Jurnal Sains Kesihatan Malaysia **12** (2) 2014: 17-26

Kertas Asli/*Original Articles*

Effects of Catfish Oil Intervention on Lipid Profile in Female Aged Cynomolgous Monkey (*Macaca fascicularis*)

(Pengaruh Pemberian Minyak Ikan Lele dan Fermentasinya terhadap Profil Lipid Monyet Ekor Panjang (*Macaca fascicularis*) Betina Usia Tua)

ISKARI NGADIARTI, CLARA M. KUSHARTO, DODIK BRIAWAN, SRI ANNA MARLIYATI & DONDIN SAJUTHI

ABSTRACT

Degenerative process is an accumulation of free radicals that can lead to a variety of changes in the cell. This aim of the study to see the effects of different dietary lipid intervention on lipid profile and lipid peroxidation in female aged cynomolgus monkey. Twelve female Cynomolgus monkey (Macaca fascicularis) were randomly divided into 4 dietary groups of three animals. Animals were held in individual cages and placed in the position where they can interact individually. They were given a normal fat diet with 2% cholesterol and 3% of fat from soybean oil and 9% of lipid as beef tallow (BFT), catfish oil (CFO), fermented catfish oil (FCFO) and soybean oil (SBO). Evaluation of their body weights, serum lipid profile and cholesterol content consumption were done monthly except an index of lipid peroxidation were performed before and after 12 weeks intervention. Animal group that consumed the feed more than 82% has body weight gain, such as CFO and BFT. On the other side, animal group that consumed less than 70% has body weight loss, such as FCFO. There was no effect ($p > 0.05$) of the experimental diets on decreasing triglyceride levels and increasing HDL cholesterol level. Cynomolgous given BFT, CFO, and FCFO diet for 3 months showed significantly increased ($p < 0.05$) in total cholesterol and LDL cholesterol level, but the monkeys fed with SBO showed lower levels. Statistically, All of diet interventions do not significantly affect on lipid peroxidation in LDL ($p < 0.05$). In general it can be concluded that catfish oil significantly cause elevated levels of total cholesterol and LDL cholesterol in blood serum, but not in the HDL plasma.

Keywords: Catfish oil; serum cholesterol; lipid profile; lipid peroxidationa

ABSTRAK

*Proses degeneratif adalah pengumpulan radikal bebas yang boleh membawa kepada pelbagai perubahan dalam sel. Tujuan kajian adalah melihat pengaruh pemberian dari berbagai sumber lemak terhadap pada profil lipid dan pengoksidaan lipid pada monyet ekor panjang betina tua. Dua belas monyet ekor panjang betina (*Macaca fascicularis*) secara rawak dibahagikan kepada 4 kelompok dan setiap kelompok terdiri daripada 3 haiwan.* Macaca *diletakkan dalam sangkar individu dan diposisikan dapat saling berinteraksi dan berkomunikasi. Mereka diberi diet dengan kandungan kolesterol 2% dan kandungan lemak 12% iaitu 3% lemak berasal daripada minyak kacang soya dan 9% berasal daripada lemak yang berbeza iaitu lemak sapi (BFT), minyak ikan lele (CFO), minyak ikan lele yang difermentasi (FCFO), dan minyak kacang soya (SBO). Penilaian berat badan mereka, profil lipid serum dan asupan diet kolesterol dilakukan setiap bulan, sedangkan penilaian peroksidasi lipid dilakukan sebelum dan setelah pemberian 12 minggu. Rata-rata konsumsi* Macaca *yang mengkonsumsi diet BFT dan CFO adalah 82%, sedangkan* Macaca *yang mengkonsumsi FCFO dan SBO hanya 70%. Tidak ada kesan ($p > 0.05$) daripada diet eksperimen mengurangkan kadar trigliserida dan meningkatkan kadar kolesterol HDL.* Macaca *yang diberikan diet BFT, CFO, dan FCFO menunjukkan peningkatan ketara ($p < 0.05$) dalam total kolesterol dan LDL kolesterol, tetapi monyet diberi makan SBO menunjukkan kadar total kolesterol dan LDL kolesterol yang rendah. Semua pemberian diet secara statistik ($p < 0.05$) tidak memberi pengaruh terhadap pengoksidaan lipid dalam LDL. Oleh kerana itu, secara umumnya dapat disimpulkan bahawa pemberian minyak ikan lele hanya menyebabkan peningkatan kadar kolesterol total dan LDL kolesterol serum dalam serum darah, tetapi tidak dalam plasma HDL.*

Kata kunci: Minyak ikan lele; kolesterol serum; profil lipid; pengoksidaan lipid

INTRODUCTION

BACKGROUND

Cardiovascular diseases (CVDs) are responsible for significant mortality and morbidity throughout the world including Indonesia (WHO, 2003). Their causation is due to atherosclerosis and/or thrombosis. These process can be initiated from an unbalanced eating habits such as excessive fat consumption regardless of the type and amount that will increase the level of low-density lipoprotein (LDL) in the blood. Elevated blood cholesterol levels especially LDL is the primary target of cholesterol-lowering therapy to reduce cardiovascular diseases risk (Mc. Cance et al. 2010). Diet is one of an important thing in the prevention and treatment of CVD. To date, many studies reported that different dietary lipids can modulate plasma cholesterol levels, depending on their fatty acid composition (Cintra et al. 2006).

In a recent meta-analysis of fourteen cohort and five case-control studies, fish consumption was associated with a 20% reduction in the relative risk of fatal and a 10% reduction in non fatal atherosclerosis (Hamer & Steptoe 2006). Morise et al. 2004 has reported that high levels of saturated fatty acid (SFAs) are associated with the formation of atherosclerotic plaques. However, intake of mono unsaturated fatty acids (MUFAs) especially oleic acid and polyunsaturated fatty acids (PUFAs) especially α linolenic acid, are associated with decreased risk of cardiovascular death. Many studies have also demonstrated that MUFAs and PUFAs show similar effects on lowering blood cholesterol when SFA is substituted in the diet (Cintra et al. 2006). Although PUFA are effective at lowering blood LDL cholesterol, at intake in excess of 8-10% dietary energy they also lower HDL cholesterol concentration, necessary for atherogenic protection. PUFA are also susceptible to metabolic oxidation and high intakes can enhance lipid peroxidation and free radical production with potentially adverse effects in terms of atherogenesis and carcinogenesis (Thomas & Bishop 2007).

