

Original Article**Salivary *Streptococcus mutans* and *Lactobacilli* levels following probiotic cheese consumption in adults: A double blind randomized clinical trial\***Shiva Mortazavi<sup>1</sup>, Najme Akhlaghi<sup>2</sup>**Abstract**

**BACKGROUND:** The beneficial effects of *Lactobacillus* species have been reported but the role of these species including *Lactobacillus casei* (*L. casei*) on oral health is not well documented. The purpose of this study was to evaluate the effects of conventional or probiotic cheese containing *L. casei* on salivary *Streptococcus mutans* (*SM*) and *Lactobacilli* levels.

**METHODS:** In this double-blind controlled trial (IRCT201009144745N1), 60 adults were randomly allocated in 2 parallel blocks. *SM* and *Lactobacilli* count assessment were performed three times. Subjects consumed either cheese containing *L. casei* ( $1 \times 10^6$  Cfu /g) (probiotic block, n=29) or cheese without any probiotic (control block, n=31) twice daily for two weeks. Bacterial levels changes were compared using Wilcoxon and Mann-Whitney Tests. Logistic regression compared changes in number of subjects with lowest and highest *SM* or *Lactobacilli* levels.

**RESULTS:** Statistically significant ( $p = 0.001$ ) reduction of salivary *SM* was found in probiotic group. *SM* levels reduction was not significant between placebo and trial groups ( $p = 0.46$ , 62% in probiotic vs. 32% in placebo group). *Lactobacilli* count changes during trial were not statistically significant inter and intra blocks ( $p = 0.12$ ). Probiotic intervention was significantly effective in high levels ( $> 10^5$  cfu/ml) of *SM* (Odds Ratio 11.6, 95% CI 1.56–86.17,  $p = 0.017$ ).

**CONCLUSIONS:** Probiotic cheese containing *L. casei* was not effective in salivary *SM* levels reduction comparing to conventional cheese. Adding *L. casei* to cheese could be useful in decreasing *SM* counts in adults 18-37 years old with highest level of *SM*.

**KEYWORDS:** Cheese, *Lactobacilli*, Probiotic, *Streptococcus mutans*.

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Probiotics are living microorganisms that are able to improve host healthy intestinal microbial balance when prescribed in adequate doses.<sup>1</sup> Most common so far used probiotic strains are *lactobacilli* and *bifidobacterium*.<sup>2</sup> In addition to gastrointestinal benefits, various effects of different strains of both *lactobacilli* on oral health have been reported such as caries and periodontal disease prevention (Table 1).<sup>3-9</sup> To possess oral health positive effects a probiotic should have the capacity to adhere to dental surfaces, integrate into oral

biofilm bacterial complex and prevent pathogenic microorganisms' proliferation.<sup>10</sup> Antibacterial substances production<sup>11</sup> and competition with pathogenic microorganisms for adhesion sites and/or substrates<sup>12</sup> are suggested roles of probiotics in reducing oral pathogenic germs. However, the exact mechanisms of action of probiotics are unknown.<sup>13</sup> *Lactobacilli* showed better adherence to "saliva-coated hydroxyapatite blocks" than *bifidobacteria* in an in-vitro study.<sup>14</sup> The highly aciduric property of *Lactobacilli* gives it a binary potential caries both

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**Table 1.** Studies' characteristics, utilized probiotics and oral outcomes of probiotic randomized clinical trials

