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# PLAQUE INDEX, SALIVARY PH, AND QUANTIFICATION OF STREPTOCOCCUS MUTANS AFTER CONSUMPTION OF PROBIOTIC IN STUNTED CHILDREN

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## ABSTRACT

**Background:** Stunting is defined as short stature for the age group, with a Z score less than −2 SD (standard deviation). Stunting causes failure of body and brain growth in children, caused by chronic malnutrition. The highest proportion of stunting comes from South Asia, including Indonesia. Stunting also affects the development and integrity of the oral cavity and increases the risk of oral diseases, including caries. Probiotics are living microbes that can provide health benefits to their host organisms. Few research has been done on the effect and potential of antibiofilm from *Lactobacillus casei* on reducing *Streptococcus mutans* and increasing salivary pH. This study aims to analyze the differences in plaque index, salivary pH, and quantification of *Streptococcus mutans* before and after consumption of *Lactobacillus casei* in stunted children aged 3–5 years at Langensari 1 Health Center, Banjar City, West Java.

**Materials and Methods:** The design of this study was an experimental test, with 36 stunted children from Langensari 1 Health Center, Banjar City, West Java, as subjects to examine the dental plaque index and acquire unstimulated saliva to calculate salivary pH and quantify relative bacteria using the RT-PCR method to identify *Streptococcus mutans* bacteria.

**Results:** There was a significant difference in plaque index from before and after consuming *Lactobacillus casei* probiotics for 7 days ($p<0.05$), but there was no significant difference in salivary pH and *Streptococcus mutans* quantification between before and after consuming *Lactobacillus casei* probiotics for 7 days ($p>0.05$).

**Conclusion:** There was a significant reduction in the plaque index after the consumption of *Lactobacillus casei* probiotic for 7 days. The salivary pH showed an increase, and the quantification of *Streptococcus mutans* parameters showed a decrease, but neither of the differences was statistically significant. Thus, it can be concluded that the *Lactobacillus casei* probiotic has a beneficial impact as an additional oral healthcare regimen, but future research is recommended to incorporate additional parameters for a better understanding of probiotic efficacy.

***Keywords*****:** *Neutrophil elasatase, polycystic ovarian syndrome, periodontit*

## INTRODUCTION

Optimal fulfillment of nutritional requirements is fundamental for harmonious growth and development in children. Conversely, inadequate nutrition, compounded by unfavorable environmental factors, renders children susceptible to malnutrition and impaired growth.[1]

Malnutrition is defined as a state resulting from a deficiency, excess, or imbalance of energy and/or nutrient intake.[2] Its etiology can be multifactorial, stemming from one or several diseases that directly impact nutrient balance, or from environmental and behavioral factors associated with imbalanced nutrient provision.[3] In early childhood, malnutrition is categorized into stunting, underweight, wasting, and obesity.[4] The fundamental principle of assessing child growth involves measuring body weight (BW) and body height/length (BH) and comparing these metrics to standardized Z-scores for the appropriate age and gender.[5,6] This assessment serves to determine whether a child's growth is within the normal range or if a growth impairment is present.[5,6]

Stunting is defined as a linear growth deficit, characterized by a height-for-age Z-score of less than −2 standard deviation (SD).[5] As an indicator of chronic malnutrition, stunting is largely irreversible and exerts a significant, long-term impact on a child's physical and cognitive development.[5] Globally, an estimated 149 million children under five years of age were affected by stunting in 2020.[7] Of the 83.6 million children under five in Asia, the greatest proportion of stunting cases (58.7%) was reported in South Asia. [7]

In Indonesia, the 2018 Basic Health Research (RISKESDAS) survey reported a stunting prevalence of 30.8% among children under five.[8] Data from the Indonesian Nutritional Status Survey (SSGI) indicates that West Java was one of the provinces with the highest

prevalence, reporting a rate of 24.5%.[9] Within this province, the city of Banjar reported a stunting prevalence of 19.3%, in addition to a 12.8% prevalence of underweight among children.[9]

Stunting is associated with inadequate intake of both macronutrients and micronutrients, which are essential for optimal growth and development.[10] The long-term consequences of stunting include an elevated risk of infection, an increased susceptibility to non-communicable systemic diseases, a higher prevalence of obesity in adulthood, and a greater overall mortality rate.[11] Furthermore, stunting can compromise the integrity and development of the oral cavity and increase the risk of oral diseases.[12]

