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# Differences in the Effects of Remineralization Materials from Etawa Crossbreed Goat's Milk with Casein Phospopeptide-Amorphous Calcium Phosphate (CPP-ACP) on Enamel Surface Hardness (in Vitro)

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A B S T R A C T

As a natural protective mechanism, the body has a remineralization process that allows tooth enamel to repair minor damage before it progresses further. The aim of this study is to determine the effect of soaking in Etawah Crossbreed goat milk on enamel surface hardness and to compare the remineralization effect between CPP-ACP and soaking in Etawah Crossbreed goat milk on enamel surface hardness. This study used an experimental laboratory method with a posttest-only control group design. The results of this study showed a significant difference between CPP-ACP, Etawah Crossbreed goat milk, and artificial saliva in increasing enamel surface hardness. The greatest increase in enamel hardness was observed in the group soaked in Etawah Crossbreed goat milk.

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**INTRODUCTION**

Tooth enamel is the hardest part of the human body, consisting of the mineral hydroxyapatite which provides strength and resistance to various stresses. However, despite its strength, tooth enamel is still susceptible to demineralization due to exposure to acids from food, drinks, or bacterial metabolism in the oral cavity (Goel et al., 2021) . Demineralization occurs when calcium and phosphate ions are released from the enamel structure due to acidic environmental conditions. If left untreated, this process can develop into carious lesions or non-carious erosion, leading to permanent damage to the teeth.

As a natural protective mechanism, the body has a remineralization process that allows tooth enamel to repair minor damage before it progresses further. This process depends on the balance of calcium and phosphate ions in saliva (Andrini et al., 2013) . Saliva plays an important role in maintaining the balance of minerals on the tooth surface, especially with the presence of proteins and enzymes that help neutralize the pH of the mouth and support remineralization. However, in some conditions, this natural ability is not enough to prevent further demineralization, so additional interventions are needed to help increase tooth enamel remineralization.

One of the widely used remineralization materials is Casein Phosphopeptide-Amorphous Calcium Phosphate (CPP-ACP). CPP-ACP is a bioactive complex derived from milk protein, especially casein, which is combined with calcium phosphate in amorphous form. This component functions as a reservoir of calcium and phosphate ions, which can help improve remineralization by rebalancing minerals lost from tooth enamel. In addition, CPP-ACP also has the ability to inhibit the growth of caries-causing bacteria by reducing the acidity of the oral cavity environment. (Abdul Shahariyar et al., 2016)

The use of CPP-ACP in the prevention of caries and tooth erosion has been widely studied, especially in the form of toothpaste, chewing gum, and gel. Several studies have shown that CPP-ACP can increase the resistance of tooth enamel to acid and prevent the early development of caries. The combination of CPP-ACP with fluoride is known to provide a more optimal remineralization effect, because fluoride can play a role in forming fluorapatite which is more resistant to demineralization than natural hydroxyapatite of tooth enamel. (Lesmana et al., 2022)

However, despite its many benefits, CPP-ACP also has some limitations. One of its weaknesses is its stability in a highly acidic environment. In low pH conditions, the solubility of CPP-ACP decreases, so its effectiveness in releasing calcium and phosphate ions is reduced (Rizqan et al., 2019) . Because CPP-ACP is derived from milk protein, its use may be limited for individuals who are allergic to dairy products or lactose intolerant. Alternative remineralization materials are needed that can provide similar benefits but with wider availability and tolerability.

One of the natural ingredients that has the potential to be used as a remineralization agent is goat milk, especially from Etawa crossbred goats. Goat milk has long been known as a source of nutrition rich in protein, calcium,

phosphorus, and various other minerals that are important for healthy teeth and bones. Compared to cow's milk, goat milk is easier to digest because the size of its fat molecules is smaller and its protein composition is more similar to breast milk. Goat milk also has a lower level of allergenicity, making it safer for consumption by individuals with cow's milk allergies (Cholissodin et al., 2017) .

Recent studies have shown that casein gel from Etawa crossbreed goat milk can increase the hardness of primary tooth enamel in vitro. This suggests that the protein and mineral components in goat milk have the potential to help remineralize demineralized teeth (Ratya et al., 2017) . Casein gel acts as a carrier of calcium and phosphate ions that can adhere to the surface of tooth enamel, similar to the mechanism of action of CPP-ACP. Thus, the use of goat milk as a natural ingredient for dental care can be a more affordable and acceptable alternative for more people, especially for those who cannot use cow's milk-based products.

