The Influence of a *Bifidobacterium animalis* Probiotic on Gingival Health: A Randomized Controlled Clinical Trial

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Background: There is growing interest in the use of probiotics in periodontal therapy; however, until now, most research has focused on lactobacilli probiotics. The aim of this study is to evaluate the effect of 4-week use of yogurt supplemented with *Bifidobacterium animalis* subsp. *lactis* DN-173010 versus a placebo yogurt, followed by a 5-day non-brushing period.

Methods: Individuals were included in this single-mask, randomized, controlled study if probing depth (PD) was ≤3 mm and attachment loss was ≤2 mm. After professional prophylaxis, they were randomized into two groups receiving yogurt containing either placebo or *B. animalis* for 28 days, followed by a 5-day non-brushing period. Outcome measures were plaque index (PI), gingival index (GI), bleeding on probing (BOP), PD, gingival crevicular fluid (GCF) volume, and total amount and concentration of interleukin (IL)-1β in GCF. These were measured at baseline, after 28 days of study product use, and subsequently after 5 days of plaque accumulation.

Results: Fifty-one patients were analyzed. No intergroup differences could be detected before and after intake of study products. However, after plaque accumulation, significantly better results for all parameters were seen in the probiotic group compared with the control group (P <0.001): lower PI and GI, less BOP, less increase in GCF volume, and lower IL-1β total amount/concentration.

Conclusion: The use of a probiotic yogurt supplemented with *B. animalis* can have a positive effect on plaque accumulation and gingival inflammatory parameters after refraining from oral hygiene practices. *J Periodontol 2017;88:1115-1123.*

KEY WORDS

***Bifidobacterium*; *Bifidobacterium animalis*; gingivitis; probiotics; yogurt.**

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There is growing interest in the use of probiotic products for restoring dysbiotic microbiota. Probiotics are defined as microorganisms that, when administered in adequate amounts, confer health benefits on the host.[1] The beneficial impact of probiotics on certain gastrointestinal disorders is well-established.[2] More than a decade ago, probiotics were introduced for periodontal healthcare.[3] For maintenance of periodontal health, the equilibrium between the microbial challenge and host response is a determining factor.[4-6] In this context, probiotics might have a possible role by suppressing and displacing harmful bacteria and indirectly by their immunomodulatory effects.[7]

A number of in vitro and in vivo studies have been conducted focusing on the role of probiotics in prevention and treatment of periodontal diseases.[8-20] It has been shown that probiotics were useful in reducing gingival inflammation[8-11] and plaque accumulation,[8,9,13] improving periodontal health,[14-16] decreasing the number of black pigmented rods including *Porphyromonas gingivalis* in saliva and/or subgingival plaque,[14,17-20] and reducing proinflammatory cytokines in patients with gingivitis.[10] Furthermore, it has also been reported that application of probiotic bacteria as an adjunct to scaling and root planing can inhibit recolonization of pathogens in periodontal pockets and reduce plaque index (PI),

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gingival index (GI), and bleeding on probing (BOP).[14-16,18,21]

Probiotic supplements come in a variety of forms, from powders, chewing gums, and capsules to foods such as chocolates and dairy products that are supplemented with specific probiotic organisms. Most probiotic studies in the periodontal literature were performed using *Lactobacillus* species.[22] Besides *Lactobacillus* species, *Bifidobacteria* are often described as potent probiotics.[23] In the dental field, it was shown that probiotics containing *Bifidobacteria* can reduce mutans streptococci counts.[24-27] However, to the authors' knowledge, no studies have examined the effects of *Bifidobacterium*-supplemented probiotics in patients with (experimental) gingivitis or periodontitis. Hojo et al.[28] evaluated the distribution of *Bifidobacterium* species in patients with current or former periodontitis and in healthy individuals. In their study, it was suggested that bifidobacterial counts might be associated with periodontal health status. More recently, microbiologic and immunoinflammatory effects of *Bifidobacterium animalis* subsp. *lactis* HN019 were shown by Oliveira et al.[29] in experimental periodontitis in rats. Therefore, the objective of this study is to evaluate the effect of a 4-week use of *Bifidobacterium*-supplemented yogurt versus a placebo yogurt, followed by a 5-day non-brushing period. Plaque accumulation was studied together with different parameters assessing the degree of gingival inflammation.

