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INTERVENTION OF CATFISH OIL (*Clarias gariepinus*) ENRICHED WITH OMEGA 3 IMPROVING LIPID PROFILES AND OXIDATIVE STRESS MARKERS IN ELDERLY

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ABSTRACT

This study aimed to examine the effect of catfish oil intervention enriched with omega 3 on lipid profiles and ox-LDL levels in the elderly. The research design used a randomized-controlled trial (RCT) in a single blind. Subjects were divided into three groups, the first group was given soybean oil (SO), second group was given catfish oil (CO), while the third group was given catfish oil enriched with omega 3 (CO+omega 3). The lipid profile and ox-LDL measurement was performed before and after the intervention. The results showed that CO+omega 3 group had significant reduction in total cholesterol. Triglyceride levels in CO and CO+omega 3 groups decreased after intervention, while triglyceride levels in SO goups increased, but no significant differences (p>0,05). The HDL levels increased in SO and CO groups, while CO+omega 3 decreased slightly, but did not different significantly (p> 0,05). LDL levels decreased in all groups, but the highest decrease was in the CO+omega 3 group. The ANOVA test showed that delta LDL levels in each treatment group did not different significantly. The ox-LDL level in each treatment decreased, but it was significant only in the treatment of catfish oil enriched with omega 3. Therefore, catfish oil enriched with omega 3 was chosen as the best intervention in improving lipid profiles and oxidative stress of the elderly.

Contribution/ Originality: This study is one of very few studies which have investigated the effect of catfish oil intervention enriched with omega 3 on lipid profiles and ox-LDL levels in the elderly.

1. INTRODUCTION

Indonesia is among the top five countries with the largest number of elderly in the world. Over a period of 50 years (1971-2018), the percentage of Indonesia's elderly population has increased by about twice. In 2018, the percentage of the elderly reaches 9,27% or around 24,49 million people. It is estimated that in 2045, Indonesia will have around 63,31 million elderly or almost 20% of the population [1]. The increasing number of elderly every year presents a challenge to maintain the condition of the elderly to remain productive and healthy; both physically and mentally.

Elderly is an age range that naturally decreases the physiological functions of the body [2, 3]. One of health problem in the elderly is dyslipidemia [4, 5]. Dyslipidemia is a lipid fraction abnormality and a major risk factor for coronary heart disease [6, 7]. Increasing of age results in aging process, including increasing free radical

compounds in the body [8]. Free radicals are molecules or atoms that have one or more unpaired electrons, so they are very labile and reactive to attack surrounding molecules and can cause damage to cell structure and function Groopper, et al. [9]. Hall, et al. [10] stated that lipids in the body are the main target of free radicals. Free radical compounds can damage lipids, especially lipids on low density lipoprotein (LDL) cholesterol. Free radical attack on LDL cholesterol is often called oxidized LDL (ox-LDL).

Catfish is one of the fisheries commodities that have been developed by many people in Indonesia. One of the uses of catfish is catfish oil for health. Fish oil contains saturated fatty acids which are quite high. Oil derived from catfish has unsaturated fatty acids (PUFA) of 32.43% and monounsaturated fatty acids (MUFA) of 36.12%. In addition, PUFA content in present study has a low ratio between n-6/n-3 so that it could improve the quality of catfish oil. Low n-6/n-3 PUFA ratio had a beneficial effect on cardiovascular risk factors by enhancing favorable lipid profiles and anti-oxidative stress effects [11].

Based on the information above, this study aimed to examine the effect of catfish oil intervention enriched with omega 3 on lipid profiles and ox-LDL levels in the elderly.

2. MATERIALS AND METHODS

2.1. Design, Location, and Time

The research design used a randomized-controlled trial (RCT) in a single blind. The study was conducted in June 2016 until January 2017, with a duration of intervention for 90 days. The intervention was carried out at the elderly Posbindu (integrated development post), Ciherang, Dramaga, Bogor, Indonesia. This research has obtained the ethical committee permission from Faculty of Medicine, Indonesian University with Number: 991/UN2.F1/ETIK/2016.

