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The effect of different milk formulas on dental plaque pH

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Summary. *Objectives.* The purposes of this study were (1) to investigate the effect of different milk formulas on dental plaque pH after rinsing with these three categories, type of protein-based formulas (milk-based, soy-based, protein hydrolysate), type of sugar (only lactose, lactose and other sugars, only non-milk extrinsic sugars), and casein ratio (high and low casein), and (2) to observe organic acids formed by different milk formulas.

Methods. Baseline plaque pH and plaque pH at 2, 5, 10, 15, 20, 25, 30, and 60 min after rinsing with milk formulas were recorded by a combination electrode in 14 healthy subjects. Deionized water and 10% sucrose were used as a negative and positive control. The plaque sample was also analysed to identify and quantify the organic acids using a high-performance liquid chromatography. Parameters including minimum pH, maximum pH drop, and area under curve were compared by RMANOVA and paired *t*-test.

Results. The minimum pH was not significantly different among different protein-based formulas, whereas, the maximum plaque pH drop of soy-based and milk-based formula was significantly higher than that produced by protein hydrolysate formula ($P = 0{\cdot}022$ and 0·03, respectively). Area under curve produced by soy-based and milk-based formulas was significantly larger than that created by protein hydrolysate formula ($P = 0{\cdot}025$ and $< 0{\cdot}001$, respectively). Milk formulas containing only lactose caused significantly less plaque pH change in minimum pH ($P < 0{\cdot}001$), maximum pH drop ($P = 0{\cdot}003$), and area under curve ($P < 0{\cdot}001$) when compared with formulas containing lactose and other sugar but not with special formulas containing only non-milk extrinsic sugar. Similarly, special formulas containing non-milk extrinsic sugar produced significantly lower minimum pH and smaller area under curve than formulas containing lactose and other sugar did ($P = 0{\cdot}044$ and 0·009, respectively). No different results were found between high and low casein follow-on formulas. Lactic acid was produced more by rinsing with formulas containing lactose and other sugars than that produced by formulas containing only lactose.

Conclusions. This study suggests that milk formulas containing added other sugars tend to cause a decrease in plaque pH.

Introduction

Milk formulas can be categorized into three major groups comprising infant formula, follow-on formula, and whole milk formula [1]. The first group, infant formula, contains all the nutritional needs for infants during the first 4–6 months of life or until 12 months if used in conjunction with other infant foods. Infant formula can be classified by protein content into three subgroups as milk-based (cow's milk), soy-based, and protein hydrolysate formula [2]. Soy-based formula is used for children with cow's milk allergy or lactose intolerance. Protein hydrolysate formula, in which the protein is hydrolysed into fragments of proteins and amino acids, is suitable for infants with protein sensitivity including galactosemia. Both soy-based and protein hydrolysate formula contain non-milk extrinsic sugars such as sucrose and glucose syrup as carbohydrate resources. The second group, follow-on formula, is a modified cow's milk composition that covers the nutritional needs of infants during 6 months to 3 years of age. Follow-on formulas available in Thailand contain different amounts of casein: low-casein formula contains a whey:casein ratio of 60:40 as in infant

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formula, whereas high casein formula has a whey:casein ratio of 20:80, which is similar to whole milk formula. According to European Society for Pediatric Gastroenterology, Hepatology and Nutrition (ESPGAN) (1990), the manufacturers were allowed to add other sugars of no more than 20% of total carbohydrate content in follow-on formula. The last group is whole milk formula, which is basically cow's milk with addition of necessary vitamins and mineral contents without any restriction of sugars added. It is recommended for children after the first year of age.

Nursing caries or baby bottle tooth decay has been linked to prolonged and frequent daytime, naptime, and nighttime bottle feeding that contains sugary solutions [3,4]. Milk formulas have been implicated in the development of nursing caries, and controversy exists concerning the cariogenicity of milk.

Several *in vitro* studies have documented that milk is not cariogenic [5,6]. Animal experiments have shown that cow's milk is less cariogenic and may have cariostatic properties when ingested with the cariogenic substances [7–9]. In addition, a human observational study reported an inverse relationship between milk consumption and dental caries increment [10].

