

Evaluation of acidogenic potential of infant milk formula

Zahra Al-Ahmari,* BDS, MSc and Joseph O. Adenubi,** BDS, MSc, MPH

أهداف الدراسة: - أولاً: تقييم الرقم الهيدروجيني في اللويحة الجرثومية المأخوذة من سطوح أسنان الأطفال قبل وبعد غسل الأسنان بحليب الأطفال لتحديد أقل رقم هيدروجيني لكل نوع من أنواع حليب الأطفال.

ثانياً: تحديد قابلية القدرة على الحماية ضد التسوس أو (القدرة على معادلة الحمض) لكل نوع من أنواع الحليب المستخدم.

المسواد والطريقة: تتراوح أعمار الأطفال المختارين للدراسة ما بين سنة إلى ثلاث سنوات من روضة الأطفال في جامعة الملك سعود بالرياض، حسب مميزات محددة وهي أن يكون الطفل سليماً صحياً وحالي من التسوس وليس لديه حساسية لمادة الحليب أو حليب الصويا. أرسلت أوراق حطية للموافقة أو عدمها مع هؤلاء الأطفال المختارين لأسانهم، وقد تم إختيار الأطفال الذين وافق آباؤهم، على الدراسة فقط، تم تحديد ستة أنواع من حليب الأطفال المستخدم بكثرة في المنطقة الوسطى "الرياض" المملكة العربية السعودية "بعد أخذ معلومات من بعض الشركات الدوائية الكبيرة في الرياض و وجد أن الأنواع الستة هي الأكثر استخداماً من قبل الأمهات في منطقة الرياض.

وكان العدد عشرون طفلاً. تعسل أسنان كل طفل بكل أنواع الحليب المختارة كل نوع يستخدم على حدة لمدة أسبوع. تعسل أسنان عشرة من الأطفال محللول السكرورز (محللول المقارنة الإيجابي) والعشرة الأخرين بالماء (محللول المقارنة السلبي)، يتم قياس درجة الرقم الهيدروجيني لمحلول اللويحة الجرثومية المأخوذة للاختبار بالطريقة

المخبرية وتسجل تفسيرات الرقم الهيدروجيني باستخدام جهاز القطب الكهربائي لقياس الرقم الهيدروجيني (portable digital pH meter)

النتائج: أدت جميع أنواع الحليب إلى انخفاض الرقم الهيدروجيني تحت الرقم الهيدروجيني للماء وباستخدام (Tukey test) في المقارنة الإحصائية المتعددة أثبتت أهمية ذلك الانخفاض في الرقم الهيدروجيني

بعض أنواع الحليب مثل: السيميلاك الأخضر، السيميلاك الأصفر، والأيزوميل، أدت إلى انخفاض الرقم الهيدروجيني عن الرقم الهيدروجيني للويحة الجرثومية قبل غسل الأسنان بالحليب باستثناء حليب S-26 لم يحقق انخفاض في الرقم الهيدروجيني بشكل إحصائي مهم. ويعتبر حليب الأطفال قد حقق انخفاض تقريبي P = 0.046، الاستنتاجات: أظهر الإختبار الإحصائي (Pearson correlation) بأنه لا يوجد علاقة إحصائية مهمة بين كمية بروتين الكازين والانخفاض في الرقم الهيدروجيني وبالتالي في القدرة على معادلة الحمض في أنواع الحليب. ولكن قدرة بروتين الكازين على الحماية ضد التسوس في مختلف أنواع الحليب المستخدمة تتعلق بوجوده وليس بكميته.

The objectives of this study were: (1) to assess the plaque pH of children before and after an oral rinse with milk formulas, especially in relation to the minimum pH obtained in response to each formula and (2) to determine the protective effect against caries or the buffering effect of each milk formula and to assess the effect of the presence and quantity of casein protein. Six infant milk formulas were identified as the most frequently used by mothers in Riyadh, Saudi Arabia. Children aged 1 to 3 years, whose parents granted informed consent were selected from the kindergarten school at King Saud University, Riyadh according to the inclusion criteria that they were normal, caries free and in good general and oral health. Twenty children rinsed with each type of infant formula at weekly intervals. Ten children each rinsed with the positive control solution (sucrose) and the negative control solution (water) at the start of the study. An *in vivo* / *in vitro* combination technique was used in plaque sampling and pH measurement. The plaque pH changes were recorded by using touch electrode connected to a portable digital pH meter. The oral rinsing with all infant formulas reduced the plaque pH significantly below the pH obtained after rinsing with water (Tukey multiple comparison test $P < 0.0001$). Rinsing with infant milk formulas reduced the plaque pH below the pre-rinse pH value for sucrose solution, Similac Advance green, Similac Advance yellow and Isomil at significant levels for all (*t* tests, $P < 0.0001$). There was no significant Pearson correlation between the quantity of casein and the drop in pH ($P = 0.54$) nor with its buffering effect ($P = 0.69$), but this protective effect of casein against caries in the different milk formulas was however associated with its presence.

