to the experimental animals. The second, third and fourth weighing were conducted after the high-cholesterol diet was given. The fifth and sixth weighing were performed

during the catfish oil intervention. Catfish oil was administered orally for two weeks, in liquid form using force-feeding method A 1-ml catfish oil was given in each force-feeding, and the analyses of rats' lipid profiles (total cholesterol, low-density lipoprotein cholesterol/LDL-c, HDL-c, and triglycerides) were performed on the next day (Gani *et al*, 2013; Mona, 2014; Matsushita *et al*, 2008).

Blood sampling for lipid profile analysis was performed by anesthetizing the rats using xylazine and ketamine until they became unconscious. They were then dissected, and 2 ml blood was taken from the heart by using a 3-ml disposable syringe. The blood in the syringe was stored in ice container until ready to be analyzed for blood lipid profile (total cholesterol, LDL-c, HDL-c, and triglycerides). Lipid profile and MDA levels were analyzed by measuring rats' serum with a spectrophotometer at a wavelength of 530 nm (Latifa, 2015).

2.4. Data Processing and Analyses

The data were processed by using Microsoft Excel and then analyzed by SPSS version 16.0 for Windows. The results were analyzed statistically by Analysis of Variance (ANOVA).

3. Results and Discussion

3.1. Subjects' Characteristics

Rats' body weights were measured to determine the effect of high-cholesterol diet and catfish oil intervention on them. Based on weight measurement, the rats' body weights tended to increase when they were fed with high-cholesterol diet. The mean increase of weight after the feeding was 15.69%. Meanwhile, the mean weight decreased by 1.85% due to catfish oil intervention. Previous study showed that there was an association between body weight, triglyceride concentrations, and HDL-c concentrations (Hernawati *et al*, 2013). If the body weight and triglyceride concentrations decreased, the HDL-c concentrations tended to increase. HDL-c is often called as good cholesterol, because it is a lipoprotein which transports the lipid from peripheral cells to the liver. It also has antioxidant properties, thereby preventing LDL-c oxidation. Its low levels in the blood will increase the risk of atherosclerosis and CHD.

3.2. Fatty Acid Content of Catfish Oil

High-quality fish oil is the one rich in fatty acids which are beneficial for health (Maulana *et al.* 2014 [24]). Fatty acid composition shows the potency of catfish oil as a supplement. It provides information regarding unsaturated fatty acid content in catfish oil products. Analysis results of fatty acids contained in catfish oil were presented in Table 1.

The dominant fatty acids in catfish oil before and after refining were not different. The dominant saturated fatty acids (SFAs) were palmitic and stearic acids. The dominant MUFA was oleic acid, while the dominant PUFAs were linoleic and linolenic acids. The highest fatty acid content in catfish oil was oleic acid, 29.17% in the crude oil and 28.68% in the refined oil. Oleic acid is an unsaturated fatty acid that is mainly found in vegetable oils. It is also the most common unsaturated fatty acid and a precursor for the production of most PUFAs (Gumilar, 2009). Meanwhile, linoleic and linolenic acids are essential fatty acids needed by the body. Catfish oil contained 0.39% linolenic acid (omega-3) and 11.17% linoleic acid (omega-6).

3.3. Rats' Serum Lipid Profiles (Total Cholesterol, Triglycerides, HDL-c, and LDL-c)

Lipid profile was analyzed to determine the effect of intervention on the conditions of the experimental animals. It was short-term descriptions related to dietary intake (Murdiati *et al*, 2010). The bonds between lipids and proteins may form lipoprotein. Based on its composition, density and mobility, it can be divided into chylomicrons, very low density lipoprotein (VLDL), LDL and HDL. Each type of lipoprotein has different functions, and it is broken down and excreted in a slightly different way (Adam, 2009). The results of rats' serum lipid profile (total cholesterol triglycerides, HDL-c and LDL-c) analyses were presented in Table 2.

Table 1. Fatty acid content of catfish oil (Alams, 2016)

Fatty Acids	Before refining %(w/w)	After refining %(w/w)
SFA	25.13	24.01
Palmitic Acid, C 16:0	19.61	18.68
Stearic Acid, C18:0	4.17	4.05
Myristic Acid, C14:0	0.58	0.54
Lauric Acid, C12:0	0.22	0.19
Pentadecanoic Acid, C15:0	0.16	0.16
Heptadecanoic Acid, C17:0	0.12	0.12
Arachidic Acid, C20:0	0.12	0.12
Behenic Acid, C22:0	0.08	0.08
Lignoceric Acid, C24:0	0.03	0.03
MUFA	33.27	32.73
Oleic Acid, C18:1n9c	29.17	28.68
Palmitoleic Acid, C16:1	3.42	3.3
Cis-11-Eicosenoic Acid, C20:1	0.59	0.55
Myristoleic Acid, C14:1	0.05	0.05
Nervonic Acid, C24:1	0.03	0.03
Elaidic Acid, C18:1n9t	0.01	0.12
PUFA	22. 27	11.74
Linoleic Acid, C18:2n6c	9.11	8.7
γ-Linolenic Acid, C18:3n6	1.42	1.39
Arachidonic Acid, C20:4n6	0.58	0.57
Cis-8,11,14-Eicosatrienoic Acid, C20:3n6	0.54	0.51
Cis-4,7,10,13,16,19DocosahexaenoicAcid, C22:6n3	0.36	0.33
Cis-11,14-Eicosadienoic Acid, C20:2	0.19	0.18
Cis-5,8,11,14,17-Eicosapentaenoic Acid, C20;5n3	0.07	0.06
Total	70.67	68.49

