The Quality of Rat Brain Spatial Memory and Expression of Peroxisome Proliferator Activated Receptor (PPAR) Which Fed with Seluang (*Rasbora spp*.)

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***Abstract*—Seluang fish (*Rasbora spp*.) besides contain high protein also contain an essential fatty acids (PUFA), which can increase intelligence. The objective of this research was to prove that Seluang fish is one of the nutrient source to increase the spatial memory and expression of PPAR brain tissue by using *Rattus norvegicus* as a model. Rats were divided into 3 groups: control group were fed standard (C), Treatment Group 1 were fed low-protein and fat (P1), Group Treatment 2 (P2) were fed standard with Seluang Formula, for 8 weeks. The first phase was to testing spatial memory using the Morris Water Maze. The second phase, was the making brain tissue's slide preparation, immunohistochemical stained, calculated the percentage of PPARα and PPARγ expression. The data analysed using ANOVA and Kruskal Wallis test with a 95% confidence level. The first phase results showed the percentage of period time in the target area for the P2 group is the highest compared to the C and P1 group, respectively 33.52%; 29.9%; and 23.15% (p = 0.021). On PPARα and PPARγ examination showed the average percentage of PPARα neurons expression in group P2 is higher than the C and P1 group, respectively 18.5%, 3.75% and 2.5% (p = 0.002) Average percentage of total PPARγ neuronan cells expression in P2 group also higher than the C and P1 group, respectively 35.41%, 6.25% and 5.83% (p = 0.001). The study shows that rats fed with Seluang Fish Formula able to improved the brain tissue's spatial memory and PPAR expression.**

***Index Terms*—seluang fish (*Rasbora Spp*), spatial memory, PPAR expression.**

I. Introduction

South Kalimantan included in 18 provinces that have low weight toddler, above the national rank. In 2010, prevalence of bad nutrition was 6%, low nutrition was 16.8%, stunting toddler was 35.3%. It still above the RPJMN target in 2014 (32%) [1]. It is a bad news, because South Kalimantan has a lot of natural resources, like freshwater fish. The freshwater fish consumption level of South Kalimantan community was 36.84 kg/period [2]. This amount is still higher than the National freshwater fish consumption level that around 33.89 kg/period, but it still lower than Malaysia (55.4 kg/period) and Singapore.

Seluang fish (*Rasbora spp*.) is a freshwater fish which famous and common consumed among South Kalimantan community. This is a potential opportunity to do research of South Kalimantan Seluang fish to response the nutritional problems in South Kalimantan in particular and Indonesia in general.

The role of nutrition in infancy greatly affect intelligence. Children who have less or poor nutritional status and short or very short children are at risk of losing intelligence or Intelligence Quotient (IQ) by 10-15 points [3]. Nutrients, especially protein, minerals, vitamins and essential fatty acids are needed in the brain cells development. Studies showed that by giving dietary omega-3 fatty acids in rats can increase PPAR gene expression which furthermore improves the learning and spatial memory. Omega-3 fatty acids increase the membrane fluidity by replacing the omega 6 and cholesterol from the cell membrane, maintaining membrane fluidity optimaly is necessary to neurotransmitter binding and signaling in cells. Omega 3 incorporation in the nerve cells membranes also increased the synaptic proteins expression and strengthen plasticity synapses in hypocampus. This process is modulated by transcription factors which is peroxisome proliferator-activated receptors (PPARs) [4].

Omega 3 of fatty acid commonly found in fish as in Seluang fish from South Kalimantan. Furthermore

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research is need to recognize all its potentials as omega 3 fatty acid resource that can increase intelligence. The model in this study are rats (*Rattus norvegicus)*. This study aims to determine the rats spatial memory quality that fed with Seluang fish compared to standard and low protein feed.

II. Material and Method

A. Material

Material in this study were 30 male rats within age of 4 weeks old (*Rattus norvegicus*), rat's blood, aquades, deionized water, standard rats feed, rat feed with low fat and protein, Seluang fish formula, antibodi PPARγ (rat), antibodi PPARα (rat), kit imunostaining, formalin, eter, DAB chromogen, alcohol, formalin 10%, eter, Morris Water Maze (MWM) device.

