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Effects of applying anchovy *(Stolephorus insularis)* substrates on the microhardness of tooth enamel in Sprague-Dawley rats

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Abstract. Anchovies *(Stolephorus insularis)* contain high levels of fluor in the form of CaF_2. The aim of this study is to analyze changes in tooth enamel microhardness after application of anchovy substrates by feeding or as a topical fluoridation material. An in vivo study of the lower left incisors of nine Sprague-Dawley rats was conducted. The sample was comprised of baseline and treatment groups, including feeding application, topical application, negative control feeding, and negative control topical groups. The treatment groups were given 5% anchovy substrates through feeding and topical applications. After treatment, tooth samples were extracted from each of the rats for examination, and statistical analyses were performed after determining hardness numbers for enamel surfaces using Vickers microhardness tester. Vickers hardness numbers (VHNs) for anchovy substrate application and consumption by feeding (440.3 ± 24.72) were higher than for the negative control (315.80 ± 17.51). VHNs for the topical application group were higher than for the negative control (347.28 ± 28.56) and for the feeding group. The use of anchovy as a fluoridation material in form of topical application is potentially an effective method for increasing the microhardness of the tooth enamel surface

1. Introduction

According to the Household Health Survey 2004 by the Indonesian Health Department, dental caries are the most frequent oral disease, with a national prevalence of 90% [1]. Dental caries occur due to demineralization of dental hard tissue, which is the loss of mineral components due to diminished hydroxyl groups in the hydroxyapatite that tooth enamel is composed of, without remineralization [2]. Fluoridation is one method used to prevent dental caries and improve tooth resistance to acid [3]. This method changes the hydroxyapatite compound in enamel into fluorapatite, which has higher resistance to acidic environments. Fluorapatite creates more solid crystals, resulting in the tooth surface reacting with and reducing acid, while increasing enamel microhardness [4,5].

In dentistry, the two most common fluoridation techniques are systemic and topical [6]. Systemic fluoridation is beginning to be replaced because it has been found that the addition of fluoride during the process of tooth development is not sufficient for preventing caries [7]. Additionally, excessive fluoride intake is absorbed by the body into the bloodstream, which prevents enamel cell formation (ameloblast), resulting in dental fluorosis [8]. Prolonged use of swallowed fluoride results in the fluoride returning to the oral cavity in saliva secretions in very low concentrations (0.02 ppm F); thus, it is not as effective for preventing caries as topical application (500 ppm F) [9]. Fluoride that enters the digestive system binds to organic components to be excreted in feces or urine (2.5 ppm F) [5]. Topical fluoridation, such as fluoride toothpaste, has been proven effective for increasing enamel hardness post tooth eruption [10] and can also be applied during the mastication process, which provides prolonged contact between fluoride and the teeth [4].

Fluoride compounds often used in topical dentistry applications are sodium fluoride (NaF) [11] andCaF_2, which is more commonly used as a fluoride reservoir, binds more closely with enamel, and provides slowly and continuously releasing fluoride ions. CaF_2 also decreases enamel porosity, solidifies enamel composition, and increases the hardness of the teeth [12]. However, synthetic CaF_2 is expensive and availability in liquid form is limited; thus, this compound is rarely used as a topical fluoride [5]. A potential fluoride source is anchovy *(Stolephorus insularis)*, which is easily obtainable and affordable in Indonesia due to it being a maritime country. In vitro research has proven that anchovy solutions are natural topical fluoridation materials that increase tooth enamel microhardness [5]. Fluoridation in previous research has been accomplished by smearing, while community consumption of anchovies is done by masticating. Therefore, this research compares the effectiveness of topical fluoridation in anchovy substrates applied by smearing and by masticating, in vivo, to observe the effects on enamel hardness. To imitate the human oral cavity, Sprague-Dawley rats will be used because they have similar enamel to humans [13].