Researcher	Study characteristics			Species	Oral outcome
	Number/age of subjects	Vehicle	trial duration		
Nase et al. <sup>3</sup>	594/1–6 years	Milk	7 months	<i>L. rhamnosus GG</i>	Decreased salivary Streptococcus mutans , reduced caries
Ahola et al. <sup>4</sup>	74/18–35 years	Cheese	3 weeks	<i>L. rhamnosus</i>	Decreased salivary yeast and Streptococcus mutans
Montalto et al. <sup>5</sup>	35/ 24–33 years	Liquid capsules	45 days	<i>Lactobacillus spp.</i>	Increased salivary Lactobacilli, Streptococcus mutans unchanged
Nikawa et al. <sup>6</sup>	40/20 years	Yoghurt	2 weeks	<i>L. reuteri</i>	Decrease salivary Streptococcus mutans
Caglar et al. <sup>7</sup>	120/21–24 years	Water/straw Lozenges	3 weeks	<i>L. reuteri</i>	Decreased salivary Streptococcus mutans
Stecksen-Blicks et al. <sup>8</sup>	248/1-5years	Fluoridated milk	21months	<i>L. rhamnosus LB21</i>	Decreased salivary Streptococcus mutans
Chuang et al. <sup>9</sup>	78/20-26years	Tablet	2weeks	<i>L. paracasei GMNL-33</i>	Decreased salivary Streptococcus mutans in 2 weeks follow up

progression and inhibition. The beneficial effects of *Lactobacillus* species have been reported<sup>15,11</sup> but the role of these *Lactobacillus* species including *L. casei* on oral health is not well documented. *L. casei* was claimed to be present in more than 1% of dental microflora and to reduce the number of attached *Streptococcus mutans* of tooth surface in rats.<sup>16</sup> However, no study has been conducted to examine the effects of *L. casei* containing cheese in controlling the microbial counts of *Streptococcus mutans* and lactobacilli. The effect of *L. casei* on *Streptococcus mutans* adhesion level was confirmed by two in-vitro studies too.<sup>14,17</sup> Different strains of *Lactobacilli* including *L. casei* decreased *Streptococcus mutans* adhesion potential to tooth surface by altering pellicle protein structure.<sup>14,17</sup> *L. acidophilus* and *L. casei* showed high adhesion and retention rates on enamel surface even comparable with early colonizing microorganisms such as *Streptococcus Sobrinus*, *Streptococcus Sanguis* or *Actinomyce*. *L. casei* de-bonding needed less shear force than *L. acidophilus*.<sup>18</sup> Various probiotic products are available in market such as dairy stuff, tablets, lozenges, straw, juice and chewing gum.<sup>19</sup> Dairy products could be advantageous vehicles for

probiotics due to their acid buffering capacity.<sup>20</sup> In a randomized double blind control trial caries risk reduction has been reported with administration of *Lactobacillus rhamnosus GG*, given in milk to 594 preschool children over a seven month period.<sup>3</sup> Among dairy products, cheese is known as a cariostatic food due to its ability in stimulating salivary flow, increasing rate of sugar clearance, plaque calcium concentration and pH.<sup>20-22</sup> Feeding cheese to rats reduced SM and dental caries.<sup>22</sup> These observations suggest that cheese consumption reduces enamel demineralization and promotes remineralization.<sup>20-22</sup> *Streptococcus mutans* level following three weeks consumption of probiotic cheese containing *Lactobacillus rhamnosus GG* and *Lactobacillus rhamnosus LC 70*.<sup>4</sup> The oral administration of probiotics, both in capsules and in liquid forms for 45 days, significantly increased salivary counts of *Lactobacilli*, while the *Streptococcus mutans* populations did not change.<sup>5</sup> Three weeks ingestion of water/straw or lozenge containing *L. reuteri* statistically decreased salivary *Streptococcus mutans* M.<sup>7</sup> Following administration *L. paracasei* GMNL-33 in the form of tablet for 2 weeks, the counts of *Streptococcus mutans* in saliva did not altered

although 2 weeks after completion this count significantly decreased.<sup>9</sup> Due to approved role of probiotic products on gastrointestinal health, several types of probiotics have been introduced and are being used in many parts of the world.<sup>2</sup> It would be advisable that before introduction of any type of probiotic added product its effects on oral cariogenic micro flora be evaluated through purpose designed studies. In this way the outcomes of a randomized controlled clinical trial on *L. casei* added to cheese would be useful. This paper examined the effects of short term consumption (two weeks) of cheese containing a probiotic bacterium, *Lactobacillus casei* LAFTI-L26, on the levels of salivary *Streptococcus mutans* & *Lactobacilli* in adults.