MATERIALS AND METHODS

This examiner-masked, randomized controlled study with two parallel groups was approved by the Marmara University Health Sciences Ethical Committee, Marmara University, Istanbul, Turkey (MAR-2011-11/14) and registered at ClinicalTrails.gov as NCT02546206. Written informed consent was obtained from all participants at the start of the study. Potential participants were recruited from the Oral Diagnosis and Radiology Department, School of Dentistry, Marmara University, Istanbul, Turkey, where patients were admitted first and screened for oral health problems to be referred to specialty clinics. Of those screened, 51 patients (19 males and 32 females, aged 16 to 26 years; mean age: 21 years) were selected for the study. This selection was conducted according to the following inclusion criteria: 1) periodontally healthy patients[30] with at least 24 natural teeth (excluding third molars); 2) probing depth (PD) ≤3 mm; and 3) without predisposing oral factors causing local irritation and plaque retention. Individuals were further evaluated periodontally and were solely included if attachment loss (AL) was ≤2 mm and GI ≤1.[31] Exclusion criteria were as follows: 1) presence of systemic diseases; 2) pregnancy or breastfeeding; 3) history of drug abuse; 4) previous probiotic supplements in diet; 5) medications, in particular current ingestion of non-steroidal or steroidal anti-inflammatory drugs or antibiotics within 3 months before entering the study; 6) mouth breathing; 7) allergic reactions to lactose or fermented milk products; and 8) current smoker or smoker during the past year.

The study was performed between November 2011 and May 2012.

Sample Size Calculation and Randomization

Sample size was calculated based on the study by Slawik et al.[32] Considering 95% power and α of 0.05, with a mean difference of 15.51 and standard deviation (SD) of 12.72 for BOP score between groups, the number of patients needed was at least 16 for each group. A 10% dropout rate was considered.

A computer-based randomization program[33] was used for assigning patients randomly into two groups (BEK). Every patient was sequentially numbered (1 to 51) and coded as 1 (test) or 2 (control). Sampling and measurements were done by an examiner (TY) who was unaware of patients' yogurt type.

Treatment Protocol

This study consisted of a period of 28 days of probiotic or placebo yogurt consumption followed by a 5-day plaque accumulation period by refraining from any oral hygiene measurement, as seen in Figure 1. Seven days before the start of the study, participants were given verbal reinforcement of oral hygiene, and professional tooth cleaning was carried out using abrasives[§] and brushes.[‖] All patients were given the same toothpaste.[¶] On day 0 (D0), patients were randomly assigned to one of the two groups as described above, and the study started with use of the probiotic and placebo yogurt.

The yogurts were handed out by BEK. Half the number of participants were given 110 g probiotic plain yogurt daily,[#] containing ≥10^8 colony-forming units (cfu)/g of *B. animalis* subsp. *lactis* DN-173010. Participants in the control group received 110 g of plain yogurt without probiotic bacteria.[**] The type of yogurt was masked as much as possible for the patients; the paper covering the body of the container was removed. It was recommended to use the study products in the morning between breakfast and lunchtime and to not eat or brush teeth for at least 1 hour after yogurt consumption.

§ Detartrine, Septodont, Cedex, France.
‖ Stoddard, Letchworth, Hertfordshire, U.K.
¶ Colgate Triple Action, Colgate-Palmolive, New York, NY.
# Activia Sade, Danone, Istanbul, Turkey.
** Naturel Yogurt, Danone.

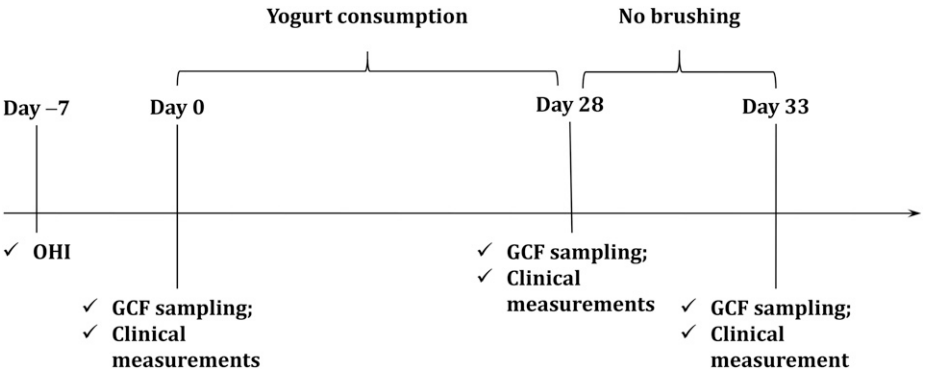

Figure 1.
Course of the study OHI = oral hygiene instruction.

Outcome Variables of Interest

At the start of the study on D0, gingival crevicular fluid (GCF) was collected and clinical measurements (PI, GI, PD, and BOP) were taken. GCF sampling and clinical measurements were repeated at the beginning and end of the non-brushing period, on days 28 (D28) and 33 (D33), respectively. Clinical measurements and GCF sampling were done from eight selected teeth: maxillary incisors and canines and lower canines. At each experimental time-point (D0, D28, D33), GCF samples and clinical measurements were taken from the same teeth and same periodontal sites.