On the other hand, negative features of milk have been described. Birkhed *et al*. (1981) showed that acidity of human dental plaque increased after frequent use of either lactose or milk [11]. A human experimental study revealed that, although milk is slightly acidogenic in plaque, it produced less acid than lactose or sucrose alone [12].

A plausible explanation of the conflicting results concerning the cariogenicity of milk is that milk itself is a combination of nutrients. Not only the ingredients that possess caries-protective effect such as calcium, phosphorus [13,14], protein casein, and whey that are capable of providing a protective organic coating on the enamel surface and enhancing remineralization potential of calcium and phosphate in plaque [6,15–17], but also cariogenic contents such as lactose or other sugars added to improve the taste and provide energy resource existing in milk formulas in different ratios. These sugars are utilized by oral microorganisms resulting in production of organic acids that demineralize the teeth. Different types of sugar can produce different amounts of organic acid, among which, sucrose is the most cariogenic and lactose is the least [5,18–23]. Soy-based and protein hydrolysate formula are lactose free, but do contain other non-milk extrinsic sugars that are more cariogenic than lactose [24].

Erickson (1998) reported that most milk formulas except whole milk and some special formulas did have the ability to significantly lower plaque pH comparable to rinsing with water [25]. This study, however, considered minimum pH, maximum pH drop, pH at 1 h, and pH drop at 1 h, which reflects only the magnitude of pH change without time scale. The area of plaque pH curve might be more appropriate to determine plaque pH change as it also reflects the length of pH drop as well as the magnitude.

Apart from nutritional properties, cariogenicity is an important factor in determining appropriate milk formulas for children. The objectives of this study were to investigate the effect of different milk formulas on dental plaque pH change and to observe organic acids formed by different milk formulas.

Subjects and Methods

This randomized, cross-over study was carried out using 14 healthy dentists/dental assistants who were willing to participate in this study. Exclusion criteria included subjects on antibiotic therapy, with xerostomia, with lactose intolerance, allergy to milk or soy, or wearing orthodontic appliances. Their mean DMFS was $25{\cdot}1 \pm 13{\cdot}7$ and the mean DFS was $8{\cdot}6 \pm 7{\cdot}5$. Following approval of the research protocol by the Mahidol University Human Subjects Committee, informed consent was obtained before admission to the study. All subjects received thorough oral examination including bitewing radiographs for detection of proximal caries, and DMFS and DFS were recorded. Appropriate dental treatment was rendered for all subjects as needed.

Twenty-four hours prior to each experiment, participants received full-mouth prophylaxis with pumice and rubber cup. They were then asked to abstain from oral hygiene in the following 24 h to allow accumulation of plaque and to fast overnight prior to sampling procedure. The experiment was carried out in the early morning before the breakfast time to avoid the effect of food on plaque pH. The test solutions were 10% sucrose as positive control, deionized water as negative control, and nine milk formulas as shown in Table 1. Each milk solution was freshly prepared according to the manufacturer's instruction in the morning and was stored at 4 °C until the experiment time. It was arranged for

Table 1. Milk formulas used in this study.

			Percentage of carbohydrate and sugar contents							
Product	Manufacturer	Type	Lactose	Glucose	Sucrose	Gluco syrup	Malto dextrin	Honey	Oligo-fructose	Isomalt oligosac-charide
Bear 2	Nestle Food (Thailand) Co., Ltd.	Follow-on /High casein	20·52	–	–	–	–	–	–	–
Snow F Plus Beta	Snow Brand Siam	Follow-on /Low casein	2·6	–	–	–	–	–	–	–
Meiji FU	Meiji MCG Daily product	Follow-on /High casein	26·5	6·8	–	–	–	–	–	1·5
Gain Advance	Abbott Laboratories Ltd.	Follow-on /Low casein	1·98	–	1·61	–	–	–	–	–
Dumex 1 Plus	Dumex Co., Ltd.	Whole milk	5·01	–	–	–	–	–	–	–
Honey Bear	Nestle Food (Thailand) Co., Ltd.	Whole milk	2	–	10	–	7	5·6	2·3	
Dumex Dumilk	Dumex Co., Ltd.	Whole milk	–	–	18	–	–	–	–	–
Isomil	Abbott Laboratories Ltd.	Soy-based	–	–	10·4	42·8	–	–	–	–
Preges timil	Mead Johnson Co., Ltd.	Protein hydrolysate	–	–	–	31	3·8	–	–	–

