International Journal of Sciences: Basic and Applied Research (IJSBAR)

ISSN 2307-4531
(Print & Online)

http://gssrr.org/index.php?journal=JournalOfBasicAndApplied

Catfish (Clarias gariepinus) Biscuit and Oil Supplementation and its Effect on Lipid Profile, Oxidative Stress Markers and Cognitive Function of the Elderly

Nunung Cipta Dainy[a], Clara M Kusharto[b], Siti Madanijah[c], Martina Wiwie Setiawan Nasrun[d*]

[a]Doctoral Student in Bogor Agricultural University Graduate School
[b, c]Department of Community Nutrition, Faculty of Human Ecology, Bogor Agricultural University
[d]Department of Psychiatry, Faculty of Medicine, University of Indonesia
[a]E-mail: nciptadainy@gmail.com
[b]E-mail: kcl_51@yahoo.co.id
[c]E-mail: smadanijah@yahoo.com
[d]E-mail: martina_wiwie@yahoo.com

Abstract

Cognitive impairment is a classic problem among the elderly. One of the risk factors of cognitive decline is the high levels of lipid profile. Deep-sea fish oil has been studied, and it has beneficial effect for improving lipid profiles. However, no studies have evaluated the beneficial effects of freshwater fish oil for improving lipid profiles. Catfish biscuit and oil supplementation is expected to improve the level of lipid profiles and maintain cognitive function of the elderly. We used a pre-post, single-blinded, randomized controlled trial (RCT) design. A total of 67 subjects with dyslipidemia aged 45-74 years were randomly selected and divided into four groups, as follows: Placebo (P); Catfish Biscuit (CB); Catfish Oil (CO); and Catfish Biscuit and Catfish Oil (CBCO). The intervention was conducted for 60 days (January – March 2015). Venous blood sample was collected and used to determine the pre- and post-intervention lipid profiles.

---

* Corresponding author.

Cognitive function was assessed by using MMSE, digit span test, ROCF test and TMT-B. The results showed that there were significant decreases in triglyceride and MDA levels ($p<0.05$) and significant improvement in cognitive function ($p<0.05$) in CBCO group. Therefore, CBCO supplementation for 60 days may be able to suppress the increase in cholesterol, LDL and ox-LDL levels; lower the triglyceride and MDA levels; and improve the cognitive function of the elderly.

Keywords: catfish biscuit; oil; cognitive; elderly; lipid profile; oxidative stress.

1. Introduction

Along with the advances in healthcare levels and decreased number of birth, the number of elderly population also increased. This situation does not only happen in developed countries, but also in developing countries. As stated in Indonesian Law No. 13/1998 on Welfare of The Elderly, those above 60 years of age are defined as the elderly. Meanwhile, according to WHO, the elderly are classified into four groups as follows: middle age (45-59 years), elderly (60-74 years), old (75-90 years) and very old (above 90 years).

Elderly is the age group vulnerable to malnutrition, due to the physical changes and the decreased ability of organ functions occuring at this age group. Energy requirement in elderly people declines because of the changes in body composition. Energy adequacy rate recommended for the elderly in Indonesia is 1500-1900 kcal [1]. Their protein requirement is relatively constant, 55-57 g/day for elderly women and 60-65 g/day for elderly men. However, it is quite difficult to fulfilled due to the decreased total energy requirement and decreased gustatory function.

Cognitive impairment (especially memory) is a classic problem among the elderly, and it was one of the states of preclinical Alzheimer's disease (AD). AD is the most common form of dementia, and is found in 50% to 70% of all dementia cases. Elderly person with dementia will experience a gradual decrease in brain function. The risk factors associated with dementia are dyslipidemia, diabetes mellitus and depression [2]. Elderly people with Alzheimer's disease will lose their independence in doing activities of daily living, thus becoming a burden to the family and people around them. Therefore, a study on the healthy food supplementation that can reduce the risk of Alzheimer's disease is urgently needed, to achieve the better health for the elderly in the future.

