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THE EFFECT OF SALTWATER FISH NANOPARTICLE POWDER CONSUMPTION ON TOOTH ENAMEL DENSITY In Vivo Study of Mice *(Mus musculus)*

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ABSTRACT
Background: *Dental caries is rarely found in children who eat saltwater fish. Overfished fish can be used as powder of saltwater fish nanoparticles, which is useful as an alternative material for the prevention of dental caries in dentistry can be used as a powder for saltwater fish nanoparticles, which is useful as an alternative material for preventing dental caries in dentistry. A Cone Beam Computed Tomography (CBCT), commonly known as a μ-CT Scanner, is a device used to quantify the increase in tooth enamel density* ***Purpose:*** *This study aimed to determine the effectiveness of saltwater fish nanoparticle powder consumption on tooth enamel density of Mus musculus.* ***Method*** *This study used an experimental research method with a randomized posttest only control group design. The research subjects were 16 mice taken from 2 pregnant female mice which were divided into two groups, namely the treatment group with 2.17 mg / 0.5 mL of saltwater fish nanoparticle powder and the control group which was given distilled water. Administration was carried out during the intrauterine period until the teeth of the mice grew, then observed the tooth enamel density with CBCT OP 3D Pro (KaVo, Germany). The data obtained were analyzed by Levene and continued with the Independent T-test.* ***Result:*** *The average value of enamel density in mandibular incisors in the treatment group was greater than the control group ($p < 0.05$).* ***Conclusion:*** *Saltwater fish nanoparticle powder can increase tooth enamel density of Mus musculus.*

Keywords: *CBCT, CT nanoparticle powder, saltwater fish, tooth enamel density,*
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INTRODUCTION

Dental caries is a problem in dental and oral health caused by the activity of microorganisms in fermented carbohydrates and undergoes a demineralization process so that can damage the hard tissues of the teeth, especially the enamel.[1] The prevalence of tooth decay in Indonesia is quite high. According to the Basic Health Research (2018), it shows that as many as 57.6% of Indonesia's population experiences dental and oral health problems, and only 10.2% gets services from dental personnel. [2]

Dental caries is rarely found in children who consume saltwater fish. Based on data from the Jepara District Health Office in 2011, the prevalence of caries in elementary school-aged children in the coastal area of Jepara in 2010 was 46.11%, and the prevalence of periodontal disease was 10.43% of 2157 elementary school students.[4]

The anatomical structure of the teeth consists of enamel, dentin, pulp, and cementum. Tooth enamel is composed of complex chemical compounds with 97% mineral protein in the form of calcium hydroxyapatite with the chemical formula $Ca_{10}(PO_4)_6(OH)_2$. The outer part of the enamel undergoes a complete mineralization process and contains some fluoride, phosphate, and nitrogen as well as a small amount of carbonate and water.[3]

Saltwater fish powder, commonly known as fish flour, is obtained from overfishing such as sardines

(*Sardinella Fimbriata*), cobs (*Euthynnus Affinis*), and splendid ponyfish (*Leiognathus Splendens*) as well as scraps from processed fisheries in the form of scales, skin, bones, gills and all the internal organs of the fish. The use of these three types of saltwater fish, namely sardines, cobs fish, and splendid ponyfish, is caused by the excess production of these three fish, which will cause a large-scale accumulation of fish processing waste without any further use. Processed fish products are obtained from fish processing factories such as canning and frozen fish flour and fish oil factories. The fish flour is usually used as a raw material for making fish, livestock, poultry, and shrimp feed. Apart from using these three types of fish, a mixture of various types of fish that is still feasible can also be processed into alternative materials to prevent dental caries.[5]

Nanoparticles are materials that are less than 100 nm in size and can change into new properties or functions.[6] Nanoparticles are made using a ball milling tool. Particle characterization is expressed by particle size, particle morphology, and the content present in these particles.[7] Nanometer-sized particles are expected to be easily absorbed by the intestine and can penetrate the placenta barrier. Several studies have described the role of saltwater fish consumption in the tooth formation process. Based on the above background, this research aims to observe the effect of the saltwater fish nanoparticle powder consumption on mice's tooth enamel density (*Mus musculus*).

