

# Effect of Prebiotics Supplements on Salivary pH and Salivary Buffer Capacity in Children with Early Childhood Caries: An *In Vivo* Study

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## ABSTRACT

*Streptococcus mutans* (*S. mutans*) is the major pathogen involved in caries development during the first few years of life. Prebiotics represent a breakthrough approach to maintaining oral health by utilizing naturally beneficial bacteria against harmful bacteria.

**Aim:** To assess the effects of prebiotics supplements on salivary pH and salivary buffer capacity in children with early childhood caries (ECC).

**Materials and methods:** This study was performed on 23 samples, aged 3–6 years who received prebiotics supplements for 14 days, followed up to 3 and 6 months. Unstimulated saliva samples were collected to assess salivary pH and salivary buffer capacity using salivary check buffer kit.

**Statistical analysis used:** The data showed normal distribution; hence, parametric tests were applied. Repeated measures of analysis of variance were applied to compare the mean at the three different time intervals, followed by paired *t*-test for pairwise comparison.

**Results:** There was a highly significant difference on comparing the different parameters. Salivary pH was lowest at baseline with a mean value of 6.65 which increased at the 3–6-month follow-up to 7.00 and 7.33. Similarly, salivary buffer capacity which was 4.73 at baseline, increased at 3 months to 8.17, and at 6 months to 10.34.

**Conclusion:** It can be suggested that prebiotics supplements can be adopted as a novel approach in children with ECC as a form of preventive measure to facilitate a better lifestyle.

**Clinical significance:** These prebiotics when taken for a period of 2 weeks increased the salivary pH as well as salivary buffer capacity when the data was compared at different time intervals emphasizing its importance, especially in children with ECC.

**Keywords:** Early childhood caries, Prebiotics, Pretest–posttest design, Salivary buffer capacity, Salivary pH.

*International Journal of Clinical Pediatric Dentistry* (2024): 10.5005/jp-journals-10005-2747

## INTRODUCTION

According to the Global Burden of Disease Study, <530 million children have dental caries. The prevalence of early childhood caries (ECC) in India among 8–48-month-olds has been reported to be 44%. In rural South India, the prevalence among 3-year-old children is 40.6%.<sup>1</sup> The disease initializes early in life, has rapid progress in children at high risk, and often goes neglected and untreated. The drift in the environment of the oral cavity, due to consumption of carbohydrates has been favorable for the growth of acidogenic and aciduric microbes, predominantly *Streptococcus mutans* (*S. mutans*).<sup>2</sup>

Probiotics growth is embellished by prebiotics which were first introduced in 1995 by Glenn Gibson and Marcel Roberfroid. Recently, Ricke refined the definition of a prebiotic, stating, “Prebiotics cannot be hydrolyzed in the gut by the host, but rather serve as a substrate to encourage the growth of beneficial bacteria.”<sup>3,4</sup>

Saliva enhances the oral cavity by its cleansing action, lubrication, buffering capacity, and ion repository of calcium and phosphate for remineralization of incipient carious lesions. Thus, these characteristics of saliva can be examined and evaluated at chairside with pH test kit and buffer kit which provides an extensive approach to the patient’s assessment.<sup>5,6</sup>

To date, prebiotics have been used on a major scale in the medical field, and its effects have been minimally evaluated in children having ECC. Hence, in this study, we intend to check for the effects of prebiotics on salivary pH and salivary buffer capacity in children with ECC.

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**How to cite this article:** Fernandes VA, Mata DB, Nadig B, *et al.* Effect of Prebiotics Supplements on Salivary pH and Salivary Buffer Capacity in Children with Early Childhood Caries: An *In Vivo* Study. *Int J Clin Pediatr Dent* 2024;17(1):54–58.

