The effect of anchovy substrate application to fluor retention rate on Sprague Dawley rat tooth email (in vivo)

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Abstract. Usage of anchovies (Stolephorus insularis), which contain a high fluoride concentration in a CaF$_2$ compound, needs to be examined as a topical fluoridative agent. Aim: To study the effects of an anchovy substrate application, either by chewing or smearing, in increasing fluoride retention of enamel. Fourteen Sprague Dawley rats were divided into five groups: baseline, experimental (feeding and smearing), and negative controls. After 15 days, lower incisor teeth were extracted and fluoride retention on the enamel surface was measured using EDX. Data were analyzed by the independent samples t-test, the Mann–Whitney U test, and the Kruskal–Wallis test. There was a significant increase in fluoride retention on enamel from the experimental groups compared to the negative control group (p < 0.05). Fluoride retention levels of the experimental feeding group (6.823%) were slightly higher than those of the experimental smearing group (6.783%), though the difference was not statistically significant (p < 0.05). Anchovy substrate application, either by chewing or smearing, increases fluoride retention on tooth enamel.

1. Introduction
Dental caries is a demineralization process of dental hard tissue [1]. According to Indonesian Health Survey 2007, caries is one of the most common complaints in dental and oral disease, with a prevalence of 73.3% [2]. Because of the high rate, preventive efforts are necessary, such as fluoridation [3,4]. This method can transform the hydroxyapatite compound in enamel into fluorapatite or hydroxyfluorapatite. Most of the fluoride that is incorporated in dental tissue is located in the outer portion of dental enamel. The amount of fluoride concentration on the tooth surface is stated as fluoride retention ability, one of the parameters for successful dental fluoridation. The higher the fluoride retention rates the more surface area of the tooth that is composed of fluorapatite, which is more resistant to low pH in the oral cavity. Fluoridation may be given systemically or topically. Systemic fluoridation is done through the consumption of foods, drinks, or fluor tablets. However, the administration of systemic fluoridation has a risk of excess intake because the dose is difficult to measure. In addition, systemic fluoridation only has a significant effect during pre-eruption, when fluoride ions affect development of the tooth bud.

On the other hand, topical fluoridation also occurs through the process of food mastication, when there is local contact between fluoride and tooth tissue. Topical fluoridation applications are effective when administered after dental eruption [3]. In dentistry, there are various topical fluoridation materials such as toothpaste; mouthwash; and NaF, SnF, APF, and CaF$_2$ gel. CaF$_2$ gives the best fluoridation results because of its ability to release fluoride gradually [5,6]. However, this compound
is not widely used in dentistry because it is a difficult material to get and is expensive. One of the potential food ingredients as a source of fluor is anchovy (*Stolephorus insularis*). Anchovy is a fish food source that is easy to get in Indonesia because Indonesia is a maritime country. The fluor in *Stolephorus insularis* is contained in a CaF$_2$ compound in greater concentrations than in other anchovies. In vitro preliminary studies have proven that application of an anchovy solution may increase fluor retention in the enamel surface [5]. However, application of anchovy solution as a topical fluoridation material needs to be further investigated at the in vivo level.

In vivo research has the advantage that the oral cavity is similar to the actual state in humans with regard to the presence of salivary flow and oral pH. However, this study cannot be conducted in humans due to the necessity of cutting enamel for laboratory examination. Therefore, an animal model is used with Sprague Dawley rats. These rats have enamel structure that is similar to human tooth enamel. This study was conducted to prove the effectiveness of topical fluoridation through the application of an anchovy substrate in vivo. One method of application used in the study was smearing. The effectiveness of this method is compared with administration by mastication, which is the way the general public consumes anchovies.

2. Materials and Methods
This was an experimental laboratory in vivo study using 14 Sprague Dawley rats. The study sample was composed of lower incisor teeth of the rats. The research variables were the administration method of the anchovy (feeding or smearing) as the independent variable, and fluor retention as the dependent variable. The research flow was as follows:

2.1. Subject Preparation
This study used rats that had been selected according to the inclusion criteria (male Sprague Dawley rats, 2 months old, body weight 120–140 grams, no dental anomalies, and good general health).