## Methods

This study was approved by Isfahan University of Medical Science Ethics Committee (no. 39178). Written informed consent form was signed by volunteers' intervention. Sixty subjects aged 18-37 years (mean age 28) participated in the experiment. Sample size calculation per group at the 0.05 significance level with 80% power ( $\alpha=0.05$ ,  $\beta=0.20$ ) resulted in 29. The sample size was estimated to show a 40% reduction of *Streptococcus mutans* count in intervention group and 10% in the control and 35% differences between two groups.<sup>6</sup> By a public invitation letter that was posted on the bulletin board of university's faculties and clinics, volunteers were invited to participate in the study. Examinations and samplings were performed at Pediatric Dentistry Department, Dental School of Isfahan University of Medical Sciences from May to September 2010. Volunteers with a history of systemic antibiotic or topical fluoride treatments within 4 weeks prior to first saliva sampling day were not included in the study. Pregnant women, smokers and regular Xylitol chewing gum users (because xylitol inhibits *SM* growth and might be a confounder factor)<sup>23</sup> and probiotics consumers were not eligible. Healthy subjects without compromised oral health, untreated active carious lesions and signs of gingivitis or peri-

odontal disease were included.

In this double-blind controlled trial study (registered at Iranian Registry of Clinical Trials code: IRCT201009144745N1), bacterial counts were performed three times; before randomization, before intervention and after it (two weeks). Subjects were randomized into two intervention and control blocks (one stratum). The other stratum was subjects' *Streptococcus mutans* count. For each subject both *Streptococcus mutans* and lactobacillus count was performed three times. The first *Streptococcus mutans* count was utilized for stratifying subjects into 5 scores from 0 cfu/ml to  $\geq 10^5$  cfu/ml as randomization unit to have equal numbers of subjects in each block per score (randomization ratio 1:1). Subjects in each score received codes 1 or 2 according to odd or even random figures respectively. Then by balloting those who received code 1 allocated in control block and code 2 in probiotic block. The procedure was carried out by one who was not aware about the study. Each participant as well as both probiotic and conventional cheese plastic containers were given codes. Both cheese types were weighted and given to subjects into sealed up coded containers with similar shape. Neither the practitioner who enrolled participants nor subjects were aware about codes. The staffs who were involved in intervention, microbiologic analyses, and data entry and analyses were not aware about participants and containers' codes too. The investigation comprised 2 consecutive parts of 1 and 2 week periods. The second *Streptococcus mutans* and *lactobacillus* count (immediately before intervention) was performed following a one week run-in period. During this period, subjects were asked not to consume any type of cheese in any way. Within a 2-week intervention, subjects were instructed to intake 50 g white cheese containing either probiotic *L. casei* LAFTI-L26 ( $1 \times 10^6$  CfU /g) (DSM Rosell-Lallemand, Eveleigh, Australia) or white cheese without probiotic strain twice daily with breakfast and dinner meals (they were prohibited from consumption extra cheese other than they were given). Both cheese types were produced by

same dairy plant. At first, it was designed that subjects consume cheese samples at university catering centers with similar breakfast ingredients; however, due to needing daily transportation to university, the design was changed providing cheese samples every other day at their home or work and taking a written report form about complete cheese consumption. They were supervised by phone too. The third saliva sample counts were conducted at the day after the fortnight intervention. Throughout all three weeks of experiment, subjects were under close supervision to brush their teeth with similar brand of fluoride tooth paste containing 0.321% sodium fluoride (Crest Procter & Gamble, Ohio, United States of America) only twice daily and one hour after breakfast and dinner. Subjects were asked not to use probiotic or Xylitol containing products, systemic antibiotic and topical fluoride during this period. Subjects with any change in health status or medication use were excluded from the study. The study was carried out from May to September 2010.

