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ORIGINAL ARTICLE

The Beneficial Effect of Yoghurt Containing *Lactobacillus Rhamnosus* on Caries Prevention in Children With Diabetes Mellitus Type 1

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Abstract

Background / Aim: Children with type 1 diabetes mellitus are thought to have an increased risk of caries. This study aimed to examine the short-term effect (sixty days long) of commercially available yoghurt consumption containing the *Lactobacillus rhamnosus* probiotic culture (LGG yoghurt) on the oral *Streptococcus mutans* count and saliva buffer capacity in children with type 1 diabetes mellitus.

Methods: Children were divided into two groups: the experimental group and the placebo group. Both groups consisted of 50 (N = 50) children with juvenile diabetes, aged 10-15 years, with controlled glucose levels and irregular oral hygiene. At the first examination, every child was evaluated for the caries risk. A sample of unstimulated saliva before yoghurt consumption and after washout of the teeth was inspected for *S mutans* count. The samples were tested for Saliva buffer capacity (Saliva-Check Buffer Testing Mat GC America). The same procedure was repeated after 14 days, 30 days and 60 days after the treatment with probiotic yoghurt.

Results: The results showed decreased number of *S mutans* colonies at the 60-day control examination in the probiotic group. The study also proved a significant increase in saliva buffer capacity in both groups after 60 days.

Conclusion: It could be concluded that daily consumption of LGG yoghurt can improve caries prevention in children with diabetes mellitus type I.

Key words: Probiotics; Diabetes mellitus type 1; *Streptococcus mutans*; Saliva buffer capacity.

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Introduction

Diabetes mellitus is one of the most common endocrinological diseases of modern times and as a chronic, non-infectious disease, has its manifestations in the oral cavity. Children with type 1 diabetes mellitus are thought to have an increased risk of caries, among others.[1, 2] According to recent studies, the role of probiotics in these patients has previously proven to be

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effective, especially in the oral cavity tract and they can be administrated in the form of probiotic milk, yoghurt, kefir, chewing gums, toothpaste that affect the reduction of cariogenic microorganisms and accumulation of dental plaque.[3, 4] Prevalence of gingivitis and caries have been demonstrated in patients with poor glycaemic control.[5] Competence with pathogenic microorganisms for the dental surface, aggregation with oral bacteria, interaction with the oral epithelium and consequent modulation of the oral biofilm together with increased IgA levels and thus the immune response, as well as inhibition of anti-inflammatory cytokine production, are characteristics of probiotic strains based on *in vitro* studies.[6] Children with uncontrolled diabetes are considered at high caries risk, lower saliva buffer capacity and lower pH.[7] Milk and yoghurt products have beneficial effects on saliva and inhibition of cariogenic bacteria due to the present casein, sodium, potassium, calcium and phosphorus.[8] The literature proved that yoghurt can increase saliva secretion,[9] and that was the main reason for the hypothesis about the use of LGG yoghurt in this study, where the buffer capacity increased in both groups. Continuous use of probiotic strain seems to be most promising due to its anti-cariogenic effects and a high buffer capacity.[10]

In previous research, it was found that the manifestations of diabetes mellitus in the oral cavity, such as oral candidiasis, halitosis and dental caries have been reduced by using probiotic strains.[5] Daily administration of probiotics is well needed to improve oral health and reduce caries development.[11, 12]

In this study, a randomised clinical trial aimed to determine the efficacy of LGG yoghurt on *Streptococcus mutans* (*S mutans)* levels and modify saliva buffer capacity over two months in children with type 1 diabetes mellitus diagnosed one year before, by comparing two groups, experimental and placebo.

Methods

The study included 50 children with type 1 diabetes mellitus diagnosed one year before, age 10-15, who received insulin as regular therapy and with no other comorbidities. All participants were without oral diseases and antibiotics use over the last two months.