Catfish oil was the 'by product' in the production of catfish flour can be used as the source of a healthy fat, which is still under utilized. Our preliminary study had shown that the catfish oil shows a high content of saturated fatty acid (SFA), monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA). The dominant content of unsaturated fatty acids such as oleic acid (C18:1) has about 22.82% and 17.8% of linoleic acid (C18:2). The excess of linoleic acid content could be desaturated and elongated to be other types of essential fatty acid such as arachidonic and gamma linolenic and if fermented with lactic acid bacteria the content of conjugated linoleic acid (CLA) increased (Hidayati 2005; Xu et al. 2004; dan Ogawa et al. 2001). This study aims to look at the effect of cat fish oil fish (CFO), fermented catfish oil (FCFO), beef tallow (BFT), and soybean oil (SBO) on the lipid profile and lipid peroxidation in cynomolgous monkey.

Cynomolgous monkey (*Macaca fascicularis*) is one of Indonesian native primates which belongs to the kingdom of Animalia, Phylum: chordates, Subphyllum: Vertebrates, Class of Mammals, Order of: Primates and Suborder : Anthropoida. Cynomolgous monkey has the advantage to be used in biomedical research due to in their the present of antibodies to certain types of viruses (Adyanto, 2010).

This monkey also has a very close phylogenetic relationship to humans that they have physiological and anatomical similarities (Smith et al. 2010). They also had a small bodies that complete information about the reactions to hormonal diets; e.g. if the ovarectomized cynomolgus are given atherogenic food feeding for at least 3 months, there will be an increase of total cholesterol concentration in the plasma and the decrease of high-density cholesterol (Williams & Suparto 2004). Various studies had suggested that animal studies using monkeys as experiment model has the best similarities with humans.

MATERIALS & METHODS

EXPERIMENTAL DESIGN

Experimental studies with a completely randomized design (CRD), which analyzes the effect of catfish oil (CFO) and fermented catfish oil (FCFO) on lipid profile in female aged cynomolgus monkey (*Macaca fascicularis*). The animal welfare committee of ethics PT. Bimana Indomedical Bogor with ACUC number of P.03.12_IR approved all animal care and experimental protocols. Twelve female aged cynomolgus (*Macaca fascicularis*) which age are over 10 years old, and body weight in the range of 2.780 to 3.3 kg. All animals have been ovarectomized and were purchased from PT IndoAnilab Bogor, Indonesia. Cynomolgous were held in individual cages placed in the position where it can interact individually. The adaptation process needed 2 months period and the animals had been given 100 grams each per day of standard feeding of Purina Monkey Chow. During this time, cynomolgous consumed feed and water ad libitum. Cynomolgous were then randomly assigned to 1 of 4 treatment groups for 12 weeks (n=3/group/period). Each group was given food intervention containing 0.2% cholesterol and isocaloric.

The amount of feeding given was 120 gram/day, 60 grams in the morning and 60 grams in the afternoon The leftover feeding were weight everyday and water were consumed ad libitum. Bodyweight and waist circumference were measured every month. Blood sample also was taken every month. Prior to the blood sampling, subjects were anasthesized using 10 mg per 10 kg body weight. Blood was taken through the femoral vein, put it into the tube and maintained at a temperature of 4C for the analysis of lipid profile and lipid peroxidation.

DIET FORMULATION

The raw material of Catfish oil obtained from PT Carmelitha Lestari Bogor. Beef tallow oil obtained from PT. Garuda Mas Lestari Bekasi and soybean oil is obtained from PT. Indofood Jakarta. The intervention diet (Table 1) were prepared and pelleted by PT. Carmelitha Lestari Bogor. Total fat content of the diets was 12% by weight, 9% of which was supplied by respective treatment fats. All diets were supplemented with 0.2% cholesterol by weight. The addition of cholesterol to the diets was to induce hyperlipidemia, thereby enhancing the detectable effects of treatments. The composition of feeding was based on food composition table per 100 grams can be seen on Table 2. All diets were designed to have the same contain of nutrient such as protein, fat and CHO, but fatty acid composition are different between each other depend on type of oil given. All feeding has both saturated fatty acid (SFA), unsaturated fatty acids either mono-unsaturated fatty acids (MUFA) or poly-unsaturated fatty acids (PUFA). The BFT and SBO treatments served as appropriate controls, because they are different, such as the BFT is source of SFA, but the SBO is source of PUFA. Fatty acids content in several types of feeding was illustrated in Table 3.

TABLE 1. Food intervention composition

Raw food material	BFT	CFO	FCFO	SBO
Flour (gram)	38	38	38	38
Maizena (gram)	10	10	10	10
Skim milk powder (gram)	10	10	10	10
Fish powder (gram)	7	7	7	7
Soy paste (gram)	5.8	5.8	5.8	5.8
Rice bran (gram)	2	3	2	3
Sugar (gram)	1	10	10	10
Soybean oil (gram)	3	12	3	3
Beef tallow (gram)	9	-	-	-
Catfish oil (gram)			-	9
Fermented catfish oil (gram)			9	-
Margarin (gram)	1	-	2	-
Agar-agar (gram)	1	1	0.5	1
CMC (gram)	1	1	0.5	1
Mineral mix (gram)	1	1	1	1
Mineral (gram)	1	1	1	1
Cholesterol (gram)	0.2	0.2	0.2	0.2
Total (gram)	100	100	100	100

Note: BFT = beef tallow, CFO = catfish oil, FCFO = fermented catfish oil, SBO = soybean oil

