

DENTINO
JURNAL KEDOKTERAN GIGI
Vol VIII. No 1. March 2023

**THE IMPACT OF CONSUMING SALMON FISH (*SALMO SALAR*) ON
DENTAL CALCIUM LEVELS IN MICE (*MUS MUSCULUS*)**

Sandy Christiono¹⁾, **Naila Salsabila**²⁾, **Rizki Amalina**³⁾, **Welly Anggarani**¹⁾, **Islamy Rahma Hutami**⁴⁾

¹⁾ Department of Pediatric Dentistry, Faculty of Dentistry, Universitas Islam Sultan Agung

²⁾ Faculty of Dentistry, Universitas Islam Sultan Agung, Semarang, Indonesia

³⁾ Department of Biology Oral, Faculty of Dentistry, Universitas Islam Sultan Agung

⁴⁾ Department of Ortodonsi, Faculty of Dentistry, Universitas Islam Sultan Agung

ABSTRACT

Background: Pregnant women require calcium as a vital vitamin. Insufficient calcium intake during pregnancy can result in enamel hypoplasia and cavities in deciduous teeth. It can boost pregnant women's calcium intake with calcium-containing pill formulations and vitamin D; however, this has negative effects such as kidney stone issues. Salmon can be used as a substitute because it has no negative side effects and contains omega 3, which can enhance calcium absorption. **Purpose:** This study aimed to determine the effect of salmon (*Salmo salar*) consumption on calcium levels in the teeth of mice (*Mus musculus*). **Method:** This study used pregnant mice and a post-test-only control strategy. Pregnant mice were fed salmon combined with CMC (Carboxy Methyl Cellulose) and CMC (Carboxy Methyl Cellulose) alone as a control. The salmon dose administered is 2.17 mg/0.5 ml. Using atomic absorption spectrophotometry, mice dental calcium levels can be determined (AAS). **Result:** The findings of hypothesis testing with an unpaired t-test of 0.041 revealed a significant difference between the salmon-eating and CMC-consuming groups at p 0.05. (Carboxy Methyl Cellulose). **Conclusion:** Consuming salmon can significantly increase the calcium content of mouse teeth.

Keywords: Dental calcium, Mice (*Mus Musculus*, Pregnant mice, Salmon (*salmo nalar*)

Correspondence: Sandy Christiono; Faculty of Dentistry, Universitas Islam Sultan Agung, Jl. Kaligawe Raya No.Km.4, Terboyo Kulon, Kec. Genuk, Kota Semarang, Jawa Tengah; E-mail: Sandy@unissula.ac.id

INTRODUCTION

Dental caries is a prevalent disease of dental tissue in the community, particularly among children aged 6 to 9 years.¹ Calcium is one of the essential minerals or nutrients required for human metabolism. Calcium is essential for regulating the body's biological processes, growth, and the formation of bones and teeth.² In humans, approximately 98% of calcium (Ca) is deposited in the bones, 1% in the teeth, and the remainder in the blood and other tissues.³ Calcium deficiency causes enamel hypoplasia and increases the incidence of dental caries; it can also exacerbate the condition of pregnant women, inadequate tooth calcification, and tooth decay.^{4,5}

Calcium in the teeth can be increased through nutritional supplementation. Vitamin D is essential for calcium absorption and

metabolism, so calcium supplements can be combined with vitamin D.^{6,7} Vitamin D and calcium supplements also have side effects. Therefore, natural materials and natural resources require creativity. The benefits of natural resources include easier and more affordable access to raw materials.⁸ Fish is one natural resource that includes calcium. Potential marine species is salmon.^{9,10}

Salmon (*Salmo salar*) is a rich source of essential nutrients, including protein, fats, vitamins, and minerals.¹¹ Salmon (*Salmo salar*) is renowned for its nutritional value and beneficial health effects. Salmon (*Salmo salar*) has a variety of health benefits, including enhancing brain and memory function, reducing inflammation, enhancing metabolism, enhancing visual acuity, reducing depression, preventing heart attacks,

promoting tooth and bone growth, and containing omega 3 fatty acids, which can enhance the absorption of tooth calcium.^{10,12-14}