The parents of all patients were informed about the purpose of the study and signed informed consent. The study followed the Declaration of Helsinki and has been approved by the Ethics Committee of the Public Health Institute of Dentistry of Banja Luka (protocol number 01-461-2/18), as well as by the Public Health Institute for Public Health of the Republic of Srpska. At the first examination, each subject was assigned a caries risk according to the Klein-Palmer (Decayed / Missed / Filled – DMF score) epidemiological index. Subjects caries risk was evaluated based on DMF score (low < 1, medium 0-3, high > 3), oral health behaviour and periodical checking. The samples of saliva were taken during the clinical examination, after tooth brushing at home and before starting using yoghurt, by collecting 1 mL in sterile graduated plastic containers. The samples were immediately transferred to a microbiology service for further processing. Each patient consumed 200 mL of yoghurt daily. Experimental group consumed "LGG Dukat, Croatia" and the placebo group (25 children) also consumed 200 mL of "standard Dukat, Croatia youghurt" for 60 days. Samples of saliva were treated with a Vortex mixer before seeding. The 0.1 mL of saliva sample was seeded onto prepared *Mitis Salivarius* agar (manufacturer's recommendation) with a smear (COPAN Spreaders, Italy).

Saliva samples were placed in an incubator (Memmert, 5 % CO_2) at 36 °C for a period of 36 to 48 h. Counting of *S mutans* (CFU/mL) colonies was performed by visual method. The saliva was sampled again after 14 days, one month and given to the microbiology laboratory for re-analysis and results readout after two months. Saliva buffer capacity was determined using Saliva-Check Buffer Testing Mat GC America tests before yoghurt consumption and after the washout period following the manufacturer's instructions.

Frequencies, percentages, the sample mean value with standard deviation were used to describe the parameters of importance depending on their nature. The Chi-square test was used to test the differences between the two categorical variables. ANOVA Repeated Measures was used to test the differences on variables measured in three-time intervals. The influence of the experimental factor on the change of the values of the parameters of importance (first examination,

30 days and 60 days after the intervention) as well as the influence of gender were tested with the SPANOVA test (Split-Plot ANOVA). The probability level was set at $p < 0.05$. Statistical processing and analysis were performed in the Statistical Package for the Social Sciences (SPSS) v 24 for Windows. The tabular and graphical presentation was done in Excel.

Results

ANOVA Repeated Measurements was applied to determine whether the number of colonies was statistically significantly different when comparing three-time intervals of measurement. Within the placebo group, there was no statistically significant difference in the number of *S mutans* colonies during the three-time intervals ($F = 1.683$, $p = 0.208$). By testing the differences within the experimental group, it was determined that a statistically significant difference exists ($F = 27.336$, $p < 0.001$). By a subsequent comparison of all three-time intervals with one another, it was determined that the average number of *S mutans* colonies at the first examination and after 30 days was not statistically significantly different ($p = 0.627$). However, when comparing the first examination (18.00 ± 10.22) and the examination after 60 days (15.60 ± 9.73), there was a statistically significant difference ($p < 0.001$). By testing the difference between the second (18.08 ± 10.29) and the third examination (15.60 ± 9.73), a statistically significant difference ($p < 0.001$) was obtained. In other words, observing only the experimental group, the average number of *S mutans* colonies was not lower after 30 days, but it was after 60 days, noting that between 30 and 60 days there was a statistically significant decrease in the number of these colonies.

It was also examined whether the buffer capacity of saliva differed in three measured time intervals, separately within the placebo and experimental groups. Within the placebo group, the mean value of the buffer capacity of saliva did not differ if one observes the difference between the first examination (5.00 ± 2.54) and the examination after 30 days (5.16 ± 2.32), ($p = 0.212$). The increase in the average value of the buffer capacity of saliva occurred after 60 days (8.12 ± 1.81), ($p < 0.001$).

Fifty respondents participated in the study, 50 % of whom were in the experimental group, while the other half was a placebo group. Groups were equated according to gender ($p = 0.744$) and to the risk of caries ($p > 0.668$) (Table 1).

