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THE EFFECTIVENESS OF OMEGA-3 MACKEREL FISH OIL ON THE DENSITY OF TOOTH ENAMEL

In Vivo Study of White Rat (*Rattus novergicus*)

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ABSTRACT

Background: *Consuming cariogenic foods can cause calcium demineralization which is characterized by the appearance of dental caries. One effort on the prevention of dental caries is by consuming Mackerel fish oil that contains omega-3 fatty acids, omega-6 fatty acids, and vitamins that are beneficial for the body.* ***Purpose:*** *The study aimed to determine the effectiveness of omega-3 Mackerel fish oil on the density of tooth enamel in white rat (*Rattus norvegicus*).* ***Method:*** *This study applied an experimental research method with randomized posttest only control group design. Samples were comprised of 20 rats taken from 6 pregnant female rats which later divided into two groups: a treatment group administered orally with omega-3 Mackerel oil as much as 1ml / 200 mg BB and an aquadest control group. The treatment was administered during pregnancy period until the eruption of the teeth. Rats were euthanized to extract the tooth and enamel density was observed using micro-CT. The data were then analyzed with Levene's homogeneity test and were preceded to* **independent** *T-test.* ***Result:*** *The average enamel density of the treatment group was 1155.18340 while the control group was 175.91640. Based on* **independent** *t test between the two groups, a significant difference in tooth enamel density of Rattus Noevegicus was obtained (p <0.05).* ***Conclusion:*** *The administration of omega-3 Mackerel fish oil may increase the density of tooth enamel in White Rat (Rattus norvegicus).*

Keywords: *density of tooth enamel, Omega-3, Mackerel fish, Micro-CT*

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INTRODUCTION

Three indispensable factors inducing the progression of caries are cariogenic microorganisms, cariogenic substrates and host or the teeth. These factors interchange when there is a presence of demineralization and remineralization imbalance on the surface of the teeth.[1] Based on DMF-T index, tooth decay prevalence in Indonesia scores 4.6 with each category of carious or unrestored permanent teeth is 1.6; carious permanent teeth and tooth radix yet to be extracted is 2.9; and restored permanent teeth is 0.08; interpreted the number of tooth decay per 100 Indonesia citizens is 460 teeth. Basic Health Research (2018) reported that Indonesian population are presented with oral and dental problems in a total of 57.6% comprehending good tooth brushing pattern with only 2.8% among the population.[2,3]

Enamel, dentin, pulp and cementum constitute the structure of the teeth. Tooth enamel is the hardest part of human body. It is formulated by crystal apatite deposit, which contains calcium, phosphate and other elements in extracellular matrix secreted by particular cell called ameloblast. Tooth enamel includes 96% inorganic material (mineral), 1% organic material, and 3% water.[4,5]

As one of ocean fish species, Mackerel price is relatively high and it is popularly consumed by public.[5] Mackerel fish contains good nutrients such as protein, calcium and vitamin A.[6] Poly Unsatturated Fatty Acids such as DHA, EPA, AA and LA are natural ligands against Peroxisome Proliferator-Activated Receptors (PPARs) which possesses anti-inflammatory effect to suppress the expression of placenta pro-inflammatory cytokine at the end of rat pregnancy.[7]

Omega-3 Poly Unsaturated Fatty Acids (Ω-3 PUFAs) are reported to increase bone strength. Omega-3 PUFA diet may promote changes in bone,

specifically in calcium absorption, osteoblast differentiation, lipid oxidation, eicosanoid production, and inflammation.[8,9]

Several studies have outlined the role of daily intake on the process of tooth formation. The administration of Omega-3 Mackerel oil has been proven to optimize inorganic material and collagen (organic) formation during prenatal life. Based on above background, author aims to validate the effectiveness of omega-3 Mackerel oil on the density of tooth enamel in white rat (*Rattus novergicus*).

MATERIALS AND METHODS

This study was conducted at Biology Laboratory Faculty of Mathematics and Science Universitas Negeri Semarang and Micro-CT Laboratory Faculty of Mathematics and Science Institut Teknologi Bandung. It required 20 galur wistar white rats (*Rattus novergicus*) taken from 6 pregnant female rats. Samples were divided into two groups: a treatment group administered with omega-3 Mackerel oil extract and a control group given aquadest only with 10 rats in each group. Sampling technique was based on inclusion and exclusion criteria. Inclusion criteria consisted of pregnant female white rats (*Rattus novergicus*) aged 2-3 months and weighed 180-200 gr. Exclusion criteria included ill rat at adaptation period.

The study was initiated by formulating omega-3 Mackerel oil extract. A total of 6 pregnant female rats were adapted for 7 days enhancing physiological adaptation which later administered with omega-3 Mackerel oil extract at 100 ml/200 kg BB dosage for treatment group and aquadest for control group. Treatment was performed during intrauterine life until 21 days after delivery - at the end of weaning period. Cubs were randomly selected on day 35 in a total of 10 rats from each group. The cubs were then euthanized using chloroform for tooth extraction. The density of tooth enamel was observed using Micro-CT programme.