Previous study showed that the elderly people with adequate cognitive capacity (Mini Mental State Examination/MMSE score ≥ 28) consumed a greater amount of total food and fish, and had an adequate intake of fatty acids [3]. In general, elderly people with satisfying intellectual functioning have a better diet. This shows the importance of proper nutrition in the maintenance of cognitive function. One of the nutrients that plays a role in maintaining cognitive function is essential fatty acids. Essential fatty acids, such as polyunsaturated fatty acids/PUFA (omega-3 and omega-6) are mostly found in fish oil. Omega-6 serves as a precursor for the synthesis of arachidonic acid (AA), while omega-3 acts as a precursor for the synthesis of eicosapentaenoic acid (EPA). Both of them are the molecules involved in maintaining the integrity of brain cells. Several studies have revealed that high-concentration PUFA can affect neuron membranes and neurotransmission [4].

This study aimed to analyze the effect of catfish biscuit and oil supplementation on lipid profiles, oxidative stress markers and cognitive function in the elderly.

2. Material and methods

2.1. Design, Location, and Time of Study

This study used an experimental method with randomized controlled trial (RCT) design. It was conducted in Limo Sub-district, Depok. The subjects were selected from the elderly people who met the inclusion criteria. The intervention was conducted for 60 days, starting from January to March 2015. Analysis of blood lipid profiles was performed in Regional Health Laboratory, located in Bogor City. Analysis of oxidative stress markers was performed in Laboratory of Physiology, Faculty of Medicine, Brawijaya University, located in Malang.

2.2. Materials and Instruments of Study

Catfish biscuit composition consisted of wheat flour, catfish flour, sweet potato flour, soy isolate, sugar, margarine and eggs; while the placebo biscuit composition consisted of wheat flour, modified cassava flour (mocaf), sugar, margarine and eggs. The nutrient content considered superior in catfish flour was protein (18.04%), while its content in placebo biscuit was only 4.57%. Biscuit supplementation in this study was a snack substitute. The biscuits that were given 50 g/day could contribute 234 kcal of energy intake [5]. Catfish oils used in this study were prepared from catfish milling with guaranteed hygiene and sanitation. The fatty acids contained in catfish oil were as follows: SFA (26.48%), MUFA (32.53%) and PUFA (19.76%) [6].

The instruments used during intervention were microtoise; weight scale; catfish biscuit and oil distribution tools; and tools for blood sample collection and analysis of serum malondialdehyde (MDA), superoxide dismutase (SOD), and lipid profile levels of the subjects.

2.3. Research Subjects

The research subjects had to meet these inclusion criteria: 1) Men or women aged 45-74 years; 2) had one of the following blood lipid profile abnormalities, i.e. total cholesterol (TC) > 200 mg/dL, LDL cholesterol (LDL-c) > 130 mg/dL, tryglicerides (TG) > 150 mg/dL or HDL cholesterol (HDL-c) < 40 mg/dL; 3) able to perform basic activities of daily living normally; 4) signed the informed consent.

Minimum sample size required for this study, with a test power of 90% and $p<0.05$ was seven people, based on the previous study by Rajkumar *et al*. [7]. Three subjects were added to anticipate the possibility of drop-out; thus, the minimum sample size was 10 people. There were four types of intervention, i.e. 1) placebo (P); 2) catfish oil (CO); 3) catfish biscuit (CB); 4) catfish biscuit and catfish oil (CBCO).

2.4. Stages of Study

The first stage was selection of the target subjects who met the inclusion criteria, based on the membership data of Poslansia (Integrated Health Service Post for the Elderly) Dahlia Senja. The target subjects were then invited to research dissemination activities, to be given an explanation of the benefits they would acquire, and asked to sign the informed consent if they were willing to participate in the study. In the next stage, we invited the subjects to be present at the time of blood sampling. The ones who were willing to have their blood drawn would be designated as the research subjects. They were then interviewed about demographic data and food consumption, as well as having their weight and height measured. Only the subjects with complete data that would be analyzed in this study.

2.5. Data Type and Collection Method

The data collected in this study were primary data, as follows: the data concerning subjects' characteristics, which included personal information, food consumption and results of anthropometric measurements (body weight and height); cognitive function data that were obtained by using Mini Mental State Examination (MMSE) questionnaires, backward digit span test, Rey–Osterrieth Complex Figure (ROCF) test and Trail Making Test Part B (TMT-B); subjects' depression level, which were assessed by using a Geriatric Depression Scale (GDS) questionnaire; and the data obtained from biochemical analysis of blood, i.e. lipid profiles (TC, TG, LDL-c and HDL-c) and oxidative stress markers (MDA and oxidized LDL).