Calcium-rich foods broken down will go to the intestine, where some calcium will be absorbed. The calcium that gets taken in by the intestines goes into the bloodstream. The blood will send calcium to the cells, salivary glands, bones, and kidneys. The process of remineralizing teeth will use the amount of calcium in saliva.¹⁵

In the study, early researchers said that calcium absorption from the bones of Atlantic salmon and Atlantic cod was a good source of calcium (Ca) for healthy young men.¹⁶ Researchers have said that natural calcium phosphate (CaP) made from salmon bones is the most environmentally friendly and cost-effective source, can keep the element of normal oligo elements from natural bones, and can guarantee better osteointegration and biocompatibility.⁸ This study aimed to determine the effect of salmon (*Salmo salar*) consumption on calcium levels in the teeth of mice (*Mus musculus*).

MATERIAL AND METHODS

This study used a control design with only a post-test and true experimental. The lower incisive teeth of the child's mice were used as a sample. The sampling method for the child's mice was simple random sampling. Criteria for inclusion and exclusion were: mice in good health, age of 2 to 3 months, weight of 20 to 30 grams, cleanliness of the mud, and pain during adaptation. Mice that showed pain during adaptation died before treatment. The sample is made up of 16 pregnant female mice. One group was given salmon mixed with 1% cmc, and the other was given 1% cmc.

Salmon is cleaned of its stomach and any dirt that sticks to it. It is then boiled for 30 minutes at 80°C, washed, and cut into 5-10 cm pieces.^{17, 18} The salmon is boiled for 30 minutes at 100°C and then dried.¹⁸ The salmon (*Salmo salar*) is then dried at 80°C for 8 hours.¹⁹ The next step for salmon (*Salmo salar*) is to use a grinder to smooth it out until it is 100 mesh smooth.¹⁹ The powder and oil are mixed with 1% CMC (Carboxy Methyl Cellulose) to dilute it. Keeping in the fridge.^{20,21}

Mice (*Mus musculus*) spend seven days in a cage to get used to it. Estrus cycles can be found by looking at the results of vaginal removal under a microscope during the adaptation period. When the female mice are in estrus in the afternoon, put 16 males and

females together in one cage and leave them for 12 hours. If the mice have a vaginal plug, they have reproduced and are on the 0th day. Pregnant mice are moved into their cages and separated into their cages. They are then split into two treatment groups: the treatment group eats salmon mixed with 1% CMC, and the control group eats salmon mixed with 1% CMC at a dose of 2.17 mg/0.5 ml three times a day every 6 to 8 hours for 21 days of pregnancy and 14 days after birth.^{22,23}

On the 14th day after giving birth, take the baby mice and kill them by breathing in ether. Give ether into the jar, then put the baby mouse in it and close the lid until it dies. The incisor teeth were removed with a scalpel, a blade, and anatomical tweezers by having the baby mice stand still at the work table. The teeth were then put in a container with 10% formalin buffers and a lid.²⁴

The part of the mouse's lower jaw that was taken, cleaned of dirt, and rinsed with water was the dental insisivus. After the teeth have been cleaned and burned at 600°C for 4 hours, they can be pounded with a mortar and pestle to make them smoother if they are not already. Next, the cooled tooth ash was mixed with 1 ml of concentrated HNO3 and a solvent until the total volume reached 10 ml. The next step is to filter it until the filtrate and residue are separate. Then, the filtrate is diluted with aquades inside the pumpkin takar 100 ml to the maximum mark. Atomic Absorption Spectroscopy (AAS) brand perkin elmer 3110 (United States) was used to test the samples.²⁵

RESULT

The study was done on two groups: the treatment group, which ate salmon, and the control group, which didn't eat salmon (Carboxy Methyl Cellulose). The statistical analysis of 12 samples of teeth mints showed that 6 of the lower front teeth were missing in the treatment group.