B. Procedure

Rats devided into 3 groups, control (C) that were given by standard feed, treatment 1 (P1) that given low protein feed (dry rice that was made into pellet), it is equivalent to 4% low protein [5]. Treatment 2 (P2) that given Seluang fish extract powder feed, consist of 25% standard feed and 75% Seluang fish extract powder, made into pellet. The feed is given to rats from the age 4 weeks old until 12 weeks old, everyday ad libitum.

TABLE I. Nutritions in 100 Grams Seluang Fish Extract Powder

Nutrition	Consentration (%)
Ca	1,6
Fe	19,9 ppm
P	1,67
Zn	122,7 ppm
Oleat acid	11,77
Linoleat acid	7,33
DHA	1.04
Aspartat	3,71
Glutamat	4,98
Serin	1,94
Glisin	3,06
Histidin	1,82
Arginin	3,05
Threonin	2,15
Alanin	2,83
Prolin	1,92
Valin	2,41
Metionin	0,48
Isoleusin	2,07
Leusin	3,62
Phenilalanin	2,37
Lisin	4,81
Sistin	0,32
Tirosin	1.62

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1) The first phase of the study: Measuring the rats memory special using MWM

After 8 weeks of treatment, all of the rats models were examined by MWM to measure their spatial memory, in the form of cylindrical drum-shaped pool measuring 1.8 m by 0.5 m high filled with water to a depth of 0.3 m. A circular platform with a diameter of 13 cm and a height of 28 cm was placed 2 cm below the water surface. To make the platform invisible, coconut milk is added to the water. Drum surface is divided into four quadrants A, B, C, and D. The platform is placed on a particular quadrant area (and not changed its location during the experiment) (Fig. 1).

Figure 1. MWM.

Spatial memory was compared among control and treatment group. All rats were trained (escape latency) by *MWM method hidden platform test* for 8 days to calculated its time to reaches the platform. Each rat got trained twice a day [6].

MWM measurement system for the escape latency: Everytime when the experiment started set the starting point when rat is placed in the pool (e.g in position A). The platform has been placed in position between A and D quadrant area. Then the rats released at the specified starting point, facing the drum wall, rats were left to swim to reach the platform and climb onto the platform. The time that needed to reach the platform (escape latency) was recorded. After the rats reached the platform, the rats were given time to rest for 30 seconds.

After that, rats are dried and rested to prepare for the next experiments. Everyday, the rat was trained twice a day with duration 90 seconds. If it failed in 90 seconds, it would be directed into platform and let it took a rest for 30 seconds. The failed rat has its escape latency noted as 90 seconds. After that, rats are dried and rested to prepare for the next experiments. The second examination, it would be placed at the different location (e.g potition C). Rats put in place and it swimmed to the platform and up onto the platform. Time to reached the platform was noted (escape latency). After reaching platform, the rat was rested for 30 seconds.

Figure 2. Probe test

To measure the rats spatial memory, it examined by probe test. Platform was removed from the water, the other components were allowed like before. Starting place was choosen randomly The rat swimmed for 90

seconds and the percentation for swimming to target quadran was calculated (quadrant previously laid the platform) compared to overall time taken by the rat through the entire quadrant (preformance in this case 90 seconds).

The drum was given rope that connected each position (A-C) and (B-D). 2 observer observed the length of time (seconds) that rats spent in the target quadrant (in this case between quadrant A and D). Observations using the MWM probe test is performed one time in morning at 9:00 AM to finish.

2) The second phase of the study: Calculating the PPAR expression

After completing the rat spatial memory measurement, in order to make the brain tissue preparations rats is killed. The sample that cut then stained by immunohistochemical staining method. The percentage of PPARα and PPARγ expression is calculated by counting the number of cells which given a brown color to the core compared to the number of cells in each field of view. From the five visual fields were taken and then calculated the average number of cells expressing the brown color

3) Data analysis

Data was tested using Saphiro Wilk. If data in normal distribution, continued by one way Anova test with 95% significances post hoc test (LSD).

