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Effect of Mackerel Oil During Pregnancy on Synapsin Expression in Newborn Rattus Novergius Cerebrum

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Abstract

The increase in the number of cells indicates a good synapse function, so that it can be linked to human intelligence. The rapid rate of synaptogenesis, myelination occurs in the first 1000 days of life. The public has not realized the importance of nutrition during pregnancy which contributes to the intelligence of the baby that will be born later. One of them is mackerel oil which contains omega 3. This study aims to analyze the effect of mackerel oil during pregnancy on synapsin expression in the cerebrum. This type of research is true experimental with a posttest-only control group design. The research samples were 30 pregnant Rattus norvegicus mothers aged 2-3 months. The 3 groups were randomly divided namely the control group (K1), the mackerel fish oil treatment group (K2), and the treatment group who were given omega-3 supplements (K3). This research was conducted at the Experimental Animal Cages and Pathology Laboratory, Faculty of Veterinary Medicine, Airlangga University. ANOVA test results on synapsin expression in the cerebrum showed that there was a significant difference with a p-value of 0.000. Synapsin expression in the cerebrum of newborn Rattus norvegicus which was given mackerel oil in the mother of Rattus norvegicus during pregnancy showed higher results than other groups.

Keywords: cerebrum; mackerel oil; synapsin

Penambahan jumlah sel menunjukkan fungsi sinaps yang baik, sehingga dapat dihubungkan dengan kecerdasan manusia. Pesatnya laju sinaptogenesis, mielinisasi terjadi pada 1000 hari pertama kehidupan. Masyarakat belum menyadari pentingnya gizi selama kehamilan yang berkontribusi terhadap kecerdasan bayi yang akan dilahirkannya kelak. Salah satunya yaitu minyak ikan kembung yang banyak mengandung omega 3. Penelitian ini bertujuan untuk menganalisis pengaruh minyak ikan kembung selama kebuntingan terhadap ekspresi *synapsin* di cerebrum. Jenis penelitian adalah *true eksperimental* dengan desain *posttest-only control group*. Sampel penelitan adalah induk *Rattus norvegicus* bunting usia 2-3 bulan sebanyak 30 ekor. 3 kelompok dibagi secara acak, yaitu kelompok kontrol (K1), kelompok perlakuan minyak ikan kembung (K2), dan kelompok perlakuan yang diberi suplemen omega-3 (K3). Penelitian ini dilakukan di Kandang Hewan Coba dan Laboratorium Patologi, Fakultas Kedokteran Hewan, Universitas Airlangga. Hasil Uji ANOVA pada ekspresi *synapsin* di cerebrum menunjukkan bahwa terdapat perbedaan signifikan denga *p-value* 0,000. Ekspresi *synapsin* di cerebrum *Rattus norvegicus* baru lahir yang diberi minyak ikan kembung pada induk *Rattus norvegicus* selama kebuntingan menujukkan hasil lebih tinggi dibanding kelompok lain.

Kata Kunci: cerebrum; minyak ikan kembung; *synapsin*

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Introduction

Stunting is one of the nutritional problems faced by the world, especially in poor and developing countries. Stunting is a serious problem due to the high risk of morbidity and mortality and can affect brain development to suboptimal levels. This has an impact on stunting growth (Unicef, 2013). Growth failure due to the accumulation of insufficient nutrients lasts a long time from pregnancy to 24 months of age (Bloem et al., 2013)

Based on the results of basic health research Riskesdas, (2013) in Indonesia in 2013-2018, more than 30% of children under five are stunted. It is known from the total percentage, of toddlers in the very short category in 2013, namely 19.2%, increased to 19.3% in 2018. One of the factors directly causing the high incidence of stunting is the lack of nutritious food intake. People also do not realize the importance of nutrition during pregnancy. This contributes to the nutritional state of the baby who will be born later. The nutritional status of pregnant women greatly influences the state of health and development of the fetus. During pregnancy, the fetus is completely dependent on the mother's intake and storage of nutrients (Woldeamanuel et al., 2019).