Tooth eruption holds particular significance in the context of early childhood caries, as the colonization of *Streptococcus mutans* increases in proportion to the number of erupted teeth.[13] This phenomenon contributes to the high incidence of caries observed in various pediatric populations.[13] The period of primary tooth eruption typically occurs between 4 and 36 months of age.[13] This phase is critical because the subsequent health of the permanent dentition is highly dependent upon maintaining proper oral hygiene during this primary dentition stage.[13,14]

Deficiencies in calcium, phosphate, and vitamins A, C, and D can lead to defects in tooth formation (odontogenesis), delayed tooth eruption, and compromised salivary gland function, thereby increasing susceptibility to dental caries.[15,16] Studies have shown that the mean Decayed, Missing, and Filled Teeth (DMFT) index is higher in children with stunting compared to their non-stunted peers. This disparity is attributed to salivary gland atrophy associated with chronic malnutrition, which disrupts oral homeostasis.[15,16] Chronic malnutrition can lead to a decrease in salivary flow rate, a reduced buffering capacity, and diminished immune and antimicrobial components in saliva.[15,17] This imbalanced oral environment promotes the proliferation of specific bacterial strains and the formation of multispecies communities on the tooth surface, known as dental biofilm.[15] The presence of dental biofilm is a primary etiological factor for various oral diseases, including dental caries and periodontitis.[16] Furthermore, studies have found a higher prevalence of *Streptococcus mutans*, a bacterium strongly associated with dental caries, in the saliva of children with stunting.[15]

Probiotics are defined as living microorganisms that, when administered in adequate amounts, confer a health benefit on the host.[18] Given the growing concern over widespread bacterial resistance to conventional antibiotics, probiotic therapy is increasingly studied for its potential application in maintaining oral health.[18] The genus *Lactobacillus* represents one of the most widely commercialized probiotics for human consumption.[19] *Lactobacillus* species have demonstrated the ability to modulate the oral microflora balance and reduce bacterial colonization within the oral cavity.[19]

*Lactobacillus casei* has been shown to inhibit the bacterial adhesion of *Streptococcus mutans* to oral tissues, thereby preventing biofilm formation on caries-susceptible teeth.[20] This cariostatic effect of *Lactobacillus casei* is most effective when combined with other preventive measures, such as fluoride application.[20] Therefore, probiotic bacteria should be considered as an adjunctive therapy in the management of the dental caries process.[20]

Previous research has demonstrated the effects and antibiofilm potential of the isolated *Lactobacillus casei* Shirota strain, sourced from commercialized food products, against *Streptococcus mutans*.[21] These studies reported a significant reduction in both *Streptococcus mutans* counts and biofilm formation.[21] Another study utilizing the same probiotic drink investigated its effect on salivary pH.[22] This research found that a significant increase in salivary pH was achieved after a 7-day regimen of gargling with the probiotic drink containing *Lactobacillus casei*.[22]

Studies on the use of probiotics as an adjunctive healthcare regimen for managing dental caries and their role in overall oral health are currently ongoing. However, research has yet to investigate the specific effects of *Lactobacillus casei* probiotics on the oral cavity of children with stunting, particularly within the 3–5 year age group. Therefore, this study was conducted to evaluate the differences in plaque index, salivary pH, and *Streptococcus mutans* quantification following a 7-day intervention with a commercialized *Lactobacillus casei* probiotic product. The research was performed on children aged 3–5 years with stunting from the Langensari 1 Health Center in Banjar City, West Java.

## Materials and Methods

This experimental study was conducted to investigate the effects of a commercialized *Lactobacillus casei* probiotic product on stunted children. Clinical examinations were performed twice—before and after the 7-day intervention—to measure the dental plaque index, salivary pH, and *Streptococcus mutans* quantification. The research samples were saliva and DNA of *Streptococcus mutans* bacteria isolated from saliva samples of the research subjects.

The study took place from September to October 2024 at the Langensari 1 Health Center in Banjar City, West Java. Ethical approval for the research was obtained from the Ethics Committee of the Faculty of Dentistry, University of Indonesia (Ethical Approval Number: 82/Ethical Approval/FKGUI/X/2024).

