

# The Effectiveness of Probiotic Milk Tablets in Preventing Dental Caries Among Young Children: A Cluster Randomized Control Trial- 12-Months Results

Prasit Wongsupa<sup>1</sup>, Kittisak Tunkura<sup>1</sup>, Rattanaporn Jantama<sup>1</sup>

<sup>1</sup>Dental Public Health Department Phayao Provincial Public Health Office, Phayao, Thailand

## Abstract

The research aimed to assess the effectiveness of probiotic milk tablets in preventing dental caries in children attending 12 selected kindergartens in Phayao Province, Thailand, from June 2022 to May 2023. The goal was to determine if probiotic-fortified milk tablets could serve as a viable method for preventing dental caries in children aged 2-5 years. Study participants needed to meet specific inclusion criteria, including being aged 2-5 years, attending one of the designated daycare centers, and having parental consent. Exclusion criteria encompassed conditions like cow's milk allergy, serious medical issues such as heart disease or asthma, oral and jaw abnormalities, the inability to undergo oral health assessments, or developmental delays. The participants were divided into control and treatment groups, with the treatment group receiving three milk tablets daily. Both groups received oral hygiene guidance. The milk tablets contained the probiotic *Lactobacillus rhamnosus* SD11. Each participant underwent oral examinations conducted by the dentists at the beginning of the study and subsequently every four months for 12 months to detect the presence of caries. The minimum required number of study participants was calculated to be 208 based on the estimated caries prevalence in the study population. Initially, 260 participants (136 males) were enrolled in the study, with 130 in the control group and 130 in the intervention group. The mean numbers of caries per participant in the control group at 4, 8, and 12 months were 5.74, 5.78, and 7.34, respectively. In contrast, the mean numbers of caries in the intervention group at 4, 8, and 12 months were 3.56, 3.33, and 3.43, respectively. Statistical analysis indicated that the differences in the mean caries numbers between the control and intervention groups at 4, 8, and 12 months were statistically significant ( $p=0.019$ ,  $p=0.018$ , and  $p=0.001$ , respectively). In summary, the intervention group showed significantly fewer caries cases compared to the control group. Consequently, this intervention be considered for the study group to reduce caries. However, further research is needed to determine if this intervention can be extended to other populations of children of similar age, different age groups and multi-center study.

**Keywords:** Dental caries prevention, Probiotic milk tablets, Young children

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**Correspondence to:**

Prasit Wongsupa, Dental Public Health Department, Phayao Provincial Public Health Office, 602 Moo 11 Bantom, Meungphayao District, Phayao, Thailand. Tel: 087-190-7047 Email: hippies\_129@hotmail.com

## Introduction

Dental caries in primary teeth is a significant global public health concern (WHO, 2019). In Thailand, the prevalence of dental caries among children aged 3-5 years is alarmingly high, with 52.9% among 3-year-olds and a staggering 75.6% among 5-year-olds.<sup>1</sup> A study conducted in Phayao Province, Thailand, reported a dental caries prevalence of 51.2% among 3-year-old children.<sup>2</sup> Typically, dental caries treatment for children under five years of age in this region involves tooth extraction, while restorative procedures and caries control are less common. Providing dental care for young children poses several challenges, including behavioral management and the logistical difficulties parents face in taking time off work, resulting in irregular dental visits and suboptimal care.<sup>3</sup>

Given the considerable challenges in treating dental caries, effective strategies for preventing caries in primary teeth are crucial. Primary preventive measures primarily revolve around maintaining good oral hygiene.<sup>4</sup> These strategies also include patient education, dietary management<sup>5</sup>, the use of fluoride-based toothpaste or other fluoride applications<sup>6,7</sup> antibacterial agents like chlorhexidine varnish, and the application of dental sealants.<sup>8</sup> Another approach to preventing dental caries in primary teeth involves the use of probiotics. Probiotics can be incorporated into various dairy products, such as including powdered milk, yogurt, lozenges, and milk tablets.<sup>9</sup> Several studies<sup>10-12</sup> have reported successful caries prevention using probiotic products.

Despite the promising outcomes of probiotic-based interventions, there is limited published data on the effectiveness of using probiotics milk tablets, especially in preventing dental caries among children. This study aims to assess the effectiveness of employing probiotics in the form of milk tablets to prevent dental caries in primary teeth among children aged 2-5 years. The objective is to determine whether probiotic-infused milk tablets offer a viable approach to preventing dental caries in this specific population.

