

pH-value decrement of saliva-plaque solution after mixing with four customary used infant milk formulas: An *in vitro* study

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ABSTRACT

Infant milk formulas may cause tooth destruction by producing different organic acids after fermentation by oral microorganisms. The aim of this study was to investigate pH changes in children's plaque-saliva solutions after fermenting four commonly used infant milk formulas. Plaque-saliva specimens of 25 children were sampled. Infant milk formulas including Bebelac 1, Nan 1, Nan 2 and Aptamil 2 were prepared and added to four respective tubes of saliva-plaque solutions. The pH of samples were measured at 10 min intervals (10-60) at 37°C. All infant milk formulas caused significant pH drop in all of the samples. Aptamil 2 and Bebelac 1 showed nearly the same pH drop and caused more pH value decrement than Nan 1 and Nan 2 after 30, and 60 min. The difference in pH decrement in solutions may be related to their different amounts of fat content as a preventive factor in acid production, as the amounts of other ingredients such as carbohydrate and protein were nearly the same.

Key words: Infant milk formula, pH, Plaque, Saliva

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INTRODUCTION

Prolonged and recurrent consumption of the milk especially during the night when the infant sleeps is the most important factor in the onset and progression of nursing caries.^[1] Nursing caries may cause severe problems in infant's nutrition and health involving the primary and subsequently the permanent teeth.^[2,3]

According to a study by Mazhari *et al.*^[2] the prevalence of nursing caries in Iranian children considering the cavitated lesions were reported 37%, which indicate the needs to improve preventive measurements.

An important subject in occurrence of the nursing caries is the content of the milk bottle.^[4] Milk formulas are a complex combination of different ingredients including fermentable carbohydrates such as sucrose, lactose and glucose playing an important role in caries production.^[5,6]

Many studies state that the milk formulas have potential cariogenicity^[6-8] depending on the amount of their carbohydrate content,^[6-9] although they may contain some ingredients such as calcium and phosphate which have a protective effect against the caries process.^[7,10]

Some studies found that in addition to the amounts of phosphate and calcium, whey and casein, as well as fat, might also provide a protective effect against caries.^[11-14]

Most infant milk formulas manufactured by different factories are nearly similar to human milk, all of them may cause nursing bottle caries.^[11,12]

The milk formulas produced by different factories are based on milk or soya.^[12] Soya based infant formulas are lactose-free but contain different fermentable sugars such as glucose polymers and sucrose which may cause caries occurrence.^[11]

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Sheikh *et al.*^[12] showed that all of the milk formulas (soya based and milk based formulas) lowered plaque and salivary pH value to below the critical pH of 5.7 after consumption by children.

Knowledge of cariogenicity of different milk formulas may help us reducing caries incidence by selecting the safest ones.

At present, there are no available data about characteristics and cariogenicity of the different infant milk formulas which are used widely in Iranian population. Hence, this matter is an important issue for research.

MATERIALS AND METHODS

This simple randomized study was carried out on 25 healthy children 4-6 years old.

Elusion criteria

1. Subjects on antibiotic therapy in the past 6 weeks.
2. Allergy to milk or soya.
3. Subjects using drugs affecting salivary flow.

Informed consent was taken from parents to participate their children in the study. Approval of the Institutional Ethical Committee was obtained.

The children received full-mouth prophylaxis before examination. They were asked not to implicate oral hygiene for 24 h and not to have anything at least 2 h prior the study.^[11] Supragingival plaque from the buccal surface of posterior teeth was collected using a spoon excavator (API Stainless Steel Instruments, German).^[15]

Samples containing 2 ml of the non-stimulated saliva were collected in 50 ml glass centrifuge tubes for each subject using a Pasteur pipette.^[16] Supra gingival plaque from the buccal surface of posterior teeth was collected by spoon excavator (one excavator movement), then the plaque was added to the samples and stirred well. Two ml of deionized water was added in each tube, and one of the tubes was kept as the negative control. Sucrose 10% was added to another tube regarded as positive control group.

Four commercially available infant milk formulas were chosen for the study including Nan 1 and Nan 2 (Nestle, Iran), Aptamil 2 (Nutrica, Iran) and Bebelac 1 (Daroo pakhsh, Iran).

The mentioned infant milk formulas were prepared according to the manufacturer's instructions, then 2 ml of each preparation were added to each respective plaque-saliva solutions. The pH was measured at the beginning of preparation by pH-meter device (WTW Inolab PH 720, Germany). After each pH measurement, the used electrode was washed in distilled water and placed in a standard buffer solution of pH 7, then the next pH value was determined.^[15]

The pH values of plaque-saliva samples were also measured at 10, 20, 30, 40, 50, and 60 min intervals. Data were analyzed by SPSS version 17. Kruskal-Wallis test and ANOVA test applied properly. $P < 0.001$ was considered as significant.