The study participants were selected based on specific inclusion and exclusion criteria. Inclusion criteria required subjects to be children with stunting (Z score below −2 SD from the median of the WHO Child Growth Standards), aged 3–5 years, from the Langensari 1 Health Center in Banjar City, West Java, who were in

good general health and had a def-t score of ≥1. Children were excluded if they had special needs, a history of systemic diseases or allergies, had consumed antibiotics or other probiotics within one month prior to the study, or used antibacterial mouthwash. Each parent or legal guardian of these subjects was provided with a detailed explanation of the experimental procedures through both oral and written communication, and signed an informed consent form before the experiments began.

Following these criteria, a sample size calculation was performed using G*Power software (version 3.1). Based on the analysis, a minimum of 17 subjects per group was required. To account for potential dropouts, one additional subject was added to each group, resulting in a total sample size of 36 children. These subjects were then randomly divided into two groups of 18: a control group and a treatment group.

Each subject in the control as well as the treatment group, equipped with a new toothbrush and fluoride toothpaste, received a Dental Oral Health Education (DOHE) session that provided information on healthy dietary practices, proper twice-daily toothbrushing technique (Bass method). The session concluded with an evaluation of the child's plaque removal efficacy using a disclosing solution. For the treatment group, in addition to the DOHE, they received a probiotic product treatment. For the probiotic treatment, subjects were instructed to hold the *Lactobacillus casei* probiotic product in their mouths for 20 seconds and then swallow it. This regimen was to be performed once daily for seven consecutive days, specifically in the mornings immediately following toothbrushing. To ensure maximum contact and efficacy, subjects were instructed to refrain from gargling, eating, or drinking for one hour after that. In addition, subjects were prohibited from consuming or gargling with any other probiotic products throughout the entire experimental period.

This research utilized an experimental design involving clinical examinations of the plaque index, salivary pH, and the quantification of *Streptococcus mutans*. These parameters were measured at two distinct time points: baseline (initial examination) and on Day 7 following the intervention period. The plaque index examination, based on the Greene and Vermillion criteria, was conducted by two trained operators. These operators first underwent calibration by examining 10 pilot subjects to assess and confirm the reliability of their scoring, using the Kappa statistic to determine inter-rater agreement. As for the saliva samples, 1 mL of unstimulated saliva was collected for five minutes into sterile tubes, which were then labeled with the subjects' names and codes. After collection, samples were immediately stored in a cooler box and then transported to the Oral Biology Laboratory at the University of Indonesia's Faculty of Dentistry, where they were frozen at -20 ºC until the salivary pH and *Streptococcus mutans* quantification could be examined and analyzed.

The saliva samples were examined for their pH and *Streptococcus mutans* quantification. Salivary pH was measured using litmus pH paper. For the quantification of *Streptococcus mutans*, DNA was first extracted from the bacterial cells using the InstaGene Matrix Kit (Bio-Rad) according to the manufacturer's instructions, and afterwards stored at -20 ºC. The purity and concentration of the extracted DNA were tested using a NanoDrop Spectrophotometer at the integrated lab of the University of Indonesia's Faculty of Medicine, and all samples were subsequently standardized to 50 ng/µL using nuclease-free water (NFW). Real-Time PCR (RT-PCR) was then employed for detection and quantification. The resulting graphical output from the machine represented the final *Streptococcus mutans* quantity.

The data collected from the clinical examinations and laboratory tests were statistically analyzed using SPSS software (version 26). The level of significance for all tests was set at $p<0.05$ to determine the presence of a statistically significant relationship concerning the quantification of *Streptococcus mutans* in stunted children aged 3–5 years before and after 7 days of *Lactobacillus casei* probiotic consumption.

Before inferential testing, data normality was assessed using the Shapiro-Wilk test ($n\leq50$). Based on these results, the data distribution for the plaque index was found to be normal in both the probiotic treatment group and the control group. Conversely, non-normal data distributions were observed for salivary pH as well as for the quantification of *Streptococcus mutans* in both groups.