#### **Saliva samples & Microbial evaluation**

Saliva sampling from each subject was carried out three times: at the beginning of the study for randomization purposes (first) and before and after intervention (second and third respectively). To evaluate microbial changes in present study, saliva samples were collected instead of plaque samples due to less intense microbial count fluctuations in saliva comparing plaque.<sup>24</sup> To overcome saliva flow rate and composition alterations during different hours of day in one person, all three saliva samples were collected between 7:30 and 08:30 a.m. for all subjects. To avoid inter individual variation effects during saliva stimulation process, the unstimulated saliva samples were collected.<sup>24</sup> Saliva samples were collected using a sterile cotton stick that was soaked at sublingual area for 5 minutes. Then the cotton stick was transferred to a 2 ml sterile tube which was sealed immediately. Microbiological analyses were commenced within 45 min after sample collection. Saliva sampling steps for all participants

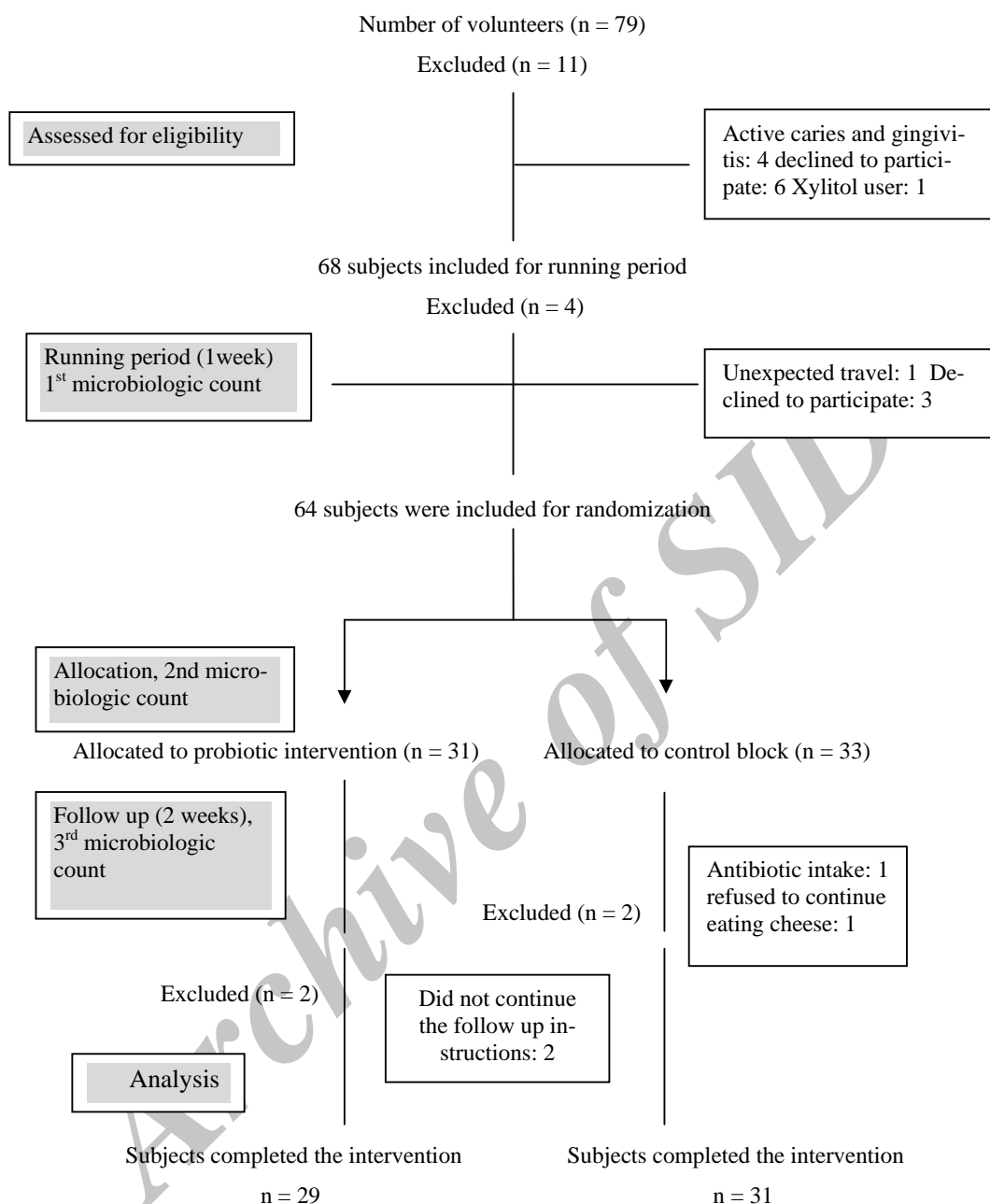
were carried out by one practitioner at Pediatric Dentistry Department, Isfahan University of Medical Sciences. For microbiological analysis, 20µl of saliva sample were spread on mitis salivarius agar (Difco Detroit, Mich, United States of America)<sup>25</sup> supplemented with 0.2 units/ml bacitracin and sucrose (15 percent w/v) for *Streptococcus mutans* count. In addition, 20µl of saliva samples were spread on Rogosa agar (Unipath, Basingstoke, UK) for the count of total *Lactobacilli*. Both groups of plates were incubated anaerobically (85% N<sub>2</sub>, 5% CO<sub>2</sub> and 10% H<sub>2</sub>) into chambers at 37°C for 3 days. The colony forming units (CFU) were identified by morphology, size and color and were counted using a stereomicroscope (Vision Engineering, Surrey, UK). *Streptococcus mutans* and *Lactobacilli* concentration in saliva was expressed as log 10 CFU/ml.