2.6. Data Processing and Analysis

The first data analysis conducted was a descriptive measurement of some parameters, such as individual and socio-economic characteristics. Some of the measures were analyzed, such as mean, standard deviation, minimum and maximum values. Statistical analyses for blood biochemical parameters were performed in several stages. In the first stage, the data were assessed for their normality by Kolmogorov-Smirnov test. If $p > 0.05$, the data were considered to be normally distributed. Paired t-test was performed to analyze the biochemical changes in blood in each intervention group before and after the intervention. Meanwhile, ANOVA test was performed to compare the intervention groups with the control group.

2.7. Ethical Considerations

Ethical clearance for this study was approved by Health Research Ethic Committee of the Faculty of Medicine, University of Indonesia, Cipto Mangunkusumo General Hospital, Jakarta. It was obtained in the form of Ethical Approval No. 94/UN2.F1/ETIK/2015 dated February 4, 2015.

3. Results and Discussion

3.1. Subjects' characteristics

Of 140 people recruited as research subject, only 72 people who met the inclusion criteria. Five subjects dropped out during the study. In the end, only 67 research subjects completed the whole intervention. Table 1 showed the data of subject's characteristics, including age, education, occupation, physical activity and body mass index (BMI). Results of statistical analyses indicated that there were no significant differences ($p>0.05$)

between the intervention groups. Mean subjects' age was 59.6±7.2 years. The most frequent daily activities performed by the subjects were household chores such as cleaning, cooking and taking care of their grandchildren. Meanwhile, most of the subjects who worked outside had occupations that did not demand much physical effort; such as teacher, office employee and stall-keeper.

Table 1: Distribution of the subjects based on their characteristics

Variable	Intervention groups				p-value
	P	CO	CB	CBCO	
Age (years)	62.6±6.92	57.9±9.02	59.2±5.93	58.6±6.59	0.24
Education	1.76±0.43	1.94±0.25	1.87±0.35	1.83±0.38	0.59
Occupation	1.41±0.50	1.22±0.44	1.44±0.62	1.39±0.60	0.77
Physical activity	1.70±0.16	1.66±0.07	1.64±0.18	1.60±0.11	0.24
BMI	27.28±4.52	26.61±4.10	23.77±5.83	26.44±2.21	0.11

Notes: P = placebo; CO = catfish oil; CB = catfish biscuit, CBCO = catfish biscuit and catfish oil; BMI = body mass index.

Most of the subjects had been through the primary education. National Socio-Economic Survey (SUSENAS) reported that most of the elderly people had low education level. More than half of the elderly citizens (56.8%) had no any academic certificate [8]. Education is strongly needed to develop the elderly's potential of life, so that they will remain productive and have active role in community. Literacy, academic certificate owned by them, and experience in formal education can be indicators of their readiness to go through the old age.

Subjects' physical activity was measured by physical activity level (PAL) values. The results showed that most of the subjects were categorized as having sedentary or moderately active lifestyles (PAL<2), and there were no significant differences found between the intervention groups. BMI was measured at the beginning and end of the study. The results indicated that there was no significant difference in BMI values among the intervention groups. Some subjects had normal BMI and the rest were overweight (BMI ≥ 18.5 kg/m^2).

3.2. Subjects' nutrient intakes

Table 2 showed that the mean percentage of energy and macronutrient adequacies among the intervention groups were not significantly different. In conclusion, subjects' daily nutrient intakes were fulfilled during the intervention period.

3.3. Effect of Intervention on Lipid Profiles

TC levels increased in all intervention groups, but the significant increase was found in groups without catfish oil intervention (P and CB) (Table 3). These results indicated that catfish oil had a tendency to suppress the increase in TC levels. However, after ANOVA test was performed, there was no significant difference in TC levels between the intervention groups.