For the normally distributed data (plaque index), a Paired T-test was conducted to assess the difference in plaque index values within each group of stunted children between the baseline and post-7-day intervention. Subsequently, an Independent T-test was done to compare the difference between before and after intervention of the plaque index between the treatment group and the control group. Meanwhile, for non-normally distributed data, the Wilcoxon test was used to analyze the difference between before and after intervention in salivary pH and *Streptococcus mutans* quantification within the treatment group and the control group. Following that, the Mann-Whitney test was conducted to compare the difference between before and after 7 days for both the plaque index and *Streptococcus mutans* quantification between the treatment group and the control group.

## RESULTS

The treatment group demonstrated measurable changes across all clinical parameters following the intervention. Before the intervention, the mean plaque index was 1.68±0.48 (Mean ± SD). After the 7-day regimen of consuming the *Lactobacillus casei* probiotic product, the mean plaque index decreased to 1.09±0.35. Salivary pH was also assessed, revealing a median value of 6.0 (ranging from 6.0 to 8.0) at baseline. Following

the 7-day probiotic consumption, the median salivary pH increased to 7.0 (ranging from 6.5 to 8.0). Finally, quantification of *Streptococcus mutans* bacterial DNA using the qPCR method yielded a median value of 4.59 (4.17–6.18) at baseline, which decreased to 4.48 (2.38–5.10) after the 7-day intervention. Detailed descriptive statistics for the treatment group are presented in Table 1. The control group also demonstrated some changes and underwent comparative analysis. At the initial examination, the mean plaque index was 1.79±0.46 (Mean ± SD). After the 7-day period (receiving DOHE only), the mean plaque index decreased to 1.35±0.58. Initial examination of salivary pH yielded a median value of 6.0 (ranging from 5.0 to 7.0). The salivary pH remained stable after 7 days, with a median value of 6.0 (ranging from 6.0 to 7.0). Quantification of *Streptococcus mutans* yielded a baseline median value of 5.01 (4.30–5.83). After the 7-day period, this value decreased slightly to 4.70 (3.96–5.56). Descriptive statistical data for the control group are summarized in Table 5.1. Subsequently, inferential statistical analysis was conducted to determine the significance of the observed changes across both groups. A paired t-test was used to analyze the difference in the mean plaque index before and after the 7-day intervention. The results indicated a statistically significant reduction ($p<0.05$) in the plaque index from baseline to post-intervention for both the probiotic treatment group and the control group. The Wilcoxon test was performed to analyze the changes in salivary pH and *Streptococcus mutans* quantification. For salivary pH, although the median value increased in the treatment group, this change was not statistically significant ($p>0.05$). The control group likewise showed no statistically significant change ($p>0.05$). For *Streptococcus mutans* quantification, the *Streptococcus mutans* levels decreased in the treatment group, but the change was not statistically significant ($p>0.05$). Conversely, the control group unexpectedly demonstrated a statistically significant decrease in *Streptococcus mutans* levels ($p<0.05$).

**Table 1.** Descriptive Data and Comparative Analysis Results for Plaque Index, Salivary pH, and *Streptococcus mutans* Quantification in Saliva Before and After 7 Days of *Lactobacillus casei* Probiotic and Non-Probiotic Treatment

| Variables | Intervention | Treatment Group n=18 | p-value | Control Group n=18 | p-value |
|---|---|---|---|---|---|
| Plaque Index | Before | 1.68 ± 0.48[a] | <0.001* | 1.79 ± 0.46[a] | 0.039* |
| | After | 1.09 ± 0.35[a] | | 1.35 ± 0.58[a] | |
| Saliva pH | Before | 6.0 (6.0-8.0)[b] | 0.210 | 6.0 (5.0-7.0)[b] | 0.317 |
| | After | 7.0 (6.5-8.0)[b] | | 6.0 (6.0-7.0)[b] | |
| *Streptococcus mutans* Quantifications | Before | 4.59 (4.17-6.18)[b] | 0.133 | 5.01 (4.3-5.83)[b] | 0.002** |
| | After | 4.48 (2.38-5.10)[b] | | 4.70 (3.96-5.56)[b] | |

[a] Data normally distributed, presented as mean ± standard deviation (SD)
[b] Data not normally distributed, presented as median (minimum–maximum)
** Paired T-test
** Wilcoxon signed-rank test

To compare the plaque index and *Streptococcus mutans* bacterial quantification between the treatment and control groups, the delta (Δ) value (the difference between the baseline and post-7-day intervention values) was used. Before the main comparison, homogeneity testing was performed. The homogeneity test for the plaque index between groups yielded a p-value less than 0.001, indicating the data were not homogeneous. Similarly, the homogeneity test for Streptococcus mutans quantification between groups showed a p-value of 0.048, also indicating the data were not homogeneous. Subsequently, a normality test was performed on the delta values for both groups using the Shapiro-Wilk test ($n\leq50$). The results of the normality tests for the difference values (before and after) in the probiotic and non-probiotic groups are presented in Table 2.

