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ORIGINAL ARTICLE

EFFECTIVENESS OF *LACTOBACILLUS REUTERI* PROBIOTICS ON THE DENTAL PLAQUE INDEX, SALIVARY PH, AND *STREPTOCOCCUS MUTANS* QUANTIFICATION OF PRESCHOOL STUNTED CHILDREN IN BANJAR CITY, WEST JAVA, INDONESIA

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Stunting is a linear growth failure which can affect dental plaque, salivary pH, and *Streptococcus mutans* counts. *Lactobacillus reuteri* probiotics have benefit in preventing plaque formation, reducing *S. mutans*, and balancing salivary pH. This research evaluates the effectiveness of consuming *Lactobacillus reuteri* probiotics for 7 days on dental plaque index, salivary pH, and *Streptococcus mutans* quantification of preschool-stunted children. A total of 54 children aged 3-5 years in Banjar City participated in the research and were divided into 3 groups. The test and the negative control group each consisted of 18 stunted children, while the positive control group included 18 children with normal growth. Both test group and positive control groups were given dental health education (DHE) and *L. reuteri* probiotics lozenges for 7 days. In the process, the negative control group was administered only DHE. Dental plaque index, salivary pH, and *S. mutans* quantification were evaluated before and after intervention using Greene and Vermillion plaque index, litmus paper, and Real-Time Polymerase Chain Reaction (RT-PCR). Moreover, T-test dependent and Wilcoxon signed-rank were performed to compare parameters. A significant difference in dental plaque index and *S. mutans* quantification was observed before and after consumption of *L. reuteri* probiotics lozenges for 7 days ($P < 0.05$). However, no significant changes were discovered in salivary pH ($P > 0.05$). Consuming *L. reuteri* probiotics for 7 days effectively reduced dental plaque index and *S. mutans* quantification of preschool-stunted children in Banjar City, West Java, Indonesia. This research has implications for the use of Lactobacillus reuteri as a simple intervention to improve oral health in stunted children. These findings may support caries prevention strategies in areas with high stunting prevalence and encourage further research with longer intervention durations.

Keywords**:** Lactobacillus reuteri; dental plaque index; salivary pH; Streptococcus mutans; stunted children.

INTRODUCTION

Stunting is a linear growth failure, measured by a height for age z-score of <-2 standard deviations (SD) below the median of the World Health Organization (WHO) Child Growth Standards (Organization, 2018). In 2016, approximately 22.9% (154.8 million) of preschool children worldwide suffered from the disorder (Organization, 2018). According to the 2018 Indonesia's Basic Health Research, the stunting and severe stunting prevalence in Indonesia reached 30.8%[3,4].

Stunting can lead to long-term impacts, including poor cognitive abilities, reduced productivity, and increased risk of chronic disease (Soliman et al., 2021). It may also cause salivary gland atrophy, which reduces secretion reduction (Abdat et al., 2020). The reduction contributes to biofilm lack of resistance, decreased salivary pH, lower flow rate, and weakened buffer capacity (Abdat et al., 2020); (Sadida et al., 2022).These imbalances promote an increase in *Streptococcus mutans* quantification and a bacteriumsignificant in the initial colonization of biofilm formation (Theodorea et al., 2022). Dental plaque associated with caries may lead to chronic infections and pain in stunted children, potentially impairing nutritional intake (Abdat et al., 2020), and impacting child's oral health-related quality of life (Khairani et al., 2017). Adequate nutrition is important to enhance linear growth and lowering the stunting prevalence (Sukmawati et al., 2023).

Lactobacillus reuteri is a potential probiotic composed of obligate heterofermentative species capable of surviving in low pH and enzyme-rich environments (Mu et al., 2018). It adheres to the epithelium for host-probiotic interactions and competes effectively with pathogenic microorganisms (Mu et al., 2018). The benefits of this probiotic in maintaining the oral cavity are well documented (Mu et al., 2018). A study showed that *L. reuteri* reuterin isolates had an inhibitory effect on *S. mutans* count (Widyarman & Theodorea, 2019). Clinical trials have signified that consuming *L. reuteri* lozenges twice daily for 56 days effectively reduced *S. mutans* count in 3-6-year-old healthy children (Almabadi et al., 2020). The present study evaluated the effects of the *L. reuteri* probiotics on dental plaque index, salivary pH, and *S. mutans* quantification of preschool-stunted children

MATERIALS AND METHODS

Study participants

The study included 54 participants, comprising 36 stunted and 18 normal children, selected through purposive sampling from Puskesmas Langensari 1, Banjar City, West Java, Indonesia, and divided into 3 groups. The test and negative control group each consisted of 18 stunted children, while the positive control group comprised 18 children with normal growth. Inclusion criteria were 3-5 years old stunted and normal children with good general health conditions. Meanwhile, the exclusion criteria were participants who had special needs, a history of systemic diseases and allergies, consumed antibiotics or other probiotics 1 month before the study, used an antibacterial mouthwash, and had no caries.