Table 1: Mean (%) and standard deviation for the control group (which got Carboxy Methyl Cellulose) and the treatment group (which got salmon)

Group	Sample	Mean (%)	Standard deviation
Treatment	6	16,94 %	2,26
Control	6	14,24 %	1,67

Table 1 shows that the average value for the salmon treatment group is 16.94%, with a standard deviation of 2.26, while the average value for the CMC group is 14.24%, with a standard deviation of 1.67. If a mouse mother eats salmon while the baby is still in the womb and after birth, the baby mice's teeth will have more calcium. Statistics were used to show that the amount of salmon eaten by the parent mice changes the amount of calcium in their offspring's teeth.

Tabel 2 Unpaired t test hypothesis

Group	P	Sig.0.05	Description
Treatment	0,041	P<0.05	Significant
Control			

Based on Table 2, the unpaired t-test showed a significant difference between the salmon-eating group and the CMC group.

DISCUSSION

Table 1 contains the mean. The amount of calcium in the teeth of the child who consumed salmon was 16.94% higher than that in the children in the control group who were fed CMC (*Carboxy Methyl Cellulose*), which was 14.24%. This happens because salmon has a lot of calcium, which can change the amount of calcium in the teeth, and a lot of omega-3, which can help the body absorb calcium from the teeth.²⁶

Calcium in salmon has many benefits, including preventing dental caries, making dental tissue stronger, reducing the solubility of enamel, speeding up the process of remineralization, and stopping streptococcus mutants from sticking to the surface of the tooth.^{4,27} Omega-3 also makes bones stronger, changes how calcium is absorbed, helps teeth and bones grow, improves brain and memory function, speeds up metabolism, improves eyesight, and keeps heart attacks from happening.^{10, 14} Calcium can get through the placenta and bind to calcium transport proteins (CaT1 and CaT2), which are then sent to the fetus.²⁸ Ca²⁺-sensing receptors on ameloblast cells will help them take in Ca²⁺. When Insulin Growth Factor (IGF) sends a signal, FATPs will also rise, which can help ameloblast cells move parts of proteins. Also, ameloblast cells release KLK-4, which can later break down amelogenin proteins. The release of calbindin D28 as a Ca²⁺ deposit will eventually lead to mineralization.²⁴

There is a big difference in the amount of calcium in the teeth of mice in the control group and the treatment group. This shows that eating salmon changes the amount of calcium in deciduous teeth. Previous research showed that high levels of Omega 3, especially DHA, are linked to high levels of BMD (Bone Mineral Density) all over the body and in the spine, as well as to bone growth in the spine.²⁹ Previous studies also found that calcium phosphate in salmon bones could be used to heal and grow new bones.³⁰ Salmon causes an increase in the quantity of calcium in the teeth. Additionally, salmon consumption promotes the growth of tooth tissue, which can significantly increase the calcium content of mice teeth.