In addition to its benefits in remineralizing tooth enamel, goat milk also has several other advantages in supporting oral health. Goat milk contains lower amounts of lactose than cow's milk, making it friendlier for individuals with lactose intolerance (Eka Putri Risyani et al., 2022) . Several studies have shown that goat milk has antibacterial properties that can help reduce the growth of caries-causing bacteria, such as Streptococcus mutans. This is due to the presence of bioactive compounds in goat milk that can inhibit bacterial adhesion to the surface of teeth and plaque.

Although the potential of goat milk as a natural remineralizing agent is quite promising, further research is still needed to test its effectiveness in clinical conditions. Trials in real oral environments are needed to determine the extent to which goat milk can provide protection against caries and enamel erosion. Formulations of goat milk-based products, such as toothpaste or mouthwash, also need to be developed to make them easier to use in daily dental care. (Novianty & Ginawangsasih, 2020)

## THEORETICAL REVIEW

Casein Phosphopeptide-Amorphous Calcium Phosphate (CPP-ACP) is a bioactive compound derived from milk protein and is used in dentistry, especially to prevent and repair tooth decay (remineralization of tooth enamel). In short, CPP-ACP is a natural tooth-protectant from milk that helps strengthen enamel and prevent cavities.

## METHODOLOGY

This study used a laboratory experimental method with a posttest-only control group design to test the effectiveness of a treatment. The study was conducted at the Biochemistry Laboratory of the Faculty of Medicine, University of X from July to September 2024. The population in this study were blood samples obtained from healthy volunteers, with a purposive sampling technique to determine samples that met the inclusion and exclusion criteria.

The research variables consist of independent variables in the form of the type of treatment given, dependent variables in the form of the levels of certain substances measured, and control variables that are maintained so as not to affect

the research results. The tools and materials used include standard laboratory equipment such as micropipettes, spectrophotometers, and chemical reagents needed for testing. The research procedure involves taking blood samples, certain treatments according to the research group, incubation, and analysis using the spectrophotometric method. The data obtained are analyzed using statistical tests to see significant differences between groups.

## RESEARCH RESULTS

### *Surface Hardness Measurement Results of Email*

This study consisted of three main stages. First, before being given treatment, 30 tooth samples were tested for enamel surface hardness using a Microvickers Hardness Tester. Next, a demineralization process was carried out by soaking the tooth samples in a citric acid solution. After undergoing demineralization, the enamel surface hardness was tested again. The 30 tooth samples were then divided into three treatment groups, each consisting of 10 teeth. In the first group, the tooth samples were treated with CPP-ACP paste. The second group was soaked in Etawa crossbreed goat milk, while the third group was soaked in artificial saliva.

The results of the average enamel surface hardness measurements in each group can be seen in Table 1.

Table 1. Average values of tooth enamel surface hardness after immersion

| Treatment | Group | N | Mean | SD |
|---|---|---|---|---|
| Before Treatment | CPP-ACP | 10 | 339.50 | 6.02 |
| | PE Goat Milk | 10 | 336.21 | 4.25 |
| | Artificial Saliva | 10 | 338.89 | 5.24 |
| After Demineralization | CPP-ACP | 10 | 85,850 | 1.57 |
| | PE Goat Milk | 10 | 63,740 | 2.46 |
| | Artificial Saliva | 10 | 178.85 | 6.59 |
| After Being Given Test Materials | CPP-ACP | 10 | 248.34 | 4.82 |
| | PE Goat Milk | 10 | 284.95 | 13.1 |
| | Artificial Saliva | 10 | 214.29 | 6.22 |

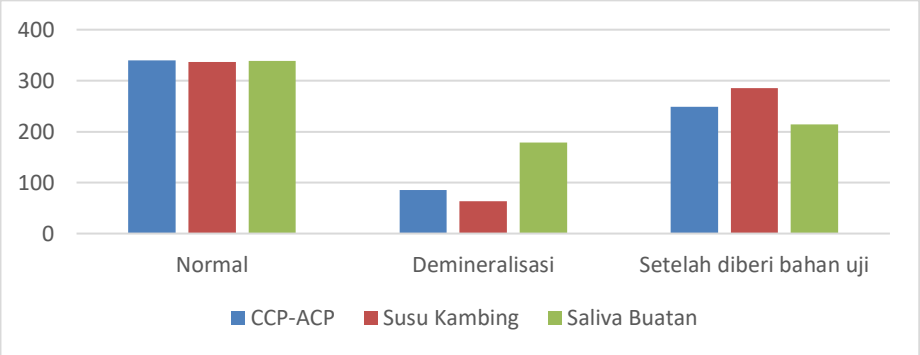


Figure 1. Graph of average enamel surface hardness values

Based on Table 1 and Figure 1, before treatment, the average enamel surface hardness in the CPP-ACP group was 339.5, the Etawa Crossbred Goat Milk group was 336.21, and the artificial saliva group was 338.89. After the

demineralization process, the average enamel surface hardness in the CPP-ACP group decreased to 85.85, the Etawa Crossbred Goat Milk group to 63.74, and the artificial saliva group to 178.85.