## Materials and Methods

### Study design

The research employed a single-blinded, cluster-randomized controlled design.

### Study subject inclusion and exclusion criteria

The study included children aged 2-5 years who attended one of the 12 kindergartens in Phayao Province, northern Thailand, during June 2022-May 2023, and whose parents or guardians provided consent for their participation. Exclusion criteria consisted of a history of cow's milk allergy, cardiac conditions, asthma, craniofacial abnormalities, inability to undergo oral health examination, or developmental disabilities

### Participants

The minimum number of participants required for the study was determined using the following formula:

$$n/\text{group} = \frac{(Z_{\alpha/2} + Z_{\beta})^2 2\sigma^2 \times IF}{(\mu_1 - \mu_2)^2}$$

- where
- $Z_{\alpha}$  = Z-score at the desired level of statistical significance ( $\alpha$ ) = 0.05;  $Z_{\alpha} = 1.96$ .
  - $Z_{\beta}$  = Z-score for the desired statistical power ( $1 - \beta$ ) = 0.2;  $Z_{\beta} = 0.84$ .
  - $\sigma^2$  = Variance of the group of 3-year-old children in the province of Phayao = 1.00 (assumed to be the same for both intervention group and control groups).
  - $\mu_1$  = Expected mean number of decayed, missing or filled tooth surfaces (dmfs) among those receiving probiotics. This was estimated by expert opinion to be = 0.84.
  - $\mu_2$  = Expected mean number of dmfs among those in the control group. This was based on data collected from children aged 3 years in Phayao Province = 1.34.
  - IF = Inflation factor (IF) = 1.65.
  - n = Minimum sample size for each study group (control and intervention groups) = 104 (total of 208 for the 2 groups).

This research assumed a dropout rate of 20%, resulting in a total sample size of 260 participants, with 130 children in each group. The intervention group participants were provided with 3 milk tablets daily by their caregivers,

which contained the probiotic *Lactobacillus rhamnosus* SD11, and they also received instructions on maintaining oral hygiene. In contrast, the control group received only oral hygiene instructions. (Fig. 1).