MATERIAL AND METHOD

This study that included the use of experimental subjects was approved by the Ethics Committee. Faculty of Dentistry, Islamic University of Sultan Agung with certificate number 212/B.1-KEPK/SA-FKG/VII/2020. This research was conducted experimentally in vivo with a post-test only control group design. This study used 16 young mice (*Mus musculus*) which were divided into two groups, namely the treatment group, which was given saltwater fish nanoparticle powder, and the control group, which was given distilled water, with each group of 8 mice (IBL, Faculty of Medicine Sultan Agung, Indonesia). The sampling technique was based on the inclusion and exclusion criteria. The inclusion criteria consisted of pregnant adult female mice aged 2-3 months, weighing 20-30 grams, and looking healthy (agile, not lethargic, clean skin without injury, and bright eyes). The exclusion criteria included mice that were sick during the adaptation period, mice that died before treatment, pregnant female mice that did not want to eat or drink.

The study was initiate with the manufacture of saltwater fish nanoparticle powder (Faculty of Chemistry, UGM, Indonesia). All pregnant female mice were separated into two cages measuring 40×30 cm and divided into two groups, i.e., the treatment group given saltwater fish nanoparticle powder at a dose of 2.17 mg / 0.5 mL, and the control group, which was given distilled water. The treatment or intervention was carried out from 16 days of gestation until 14 days after delivery. The pups were randomly selected on the 30th day, with a total of 8 mice from each group. The 16 young mice were euthanized using chloroform to cut their upper and lower jaws. Incisor enamel density was observed using CBCT OP 3D Pro (KaVo, Germany) and further analyzed using CT-Analyzer software (Bruker, Köntich, Belgium). The scan was performed at 89.8 kVp, with an isotropic voxel size of 350 µm. The density is represented in terms of a mean grayscale index calculated for a particular Volume of Interest (VOI) enclosing the enamel.

Data analysis was performed using the Shaphiro-Wilk normality test and Levene's homogeneity test. The available data were normally distributed and homogeneous, so an Independent-T Test analysis was carried out to determine the differences between the two study groups.

RESULTS

After the research data was obtained, a statistical test was then carried out to determine the effect of saltwater fish nanoparticle powder consumption on tooth enamel density in mice (*Mus musculus*). The normality test showed a significant value for the saltwater fish nanoparticle powder group (0.265) and the control group (0.931). Each group obtained a significant value greater than 0.05, which led to a normal data distribution in each group. The homogeneity test obtained a significance value of 0.827, which means it is greater than 0.05 to present a homogeneous data distribution. Normality and homogeneity tests produced parametric data, namely data that were normally distributed and homogeneous. An Independent T-test was conducted to analyze the average difference between treatment and control groups.

Table 1. Grayscale Index of maxillary incisor tooth enamel density

Variable	Min	Max	Mean	Standard Deviation	Sig.
Saltwater fish nanoparticle powder	117.10	124.07	119.82	2.55	.012
Aqua dest	112.94	119.12	116.28	1.95	.012

Based on Table 1, it can be seen that the average value of tooth enamel density treated with saltwater fish nanoparticle powder was greater in the maxillary incisors.

Table 2. Grayscale Index of mandibular incisor tooth enamel density

Variable	Min	Max	Mean	Standard Deviation	Sig.
Saltwater fish nanoparticle powder	119.90	127.16	123.43	2.47	.012
Aqua dest	119.71	121.83	120.84	0.77	.012

Based on Table 2, it can be seen that the average value of tooth enamel density treated with saltwater fish nanoparticle powder was greater in the mandibular incisors. The enamel density in the control group with distilled water was greater in the mandibular incisors than the maxillary incisors.