Methodology

The study was conducted in August 2024. This clinical experimental study used L. reuteri DSM17938/ATCCPTA5289 probiotics lozenges as an intervention. Ethical approval was obtained from the Dental Research Ethics Commission (KEPKG) of the Faculty of Dentistry, Universitas Indonesia, Jakarta, Indonesia, with approval number 40/Ethical Approval/FKGUI/VII/2024. A certificate of good clinical practice was received from the National Institute on Drug Abuse (NIDA), North Bethesda, United States of America (USA). Previously, participants and parents were provided with a comprehensive explanation of the study procedures both verbally and in writing. A consent form was then signed by a parent or guardian who agreed to participate. Before the intervention (day 0), dental plaque was examined using the Greene and Vermillion plaque index. Salivary pH was measured with pH litmus paper, and minimally 1 ml unstimulated saliva was collected from each participant between 8-12 AM. The samples were placed in a tube, labeled with a subject code, and stored in a cooler box before being sent to the Oral Biology Laboratory for RT-PCR analysis. RT-PCR was then conducted to measure the quantification of S. mutans.

All participant groups were given DHE before the intervention. This covered guidance on maintaining a healthy diet and practicing proper oral hygiene, including brushing teeth twice daily, after breakfast in the morning and before bed at night. Participants were also educated on how to brush using the bass method with the help of a tooth model. The success of the education was evaluated using a disclosing solution. All children were provided with the same type of toothbrush and fluoride toothpaste to ensure consistency. Both the test and positive control group were given L. reuteri probiotics lozenges once daily for 7 days after the night toothbrush as an intervention. Meanwhile, the negative control group did not receive any probiotics. Participants were instructed not to eat or drink for 1 hour after taking probiotics and were advised to avoid consuming any other products during the study period. After the intervention (day 7), the same procedures conducted before the intervention were repeated to examine dental plaque, salivary pH, and S. mutans quantification across all groups.

Statistical analysis

Reliability testing was performed using the kappa agreement test. Data normality was examined with the Shapiro-Wilk test. For normally distributed data (P 0.05), comparisons within groups were conducted using the paired t-test. In cases where Data was not normally distributed P < 0.05), the Wilcoxon signed-rank test was applied. The hypothesis assumed that a significant difference existed between the mean values of the two groups. A significance level of α = 0.05 was used, and the hypothesis was considered accepted when P < 0.05.

RESULT AND DISCUSSION

Clinically, the mean dental plaque index decreased in all groups after the intervention. In the test group, the mean dropped from 1.87 (±0.45) to 0.95 (±0.42). In the positive and negative control group, it declined from 1.75 (±0.41) to 0.74 (±0.34) and 1.79 (±0.58) to 1.35 (±0.47), respectively. Salivary pH showed a modest increase across all groups, but the median remained unchanged. In the test group, the median value shifted from 6.0 (range 5.0–7.0) before intervention to 6.0 (range 5.0–6.0) after. The positive control group maintained a consistent median of 6.0 (range 5.0–7.0) both before and after. Similarly, the negative control group retained a median of 6.0, but the range changed from 5.0–7.0 to 6.0–7.0. In the quantification of S. mutans, a clinical reduction was observed in the test group, decreasing from 4.6 (range 1.53–12.1) to 1.71 (range 0.12–10.75). The negative control group also showed a decrease, from 5.25 (range 1.02–33.8) to 2.82 (range 1.10–18.65). Conversely, the positive control group recorded an increase in *S. mutans* levels from 1.00 (range 1.00–1.00) to 3.36 (range 1.22–60.0). Statistical analysis presented significant differences in dental plaque index and *S. mutans* quantification before and after intervention in all groups (P < 0.05). However, the changes in salivary pH were not statistically significant (P > 0.05) (Table 1).

