



The effect of four infant milk formula on dental plaque pH: A double blinded randomized clinical trial

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Abstract

Nursing bottle caries is caused mainly due to bottle feeding of infant formulas. This study was done to estimate the cariogenic potential of commercially available infant formulas with regard to their ability to alter the dental plaque pH. The study was carried out among 120 caries free adults aged 18 to 24 years. The participants were grouped randomly into 4 groups to test Aptamil 1, Nan pro1, Lactogen 3 and Dexolac 3. The pH of the dental plaque was estimated at different timings (baseline, 5, 10, 15, 30,45 and 60 minutes) by diluting the dental plaque with distilled water and recording pH using digital pH meter. One way ANOVA test was done to compare the pH between the intervals for each sample and those samples that showed significance was further analyzed using the paired sample T test to check in which particular interval the pH significantly varies. Level of significance was set as 0.05. Aptamil 1 and Nan pro 1 showed a significant change in dental plaque pH. Pairwise test showed a significant reduction in dental plaque pH in all intervals for Aptamil 1 and Nan Pro1 significant reduction was seen up to 30 minutes. Lactogen 3 and Dexolac 3 showed no significant changes. Nan pro1, Lactogen 3 and Dexolac 3 are safer for consumption and can be used for infants. New parents and caregivers should be advised by the health care providers to carry out proper oral health care maintenance for the infants to prevent demineralization of the newly erupted tooth.

Keywords: Dental caries, Demineralization, pH meter, Lactose.

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1. Introduction

Nursing bottle caries (Nbs), also called as the baby bottle tooth decay, is one among the most prevalent oral disease affecting children caused due to prolonged bottle feeding of infant formulas [1-2]. It appears suddenly and spreads rapidly resulting in early involvement of the pulp. Studies have shown that milk formulas contribute to the development of nursing bottle caries [3-5]. They contain various ingredients including glucose, sucrose and lactose play a vital role in caries development [6-7]. However, there exists a controversy as some studies report that milk formulas have calcium, phosphate, whey and fat that provide a protective effect against dental caries [8-10]. This study explores the cariogenic potential of commercially available

infant formulas with their specific ability to alter dental plaque pH.

2. Methodology

A double-blinded randomized controlled study was conducted among 120 (60 males and 60 females) caries-free participants aged 18 years and above. The participants who had morphologically healthy teeth that are devoid of any congenital and developmental anomalies were included. The study was conducted after obtaining ethical clearance from the Institutional Human Ethical Committee Sathyabama Institute of Science and Technology (Ref No: Sathyabama University/IHEC/ study no 036). The participants who signed the informed consent were included in the study. The

study procedure was explained individually to each participant before the start of the study.

2.1. Procedure

The participants were instructed not to brush their teeth or practice any other oral hygiene measures for 24 hours. The supragingival plaque was collected from the buccal surface of the right molars and premolars using spoon excavator. The baseline pH of the supra-gingival plaque was recorded first by collecting and diluting the dental plaque in 20ml of distilled water and then pH was estimated using a digital pH meter (PARIJATA® digital LPD pocket pen type pH meter). The study participants were then divided randomly into four groups by the chief investigator, each group comprising 30 (15 males and 15 females each) participants. The first group was given Aptamil 1 milk product, the second group was given Nan pro 1, the third group was given Lactogen 3 and the fourth group were given Dexolac 3. The study participants and the investigators who carried out the observation were blinded. The participants were asked to swish their mouth for 1 minute using the diluted milk products subsequently and then spit it out. The supra-gingival plaque was collected from the maxillary second premolar, diluted in 20 ml of distilled water and then the pH was estimated. The pH meter was then placed in the buffer solution provided by the manufacturer for neutralization. This procedure was carried out after 10, 15, 30, 45, and 60 minutes. For each of the predetermined timings, the plaque was collected, diluted in distilled water and the plaque pH was recorded.