TABLE 2. Nutrition content in food *)

Component	BFT	SBO	CFO	FCFO
Energy	398	397	396	400
Fat	13.5	13.5	13.5	13.5
Protein	13.4	13.4	13.4	13.4
Carbohydrate	55.8	55.8	55.9	55.8
% protein to energy	13.5	13.5	13.5	13.4
% fat to energy	30.	30.1	30	30.8
% CHO to energy	56	56.4	56.5	53.8

Note: *) based on food composition table
BFT = beef tallow, CFO = catfish oil, FCFO = fermented catfish oil, SBO = soybean oil

TABLE 3. Fatty acid composition of BFT, CFO, FCFO, and SBO in food (% fatty acid)

Fatty acid	BFT	CFO	FCFO	SBO
C8:0	-	-	-	-
C10:0	-	-	-	-
C12:0	0.1	-	-	-
C14:0	0.5	0.2	0.3	-
C16:0	2.9	3	2.9	1.3
C18:0	3.4	0.7	1	0.4
∑SFA	6.8	3.2	4.2	1.7
C14:1	-	-	-	-
C16:1	0.2	1.1	0.3	-
C18:n9c	2.7	3.0	4.2	2.7
∑MUFA	2.9	4.1	4.5	2.7
C18:2n6c	1.9	4	2.6	6.6
C20:0	-	-	-	-
C18:3n3	0.2	0.3	0.3	0.8
C22:0	0.1	0.1	0.05	0.1
C22:1n9	-	-	0.1	-
C20:4n6	-	-	-	-
C24:0	-	-	-	-
C20:5n3	-	0.1	0.1	-
C22:6n3	-	0.4	0.1	-
CLA	0.1	-	0.05	-
∑PUFA	2.3	4.8	3.3	7.5
∑ MUFA+PUFA	5.2	8.9	7.8	10.2
∑SFA+MUFA+PUFA	12	12.	12	11.9
P/S	0.3	1.5	0.8	4.4
W6:W3	-	15 : 1	13:1	9:1

Note: BFT = beef tallow, CFO = catfish oil, FCFO = fermented catfish oil, SBO = soybean oil

PLASMA LIPID AND LIPID PEROXIDATION

Materials for lipid profile analysis is Liquicolor Bavaria Cholesterol Test Kit, while the materials needed for lipid peroxidation analysis are bovine serum albumin (BSA), sodium carbonate (Na2CO3), sodium bicarbonate (NaHCO3), copper sulfate (CUSO4.5H2O), potassium tartrate, sodium hydroxide

(NaOH), acid ethylenediamine - tetrasetat (EDTA), sodium chloride (NaCl), Folin-phenol reagent, potassium bromide (KBr), 1,1,3,3-tetrametoksipropana (TMP), trichloroacetic acid (TCA), tiobarbiturat acid (TBA), acetic acid, glasiat, ethanol and the dialysis bag.

The tools used for the analysis of lipid profile and lipid peroxidation is a low-speed centrifuge with rotor Beckman Bucket Swing 3750 rpm, ultrasentrifus Beckman XL-90 to SW-40 rotor, polialomer tube (14 × 95 mm), tube slicer knife, glass tools, UV-VIS spectrophotometer DMS 100 (Varian), vortex, milipore 0.45 um filter, water bath and pipette tip.

STATISTICAL ANALYSIS

The data obtained are presented in the form of the average and standard deviation. All data were analyzed with analysis of variance test at 5% level test. Duncan difference test is only done when a real treatment effect ($p < 0.05$).

RESULTS

CONSUMPTION AND PHYSICAL CHANGES

Various responses to the feeding consumption was shown during the intervention. Table 4 illustrates the average and percentage of various feeding consumption during the intervention. Data showed that the acceptance to CFO was pretty good. Consumption of CFO reached more than 80% of the served food was not much different from the consumption of the feeding containing BFT. Fat functioned as something that improved the palability of meal on CFO. Different chemical structures can also affect the taste of fat (Piliang 2006). Treatment of various types of feeding caused the physical changes to subjects that include the body weight, waist circumference, and body mass index (BMI). Figure 1 presented graph the percentage of physical changes in Subjects.

TABLE 4. Feed consumption and precentage in all groups

Intervention	Food weight (gram)	Average consumption (gram)	Precentage consumption (%)
BFT	120	107.00 ± 5.90	89.17
CFO	120	98.78 ± 16.48	82.32
FCFO	120	75.08 ± 11.53	62.57
SBO	120	85.89 ± 18.63	71.57

Note: BFT = beef tallow, CFO = catfish oil, FCFO = fermented catfish oil, SBO = soybean oil

Graph in Figure 1 shows that the intervention group treated with BFT and CFO have increased body weight respectively by 10% and 8%, while the group fed by SBO and FCFO have decreased body weight respectively by 17% and 2%.

All treated group had increased waist circumference by more than 5%. IMT changes happened to all groups but not for subjects which given SBO feeding. The group given the BFT and CFO got increasing of 12% and 2% respectively while the group given the FCFO decrease of 17%.