Table 1. Comparative analysis of dental plaque index, salivary pH and *S. mutans* quantification before and after consuming *L. reuteri* probiotics for 7 days

Group Parameter		Negative control group n=18	*P*-value	Test group n=18	*P*-value	Positive control group n=18	*P*-value
Dental Plaque Index	Before	1.79 ± 0.58[a]	<0.001*	1.87 ± 0.45[a]	<0.001*	1.75 ± 0.41[a]	<0.001*
	After	1.35 ± 0.47[a]		0.95 ± 0.42[a]		0.74 ± 0.34[a]	
Salivary pH	Before	6.0 (5.0-7.0)[b]	0.317	6.0 (5.0-7.0)[b]	0.655	6.0 (5.0-7.0)[b]	0.782
	After	6.0 (6.0-7.0)[b]		6.0 (5.0-6.0)[b]		6.0 (5.0-7.0)[b]	
S. mutans quantifica-tion	Before	5.25 (1.02-33.8)[b]	0.001*	4.6 (1.53-12.1)[b]	0.002*	1.00 (1.00-1.00)[b]	<0.001*
	After	2.82 (1.10-18.65)[b]		1.71(0.12-10.75)[b]		3.36 (1.22-60)[b]	

[a]Normal distribute data, presented in mean SD [b]Abnormal distribute data, presented in median (minimum-maximum)
*Wilcoxon signed-rank test, P < 0.05

DISCUSSION

Stunting can cause problems in the oral cavity, such as reduced resistance to biofilms and a low salivary flow rate (Sadida et al., 2022). This is attributed to salivary glands atrophy, lowering secretion and a reduction in pH, flow rate, and salivary buffer capacity (Abdat et al., 2020); (Sadida et al., 2022). Reduced saliva function facilitates the *S. mutans* bacteria colonization in the oral cavity, promoting plaque and biofilm formation (Folayan et al., 2020). Furthermore, malnutrition in stunted children contributes to dysbiosis in the oral cavity, causing *S. mutans* elevated levels (Folayan et al., 2020). This microbial imbalance serves as a significant risk factor for caries' high incidence (Folayan et al., 2020). Therefore, the study examined plaque index, salivary pH, and quantification of S. mutans.

The subjects in this study were stunted and normal children who had been registered in the Integrated Health Service Post (Posyandu) program under Puskesmas Langensari 1, Banjar City, West Java, Indonesia. Subjects in this study were categorized as stunted (z-score -3 SD to -2 SD) and normal (z-score -2 SD to 3 SD) based on height for age indicators from the Child Anthropometric Standard Regulation issued by the Indonesian Ministry of Health (Indonesia, 2020). This is in line with the WHO definition of stunting, which refers to the percentage of children whose length or height for age falls <-2 SD as evaluated according to the WHO Child Growth Standards in 2006 (Organization, 2018).

All subjects in this study were between the ages of 3 and 5. Stunting can be caused by chronic, repeated malnutrition that often begins in utero and continues through the first 2 years of life (De Onis & Branca, 2016). Growth failure in those years is appropriate to the linear growth failure observed between the ages of 2 and 5 (De Onis & Branca, 2016). Approximately 70% of height deficits at ages 2-5 are caused by linear growth failure in the first 1000 days, with the remaining 30% developing as deficits accumulate (De Onis & Branca, 2016). The age range is a potential period to restore linear development deficits (catch-up) in stunted children (De Onis & Branca, 2016). In addition, toddlers (under 5 years) fall within the primary target group for routine monitoring of growth and development status by health workers through the Posyandu program. This makes early detection and timely intervention for stunting more feasible (Posyandu, 2011).

This study was conducted in Puskesmas Langensari 1, Banjar City, West Java, Indonesia. The location was selected according to the Study and Dissemination of Stunting Case Audits in Banjar City Semester I of 2023, where the prevalence of stunting in Banjar City remained above the national target, reaching 19% in 2023.3 Furthermore, data from the Nutritional Status of the Banjar City Health Office, collected in February 2024, identified the Puskesmas Langensari 1 as a facility with the highest rate of stunting in the city (D. K. K. Banjar, 2024).