Introduction

The dental profession continues to be concerned about the condition variously called nursing caries, baby bottle tooth decay, rampant caries in infants and toddlers or more recently early childhood caries (ECC).¹⁻³ This serious pattern of dental caries begins with the very young child's maxillary incisors where the nipple of baby bottle is placed. Initially, the maxillary incisors develop a band of dull white demineralization along the gum line

that goes undetected by the parents.⁴ In advanced cases, the crowns of the four maxillary incisors may be destroyed completely, leaving decayed brownish root stumps. Conversely, the four mandibular primary incisors remain unaffected.⁴

From studies in different countries, it has been estimated that 3 to 8 percent of children younger than age 4 years have this disease,^{1,5,6} although in some populations, it may affect as many as 10-13 percent of young children.⁷ In the Kingdom of Saudi Arabia, Al-Amoudi *et al.*⁸ and Wyne *et al.*⁹ reported the prevalence figures of 20% and 27.3% in preschool children from Jeddah and Riyadh

Received 15 May 2002; Revised 16 November 2002

Accepted 26 January 2003

*Specialist in Pediatric Dentistry

Public Security Training Center

Ministry of Interior

P.O. Box 59142, Riyadh 11525, KSA

** Professor, Department of Preventive Dental Sciences

College of Dentistry, King Saud University, Riyadh, KSA

Address reprint requests to:

Prof. Joseph O. Adenubi

Department of Preventive Dental Sciences

College of Dentistry, King Saud University

P.O. Box 60169, Riyadh 11545, KSA

E-mail: adenubi21@hotmail.com

respectively. Thus, prevalence rates vary greatly since risk factors do vary from population to population due to socio-cultural differences in different geographical locations.

It is widely accepted that the group of cariogenic microorganisms responsible for ECC is *Streptococcus mutans* group. Children with ECC reportedly have elevated oral levels of mutans streptococci¹⁰ which generally are acquired from their mothers.¹¹ *Streptococcus mutans* is the principal microorganism responsible for coronal caries in humans and averaged about 60% of the total cultivatable flora of dental plaque of ECC from white spot margins of these lesions, or from clinically sound areas of upper anterior teeth, and averaged about 27% in plaque from mostly clinically sound areas of posterior teeth.¹⁰ Such high concentration of acidogenic microorganisms combines with frequent carbohydrate intake to produce abundant acid that lowers plaque pH for extended periods and demineralizes the child's teeth.^{3,4}

Infant formulas in the nursing bottle have been implicated in the development of ECC.^{12,13} Bovine and human milk contain the carbohydrate lactose. Ripa⁴ and Moynihan *et al.*¹⁴ reported the nutrient content of different milk sources. The concentration of the constituents in infant milk formulas are similar to those of human milk, including the lactose content. Soy-based formulas and protein hydrolyzate formulas are free of lactose but contain an equivalent concentration of total carbohydrate, which, depending upon the brand of the infant formula, may be mostly sucrose⁴ and perhaps glucose polymers and corn syrup. Jenkins and Ferguson¹⁵ after studying the effects of bovine milk, concluded that milk did not promote caries and that probably milk might provide a protective effect against cariogenic foods in spite of the modest acid produced. On the contrary Birkhed *et al.*¹⁶ showed that, in humans, acid production in dental plaque increased after frequent ingestion of either lactose or milk. Other laboratory studies also have shown that, in animals, lactose did enhance oral implantation of bacteria, produce dental caries and demineralize tooth enamel.^{17,18} These conflicting views make it essential to evaluate the acidogenic potential and the buffering effect or later increase in pH of the readily available and most commonly used infant milk formulas in the Kingdom of Saudi Arabia.