#### **Statistical methods**

Comparisons of bacterial scores within groups from baseline to follow up were performed with Wilcoxon Signed Ranks test. Mann-Whitney Test was used to compare changes in the bacterial levels during the intervention between groups. In each block subjects were divided into 2 groups according to their first *Streptococcus mutans* or *Lactobacilli* counts ( $\geq 10^5$  or  $< 10^5$ ). These binary groups considered as dependent variables. Other variables (DMFT, age, gender, probiotic/conventional cheese consumption and educational status) were considered as independent variables. Backward logistic regression with a stepwise selection procedure was utilized to investigate the influence of factors to the outcome of salivary bacterial levels. Intra block statistical analyses were repeated while subjects with zero level of *Lactobacilli* were excluded. The analyses were processed by SPSS software (version 11-5 16 Chicago, IL, USA). The level of confidence interval was considered at 95%.

#### **Results**

In general, 60 subjects (17 males, 43 females) completed the trial (Figure 1). Demographic characteristics of participants were as follow:



**Figure 1.** Flowchart of the study design

mean age  $28.3 \pm 4.1$  years (19 female, 12 male) and  $28.4 \pm 4.4$  years (24 female, 5 male) in control and probiotic block, respectively. Educational levels of subjects participated in probiotic block were: 2 subjects with less than eight years at school, 17 subjects with high school diploma, 2 subjects with two years college and 8 subjects with some years at university. The

conventional cheese block educational levels were 2 subjects less than five years at school, 1 less than eight years school, 15 high school diplomas, 4 subjects two years college and 9 subjects some years at university. Mean DMFT of subjects in conventional cheese block was  $8.16 \pm 4.07$  and in probiotic cheese block was  $8.2 \pm 3.88$ .