Table 2: Percent daily nutrient adequacy among the intervention groups

Variable	Intervention groups				p-value
	P	CO	CB	CBCO	(ANOVA)
Energy (kcal)	69.75±24.15	69.45±17.45	66.75±14.20	69.35±19.00	0.794
Protein (g)	66.55±23.90	65.75±20.75	62.95±19.80	78.90±34.10	0.280
Fat (g)	86.35±38.75	86.75±42.15	80.50±33.00	80.25±34.40	0.798
Carbohydrates (g)	64.45±24.20	63.70±17.15	64.10±21.95	66.45±22.30	0.856

Table 3: Results of lipid profile test

Variable	Intervention groups				p-value
	P	CO	CB	CBCO	(ANOVA)
TC					
Pre	213.06±41.06	218.93±31.04	196.88±44.38	210.89±32.77	
Post	238.06±46.91	228.07±48.73	217.56±48.07	225.56±19.55	
p-value (paired t-test)	0.002*	0.284	0.023*	0.058	
delta	25.00	8.67	11.42	14.67	0.354
TG					
Pre	132.43±61.55	118.31±66.08	108.71±52.73	135.13±54.64	
Post	146.14±51.64	113.50±32.59	137.50±73.09	125.50±51.35	
p-value (paired t-test)	0.037*	0.383	0.082	0.049*	
delta	30.85[b]	-15.42[a]	15.23[ab]	-14.29[a]	0.014*
LDL-c					
Pre	125.94±39.00	136.13±24.83	113.56±35.18	127.39±27.14	
Post	140.25±43.06	146.94±39.92	123.27±42.89	139.28±19.60	
p-value (paired t-test)	0.031*	0.147	0.105	0.086	
delta	16.80	6.61	10.33	11.88	0.734
HDL-c					
Pre	53.12±8.09	55.13± 8.72	56.25±11.47	57.00±9.01	
Post	60.00±10.31	54.06±14.72	58.75±9.34	59.94±8.05	
p-value (paired t-test)	0.109	0.841	0.485	0.449	

TG levels decreased in the groups receiving catfish oil intervention (CO and CBCO) and increased in those without catfish oil intervention (P and CB). ANOVA test showed that TG levels were significantly different. Based on Duncan’s post hoc test, TG levels were significantly different between placebo group (P) and groups with catfish oil intervention (CO and CBCO). This indicated that catfish oil supplementation significantly lowered the TG levels (p=0.014).

LDL-c levels increased in all intervention groups, but the significant increase was only found in placebo group. ANOVA test showed that the differences in LDL-c levels in each group were not significantly different. HDL-c increased in all intervention groups, but the increase was not significant.

3.4. Effect of Intervention on Oxidative Stress Markers

MDA levels decreased significantly in the groups with catfish oil or catfish biscuit interventions (CO, CB and CBCO), and increased in the placebo group. However, results of ANOVA test demonstrated that the values were not significantly different between interventions. The opposite effects were found in oxidized LDL (ox-LDL) levels. Ox-LDL levels increased significantly in the groups with single intervention (P, CO and CB), but the increase in CBCO group was not significant. These results showed that CBCO intervention had a tendency to suppress the increased ox-LDL levels. ANOVA test indicated that ox-LDL levels were not significantly different between the intervention groups (Table 4).

Table 4: Results of MDA and ox-LDL analyses

Variable	Intervention groups				p-value
	P	CO	CB	CBCO	(ANOVA)
MDA (ng/ml)					
Pre	232.96±38.90	261.33±41.97	251.71 ±31.91	266.78±47.90	
Post	222.25±19.28	219.33±19.42	223.14±22.52	227.25±24.23	
p-value (paired t-test)	0.308	0.003*	0.024*	0.022*	
delta	-10.71	-42.00	-28.57	-39.53	0.293
Ox-LDL (pg/ml)					
Pre	643.01±124.08	576.13±146.67	591.28±122.96	681.27±169.58	
Post	801.43±144.67	759.91±152.61	792.23±140.38	761.14±139.33	
p-value (paired t-test)	0.007*	0.009*	0.000*	0.153	
delta					0.369

MDA is a derivative product resulting from the presence of ox-LDL. However, our study showed that despite the increased ox-LDL levels, the amount of MDA decreased. This might occur because the body had natural defenses, in the form of antioxidants. Previous researchers states that an antioxidant is a molecule which can prevent the negative effects of oxidation and protect the cells and tissues in the body from the damage caused by free radicals [9]. Catfish oil in our study contained monounsaturated fatty acids (MUFA) and PUFA, which also acted as antioxidants. Previous study showed that fish oil could reduce oxidative stress levels in adult subjects [10]. These results were consistent with another study, which indicated that rich-in-antioxidant diet could decrease the MDA levels [11].