Data analysis was performed using Saphiro-Wilk normality test and Levene's homogeneity test. Normal distribution was presented by the data so comparative Independent T-test was conducted for two study groups.

RESULTS

After data collection, statistical analysis was conducted to identify the effect of Mackerel fish extract (*Rastrelliger spp*) on tooth enamel density of Galur Wistar white rat (*Rattus novergicus*). Normality test demonstrates a significant value for omega-3 group (0.804) and control group (0.992). Each group obtained significant value which is greater 0.05 resulted in normal data distribution in respective group. Homogeneity test illustrates a significant value of 0.03 which is less than 0.05 thus presenting non-homogenous data distribution. Normality and homogeneity test resulted in normal yet non-homogenous data distribution. Independent t-test was performed to analyze the presence of mean difference between treatment and control group.

Variable	n	Min	Max	Mean	Standard Deviation	Sig.
Omega-3	10	942.423	1321.290	1155.18340	118.064867	0.00
Aquadest	10	158.663	190.508	175.91640	9.739336	

Table 1. The result of *Independent t-test* analysis

The result of data analysis using independent t test depicts a significant value of 0.000 which is less than 0.05. It can be concluded that there is a significant difference on the density of tooth enamel between treatment group and control group.

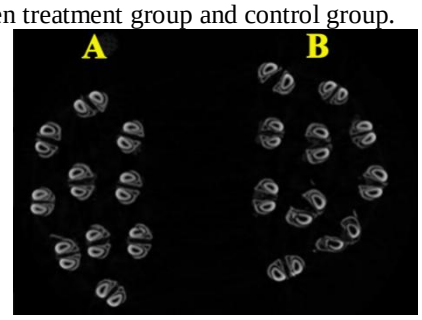

Figure 1. The density of enamel using micro CT
A: Enamel density after Mackerel fish administration;
B: Enamel density after aquadest administration

It is obtained from the data that there is a significant difference on the density of enamel between treatment group and control group. Mean value for treatment group administered with omega-3 oil was 1155.18340 while control group administered with aquadest was 175.91640.

DISCUSSION

The development of enamel (amelogenesis) can be divided into several stages namely pre-secretory, secretory, transition and maturation. These stages are presented according to the morphology and the function of ameloblast. Ameloblast is a single cell layer which covers enamel development and is responsible for enamel formation. Ameloblast is a

part of enamel organ including outer epithelial layer, stellate reticulum, stratum intermedium and inner enamel epithelium. Pre-secretory stage is initiated in the commence of mineralization process around area which later known as dentinoenamel junction (DEJ).[10,11]

An increase in enamel density was discovered at the treatment group of this study. It is in accordance with a study from Rusu *et al* (2014) which mentioned that Micro CT method is proven as a measurement to evaluate new bone formation in rats. This method enables numerical quantification for new regenerative bone and identification of bone healing process. Calcium is the main component for enamel and dentin formation which is also the highest inorganic material to be found during calcification process. Calcification process begins on the fourth month intrauterine where dental calcification is dependent on calcium metabolism process. Blood and intracellular fluid are known to deposit a sufficient amount of calcium ion. One factor affecting ion addition is calcium intake during calcification process.[12,13]

Mackerel fish is a source of nutrient which contains Omega-3Poly Unsaturated Fatty Acid. A study from different field has reported that Omega-3 Poly-Unsaturated Fatty Acids (omega-3 PUFAs) are able to increase bone strength. Omega-3 PUFA diet in bone affects the change of calcium absorption, osteoblast differentiation, lipid oxidation, eicosanoid production, and inflammation. They are also spotted to induce changes in pregnant female rat. Omega-3 Poly-Unsaturated Fatty Acids in the form of NEFA is a natural ligand which activates PPARS to signal FATPs so that it can penetrate placenta membrane to be later transported by FABP. FATPs will be increased in the presence of Insulin Growth Factor (IGF) signaling. Insulin Growth Factor (IGF) will send a transduction signal to cell nucleus thus activating P38 MAP (Mitogen Activated Protein Kinase) and p44/p42 (Mitogen Activated Protein Kinase). Both signals enhance the production of HSP 27 as a chaperone molecule to protect the continuity of the cell. Insulin Growth Factor (IGF) will assist protein migration secreted by ameloblast at secretory stage, such as amelogenin, ameloblasin, enamielin and Matrix Metalloproteinase-20. An increase of these proteins will strengthen tooth enamel.[8,14-16] Optimal maternal nutrition is essential for matrix formation and mineralization of deciduous teeth. It is also pivotal to maintain optimum mother nutrition during infancy and childhood for the calcification of permanent teeth. Calcium, omega-3 and other proteins deficiency during maternal period will disrupt ameloblast activity at calcification stage resulting in lesser production of enamel matrix protein which increase the feasibility of hypoplasia.[17,18]

Limitation of this study includes the absence of control for breastfeeding amount and frequency at postnatal period provided by the mother to its cub in a day. Based on the study performed, it can be inferred that omega-3 Mackerel fish oil may increase the density of tooth enamel in white rat (*Rattus novergicus*).

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