***Streptococcus mutans***

At baseline, 70% of all subjects exhibited  $\geq 105$  CFU of salivary *Streptococcus mutans*. There was no significant difference between frequency of subjects allocated in each *Streptococcus mutans* score between two probiotic/conventional cheese blocks ( $p = 0.99$ ). During the 2 weeks intervention, 18 subjects (62%) exhibited decreased levels of the *Streptococcus mutans* count, 8 subjects (27.6%) had unchanged levels and 3 subjects (10.4%) displayed increased levels in the probiotic block. In conventional cheese group the *Streptococcus mutans* count decreased in 10 subjects (32.2%), remained unchanged in 16 subjects (51.6%) and increased in 5 subjects (16.2%). According to Wilcoxon Signed Ranks test a statistically significant ( $p = 0.001$ ) reduction of salivary *Streptococcus mutans* was registered after 2 weeks consumption of probiotic cheese. A certain decline of *Streptococcus mutans* counts was also evident after ingestion of conventional cheese, but the difference compared to baseline was not statistically significant ( $p = 0.157$ ). Significant reduction of salivary *Streptococcus mutans* was not registered following 2-week intervention between the study groups according to Mann-Whitney test ( $p = 0.46$ ). During the intervention, the *Streptococcus mutans* count decreased in 46.6% and remained unchanged in 40% and increased in 13.4% of all the subjects regardless of intervention group. Distri-

bution of salivary *Streptococcus mutans* at before and after 2-week consumption of probiotic ( $n = 29$ ) and control cheese ( $n = 31$ ) are given in table 2 (second and third counts).

***Lactobacilli***

At first, 83% of all subjects exhibited detectable levels of salivary *Lactobacilli*. Changes in the salivary *Lactobacilli* counts at baseline and after 2-week probiotic ( $n = 29$ ) and control cheese ( $n = 31$ ) intake are given in table 3. The first *Lactobacilli* levels were approximately similar in the control group and probiotic group, and the difference was not statistically significant ( $p = 0.64$ ). After the 2-week ingestion of probiotic cheese, decreased *Lactobacilli* levels were found in 6 subjects (20.7%), while 17 subjects (58.6%) had unchanged levels and 6 subjects (20.7%) displayed increased levels. After eating the control cheese, 4 (12.9%) subjects displayed decrease, 21 (67.7%) had unchanged levels, while 6 (19.4%) were registered with an increased levels. According to Mann-Whitney test, the changes in the *Lactobacilli* counts during the intervention were not statistically significant between the study groups ( $p = 0.12$ ). During the trial, the *Lactobacilli* count decreased in 15% and increased in 20% of all subjects regardless of intervention group. No statistically significant changes appeared between the second and third levels in both groups ( $p > 0.05$ ).

**Table 2.** Numbers and percentages of subjects with different salivary *Streptococcus mutans* scores before and after 2-weeks consumption of probiotic or control cheese

	Streptococcus mutans scores (cfu/ml)				
	1 n (%)	2 n (%)	3 n (%)	4 n (%)	5 n (%)
Probiotic cheese (n = 29)					
Before intervention	4 (13.8)	2 (6.9)	4 (13.8)	11 (37.9)	8 (27.6)
After intervention	5 (17.2)	6 (20.7)	10 (34.5)	6 (20.7)	2 (6.9)
Control cheese (n = 31)					
Before intervention	3 (9.7)	3 (9.7)	6 (19.3)	10 (32.3)	9 (29)
After intervention	2 (6.5)	5 (16.1)	9 (29)	8 (25.8)	7 (22.6)

1: 0 (cfu/ml), 2: 100-<10000 (cfu/ml), 3: 10000-<100000 (cfu/ml), 4: 100000-<1000000 (cfu/ml), 5:  $\geq 1000000$  (cfu/ml)

**Table 3.** Numbers and percentages of subjects with different salivary *Lactobacilli* scores before and after 2-week consumption of probiotic or control cheese

	<i>Lactobacilli</i> scores(cfu/ml)				
	1 n (%)	2 n (%)	3 n (%)	4 n (%)	5 n (%)
Probiotic cheese (n=29)					
Before intervention	3 (10.4)	6 (20.7)	7 (24.1)	8 (27.6)	5 (17.2)
After intervention	3 (10.4)	9 (31)	4 (13.8)	5 (17.2)	8 (27.6)
Control cheese(n=31)					
Before intervention	5 (16.1)	10 (32.3)	4 (12.9)	4 (12.9)	8 (25.8)
After intervention	7 (22.6)	6 (19.3)	3 (9.7)	6 (19.3)	9 (29)