3.5. Effect of Intervention on Cognitive Function

Cognitive function was assessed by using MMSE, backward digit span test, ROCF test and TMT-B.

3.5.1. Mini Mental State Examination (MMSE)

Table 5 showed that MMSE scores increased in all intervention groups, but the significant increase (p=0.010) was only found in CBCO group. However, based on ANOVA test, there were no significant differences between the intervention groups (p>0.05).

Table 5: MMSE results

Variable	Intervention groups				p-value
	P	CO	CB	CBCO	
Pre	20.00±4.50	24.46±4.48	24.23±4.72	22.53±5.24	
Post	22.43±3.18	26.08±3.96	23.77±4.86	25.65±2.84	
p-value	0.086	0.127	0.684	0.010*	
delta	2.429	1.615	0.462	3.118	0.316

The increase in MMSE scores was in line with preclinical studies, which showed that fish oil containing PUFA could maintain cognitive function by maintaining membrane fluidity, improving synaptic and neurotransmitter functions, improving academic performance and memory. It also had neuroprotective effects. Besides, PUFA also reduced β-amyloid accumulation in blood vessels [12]. Initial studies of Rondanelli *et al*. [13] showed that there was a significant improvement in semantic verbal fluency test of the subjects who received an intervention. Significant differences in values in CBCO group indicated that there was a synergistic effect of catfish biscuit and catfish oil on cognitive function improvement.

3.5.2. Backward Digit Span Test

Digit span is a test to assess attention, concentration and short-term verbal memory. It is routinely used in psychological studies, either as a stand-alone test or as a part of a series of psychological assessment [14].

Table 6 showed the subjects' digit span test results. It could be seen that there were no significant differences between digit span scores at the baseline and the ones at the endline. Short-term memory is used for temporary information, which usually occurs within a few seconds. Conceptually, short-term memory is an active information storage, while long-term memory is a passive information storage.

3.5.3. Rey-Osterrieth Complex Figure (ROCF)

Rey-Osterrieth Complex Figure (ROCF) test is widely used in neuropsychological assessment, especially in assessing perceptual organization, visuospatial constructional ability and visual memory. Complex images in ROCF test reflects the executive functions such as planning and organizational capabilities, besides the visual cognitive function [15]. There are two stages of drawing test in ROCF test [16], in which the subject has previously practiced copying the figures. In the first stage of the test, the subject is asked to draw ROCF on a separate sheet, immediately after figure-copying practice. In the second stage, after 30-minute delay, the subject is asked to reproduce the figure.

Table 6: Results of digit span test

Variable	Intervention groups			
	P	CO	CB	CBCO
Pre	2.7±1.3	3.3±1.3	2.4±1.2	3.0±1.2
Post	2.8±1.1	3.2±1.2	2.6±1.0	3.1±0.9
p-value	0.855	0.792	0.333	0.750

Table 7: Results of ROCF test

Variable	Intervention groups				p-value
	P	CO	CB	CBCO	
Immediate recall					
Pre	8.10±7.76	11.90±8.86	8.31±7.71	7.76±5.87	
Post	9.26±8.41	15.20±10.72	12.06±8.57	13.58±6.48	
p-value	0.415[a]	0.016*	0.014*	0.000*	
delta	1.167[a]	3.300[ab]	3.750[ab]	5.824[b]	0.072
Delayed recall					
Pre	8.20±7.53	11.66±9.06	8.31±6.85	7.58±4.93	
Post	8.72±8.51	14.63±11.38	11.09±7.41	12.14±6.08	
p-value	0.718	0.022*	0.037*	0.000*	
delta	0.520 [a]	2.967 [ab]	2.781 [ab]	4.559 [b]	0.108

Table 7 showed that there were significant changes between pretest and posttest scores. There was a significant improvement ($p<0.05$) in ROCF scores in all intervention groups, except placebo group. However, after the results were analyzed by Duncan's post hoc test, there were no significant differences in ROCF score improvement between the intervention groups. These findings suggested that there was a tendency that catfish oil and catfish biscuit supplementation could improve the subjects' ROCF scores, either given separately or simultaneously.