the subject to rinse one of the solutions randomly each visit, with at least 1-week interval between each solution. Each subject attended the experiment on 11 occasions in total.

Pre-rinsing supragingival plaque from buccal surfaces of maxillary posterior teeth was sampled randomly by a spoon excavator for plaque pH baseline data. Then, the subjects rinsed the mouth thoroughly for 2 min with 10 mL of the solution and then spitted out. The plaque was sampled immediately and transferred into 0·5-mL microcentrifuge tube dispersed in 50 µL deionized water. The pH of the plaque samples was measured by a combination electrode (PHR-146 microcombination pH electrode, LAZAR (CA, USA)), which was calibrated earlier in each visit with buffer solution at pH 4·01 and 7·0 to provide the prerinse plaque control. Subsequently, the pH of pooled plaque samples was measured at 5, 10, 15, 20, 25, 30, and 60 min after rinsing. As soon as the pH of the plaque solution was recorded, the plaque sample was kept on ice in a microcentrifuge tube sealed with parafilm and stored at –20 °C for further analysis of the type and concentration of organic acids.

Regarding the analysis of organic acids, the plaque samples of all solutions at minimum pH from five subjects were centrifuged at 12,000 × ***g*** at 4 °C for 15 min. After centrifugation, the supernatant was drawn from each tube with a microsyringe, pooled into a single centrifuge tube, and recentrifuged at 12,000 × ***g*** at 4 °C for 10 min. Then the plaque sample was analysed to identify and quantify the organic acids using high-performance liquid chromatography (HPLC; Water™ 600, MA, USA) connected to UV detector. The temperature of the column (Aminex, Bio-Rad Laboratories, CA, USA) was maintained at 30 °C. The eluant was 0·004 M sulphuric acid and the flow rate was 0·6 mL/min. Mixtures of standard acids were used to prepare calibration curves from which concentrations of the separated plaque sample were determined.

The plaque pH of each subject was plotted against time to create pH curve for each solution and three parameters were extracted: the minimum pH, the maximum pH drop from baseline, and the area under curve between the time pH curve and baseline. For the statistical analyses, the nine milk formulas were grouped in three different ways, type of protein (milk-based, soy-based, protein hydrolysate), type of sugar (only lactose, lactose with other sugars, only non-milk extrinsic sugars), and casein ratio (high and low). The mean and standard deviation of all indexes after rinsing with different protein-based formulas and different sugary content formulas were compared using RMANOVA. Follow-on formulas containing high or low casein were compared by paired *t*-test. A significance level of $P = 0·05$ was used in all statistical tests.

Results

The mean plaque pH change after rinsing with the test and control solutions are presented in Fig. 1. Minimum pH was reached after 10–20 min in most milk formulas and then gradually returned towards the baseline within 60 min. However, 10% sucrose and milk formulas with added non-milk extrinsic sugars (Meiji FU™, Meiji MCF, Bangkok, Thailand; Dumex Dumilk™, Dumex Co. Ltd, Bangkok, Thailand; and Honey Bear™, Nestle, Bangkok, Thailand) produced a pH drop of longer duration that did not return to baseline pH within 60 min.

Considering different protein-based formulas, soy-based formula yielded the greatest dental plaque pH change. There was no difference with regard to minimum pH between groups, whereas maximum plaque pH drop of milk-based and soy-based formula were significantly higher than that produced by protein hydrolysate formula ($F(1,13) = 5{\cdot}942$, $P = 0{\cdot}03$ and $F(1,13) = 6{\cdot}759$, $P = 0{\cdot}022$, respectively). The area under curve produced by milk-based and soy-based formulas were significantly larger than that created by protein hydrolysate formula ($F(1,13) = 19{\cdot}507$, $P < 0{\cdot}001$ and $F(1,13) = 6{\cdot}375$, $P = 0{\cdot}025$, respectively) as shown in Table 2 and Fig. 2.