1: 0 (cfu/ml), 2: 100-<10000 (cfu/ml), 3: 10000-<100000 (cfu/ml), 4: 100000-<1000000 (cfu/ml),

5:  $\geq 1000000$  (cfu/ml)

The backward logistic regression showed that the probiotic intervention reduced the risk of the high level ( $\geq 100000$  cfu/ml) of *Streptococcus mutans* counts (OR = 11.6, 95% CI 1.5–86.1,  $p = 0.017$ ) and the difference was statistically significant ( $p = 0.017$ ). Salivary *Lactobacilli* counts did not change neither in probiotic nor conventional cheese groups by this new set of data (OR = 2.84, 95% CI 0.49–16.06,  $p = 0.24$  in subjects with *Lactobacilli* high levels in probiotic block). No side or adverse effects were reported during the course of the study.

## Discussion

Dental caries risk factors such as saliva buffering capacity were not compared in this study since subjects were matched for some other risks like oral hygiene status. On the other hand, consuming cheese affects similarly saliva composition and buffering potential in both groups. Cheese was selected as the vehicle for *L. casei* to evaluate whether adding a probiotic strain to cheese (as a literally cariostatic agent)<sup>20-22</sup> has synergic effect on its caries reducing potential. Furthermore, probiotic cheese containing *L. casei* LAFTI- L26 has been recently available in market. According to our findings, a significant reduction of *Streptococcus mutans* after intervention was seen but the reduction was not significant between probiotic and conventional cheese groups. However, authors suggested that *L. casei* was capable of reducing *Streptococcus mutans* in subjects with high initial bacterial counts ( $\geq 100000$  cfu/ml).

If higher number of subjects were included in conventional cheese group, perhaps more powerful statistical evidence about *Streptococcus mutans* reducing potential of probiotic cheese would be respectable. To increase the strength of study in addition to applying defined inclusion and exclusion criteria for sampling which was not considered in other studies, backward logistic regression was performed. It was conducted to evaluate *Streptococcus mutans* count changes in a binary group with high and low *Streptococcus mutans* levels. The analyses showed that cheese containing *L. casei* LAFTI® L26 is capable of reducing *Streptococcus mutans* in younger individuals at higher levels of *Streptococcus mutans* when controlled for age and baseline *Streptococcus mutans* counts. Similar findings were reported with probiotic cheese containing *L. rhamnosus* GG and *Lactobacillus rhamnosus* LC 705.<sup>4</sup>

*Streptococcus mutans* level reducing effect with *Lactobacilli*-derived probiotics was observed in some studies,<sup>3,4,6,8,17</sup> while different results were reported by others.<sup>5,9</sup> To be effective in limiting or preventing dental caries, probiotic bacteria should be able to disaffect the cariogenic pathogens.<sup>10</sup> Such event would be likely in the presence of *L. casei* strains. In vitro and animal studies revealed that *L. casei* diminished cariogenic bacterial population by different mechanisms.<sup>14,16,17</sup> Furthermore *L. acidophilus*, *L. casei* and *bifidobacterium bifidum* presented in yoghurt can adhere to enamel and inhibit *Streptococcus mutans* in vitro.<sup>18</sup> Howev-

er, powerful evidence of colonization of these microorganisms (provided in yoghurt) in the human mouth after 1 week consumption of this product was not achievable.<sup>18</sup>