3.5.4. Trail Making Test (TMT)

Trail Making Test (TMT) is one of the most common neuropsychological test used in clinical practice to assess executive function, because this instrument is sensitive to brain damage [17]. TMT test is related to the role of frontal lobe. The frontal lobe plays a role in working memory, complex attention, planning, cognitive set shifting, response inhibition, problem solving and executive functions. There are two types of TMT, i.e. TMT-A and TMT-B. In TMT-B, subjects are assigned to connect the lines alternating between numbers and letters (1, A, 2, B, 3, C, etc.) properly as instructed. The time taken to complete the test is recorded as TMT-B score.

The test conducted in this study was TMT-B, because the subjects were classified as adult age group. TMT-B scores were categorized into normal and abnormal (Table 8). Status parameters of the subjects categorized as having normal cognitive function were determined by age and the time spent to complete TMT-B, as follows: a) age group of 40-49: ≤ 100 seconds; b) age group of 50-59: ≤ 135 seconds; c) age group of 60-69: ≤ 170 seconds; d) age group 70-79: ≤ 280 seconds [18].

Table 8: TMT-B results

Variable	Intervention groups				p-value
	P	CO	CB	CBCO	
Pre					0.89
Normal	0	1	1	0	
Abnormal	12	14	12	14	
Post					0.82
Normal	1	4	3	2	
Abnormal	11	11	10	12	

TMT-B results showed that there were no significant differences in TMT-B scores, based on the intervention given. However, there was a correlation between demographic factors and TMT-B scores. Age was significantly correlated with baseline scores (r=-0.24; p=0.05), while education had a significant correlation with endline scores (r=0.411; p=0.00). These results were in accordance with the previous study [19], which suggested that demographic variables (age and education) were significantly correlated with TMT scores.

In general, our findings showed that with relatively homogenous subjects' characteristics, CBCO intervention was able to improve TG levels significantly and synergistically with the decrease in MDA levels and cognitive function improvement characterized by the improvement of MMSE and ROCF scores. The decreased TG levels in this study was consistent with the results in previous study, suggesting that supplementation with fish oil containing omega-3 PUFAs was effective to decrease plasma TG [20]. Supplementation with 1 g fish oil/day decreased plasma TG levels by 15% after 60-day consumption. The decrease in TG levels was a result of decreased production of very-low-density lipoprotein (VLDL) in the liver, and the increased VLDL clearance.

Fish oil can decrease VLDL production through several mechanisms, one of which is by reducing the transport of non-esterified fatty acids (NEFAs) to the liver [21]. NEFAs are the main fatty acids in VLDL production, besides fatty acids from diet and de novo lipogenesis [22,23]. Fish oil plays a role in preventing intracellular lipolysis, one of which is by suppressing adipose tissue inflammation. Fish oil increases extracellular lipolysis by the action of lipoprotein lipase (LPL) in adipose tissue, heart and skeletal muscles. It also increases βoxidation in the liver and skeletal muscles, which contributes to reducing fatty acid transport to the liver [24]. Decreased VLDL production is associated with improved cognitive function, because cognitive function improvement is initiated by the decreased LDL and VLDL oxidation; thereby reducing amyloid plaque accumulation that inhibits the transfer of information between neurons.

Our study has strength and limitations. To the best of our knowledge, our study is the first one to analyze the effect of catfish biscuit and catfish oil supplementation on lipid profiles and oxidative stress markers, and its association with cognitive function; thus, becoming the strength of this study. One of this study's limitations is small number of subjects. Subjects were selected from one Poslansia only so that we did not get a great number of subjects, but still met the minimum sample size requirement. Sampling site was chosen purposively, thus the results of this study could not be generalized. Besides that, the education variable in this study was only categorized as "attending formal education" and "not attending formal education", because there was not a lot of variation in subjects' education level (most of them were primary school graduates). If similar study will be conducted in the future, the data obtained from cognitive function test should be grouped based on subject's education level.