2.2. Statistical analysis

The data was entered in Windows Excel 2008 and then transferred to SPSS version 11 for statistical analysis. Descriptive statistics were carried out. One-way ANOVA test was done to compare the pH between the intervals for each sample. Samples with significantly different pH between the intervals identified from the ANOVA test were further analyzed using the paired sample T-test to check in which specific interval the pH significantly varies. The level of significance was set as 0.05.

3. Results

From table 1, we observe that the significance value is less than 5% for Lactogen 3 and Dexolac 3 samples. Hence, there is no significant difference in pH level in the Lactogen 3 and Nutricia samples. However, the pH levels in the other two samples (Aptamil and Nan Pro 1) differ significantly between the intervals. These two samples were analyzed further using the paired sample T-test to compare each interval. Table 2, shows the mean and standard deviation of pH values of all four samples. From table 3, we observe that the p-value is less than 5% in all the pairs which indicates that the pH value significantly deteriorates over time in the Aptamil 1 sample. However, Nan pro 1 sample pH level deteriorates till 30 minutes and remains approximately in the same level after 30 minutes till 60 minutes.

4. Discussion

Dental caries is caused by the dissolution of hard tissues by the acids produced by caries-causing bacteria. The pH of dental plaque is commonly used to estimate the cariogenic potential of foods. Dental plaque from smooth enamel

surfaces is commonly excavated to assess the plaque pH [11-12]. Early Childhood caries (ECC) is a major public health problem with increased prevalence globally. It has been reported that the prevalence of ECC in India is 49.6% [13]. Nursing bottle caries is a form of ECC commonly seen in infants. Dr. Elias Fass (1962) was the first to describe that nursing caries is caused due to stagnation of milk around the infant's teeth [14]. Bernick, (1971) reported that rampant caries is caused due to prolonged bottle feeding with milk [11]. Nutrients that produce acid after fermentation of dental plaque are said to be cariogenic. This study was undertaken to assess the cariogenic potential of 4 milk formulas, Aptamil 1, Lactogen 3, Nutricia Dexolac 3, Nan Pro 1, commonly available commercially. The results of our study showed that, of the four milk formulas analyzed, Aptamil 1 and Nan pro 1 showed a significant reduction in dental plaque pH when compared to pre-rinsed pH. Pairwise distribution showed a significant difference at regular intervals (baseline to 5 minutes, 5 to 10 minutes, 10 to 15 minutes, 15 to 30 minutes, 30 to 45 minutes and 45 to 65 minutes) for Aptamil 1 whereas in Nan pro 1 there was a significant pH drop up to 30 minutes after which there was no significant difference. Our study results differ from those of other studies. Pandey, A. et al. (2022) in their study showed a decrease in plaque pH in Lactodex 2, Lactogen 1, Similac 1 and Nan Pro 2 after 30 and 60 minutes [15]. Raju, A.S. et al. (2012) found that the pH of lactogen, Nestogen, Nan, Farex, Lactodex and Amulspray reduced significantly within 5 minutes and then returned to pre-rinse pH in 60 min [12]. The reduction in pH of dental plaque will lead to demineralization of enamel surface. We hypothesize that bacteria present in the dental plaque, ferment the carbohydrates present in the milk formula into acids that could have caused the reduction of plaque pH after rinsing the formulas. The reduction of pH below 5, will lead to demineralization of enamel surfaces [16]. Our study showed that the pH value of the plaque did not reduce below 5 for all four samples during our study timings. Also, the pH values were found to increase for Nan pro1, Dexolac 3 and Lactogen 3 at 60 minutes. This might be because, the form of carbohydrates used in the tested formulas had no sucrose. Aptamil 1, Nan pro 1 and Dexolac 3 had lactose while lactogen 3 had maltodextrin. The infant's oral cavity is introduced to various microorganisms from the time of birth. The bacteria and fungi find its way to the oral cavity through the maternal transmission during the time of delivery, diet and transmission from the caregivers [17]. Along with the eruption of primary dentition in early infant to the eruption of permanent dentition in the children, these organisms also continue to grow forming a complex microbiota [18]. ECC is caused mainly due to *Streptococcus mutans* because of its acidogenic, aciduric properties and also, its ability to form extracellular mutans [19]. Linds, L.M. et al. (2016) conducted a study to investigate the effects of infant formulas on the formation of biofilm by *S. mutans* [20]. The results showed that infant formulas which had sucrose showed significant increase in the growth of biofilm at various concentrations. Lactose containing milk formulas, though showed no significant changes statistically, there was a visible change in biofilm formation. Assaf, D. et al. (2015) also reports that lactose present in the milk induces the formation of biofilm by *S. mutans* [21].