2. Materials and Methods

This study was conducted on nine Sprague-Dawley rats. Prepared subjects were pre-examined, which included weighing, examining teeth, examining sex, and measuring eating behaviors times by observing the rats as they ate. Mastication duration was used as the basic measurement to determine the duration of solution application per day. Based on initial observations, the rats took 30 minutes to chew 20 grams of pellets. Three types of rat food were given in the study: commercial, control, and treatment. Commercial rat food was available through retailers with a composition provided by the factory that made it. The control food was made by mashing sweet corn in a blender, which was then mixed with wheat flour to form dough that was shaped similar to pellets and dried in the sun for one day. Rat food with a mixture of 5% anchovy (i.e., the treatment food) was made by mashing sweet corn in a blender, drying the anchovy in the sun for two days, heating the anchovy in an oven to a temperature of 80 °C for one hour and blending to a powder, and mixing the corn and anchovy powder with wheat flour. The resulting dough was formed into small balls similar to pellets and dried in the sun for one day. For the topical treatment, an anchovy substrate of 5% was made fresh daily by mixing 0.5 grams of anchovy powder and 10 ml of distilled water in a centrifuge tube. The amount of

Table 1. Sprague-Dawley rat food

Commercial		Control			Treatment		
Component	Percentage	Component		Percentage	Component		Percentage
Water	13%		Carbohydrate			Carbohydrate	
Protein	19–21%	Sweet Corn [28]	Protein	52.50%	Sweet Corn [28]	Protein	52.50%
Fat	5%		Sugar			Sugar	
Fiber	5%		Mineral			Mineral	
Ash	7%		Carbohydrate			Carbohydrate	
			Protein			Protein	
Calcium	0.90%		Water			Water	
Phosphor	0.60%	Wheat Flour	Sugar	47.50%	Wheat Flour	Sugar	45%
			Fat			Fat	
M.E.	3,000–3,100 Kcal/kg		Mineral			Mineral	
					Anchovy	Protein, F (CaF_2)	5%

anchovies contained in the food and the solution was determined based on the assumption that in one serving of human food, anchovy makes up 5% of rice and side dishes, especially in Indonesia as an archipelago.

The Sprague-Dawley rats used in the study were divided into five treatment groups. Group 1 (1 rat, baseline) was a group given commercial pellets with a factory-made composition and the method of feeding at the place of breedingwas maintained. Group 2 (2 rats, feed control) was fed thecorn pellets with a basic composition. Group 3 (2 rats, aquadest control) was smeared with aquadest. Group 4 (2 rats, anchovy feed) was fed pellets containing 5% anchovy substrate. Group 5 (2 rats, treated with the anchovy solution) was smeared with aquadest solution containing 5% anchovy. Application occurred twice a day for 15 minutes, and pellet feeding occurred once a day and the rats were given 20 grams of feed. Treatment was performed for 15 days. After treatment, the lower left incisors from the rats were cut to see the value of tooth enamel surface hardness.

Tooth samples were taken and all rats were terminated using ether. The lower jaws were separated from the rest of the bodies using a scalpel, tweezers, and tissue scissors. The remaining soft tissue was cleansed from the lower jaw, whichwas washed with 90% alcohol, soaked in 70% formalin, and dried. Dry jaws were stored in plastic pots with silica gel.Preparation of the test specimens was done by stirring acrylic powder and liquid to a dough consistency and fully inserting this solution into a 1.5 cm diameter paralon pipe to be used as a medium for the test specimens. The separated lower jaws were put into a mold containing acrylic with the tip of the rat teeth resting on the shoulder of the mold. The flatest tooth position was set for hardness testing. Excessive acrylic was removed, and the paralon surface was set so that no acrylic exceeded the tooth. After the acrylic hardened, the bottom part was trimmed flat and made smooth with a grinding and polishing machine. Prepared samples were tested with a Vickers microhardness tester. The load selection was based on previous research and set to 50 grams. The microhardness results for tooth enamel surfaces were statistically tested using the one-way ANOVA method and post-hoc Tukey and independent sample t-tests were performed to determine the significance of the differences in Vickers hardness values for each group.

3. Results and Discussion

3.1 Results

As many as five marks were assigned to each dental specimen using the Vickers microhardness tester. The results of rat tooth enamel hardness are given in Table 2.