Table 2. Lipid profile of Sprague-Dawley Rats

Type of intervention	Total cholesterol	Triglycerides	HDL-c	LDL-c
Negative control (high-cholesterol diet)	58.3±9.93	156.00±47.65	42.67±11.43	12.67±7.23
Catfish oil	55.00±11.5	101.17±59.55	39.17±9.37	10.50±1.90
Catfish oil + omega-3 concentrate	56.60±4.34	117.80±73.82	36.20±4.15	11.00±3.39
Catfish oil + omega-3 + vitamin E	57.83±11.21	129.33±62.89	54.67±39.32	9.67±4.07

Based on the analysis results of rats' lipid profile in Table 2, it could be seen that catfish oil intervention had a tendency to decrease rats' total cholesterol levels. It was indicated by the decrease in total cholesterol levels, from 77 mg/dL before intervention to 58.3±9.93 mg/dL after the intervention. Moreover, the tendency of catfish

oil to suppress the increase in cholesterol levels was indicated by mean total cholesterol levels of the rats in the negative control group, which had the highest value (58.3±9.93 mg/dL) among the ones in other intervention groups. These findings were consistent with the recent study, which reported that catfish oil had the ability to suppress the increase in cholesterol levels in the body (Srimiati, 2016). Nevertheless, the statistical analyses indicated that there was no significant difference ($p<0.05$). In this regard, the previous study suggested that in normal and healthy individual, the body was able to control cholesterol synthesis so that the cholesterol pool in it was always relatively constant (Murdiati *et al*, 2010). This phenomenon was thought to occur in our study; thus, the rats tended to maintain their cholesterol within the normal levels. Biological factors, in which LDL receptors increased due to cholesterol diet intervention from outside the body, were also thought to affect the acquired results. Several researchers suggested that the younger the rats, the higher the chances of mortality, and vice versa (Wongso dan Iswahyudi, 2013). Therefore, it could be presumed that older rats were more likely to have low sensitivity to intervention, thereby affecting the results of the study. Other researchers stated that there were no significant differences in the effect of oleic and linoleic acids on HDL-c concentrations (Thijssen and Mensink, 2005).

3.4 Sprague-Dawley Rats' Serum Malondialdehyde Levels

Addition of vitamin E (α-tocopherol) in fish oil products aimed to prevent oxidation that might lead to decreased quality of fish oil; and to observe the effectiveness of the antioxidants on rats' MDA levels. Low antioxidant levels might increase lipid peroxidation products such as MDA (Siswanto and Purwaningsih, 2012) MDA was one of the most commonly used biomarkers and reliable in providing an overview of oxidative stress in clinical situations (Giera *et al*, 2012). Meanwhile, vitamin E was the antioxidant used in fish oil in this study. Previous study reported that it was the most effective antioxidant that could be used on animals (Musalmah *et al*, 2002). The results of rats' serum MDA analysis were presented in Table 3.

Table 3. Rats' MDA levels

Type of Intervention	Mean MDA levels±SD (nmol/L)
A1 (negative control)	0.616±0.071
A2 (catfish oil)	0.835±0.223
A3 (catfish oil + concentrate)	0.832±0.160
A4 (catfish oil + concentrate + vitamin E)	0.702±0.113

Table 3 showed the results of Sprague-Dawley rats' MDA levelsthat received different interventions. Based on the results, it was known that the MDA levels were very low, but still within the normal value. This finding was supported by earlier study suggesting that MDA level was defined as normal if it was less than 4 nmol/L (Siswanto dan Purwaningsih, 2012). ANOVA results indicated that there was no significant difference in MDA levels between the intervention groups, with a significance value of 0.085 ($p<0.05$). Most of the α-tocopherol in rats is transported through cholesterol; i.e. 70%-80% through HDL-c, 18%-22% through LDL-c and less than 8% through VLDL. If it is associated with cholesterol metabolism as described in other studies (Katar (1995) in Yuliani *et al*, 2002), rats have high fractional catabolic rates. They can clean $6ml^{-}.h^{1}.kg^{-1}$of their LDL-c contents (10 times larger than human). Thus, their LDL-c levels in serum are decreased and affecting their serum MDA levels. As an oxidation product of radical oxygen species (ROS), MDA gives an overview of the damage that may occur if the antioxidants in the body cannot cope with the increased amount of oxidants.

Our study showed that MDA levels in all intervention groups were not significantly different, indicating that the body's antioxidants can still overcome the increased ROS. In addition, carotenoids contained in catfish oil were also presumed to act as antioxidants. Earlier research indicated that β-carotene could increase the activity of antioxidant enzyme (catalase) in acetaminophen-induced rats (Morakinyo *et al*, 2012). Carotenoids are thought to act as antioxidants, because these compounds can increase gene expression of antioxidant enzymes (e.g. catalase) and superoxide dismutase (SOD) by increasing the amount of mRNA (Junior *et al*, 2012).

4. Conclusion and Recommendations

Catfish oil supplementation is considered potential in lowering cholesterol levels. It can be concluded by the lipid profile values of all intervention using catfish oilrats tend tolowering cholesterol levels, although it is not significantly significant.All intervention groups had very low MDA values (A1: 0.616±0.071, A2: 0.835±0.223, A3: 0.832±0.160, A4: 0.702±0.113 nmol/L), indicating that their MDA values were in normal value (<4nmol/L).

Clinical studies on catfish oil as a source of antioxidants are still needed to analyze the effect of antioxidants contained in catfish oil on human, especially its vitamin E content.

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