2.2. Initial Inspection
Examinations were conducted, including examination of tooth condition and measurement of the Sprague Dawley rats’ feeding duration. Observations were done on the duration of chewing of 20 grams of pellets. The chewing duration was used to determine the basic measurement of solution smearing to be done per day.

2.3. Making the Rat Feed
This study used three kinds of rat feed, namely, commercial feed, feed as a control (without a mixture of anchovy), and treatment feed (with a mixture of 5% anchovy). Commercial rat feed is a pellet-shaped rat feed with a factory-made composition that is available in the market. Feed control and feed treatments are feeds that are tailored to the needs of the study. The rat feed is made by mixing sweet corn that has been shaved and mashed with flour. The dough is shaped into small round pellets and then dried for one day. In feed treatment, the feed is mixed with anchovy powder before it is shaped into a pellet. The anchovy powder was manufactured by drying anchovies in the sun for two days and then putting them in the oven at 80 °C for one hour, after which they are placed in a blender until a powder consistency is achieved.

2.4. Preparation of 5% Anchovy Fish Solution
A 5% jengki anchovy solution was made fresh daily by mixing 0.5 grams of anchovy powder with 10 ml of Aqua Dest in a centrifuge tube.

2.5. Grouping and Treatment of the Subjects
Treatments were given for 15 days. After treatment, lower incisors were taken from each rat subject. The Sprague Dawley rats were divided into five treatment groups: the baseline group, the negative control group for feeding, the negative control group for smearing, the feeding group, and the
smearing group. The baseline group was fed once per day. The negative control group for feeding was given a control pellet once a day. The negative control group for smearing was given a control pellet once a day and was smeared with Aqua Dest twice daily for a duration of 15 minutes for each application. The treatment group for feeding was given pellets containing 5% anchovy once a day, while the treatment group for smearing was given a control pellet once a day and smeared with a 5% anchovy solution twice daily with a duration of 15 minutes for each application.

Table 1. Sprague Dawley rat feed

| Composition   | Control Percentage | Composition | Treatment Percentage | Composition | Treatment Percentage |
|---------------|--------------------|-------------|-----------------------|-------------|-----------------------|
| Water         | 13%                | Carbohydrate | 52.50%                | Carbohydrate | 52.50%                |
| Protein       | 19–21%             | Sweet Corn [28] | Protein               | Sweet Corn [28] | Protein               |
| Fat           | 5%                 | Sugar       | Mineral               | Mineral     |                       |
| Fiber         | 5%                 |             |                       |             |                       |
| Ash           | 7%                 | Carbohydrate | Protein               | Protein     |                       |
| Calcium       | 0.90%              | Water       | Wheat                 | Water       |                       |
| Phosphor      | 0.60%              | Wheat flour | Sugar                 | Sugar       | Fat                   |
| M.E.          | 3000–3100 Kcal/kg  | Mineral     | Anchovy               | Mineral     | Protein F (CaF2)      |

2.6. Tooth Sampling
After 15 days of treatment, a lower incisor was taken from each rat in each treatment group. Rats in the baseline group, the negative control group for feeding, the negative control group for smearing, the treatment group for anchovy feeding, and the treatment group for smearing of anchovy solution were terminated using ether. The lower jaw was separated from the rest of the body by using a scalpel, tweezers, and tissue scissors. The remaining soft tissue was cleansed from the lower jaw that had been cut from the tissue attachment. The lower jaw was cleansed with 90% alcohol, soaked in 70% formalin, and dried. Dry jaws were stored in plastic pots using silica gel. For the next step, element analysis using EDX, the jaw had to be completely dry.

2.7. Interpretation of Results
Observations of fluorine retention rates on the surface of the enamel were performed using a Carl Zeiss EVO MA 10 scanning electron microscope and a Bruker Nano XFlash Detector 5010 energy dispersive X-ray. Fluor retention was measured in percentages according to specified EDX standards. The measured element concentrations were Ca, P, O, and F, which are the constituents of fluorapatite (Ca10 (PO4)6F2). The results were found using normalized data methods in which the number of all four elements measured is 100% by excluding other elements such as Mg, N, Cl, and H. The shot of a backscattered ray emission aimed at the 1/3 labial center area, with the same area sampled on each tooth.

