The Influence of Chewing Casein Gum Made from Buffalo Milk Toward the Number of Streptococcus Mutans Bacteria and Saliva Profile

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Abstract

To reviewing the influence of chewing casein gum from buffalo milk to the amount of bacterium Streptococcus mutans and saliva profile. The research type used is true experimental. Samples are buffalo milk, Streptococcus mutans, saliva pH, and salivary volume. The data were processed using the ANOVA test.The number of Streptococcus mutans bacteria decreased in the treatment group from 63.40 CFU on day 0 to 10.80 CFU on day 9. Salivary pH in the day 0 to 9 treatment group was in neutral pH. In the salivary volume in the treatment group increased from 3.30 mL on days 0 to 6.60 mL on day 9. Furthermore, the independent test obtained the value of p <0.005 which means there is a significant difference in casein chewing gum in reducing the amount of Streptococcus mutans bacteria compared with xylitol gum. Chewing casein gum from buffalo's milk is effective in lowering the amount of Streptococcus mutans.

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Introduction

Streptococcus mutans (S. mutans) are cariogenic and are a major cause of dental caries. One characteristic of this bacterium is having the ability to attach to all the location of its habitat in the oral cavity. The activity of S. mutans attachment to the host through its receptor, in this case, is that the saliva particle that has several receptors for S.mutans attachment, it is also said that the saliva particle is a mediator where oral bacteria is inserted.[1-3]

Saliva plays an important role in the defence process against caries attacks. The mechanisms of saliva protection function include: bacterial cleansing, buffer action, antibacterial action, and remineralization. Saliva also contains urea and other buffers that help dissolve acid in plaque. Acidity (pH) of saliva is strongly influenced by circadian rhythms, diet, and stimulation of salivary secretions. Diets that contain carbohydrates will cause a decrease in salivary pH that can accelerate the demineralization of tooth enamel. Ten minutes after eating carbohydrates a spike in acid production will occur through the process of glycolysis and saliva pH will decrease until it reaches the critical pH (5.5-5.2) and return to normal after30-60 minutes. Plaque control can be performed by mechanical plaque cleaning and possible use of anti-bacterial agents primarily to suppress S. mutans. Chewing gum is useful for stimulating salivary secretions, increasing plaque and salivary pH, so it should be used as an oral cleanser.[4-6]

Several studies have shown that casein also has an influence in the ecology of the oral cavity. There is a study in Finland showing that there is an increase in dental hygiene and a decrease in the number of S. mutans in school children who consume processed products in the form of casein. Even some have a positive effect in reducing the number of S. mutans in the saliva of the human oral cavity.[7-9]

Materials and methods

The type of research that will be conducted is true experimental research and pre- and post-test research design. A total of 20 research subjects who met the study criteria of

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age 14-16 years, found no caries in the teeth, did not consume any other gum, did not eat 60 minutes before the study, were not undergoing drug therapy, were healthy and were willing to be a research sample by providing informed consent. This study has obtained the approval of the ethics commission with letter of Recommendation of Ethical Agreement No. 605 / H4.8.4531 / PP36-KOMETIK / 2017.

The casein chewing gum of buffalo milk is made from buffalo milk extract will be chewed bythe subjects as the treatment and xylitol chewing gum will be chewed by the research subjects as a control. Streptococcus mutans were calculated in CFU units, saliva pH was measured using pH meters, and salivary volume measured the amount of saliva produced by the stimulation of chewing gum.

The casein gum was produced in the pharmacy laboratory of the Faculty of Pharmacy Unhas. The production of casein gum requires 58 grams of casein extracted from buffalo milk, 75 grams of gum base, 150 grams of mannitol, 6 grams of glycerin, and 6 grams of sorbitol to produce 100 gum seeds. The gum base was melted in a water bath at 60 °C before being combinedwith casein, mannitol, glycerin, and sorbitol and stirred until evenly distributed.The mixture was separated into 3 gram aliquots to form the individual gum seeds.

Scaling was done on the subject first followed by sampling using the swab technique and spitting method. Sampling with the swab technique was used to calculate the amount of Streptococcus mutans bacteria while the spitting method was used to calculate salivary pH and salivary volume of the sample. This sampling is considered to be the initial state of the oral cavity of the subject and will be referred to as Day 0 sampling. The subjects were divided into 2 groups and instructed to chew xylitol gum (as control group) orcasein gum (as treatment group) for 9 days. The subjects were instructed to chew the gum three times daily,in the morning between 08.00-09.00; at noon between 12:00-13:00; and in the afternoon between 17:00-18:00. Sampling was performed again on the 3rd, 6th, and 9th days with the same techniques and methods as the 0th-day sampling. At the end of this procedure the subjects are asked to rinse using mineral water to clean the oral cavity.