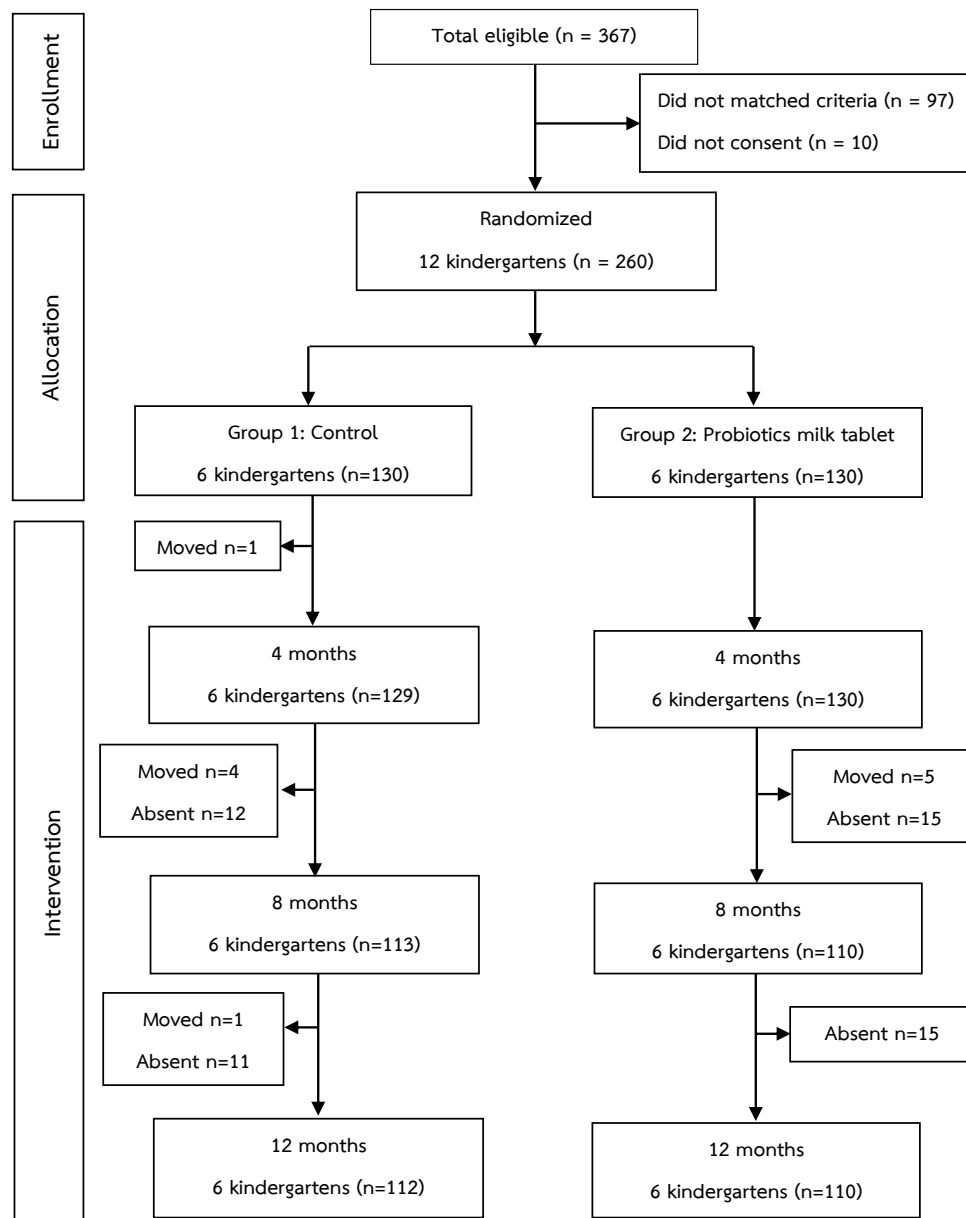


Figure 1 Flow of study, the random allocation and attrition of participants over the course of the 12-months intervention duration

### Clinical examination

Each study participant underwent dental examinations conducted by a dentist at the beginning of the study and subsequently at 4, 8, and 12 months after the study initiation. These examinations followed the standard criteria set by the World Health Organization (WHO) in 2013 and involved the use of a dental mirror

and probe. Prior to the examination, each participant brushed their teeth.

To assess the reliability of the dental examinations, inter-examiner reliability between the two study dentists was evaluated before the study, yielding a weight Kappa value of 0.89. Additionally, the intra-examiner reliability of the two study dentists was assessed, resulting in Kappa

values of 0.91 and 0.92. Importantly, the dentists conducted their examinations without knowing which of the groups each participant belonged, ensuring blinding.

### Ethical clearance

The study obtained ethical clearance from the Research Ethics Review Committee for Studies Involving Human Research Participants at Chulalongkorn University Faculty of Medicine. The Institutional Review Board reference number is IRB No.721/64 COA No.1613/2022. Additionally, the study was registered with the Thai Clinical Trial Registry under reference number TCTR20220329003. All children included in the study had written informed consent obtained from their parents or guardians prior to their participation in the study.

### Statistical analysis

The age and gender of the participants were recorded as well as the number of dmfs and carious surfaces at baseline, 4 months, 8 months and 12 months. These data were presented using means and standard deviations. Comparisons of the mean numbers of dmfs at baseline, 4 months, 8 months and 12 months were made using difference analyses and relationship comparisons were made using the Fisher's exact test and generalized linear mixed models. This study was conducted as a randomized controlled trial, and the statistical analysis used adheres to the intention-to-treat (ITT) principle, which measures all participants who were part of the study, regardless of whether they dropped out.<sup>13</sup>

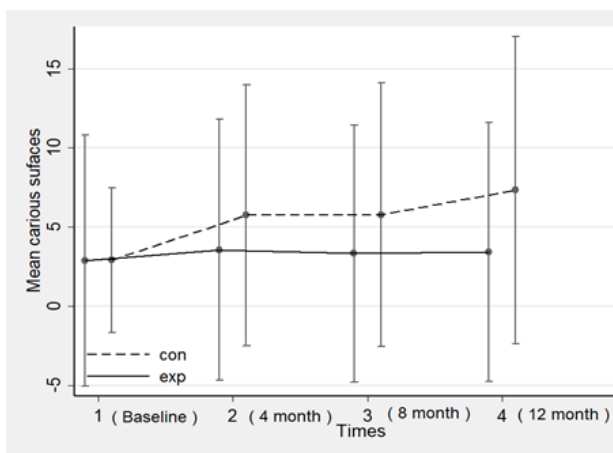


Figure 2 Mean carious surfaces values at baseline, 4 months, 8 months and 12 months