Table 2. Rat tooth enamel hardness

Group	N Sample	N Marks	Mean	SE	SD	Min.	Max.
Baseline	1	5	326.89	6.54	14.61	311.53	340.49
Feed Control	2	10	315.80	5.54	17.51	294.23	351.05
Aquadest Control	2	10	347.28	9.03	28.56	311.53	385.85
Anchovy Food Treatment	2	10	440.30	7.82	24.72	412.00	490.31
Smearing Treatment	2	10	510.32	11.30	35.72	456.51	570.24

Vickers hardness value in Vickers hardness number (VHN)

3.2 Discussion

The study included groups consisting of a baseline, treatments with smearing and feeding, and controls for each method (i.e., feed control and aquadest control). This grouping was used to determine the effect of anchovy application, either through feeding or topically, on increasing tooth enamel surface hardness. In addition, this study compared the most effective methods of application of fluoride for improving the surface enamel microstructure. The duration of fluoride-smearing component preparation for rat teeth was determined based on observations of experimental rats that spent 30 minutes eating an average of 20 grams of rat food per daily. Thus, each day, food came into contact with the rat's teeth due to the mastication process for 30 minutes. Because of the topical effect of fluoride contained in anchovies on enamel results from the chewing process, feedings were carried out for 15 minutes two times a day to obtain the duration of enamel contact with fluoride components that resembled the duration of contact of human tooth enamel with food consumed in a day [14].

The factory-made commercial feed for laboratory rats contains calcium and phosphorus content, which are tooth-shaping minerals [15,16]. This was the basic consideration of the researchers while preparing artificial food for the rats used in this study, which consisted of sweet corn and wheat flour to control treatment content and ensure the results were not affected by mineral elements that may affect tooth formation. In addition, commercial food could not be used because of its raw composition, which could not be mixed with anchovy powder. Like rat food, the aquadest solution used in this study was intended to control the aquadest content to ensure that teeth were unaffected by other mineral deposits. The selection of 5% anchovy for the treatment groups (feeding and smearing) was based on the assumption that in one portion of human food there is 5% anchovy in rice and side dishes [5]. Previous studies using topical fluoride applications increased fluoride retention after 14 days of treatment; therefore, it was decided in this study to administer fluoride through anchovies to experimental animals for 15 days [14,17].

The surface enamel microstructure measurements used VHNs, which take into account small and very hard properties of teeth but prevent the use of other tests [18]. One third of the rat incisors were designated for tests, which were performed by calculating the length of each rat tooth (±7 mm) and extracting the entangled tooth length (±0.4 mm/day x 15 days = ±6 mm) to estimate the portion continuously exposed to fluoride exposure during treatment.13 From the results of this research, no significant difference was found for average hardness values between the baseline group and the food and aquadest control groups. In accordance with the purpose of the baseline group, the results showed no effect of corn food or aquadest on teeth. The surface hardness of tooth enamel among rats in the treatment groups (feeding and topical) was significantly different from the control group. For the specimen group smeared with anchovy solution, the mean hardness value was higher than the mean hardness value of the aquadest control specimens.

Data analysis revealed that feeding anchovies increased surface enamel microhardnessby 13.9%, while smearing teeth with anchovy solution increase surface enamel microhardness by 14.7%. Based on previous theories, it was assumed that this increase was due to an influx of fluoride from the CaF2 contained in anchovies resulting in apatite crystals that formed fluoroapatite compounds in treatment specimens [5,12]. In the fluoroapatite, there was a decrease in the length of the lattice parameter, which increased tensile strength between fluoride and apatite ions and resulted in increased binding force [19]. Thus, apatite crystals (i.e., enamel) became denser, which increased surface enamel hardness [12]. It was concluded that both feeding anchovies and topically applying an anchovy solution increased tooth enamel hardness.

The results of this study showed that the mean enamel surface hardness of the smearing treatment group specimens (510.32 ± 35.72) was higher than the mean enamel surface hardness of the feeding treatment group specimens (440.30 ± 24.72). The advantage oftopical application over feeding is improved surface enamel microstructures due to more consistent fluoride quantities and contact with enamel surfaces. In contrast, the feeding of fluoride-containing anchovies caused contact between fluoride and enamel to occur only during the process of chewing food. Thus, the quantity of fluoride in contact with the enamel and the duration of this contact were inconsistent and based on the amount of food consumed. In addition, feeding increased the likelihood of fluoride being swallowed and creating a systemic effect compared to topical application.

4. Conclusion

The both feeding anchovies and topical application of an anchovy solutionincrease tooth enamel surfacemicrohardness. However, this research showed that the topical method was more effective in improving tooth enamel surface microhardness than feeding.

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