The rate of synaptogenesis in the first 1000 days of life is very rapidly developing in the brain (Burciu et al., 2014). The brain is a cognitive organ (Norouzkhani et al., 2022). An increase in the number of cells shows good synapse function, so it can be linked to human intelligence (Joewono, 2020). This process is supported by synapsin as a marker of synaptogenesis. Synapsin is a synaptic vesicle phosphoprotein produced from a single primordial syn gene located presynaptically. Synapsin is regulated by BDNF, a neurotrophin that helps stimulate and control the most active neurogenesis in the hippocampus (Fornasiero et al., 2010). Synapsin is a specific phosphoprotein of nerve terminals and plays a role in axon elongation (regulation of axonogenesis), maintenance of synaptic contacts (synaptogenesis), and neurotransmitter release (Evergren et al., 2007). The more neurotransmitters that are formed and the connections between neurons that are formed, the more synapses are formed so that information is processed faster, it is hoped that the smarter it will be (Joewono, 2019).

The gestation period is a good opportunity to provide optimal stimulation and nutrition to ensure normal development and support the speed of processing in the brain so that the quality of the brain increases so that it can influence future behavior such as influencing emotions, learning abilities, and memory (Joewono, 2019). Appropriate strategies and responses are needed to overcome and reduce the prevalence of stunting (RI, 2019).

Interventions carried out in order to accelerate the reduction of stunting are increasing the availability and access to nutritious food, especially during pregnancy (Mitra, 2015). Fulfillment of adequate nutrition, both macro-nutrients and micro-nutrients is urgently needed to avoid or minimize the risk of stunting (Branca & Ferrari, 2002). Pregnant women are encouraged to provide brain nutrition or brain booster during pregnancy (Fitriyani et al., 2017). You can fulfill your nutritional needs during pregnancy by consuming nutritious foods, one of which is fish, which contains lots of omega-3. The omega-3 index is higher in children who are not stunted (Adjepong et al., 2018).

The level of omega-3 fatty acids in mackerel is quite high, around 8.5 g/100 g of meat, with EPA content of 0.93 g/100 g of meat and DHA of 5.7 g/100 g of meat (Latupeirissa & Rumahlatu, 2016). Mackerel is a type of local fish that is easy to obtain in Indonesian waters in large enough quantities and is easy to obtain and has important economic value (Prahadina et al., 2015). Omega-3 is a polyunsaturated fatty acid (PUFA) that contains Docosahexaenoic acid (DHA) and Eicosapentaenoic acid (EPA) (Siriwardhana et al., 2012). DHA and EPA are found in abundance in the cerebrum which is considered important for their capacity (Simopoulos, 2016). DHA rapidly accumulates in the brain during gestation and the availability of DHA via transfer from maternal stores impacts the degree of incorporation of DHA into neural tissue (Weiser et al., n.d.).

This research was conducted on experimental white rats (Rattus norvegicus) because this experimental research has limited ethical constraints that are not possible in humans. This research is a series of studies to educate humans since the fetal period. The results of the study are expected to show efforts to increase the biopsychosocial potential of the fetus in the womb through the nutrition of mackerel oil containing omega 3 on the expression of synapsin in the cerebrum of newborn Rattus norvegicus.

Methods

This research is a laboratory experiment by conducting experiments using female Rattus norvegicus animals. The research design is true experimental with a posttest-only control group design. The research sample was randomly divided into three groups. Group 1 was the control group which did not receive any treatment. Group 2 is the treatment group that was given mackerel oil which contains omega-3. Group 3 is the treatment group that was given supplements containing omega-3.

The samples used were female rats (Rattus norvegicus) aged 2-3 months and weighing 120-130 grams. All samples taken previously had been acclimatized for 1 week. Mother Rattus norvegicus who was sick and her child Rattus norvegicus died in the womb and were not used in this study. Samples were given treatment at the age of 1-17 days of gestation. Each group consisted of 10 Rattus norvegicus broodstock.

The study was conducted at the Experimental Animal Cage and Pathology Laboratory, Faculty of Veterinary Medicine, Airlangga University, Surabaya from March to September 2020. The dosage was determined based on Global recommendations for EPA and DHA for pregnant women which had been converted for experimental animals so that a dose of 5.4 mg was obtained. /200 gr BW/ day. Experimental animals Rattus norvegicus female estrus synchronization using PMSG and hCG. Furthermore, Rattus norvegicus females mated with male rats aged 2-3 months. After mating, vaginal plugs were observed as a sign of pregnancy.