RESULTS

The results of the present study showed that all of the four infant milk formulas decreased the mean pH values in saliva-plaque solutions significantly after 30 min and 60 min of preparation $P = 0.0023$.

Bebelac 1 and Aptamil 2 decreased the mean values of pH in all samples more than the two other milk formulas nearly at the same rate $P = 0.0012$.

The mean pH decrement made by Nan 1, and Nan 2 in the samples were also nearly the same [Table 1].

DISCUSSION

It is generally accepted that the nutrients with more acid production after fermentation by plaque microorganisms have more cariogenicity. The methods which measure plaque pH values following food consumption are used in many researches because of their valuable guide to determine the potential cariogenicity of foods.^[16,17]

In the current study, we used an experimental method for pH change measurements of saliva-plaque solution after using different infant milk formulas.

Table 1: The mean of pH values and pH decrements of samples at 30 and 60 min after preparation

Samples	pH at the beginning	pH after 30 min	pH after 60 min	pH decrement after 30 min	pH decrement after 60 min
Bebelac	6.75±0.14	6.41±0.12	6.08±0.09	0.34± 0.05	0.69±0.08
Nan 1	6.96±0.15	6.63±0.13	6.52±0.11	0.24±0.03	0.43±0.05
Aptamil 2	6.71±0.15	6.34±0.12	6.00±0.06	0.44±0.06	0.67±0.07
Nan 2	6.67±0.14	6.35±0.13	6.24±0.10	0.29±0.03	0.48±0.07
Positive control	6.51±0.13	6.07±0.14	5.54±0.13	0.52±0.07	0.93±0.09
Negative control	6.96±0.13	6.93±0.11	6.96±0.10	0.01±0.009	0.02±0.007

Some studies reported that the presence of protein and casein in milk has a buffering capacity.^[18-23]

Proteins such as lactoferrin can prevent streptococcus mutans from binding to enamel the surface as well as dental plaque matrix.^[24,25]

Chaundhary *et al.*^[19] showed that the presence of protein in milk has a protective effect against caries. Their findings showed that soya-based milk formulas lowered the plaque pH value more than milk-based ones.^[19]

However, Moynihan *et al.*^[20] showed no significant difference between pH value decrement made by soya based and milk based formulas.^[20] This disagreement may be due to the different sugar contents of soya-based milk formulas made by different factories.^[20]

All of four milk formulas used in the current study were milk-based formulas and the protein and casein, as well as the carbohydrate and mineral content of them, were nearly the same [Table 2].

The results of the current study showed that all of these milk formulas lowered the mean pH value of saliva-plaque solutions significantly.

Bebelac 2 and Aptamil 1 decreased the mean plaque pH value significantly more than Non 1, Non 2 in all of the samples.

Chaundhary *et al.*^[19] found no significant effect of casein in preventing the decrement of pH value. They suggested that the buffering capacity of casein was not able to neutralize the acid produced by fermentation of milk carbohydrates.

Minerals may have some cariostatic effects in milk products.^[26] According to many researchers, iron is considered to inhibit the caries process.^[27,28] Masih *et al.*^[29] who studied on three different milk formulae, showed that lactodex 2 decreased the salivary pH value less than lactogen 2 and Amulspray. They stated that it may be related to different iron content of the three milk products. Lactodex 2 had the highest iron content (8.0 mg) in comparison with Lactogen 2 (7.9 mg) and Amulspray (5.0 mg).^[29]

Table 2: The amounts of different ingredients in four infant milk formulas (per 100 g milk powder)

Ingredient/100 g powder	Bebelac 1	Aptamil 2	Nan 1	Nan 2
Carbohydrate (g)	54.3	52.7	58.4	57.2
Protein (g)	14.7	15.4	13.6	12.8
Fat (g)	20.3	20.7	27.7	27.4
Calcium (mg)	450	500	427	432
Phosphate (mg)	309	324	289	288

Fat can prevent dental caries by different mechanisms.^[14,30,31] The fatty acids and monoglycerides can show antimicrobial effects.^[30,31] Fat can also have a physical effect, reducing the contact of carbohydrates with microorganisms of plaque and saliva. It may play a preventive role as a barrier, reducing the fermentation process.^[14,30]

The amount of fat in milk formulas used in our study was different [Table 2].

The fat content in Nan 1 and Nan 2 was nearly 25% higher than Aptamil 1 and Bebelac 2 [Table 2].

CONCLUSION

Bebelac 1 and Aptamil 2 decreased the mean values of pH in all samples more than the two other milk formulas nearly at the same rate.

The different pH decrease in solutions may be related to their different amounts of fat content (The fat content in Nan 1 and Nan 2 was higher than Aptamil 1 and Bebelac 2) as the carbohydrate and protein content as well as the amounts of casein and whey of four milks were nearly the same.

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