Table 1: Comparison between intervals for each sample

| Samples | | Sum of Squares | Df | Mean Square | F | Sig. |
|-------------------|----------------|----------------|-----|-------------|---------|------|
| Aptamil 1 | Between Groups | 17.310 | 6 | 2.885 | 182.540 | .000 |
| | Within Groups | 3.208 | 203 | 0.016 | | |
| | Total | 20.518 | 209 | | | |
| Lactogen 3 | Between Groups | 4.124 | 6 | 0.687 | 1.873 | .087 |
| | Within Groups | 74.488 | 203 | 0.367 | | |
| | Total | 78.613 | 209 | | | |
| Dexolac 3 | Between Groups | 2.188 | 6 | 0.365 | 1.640 | .138 |
| | Within Groups | 45.154 | 203 | 0.222 | | |
| | Total | 47.342 | 209 | | | |
| Nan pro 1 | Between Groups | 9.942 | 6 | 1.657 | 8.807 | .000 |
| | Within Groups | 36.874 | 196 | 0.188 | | |
| | Total | 46.815 | 202 | | | |

Table 2: pH values of all samples at different intervals

| | | N | Mean | Std. deviation | Minimum | Maximum |
|-------------------|---------------------|------------|--------------|----------------|----------|------------|
| Aptamil 1 | pH before rinsing | 30 | 7.067 | 0.1398 | 6.9 | 7.4 |
| | pH after 5minutes | 30 | 6.777 | 0.1278 | 6.4 | 7 |
| | pH after 10 minutes | 30 | 6.657 | 0.1104 | 6.5 | 6.9 |
| | pH after 15 minutes | 30 | 6.483 | 0.1464 | 6.2 | 6.8 |
| | pH after 30 minutes | 30 | 6.36 | 0.0932 | 6.2 | 6.6 |
| | pH after 45 minutes | 30 | 6.29 | 0.1094 | 6.1 | 6.5 |
| | pH after 60 minutes | 30 | 6.177 | 0.1431 | 6 | 6.5 |
| | Total | 210 | 6.544 | 0.3133 | 6 | 7.4 |
| Lactogen 3 | pH before rinsing | 30 | 6.81 | 0.1155 | 6.6 | 7 |
| | pH after 5minutes | 30 | 6.5 | 1.12 | 6.6 | 6.9 |
| | pH after 10 minutes | 30 | 6.64 | 0.1102 | 6.5 | 6.9 |
| | pH after 15 minutes | 30 | 6.59 | 0.0995 | 6.4 | 6.8 |
| | pH after 30 minutes | 30 | 6.557 | 0.104 | 6.4 | 6.8 |
| | pH after 45 minutes | 30 | 6.317 | 1.122 | 6.4 | 6.8 |
| | pH after 60 minutes | 30 | 6.49 | 0.0923 | 6.3 | 6.7 |