2.8. Data Processing
To find the distribution of data in each group, a Lilliefors normality test was done using the Shapiro–Wilk test. To compare the fluoride retention rate on the baseline group with the negative control group, the Kruskal–Wallis method of statistical test was used. To compare the fluoride retention rate on the group treated with anchovy smearing to the negative control group for smearing, a Mann–
Whitney statistical test was performed. To determine the ratio of fluoride retention rates in the feeding treatment group with the negative control group for feeding and to compare between the treatment groups, the Independent Samples T-test statistical test was performed.

3. Results and Discussion

3.1 Results

In this study, area mode evaluation was used, in which fluor concentrations on the enamel surfaces (fluoride retention) is stated as a percentage of the element standard of EDX tools, as follows:

| Group                | N  | Mean   | SE    | SD    | Min  | Max  |
|----------------------|----|--------|-------|-------|------|------|
| Baseline             | 2  | 4.13   | 2.74000 | 3.87495 | 1.39 | 6.87 |
| Feed control         | 4  | 4.295  | 0.93504 | 1.87009 | 2.28 | 6.73 |
| Aqua Dest control    | 4  | 2.25   | 0.94366 | 1.88733 | 1.02 | 5.06 |
| Feeding treatment    | 4  | 6.823  | 0.52879 | 1.05759 | 5.45 | 8.03 |
| Smearing treatment   | 4  | 6.783  | 1.03221 | 2.06442 | 4.76 | 9.29 |

Note: fluor retention in (%) of the EDX standard

It appears that the highest fluoride retention was obtained in the feeding treatment group, and the lowest fluoride retention was found in the negative control group for smearing. From the normality test results, the distribution of data across the whole group was found to be normal except for the baseline group and the negative control group for smearing.

![Figure 1](https://example.com/figure1.png)

**Figure 1.** The mean comparison of fluor rates between baseline and the negative control group

The baseline group had approximately the same fluor retention (4.13%) as the negative control group for feeding (4.295%), and both were higher than the negative control group for smearing (2.25%). However, there was no statistically significant difference (p > 0.05).
Figure 2. The mean comparison of fluor retention rates in feeding treatment group

The anchovy feeding treatment group had higher fluor retention (6.823%) than the negative control group for feeding (4.295%) or the baseline group (4.13%). Statistically, there was a significant difference between fluoride retention rates in the anchovy feeding treatment group and the negative control group for feeding.

Figure 3. The mean comparison of fluor retention rates in smearing group

The group receiving a smearing treatment of anchovy solution had higher fluor retention (6.783%) than the negative control group for smearing (2.25%) or the baseline group (4.13%). Statistically, there was a significant difference between fluoride retention in the anchovy smearing treatment group and the negative control group for smearing.

Figure 4. The mean comparison of fluor retention rates between feeding group and smearing group

The anchovy feeding group had higher fluor retention (6.823%) than the anchovy solution smearing group (6.783%). However, there is no statistically significant difference.
3.2 Discussion

The Sprague Dawley rat was chosen as the subject of the study because the rat has a similar enamel structure as humans. The rats used as research subjects were treated with feeding and smearing. This type of treatment is based on the consideration that people generally eat anchovies in the form of food, while preliminary research stated that the fluoridation of the anchovy substrate occurs through application by means of smearing [5].

The duration of treatment was determined by Lennon et al.'s study on the effects of application of a casein–calcium phosphate and fluor tooth cream on cow teeth. In the study, the application was performed for 14 days (twice a day) [7]. The results indicated that within two weeks, topical gel applications containing fluoride could improve the resistance of enamel to erosion [6]. Therefore, in this study, treatment was given for 15 days with the assumption that length of time is sufficient to produce significant fluoride retention to protect the enamel.

To ensure that the measured fluoride retention value in this study was purely due to the treatment, the baseline and negative control groups were used as controls for rat food composition, while the negative control group for smearing was to confirm the possibility of fluoridation occurring due to the solvent used (Aqua Dest). It is known that Aqua Dest contains certain minerals.