All the research results were collected and recorded followed by processing and data analysis using SPSS version 22.0.

Results

As shown in (Table 1), the difference in the number of Streptococcus mutans bacteria in the control group and treatment group on the 0th, 3rd, 6th and 9th days is significant because the p-value<0.05. In the control group, the average number of bacteria on day 0 was 82.00 and continued to decrease the number of bacteria to 38.00 on the 3rd day, 32.86 on the 6th day, and increased to 35.60 on the 9th day. In the treatment group, the average number of bacteria on day 0 was 63.40 and continued to decrease the number of bacteria to 33.00 on the 3rd day, 17.00 on the 6th day, and 10.80 on the 9th day.

As shown in (Table 2), the difference of salivary pH average between control group and treatment group on day 0, 3rd, 6th, and 9th day is not significant because thep-value<0.05. In the control group, the mean salivary pH on day 0 was 7.15 and continued todecrease on days 3 and 6 before increasing again on the 9th day. The same was true in the treatment group, the mean pH of saliva treatment group on day 0 was 7.16 that continued to decrease on days 3 and 6 before increasing again on the 9th day. A comparison between the control group and the average treatment group salivary pH did not show significant differences however, the salivary pH in the control group and treatment group was still in a normal state of 5.6 to 7.0.

As shown in (Table 3), the difference of average salivary volume between the control and treatment groups on days 0, 3, 6, and 9 is not significant because p <0.05.

Groups	Days	Streptococcus mutans (CFU)	Normality test	Comparison test
		Mean ± SD	p-value*	p-value**
Xylitol	0	82.00 ± 11.77	0.51	0.00
	3	38.00 ± 6.63	0.27	
	6	32.86 ± 4.60	0.99	
	9	35.60 ± 4.16	0.98	
Casein	0	63.40 ± 10.53	0.27	0.00
	3	33.00 ± 5.57	0.84	
	6	17.00 ± 2.55	0.21	
	9	10.80 ± 2.77	0.66	

Table 1. Comparison of the amount of Streptococcus mutans in the control group and the treatment group.

In the control group, the average salivary volume on day 0 was 3.30 mL that continued to

increase to 4.20 mL on the 3rd day, 5.60 mL on the 6th day, and 6.70 mL on the 9th day. In the treatment group, there was also an increase of daily salivary volume of 3.26 mL on day 0 to 4.20 mL on day 3, 5.60 mL on day 6, and 6.60 mL on day 9. When compared between the control group and the treatment group, There was no significant difference in salivary pH increase because saliva volume on the 9th day of the control group was 6.70 mL and the treatment group was 6.60 mL.

Groups	Days	Streptococcus mutans (CFU)	Normality test	Comparison test
		Mean ± SD	p-value*	p-value**
Xylitol	0	7.15 ± 0.81	0.36	0.00
	3	6.96 ± 0.11	0.90	
	6	6.89 ± 0.05	0.51	
	9	6.96 ± 0.04	0.64	
Casein	0	7.16 ± 0.08	0.52	0.00
	3	6.90 ± 0.09	0.87	
	6	6.85 ± 0.06	0.55	
	9	6.92 ± 0.04	0.34	

Table 2. Comparison of Saliva pH in the control group and treatment group.

Groups	Days	Saliva volume (mL)	Normality test	Comparison test
		Mean ± SD	p-value*	p-value**
Xylitol	0	3.30 ± 0.36	0.25	0.00
	3	4.20 ± 0.84	0.31	
	6	5.20 ± 0.15	0.69	
	9	6.70 ± 0.15	0.69	
Casein	0	3.26 ± 0.49	0.34	0.00
	3	4.20 ± 0.57	0.81	
	6	5.60 ± 0.84	0.78	
	9	6.60 ± 0.82	0.31	

Table 3. Comparison of salivary volume in the control group and treatment group.