Descriptive statistics for the difference or delta values showed variability between groups. For the plaque index, the treatment group's delta mean was −0.589±0.48 (Mean ± SD). In contrast, the control group's delta median was −0.33

(ranging from −1.17 to 0.00). For *Streptococcus mutans* quantification, the treatment group had a delta median of −0.206 (ranging from −1.95 to 0.42), while the control group had a delta mean of −0.28±0.358. Descriptive data for the delta values are summarized in Table 2. The Mann-Whitney test was then used to compare the delta value in plaque index and *Streptococcus mutans* quantification between the treatment and control groups. The analysis revealed no statistically significant difference (p>0.05) between the two groups for either the plaque index or the quantification of *Streptococcus mutans* (Table 2).

**Table 2.** Descriptive Delta Data and Comparative Analysis Results for Plaque Index and *Streptococcus mutans* Quantification Between Groups Before and After 7 Days of *Lactobacillus casei* Probiotic and Non-Probiotic Treatment

| Variable | Δ Before and After 7 Days<br>Treatment Group<br>n=18 | Control Group<br>n=18 | P-value |
|---|---|---|---|
| **Plaque Index** | -0.589 ± 0.48[a] | -0.33 (-1.17-0.00) [b] | 0.962 |
| Relative *Streptococcus mutans* Quantifications | -0.206 (-1.95-0.42) [b] | -0.28 ± 0.358[a] | 0.681 |

[a] Data normally distributed, presented as mean ± standard deviation (SD)
[b] Data not normally distributed, presented as median (minimum–maximum)
[*] Mann-Whitney test, p<0.05

## DISCUSSION

Stunting, primarily caused by chronic malnutrition, can significantly impact the oral cavity, leading to an increased risk of oral diseases such as dental caries. [12] This heightened caries risk in stunted children may be attributed to three possible mechanisms: defects in tooth formation (odontogenesis), delayed tooth eruption, and salivary gland atrophy. [17] This atrophy, in turn, influences the oral environment by reducing the salivary flow rate, impairing buffer function, and decreasing the immune and antimicrobial components that are naturally present in the saliva. [15,17]

Maintaining an optimal salivary pH of 7 is essential for a healthy oral environment. However, children affected by stunting often exhibit salivary pH values lower than this ideal level. [23] Saliva significantly influences the pH of both the oral cavity and the dental plaque. When the pH drops below the critical level, it leads to the demineralization of the tooth surface, which can result in the formation of dental caries and tooth erosion.[15,16,17] This disruption to the oral equilibrium in stunted children, directly attributed to the compromised salivary function, also promotes an increase of certain bacteria and the formation of a pathogenic biofilm, consequently driving caries development. Studies have reported that the quantity of *Streptococcus mutans* in the saliva of stunted children is higher compared to that found in non-stunted children.[15] Therefore, this study focused on examining the parameters of plaque index, salivary pH, and *Streptococcus mutans* quantification.

The subjects for this study were stunted children aged 3–5 years who were registered in the Langensari 1 Health Center program. Stunting was defined using the indicator of height/length-for-age (H/A) measurements, with the score of below −2 standard deviations from the median, in accordance with the Minister of Health Regulation on Child Anthropometric Standards and based on the WHO 2006 child growth charts.[6] Stunting serves as a critical indicator of a general deficit in child growth and development, primarily resulting from chronic malnutrition. [5]

The first 1,000 days, spanning from conception to the child's second birthday, represent the golden period for human growth and development.[24] During this time, brain volume increases rapidly, reaching 60% of adult volume by age one and 80% by age two, ultimately growing to 90% by five years old.[25] The consequences of stunting have a major impact on a child's overall physical, motor, and cognitive development, which carries long-term implications for their general health.[5,11] Furthermore, this developmental deficit negatively affects the oral cavity by compromising oral motor coordination, thereby disrupting the stomatognathic system functions (including chewing, swallowing, and speech).[26] Inadequate psychomotor skills also contribute to plaque accumulation due to the child's inability to perform effective toothbrushing.[27]