Testing the surface hardness of the enamel after soaking and administering the test material using the Microvickers Hardness Tester, then analyzed using the One Way ANOVA test to assess the effect of each material on the surface hardness of the enamel between groups. Before the One Way ANOVA test was carried out, a normality test was first carried out using the Shapiro-Wilk method. The results of the normality test showed that all p values > 0.05, so it can be concluded that the data are normally distributed. The study was continued with the LSD test to compare the effect of administering test materials on the surface hardness of the enamel between the CPP-ACP, Etawa Crossbred Goat Milk, and artificial saliva groups, as presented in Table 5.4 .

The results of this study were continued by comparing the enamel surface hardness at three stages, namely before treatment, after the demineralization process, and after administration of the test material. This analysis was carried out using a paired sample t-test. Table 5.4 presents the results of the comparison of the enamel surface hardness between before treatment and after the demineralization process, as well as the comparison between before treatment and after administration of the test material. In addition, an analysis of the difference in enamel surface hardness between the conditions after demineralization and after administration of the test material was also carried out in each group, namely CPP-ACP, Etawa Crossbred Goat Milk, and artificial saliva.

Table 2. Results of the One Way ANOVA test of the effect after administration of test materials on the hardness of tooth enamel surfaces between groups (α=0.05)

| | N | Mean ± SD | P |
|---|---|---|---|
| Before being given treatment | | 338.20 ± 5.240 | 0.340 |
| Demineralized | | 109.48 ± 50.88 | 0,000 |
| After Being Given Test Materials | | 249.19 ± 30.56 | 0,000 |

Based on the analysis results in Table 2, the ANOVA test shows that before treatment, the p value = 0.340 > 0.05, so it can be concluded that there is no significant difference in hardness between the CPP-ACP, Etawa Crossbred Goat Milk, and artificial saliva groups. After the demineralization process, the ANOVA test results showed p = 0.000 < 0.05, which indicates a significant difference in hardness between the three groups. The same thing also happened after the administration of the test material, where the p value = 0.000 < 0.05 was obtained, so it can be concluded that there is a significant difference in hardness after treatment. This study was continued with Post Hoc statistical analysis (LSD) to evaluate the effect of each test material on the hardness of the tooth enamel surface between the CPP-ACP, Etawa Crossbred Goat Milk, and artificial saliva groups, as presented in Table 3.

Table 3. Results of the Post Hoc (LSD) test of the effect after administration of test materials on the hardness of tooth enamel surfaces between groups (α=0.05)

| | Post Hoc (LSD) | Average Difference | P |
|---|---|---|---|
| Before Treatment | CPP-ACP | 3,290 | 0.171 |
| | Etawa Crossbred Goat Milk | | |
| | CPP-ACP | 0.610 | 0.798 |
| | Artificial Saliva | | |
| | Etawa Crossbred Goat Milk | -2,680 | 0.260 |
| | Artificial Saliva | | |
| Demineralization | CPP-ACP | 22.11 | 0,000 |
| | Etawa Crossbred Goat Milk | | |
| | CPP-ACP | -93.00 | 0,000 |
| | Artificial Saliva | | |
| | Etawa Crossbred Goat Milk | -115.1 | 0,000 |
| | Artificial Saliva | | |
| After Being Given Test Materials | CPP-ACP | -36.61 | 0,000 |
| | Etawa Crossbred Goat Milk | | |
| | CPP-ACP | 34.05 | 0,000 |
| | Artificial Saliva | | |
| | Etawa Crossbred Goat Milk | 70.66 | 0,000 |
| | Artificial Saliva | | |

Based on the results of the LSD test in Table 3, there was no significant difference in hardness between CPP-ACP and Goat Milk ($p = 0.171 > 0.05$, average difference 3.29), CPP-ACP and artificial saliva ($p = 0.798 > 0.05$, average difference 0.61), and Goat Milk and artificial saliva ($p = 0.260 > 0.05$, average difference -2.68). After administering the test material, there was a significant difference in hardness between CPP-ACP and Goat Milk ($p = 0.000 < 0.05$, difference 22.11), CPP-ACP and artificial saliva ($p = 0.000 < 0.05$, difference -93), and Goat Milk and artificial saliva ($p = 0.000 < 0.05$, difference -115.11). Significant differences were also found between CPP-ACP and Goat Milk ($p = 0.000 < 0.05$, difference -36.61), CPP-ACP and artificial saliva ($p = 0.000 < 0.05$, difference 34.05), and Goat Milk and artificial saliva ($p = 0.000 < 0.05$, difference 70.66).