Section Caesarea is done to give birth to Rattus norvegicus. Immediately after birth, the brain tissue of Rattus norvegicus children was taken to examine synapsin expression by immunohistochemistry. The data for each sample is the average value *Immuno Reactive Score* (IRS) observed at 5x visual field (LP) at 400x magnification. Analysis of synapsin expression in the cerebrum of newborn Rattus norvegicus between groups was tested for data normality. Normally distributed data were analyzed using a one-way Analysis of Variance (ANOVA) followed by a different test. This research has received approval for ethical feasibility from the Ethics Committee of the Faculty of Veterinary Medicine, Airlangga University with number 2.KE.036.04.2020.

Results and Discussion

All pregnant Rattus norvegicus parents were weighed before being sacrificed. Based on Table 1, the results of the descriptive analysis of the characteristic data showed that the largest mean pregnant weight of the main Rattus norvegicus came from the group that was given the treatment in the form of mackerel oil, namely 253.70 grams.

All children of Rattus norvegicus were born by sectio caesarea on the 18th day of gestation which was then weighed and selected three children of Rattus norvegicus were from one parent with one largest weight, one medium weight, and one smallest weight, then decapitation was carried out. Each of the three brains of Rattus norvegicus children from one parent was made into one preparation and stained. Based on Table 2, it shows that the lowest average body weight, body length, and head weight of Rattus norvegicus children came from the control group with respective averages of 2.78 grams, 3.55 cm, and 0.85 grams.

Table 1. Characteristics of the initial and final body weight of Rattus norvegicus broodstock

Experimental Animal Group	Mean ± SD Initial Weight	Mean ± SD Final Weight
No Treatment	124.50±3.60	231.60±45.87
Mackerel Oil	127.00±2.75	253.70±94.49
Omega 3 supplements	125.60±3.17	240.00±52.01

Table 2. Characteristics of newborn Rattus norvegicus children

Experimental Animal Group	Mean ± SD Weight	Mean ± SD Body Length	Mean ± SD Head Weight
No Treatment	2.78±1.26	3.55±0.71	0.85±0.26
Mackerel Oil	4.27±1.29	4.59±0.41	1.22±0.40
Omega 3 supplements	3.66±1.27	3.88±0.72	1.01±0.41

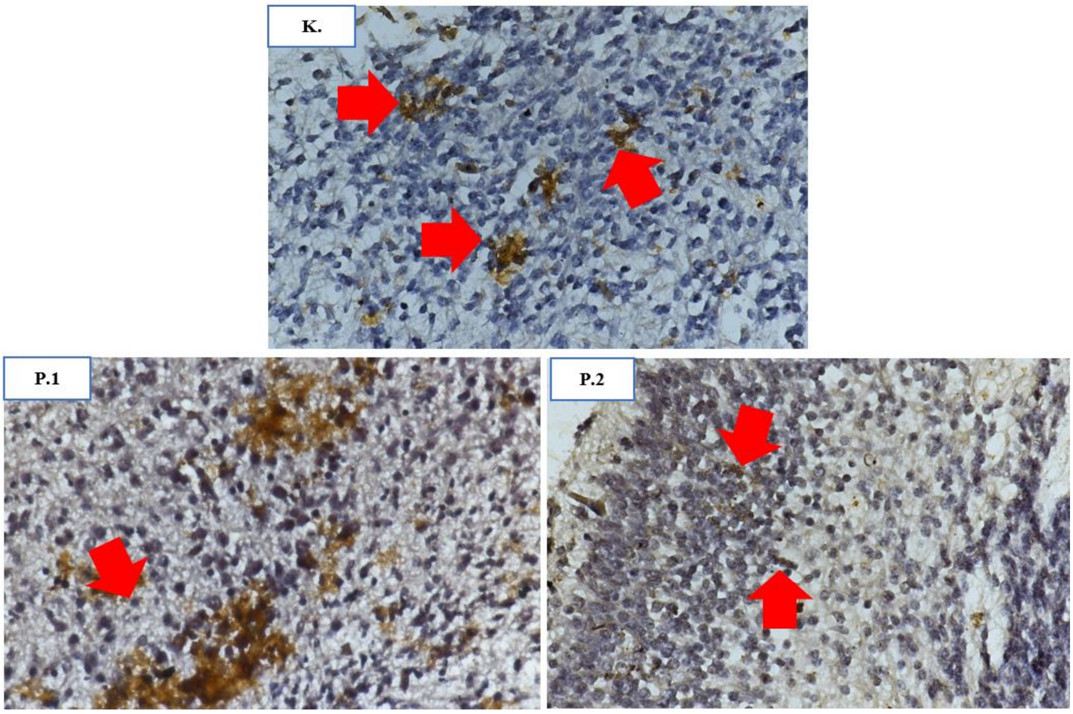

Figure 1. Comparison of synapsin expression in the brain of Rattus norvegicus children (K1, K2, K3). IHC. 400x.