**Source of support:** Nil

**Conflict of interest:** None

## MATERIALS AND METHODS

The present *in vivo* study was conducted on 23 children aged 3–6 years with type I and type II ECC satisfying the inclusion and exclusion criteria who visited the Outpatient Department of Pedodontics and Preventive Dentistry. The study design was a one group pretest–posttest design, prospective, experimental, and *in vivo* study carried out over a period of 6 months.

The ethical approval was taken from the Research Development and Sustenance Committee (RDSC) BDC/Exam/509/2019–20. All of the participating children’s parents/carers were asked to provide informed consent after being fully informed about the entire treatment procedure.

### Inclusion Criteria

- Children aged 3–6 years.<sup>7,8</sup>
- Children with type I and type II ECC.
- Children with ASA 1 physical status.
- Parents who have given written consent and children's assent for the treatment.

### Exclusion Criteria

Children suffering from diarrhea or other stomach illness.<sup>3</sup>

- Children with dry mouth.
- Children with any known history of milk allergy.
- Children with any known history of allergy to proteins.
- Children who are receiving antibiotic treatment.

A study of 6 months duration was initiated in children with ECC. Children were asked to continue their normal hygiene regimens, which included brushing with a recommended nonmedicated toothpaste.

### Screening for Early Childhood Caries

Caries examinations of children with type I and type II ECC at the first appointment were performed by a single calibrated examiner using #5 dental mirrors and #23 explorers (Fig. 1).

After the clinical examination was done, children satisfying the inclusion criteria were selected. Baseline data of salivary pH levels and salivary buffer capacity were recorded using the methods mentioned below.

### Sample Collection

- Before the initiation of the experimental procedure, baseline samples were collected from the selected children.<sup>9</sup>
- Children were asked to abstain from oral hygiene procedures overnight and refrain from having food 2 hours before sample collection.
- Prior to the sample collection trial, children were instructed to thoroughly cleanse their mouth with distilled water in order to collect unstimulated saliva.
- Children were made to sit comfortably, eyes open, head tilted slightly forward, and advised to relax for 5 minutes with little orofacial movement.



Fig. 1: Instruments used for oral examination

- The children were then instructed to spit out any saliva that had accumulated inside their mouth into the sterile collecting cup every 60 seconds until a total of 2 mL of saliva had been collected (Fig. 2).

### Evaluation of Salivary pH in Children with Early Childhood Caries

After the collection of the saliva sample, A pH strip (Saliva-Check Buffer Kit, China) was immersed in the resting saliva for 10 seconds.<sup>6</sup> The color change of the strip was then assessed by comparing it to the color testing chart. The red section was represented by highly acidic resting saliva, which had a pH between 5.0 and 5.8, the yellow section by moderately acidic saliva with a pH between 6.0 and 6.6, and the green section by healthy saliva, which had a pH between 6.8 and 7.8 (Fig. 3).

### Evaluation of Salivary Buffer Capacity in Children with Early Childhood Caries

The saliva check buffer test yields results consistent with the titration methods described by Ericsson's 1959 method.<sup>10</sup>

The testing side of the buffer strip (Saliva-Check Buffer Kit, China) was placed on the absorbent tissue. Using a pipette, enough saliva was removed from the collecting cup so that a drop could be applied to each test pad side. The extra saliva was immediately absorbed on the absorbent tissue by turning the strip 90°, which prevented excess saliva from swelling on the test pad and compromising the accuracy of the test result. The test pads started to change color right away, and the final results were ready after 2 minutes (Fig. 3).

After the collection of baseline sample, using nonfluoride-releasing restorations, the Carious involved teeth were restored.

Children then received a 2-week allotment of prebiotic supplements (Essens Soluble Fibre, France) and parents or guardians were instructed about its usage. The content of this prebiotics sachet is dietary fiber (100% wheat dextrin). Parents were also given a checklist to make sure that prebiotics are being taken on an everyday basis. Children took one sachet containing 5 gm of prebiotics supplement mixed with water once in the morning while having breakfast for a time span of 2 weeks (Fig. 4).