Discussion

The results of this study showed that there was a decrease in the amount of Streptococcus mutans bacteria, increased salivary volume, and increased salivary pH seen on the 3rd, 6th and 9th days. Microorganisms (Streptococcus mutans) and hosts (salivary pH and salivary volume) are some of the contributing factors of caries. According to research, Streptococcus mutans play a role in the initiation of dental caries. In fact, Streptococcus mutans was first isolated by Clark in 1924 from a carious human tooth.[2,10-12]

The flow rate of salivary secretions varies with the individual andis conditional according to the function of time, ie, salivary secretion reaches at least when not stimulated and reaches maximum at the time of stimulation. Saliva is also not produced in large quantities regularly, only at certain times alone increased salivary secretion. The degree of saliva acidity is normally under 6.5-7.0 with an average pH of 6.7. Some of the factors that cause changes are salivary pH among others are average saliva flow rate, oral microorganisms, and salivary buffer capacity.[13,14] The number of Streptococcus mutans bacteria in the control group and the treatment group showed the benefits of chewing buffalo milk casein gum because it was able to decrease the amount of Streptococcus mutans bacteria on the 3rd, 6th and 9th day. In addition, the figure also shows that casein gum is more effective than xylitol gum in reducing the amount of Streptococcus mutans. The number of bacteria on the 9th day in the samples chewing casein gum was less than that of the control group chewing xylitol gum. This is consistent with other studies suggesting that casein not only has the ability to bind and stabilize calcium and phosphate, but it can also protect tooth enamel from plaque. Casein can even improve the surface quality of enamel to be more resistant to organic acids as a result of fermentation of carbohydrate substrate caused by bacteria. The calcium and phosphate ions contained in the casein, if routinely administered, can replace minerals from dissolved enamel creating the potential for remineralization.[15,16]

There is the influence of chewing buffalo milk casein gum on saliva pH because it can decrease and increase salivary pH on day 3, 6 and 9. On the 3rd day, there was a decrease of saliva pH from 7.16 to 6.90. On the 6th day, there was another decrease in pH to 6.85 and finally, on day 9, there was an increase in pH to 6.92. The mean values of salivary pH of the treatment group remained at normal salivary pH values of 6.5-7.0. The same thing was observed in the control group chewing xylitol gum. There was a decrease and the increase of salivary pH on the 3rd, 6th and 9th day. On the 3rd day, the saliva pH decrease from 7.15 to 6.95 and again decreased on the 6th day to 6.89. Finally, there was an increase to 6.92 on the 9th day. Saliva pH values in the control group remained at normal conditions of 6.5-7.0. There are several factors that could contribute to the changes in saliva pH including average saliva flow rate, oral

cavity microorganisms, and salivary buffer capacity. The optimum saliva acidity (pH) level for bacterial growth is 6.5 to 7.5 and if the pH of the mouth is low (between 4.5-5.5), acidogenic germs such as Streptococcus mutans will begin to thrive. There is also research that suggests that increased saliva pH (basic) will result in the formation of tartar.

The effect of chewing the buffalo milk casein gum on salivary volume was able to increase salivary volume on the 3rd, 6th and 9th day. On day 0, the volume of saliva 3.26 mL that increased to 4.20 mLon day 3, 5.60 mL on day 6, and 6.60 mL on day 9. The same is seen in samples chewing xylitol gum. The salivary volume increased on day 3, 6 and 9. On day 0, the volume of saliva was 3.30 mL that increased to 4.20 mL on day 3, 5.20 mL on day 6, and 6.70 mL on day 9.

Based on this research, the flow rate of salivary secretion varies in individuals and is conditional in accordance with the function of time, ie saliva secretion reaches a minimum when not stimulated and reaches the maximum at the time of stimulation. Saliva is also not produced in large quantities regularly, only at certain times alone increased salivary secretion. The average saliva flow rate is 20 mL/hr at rest, 150 mL/hr at meal time, and 20-50 mL/hr during sleep. Other studies suggest that saliva expenditure in adults ranges from 0.3-0.4 mL/min but when stimulated, the amount of normal saliva expenditure increases to 1-2 mL/min.[11,15,17]

Conclusions

Based on the research that has been done then it can be concluded that buffalo milk casein gum is effective in reducing the amount of Streptococcus mutans bacteria. It can be seen that there was a decrease in the number of bacteria from 63.40 CFU on day0 to 10.80 CFU on day 9. Casein gum has no significant influence on salivary pH compared to xylitol gum, so it can be concluded that casein gum and xylitol gum have the same ability to maintain salivary pH. Casein gum has no significant influence on increasing salivary volumecompared to xylitol gum, so it can be concluded that casein gum and xylitol gum have the same ability to increase salivary volume.

Declaration of Interest

The authors report no conflict of interest.

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