The period of deciduous teeth eruption typically begins at four months and concludes by 36 months of age. The health of the permanent teeth depends upon maintaining adequate hygiene during that period.[13,14] *Streptococcus mutans* is recognized as a major contributor to microbial dysbiosis within the dental plaque.[28,29] Given the high risk of oral diseases, particularly caries, in this vulnerable population, prevention and intervention must be initiated as early as possible. The use of probiotics represents one such form of early intervention.[12,30]

According to the WHO, probiotics are defined as live microorganisms that, when administered in adequate amounts, can impart health benefits to the host.[31] Probiotics function as functional food ingredients that are commercially available as dietary supplements or food products.[31] The selection criteria for a probiotic to be utilized as a supplement are strongly emphasized by its ability to modulate the immune response, both specific and non-specific immunity responses.[31,32] Additionally, probiotics are capable of producing antimicrobial substances, such as bacteriocins, and possess the critical ability to adhere to mucosal and epithelial surfaces.[32,33]

The Lactobacillus genus represents one of the most commonly available commercial probiotics for human consumption.[34] This study specifically utilized *Lactobacillus casei* due to previous studies that have examined its role in managing dental caries and mitigating *Streptococcus mutans* activity.[35] This probiotic has been investigated as an adjunctive therapy for addressing biofilm formation on teeth. *Lactobacillus casei* is recognized as one of the most effective and widely used probiotic strains in caries management research.[35] Specifically, the short-term consumption of the Lactobacillus casei Shirota strain has been shown to increase salivary pH, leading to a subsequent decrease in oral biofilm acidogenicity and an improved pH recovery time.[36]

The findings of this study revealed a significant reduction in the plaque index after the 7-day intervention, observed in both the probiotic treatment group and the non-probiotic control group. However, the comparative analysis of the delta plaque index between the treatment and control groups demonstrated no statistically significant difference. Despite this, the mean reduction in plaque index was numerically greater in the treatment group than in the control group. This numerical trend aligns with previous research suggesting a potential cariostatic effect from short-term consumption of commercial food products containing *Lactobacillus* casei in reducing the oral biofilm.[37] Another study also reported similar findings where a statistically significant decrease in plaque index scores was observed when comparing baseline and post-7-day consumption of *Lactobacillus casei* probiotics.[38]

In the present study, the pH values in both the treatment and control groups did not demonstrate a statistically significant difference. However, the median pH value did increase in the probiotic group. Notably, the salivary pH, which was initially categorized as acidic, shifted to a balanced salivary pH category following the use of *Lactobacillus casei*. This descriptive shift aligns with previous research utilizing *Lactobacillus casei* for a 7-day period, which reported a significant 5.2% increase in the mean lowest pH value.[37] Furthermore, the findings are consistent with a similar 14-day probiotic study where salivary pH levels showed an increase after 7 days of treatment, and continued to rise after 14 days, although the increase remained statistically nonsignificant. That study also analyzed salivary flow rate, finding a significant increase, which was suggested to be a contributing factor to the observed improvement in pH values.[22]

The results for Streptococcus mutans quantification showed no statistically significant difference between the baseline and post-7-day intervention in the treatment group, despite the median value exhibiting a decrease. Interestingly, the control group demonstrated a statistically significant difference in the reduction of *Streptococcus mutans* levels. One potential explanation for the lack of significant change in the probiotic group relates to the product's nutritional composition. The probiotic liquid (65 mL) used in this study contained $6.5\times10^9$ *Lactobacillus casei* Shirota strain along with a total sugar content of 10 g and 11 g of total carbohydrates. Notably, sugar is the second most abundant ingredient in this formulation. The sugar content must be highly reconsidered, as a single bottle provides nearly half of the American Heart Association's recommended limit of ≤25 g (6 teaspoons) of added sugar per day for children.[39] A study investigating factors that influence the antagonistic capacity of commercial *Lactobacillus casei* products found that sugar plays a role. That research demonstrated that when *Lactobacillus casei* was cultured with sucrose, an inhibitory effect on *Streptococcus mutans* was observed after 24 hours. However, after 48 hours, the growth of *Streptococcus mutans* actually increased. This suggests that the high sucrose content in the probiotic product may have attenuated the beneficial effects of *Lactobacillus casei* over the full 7-day treatment period.[40]