| | | | | | | |
|---------------------------|---------------------|------------|--------------|---------------|------------|------------|
| | Total | 210 | 6.558 | 0.6133 | 6.4 | 7 |
| Nutricia Dexolac 3 | pH before rinsing | 30 | 6.717 | 0.296 | 6.1 | 7.3 |
| | pH after 5minutes | 30 | 6.617 | 0.2365 | 6 | 6.9 |
| | pH after 10 minutes | 30 | 6.497 | 0.2297 | 6.1 | 6.9 |
| | pH after 15 minutes | 30 | 6.567 | 0.2123 | 6.1 | 6.9 |
| | pH after 30 minutes | 30 | 6.363 | 1.1125 | 6 | 6.9 |
| | pH after 45 minutes | 30 | 6.503 | 0.1884 | 6.2 | 6.9 |
| | pH after 60 minutes | 30 | 6.513 | 0.2063 | 6.1 | 6.9 |
| | Total | 210 | 6.54 | 0.4759 | 6 | 7.3 |
| Nan pro 1 | pH before rinsing | 30 | 7.114 | 0.1941 | 6.8 | 7.4 |
| | pH after 5minutes | 30 | 6.866 | 0.0614 | 6.8 | 7 |
| | pH after 10 minutes | 30 | 6.824 | 0.0786 | 6.7 | 7 |
| | pH after 15 minutes | 30 | 6.748 | 0.1022 | 6.6 | 7.1 |
| | pH after 30 minutes | 30 | 6.69 | 0.0976 | 6.6 | 7 |
| | pH after 45 minutes | 30 | 6.386 | 1.1151 | 6 | 6.7 |
| | pH after 60 minutes | 30 | 6.51 | 0.0772 | 6.3 | 6.6 |
| | Total | 210 | 6.734 | 0.4814 | 6.5 | 7.4 |

Table 3: Pairwise comparison of Aptamil 1 and Nan pro 1.

| Samples | | Paired differences | | | | | T | df | Sig. |
|-----------|--|--------------------|----------------|-----------------|-----------------|--------|-------|----|-------|
| | | Mean | Std. deviation | Std. Error Mean | Interval of the | | | | |
| | | | | | Lower | Upper | | | |
| Aptamil 1 | pH before rinsing pH after 5 minutes | 0.29 | 0.2139 | 0.0391 | 0.2101 | 0.3699 | 7.425 | 29 | 0.000 |
| | pH after 5 minutes pH after 10 minutes | 0.12 | 0.1495 | 0.0273 | 0.0642 | 0.1758 | 4.397 | 29 | 0.000 |
| | pH after 10 minutes pH after 15 minutes | 0.1733 | 0.0785 | 0.0143 | 0.144 | 0.2026 | 12.09 | 29 | 0.000 |
| | pH after 15 minutes pH after 30 minutes | 0.1233 | 0.1194 | 0.0218 | 0.0787 | 0.1679 | 5.656 | 29 | 0.000 |
| | pH after 30 minutes pH after 45 minutes | 0.07 | 0.0596 | 0.0109 | 0.0477 | 0.0923 | 6.433 | 29 | 0.000 |

| | | | | | | | | | |
|-----------|--|---------|--------|--------|---------|--------|--------|----|-------|
| | pH after 45 minutes pH after 65 minutes | 0.1133 | 0.073 | 0.0133 | 0.0861 | 0.1406 | 8.5 | 29 | 0.000 |
| Nan pro 1 | pH before rinsing pH after 5 minutes | 0.2483 | 0.1883 | 0.035 | 0.1767 | 0.3199 | 7.102 | 28 | 0.000 |
| | pH after 5 minutes pH after 10 minutes | 0.414 | 0.0946 | 0.0176 | 0.0054 | 0.0773 | 2.357 | 28 | 0.026 |
| | pH after 10 minutes pH after 15 minutes | 0.0759 | 0.1057 | 0.0196 | 0.0356 | 0.1161 | 3.863 | 28 | 0.001 |
| | pH after 15 minutes pH after 30 minutes | 0.0586 | 0.115 | 0.0214 | 0.0149 | 0.1024 | 2.745 | 28 | 0.010 |
| | pH after 30 minutes pH after 45 minutes | 0.3034 | 1.1185 | 0.2077 | -0.122 | 0.7289 | 1.461 | 28 | 0.155 |
| | pH after 45 minutes pH after 60 minutes | -0.1241 | 1.1376 | 0.2112 | -0.5568 | 0.3086 | -0.588 | 28 | 0.561 |