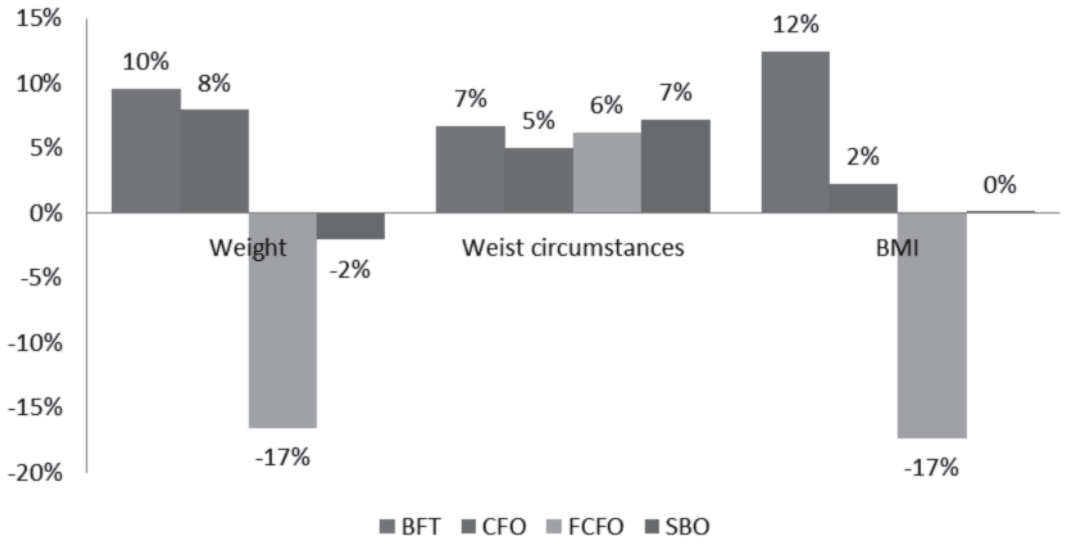

FIGURE 1. Precentage of weight, weist circumstances and BMI changes

LIPID PROFILE

Lipid profile is one of the tools to determine the presence of or absence of lipid disorders in the body which is known as dyslipidemia. Dyslipidemia is a major risk factor for atherosclerosis. Lipid profile includes triglycerides, total cholesterol, LDL and HDL. Table 5 illustrates the Subjects lipid profile during intervention.

TRIGLYCERIDE PROFILE

Table 5 shows that the average level of triglyceride before the intervention in the group that was given CFO, the average levels increase continuously. Before the intervention the triglyceride average level was 52.67 ± 16.92 mg/dl. Triglyceride levels increased continuously during intervention to reach 97.00 ± 67.51 mg/dl in the 3rd month. Intervention using BFT and SBO feeding in cynomolgous showed the same modulation of the level of triglyceride. The average level of triglyceride before the intervention in the group given the BFT and SBO feeding in row is 56.00 ± 1.00 mg/dl and 67.00 ± 42.58 mg/dl. The average of triglyceride level then increased to 152.50 ± 5.50 mg/dl (BFT) and 84.00 ± 41.68 mg/dl (SBO) in the 3rd month.

Intervention using FCFO feeding showed different modulation by other group. In this group average of triglyceride levels increased from 42.7 ± 9.29 mg/dl before intervention to 72.67 ± 68.73 mg/dl in the 1st month. However in the 2nd month the average of triglyceride levels decreased to 57.00 ± 19.28 mg/dl. This decline didn't continue because in the 3rd month the average of triglyceride levels increased sharply to reach back to 108.67 ± 23.80 mg/dl.

TOTAL CHOLESTEROL LEVELS

Profile of total cholesterol in the group that were given BFT and CFO feeding showed an almost similar modulation. It can be seen in Table 5 that the average cholesterol levels in both groups are increasing. The average total cholesterol in the group which was given BFT before intervention was 173.50 ± 12.50 mg/dl and group given CFO was 157.67 ± 19.55 mg/dl. After 3 month intervention, the average of cholesterol level has increased sharply to reach 276.00 ± 111.00 mg/dl in the group fed by BFT, and the average total cholesterol levels of group given CFO increased from 157.67 ± 19.55 to reach 372.67 ± 10.97 mg/dl. The total cholesterol levels that continued to decline was shown by the group fed SBO. In this group, the declined of the average of total cholesterol levels continued from 139.33 ± 15.04 mg/dl before the intervention to 117.67 ± 23.44 mg/dl in the 3rd month of intervention.

LDL PROFILE

LDL profile of the group that were fed with BFT and CFO showed an increase of LDL levels continuously from the beginning until the end of intervention. Table 5 showed that the average of LDL of pre-intervention level of the BFT

TABLE 5. Average lipid profile (mg/dl)

Lipid profile	Month	CFO	FCFO	BFT	SBO
Triglyceride	0	52.67±16.92[1]	42.67±9.29[1]	56.00±1.00[23]	67.00±42.58[1]
	1	61.67±20.50[1]	72.67±68.73[1]	50.00±3.00[3]	61.33±23.18[1]
	2	84.33±66.86[1]	57.00±19.28[1]	74.00±18.00[2]	69.00±24.63[1]
	3	97.00±67.51[1]	108.67±23.80[1]	152.50± 5.50[1]	84.00±41.68[1]
Average		83.42±53.99[a]	80.29±26.71[a]	97.44±8.06[a]	72.71 ±29.88[a]
Total Chol.	0	157.67±19.55[3]	141.00±40.78[1]	173.50±12.50[2]	139.33±15.04[1]
	1	255.33±62.68[2]	263.33±87.95[1]	266.00±12.50[12]	119.00±50.69[1]
	2	260.33±72.88[2]	228.67±33.85[1]	271.00±92.00[1]	121.67±37.87[1]
	3	372.67 ±10.97[1]	211.67±79.00[1]	276.00±111.00[1]	117.67±23.44[1]
Average		301.21±39.8[a]	230.9±21.02[ab]	271.63±84.13[a]	119.5±34.56[b]
LDL Chol.	0	37.00±10.00[3]	36.33±30.11[1]	53.50±11.50[2]	38.33±27.32
	1	86.33±56.70[3]	152.00±123.261	78.50±13.50[12]	10.67±10.02
	2	164.33±85.10[2]	170.00±73.18[1]	112.50±6.5[12]	40.6±1.27
	3	306.00+±18.08[1]	133.33±98.74[1]	194.00±16.0[1]	39.20±13.00
		197.96±9.95[a]	151.75±49.98[a]	134.56±49.32[a]	32.59±10.56[ab]
HDL Chol	0	120.0±22.27[2]	102.33±23.8[1]	109.00±24.00[12]	87.33±33.25[1]
	1	171.00±22.00[1]	97.00±51.2[1]	177.5±19.50[1]	112.67±55.72[1]
	2	79.00±26.89[3]	49.33±47.43[1]	144.00±82.00[1]	106.67±50.33[1]
	3	47.00±16.46[4]	56.3±27.54[1]	51.50±6.50[2]	61.67±21.50[1]
		90.00±19.55[a]	63.8±35.41[a]	117.69±33.19[a]	91.29±33.60[a]