Table 1: General data on respondents

	Placebo group (N = 25)	Experimental group (N = 25)	p	All respondents (N = 50)
Gender, N (%)				
male	15 (60 %)	14 (56 %)	0.744[a]	29 (58 %)
female	10 (40 %)	11 (44 %)		21 (42 %)
Caries risk, N (%)				
low	6 (24 %)	7 (28 %)		13 (26 %)
medium	9 (36 %)	11 (44 %)	0.668[a]	20 (40 %)
high	10 (40 %)	7 (28 %)		17 (34 %)

[a] Chi-square test; p = statistical significance.

Table 2: Number of Streptococcus mutans colonies during three-time intervals

Group	Time	M	SD	F	p	Partial Eta²	LSD post hoc test
Placebo group	Time 1: First examination	17.28	9.15	1.683	0.208	0.128	Time 1 : Time 2 (p = 0.298)
	Time 2: 30 days	17.52	9.18				Time 1 : Time 3 (p = 0.103)
	Time 3: 60 days	17.12	9.10				Time 2 : Time 3 (p = 0.134)
Experimental group	Time 1: First examination	18.00	10.22	27.336	**0.000**	0.704	Time 1 : Time 2 (p = 0.627)
	Time 2: 30 days	18.08	10.29				Time 1 : Time 3 (p = 0.000)
	Time 3: 60 days	15.60	9.73				Time 2 : Time 3 (p = 0.000)

ANOVA Repeated Measurement was applied; M = mean, SD = standard deviation, F = Repeated Measure ANOVA, p = statistical significance, bold: statistically significant.

Table 3: Buffer capacity of saliva during three-time intervals

Group	Time	M	SD	F	p	Partial Eta²	LSD post hoc test
Placebo group	Time 1: First examination	5.00	9.15	125.68	**0.000**	0.916	Time 1 : Time 2 (p = 0.212)
	Time 2: 30 days	5.16	9.18				Time 1 : Time 3 (p = 0.000)
	Time 3: 60 days	8.12	9.10				Time 2 : Time 3 (p = 0.000)
Experimental group	Time 1: First examination	5.68	10.22	107.05	**0.000**	0.903	Time 1 : Time 2 (p = 0.029)
	Time 2: 30 days	6.00	10.29				Time 1 : Time 3 (p = 0.000)
	Time 3: 60 days	8.80	9.73				Time 2 : Time 3 (p = 0.000)

ANOVA Repeated Measurement was applied; M = mean, SD = standard deviation, F = Repeated Measure ANOVA, p = statistical significance, bold: statistically significant.

When the differences within the experimental group were tested, the statistically significant difference between any measurements could be confirmed. Thus, at the first examination, the buffer capacity of saliva was the lowest (5.68 ± 2.05), at the measurement after 30 days it was statistically significantly higher (6.00 ± 2.19), while it had the highest value after 60 days (8.80 ± 1.70).

Table 4: Influence of group and gender on the number of Streptococcus mutans colonies during three measurements

Measurement	Group	Gender	M	SD
First examination	Placebo	Male	17.87	10.12
		Female	16.40	7.92
		Total	17.28	9.15
	Experimental	Male	19.14	8.93
		Female	16.55	11.97
		Total	18.00	10.23
	Total	Male	18.48	10.23
		Female	16.48	9.41
		Total	17.64	9.99
30 days	Placebo	Male	18.20	10.06
		Female	16.50	8.11
		Total	17.52	9.19
	Experimental	Male	19.29	8.84
		Female	16.55	12.17
		Total	18.08	10.30
	Total	Male	18.72	9.34
		Female	16.52	10.18
		Total	17.80	9.66
60 days	Placebo	Male	17.67	10.15
		Female	16.30	7.73
		Total	17.12	9.11
	Experimental	Male	16.64	8.18
		Female	14.27	11.71
		Total	15.60	9.74
	Total	Male	17.17	9.10
		Female	15.24	9.83
		Total	16.36	9.36

Group: F = 18.77, p = 0.000, Partial Eta² = 0.455.
Gender: F = 0.352, p = 0.705, Partial Eta² = 0.015.
Group x Gender: F = 0.024, p = 0.976, Partial Eta² = 0.001.
SPANOVA was applied (Split-Plot ANOVA); M = mean, SD = standard deviation.