III. RESULT

The first phase study showed the percentage of the time length in target area from the P2 group the highest compared to the K and P1 group, respectively 33.52%, 29.9%, and 23.15%

The result showed that there were significant differences for rats to stay at platform among control and treatment group (p=0,021).

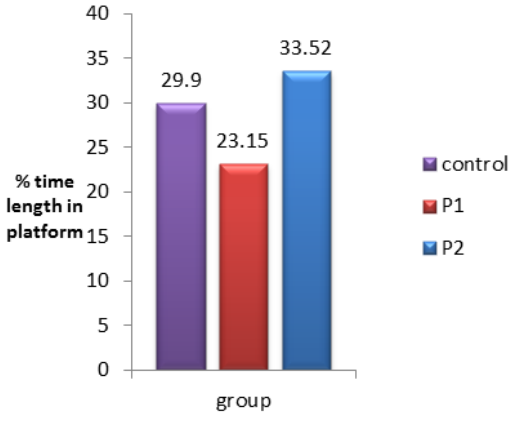

Figure 3. The average percentage of time in the target quadrant (probe test) after treatment for 8 weeks.

Advanced analysis test used post hoc test (LSD), it showed significant differences between P1 and P2 (p=0,006). There was no significant differences between K and P1. It means that Seluang fish formula increasing rat's memory showed with the highest time length in target area.

On the PPARα and PPARγ examination showed the average percentage of neuron cells that express PPARα in P2 group is higher than K and P1 group, respectively 18.5%, 3.75% and 2.5%. The mean percentage of neuron cells that express PPARγ in P2 group also higher than K and P1 group, respectively 35.41%, 6.25% and 5.83%.

The Kruskal Wallis Statistical test results showed that there were significant differences in average percentage of neurons that express PPARα were examined between 3 groups (p = 0.002). The same test results the percentage of neuronal cells that express PPARγ showed significantly different in average percentage of neuron cells that express PPARγ were examined between 3 groups (p = 0.001)

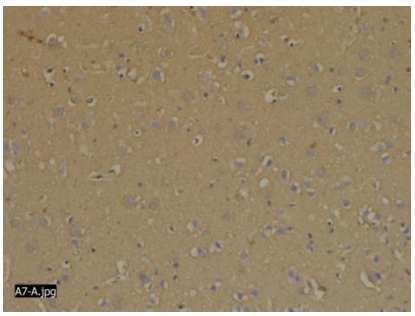

Figure 4. Neuron cells expressing PPARα.

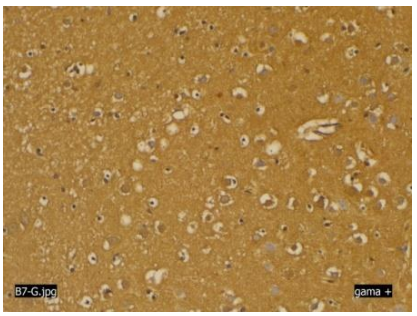

Figure 5. Neuron cells expressing PPARγ

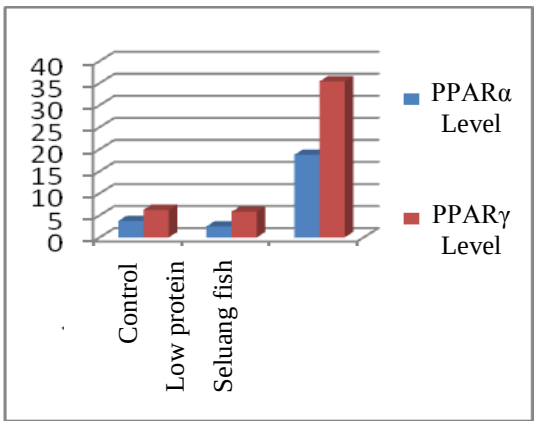

Figure 6. The average percentage of neuron cells expressing PPARα and PPARγ

IV. DISCUSSION

Hajjar *et al* [4] reported with administrating higher dietary omega-3 fatty acids from the omega-6 (ratio of omega-6 : omega-3 low) improving spatial memory and