Formulas containing only lactose caused significantly less plaque pH change in all parameters, minimum pH ($F(1,13) = 27{\cdot}928$, $P < 0{\cdot}001$), maximum pH drop ($F(1,13) = 13{\cdot}348$, $P = 0{\cdot}003$), and area under curve ($F(1,13) = 22{\cdot}796$, $P < 0{\cdot}001$), than the ones containing lactose and other sugars. Formulas containing lactose and other sugars had significantly lower minimum pH (($F(1,13) = 4{\cdot}951$, $P = 0{\cdot}044$) and area under curve ($F(1,13) = 9{\cdot}260$, $P = 0{\cdot}009$)) when compared with special formulas containing non-milk extrinsic sugars. In contrast, formulas containing only lactose showed no significant difference in any parameter when compared with special formulas containing non-milk extrinsic sugars, as shown in Table 2 and Fig. 3. Follow-on formulas with high and low casein were not statistically significantly different in any criteria as presented in Table 2 and Fig. 4.

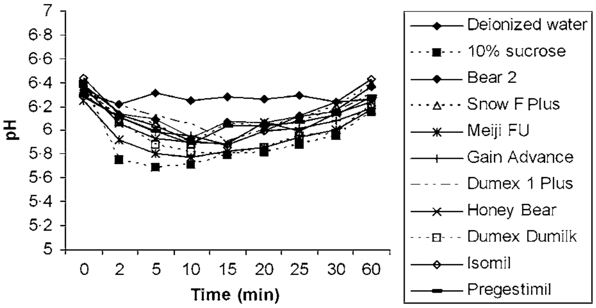

Fig. 1. Dental plaque pH change by deionized water, 10% sucrose, and nine brands of milk formulas.

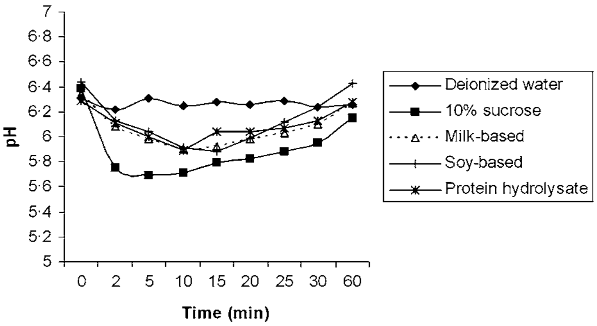

Fig. 2. Dental plaque pH change by different protein-based formulas.

Lactic acid was produced more by rinsing with formulas containing lactose and other sugar including 10% sucrose than formulas containing only lactose. The mean concentration of acids is shown in

Table 2. Mean (SD) of dental plaque pH change after rinsing with milk formulas classified by protein based, sugar and casein component.

	Minimum pH	Maximum pH drop	Area under curve
Protein based			
Milk-based	5·79 (0·29)	0·59 (0·21)*	2·54 (1·26)*
Soy-based	5·79 (0·35)	0·70 (0·28)†	3·03 (1·36)†
Protein hydrolysate	5·85 (0·28)	0·49 (0·14)*,†	1·81 (0·72)*,†
Sugar component			
Only lactose	5·91 (0·26)‡	0·51 (0·17)‡	2·00 (0·96)‡
Lactose and other sugars	5·71 (0·28)‡,§	0·63 (0·21)‡	2·93 (1·31)‡,§
Only non-milk extrinsic sugars	5·83 (0·32)§	0·58 (0·23)	2·34 (1·17)§
Casein component			
High casein	5·77 (0·26)	0·58 (0·19)	2·50 (1·13)
Low casein	5·83 (0·30)	0·57 (0·16)	2·18 (1·15)

*,†,‡,§The same symbol shows significant difference ($P < 0{\cdot}05$).