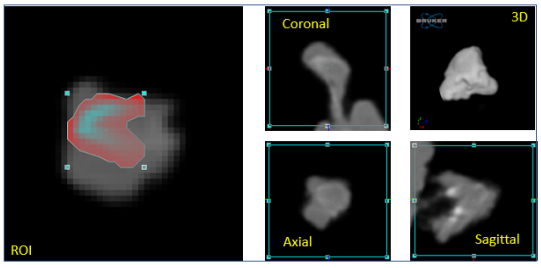

Figure 1. The density of enamel using CBCT, a grayscale index calculated for a particular Volume of Interest (VOI)

Data analysis results using the Independent-T Test obtained a significance value of 0.012, which means less than 0.05. It can be concluded that there was a significant difference in tooth enamel density between the treatment and control groups.

DISCUSSION

In this study, saltwater fish nanoparticle powder contains omega-3 fatty acids (PUFA), which are one of the primary nutrients for the body. Omega-3 is a polyunsaturated fatty acid that has two or more double bonds.[8] Omega-3 can penetrate the placenta barrier. The content of omega-3 in the saltwater fish powder can increase enamel density through the apposition stage by forming a tissue matrix resulting from the deposition of ameloblasts, odontoblasts, and formative cells. The effect of omega-3 content at the apposition stage is to assist the migration of proteins secreted by ameloblasts such as amelogenin, ameloblastin, enamielin, and Matrix Metalloproteinase-20. Amelogenin protein is a protein enamel matrix that plays an essential role in the amelogenesis process. [9]

Omega-3 consists of several parts, namely ALA (*alpha-linolenic acid*), EPA (*eicosapentaenoic acid*), and DHA (docosahexaenoic acid). ALA is the simplest form of omega-3 fatty acids among others so that ALA can be reconstructed and modified by the body to become DHA or EPA. *Docohexasonoic acid* (DHA) and *eicosapentaenoic acid* (EPA) are known to reduce bone resorption mediators, namely prostaglandins (PGE2) and proinflammatory cytokines, so they are said to increase bone formation. The contents of EPA and DHA have the potential to regulate the formation and activity of osteoblasts and osteoclasts. EPA-DHA can affect osteoclasts by reducing osteoclasts formation during the osteoclastogenesis process, especially DHA, which further affects RANKL production induced by proinflammatory cytokines that cannot bind to RANK, which produces osteoclasts.[10]

Research conducted by Indahyani (2013) on female rats who consumed omega-3 fatty acids during pregnancy and weaning showed that there had been an increase in plasma omega-3 fatty acids and an increase in the bone formation process, osteoblast differentiation, and a decrease in proinflammatory cytokine receptors such as TNF-α and IL-6.[11]

Calcium in the saltwater fish powder can also increase enamel density through the calcification stage by increasing enamel mineralization in the stratum intermedium and ameloblast cells in enamel formation as well as deposition of calcium salts and mineral deposition of hydroxyapatite crystals.[12] Calcium and bone mineral metabolism in Bone Mineral Density are closely related and integrated. Research conducted by Lukas et al. (2011) have proven that five-weeks old male Spraque-Dawley rats which consume omega-3s

have an increased absorption of calcium ions, decreased levels of Ca2+ in urine, and increased calcium ions in bones.[13]

Vitamin D's content in saltwater fish powder functions in fetal growth through calcium metabolism, maintaining cell integrity, bone mineral metabolism, and maintaining placental function. Vitamin D has two active forms, namely calciferol D2 and D3. Sufficient levels of Vitamin D during pregnancy can also aid in developing skeletal muscles and adipose tissue, which are needed for the growth and development of the fetus once it is born. Vitamin D maintains levels of calcium and phosphorus in the blood, a mineral that functions in mineral ion homeostasis, myoblast activity, and bone formation during early growth.[14]

In this study, the mean Grayscale value for enamel density was higher in the treatment group. This study aimed to determine the degree of remineralization that occurs in primary tooth enamel as well as build up by Rusu (2014) which proves that the MicroCT method can be an effective tool for evaluating new bone growth in mice. This method enables both the numerical quantification of new bone regeneration and a determination of the bone healing process. In the tooth calcification process, calcium is an inorganic material present in the highest concentration and is a major component of enamel and dentin formation. A factor that influences the addition of these ions is calcium intake during the calcification process.[15]

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