Table 5: Influence of group and gender on saliva buffer capacity during three measurements

Measurement	Group	Gender	M	SD
First examination	Placebo	Male	5.80	2.88
		Female	3.80	1.32
		Total	5.00	2.55
	Experimental	Male	6.57	2.06
		Female	4.55	1.44
		Total	5.68	2.06
	Total	Male	6.17	2.51
		Female	4.19	1.40
		Total	5.34	2.32
30 days	Placebo	Male	6.00	2.59
		Female	3.90	0.99
		Total	5.16	2.32
	Experimental	Male	7.07	2.02
		Female	4.64	1.63
		Total	6.00	2.20
	Total	Male	6.52	2.35
		Female	4.29	1.38
		Total	5.58	2.28
60 days	Placebo	Male	8.67	2.02
		Female	7.30	1.06
		Total	8.12	1.81
	Experimental	Male	9.36	1.78
		Female	8.09	1.38
		Total	8.80	1.71
	Total	Male	9.00	1.91
		Female	7.71	1.27
		Total	8.46	1.78

Group: F = 0.417, p = 0.662, Partial Eta² = 0.018.
Gender: F = 6.876, p = 0.002, Partial Eta² = 0.234.
Group x Gender: F = 0.576, p = 0.566, Partial Eta² = 0.025.
SPANOVA was applied (Split-Plot ANOVA); M = mean, SD = standard deviation, F = Repeated Measure ANOVA, p = statistical significance

After examining the differences in the average number of *S mutans* colonies in three-time intervals in each group separately (placebo and experimental), the idea was also to examine whether the group affects the change in results over time. In addition to the influence of the group the separate influence of participants gender was examined and their interaction (Group x Gender). The effect was examined by the Combined Analysis of Variance (SSPANOVA). The results imply that the group statistically significantly contributed to the decrease in the number of *S mutans* ($F = 18.77$, $p < 0.001$). Gender did not show a statistically significant influence ($F = 0.352$, $p < 0.705$) and the interaction between gender and the group was not statistically significant either ($F = 0.024$, $p = 0.976$). Thus, the influence of the experimental factor was statistically significant and it did not depend on gender.

After examining the differences in saliva buffer capacity at three-time intervals in each group separately (placebo and experimental), the idea was also to examine whether the group affects the change in results over time. In addition to the influence of the group, the separate influence of gender was examined, as well as their interaction (Group x Gender). The effect was examined by the Combined Analysis of Variance (SSPANOVA). The results showed that the group did not statistically

significantly contribute to the increase in saliva buffer capacity (F = 0.417, p = 0.662). Gender showed a statistically significant effect (F = 6.876, p = 0.002). The interaction between gender and the group was not statistically significant (F = 0.576, p = 0.566). Thus, the influence of the experimental factor was not a statistically significant factor that was contributing to the change in the buffer capacity of saliva. On the other hand- gender was found to be a statistically significant factor for buffer capacity change. Namely, in men at the first examination, the average buffer capacity of saliva was 6.17 ± 2.50. After 30 days it was 6.51 ± 2.35 and after 60 days 9.00 ± 1.90. The buffer capacity of saliva measured at the first examination in women was: 4.19 ± 1.40. After 30 days it was 4.28 ± 1.38 and after 60 days 7.71 ± 1.27.

Disscusion

Probiotics demonstrated efficiency to reduce *Mutans streptococci* colonies in plaque in the short term studies.[13] This study determined whether the daily intake of probiotic yoghurt drink decrease the CFU/mL of *S mutans* and its influence on the saliva buffer capacity of diabetic children. Results showed a reduction of *S mutans* after two months of probiotic administration in the experimental group and a significant increase of salivary buffer capacity in both groups. Additionally, some previous studies also demonstrated the benefits of milk and yoghurt products for anti-cariogenic effects, increase salivary buffer capacity.[14]