2.2. Number and Sampling Technique

The population in this study were men or women aged ≥60 years who lived in the Dramaga-Bogor area. The study population was the elderly who were assisted by integrated development post at Ciherang, Dramaga, Bogor. Subject inclusion criteria were elderly men or women aged ≥60 years, had dyslipidemia, i.e., one of blood lipid profiles was abnormal (cholesterol >200 mg/dl, low density lipoprotein (LDL) cholesterol >130 mg/dl, triglycerides >150 mg/dl, and high density lipoprotein (HDL) cholesterol <40 mg/dl), physically independent, not taking lipid-lowering drugs (cholesterol), and signed an informed consent. The exclusion criteria were participated in other studies and routinely consumed supplements that have similarities with the test material.

Minimum sample size required for this study, with a test power of 90% and p<0,05 was nine people, based on the previous study by Rajkumar, et al. [12]. Three subjects were added to anticipate the possibility of drop-out; thus, the total number of respondents involved was 30 elderly. The intervention applied in this study were 3 groups, i.e., control group (soybean oil, SO), catfish oil group (CO) and catfish + omega 3 oil group (CO+omega 3). The ratio of omega-3 added in CO+omega 3 group was based on a ratio of 1:2 (n-3:n-6).

2.3. Types and Modes of Data Collection

The data collected in this study were primary data, which included subject's characteristics, levels of lipid profiles and ox-LDL subjects. Data collection of subject characteristics was obtained through direct interviews using a questionnaire. Subject blood collection for analysis of blood lipid profiles and ox-LDL was carried out after the subjects were fasted for 10-12 hours and performed twice, i.e. before and after intervention. Blood collection is carried out by officers from the Regional Health Laboratory (Labkesda), Bogor. Blood samples were taken through a 5 ml mediana cubital vein. Examination of blood lipid profile consists of total cholesterol, triglycerides, LDL, and HDL. Measurement of total cholesterol levels used the total cholesterol analysis method, triglyceride levels used the triglyseride analysis kit method, LDL levels used the indirect method, and HDL levels used the cholesterol

HDL analysis kit method. The examination of ox-LDL subjects used the ELISA method (enzyme-linked immunosorbent assay).

2.4. Processing and Analysis of Data

Data processing and analysis was done by used Microsoft Excel 2010 and SPSS 20. The initial stage of the analysis were assessed for their normality by Kolmogorov-Smirnov test (K-S test) to see the distribution of research data and determine the type of analysis to be used. Analysis to show differences in the subject's characteristics, lipid profiles and ox-LDL levels between groups used the One Way Anova test (normally distributed data) or Kruskal-Wallis (if the data was not normally distributed). Paired samples-t test was performed to analyze the differences in lipid profiles and ox-LDL in each group before and after the intervention.

3. RESULTS AND DISCUSSION

3.1. Subject's Characteristics

Subject's characteristics observed in this study included gender, age, education level, occupation, and marital status. Subject characteristics were presented in Table 1. The subjects involved in this study were mostly female, consisted of 80% in the SO group, 90% in CO group and 66,67% in the CO+omega 3 group. Mean subject's age was 64,70±4,88 years in SO group, 64,90±4,51 years in CO group and 67,44±6,52 years in the CO+omega 3 group. Based on the World Health Organization (WHO), subjects were included in the elderly group, with an age range of 60-74 years.

Most of the subjects had been through the primary education (51,72%). Subjects who did not complete primary education were 24,14%, and only a small proportion (6,90%) had completed junior secondary education. National Socio-Economic Survey (SUSENAS) reported that most of the elderly people had low education level. Most of young elderly (60-69 years) did not complete primary education (33,98%) and only 7,37% completed junior secondary education [1]. The level of education influences the attitude and behavior of healthy living. A high level of education will make it easier for someone to receive and absorb information, including nutrition and health information. The level of education can be used as a benchmark for knowledge in healthy behavior and manage the illness.

Table-1. Subject distribution based on subject's characteristics.