Data analysis was conducted using the STATA program, version 18.0 (StataCorp 2023. Stata Statistical Software: Release 18. College Station, TX: StataCorp LLC)

### Definitions

In this study, specific terms are defined as follows:

1. Normal Teeth: These refer to teeth that do not have any cavities or carious lesions.
2. Carious surfaces: These are teeth surfaces that exhibit soft or softened enamel or dentin, and where the tooth structure can be displaced or effected using a dental probe and not including inactive or non-cavitated caries. Carious surfaces essentially have cavities or lesions.
3. Effectiveness: This term measures the ability of a particular intervention to prevent the formation of cavities, and it is evaluated by comparing the outcomes of the intervention group with those of a control group that receives no intervention. In essence, effectiveness assesses how well the intervention can prevent the development of dental cavities.

## Results

### Clinical examination

The study included a total of 260 participants, with 130 in the control group and 130 in the intervention group, comprising 136 males and 124 females in both groups. There were no significant differences between the control and intervention groups in terms of mean age ( $3.01 \pm 0.57$  and  $3.04 \pm 0.61$  years, respectively), gender distribution (68 males and 62 females in both groups), mean dmfs scores ( $3.52 \pm 4.09$  and  $3.24 \pm 3.83$ , respectively), and the mean numbers of caries surfaces at baseline ( $2.92 \pm 4.57$  and  $2.88 \pm 7.93$ , respectively) (Table 1).

The mean numbers of carious surfaces at 0, 4, 8, and 12 months in the control group were  $2.92 \pm 4.57$ ,  $5.74 \pm 8.24$ ,  $7.34 \pm 9.70$ , and  $7.34 \pm 9.70$ , while in the intervention group, they were  $2.88 \pm 7.93$ ,  $3.56 \pm 8.25$ ,  $3.33 \pm 8.13$ , and  $3.43 \pm 8.17$ . The p-values for differences were 0.96, 0.019, 0.018, and 0.001, respectively (Table 2). These results indicate that the number of carious surfaces increased over time in both study groups, but the increase was more rapid in the control group than in the intervention group.

The mean dmfs scores at 0, 4, 8, and 12 months in the control group were 6.88±8.92, 10.16±10.79, 9.68±11.09, and 11.36±12.10, while in the intervention group, they were 5.05±9.82, 6.30±9.98, 5.78±10.18, and 5.93±10.29. The p-values were 0.14, 0.002, 0.002, and <0.001, respectively. The reduction in dmfs observed at the 8-month examination might be due to the possibility that dental caries lesions may have transitioned from an active to an inactive state. (Table 3).

These findings indicate that there was a significant difference in dmfs scores between the control and intervention groups, with the intervention group showing lower scores, particularly at 4, 8, and 12 months.

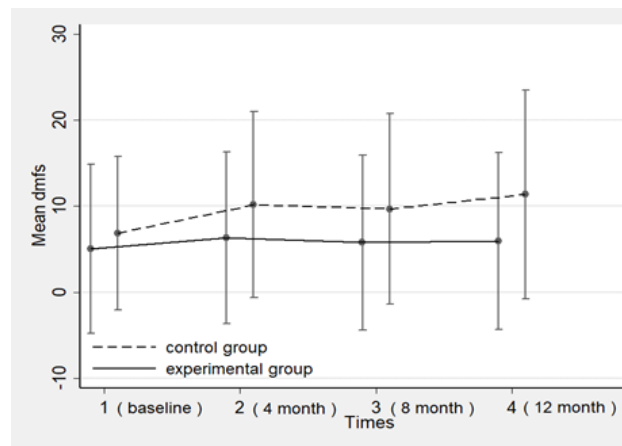


Figure 3 Mean of decayed missing filled surfaces of deciduous teeth (dmfs) values over the course of 12 months

Table 1 Baseline characteristics of study participants by study group (n=260)

Characteristics	Groups	
	Control	Intervention
Number of children	130	130
Age in years (Mean ± SD)	3.01 ± 0.57	3.04 ± 0.61
Gender		
Male, n	68	68
Female, n	62	62
dmfs score (Mean ± SD)	3.52 ± 4.09	3.24 ± 3.83
Numbers carious surfaces (Mean ± SD)	2.92 ± 4.57	2.88 ± 7.93

Note: no difference in Baseline characteristics data.