REFERENCES

1. Marthinu LT and Bidjuni M. Penyakit Karies Gigi pada Personil Detasemen Gegana Satuan Brimob Polda Sulawesi Utara Tahun 2019. *Jurnal Ilmiah Gigi dan Mulut* 2020; 3: 58-64.
2. Yusmiati SNH and Wulandari ER. Pemeriksaan Kadar Kalsium Pada Masyarakat Dengan Pola Makan Vegetarian. *Jurnal SainHealth* 2017; 1: 43-43. DOI: 10.51804/jsh.v1i1.77.43-49.
3. Hossain MA and Yoshimatsu T. Dietary calcium requirement in fishes. *Aquaculture Nutrition* 2014; 20: 1-11. DOI: 10.1111/anu.12135.
4. Damayanti AY, Darni J and Octavia R. Asupan Kalsium dan Fosfor Berkaitan dengan Karies Gigi pada Anak Sekolah. *Nutri-Sains: Jurnal Gizi, Pangan dan Aplikasinya* 2020; 4: 67-76. DOI: 10.21580/ns.2020.4.1.5260.
5. Pflipsen M and Zenchenko Y. Nutrition for oral health and oral manifestations of poor nutrition and unhealthy habits. *General Dentistry* 2017; 65: 36-43.
6. Rathee DM, Singla DS and Tamrakar DAK. Calcium and Oral Health: A Review. *International Journal of Scientific Research* 2013; 2: 335-336. DOI: 10.15373/22778179/sep2013/116.
7. Van Der Velde RY, Brouwers JRBJ, Geusens PP, Lems W and Van den Berg JP. Calcium and vitamin D supplementation: State of the art for daily practice. *Food and Nutrition Research* 2014; 58: 1-12. DOI: 10.3402/fnr.v58.21796.
8. Bas M, Daglilar S, Kuskonmaz N, Kalkandelen C, Erdemir G, E. Kuruca S, Tulyaganov D, Yoshioka T, Gunduz O,

- Ficai D and Ficai A. Mechanical and biocompatibility properties of calcium phosphate bioceramics derived from salmon fish bone wastes. *International Journal of Molecular Sciences* 2020; 21: 1-14. DOI: 10.3390/ijms21218082.
9. Aryati E E and Dharmayanti AWS. Manfaat Ikan Teri Segar (*Stolephorus sp*) Terhadap Pertumbuhan Tulang dan Gigi. *ODONTO Dental Journal* 2014; 1: 52-56.
 10. Ramli. Karakteristik Organoleptik Biskuit dengan Fortifikasi Abon Ikan Salmon (*Oncorhynchus nerka*). *Jurnal Ilmu Perikanan* 2014; 5: 73-79.
 11. Colombo SM and Mazal X. Investigation of the nutritional composition of different types of salmon available to Canadian consumers. *Journal of Agriculture and Food Research* 2020; 2: 100056-100056. DOI: 10.1016/j.jafr.2020.100056.
 12. Eltokhey HM and Zahran DH. Evaluation of the Effect of Omega 3 Fatty Acid (N-3) on Socket Healing in Orchiectomized Rats. *Journal of American Science* 2011; 7: 263-271.
 13. Atanasoff A, Nikolov G, Staykov Y, Zhelyazkov G and Sirakov I. Proximate and mineral analysis of Atlantic salmon (*Salmo Salar*) cultivated in Bulgaria. *Biotechnology in Animal Husbandry* 2013; 29: 571-579.
 14. Septiarsih NT, Hadi S and Hidayati S. Hubungan Pola Konsumsi Ikan Laut Dengan Karies Gigi Pada Nelayan Di Pelabuhan Perikanan Pasongsongan Tahun 2020. *Jurnal Ilmiah Keperawatan Gigi* 2020; 1: 23-30. DOI: 10.37160/jikg.v1i3.545.
 15. Ahassa U, Kusuma Wardhani P and Titien Soeprihati I. The Effect of Cow and Soy Milk Consumption on Calcium Levels of Wistar Rat Teeth. *ODONTO Dental Journal* 2021; 8: 40-51.
 16. Malde MK, Bügel S, Kristensen M, Malde K, E Graff I and I Pedersen J. Calcium from salmon and cod bone is well absorbed in young healthy men: a double- blinded randomised crossover design. *Nutrition & Metabolism* 2010; 7: 1-9.