4. Conclusion and Recommendations

Supplementation with 50 g catfish biscuit/day and 1 g catfish oil/day for 60 days could significantly reduce TG levels by 15%, MDA levels by 14%, and tended to suppress the increased LDL-c and ox-LDL levels if compared with placebo. Cognitive function could also be well maintained in subjects receiving CBCO intervention. It is proven by the improvement in their MMSE and ROCF scores that was significantly different than the placebo group. Based on our findings, CBCO supplementation was chosen as the best intervention in improving lipid profiles and cognitive function of the elderly.

Acknowledgement

We would like to thank The Ministry of Research, Technology and Higher Education for the financial support in conducting this research, as part of Competitive Research Grant entitled "*Functional food with high protein and mineral enriched by fish oil by-product of catfish (Clarias gariepinus) flour as nutritious and emergency food for elderly*" with Prof. Dr. Clara M Kusharto, MSc as the principal investigator.

References

[1]. Kementerian Kesehatan Republik Indonesia (Ministry of Health, Republic of Indonesia). Angka Kecukupan Gizi (AKG) 2013 (Recommended Dietary Allowance in 2013). Jakarta: Kementerian Kesehatan Republik Indonesia (Ministry of Health, Republic of Indonesia), 2014.

[2]. M.W.S. Nasrun. Buku Pedoman Skoring: Hendaya Kognitif Non Demensia (HKND) pada Populasi Brain at risk" (Scoring Guidebook: Cognitive Impairment with No Dementia in "Brain at Risk" Population). Jakarta: Interna Publishing - Pusat Penerbitan Ilmu Penyakit Dalam (Publication Center of Internal Medicine), 2009.

[3]. R.M. Ortega, A.M. Requejo, F. Robles, B. Navia, M. Faci and A. Aparicio. “Influence of nutrition on cognitive function in a group of elderly, independently living people”. European Journal of Clinical Nutrition, vol. 57 (Suppl), pp. S54–S57, Sep. 2003.

[4]. J.L. Cooper. “Dietary lipids in the aetiology of Alzheimer’s disease: implications for therapy”. Drugs Aging, vol. 20 (6), pp. 399-418, 2003.

[5]. C.M. Kusharto, S.A. Marliyati, I.Surono. ”Makanan fungsional kaya protein, mineral dan minyak by-product penepungan ikan lele (Clarias gariepinus) sebagai nutritious and emergency food untuk lansia” (Functional food with high protein and mineral enriched by fish oil by-product of catfish [Clarias gariepinus] flour as nutritious and emergency food for elderly). Laporan Penelitian Hibah Kompetensi. LPPM IPB. Bogor. 2012.

[6]. M. Srimiati, C.M. Kusharto, I. Tanziha and S.H. Suseno. “Pengaruh pemberian minyak ikan lele (Clarias gariepinus) yang diperkaya omega 3 terhadap profil lipid lansia (Effect of catfish oil intervention [Clarias gariepinus] enriched with omega-3 on the elderly’s lipid profile)”. Master’s Thesis, Graduate School of Bogor Agricultural University, Bogor, 2016.

[7]. H. Rajkumar, N. Mahmood, M. Kumar, S.R. Varikuti, H.R. Challa, and S.P. Myakala. “Effect of probiotic (vsl#3) and omega-3 on lipid profile, insulin sensitivity, inflammatory markers, and gut colonization in overweight adults: a randomized, controlled trial”. Mediators of Inflammation, vol. 2014, Article ID 348959, 8 pages, Mar. 2014.

[8]. Badan Pusat Statistik (Statistics Indonesia). “Statistik Penduduk Lanjut Usia 2014 Hasil Survei Sosial Ekonomi Nasional (Statistics of Elderly Citizen in 2014, National Socio-Economic Survey Results)”. Jakarta: Badan Pusat Statistik (Statistics Indonesia), 2015.

[9]. S. Valtaena, D. Del Rio, N. Pellegrini, D. Ardigo, L. Franzini, S. Salvatore, et al. “The total antioxidant capacity of the diet is an independent predictor of plasma β-carotene”. European Journal of Clinical Nutrition, vol. 61, pp. 69-76, Jan. 2007.

[10]. A. Pipingas, S. Andrew, D.C. Kevin, S.J. Andrzej, J.J. Alicia, A.M. Trevor, et al. “Fish oil and multivitamin supplementation reduces oxidative stress but not inflammation in healthy older adults: A randomised controlled trial”. Journal of Functional Foods, vol. 19, pp. 949–957, Dec. 2015.