Casein (bovine milk phosphoprotein) has been shown to reduce the caries activity of animals where the level of protein was increased in cariogenic diet.¹⁹ Later, studies²⁰ suggested that,

with casein protein, it should be possible to reduce the cariogenicity of confectionaries. Reynolds and Black²⁰ also demonstrated an inverse correlation between the casein level of certain confections and their cariogenicity in the rat with monitored *Streptococcus mutans* infection and feeding frequency. It should therefore also be of interest to assess the role of casein in the cariogenicity of infant milk formulas. The objectives of this study were: (1) to assess the plaque pH of children before and after an oral rinse with infant milk formulas, especially in relation to the minimum pH obtained in response to each formula and (2) to determine the protective effect against caries or the increase of pH as the buffering effect of each milk formula and to assess the effect of the presence and quantity of casein protein.

Materials and Methods

After obtaining information from the pharmacists in several large pharmaceutical companies in Riyadh, Saudi Arabia, it was established that the following six infant milk formulas were the most frequently used by mothers in Riyadh and are readily available in most pharmacies in the country: Similac Advance yellow, S-26, Similac Advance green, Isomil, Progress and Saudia Junior Milk. The composition of the 6 selected infant milk formulas is shown in Table 1 (Source: Manufacturers).

Twenty children (13 male and 7 female) aged 1-3 years, whose parents granted informed consent were selected from the Kindergarten at King Saud University (KSU) in Riyadh to participate in the study. The inclusion criteria were normal caries-free children in good general and oral health while the exclusion criteria were presence of xerostomia, soy allergy, lactose intolerance or general allergy to milk. The study was carried out in the nursery room of the school to facilitate regular attendance of the children in early morning sessions. Each child attended the nursery room 8 times, one for each of 6 milk formulas as well as one for the negative and one for the positive control solutions. Each parent was requested to abstain from oral hygiene for his or her child for 24 hours and avoid giving the child food and water for 2 hours prior to plaque sampling. Saudi Junior milk was a premixed, ready-to-feed solution. The other five infant formulas were in powder form. These were mixed fresh daily with sterile bottled water using sterile glass nursing bottle according to the manufacturer's direction.

A pilot study on six patients in the pediatric

Table 1. The names and composition of the six selected infant milk formulas according to the manufacturers.

Formulas	Brand Name	Carbohydrates	Protein	Fat	Calcium	Phosphate
A. Formula with High Iron	1. Similac Advance yellow *		10.9 g	28.8 g	410 mg	
	2. S-26**	55.2 g	2.25 g	5.4 g	86 mg	221 mg
B. Formula with Low Iron	3. Similac Advance green *	10.8 g	10.9 g	28.8 g	410 mg	221 mg
	4. Isomil*	55.2 g	16 g	36 g	700 mg	500 mg
D. Follow-up formula	5. Progress**	69 g	32 g	15.1 g	1000 mg	8000 mg
	6. Saudia Junior Milk***	64.1 g	3.6 g		145 mg	

*Abbot Laboratories Health Care Products North Chicago, USA

**Wyeth-Ayerst International Philadelphia, USA

***Saudi Dairy & Foodstuff Co. Ltd. Jeddah, KSA

clinics of the College of Dentistry at King Saud University showed that the plaque sample when dispersed in 0.05 ml of deionized water as described by Sheikh and Erickson¹² proved inadequate. The reported technique was therefore modified to have enough plaque solution to last the one-hour study. Accordingly, the plaque pH was determined by this modification of the technique earlier described by Sheikh and Erickson.¹² Supragingival plaque was sampled from labial surfaces of maxillary incisors. Alternating surfaces were sampled by using sterile, stainless steel explorer prior to rinsing, to provide the pre-rinse plaque as control. The labial surfaces of alternating maxillary incisors were sampled after bathing all the anterior teeth with 10 ml of each of the six infant milk formulas or control solutions by using disposable syringe around the mouth for 1-2 minutes and the child spat it out or swallowed it.

The pH value of the deionized water in the study was 6.89-7.98 and 0.15 ml of the deionized water was transferred to the dappen dish using a sterile micropipette and the plaque sample dispersed in it. For each child, two samples were collected. The first sample was the pre-rinse plaque as control, and the second sample was the post-rinse plaque. The pH measurement was done with a portable Fisherbrand Hydrus 100 pH meter, battery operated microprocessor based digital meters¹ which offered simultaneous display of pH, automatic calibration, automatic temperature compensation and manual buffer calibration option. The combination pH electrode with silver/silver chloride references was connected to pH meter for pH measurement as shown in Figure 1. The pH was monitored for the

¹ Fisher Scientific UK Ltd., Leicestershire, U.K.

pre-rinse plaque sample one time only, while the post-rinse plaque sample was monitored every 5 minutes for a period of one hour. For each type of milk formula, there were 20 children for plaque sampling and the same children were sampled for another type of milk formula on another day. This continued until the 6 infant formulas had been used in the study on different days at weekly intervals.