 17. Asikin AN and Kusumaningrum I. Uji Organoleptik Amplang Ikan Bandeng (*Chanos Chanos*) yang Difortifikasi dengan Tepung Tulang Ikan Belida. *Media Sains* 2016; 9: 152-161.
 18. Adawiyah A and Selviastuti R. Serburia Suplemen Tulang Ikan Bandeng Dengan Cangkang Kapsul Alginat Untuk Mencegah Osteoporosis. *Jurnal Ilmiah Mahasiswa* 2014; 4: 97088-97088.
 19. Harmain RM, Dali F, Nurjannah and Jacob AM. Karakteristik Organoleptik dan Kmia Ilabulo Ikan Patin Fortifikan. *Jurnal Pengolahan Hasil Perikanan Indonesia* 2017; 20: 329-338.
 20. Christiono S, Pradopo S and Sudiana IK. The Effect of Saltwater Fish Consumption by Mother Mice (*Mus Musculus*) on the Expressions of FABPs and Type 1 Collagen regarding Increase in Enamel Density. *Journal of International Dental and Medical Research* 2022; 15: 1535-1540.
 21. Christiono S, Ardiani FP, Anggarani W and Eljabbar FD. The effect of saltwater fish nanoparticle powder consumption on tooth enamel density In Vivo Study of Mice (*Mus musculus*). *Dentino : Jurnal Kedokteran Gigi* 2021; 6: 1-1. DOI: 10.20527/dentino.v6i1.10724.
 22. Kurniasari S, Yanti AH and Setyawati TR. Kadar Malondialdehyde Induk dan Struktur Morfologis Fetus Mencit (*Mus musculus*) yang Diperengarkan Murottal dan Musik Rock pada Periode Gestasi. *Jurnal Protobiont* 2017; 6: 89-97.
 23. Sulastris S, Wiratmini NI and Luh Suriani N. Panjang siklus estrus mencit (*mus musculus l.*) yang diberi pemanis buatan aspartam secara oral. *Jurnal Biologi* 2014; 18: 69-72.
 24. Samia S, Christiono S and Sari RK. Pengaruh Susu Ibu Hamil Terhadap Ekspresi Kallikrein-Related Peptidase-4 (Klk-4) Pada Sel Ameloblas Dalam Tumbuh Kembang Gigi. *ODONTO : Dental Journal* 2020; 7: 98-98. DOI: 10.30659/odj.7.2.98-103.
 25. Dewi LK, Supriadi and Aminah S. Analysis of Calcium (Ca) Levels in Milkfish's (*Chanos chanos*) Bone Using Atomic Absorption Spectrophotometry (AAS). *Jurnal Akademika Kimia* 2021; 10: 15-19. DOI: 10.22487/j24775185.2021.v10.i1.pp15-19.
 26. Tompkins YH, Chen C, Sweeney KM, Kim M, Voy BH, Wilson JL and Kim WK. The effects of maternal fish oil supplementation rich in n-3 PUFA on offspring-broiler growth performance, body composition and bone microstructure. *PLoS One* 2022; 17: e0273025. 2022/08/17. DOI: 10.1371/journal.pone.0273025.

27. Hendaro A. Nutrisi dan Kesehatan Gigi-Mulut pada Anak. *Sari Pediatri* 2015; 17: 71-71. DOI: 10.14238/sp17.1.2015.71-5.
28. Goodfellow LR, Cooper C, Harvey NC and Javaid MK. Regulation of Placental Calcium Transport and Offspring Bone Health. *Frontiers in Endocrinology* 2011; 2: 1-7. DOI: 10.3389/fendo.2011.00003.
29. Höglström M, Nordström P and Nordström A. n-3 fatty acids are positively associated with peak bone mineral density and bone accrual in healthy men: The NO2 study. *American Journal of Clinical Nutrition* 2007; 85: 803-807. DOI: 10.1093/ajcn/85.3.803.
30. Bas M, Daglilar S, Kuskonmaz N, Kalkandelen C, Erdemir G, Kuruca SE, Tulyaganov D, Yoshioka T, Gunduz O, Fıcaı D and Fıcaı A. Mechanical and Biocompatibility Properties of Calcium Phosphate Bioceramics Derived from Salmon Fish Bone Wastes. *International Journal of Molecular Sciences* 2020; 21: 1-14. DOI: 10.3390/ijms21218082.