Table 4. Results of the T-Paired statistical test comparing the hardness of the enamel surface before treatment, after demineralization and after administration of the test material to each group.

| | Treatment of Test Materials | P |
|---|---|---|
| CCP-ACP | Before Treatment<br>After Demineralization | 0,000 |
| | Before Treatment<br>After Being Given Test Materials | 0,000 |
| | After Demineralization<br>After Being Given Test Materials | 0,000 |
| Etawa Crossbred Goat Milk | Before Treatment<br>After Demineralization | 0,000 |
| | Before Treatment<br>After Being Given Test Materials | 0,000 |
| | After Demineralization<br>After Being Given Test Materials | 0,000 |
| Artificial Saliva | Before Treatment<br>After Demineralization | 0,000 |
| | Before Treatment<br>After Being Given Test Materials | 0,000 |
| | After Demineralization<br>After Being Given Test Materials | 0,000 |

Based on Table 4, there were significant differences in hardness in all test groups (CPP-ACP, Etawa Crossbred Goat Milk, and artificial saliva) with $p = 0.000 < 0.05$. In the CPP-ACP group, significant differences were found between before treatment and after demineralization, before treatment and after administration of test materials, and after demineralization and after administration of test materials. The same thing also happened in the Etawa Crossbred Goat Milk group and the artificial saliva group, where each stage of treatment showed significant changes in hardness.

## DISCUSSION

This study is a laboratory experiment with a Post Test Only Group Design, which aims to determine the effect of Etawa Crossbred Goat's milk immersion on the hardness of tooth enamel surfaces. The research sample consisted of 30 extracted human premolar teeth, with the approval of the Health Research Ethics Commission (KEPK) of the Faculty of Medicine, University of North Sumatra through the Ethical Clearance letter No: 266/KEPK/USU/2022.

Hardness testing was carried out using the Microvickers Hardness Tester at three stages: before treatment, after demineralization, and after administration of the test material. This tool was chosen because it is able to measure the hardness of the enamel surface without damaging the sample, suitable for the fine and crack-prone enamel microstructure. In addition, the Microvickers Hardness Tester is considered a simple, fast, economical, and reliable method in

detecting mineral changes in enamel, which reflects the remineralization process (Kuswanto et al., 2023) .

In the oral cavity, there is a balance between demineralization and remineralization. Factors such as diet, oral hygiene, and microbial activity can increase demineralization. Remineralization is assisted by salivary buffering, which allows calcium and phosphate ions to precipitate and form new minerals, playing an important role in preventing dental caries (Krismaningrum & Rahmadhia, 2023) .

In this study, the remineralizing agent was used in the form of a paste, because it is more stable in the oral cavity than a gel. Fernandes R et al. found that paste can increase salivary pH, while gel actually decreases it. AJM Ligtenberg also stated that although gel increases pH in the first 5 minutes, paste is more effective in maintaining pH stability for up to 60 minutes after brushing (Wihandoyo et al., 2022) .

In this study, the test material was given for 30 minutes every day for 7 consecutive days. The duration of 30 minutes was chosen based on the research of Wiryani F et al., which showed a significant increase in enamel surface hardness with an optimal application of 30 minutes. According to the Ostwald Ripening theory, the longer the remineralization process, the more stable and larger the formation of apatite crystals, which contributes to increased enamel hardness. The duration of 7 days is based on the research of Salista Daysa, which found significant differences before and after treatment, with the Oneway ANOVA test showing a p value <0.05, indicating a significant remineralization effect (Sutanti et al., 2021) . This study consisted of three groups:

1. CPP-ACP Group: Ten samples were smeared with CPP-ACP paste using a microbrush on the buccal surface, left for 30 minutes, washed with distilled water, then soaked in artificial saliva at 37°C in an incubator. This process was repeated for 7 days.
2. Etawa crossbred goat milk group: Ten samples were soaked in milk for 30 minutes in an incubator at 37°C, then washed and soaked in artificial saliva until the next day. This process was also repeated for 7 days.
3. Control group: Ten samples were soaked in artificial saliva at 37°C for 30 minutes, then stored in an incubator with the same procedure for 7 days .