Table 4: Amount of various ingredients present per litre in prepared formulas

| Per liter prepared formula | Aptamil 1 | Nan Pro 1 | Dexolac 3 | Lactogen 3 |
|----------------------------|-----------|-----------|-----------|------------|
| Carbohydrates(grams) | 79.7 | 80.2 | 73.4 | 84.9 |
| Proteins (Grams) | 14.9 | 14.6 | 21.6 | 20.1 |
| Calcium (milligrams) | 675 | 367.9 | 911.3 | 784.8 |
| Phosphorus(milligrams) | 405 | 183.95 | 560.3 | 442.39 |
| Sodium(milligrams) | 216 | 134.9 | 405 | 141.43 |
| Potassium (milligrams) | 554 | 545.04 | 884.3 | 642.77 |

From these studies we can assume that lactose present in the milk formulas might influence the formation of biofilm that helps *S. mutans* survive in the oral environment, thus, increasing the risk of demineralization of newly erupted teeth. Studies have shown that, calcium rich diet such as milk, cheese and yogurt are considered non cariogenic because of the presence of high amount of calcium and phosphate content, lactose, casein and casein phosphopeptides [22-24]. These products when present in the diet reduce the chances of enamel demineralization. Table 4 shows that Dexolac 3 and lactogen 3 milk formulas have higher content of milk proteins, calcium and phosphorous when compared to stage 1 Aptamil and Nan pro. The protective effect of the minerals and proteins can be the reason for the insignificant reduction in dental plaque pH after rinsing with Dexolac 3 and Lactogen 3.

It should be noted that Aptamil 1 and Nan Pro 1 are stage 1 milk formulas recommended for infants 0 to 6 months and Dexolac 3 and Lactogen 3 are Stage 3 formulas recommended for babies over 12 months. Therefore, the content of protein, calcium and phosphorus will increase in stage 3 formulas when compared to stage 1 formulas to facilitate the rapid physiologic growth and cognitive development of the infant.

Furthermore, sodium and potassium content, shown in Table 4, present in the milk formulas play a major role as a buffer to neutralize the dental plaque pH. It is the buffering capacity of dental plaque fluid that determines the amounts of acids and bases needed to neutralize dental plaque pH. Studies have shown that the buffering capacity of dental plaque fluid is approximately twice that of saliva [25]. This could also be a reason for dental plaque pH not reducing to critical pH (5) in our study.

This study had certain limitations. First, only four infant formulas, Stage 1 Aptamil, Nan Pro and Stage 3 Dexolac and Lactogen were selected for this study. The result of this study cannot be generalized for all milk formulas as each milk formula has different composition at different stages. Secondly, estimating the salivary pH in addition to the dental plaque pH may have given a more accurate estimation of the cariogenic potential of the studied milk formulas.

Given the fact that pH values of the dental plaque did not reduce below 5 in any of the study samples and the mean pH of all samples except Aptamil 1, increased at 60 minutes when compared to 45 minutes, this study highlights that Nan Pro 1, Dexolac 3 and Lactogen 3 milk infant formulas are safer for consumption. As health care provider, we should recommend the new parents the need of oral hygiene maintenance after each feed of infant formulas to reduce the formation of biofilm by the *S. mutans*.

5. Conclusion

This study shows that stage 1 Aptamil and Nan pro showed a significant reduction in plaque pH. In Nan pro 1, there was an increase in mean dental plaque pH at 60 minutes when compared to 45 minutes. Stage 3 Lactogen and Dexolac 3 showed no significant changes. Therefore, it is suggested that Dexolac 3, Lactogen 3 and Nan Pro 1 can be used to feed infants. It is recommended from this study that new parents and caregivers should be advised by the health care providers to carry out proper oral health care maintenance for the

infants to prevent demineralization of the newly erupted tooth.

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