Calibration of the pH meter was carried out

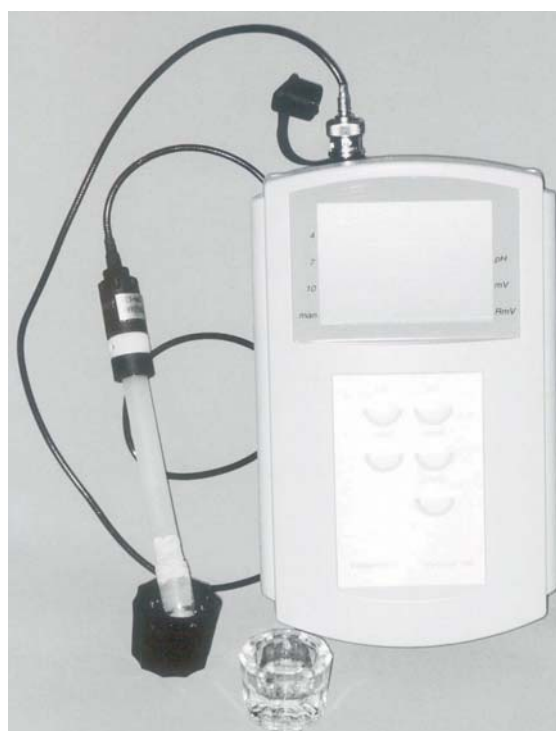


Fig. 1. Touch electrode in dappen dish containing plaque / deionized water connected to Fisherbrand Hydrus 100pH meter.

using standard solutions of pH 7.0 and 4.0, and the test of standards ran randomly between the plaque pH readings. The pH was recorded each time after the pH reading had stabilized usually between 10 and 15 seconds. The positive control was plaque sample collected after 10% sucrose rinse as a post-rinse plaque control and plaque sample collected before the sucrose rinse as pre-rinse plaque control for each of 10 children. The negative control was plaque collected after water rinse as post-rinse plaque control and the plaque sample collected before the water rinse as pre-rinse plaque control for each of the other 10 children. Due to poor attendance of the subjects in the clinic at KSU, it became necessary to shift the site of the research to the Kindergarten where attendance of the desired age group was guaranteed hence the study could not be carried out blind. The collection of the plaque sample and the determination of the plaque pH were carried out by the principal investigator (ZA).

For all the samples collected, the following three plaque pH measurements were recorded:

1. Minimum pH: the lowest pH recorded in the one-hour period of the study.
2. pH at 1 hour: the post rinse plaque pH at 60 minutes.
3. pH drop: The drop in pH was calculated as the difference between the initial pre-rinse plaque pH and the minimum plaque pH obtained in the study.

The presence or absence of casein protein (milk phosphoprotein) and the amount when present in the six milk formulas were obtained from the manufacturers' literature.* These were later related to the protective effect against caries or the buffering capacity of each milk formula, if any.

Reproducibility of the pH Readings

Each morning of the study, before starting the pH measurement of plaque solutions, the pH of deionized water was determined twice. This was to monitor the accuracy of the pH meter and the reliability of the pH readings. On all the eight occasions of the study, the two pH readings were identical.

Statistical Analysis

!! Abbot Laboratories Health Care Products, North Chicago, USA
WyethAyerst International Philadelphia, USA.
Saudia Dairy & Foodstuff Co. Ltd., Jeddah, KSA.

Data obtained were entered into a computer using the Statistical Package for Social Sciences (version 9.0). Tukey test, paired *t* tests (two-tail) non-parametric Wilcoxon Signed Rank test and Pearson correlation test were used as appropriate.

Results

Table 2 shows the means of pre-rinse and lowest post-rinse pH values of different selected milk formulas and the controls. The pre-rinse pH values ranged from 6.09 to 7.27. The lowest post-rinse plaque pH values during the one-hour period ranged from 5.19 of Similac Advance green to that of Progress at 7.21. In comparison to the negative control solution (water) 7.46 and the positive control solution (sucrose) 5.51, these findings indicated that all the infant milk formulas had the ability to reduce the pH value below the pre-rinse pH value.