Casein Phosphopeptide-Amorphous Calcium Phosphate (CPP-ACP) is a remineralizing agent containing casein phosphoprotein (CPP), calcium, and phosphate in high concentrations, so it can inhibit demineralization and maintain ion balance on the surface of tooth enamel. CPP-ACP works by localizing calcium and phosphate ions, which then enter the enamel rod to form apatite crystals, increasing tooth hardness. CPP-ACP paste contains 18% calcium and 30% phosphate, which play a role in the remineralization process (Aziz et al., 2020) .

Milk is an important source of nutrition rich in protein, vitamins, minerals, and calcium, which are essential for dental health (Handayani et al., 2021). Casein in milk helps stabilize calcium and phosphate ions through the release of caseinophosphopeptides (CPP), which form amorphous calcium phosphate complexes, promoting enamel remineralization. Research (Risyani et al., 2022)

shows that casein gel from Etawa crossbreed goat milk is effective in increasing the hardness of primary tooth enamel in vitro, especially at concentrations of 5%, 10%, and 20%.

The untreated group used artificial saliva to simulate oral conditions. This solution contains the main minerals hydroxyapatite, such as calcium and phosphate, which play a role in enamel remineralization. Saliva also functions as a natural buffer, helping to neutralize acids, form a protective pellicle, and prevent tooth demineralization. Saliva pH has a direct effect on remineralization, as the amount of calcium and phosphate ions available determines the effectiveness of this process. Artificial saliva has been shown to stimulate enamel remineralization, as shown in the study by Amaechi et al., where the One-way ANOVA statistical test showed a significant increase in enamel surface hardness ($p < 0.05$).

ANOVA test showed that before demineralization, there was no significant difference in enamel hardness between groups ($p = 0.340 > 0.05$). LSD results also showed that there was no significant difference between CPP-ACP and goat milk ($p = 0.171$), CPP-ACP and artificial saliva ($p = 0.798$), and goat milk and artificial saliva ($p = 0.260$). After demineralization, ANOVA test showed a significant difference in hardness ($p = 0.000 < 0.05$) in all groups. LSD test also confirmed significant differences between CPP-ACP and goat milk ($p = 0.000$), CPP-ACP and artificial saliva ($p = 0.000$), and goat milk and artificial saliva ($p = 0.000$). After administration of the test material, the ANOVA results again showed significant differences ($p = 0.000 < 0.05$) in all groups, as confirmed by the LSD test on each comparison ($p = 0.000$).

This study is in line with a study conducted by Aziz et al. (2020), which showed that casein gel from Etawa crossbreed goat milk is effective in increasing the hardness of primary tooth enamel in vitro. This increase in enamel hardness was more significant in groups with concentrations of 20%, 10%, and 5%, with a higher increasing trend as the concentration of casein in the gel increased.

The results of this study indicate that the treatment group had a lower enamel hardness value compared to the positive control group (normal), but higher than the negative control group (demineralization). This finding indicates that the administration of Etawa crossbreed goat milk contributes to the remineralization process and increases the surface hardness of tooth enamel compared to the group that experienced demineralization. The results of this study also indicate that Etawa crossbreed goat milk has a higher remineralization effectiveness compared to CPP-ACP paste. One factor that may influence these results is the possibility that the effectiveness of CPP-ACP in remineralizing teeth is not optimal. Therefore, further research is needed regarding the concentration of test materials and the duration of application that is most effective in supporting tooth remineralization.

## CONCLUSIONS AND RECOMMENDATIONS

Based on the results of research on the differences in tooth enamel surface hardness on immersion with Etawa crossbreed goat's milk, it was found that there was an effect of immersion with Etawa crossbreed goat's milk on the hardness of

the enamel surface. There was a difference in the effect of giving remineralizing materials between CPP-ACP and immersion with Etawa crossbreed goat's milk on the hardness of the enamel surface, where Etawa crossbreed goat's milk had a higher level of enamel surface hardness than CPP-ACP and artificial saliva.

Given these findings, several recommendations can be made. First, further studies should explore the clinical application of Etawa crossbreed goat's milk as a natural remineralizing agent, particularly in preventing enamel demineralization. Additionally, product development initiatives could focus on formulating dental care products, such as mouth rinses or toothpaste, incorporating Etawa crossbreed goat's milk as an alternative or complementary treatment for enamel remineralization.

## FURTHER STUDY

Future research should also investigate the long-term effects of Etawa crossbreed goat's milk on enamel hardness, its mechanism of action, and its effectiveness in various conditions, such as exposure to acidic environments. Moreover, raising public awareness about the potential benefits of Etawa crossbreed goat's milk for oral health could encourage its incorporation into daily dental care practices. Finally, further comparative studies involving other natural remineralizing agents and commercial products would be beneficial in establishing the efficacy and practical applications of Etawa crossbreed goat's milk in dentistry.

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