Another in vitro study involving the amount of *Streptococcus mutans* in biofilms before and after consuming *Lactobacillus casei* product for 7 days also reported no decrease in *Streptococcus mutans* quantity. However, the study did find that there was a decrease, albeit nonsignificant statistically, in acid production of the *Streptococcus mutans* through the lessening of the gtfB, gtfC, and ldh gene expressions. Therefore, the

amount of bacteria in the saliva or biofilm might not be one of the best parameters to judge the effects of probiotics in terms of evaluating their anti-caries effect; instead, other parameters, such as gene expression, could provide better information regarding the effects.[37]

A research investigating the effects of 7-day *Lactobacillus casei* probiotic consumption in children aged 7–11 years, using a cariogram as one of the caries risk parameters, demonstrated highly variable outcomes from the probiotic treatment. One of the outcomes was a cariogenic effect on low-caries-risk children. The proposed mechanism behind this observation is that, theoretically, *Lactobacillus casei* delivered via commercial food products can act as a cariogenic agent precisely because of its added sugar content.[37] Therefore, even if a numerical decrease in *Streptococcus mutans* quantification was observed in the probiotic group of the present study, the cariogenic effect of the sugar in the probiotic drink product likely inhibited or masked this potential beneficial reduction.

The comparative analysis of the delta values for both the plaque index and *Streptococcus mutans* quantification between the treatment and control groups in this study showed a numerical decrease, but no statistically significant difference. This lack of significant difference aligns with research suggesting that if bacterial cells are not effectively removed from the tooth surface and if cariogenic plaque continues to form, the ongoing interaction between cariogenic microbial cells, such as *Streptococcus mutans*, salivary proteins, and food particles, can actually lead to greater plaque retention.[41] This outcome in our study may also be significantly influenced by the added sugar content present in the probiotic drink product that was used in this research.

The chronic lack of optimal nutrient intake causes stunted children to be highly vulnerable to malnutrition and poor growth outcomes.[1] Children in this population who consume sweet foods and beverages often displace the intake of nutritious foods and drinks, which consequently contributes to both dental decay and nutritional deficiency. Therefore, alternative products for the delivery of probiotics have been proposed, including cheese, tablets, yogurt, and specialized straws. These alternative products may offer a more effective route for administering *Lactobacillus casei* as an adjunctive caries preventive treatment, particularly for vulnerable groups with high caries risk.[37]

Several limitations should be noted regarding this study. First, there was limited supervision of probiotic consumption over the 7-day intervention period. This challenge arose primarily due to restricted communication access with several subjects who did not own communication devices. Furthermore, the participants' residences were dispersed across three separate villages with significant distances between them, preventing the researchers from supervising the probiotic use directly. Second, the parameters evaluated in this study were limited to three variables: plaque index, salivary pH value, and *Streptococcus mutans* quantification. Future research is therefore recommended to incorporate additional parameters, such as caries risk assessment tools (e.g., Cariogram) and analyses of gene expression in *Streptococcus mutans*, which may provide more valid data for understanding probiotic efficacy.

## CONCLUSION

There was a significant reduction in the plaque index after the consumption of *Lactobacillus casei* probiotic for 7 days. The salivary pH showed an increase, and the quantification of *Streptococcus mutans* parameters showed a decrease, but neither of the differences was statistically significant. Thus, it can be concluded that the *Lactobacillus casei* probiotic has a beneficial impact as an additional oral healthcare regimen, but future research is recommended to incorporate additional parameters for a better understanding of probiotic efficacy.

## DECLARATION

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**Conflict of Interest**

The authors declare that they have no competing interests. None of the authors has relevant financial relations with a commercial interest.

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**Consent for publication**

Patients were informed verbally and in writing about the study and gave written informed consent.

**Authors' contributions**

NAA contributed: conceptualization, methodology, and resources; investigation and data collection (clinical and laboratory); formal analysis and interpretation of results; and writing—original draft preparation. EF and MS provided supervision and critical review. All authors have read and agreed to the published version of the manuscript.

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