In this study, the percentage of fluoride retention in the baseline group was 4.13%. This value indicates fluoride retention in enamel in a condition without any intervention. The results showed the tendency of the fluor retention rate in the baseline group (4.13%) was equivalent to the negative control group for feeding (4.295%). However, both were higher than the negative control group for smearing (2.25%). This result is probably due to the effect of Aqua Dest smearing, which can dissolve natural fluoride that is fixed on the enamel surface of the tooth.

Commercial feed could not be used because it is available in fixed form (pellet) so it cannot be mixed with anchovy fish powder to get the appropriate concentration. Therefore, feed alternatives were manually made using ingredients that contain nutrients more or less the same as commercial feeds [8]. The making of corn feed refers to the Nurdin et al. study that used Sprague Dawley rats that were fed various types of Indonesian corn [9]. Because artificial corn feed does not affect fluoride retention rates, the feed could be used in this study.

Fluoride retention of both treatment groups was higher than that of the two negative control groups. This proves the existence of a fluoridation process due to the addition of an anchovy substrate, both in feed form and as a solution. This result eliminates the possibility of fluoridation occurring due to food composition (in this case, corn and flour) or solvent (in this case, Aqua Dest).

Statistical analysis ensures that fluoride administration through mastication of food may improve fluoride retention in rat tooth enamel. The pH of the oral cavity decreases after eating due to exposure to carbohydrates. The cariogenic bacteria will ferment the carbohydrate to acid, which causes a decrease in pH within minutes after the process of chewing food [10]. This acidic condition causes the concentration of H⁺ ions to increase so that the polarity of the enamel surface is more positive. H⁺ ions will draw OH⁻ ions from the hydroxyapatite group so that the crystal structure is released. Consequently, the negatively charged F⁻ ion is more ready to react with the surface of the enamel. In addition, the CaF² compound in anchovies is easily decomposed at low pH, so there are enough fluoride ions available in the saliva to bind to apatite crystals [5].

The analysis result in the treatment group showed that the application of anchovy fish solution can increase fluoride retention on the enamel surface of the tooth. When smearing the anchovy solution, there is no F⁻ binding with tooth enamel structure because the pH of the oral cavity does not decrease. The pH level remains the same due to the absence of chemical reactions at the time of mastication, so acidic conditions are not formed in the oral environment. Therefore, the reaction that occurs is an interstitial reaction. The fluoride ions will enter the gap between the apatite crystals (microtunnel). Good retention results are also caused by the fluor carrier media in the form of liquid so that it can more easily penetrate into the microtunnel.
Statistically (p > 0.05), fluoride retention in the anchovy feeding group (6.823%) did not differ from the group treated with the anchovy solution (6.783%). These results indicate that whether the method of administering anchovies is through feeding or smearing the same fluoride retention results in rat tooth enamel. Both methods have their own advantages and disadvantages, but the result of fluorine retention is not different.

In the feeding method, the effect of topical fluoridation is obtained through local contact between the tooth surface and food. During mastication, the fluoride will bind specifically (chemically) with tooth enamel by replacing the OH- group. However, the attached fluoride in this surface can be detached due to factors such as drinking activity of the rats or biting the cage between mastication times. With the smearing method, contact duration and the amount of fluoride exposure to enamel is more controlled. However, most fluoride ions will enter the gap between the enamel prisms so that the bonds formed are not as strong as the substitution reactions. The fluoride ions are easily removed by brush strokes during smearing or by eating and drinking activity after application.

This research can be improved upon with an analysis of compounds to see the formation of fluorapatite crystals. Prior to additional clinical research, it is necessary to examine the most effective form of the anchovy as a topical fluoridation material. There could also be a comparative study of fluoridation through the administration of an anchovy substrate with topical chemical fluoridation materials available on the market.

4. Conclusion
The administration of fluoride by either feeding or smearing an anchovy (*Stolephorus insularis*) substrate solution can increase fluoride retention in tooth enamel. The two methods of substrate administration showed no difference in fluoride retention in tooth enamel. Therefore, in vivo tests in Sprague Dawley rats indicate that anchovies (*Stolephorus insularis*) can be used as a topical fluoridation material.

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