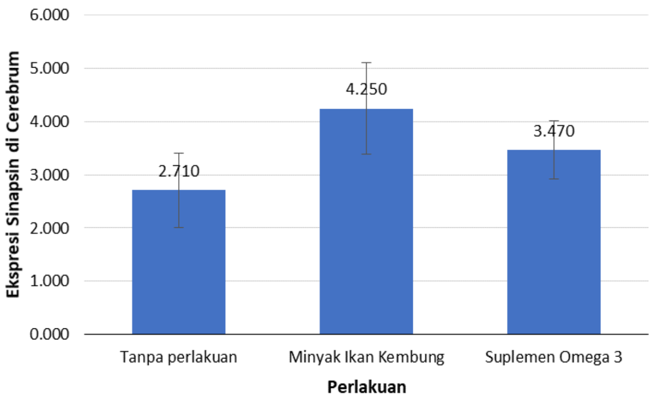

Figure 2. Graph of mean synapsin expression in the cerebrum of Rattus norvegicus children

Table 3. Statistical test results of synapsin expression in the cerebrum of Rattus norvegicus children

Experimental Animal Group	Normality test	ANOVA test	BNT test
No Treatment	0.941		2.710±0.705
Mackerel Oil	0.465	0.000	4.250±0.864
Omega 3 supplements	0.799		3.470±0.560

A comparison of the synapsin expression in the fetal brain between groups can be seen in Figure 1. The red arrows indicate the presence of apoptotic expression in the cerebrum which is indicated by the presence of chromogen brown color. The results of observations in each group per 5x field of view then the average synapsin expression in the cerebrum was tested for normality using the Shapiro-Wilk. The results of the normality test showed that the data distribution was normal in all groups. A statistical analysis followed by an ANOVA parametric test with significant results with a p-value of 0.000. Figure 2 shows the average synapsin expression in the cerebrum in the control group was 2.710, the mackerel oil group was 4.250, and the omega 3 supplement group was 3.470. The test continued with BNT and showed that there were differences in the results of synapsin expression in the cerebrum.

Omega-3 fatty acids are essential for proper brain development and function whereas DHA is maintained by the nerve membranes. Pregnant rats were fed adequate omega-3 fatty acids or a diet less than 14 days gestation and their pups were reared on their respective diets. Continuing this diet for three generations resulted in a loss of about 70% of DHA in the brain (Desai et al., 2014). DHA has been shown to be a major component of neuronal membranes that enhance synaptogenesis. The provision of DHA improves nerve function by supporting synaptic membranes. One source of supporting food is mackerel (Wingate D.S., 2019). DHA supplementation uniquely enhances neurite outgrowth, synapsin puncta formation, and expression of synaptic proteins, particularly synapsin and glutamate receptors. In DHA-supplemented neurons,

Nutritional status in the womb is often used as an indicator of weight at birth. In children over 5 years old, stunting, thinness, and low body weight are the main indicators used to measure the nutritional status of individual children (Lutter et al., 2011). Among these indicators, stunting and wasting are used as nutritional indexes for cumulative and acute malnutrition. Low body weight is a composite indicator that can reflect acute weight loss, stunting, or both. Lynn, (2009) showed that the average score on intelligence tests increased with improved prenatal nutrition. These results reflect the important role of prenatal nutrition on intellectual development (Li et al., 2016). Mackerel oil is one of the treatments that can be used to increase synapsin expression in the cerebrum of Rattus norvegicus children.

Conclusion

Mackerel oil has a significant effect on synapsin expression. Synapsin expression in the cerebrum of Rattus norvegicus newborns who were given mackerel oil during pregnancy was higher than those who were not given mackerel oil and omega-3 supplements.

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