Table 2 also shows the mean pH drop or the difference between the initial pre-rinse plaque pH values and the lowest post-rinse plaque pH values during the one-hour follow-up period as well as the mean post-rinse pH values recorded after one hour. The mean drop in pH values indicated the amount of acid production within the one-hour period. The highest acid production was associated with Isomil with a pH drop of 0.96. The drop in pH, which reflected the amount of acid production from all the milk formulas and their cariogenicity is statistically significant except in Progress and S-26 whose paired *t* tests showed non-significant two-tailed *P* values of 0.130 and 0.185, respectively. The pH changes in dental plaque solution after rinse with different milk formulas, water and sucrose solution with time is indicated in Stephan's curve (Fig. 2).

The formula with the least buffering effect was Similac Advance yellow with a drop in pH of 0.84, followed by Isomil (0.55), sucrose solution (0.55) and Similac Advance green (0.53). Progress (-0.32), Saudia Junior milk (-0.39), water as negative control solution (-0.76) and S-26 (-1.21) did not show drops in pH. Instead, there was a rise in pH. The *P* values (0.000 to 0.003) from paired *t* tests of pre-rinse and one hour post-rinse pH values indicate that each milk formula has a significant buffering effect but that the buffering effect was variable. Table 3 shows the *P* values for Tukey's multiple comparison of the acidogenic or cariogenic potential of the milk formulas and controls.

Table 4 shows the relationship between the

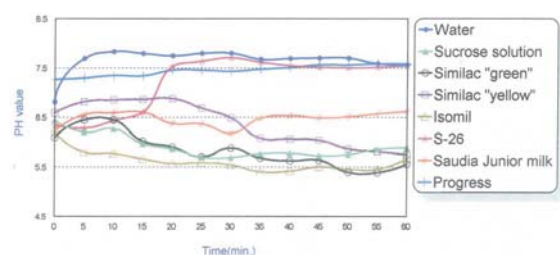
Table 2. Plaque pH values: Pre-rinse, 1 hour post rinse and lowest post rinse pH throughout study period

Milk formulas and controls (SD)	N	Mean pre-rinse pH (SD)	Mean pH after 1 hour (SD)	Mean of lowest post-rinse plaque pH (SD)	Mean drop in pH (SD)	P value for t test
1. Water	10	6.81(0.101)	7.56(0.223)	7.46(0.194)	-0.65(0.199)	0.000*
2. Sucrose	10	6.44(0.281)	5.89(0.268)	5.51(0.307)	0.93(0.384)	0.000*
3. Similac Advance green	20	6.09(0.273)	5.55(0.377)	5.19(0.239)	0.89(0.394)	0.000*
4. Similar Advance yellow	20	6.58(0.671)	5.74(0.434)	5.71(0.408)	0.87(0.548)	0.000*
5. Isomil	20	6.23(0.429)	5.67(0.216)	5.26(0.237)	0.96(0.429)	0.000*
6. S-26	20	6.34(0.241)	7.54(0.093)	6.22(0.403)	0.12(0.380)	0.185
7. Saudi Junior	20	6.23(0.165)	6.61(0.398)	6.01(0.427)	0.21(0.449)	NS
8. Progress	20	7.27(0.216)	7.58(0.125)	7.21(0.151)	0.049(0.137)	0.046*

SD Standard Deviation

NS not significant

*Significant

**Fig. 2.** Stephan's curve showing pH changes in dental plaque with time after rinse with different milk formulas, water and sucrose.

presence and quantity of casein protein to the ability to produce acid (pH drop value) and the acid clearance or buffering effect within one-hour period of different milk formulas. The highest casein content of 80% of total protein was found in Progress and the formula's mean pH drop was the lowest. Isomil had no casein protein and the mean pH drop was the highest at 0.962. The highest buffering effect, as later increase in pH, was found in S-26 with a casein protein content of 40% followed by Saudia Junior milk and Progress milk formulas, whose casein protein contents are 9.35% and 80%, respectively. The Pearson correlation of casein protein content of the infant formulas to the drop in pH values and to the buffering effects were 0.510 and -0.033 with *P values* of 0.546 and 0.696, respectively. This shows that there was no statistically significant correlation.