Note: Numbers followed by different numbers of test results indicate significantly different between months ($p < 0.05$), while the letter shows the test results were significantly different between treatments

BFT = beef tallow, CFO = catfish oil, FCFO = fermented catfish oil, SBO = soybean oil

and CFO group are 53.50 ± 11.50 mg/dl and 37.00 ± 10.00 mg/dl. The average LDL levels in both groups increased sharply to reach 194.00 ± 116.00 mg/dl (BFT) and 306.00 ± 18.08 mg/dl (CFO) in the 3rd month.

An increase in the average of LDL levels after 3 months of intervention also occurred in the group that were fed with FCFO and SBO. However both groups showed different modulations compared to other groups. In the group of Subjects fed with FCFO the average of LDL levels before the intervention were 36.33 ± 30.11 mg/dl. The average LDL levels then increased sharply up to 152.00 ± 123.26 mg/dl in the 1st month but decreased to 102.00 ± 19.28 mg/dl in the 2nd month. At the 3rd month the average levels of LDL increased again to 133.33 ± 98.74 mg/dl. Despite the decline, levels of LDL is still higher than the LDL levels before the intervention.

The group that were fed with SBO showed different LDL modulation compared to other groups. Before the intervention, the average levels of cholesterol was 38.33 ± 27.32 mg/dl. The average levels of LDL decrease until 10.67 ± 10.02 mg/dl in the 1st month but the average increased again in the 2nd month to 40.6 ± 1.27 mg/dl and increased again to 39.20 ± 13.00 in the 3rd month.

HDL LEVELS

In Table 5 it can be seen that the average HDL levels in all groups decreased after intervention by giving them all types all of feeding. The group that were given BFT, CFO, and SBO showed a similar modulation. Before the intervention, the average level of HDL in the groups that were given BFT, CFO and SBO feeding in a row were 109.00 ± 24.00 mg/dl, 120.00 ± 22.27 mg/dl and 87.33 ± 33.25 mg/dl.

In the 1st month the average levels of HDL in the group given BFT feeding peaked at 177.50 ± 19.50 mg/dl and decreased in the 3rd month to 51.50 ± 6.50 mg/dl. The same modulation occurred in the group fed with CFO and SBO. In the group fed by CFO feeding, the average of HDL levels peaked at the 1st month to 171.00 ± 22.00 mg/dl then declined again to 47.00 ± 16.46 mg/dl in the 3rd month.

In the group fed SBO feeding, the average HDL levels peaked at the 1st month to 112.67 ± 55.72 mg/dl and then declined again until 61.7 ± 21.50 mg/dl in the 3rd month. The groups fed with FCFO feeding showed different modulation with other groups. The average levels of HDL before intervention was 102.33 ± 23.80 mg/dl. Average of HDL decreased continuously until 49.33 ± 47.43 mg/dl and a slight increased to 56.33 ± 27.54 mg/dl in the 3rd month.

LIPID PEROXIDATION

Lipid peroxidation is a mechanism of cellular injury which is used as an indicator of oxidative stress in cells and tissues. LDL oxidation in principle a chain reaction of lipid peroxidation induced by free radicals. One of the products of lipid oxidation is malondialdehida (MDA). In Figure 2, it can be seen that the levels of MDA in LDL change after the intervention. All treatment groups were seen to have elevated levels of MDA in LDL. Increased levels of MDA in LDL highest experienced by the group fed CFO is from 0.21 nmol/mg before intervention into 0.815 nmol/mg after the intervention. In contrast, the lowest increase experienced by the group fed SBO of 0.11 nmol/mg before the intervention to be 0.155 nmol/mg after the intervention. However, no statistically significant treatment effect with increased levels of MDA in LDL.

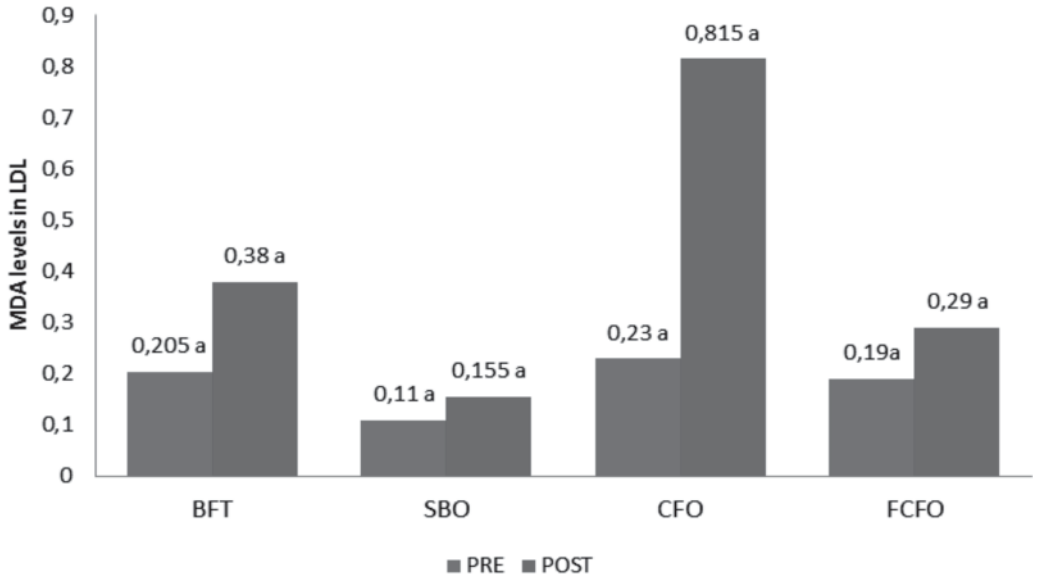

FIGURE 2. MDA levels pre and post intervention

DISCUSSION

PHYSICAL CHANGES

Oil catfish and catfish oil fermentation can be considered as a source of energy because it gives energy equals to other oil that is ± 9 kcal for every 1 gram of oil. Pekenpaugh (2010) stated that fat accounts for higher energy than protein and carbohydrate because the have more carbon bond in its structure. The evidence showed that the group that consumed more feeding got an increasing and waist circumference compared to the groups which got less feeding (< 70%).