Surprisingly, no statistical significant change in salivary *Lactobacillus* bacterial count was observed in subjects before and after the intervention despite exposing saliva to 50 gram cheese containing at least  $1 \times 10^6$  CFU/gram *L. casei*. Even in subjects with highest level ( $\geq 100000$  cfu/ml) of salivary *Lactobacilli* counts, probiotic strain exposure was not effective. This result was against what was expected based on the in vitro study that showed highly adhesion rate of *Lactobacilli* to enamel surface.<sup>14</sup> Reducing saliva agglutinin gp340 and peroxidase concentrations and decreasing numbers of attached *Streptococcus mutans* by altering pellicle composition are two suggested mechanisms that probiotic strains including *L. casei* affected oral microflora. These ways of action could interpret *Streptococcus mutans* count reduction but not change in *Lactobacilli* count. Furthermore, some individuals might have inherent trait of *Lactobacilli* uninstillation due to missing *Lactobacilli* retention sites.<sup>18</sup> These people might act as a confounder when results are being compared. To be confident about *Lactobacilli* count changes, the data of subjects with 0 score in all three steps of saliva sample *Lactobacilli* counts were excluded (3 in probiotic block and 5 in conventional cheese block and 8 in total). The statistical analyses still did not show significant change in both groups in *Lactobacilli* counts. Oral microbial pattern in adequate numbers of subjects with 0 *Lactobacilli* count in comparison with individuals showing positive counts of *Lactobacilli* might lead us to remarkable results. Variations in study design, dosage, and ways of administration, age of participants and strains of *Lactobacilli* make comparison of this study with other researches complicated. It is not possible to absolutely determine the results of probiotic studies on oral health. However, in majority of probiotic *Lactobacilli* using dairy products vehicles more encouraging outcomes were found. Valuable effects of long-term (7

months) consumption of milk containing probiotic *L. rhamnosus* GG bacteria on dental<sup>8</sup> *L. rhamnosus* LB21 and fluoride reduced dental caries in preschool children. A cross over randomized control trial of 2 weeks placebo or yoghurt containing probiotic *L. reuteri* caused significant reduction counts of *Streptococcus mutans* in young adults.<sup>6</sup> During the "post treatment period" of 3 weeks consumption of probiotic cheese containing *L. rhamnosus* GG and *Lactobacillus rhamnosus* LC 705, significant reduction of *Streptococcus mutans* was seen comparing to subjects receiving conventional cheese as placebo but salivary *Streptococcus mutans* count did not change immediately after 3-week intervention.<sup>4</sup> In our study, probiotic group solely caused a reduction in *Streptococcus mutans* population while intergroup comparing test did not show expected results. Cheese per se was beneficial in reducing salivary *Streptococcus mutans* counts although this decrease was not significant. This finding was in agreement with other researches.<sup>4,22</sup>

The synergism between caries reducing effect of cheese and oral health promoting role of probiotics could help individuals with higher levels of *Streptococcus mutans* to promote their oral health.<sup>21,22</sup> Although significant results were not seen between placebo and trial groups, the encouraging observation in *Streptococcus mutans* levels reduction in probiotic block reinforced the value of further researches. Particularly, long-term effects of probiotic cheese on incipient caries development (as a known role of *Streptococcus mutans* in dental caries etiology) would be valuable. The residual effects following withdrawal of these products should also be taken into account. It should be noted that the present study comprised healthy adults without active dental caries. Due to possibility of not completely matured children's oral flora and the fact that they affect more easily compared to adults<sup>4</sup> and ethical limitations, it was decided not to include children at this step. However, the findings may suggest performing an intervention study on children at high risk for dental caries.



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## Conflict of Interests

No one of those who were involved in conception, designing, planning, running trial, interpretation, drafting manuscript and submission are funded or supported by any dairy plant, probiotic product manufacturer or any other related affairs organization. No other type of relation (patent holding, employment, share holding and so on) is declared. The funder had no role in study design, data collection and analysis and decision to publish or preparation of the manuscript. All the above mentioned steps were conducted by both authors.

## Authors' Contributions

Shiva Mortazavi and Najme Akhlaghi coordinated and carried out the design of the study, participated in most of the experiments and prepared the manuscript. Both authors have read and approved the content of the manuscript.

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