[11]. J. Beltowski, G. Wojcicka, D. Gorny and A. Marciniak. “The effect of dietary-induced obesity on lipid peroxidation, antioxidant enzymes and total plasma antioxidant capacity”. Journal of Physiology and Pharmacology, vol. 51, pp. 883-896, Dec. 2000.

[12]. D.P. de Souza Fernandes, F.A.C. Rezende, G.R. Pereira, M.D.S. Filgueiras, P.R.S. Moreira and R.C.G. Alfenas. “Effect of eicosapentaenoic acid and docosahexaenoic acid supplementations to control cognitive decline in dementia and Alzheimer’s disease: a systematic review”. Nutrición

Hospitalaria, vol. 32, pp. 528-533, Aug. 2015.

[13]. M. Rondanelli, A. Opizzi, M. Faliva, M. Mozzoni, N. Antoniello, R. Cazzola, et al. “Effects of a diet integration with an oily emulsion of DHA-phospholipids containing melatonin and tryptophan in elderly patients suffering from mild cognitive impairment”. Nutritional Neuroscience, vol.15, pp. 46-54, Mar. 2012.

[14]. G. Jones and B. Macken. “Questioning short-term memory and its measurement: Why digit span measures long-term associative learning”. Cognition, vol. 144, pp. 1–13, Nov. 2015.

[15]. T. Ogino, K. Watanabe, K. Nakano, Y. Kado, T. Morooka, A. Takeuchi, et al. “Predicting executive function task scores with the Rey-Osterrieth Complex Figure”. Brain and Development, vol. 31, pp. 52–57, Jan. 2009.

[16]. L.B. Taylor. “Localization of cerebral lesions by psychological testing”. Clinical Neurosurgery, vol. 16, pp. 269–287, 1969.

[17]. B. Cangoz, E. Karakoc, K. Selekler. “Trail Making Test: normative data for Turkish elderly population by age, sex and education”. Journal of the Neurological Sciences, vol. 283, pp. 73–78, Aug. 2009.

[18]. M.W.S. Nasrun. “Deteksi dini hendaya kognitif non demensia pada penyandang diabetes melitus tipe 2: pendekatan epidemiologi klinis, psikometrik dan spektroskopi resonansi magnetic (Early detection of cognitive impairment with no dementia in patients with type 2 diabetes mellitus: clinical epidemiology, psychometric and magnetic resonance spectroscopy approaches)”. Dissertation, Doctoral Study Program of Medical Science, Faculty of Medicine University of Indonesia, Jakarta, 2007.

[19]. T.N. Tombaugh. “Trail Making Test A and B: Normative data stratified by age and education”. Archives of Clinical Neuropsychology, vol.19, pp. 203–214, Mar. 2004.

[20]. G.C. Shearer, O.V. Savinova and W.S. Harris. “Fish oil -- how does it reduce plasma triglycerides?” Biochimica et Biophysica Acta, vol. 1821, pp. 843–851, May 2012.

[21]. B.R. Barrows, M.T. Timlin and E.J. Parks. “Spillover of dietary fatty acids and use of serum nonesterified fatty acids for the synthesis of VLDL-triacylglycerol under two different feeding regimens”. Diabetes, vol. 54, pp. 2668–2673, Sep. 2005.

[22]. M.K. Hellerstein, M. Christiansen, S. Kaempfer, C. Kletke, K. Wu, J.S. Reid, et al. “Measurement of de novo hepatic lipogenesis in humans using stable isotopes”. The Journal of Clinical Investigation, vol. 87, pp. 1841–1852, May 1991.

[23]. A. Vedala, W. Wang, R.A. Neese, M.P. Christiansen and M.K. Hellerstein. "Delayed secretory pathway contributions to VLDL-triglycerides from plasma NEFA, diet, and de novo lipogenesis in humans". Journal of Lipid Research, vol. 47, pp. 2562–2574, Nov. 2006.

[24]. J.M. Miles, Y.S. Park, D. Walewicz, C. Russell-Lopez, S. Windsor, W.L. Isley, et al. "Systemic and forearm triglyceride metabolism: fate of lipoprotein lipase-generated glycerol and free fatty acids". Diabetes, vol. 53, pp. 521–527, Mar. 2004.