Bowman et al. (2006) stated that the ± 70% of energy intake in the elderly, particularly on animal study, may slow the aging process, prolong the life expectancy and inhibit chronic diseases associated with age. Though mechanism in the body is unclear but it is suspected that the reduction of 30% energy can reduce the metabolic rate in oxidative stress, improve insulin sensitivity and change the neuro endocrine and symphatetic nervous system function.

Weight loss will not happen continuously and will stop at some point. Results show that within 3 months the weight change will already fix. It means that within the period of 3 months the cynomolgous have been able to adapt the diet so that the body weight can be maintained.

TRIGLYCERIDE LEVELS

Triglycerides are the form of fat stored in the body that are found in the adipose tissue. Some triglycerides circulate in the blood and they are used as energy in the muscles to work. All treated groups experienced increasing triglyceride levels ($p > 0.05$). The increased of triglyceride levels in the blood of all treated groups ranged from 4.44 – 39.17 mg/dl. Oberman (2000) suggested that the increased levels of triglycerides by 90 mg/dl may increase the risk of cardiovascular disease by 32%.

The increase in triglyceride serum is often associated with VLDL synthesis and its distribution**.** Triglyceride synthesis in the liver is regulated by the availability of both free fatty acid derived from the amount of glycogen in the liver and the status of insulin and glucagon hormones in particular. No single factor is affecting the increase of triglycerides. Other factors are the relationship with the disorders of bile acid metabolism and the production of abundant triglycerides, obesity, carbohydrate Intake and excess of saturated fatty acid, less activity and hyperglycemic (Stipanuk 2000).

TOTAL CHOLESTEROL LEVELS AND LDL CHOLESTEROL

Total and LDL cholesterol profiles showed that all groups showed classic modulation levels of hypercholesterolemia towards atherogenic feeding with the exception of the SBO group. Results showed that the total and LDL cholesterol levels in the cynomolgous which were given BFT, CFO and FCFO during intervention did not show significant differences, but the groups given SBO feeding got a significant different result with the other groups ($p < 0.05$).

Mitruka and Rawnsky (1977) stated that normal levels of total cholesterol serum in cynomolgous ranged between 100-150 mg/dl, while normal levels of LDL cholesterol was 68 ± 9 mg/dl (Lubis 1993). Results showed that only the group given with SBO showed that the levels of cholesterol and LDL were considered normal which is 117.67 ± 23.44 mg/dl for cholesterol and 39.20 ± 13 mg/dl for LDL levels.

Based on the ratio of P/S, the CFO, FCFO, BFT, and SBO are 1.5, 0.8, 0.3 and 4.4, while Kang et al. (2005) stated that P/S Ratio between 1-1.5 is the best to reduce the risk of atherogenesis. This opinion is supported by Muller et al. (2003) which stated that to lower the total SFA without balancing the P/S Ratio will not decrease the LDL Cholesterol level. The P/S ratio is the maximal point which can affect the regulation of total cholesterol serum. This research showed that the increasing total Cholesterol serum was not influenced by the P/S ratio., e.g. catfish oil with P/S ratio 1.5 is not able to suppress the increasing total cholesterol and LDL. This presumably because there are other affecting factors such as the high amount of SFA. IOM (2002) stated that the limit of SFA is 7%, and the only one that has SFA < 7% is only SBO.

The increasing levels of serum cholesterol and LDL is one strong indication of the risk of coronary heart disease (Garg & Simha 2007). LDL function is to carry cholesterol from the liver to the tissues. Normal levels of LDL Cholesterol are controlled by the hepatic LDL receptor that binds LDL and limits Lipoprotein synthesis the liver. The reduced formation of LDL receptors will cause the circulating amount of cholesterol in the blood above normal (Kasim et al. 2006).

There are several types of LDL. The most atherogenic is the LDL particles with low density, but it was not measured in the study. The menopause female subjects generally have smaller LDL particles compared to premenopause women (Krummel & Krisetherton 1996). Moreover the absence of estrogen hormone also affects the increasing rate of LDL serum and total cholesterol. This will result in hyperglycemic condition that can not be controlled therefore the lipid and lipoprotein metabolism is not normal (Peckenpaugh 2007).

Another factor that is suspected in influencing the value is the internal factor of Subjects such as abdominal obesity. Results of this study indicated that the increased waist circumference in Subjects is apparently has positive effect with the increased levels of cholesterol, LDL Cholesterol and triglycerides ($p < 0.05$). It is in accordance with the opinion of Jalal et al. (2006) who stated that the waist circumference is one of the best indicator metabolic syndrome including dyslipidemia.

Binkoski et al. (2005) reported that PUFA from vegetable sources can lower total cholesterol and LDL

levels. CFO, FCFO, BFT are oil derived from animal. Binkoski et al. (2005) also stated that although the amount and types of fat is similar but if the source of saturated and fats unsaturated is different then the results will be different too. Kromhout et al. (2011) added that fat restriction did not give important effect. What is were important is the low content of saturated fatty acids. Therefore it proved that only SBO feedings are able to maintain the levels of total cholesterol and LDL levels to remain at normal level.