PI was determined using the Silness and Löe[34] index at four surfaces of the teeth (mesio-buccal, mid-buccal, disto-buccal, and mid-lingual). Plaque was assessed visually without staining and graded by four degrees: 0 = no plaque; 1 = little accumulation of plaque adhering to the free gingival margin and adjacent area of the tooth, which can only be seen with use of a probe; 2 = moderate accumulation of plaque adhering to the free gingival margin and adjacent area of the tooth, which can be seen with the naked eye; and 3 = pronounced accumulation of soft matter.

GI was recorded at the same four surfaces per tooth as done for PI, according to the Löe and Silness index: 0 = normal gingiva; 1 = mild inflammation, slight change in color, mild alteration of gingival surface structure, and no BOP; 2 = moderate inflammation, redness, edema and swelling, and BOP; and 3 = severe inflammation, marked redness and edema, ulceration, and tendency to spontaneous bleeding.[31]

PD, measured at six sites per tooth, was defined as the distance between base of the sulcus and margin of the gingiva. Presence or absence of bleeding was measured at six sites per tooth after probing.††

GCF was collected with an absorbent paper strip.‡‡ Selected teeth were isolated by using cotton rolls. After elimination of supragingival plaque and saliva, paper strips were gently inserted in the gingival sulcus for 30 seconds and the volume of the GCF sample was immediately recorded,§§ expressed in the measuring device units and followed by calculation of volume of each sample using a standard curve. The paper strips were transferred to plastic tubes‖‖ and stored at –70° C until analysis. For this, the strips were allowed to thaw at room temperature for 30 minutes. Then, pooled GCF samples were eluted from the eight paper strips per patient by placing them in 150 µL phosphate-buffered saline and stored for ≤24 hours at 4°C prior to use.[35] Levels of interleukin (IL)-1β in GCF samples were analyzed by enzyme-linked immunosorbent assay (ELISA), using a commercially available kit¶¶ according to manufacturer's instructions. Concentrations of IL-1β in each of the GCF samples were calculated from the standard curve and presented as picograms per milliliter per site.

Examiner Calibration

A calibration exercise was performed for the examiner (TY) to determine acceptable intraexaminer reproducibility. Five patients with gingivitis (with both bleeding and non-bleeding sites on probing) not included in the study were evaluated by the examiner on two separate occasions 48 hours apart. PI, GI, PD, and BOP were measured. Calibration was accepted if measurements at baseline and at 48 hours were consistent in ≥90% of the measurements.[36]

Compliance and Adverse Effects

Compliance was checked and confirmed verbally at D14 and D28 (BEK). For checking adverse effects, patients were asked whether they got any of the following symptoms: 1) stomach gas; 2) diarrhea; 3) signs of infection (fever, chills); 4) allergic reactions (rash, hives, itching, difficulty in breathing, swelling of mouth/lips/face/tongue); and/or 5) dizziness.

Statistical Analyses

For all statistical evaluations, the patient was maintained as the unit of measurement. Quantitative data were taken as mean ± SD of eight periodontal sites from eight teeth in each individual for all parameters. Kolmogorov-Smirnov test was used to check normality of the distribution. Two-way Friedman test was

†† PCP-15 UNC, Hu-Friedy, Chicago, IL.
‡‡ PerioPaper Strip, Pro Flow, Amityville, NY.
§§ Periotron 8000, Proflow, New York, NY.
‖‖ Eppendorf, Millipore, Billerica, MA.
¶¶ Solid Phase Sandwich ELISA kit, Quantikine human interleukin-1β HSLB00C, R&D Systems, Minneapolis, MN.

used for multiple intragroup comparisons for all parameters at three different time-point measurements (D0, D28, and D33). If this was found statistically significant, intragroup comparisons in pairs (Wilcoxon test) between two time points (D0 and D28; D28 and D33; D0 and D33) were done. Afterward, these intragroup differences, as well as mean values of the parameters at each time point, were compared between the two groups (Mann-Whitney *U* test).

For all measurements, statistical significance was set as $P<0.05$. In paired comparisons, *P* values were corrected for multiple comparisons with Bonferroni correction and statistical significance was set as $P<0.017$.

RESULTS

As shown in Figure 2, 63 patients were screened and 51 patients were found eligible to participate in the study. These 19 males and 32 females completed the study; other demographic data can be found in Table 1. When compliance of the use of study products was checked, all patients declared that they consumed the yogurt without missing a day. Adverse effects were checked verbally with a list of possible side effects, but none of the patients reported a problem from this list.

Table 2 shows all intragroup and intergroup comparisons for the examined parameters. *P* values for intragroup differences in pairs are shown in Table 3.