Subject's characteristics	SO	CO	CO+omega 3	P-value[1]
	n (%)	n (%)	n (%)	
Gender[2]				
Men	2 (20)	1 (10)	3 (33,33)	0,467
Female	8 (80)	9 (90)	6 (66,67)	
Age (years)	64,70 ± 4,88	64,90 ± 4,51	67,44 ± 6,52	
60-74	9 (90)	10 (100)	7 (77,78)	0,474
75-90	1 (10)	0	2 (22,22)	
Education[2]				
Never got an education	2 (20)	1 (10)	2 (22,22)	0,619
Did not graduate	3 (30)	2 (20)	2 (22,22)	
Elementary school	4 (40)	6 (60)	5 (55,56)	
Junior high school	1 (10)	1 (10)	0	
Occupation[2]				
Worked	3 (30)	2 (20)	3 (33,33)	0,799
Did not work	7 (70)	8 (80)	6 (66,67)	
Marital status[2] Married	4 (40)	3 (30)	2 (22,22)	0,711
Divorced died	6 (60)	7 (70)	7 (77,78)	

[1]One way Anova; [2]Kruskal-Wallis.

Most of the subjects were unemployed (72,41%), only take care of the household or grandchildren, while 27,59% were still working, with the type of work as laborers, drivers or selling food. The weak physical condition of the elderly causes them to do more activities that do not really need physical strength, such as looking after grandchildren or taking care of the household [13]. The percentage of divorced dead subjects in this study were higher (68,96%) than married subjects (31,04%); whereas the proportion of divorced dead were higher in female subjects (65,52%). According to BPS [1] elderly women who divorced died were higher (54,68%) than elderly men (14,99%). This indicated that women's life expectancy was higher (9,53%) than men (8,54%). Statistical tests of the subject's characteristics showed no significant difference (p> 0,05) between each group. This showed that the subject's characteristics in this study were homogeneous and not a confounding factors in this study.

3.2. Lipid Profile before and after Intervention

The results of lipid profile analysis were presented in Table 2. Measurement of cholesterol level before intervention in SO, CO and CO+omega 3 groups showed no significant differences (p>0,05). The cholesterol levels in all groups before intervention were above normal levels (>200 mg/dl). After the intervention, cholesterol levels in the CO and CO+omega 3 decreased, but only the CO+omega 3 group had below normal cholesterol levels (193,67±28,60 mg/dl). ANOVA test showed that cholesterol levels were significantly different. Based on Duncan's post hoc test, cholesterol levels were significantly different between CO+omega 3 group with SO and CO groups. This indicated that the addition of omega 3 can improve the composition of non-saturated fatty acids in catfish oil, so it can reduce cholesterol levels significantly compared to the intervention using only catfish oil or soybean oil.

These findings were similar to a randomized-controlled trial about effect omega 3 on lipid profile in adults [12]. The study found that the omega 3 supplemented group had significant reduction in total cholesterol. Plasma cholesterol concentration has been shown to be reduced by fish oil which is rich in omega-3 through suppress sterol regulatory element binding protein-1 (SREBP-1) expression which can reduce the process of lipogenesis and decrease very low density lipoprotein (VLDL) secretion, increase cleansing of liver lipoprotein through enhancement lipoprotein lipase (LPL) expression and decrease in apoC-III levels, as well as increased cholesterol transport to the liver [14].

Table-2. The mean of lipid profile level of the subject before and after intervention.

Variable (mean±SD)	Groups			P-value[2]
	SO	CO	CO+omega 3	
Cholesterol (mg/dl)				
Pre	213,70±37,42	228,40±22,88	233,33±34,36	0,389
Post	214,00±37,92	218,00±20,02	193,67±28,60	0,187
P-value[1]	0,92	0,032*	0,014*	
Delta	0,30±9,20[a]	-10,40±12,97[a]	-39,67±37.93[b]	0,003*
Triglyceride (mg/dl)				
Pre	114,00±40,90	142,10±92,49	146,22±52,79	0,514
Post	117,00±40,60	114,80±46,51	141,56±63,09	0,457
p-value[1]	0,754	0,470	0,822	
Delta	3,00±29,40	-27,30±114,52	-4,67±60,38	0,665
LDL (mg/dl)				
Pre	137,00±26,21	134,30±34,22	135,67±28,31	0,980
Post	128,30±33,56	124,60±29,88	121,56±40,83	0,915
P-value[1]	0,473	0,150	0,352	
Delta	-8,70±36,77	-9,70±19,50	-14,11±42,84	0,253
HDL (mg/dl)				
Pre	57,70±10,81	62,10±10,35	49,11±5,82	0,019
Post	66,80±16,68	58,40±23,36	46,22±10,27	0,058
p-value[1]	0,078	0,682	0,413	
Delta	9,10±14,45	-3,70±27,68	-2,90±10,03	0,271

[1]Paired-samples t-test, [2]One way ANOVA and then continued with Duncan's post hoc test (*significant at p<0,05). The different superscripts show significant differences.