HDL LEVELS

On lipid metabolism in the body, HDL has the opposite mechanism with LDL, which is free of cholesterol transport to the liver tissue to be removed from the body or converted into bile acids through a mechanism known as reverse cholesterol transport. HDL also plays a role in endothelial repair and reduce thrombosis. HDL can be broken down into several particle density (HDL 2 and HDL 3) or by size (large, medium, and small). The most effective HDL transports cholesterol from the tissues back to the liver, and HDL 2 as a better indicator than total atherogenic HDL (Murray et al. 2003).

Statistically significant test results showed that all groups decreased markedly different to the levels of HDL are fed with different types of treatment ($p < 0.05$). One of the causes of decreased levels of HDL are high levels of CHO in the diet exceeds 60% of energy. Brehm et al. (2003), states that a low CHO diet such as 15% energy, 28% protein and 60% fat (20% of the SFA) for 6 months will increase HDL levels by 13%.

LIPID PEROXIDATION

Lipid peroxidation is a process that is closely associated with free radicals. Lipid peroxidation is generally a unification of molecular oxygen into PUFAs in biological membranes. PUFA by free radicals occurs in the H atom that is unstable, especially if the attached C atom close to the double bond, so that the newly formed free radicals are very sensitive to oxygen (Sunil & Dinesh 2009). Lipid oxidation results will form one of the wide range of products including malondialdehida.

Malondialdehida (MDA) is one of the end products of lipid peroxidation compounds formed after the radical attack of membrane lipids containing polyunsaturated fatty acids (PUFA). *Cynomolgous monkey* is the type of animal that has a very close phylogenetic to humans that have similarity of aspects of physiology and anatomy. Statistical test results showed that the value of MDA in LDL was not affected by treatment ($p > 0.05$). But descriptively seen that MDA levels in the Cynomolgous group fed BFT, SBO, CFO, and FCFO increased although the increase was within normal limits, but the highest increased were found in a group given CFO diet.

MDA levels in LDL were higher in the group with Cynomolgous feed CFO who can give bad effects on metabolic processes in the body. McCance et al. (2010) and Crawford (1993) states that oxidized LDL is toxic to endothelial cells, causes smooth muscle proliferation, and activates further immune and inflammation responses. The oxidized LDL binds to scavenger receptors of macrophages and causes the formation of foam cells. This will stimulate the gene expression of a number of cytokines and growth factors and lead to proliferation of smooth muscle cells at the intima. As a result of blood vessel walls will swell due to the accumulation of plaque in the media (Crawford 1993; Mc Cance et al. 2010).

However, in the present study, the lipid peroxidation value was higher in cynomolgous fed CFO diet rather than cynomolgous feed FCFO. This finding probably reflects the decreased susceptibility of FCFO because of its high monounsaturated fatty acid and conjugated linoleic acid (CLA) content compared with CFO. MUFA are regarded as the most beneficial type of fatty acid as they do not have a hypercholesterolaemic effect (Thomas & Bishop 2007). Egert et al. 2011 reported that a low-fat diet and a high-fat diet, both rich in MUFA, had similar effects on serum lipids and lipoprotein, LDL sizes and indices of lipid peroxidation in metabolically healthy, young and non obesity men and women.

CONCLUSION

During the intervention the groups which were given intervention with CFO and BFT increased the body weight, whereas the intervention group given SBO and FCFO had lost weight. Intervention with BFT, CFO, FCFO, were increased total cholesterol and LDL levels significantly ($p < 0.05$, but intervention with SBO was decreased them. Intervention with CFO and FCFO also has the potential increasing of triglyceride levels although not statistically significant ($p > 0.05$). Interventions with BFT and CFO significantly lowering levels of HDL ($p < 0.05$), and not significantly reduced lipid peroxidation (MDA) in the LDL ($p > 0.05$). In general it can be concluded that catfish oil significantly cause atherogenic only in blood cholesterol total, but not in the HDL plasma.

ACKNOWLEDGEMENTS

We thank to PT Carmelitha Lestari dan PT. IndoAnilab Bogor, Indonesia for providing the sample and ingredients of this study. The authors are very grateful to Politeknik Kesehatan Kemenkes Jakarta 2 for all the support towards this work. We much appreciate the comments from Ass. Prof. Dr. Nor Fadilah Rajab to improve the clarity and focus of this manuscript.

REFERENCES

Achmad, R.A. 2003. Antioksidan penemuan revolusioner bagi kesehatan. *Majalah Gizi Mindo* 2(5): 4.

Adiyanto, AH. 2010. Studi perkembangan korteks serebri fetus monyet ekor panjang pada umur 120 hari, 150 hari dan neonates. (skripsi).

Brehm, B.J., Seeley, R.J., Daniels, S.R. & D'alessio, D.A. 2003. A randomized trial comparing a very low carbohydrate diet and a calorie-restricted low fat diet on body weight and cardiovascular risk factor in healthy women. *Journal of Clinical Endocrinal Metabolism* 88: 1617-1623.

Binkoski, A.E, Kris-Etherton, P.M., Wilson, T.A., Mountain, M.L. & Nicolosi, R.J. 2005. Balance of unsaturated fatty acids is important to a cholesterol-lowering diet: comparison of mid-oleic sunflower oil and olive oil on cardiovascular disease risk factors. *Journal of American Diet Association* 105(7): 1080-1086.

Cintra, D.E.C., Costa, A.G.V., Peluzio, M.C.G., Matta, S.L.P., Silva, M.T.C. & Costa, N.M.B. 2006. Lipid profile of rats fed high-fat diets based on flaxseed, peanut, trout or chicken skin. *Nutrition* 22: 197-205. Doi:10.1016/j.nut.2005.09.003

Crawford, M.A. l993. The role of essential fatty acids in neural development: implication for perinatal nutrition. *American Journal Clinical Nutrition* 57: 703-710.

Egert, S., Kratz, M., Kannenberg, MF. & Wahrburg, U. 2011. Effects of high-fat diets rich in monounsaturated fatty acid on serum lipids, LDL size and indices of lipid peroxidation in healthy non-obese men and women when consumed under controlled conditions. *Eur. J. Nutr.* 50: 71-79.