Discussion

Acid production is reflected by a fall in the pH value in the dental plaque. This is considered as a method to measure the potential cariogenicity of foods and solutions including milk formulas.^{21,22} The plaque sampling method used¹² in the study was preferred as an *in vivo* / *in vitro* combination because it reduced the ability of saliva to buffer the pH as the samples were being monitored. Thus, the technique involved a diminished effect of saliva which would be similar to that seen at night time infant feedings due to circadian rhythm. This form of plaque sampling is most suitable for child cooperation in the age group 1-3 years. The limitation of the study was the inability to achieve a blind study.

All the infant formulas except Progress demonstrated the ability to reduce the pH significantly below the pH obtained after rinsing with water. The difference between the pre-rinse plaque pH values and the post-rinse plaque pH values after one hour measured each formula's buffering effect to increase the pH back to the pre-rinse value. The mean pre-rinse plaque pH values for all the formulas ranged from 6.09 to 6.81 except that of Progress (7.27) which was surprisingly higher than all the others. This deviation could be due to the wide range of the pH value of deionized water (6.89-7.98) used in the study. In spite of this, the mean of all 12 readings with Progress milk formula showed the lowest post-rinse pH value as 7.21. In comparison with other milk formulas, it would appear that Progress had low acidogenic potential or low cariogenicity properties. However, further study of this milk formula is recommended to confirm its low acidogenic

Table 3. *P* values for Tukey multiple comparison test of the acidogenic potential for milk formulas and controls

Milk Formulas	Sucrose	Similac Advance green	Similac Advance yellow	Isomil	S-26	Saudia Junior Milk	Progress yellow
Water	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	.975
Sucrose	-	.038	.733	.392	.083	.891	<.0001
Similac advance green	.039	-	<.0001	.932	<.0001	<.0001	<.0001
Similac advance yellow	.733	<.0001	-	<.0001	.811	1.00	<.0001
Isomil	.392	.932	<.0001	-	<.0001	<.001	<.0001
S-26	.083	<.0001	.811	<.0001	-	.570	<.0001
Saudia Junior Milk	.891	<.0001	1.00	.001	.570	-	<.0001

Table 4. The relationship between the presence and quantity of casein protein to the drop in pH values and buffering effect

Milk formulas	Casein content of protein (%)	Mean drop in pH value	Mean of buffering effect
Water	-	-.653	-.758
Sucrose	-	.933	.552
Similac Advance green	52%	.899	.532
Similac Advance yellow	52%	.873	.841
Isomil	No casein	.962	.554
S-26	40%	.117	-1.207
Saudia Junior milk	9.35%	.214	-.387
Progress	80%	.0485	-.317

*Drop in pH value i.e. Difference between the pre-rinse pH value and the lowest pH value

**Buffering effect i.e. Difference between the pre-rinse pH value and the post-rinse pH value at 1 hour after the rinse with milk formula

potential. The multiple comparison test of Tukey showed that the reductions in plaque pH of the milk formulas below those obtained after rinsing with water were statistically highly significant ($P < 0.0001$) and confirm earlier studies.^{12,23} The post-rinse pH value for sucrose confirmed reports that it is a most cariogenic substance.¹³

Four of the milk formulas and sucrose solution also significantly lowered the post-rinse pH values below the pre-rinse pH values as showed by paired *t* tests. The two exceptions are Progress ($P = 0.185$) and S-26 ($P = 0.130$). Saudi Junior milk was

marginally significant at $P = 0.046$. The average of post-rinse plaque pH values of sucrose solution, Similac Advance green, Similac Advance yellow and Isomil climbed back towards their pre-rinse values but were still lower than the pre-rinse values. This reflected their acidogenicity or less anti-cariogenic properties, while the average of post-rinse plaque pH values of S-26, Progress and Saudia Junior milk were higher than pre-rinse plaque pH values and suggested pronounced anti-acidogenic properties by the increase in pH. The finding agreed with an earlier report.¹²

The cariogenic order of the milk formulas would justify an attempt to classify them into two main categories. Those with low cariogenic potentials will include Saudia Junior Milk, S-26 and Progress while the high cariogenic potentials will include Isomil, Similac Advance green and Similac Advance yellow as shown in the histogram (Fig. 3). The order of cariogenicity in this study confirms the findings of Bowen.²⁴

The high acidogenicity of the soy-formula Isomil may be attributed to the difference in carbohydrate source and the absence of casein protein which is readily used in drinking water, milk chocolate and confectionary to reduce caries activity.^{20,25} Several studies have shown that lactose-based milk formulas are less cariogenic than sucrose-based milk formulas.^{12,14} It is therefore desirable that manufacturers of infant milk formulas should produce more lactose-based milk formulas than the sucrose-based milk formulas to reduce the effect of these infant formulas on the incidence of early childhood caries (ECC).