Dental caries is a dynamic pathological process that largely depends on the virulent biofilms development (Anil & Anand, 2017). This process includes complex interaction of host salivary constituents, oral microbes (and their products), and dietary carbohydrates (Hajishengallis et al., 2017). The current study identifies preventive solutions to reduce pathogenic bacteria and slow the progression of caries in high-risk patients (Mu et al., 2018). The caries etiology is multifactorial but a direct correlation with the presence of *S. mutans* bacteria was observed (Anil & Anand, 2017). Several analysis have examined preventive methods including the probiotic therapy use such as L. reuteri, to change the oral microflora in children (Cannon et al., 2013). In this study, *L. reuteri* was selected as the intervention due to its beneficial properties for host health (Sadida et al., 2022). These include enhancing resistance to infection, formation of biofilms that prevent the pathogenic bacteria colonization, and the reuterin production, a compound with antimicrobial activity (Cannon et al., 2013). *L. reuteri* also synthesizes vitamins B12 and B9 and contains histamine, which functions as an anti-inflammatory agent (Mu et al., 2018).

Study results showed significant differences in dental plaque index and *S. mutans* quantification before and after intervention in all groups. This is in accordance with a study that reported statistically significant differences between *S. mutans* and Lactobacilli bacteria as well as the amount of plaque, following 56 days of consumption of the probiotic lozenges in both the treatment and control group (Almabadi et al., 2020). Similarly, another study evaluating the effect of *L. reuteri* over 28 days discovered that the probiotic restrained the *S. mutans* and Lactobacilli colonies formation (Cannon et al., 2013). Significant decrease in bacteria associated with caries and plaque formation was also observed following the consumption of *L. reuteri* probiotic lozenges (Alamoudi et al., 2018).

Meanwhile, the positive control group clinically showed an increase in the median value of *S. mutans* quantification. This outcome corresponded with a study that reported a higher number of *S. mutans* at a 6 months follow-up in the test and control groups (Romani Vestman et al., 2013). The *S. mutans* number detected was higher inversely proportionate to the *L. reuteri* DNA amount in saliva. This idea suggests that *S. mutans* were regrowth due to the absence of *L. reuteri* DNA in saliva (Romani Vestman et al., 2013). An inverse relationship was identified between *S. mutans* counts and *L. reuteri* DNA levels in saliva, suggesting the occurrence of bacteria regrowth (Luo et al., 2024). These include the inhibition of microbial biofilm formation by downregulating genes such as gtfB, gtfC, and gtfD (Wasfi et al., 2018), competitive adhesion and colonization by producing EPS (Mu et al., 2018), and the secretion of reuterin (Widyarman & Theodorea, 2019).

There was no significant change in median salivary pH before and after the intervention in any group. This finding was consistent with reports indicating that 28-day consumption of *L. reuteri* DSM17938/ATCC5289 lozenges did not significantly affect salivary pH (Borrell García et al., 2021). The absence of a significant difference may be due to a lack of data on the duration of probiotic persistence in saliva following discontinuation (Flichy-Fernández et al., 2010). A study showed that the presence of *L. reuteri* in saliva is only temporary and disappears after cessation (Borrell García et al., 2021). However, another investigation observed that after the administration of LGG probiotics for 14 days, *S. mutans* count in the oral cavity gradually decreased (Borrell García et al., 2021). This signified the absence of permanent colonization and affirmed the transient nature of probiotic in the mouth (Borrell García et al., 2021).

CONCLUSION

This study concludes that the consumption of *Lactobacillus reuteri* probiotic lozenges for seven consecutive days significantly reduces the dental plaque index and the quantification of *Streptococcus mutans* in preschool-aged stunted children in Banjar City, West Java, Indonesia. Although no significant change was observed in salivary pH, the findings support the effectiveness of *L. reuteri* in improving oral health parameters related to caries risk in a vulnerable population. These results affirm the initial research objectives and highlight the potential of probiotic-based interventions as a non-invasive, low-cost strategy to manage oral health among stunted children. Future research could explore the long-term effects of probiotic consumption, its potential for permanent microbial modulation, and its integration into broader public health programs aimed at reducing the burden of early childhood caries, especially in areas with high stunting prevalence.

DECLARATIONS

Ethics Approval and Consent to Participate

Ethical approval was obtained from the Institutional Ethics Committee.

Consent for Publication

The patient provided written informed consent for the publication of this case report, including clinical images and radiographic findings.

Competing Interests

The authors declare that they have no competing interests related to this study.

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