After a period of 3 and 6 months, salivary pH levels and salivary buffer capacity were again recorded using the abovementioned methodology.



Fig. 2: Armamentarium for collection and dilution of saliva

The data showed normal distribution, hence parametric tests were applied. Repeated measures of analysis of variance were applied to compare the mean at the three different time intervals, followed by paired *t*-test for pairwise comparison. Statistical significance was fixed at or below 5% ( $p < 0.05$ ).

## RESULTS

The efficacy of the prebiotics supplements used in this study was determined by measuring the salivary pH and salivary buffer capacity. Results were expressed as mean and standard deviation; *p*-value was calculated for statistical significance.

Table 1 shows a highly significant statistical difference existed between all the parameters when compared at the various time intervals. The salivary pH was the lowest at baseline with a mean value of 6.65 and standard deviation of 0.16. The mean value at 3 months was 7.00, and at 6 months, it was 7.33. Thus, a steady increase in the pH values was noticed from baseline to 6 months.



Fig. 3: Assessment of salivary pH and salivary buffer capacity

A similar trend was also observed with the salivary buffer capacity. At baseline, the mean buffer capacity was 4.73, at 3 months it was noted as 8.17, and at 6 months, the mean buffer capacity was 10.34.

Table 2 depicts a highly significant statistical difference seen upon comparing the parameters pairwise for each of the time intervals. Based on the mean difference, it was seen that pH at 6 months > pH at 3 months > pH baseline. Similarly, the buffer capacity was highest at 6 months, followed by 3 months, and the lowest buffer capacity was observed at baseline.

## DISCUSSION

It has rightly been said that “Diseases can be eliminated through early diagnosis, but prevention can eliminate the disease.” This statement is applicable to dental caries since children are primarily affected by ECC.

In its simplest form, the human microbiome always maintains a balance between beneficial and harmful microorganisms, living in symbiosis with its host—the human body. Antibiotic use, however, appears to have had major negative impacts, such as pathogen resistance, harm to the intended oral microbiota, and an increased risk of dental caries in oral cavities.

As a result of this, prebiotic therapy—a recently developed, preventive approach—has gained popularity in recent years. These results make it necessary to develop new dental caries prevention techniques, particularly in light of the global recession that followed the coronavirus disease 2019 (COVID-19) epidemic.<sup>11</sup>



Fig. 4: Prebiotics supplements (contains 100% wheat dextrin)

Table 1: Comparative evaluation of the various parameters within the time intervals—baseline, 3 months, and 6 months

Parameter	Mean	Std deviation*	95% confidence interval		F**	Chi-square	Sig <sup>†</sup>
			Lower bound	Upper bound			
pH at baseline	6.6522	0.162	6.582	6.722	125.563	7.333	0.000*
pH at 3 months	7.0087	0.22945	6.909	7.108			
pH at 6 months	7.3348	0.23279	7.234	7.435			
Buffer capacity baseline	4.7391	0.75181	4.414	5.064	167.048	7.43	0.000*
Buffer capacity at 3 months	8.1739	1.33662	7.596	8.752			
Buffer capacity at 6 months	10.3478	1.49571	9.701	10.995			

\*Std deviation, Standard deviation; \*\*F, frequency; <sup>†</sup>Sig, significance

**Table 2:** Pairwise comparison of the various parameters within the time intervals—baseline, 3 months, and 6 months

Parameter	Parameter	Mean	Std deviation*	95% confidence interval of the difference		Sig**
				Lower	Upper	
pH at baseline	pH at 3 months	-0.3565	0.24088	-0.4607	-0.2524	0.000*
pH at baseline	pH at 6 months	-0.6826	0.23091	-0.7825	-0.5828	0.000*
pH at 3 months	pH at 6 months	-0.3261	0.14212	-0.3875	-0.2646	0.000*
Buffer capacity baseline	Buffer capacity at 3 months	-3.4348	1.12112	-3.9196	-2.95	0.000*
Buffer capacity baseline	Buffer capacity at 6 months	-5.6087	1.43777	-6.2304	-4.987	0.000*
Buffer capacity at 3 months	Buffer capacity at 6 months	-2.1739	0.88688	-2.5574	-1.7904	0.000*