Formulas with high iron had lower pH drop than

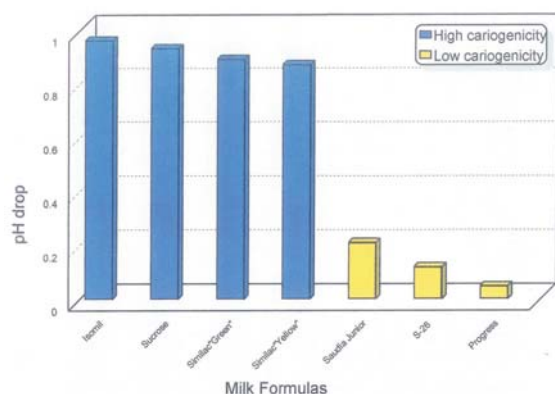


Fig. 3. Histogram of cariogenicity of the infant milk formulas as indicated by drop in pH.

formulas with low iron as previously reported¹³ and is consistent with observations that iron has cariostatic properties.²⁶ The least pH drop was associated with follow-up formula Saudia Junior Milk, high-iron milk formula S-26 and follow-up formula Progress which indicate their anti-cariogenic properties. These three are to be preferred in the feeding of infants. Parents should be advised to avoid the more cariogenic Isomil, Similac Advance green and Similac Advance yellow to reduce the impact of infant milk formulas on ECC. Isomil which contains sucrose should only be used in children who have lactose intolerance and sucrose should not be added to milk formulas during preparation.

After the initial drop in pH, the later increase in the pH by different milk formulas demonstrated significant differences which may be attributed to the differences in casein concentration as well as the carbohydrate source and content. The highest increase in pH as a buffering effect was associated with high-iron formula S-26, and follow-up formulas Saudia Junior milk and Progress which indicated the possibility that casein protein may play a role in the protection of enamel against demineralization.^{20,25} Supporting this probable role is the very low buffering effect of soy-formula Isomil which has no casein protein. The high-iron S-26 had the highest increase in pH or recovery of the lowered pH which is attributed to the presence of about 40% casein protein and the iron contents. Saudia Junior milk formula had 9.35% casein of total protein yet the buffering capacity was high while Similac Advance yellow with high iron and Similac Advance green with low iron had the same amount of casein protein (52%) but a low buffering capacity. This reflects the complex

effect of casein protein with other minerals in its buffering effect or protective effect against caries.²⁵ There was no significant Pearson correlation between the quantity of casein and the drop in pH. There have been several studies with different casein protein concentrations and all reported its protective action against caries.^{27,28} The relationship of the quantity of casein protein in milk formulas to caries reduction is not a linear relationship but the buffering capability of casein protein in the different milk formulas is related to its presence. The anti-caries role of casein is therefore a complex one and requires more detailed studies.

Conclusions

The findings in this study permit the following conclusions:

1. Oral rinse with all six infant milk formulas and sucrose significantly reduced the plaque pH below the pre-rinse pH value and also below the pH obtained after rinsing with water.
2. Each milk formula had a significant but variable buffering effect or tendency to increase the pH.
3. Saudia Junior milk, S-26 and Progress were less acidogenic and therefore appeared to have less cariogenic potentials than Isomil, Similac Advance green and Similac Advance yellow.
4. Isomil, the soy-based milk formula containing sucrose but no casein was the most acidogenic/cariogenic of the infant milk formulas.
5. The relationship between the quantity of casein protein in milk formulas and caries reduction was not a linear relationship but its buffering capability or protective effect against caries in the different milk formulas was related to its presence.

Acknowledgement

This research was funded by the College of Dentistry Research Center (CDRC) of King Saud University, Riyadh. We also express our appreciation to Dr. Nazeer Khan, Assistant Professor of Statistics at the College of Dentistry, King Saud University for his invaluable help with the statistical analysis and to Mrs. Elizabeth Posadas who graciously typed the manuscript.