Table 2 Data of Mean carious surfaces at baseline, 4, 8 and 12 months by study groups (n=260)

Time of investigation	numbers carious surfaces (Mean ± SD)		Difference in mean numbers of carious surfaces (Intervention - Control)	95% confidence interval	p - value
	Control group	Intervention group			
Baseline	2.92 ± 4.57	2.88 ± 7.93	-0.04 ± 0.86	(-1.73, 1.65)	0.96
4 months	5.74 ± 8.24	3.56 ± 8.25	-2.18 ± 0.93	(-4.01, -0.36)	0.019*
8 months	5.78 ± 8.31	3.33 ± 8.13	-2.45 ± 1.03	(-4.47, -0.42)	0.018*
12 months	7.34 ± 9.70	3.43 ± 8.17	-3.91 ± 1.16	(-6.18, -1.63)	0.001*

\* p < 0.05

Note: Analysis with generalized linear mixed model.

Table 3 Assessing the difference in mean dmfs (decayed, missing, and filled surfaces) at the initial point, 4 months, 8 months, and 12 months during the longitudinal analysis.

Time of oral investigation	dmfs (Mean ± SD)		Mean difference of dmfs (Intervention - Control)	95% confidence interval	p - value
	Control group	Intervention group			
Baseline	6.88 ± 8.92	5.05 ± 9.82	-1.84 ± 1.22	(-4.22, 0.55)	0.14
4 months	10.16 ± 10.79	6.30 ± 9.98	-3.86 ± 1.32	(-6.45, -1.28)	0.002*
8 months	9.68 ± 11.09	5.78 ± 10.18	-3.91 ± 1.47	(-6.79, -1.02)	0.002*
12 months	11.36 ± 12.10	5.93 ± 10.29	-5.43 ± 1.66	(-8.69, -2.17)	< 0.001**

\* p < 0.05

\*\* p < 0.001

Note: Analysis with generalized linear mixed model.

## Discussion

In this study, there were significant differences in caries prevention outcomes observed at 4, 8, and 12 months. The group that consumed probiotic milk tablets showed statistically significant reductions in carious lesions compared to the control group. This difference was most pronounced at 8 and 12 months, with the control group demonstrating a clear increase in carious surfaces over time. This study did not utilize the ICDAS criteria.<sup>14</sup> for evaluating caries progression. However, it is important to recognize that employing a combined approach that includes both the probing method and the ICDAS criteria could potentially provide more comprehensive data.

These discoveries align with earlier research, particularly when compared to a 2014 study that investigated probiotic tablets versus placebo tablets in adolescents aged 12 to 17 over a three-month duration. Intriguingly, that study revealed no statistically significant variances in caries prevention at the three-month<sup>15</sup>, which contrasts with our study. This discrepancy may be attributed to differences in study duration and methods of assessing carious lesions.

Furthermore, the study in 2016, which compared the effects of probiotic milk with standard milk in young children over a 10-month period, found that the probiotic milk group demonstrated significantly better caries prevention outcomes<sup>16</sup>, consistent with our results.

Another study in 2017 investigated the use of Probiotic Yogurt and Xylitol-Containing Chewing Gum for caries prevention in 50 female school students over a period of 12 months. The levels of *S. mutans*, a cariogenic bacteria, was measured and it was found that both interventions led to statistically significant reductions in *S. mutans* counts.<sup>17</sup> The outcomes of that study align with our results, that the reduction in cariogenic bacterium may be related to the caries prevention, where children receiving probiotic interventions had fewer carious lesions compared to the control group.

The study conducted in 2020, which involved using probiotics mixed with milk powder for children aged 3-4 years, yielded results consistent with our study. In this

research, participants were divided into three groups: one group received the probiotic-mixed milk powder every day, another group received it three times a week, and the control group consumed regular milk powder for six months. The outcomes were measured for 12 months, and it was found that the group receiving probiotics daily or three times a week demonstrated statistically significant improvements in caries prevention compared to the control group.<sup>18</sup> These findings align with the observations of the study that probiotic interventions can lead to better caries prevention outcomes.

## Conclusion

The study provides further evidence that probiotic interventions, such as probiotic milk tablets, can be effective in preventing dental caries in young children. However, it is important to note that while this intervention shows promise, it should be considered as part of a comprehensive approach to oral health, and further research is needed to explore its applicability to different populations and age groups, as well as its long-term effects.

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