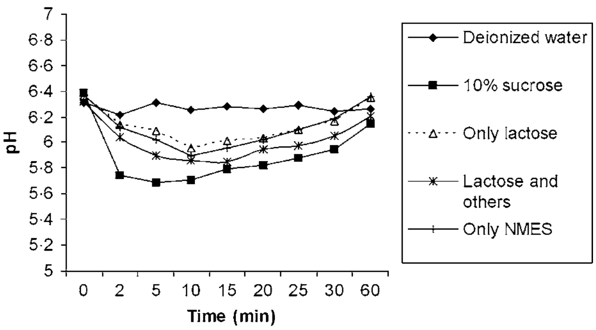

Fig. 3. Dental plaque pH change by formulas containing only lactose, those containing lactose and other sugars, and those containing other sugars.

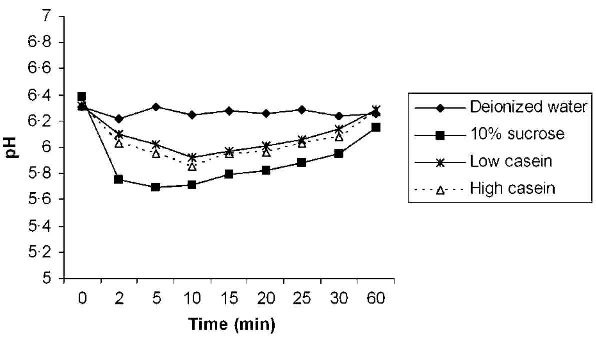

Fig. 4. Dental plaque pH change by follow-on formulas containing high and low casein.

Table 3. There was no correlation between lactic acid, pyruvic acid, and minimum pH.

Discussion

There are many methods to investigate the cariogenicity of diet. Plaque pH measurement methods has been used in many studies and widely accepted as it provides a valuable guide to the cariogenicity of food [26,27]. Plaque pH studies use four different methods: metal probes, glass probes, miniature glass electrodes built into a partial denture, and harvesting method. This study used the harvesting method, an appropriate method for ranking the cariogenicity of food by their acidogenicity [24]. It is an experimental method, however, and takes no account for habitual consumption or the presence of calcium and phosphate in saliva. Therefore, these findings should be interpreted with other forms of evidence.

It was found in this study that dental plaque pH decreased when subjects rinsed with milk formulas and did not reach minimum pH until after 10–20 min, whereas positive control (10% sucrose) reached the minimum pH within 5 min. This is probably because of the casein in milk that may act as a buffer against plaque pH depression in the initial period [23]. Buffer capacity of milk can counteract the acid produced by sugar.

The type of protein in milk seem to influence milk's protective effect based on the findings that soy-based formula lowered the plaque pH more than the others. Similar findings were found in the study by Sheikh and Erickson [28]. Nonetheless, Moynihan *et al.* [29] reported no significant difference in acidogenicity between soya infant formula and milk-based formula. The variation of results may be due to different sugar content in soy-based formula. Isomil™ (Abbott Laboratories Ltd, Bangkok, Thailand) used in this study contains 10·4% of sucrose with 42·8% of corn syrup, which is significantly higher than 7% of corn syrup in Infasoy™ (Cow & Gate, Nutricia Ltd) used in the study by Moynihan *et al.*

Protein hydrolysate formula caused the least dental plaque pH change, possibly because of some buffering effect from the hydrolysed casein. In addition, the taste of protein hydrolysate formula may have resulted in increasing saliva flow rates, which dilutes the rinse and minimizes plaque pH change

Table 3. Mean organic acid concentration (ppm) of plaque samples produced after rinsing with different milk formulas.