up-regulation of PPARα and PPARγ gene expression in the hippocampus. This suggests that PPARα and PPARγ as transcription factors can influence the synaptic proteins expression that trigger the synaptic plasticity and improves spatial memory in animals that fed high dietary in omega-3 fatty acids. Peroxisome Proliferator-Activated Receptors (PPARs) are a subset of the nuclear hormone receptor super family that has transcriptional activity, modulated by ligand-receptor interaction. There are three families of PPARs known as PPARα, PPARγ and PPARβ / δ. They bind to the peroxisome proliferator response elements are similar but show differences in transactivating function, which is partialy mediated by the distribution network, and coactivator ligand specificity [5]-[7]. Peroxisome proliferator-activated receptors expressed in various tissues, among others, liver cells, kidney, and brain. In brain tissue PPARs are expressed in a number of brain cells, especially in the hippocampus [8].

In this study, groups of rats were fed Seluang Formula showed higher neuron cells in hippocampus that express PPARα and PPARγ compared to the control group and the low-protein group. It is proven that the nutrients contained in Seluang fish especially unsaturated fatty acids such as essential fatty acids linoleic, oleic and DHA can trigger the PPARα and PPARγ expression. PPARα plays an important role in the biosynthesis regulation of acetylcholine that contribute to cognitive function, while PPARγ has a prominent role in the central nervous system inflammatory regulation and neuroprotective, triggering the cognitive performance. Several studies showed that cognitive performance can be improved through the PPAR nuclear receptors [4]. Kuang *et al* (2012) reported that preactivation of PPARβ can improve spatial memory and cognitive functions as well as changes cytomorphologi neuron cells hippocampus after sustained global cerebral ischemia-reperfusion injury (GCIRI). Meanwhile another study reported that PPARγ able to up-regulation of Bcl-2 anti-apoptotic protein in neurons and induces mitochondrial stabilization also protected from oxidative stress and apoptosis in cultured neuron cells [7].

Other studies have reported that chronic deficiency of alpha linolenic fatty acids can caused changes in cholinergic parameters by increasing spontaneous release of acetylcholine (ACh) in the hippocampus and lead to a lower release whisch associated with less binding to the muscarinic receptors. The responses can be different based on the excessive loss of neuronal DHA and n-6 PUFA, particularly by the septo-hippocampal cholinergic system which plays a role in the cognitive function regulation. Such modifications may contribute to cognitive impairment caused by the deficiency of n-3 PUFAs [8].

In a other study about the gyrus dentatus granule cells and the hippocampus CA3 pyramidal cells, suggesting that protein malnutrition effected on all of these cells, the dominant effect is on the gyrus dentatus cells [9]. In the same result associated with protein malnutrition showed a decrease in prenatal neurogenesis in the hippocampus granule cells dentatus formation [10].

Formatio hippocampus is involved in learning and memory which are ruled the construction of spatial cognitive map to surrounding environment. This map is used by the animal to identify the differences in place (situation). The information that viable will be organized into working memory as short-term memory. In the spatial cognition study, Morris water maze test is often used as a tool [11]. If an interruption occurred in the hippocampus formation caused by malnutrition, can impact on spatial memory. Another study found that malnutrition in early life caused a decrease in hippocampus neural progenitor and also deficiency recognition object in adulthood [12]. Malnutrition adverse effects on cognitive development can occur as a result of neuron structural and functional maturation disruption such as meilinization delayed and dendritic arborization decreased [13]. One theory about the involvement of protein synthesis in learning and memory is the involvement of cyclic adenosine monophosfate / Protein Kinase A (cAMP / PKA) that mediate the signaling of learning and memory process. The connection between protein synthesis and memory formation depends on synaptic facilitation. The important role of cAMP factor response binding protein (CREB) in the form of plasticity and sensitive rapamisin forming protein, is also an important role of NMDA receptors in the signaling and stimulation of gene expression to form protein will produce synaptic plasticity and long-term memory [14].

V. Conclusion

The conclusion of this study is Seluang fish has the complete nutritions compotition that could increased the spatial memory, expression of PPARα and PPARγ that lead to intelligency increased.

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