Fratiglioni, L., Mangialasche, F. & Qiu, C. 2010. Brain aging: lessons from community studies. *Nutrition Review* 68(12): 119-127.

Gaytan, CN., Shin, D., Sams, AR., Keeton, JT., Miller, RK., Smith, SB. & Plata, MXS. 2011. Lipid oxidation stability of omega 3 and conjugated linoleic acid-enriched *sous vide* chicken meat. *Poultry Science* 90: 473-480. Doi: 10.3382/ps.2010-01002.

Hamer, M. & Steptoe, A. 2006. Influence of specific nutrients on progression of atherosclerosis, vascular function, haemostasis and inflammation in coronary heart disease patients : a systematic review. *British Journal of Nutrition* 95: 849-859.

Hidayati, D. 2005. Pembentukan conjugated linoleic acid (CLA) oleh bakteri asam laktat pada fermentasi susu kedelai. Yogyakarta, Indonesia: Universitas Gadjah Mada, MSc thesis.

Komisi Nasional Lanjut Usia. 2010. Profil Penduduk Lanjut Usia 2009. Jakarta (ID).

Kromhout, D., Geleijnse, J.M., Menotti, A. & Jacob, D.R. 2011. The confusion about dietary acids recommendations for CHD prevention. *British Journal of Nutrition.* 106: 627-632

Lee, J.N., Koo & Min, D.B. 2004. Reactive oxygen species, aging, and antioxidative neutraceuticals: reviews. *Food Science and Food Safety* 3: 21-33.

Lubis, M.I. 1993. Pengaruh minyak ikan lemuru dalam pakan terhadap respons vaskuler kera ekor panjang (Macaca fascicularis) yang hipokolesterolemik. Bogor, Indonesia: Institut Pertanian Bogor. PhD dissertation.

McCance, K.L., Huether, S.E., Brashers, V.L. & Rote NS. 2010. Alterations of cardiovascular function. *Pathophysiology: The Biology Basis for Diseases in Adults and Children 6th Edition. St. Louis*: Mosby Inc.

Mitruka, B.M. & Rawnsky, H.M. 1997. Clinical, Biochemcical, and Hematological Refference Values in Normal Experimental Animals. New York (US): Masson.

Morise, A., Serogne, C., Gripois, D., Blouquit, M., Lutton, C. & Hermier, D. 2004. Effects of dietary alpha linolenic acid on cholesterol metabolism in male and female hamsters of the LPN strain. *J. Nutr. Biochem* 15: 51-61.

Muller, H., Lindman, A.S., Brantsaeter, A.L. & Pedersen, J.I. 2003. The serum LDL/HDL cholesterol ratio is influenced more favorably by exchanging saturated with unsaturated fat than by reducing saturated fat in the diet of women. *Journal of Nutrition*. 133: 78-83.

Oberman, A. 2000. Hypertriglyceridemia and coronary heart disease. *Journal of Clinic Endocrinalin Metabolism* (85)6: 2098-2105.

Ogawa, J., Matsumura, K., Kishino, S., Omura, Y. & Shimizu, S. 2001. Conjungated linoleic acid accumulation via 10-hydroxy-12-octasecaenoic acid durimg microaerobic transformation of linoleic acid by lactobacillus acidophilus. *Journal of Application Environmental Microbiolology* 67: 1246-1252.

Peckenpaug. 2010. *Nutrition Essentials and Diet Therapy*. China: Saunders Elsevier.

Piliang, W.G. & Al Haj, S.D. 2006. *Fisiologi Nutrisi Volume 1*. Bogor (ID): IPB Press.

Ringseis, R. & Eder, K. 2009. Influence of conjugated linoleic acids on functional. *Br. J. Nutr.* 102(8): 1099-116.

Sikoki, B., Kim, Y., Strauss, J. & Witoelar, F. 2011. Well being and support for the elderly in Indonesia: a challenge to policy for elderly, international conference on the population aging explosion: opportunities and challenges. Bali, Indonesia.

Stipanuk, M.H.. 2000. Nutrition, Lipids, and Cardiovascular Disease; *Biochemical and Physiological Aspects of Human Nutrition*. p927-936.

Smith. 2010. Implicit and explicit category learning by macaques (*Macaca mulatta*) and humans (*Homo sapiens*). *Journal of Experimental Psychology*: *Animal Behavior Processes*. 36: 54-65.

Sunil, K. & Dinesh, K. 2009. Antioxidant dan free radical scavenging activities of edible weeds. *Afjand* (Online) 9: 1-17.

Thomas, B. & Bishop, J. 2007. Dietary fat and fatty acids. *Manual of Dietetic Practice*: (164).

Yagi, K. l994. Lipid Peroxides In Hepatic, Gastrointestinal, And Pancreatic Diseases. New York (US): Plenum Pr.

Xu, S., Boylston, T.D. & Glatz, B.A. 2004. Effect lipid source on probiotic bacteria and conjugated linoleic acid formation in milk model systems. *Journal of the American Oil Chemists' Society* 61: 589-595.

World Health Organization. 2003. *Diet, Nutrition and the Prevention of Chronic Diseases*. Report of a Joint WHO/FAO Expert Consultation (5).

Iskari Ngadiarti
JNutrition Department
Health Polytechnic Jakarta
Jl. Hang Jebat III/F3 Kebayoran Baru Jakarta Selatan 12120

Clara M. Kusharto
Dodik Briawan
Sri Anna Marliyati
Department of Communicty Nutrition
Faculty of Human Ecology
Bogor Agricultural University, Darmaga Campus
Bogor 16680

Dondin Sajuthi
Primate Research Centre,
Bogor Agricultural University
Darmaga Campus, Bogor 16680

Correspondence author: Iskari Ngadiarti
Email address: iskaringadiarti@gmail.com
Tel: (021)-7300916

Received: April 2013
Accepted for publication: November 2013