The triglyceride levels in CO and CO+omega 3 groups decreased after intervention, while triglyceride levels in SO goups increased, but no significant differences (p>0,05). Fish oils have been widely reported as a useful supplement to reduce fasting blood triglyceride levels in individuals with dyslipidemia. A systematic review and meta-analysis by Eslick, et al. [15] from 47 studies showed that taking fish oils (weighted average daily intake of 3,25 g of EPA and/or DHA) produced a clinically significant reduction of triglyceride levels.

A study by Dainy, et al. [16] showed that giving catfish oil as much as 1 g/day for 60 days can significantly reduce triglyceride levels. The fatty acids (FAs) used in hepatic triglyceride synthesis can be derived from at least three sources: 1) the diet (i.e.chylomicron/remnant); 2) de novolipogenesis; and 3) circulating non-esterified FAs (NEFAs) [17]. FAs derived from de novolipogenesis and/or diet are first stored as triglycerides in hepatocytes whereas NEFAs can be directly incorporated into VLDL-triglyceride. Other studies confirm the primary contribution of NEFA to hepatic triglyceride production, emphasizing that changes in NEFA flux to the liver would be necessary to reduce production of VLDL-triglyceride [18].

The HDL levels increased in SO and CO treatments, while CO+omega 3 treatment decreased slightly, but did not different significantly (p> 0,05). LDL levels decreased in all treatments, but the highest decrease was in the CO+omega 3 group. The ANOVA test showed that delta LDL levels in each treatment group did not different significantly. Fish oil reduced LDL levels through inhibition of triacylglycerol and synthesis of VLDL in the liver [14].

3.3. Effects of Intervention on Oxidative Stress Marker

Oxidative stress is a condition of imbalance between free radical compounds and the amount of antioxidants in the body. This condition causes damage to cell membrane lipids, proteins and DNA which results in cell or tissue survival. Lipid oxidation or lipid hydroperoxide is the easiest measurement of free radicals. One final product due to lipid peroxidation is oxidized-LDL [19].

Table-3. The mean of ox-LDL level of the subject before and after the intervention.

Variable (Mean±SD)	Groups			p[2]
	SO	CO	CO+omega 3	
Ox-LDL (Pg/ml)				
Pre	1366,00±190,59	1281,67±284,97	1433,19±154,16	
Post	1173,00±305,41	1021,67±379,02	1059,11±454,28	
P-value[1]	0,067	0,100	0,035*	
Delta	-193,00±293,73	-260,00±448,77	-374,07±443,52	0,617

[1]Paired-samples-t test, [2]One Way Anova; *Significant at p<0,05.

Table 3 shows that the ox-LDL level in each treatment decreased, but it was significant only for CO+omega 3 treatment. The results of statistical tests showed that these values did not different significantly between treatment groups (p> 0,05). The results of this study were similar with study conducted by Kiecolt-Glaser, et al. [20] which showed that supplementation of omega 3 PUFAs (polyunsaturated fatty acids) could reduce oxidative stress by about 15% compared to placebo. Provision of salmon oil supplements containing omega 3 for 4 months can also reduce levels of oxidative stress in adults significantly [21]. Catfish oil in our study contained PUFA, which also acted as antioxidants with low ratio between n-6/n-3. Low n-6/n-3 PUFA ratio (1:1 and 5:1) had a beneficial effect on cardiovascular risk factors by enhancing favorable lipid profiles, having anti-inflammatory and anti-oxidative stress effects, and improving endothelial function. Meanwhile, a high n-6/n-3 PUFA ratio (20:1) had adverse effects [11].

4. CONCLUSION

Intervention of catfish oil enriched with omega 3 can reduce the total cholesterol level elderly significantly and improve the levels of triglycerides, HDL and LDL. The ox-LDL level in each treatment decreased, but it was significant only in the treatment of catfish oil enriched with omega 3. Based on our studies, catfish oil enriched with omega was chosen as the best intervention in improving lipid profiles and oxidative stress in the elderly.

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