Samples	Pyruvic	Lactic	Formic	Acetic	Propionic	Butyric
Deionized water	2·50	73·71	0	56·54	13·08	46·21
10% sucrose	8·73	162·59	10·93	277·79	24·61	30·79
Bear 2™	6·09	141·19	0	95·06	21·28	58·61
Snow F Plus Beta™	4·42	94·15	0	52·80	18·36	18·12
Meiji FU™	13·40	106·59	4·22	52·47	28·05	55·19
Gain Advance™	10·70	124·68	0	82·97	31·60	64·81
Dumex 1 Plus™	9·72	106·53	0·58	74·55	26·94	15·89
Honey Bear™	2·20	99·79	1·22	138·37	24·18	38·55
Dumex Dumilk™	14·82	240·94	0	165·14	24·89	42·26
Isomil™	9·25	136·89	0	52·60	27·48	33·69
Pregestimil™	6·77	154·70	10·84	152·88	29·38	47·78

[23]. In contrast, Sheikh and Erickson [28] reported that both soy-based and protein hydrolysate formulas produced a drop in plaque pH to below the critical pH of 5·7. Nevertheless, their study was *in vitro* where pH change was derived from one pre-rinse and one post-rinse plaque sample, which may potentially mask the buffering effect from saliva.

According to our result, milk formulas with other sugar added reduced plaque pH significantly more than those containing only lactose. Special formulas that contain non-milk extrinsic sugars (mainly glucosyrup), however, had no significant effect on plaque pH when compared with those containing only lactose. These two findings are not surprising as other sugars added to milk used in this study are mainly sucrose, which is more acidogenic than glucose polymer used in special formulas. These can support the effect of sugar components as in previous studies [21–23].

Follow-on formulas available in Thailand contain different amounts of casein. Our study failed to show a significant difference in plaque pH change between high and low casein follow-on formulas. Although many studies support the theory of the buffer capacity of casein in milk [15–17], it seems that the buffer capacity was not able to overcome the acid produced from sugar.

This result showed positive correlation between lactic acid and pyruvic acid after rinsing with all solutions, similar to the study by Birkhed *et al*. [21], because these two acids are reversible products of carbohydrate metabolism [30]. There was no correlation between the amounts of acid production: lactic and pyruvic acid, and minimum pH. These two organic acids were produced more after rinsing with formulas with other sugars added and 10% sucrose than those containing only lactose as the sugar component.

Only 10% sucrose and formulas with other sugars added (Meiji FU™ and Dumex Dumilk™) depressed the minimum plaque pH to 5·7, the critical pH of enamel demineralization. The plaque pH reported here was apparently higher than those of the previous reports [21,22] because a different technique was used. In our study, the plaque was dispersed in deionized water before measurement and this method has been shown to heighten pH compared to those obtained by other direct plaque pH measurements [26,27,31,32].

Although the plaque pH was higher than in previous reports, the area under pH 5·7 was significantly larger in milk formulas with other non-milk extrinsic sugars added than milk formulas containing only lactose. This result supports the acidogenic effect of the sugar component in milk formulas [21–23].

Extrapolation of the results of the study on plaque pH change in children and adults must be made with caution [25,33,34]. This study was carried out in adult because of the limitation of subjects who volunteered to participate in this project; however, the acidogenicity of milk formulas in this study can also reflect the caries-related risk associated with different milk formulas in children. In addition, it will be applicable to educate parents and caretakers about child oral healthcare in the mode of milk consumption and oral hygiene practice especially for infants who use high caries risk milk formula.

What this paper adds

- Although milk has been accepted as a noncariogenic substance, milk formulas that add non-milk extrinsic sugars are more cariogenic.

Why this paper is important for paediatric dentists

- Paediatric dentists can use this evidence to encourage parents and children to consume milk without adding free sugars.

Conclusions

From this study, it can be concluded that:

1 Soy-based and milk-based formulas produced a larger plaque pH reduction than protein hydrolysate formula.

2 Formulas containing only lactose as the sugar component caused significantly less plaque pH reduction than formulas that added other non-milk extrinsic sugars.

3 Follow-on formulas with high and low casein component did not show any significant difference in plaque pH change.

4 Rinsing with formulas containing other non-milk extrinsic sugars tends to produce more lactic acid.

5 The main determinant of the acidogenicity of milk formulas is whether non-milk extrinsic sugars are present.

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