\*Std deviation, Standard deviation; \*\*Sig, significance

With regard to management of dental caries, prebiotics are nutrients derived from microbial taxa that either produce alkali from these nutrients to increase pH and buffer capacity or inhibit acidogenic and aciduric microorganisms.<sup>12</sup>

In the present study, the two main parameters chosen were salivary pH and salivary buffer capacity: These characteristics may be related to dental caries in a number of ways: (1) aciduric/acidogenic bacteria (pH of saliva) and (2) acid-neutralizing (buffering capacity) of saliva.<sup>13</sup>

### Salivary pH and Early Childhood Caries

With regard to salivary pH and ECC, the potentiometric method which uses pH meters is the most widely used laboratory technique for measuring saliva pH.<sup>14</sup> It is recommended that this parameter be measured as soon as saliva is collected. This conclusion has been reached after comparing the various methods available for assessing salivary pH and analyzing the most recent literature reports. Although the accuracy of this approach is very good, its main drawback is that it takes a lot of time and can be a little difficult to be performed in children. Therefore, other direct diagnostic options exist, such as chairside pH diagnostic techniques. This is a very easy and quick method of assessing salivary pH. Hence, in the present study, the pH strips method was used for assessment of salivary pH in children with ECC.

The findings of this investigation align with the research conducted by Prabhakar et al.<sup>15</sup> and Preethi et al.<sup>16</sup> However, the findings of their individual investigations were not significant. When comparing children with active dental caries to those without, there was only a minor decrease in salivary pH. According to Zhou et al.,<sup>13</sup> the pH of saliva from young children with dental caries was statistically higher than that of children without dental caries. Conversely, research by Thaweboon et al. found that children with caries-free and rampant caries had similar mean salivary pH levels.<sup>17</sup>

### Salivary Buffer Capacity and Early Childhood Caries

Salivary buffer capacity can be directly diagnosed by utilizing specialized test kits in a laboratory setting or at a dental clinic. Results from the saliva-check buffer kit are readily available in 2 minutes. The test strip windows the colour in 2 minutes after it is placed in the saliva sample to assess the buffering capacity.<sup>13</sup>

The present study's findings are consistent with the research carried out by Prabhakar et al.<sup>15</sup> and Preethi et al.<sup>16</sup> Nevertheless, the findings of their investigations did not yield many notable insights. When comparing children with active dental caries to those without,

there was only a minor decrease in the salivary buffering capacity. Saliva from ECC patients had a statistically better buffering capacity than saliva from caries-free children, according to a study by Zhou et al.<sup>13</sup> Similar findings were reported in a research by Malekipour et al.,<sup>18</sup> despite the fact that the difference was not statistically significant.

Prebiotics in simple terms provide food to the probiotic microorganisms present in the human gut. Thus, in correlation with the literature and our study, it was evident that there was increase in salivary pH and salivary buffer capacity when the data was compared at baseline with 3 months and 6 months, respectively.

Within the limitations of the present study, further studies should be carried out on a larger scale in order to evaluate large number of individuals from different populations. Also, incorporation of newer technology can be useful to predict caries activity before and after the use of prebiotic supplements.

### CONCLUSION

Based on the results of this study, the following conclusion can be made:

- The prebiotics supplements used in this study can be adopted as a novel approach in children with ECC as a form of preventive measure to facilitate a better lifestyle.
- These prebiotics when taken for a period of 2 weeks increased the salivary pH as well as salivary buffer capacity when the data was compared at different time intervals emphasizing its importance, especially in children with ECC.
- As prebiotics supplements are economical, they can be recommended even in low socioeconomic status population.

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