References

1. Goose DH, Gittus E. Infant feeding methods and dental caries. *Public Health* 1968;82:72-76.
2. Centers for Disease Control and Prevention (CDCP), Conference. Atlanta, GA, September 1994.
3. Tinanoff N, O'Sullivan DM. Early childhood caries: Overview and recent findings. *Pediatr Dent* 1997;19:12-16.
4. Ripa LW. Nursing caries: A comprehensive review. *Pediatr Dent* 1988;10:268-282.
5. Winter GB, Rule DC, Mailer GP, James PMC, Gordon PH. The prevalence of dental caries in preschool children aged 1-4 years. *Br Dent J* 1971;130:271-277.
6. Holt RD, Joels D, Winter GB. Caries in preschool children. The Camden Study. *Br Dent J* 1982;153:107-109.
7. Richardson BD, Cleaton-Jones PE. Nursing bottle caries. *Pediatrics* 1977;60:748-749.
8. Al-Amoudi A, Salako NO, Linjawy A. Prevalence of nursing bottle syndrome among preschool children in Jeddah, Saudi Arabia. *Saudi Dent J* 1996;8:34-36.
9. Wyne A, Darwish S, Adenubi J, Battata S, Khan N. The prevalence and pattern of nursing caries in Saudi preschool children. *Int J Paediatr Dent* 2001;11:361-364.
10. Van Houte J, Gibbs G, Butera C. Oral flora of children with nursing bottle caries. *J Dent Res* 1982;61:382-385.
11. Brown JP, Junner C, Liew V. A study of *Streptococcus mutans* levels in both infants with bottle caries and their mothers. *Aust Dent J* 1985;30:96-98.
12. Sheikh C, Ericksson R. Evaluation of plaque pH changes following oral rinse with eight infant formulas. *Pediatr Dent* 1996;18:200-204.
13. Bowen WH, Pearson SK, Rosalen PL, Miguel JC, Shih AY. Assessing the cariogenic potential of some infant formulas, milk and sugar solutions. *J Am Dent Assoc* 1997;128:865-871.
14. Moynihan PJ, Wright WG, Walton AG. A comparison of the relative acidogenic potential of infant milk and soya infant formula: A plaque pH study. *Int J Paediatr Dent* 1996;6:177-181.
15. Jenkins GN, Fergusson DB. Milk and dental caries. *Br Dent J* 1966;120:472-477.
16. Birkhed D, Imfeld T, Edwardsson S. pH changes in human dental plaque from lactose and milk before and after adoption. *Caries Res* 1993;27:43-50.
17. Krasse B. The effect of the diet on the implantation of caries-inducing *Streptococci* in hamsters. *Arch Oral Biol* 1965;10:215-221.
18. Guggenheim B, König KG, Herzog E, Muhleman HR. The cariogenicity of different dietary carbohydrates tested on rats in relative gnotobiosis with a *streptococcus* producing extracellular polysaccharides. *Helv Odontol Acta* 1966;10:101-113.
19. Holloway PJ, Shaw JH, Sweeney EA. Effects of various sucrose: casein ratios in purified diets on the teeth and supporting structures of rats. *Arch Oral Biol* 1961;3:185-200.
20. Reynolds EC, Black CL. Reduction of chocolates cariogenicity by supplementation with sodium caseinate. *Caries Res* 1987;21:445-451.
21. Stephan RM. Changes in the hydrogen ion concentration on tooth surfaces and in carious lesions. *J Am Dent Assoc* 1940;27:218.
22. Newbrun E. Dietary carbohydrates: Their role in cariogenicity. *Med Clin North Am* 1979;63:1069-1086.
23. Erickson R, McClintock K, Green N, Lappleur J. Estimation of the caries related risk associated with infant formulas. *Pediatr Dent* 1998;20:395-403.
24. Bowen WH. Biological mechanisms of early childhood caries. *Community Dent Oral Epidemiol* 1998;26:28-31.
25. Reynolds EC. The prevention of sub-surface demineralization of bovine enamel and change in plaque composition by casein in an intra-oral model. *J Dent Res* 1987;66:1120-1127.
26. Miguel JC, Bowen WH, Pearson SK. Influence of iron alone or with fluoride on caries development in desalivated and intact rats. *Caries Res* 1997;31:244-248.
27. Dreizen S, Dreizen JOG, Stone RE. The effect of cow's milk on dental caries in the rats. *J Dent Res* 1961;40:1025-1028.
28. Reynolds EC, del Rio A. Effects of casein and whey-protein solutions on caries experience and feeding patterns of the rat. *Arch Oral Biol* 1984;29:927-933.