Children with diabetes have shown higher acidogenic of saliva and predisposition for caries development.[15] Dysfunctional salivary flow at the oral cavity of diabetic children is an appropriate environment for the establishment of *S mutans*, especially among the children with uncontrolled diabetes.[16] It is proven that oral administration of probiotics is needed daily and for a long period, because the level of *S mutans* can increase after a short period of use.[17] This study did not follow the pH parameters, because an acidogenic oral environment in diabetic children has been demonstrated previously.[18] The level of *S mutans* decreased during two months of LGG yoghurt intake among the experimental group of children. For this study, two brands of yoghurt were used: *Dukat standard* for the placebo group and probiotic yoghurt, *Dukat LGG* containing 5 x 10^7 CFU/g of *Lactobacillus rhamnosus*. A high level of a probiotic strain is previously reported in the literature and it is between 10^7 and 10^9 in some supplements.[19] Otherwise, results about pH and buffer capacity require more investigations and one of the hypothesis is that yoghurt increase salivary secretion.

Yoghurt consumption was not documented with any harmful side effects and that is the main reason why this food was chosen, enriched by the *Lactobacillus rhamnosus,* to be an experimental factor in this study. Some short-term studies are indicating that *Lactobacillus species* in the form of probiotics can help reduce *S mutans* count.[20] It is also known that *Lactobacillus sp.* can adhere to hydroxyapatite, with variable strength of adhesion to the dental surface.[21] *Lactobacillus* probiotic strain is also related to the activity of arginine deiminase.[22] In this study, after the experimental time of probiotics consumption, the number of *S mutans* slowly increased, indicating the importance of daily administration of probiotics. Additionally, it is noteworthy that the difference between the two groups was not significant in the first and second measurements, but in a longer period of two months, the number of cariogenic bacteria decreased. Long-term probiotic use benefits are reported in the literature[23] and are additionally underlined in this study.

Foods rich in casein phosphopeptides (CPPs) have positive effects on remineralisation of dental enamel and buffering effect on plaque, interfering with *S mutans* on a dental surface.[24] So far, yoghurt and milk products have limited effectiveness in their natural source because they would require large consumption of dairy products. Additionally, it cannot be claimed that in this study CPPs was directly related to an increase of salivary buffer in both groups like in some previous studies.[25] The literature has also shown that salivary buffers can stabilise pH in plaque which can be very useful for the enamel demineralisation.[26]

Dry mouth and reduced salivary flow rates can in diabetic children lead to oral complications. In this study, yoghurt was chosen as an experimental factor due to its influence on saliva secretion induction,[10] which was very useful for this study group. Some studies also suggest that hyposalivation and duration of disease are related to a reduction in the buffer capacity in diabetic children.[1]

This study demonstrated that there was a significant increase of salivary buffer between the first and the last examination. Similar results were found in earlier studies.[27] Additionally, the results demonstrated the influence of participants gender

on saliva buffer capacity. At first examination, in men, it was 6.17 and after 60 days it was 9, while in women it was 4.19 at first examination and 7.71 after 60 days. The participants' gender influence was something to be further addressed in studies about the diabetic syndrome in the children population.

It seems that *Lactobacillus rhamnosus* does not ferment sucrose.[28] Sucrose has a cariogenic effect by itself and promotes demineralisation.[29] Sucrose is important as a substrate for extracellular (EPS) and intracellular polysaccharides (IPS) formation in the oral cavity and reduces the concentration of Ca, phosphorus (P) and F in dental plaque.[30] The question of caries prevention in the oral cavity of diabetic children with controlled parameters of disease by synergistic administration of probiotic strain and anti-cariogenic food is yet to be investigated. More research in understanding bacterial metabolism is needed to develop therapeutic strategies to define acid accumulation and tooth demineralisation in diabetic children. The improvement of general health in diabetic children using probiotic strain is yet to be demonstrated.

The small sample size determined by a restricted geographical area is one of the studies limitations. All subjects confirmed that they followed study protocol, but this cannot be claimed for all children ages. Also, this study showed effects for children affected by diabetes, but for the adult population, there should be further examination.

Conclusion

Oral administration of probiotic yoghurt may have a large impact on balance in oral microbiota. This study confirmed the effectiveness of short-term use of probiotic strain with metabolic patients and could be a promising preventive factor to caries development.

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Conflict of interest

None.

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