For PI and GI, in both the probiotic and control groups, no intragroup differences could be noted at D0 and D28. However, 5 days of not brushing led to significantly higher PI and GI compared with D0 and D28, regardless of the study group (for all these intragroup measurements, $P<0.001$). Regarding intergroup comparisons, no statistically significant differences were found for the groups at D0 and D28. However, at the end of the non-brushing period, on D33, means of PI and GI were significantly lower in the probiotic group than in the placebo group. Increase in PI and GI between the measurements on D33 versus D28 and those on D33 versus D0 was statistically significantly different between the two groups; the mean increase at D33 and D28 or D0 was smaller in the probiotic group than in the control group.

Regarding BOP, the same trends can be seen as those for PI and GI. BOP was significantly higher both in the probiotic group and in the control group on D33 than on D28 ($P<0.001$ and $P<0.001$, respectively) and on D33 than on D0 ($P<0.001$ and $P<0.001$, respectively). BOP was comparable between both groups on D0 and D28. However, after plaque accumulation on D33, BOP was significantly lower in patients who consumed the probiotic yogurt compared with those who consumed the placebo yogurt. Looking at the intergroup comparison of the intragroup differences between the start of the non-brushing period on D28 and the end of this period on D33, increase in bleeding sites was significantly lower in the probiotic group than in the control group (10.45% versus 21.23%).

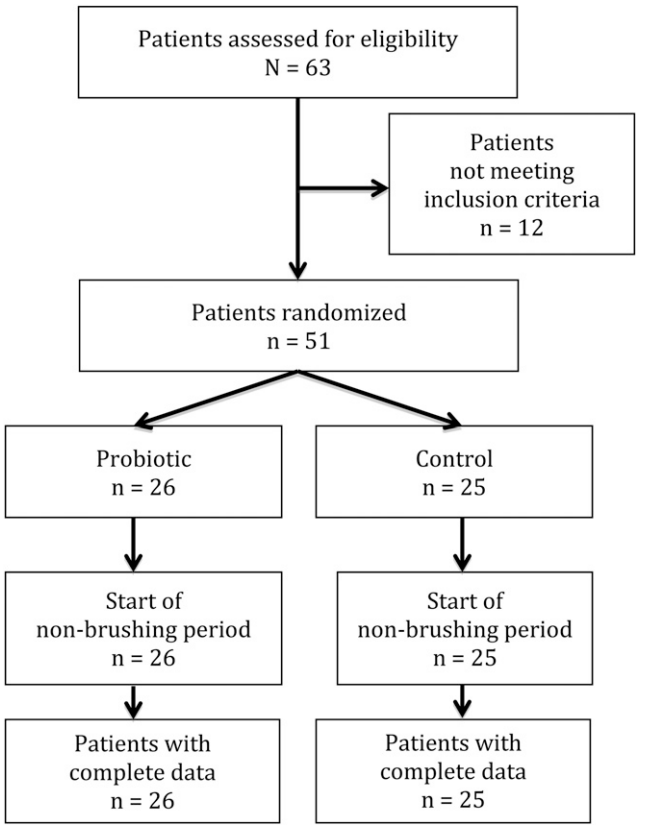

Figure 2.
Flowchart of the study.

For PD, the only statistically significant intragroup difference was that mean PD in the control group was significantly deeper on D33 than on D0 ($P=0.005$). Accordingly, the only significant intergroup difference was the higher mean PD value in the control group, reached after the non-brushing period on D33. The intergroup comparison of the intragroup differences was not statistically significantly different.

Regarding GCF measurements, no intragroup differences could be noted in the probiotic group; they were only detected in the control group. Volumes of GCF per pooled site, IL-1β concentration per pooled site, and IL-1β total amount per pooled site were significantly higher on D33 than on D0 or D28 (for all these measurements, $P<0.001$). These measurements were also significantly higher in the control group on D28 than on D0 ($P=0.009$, $P=0.001$, and $P=0.002$, respectively). Regarding differences between

Table 1.

Patient Demographics

Variable	Treatment Group		*P* Value
	Probiotic	Control	
Number of patients	26	25	
Number of males	9	10	
Number of females	17	15	
Age range (mean ± SD [years])	17 to 25 (22.8 ± 3.52)	16 to 26 (21.64 ± 4.15)	
PI	0.28 ± 0.14	0.37 ± 0.15	0.080
GI	0.19 ± 0.08	0.22 ± 0.07	0.196
BOP (%)	1.42 ± 0.92	1.36 ± 0.65	0.843
PD (mm)	1.49 ± 0.20	1.53 ± 0.13	0.434
GCF volume/pooled site (μL)	0.19 ± 0.04	0.14 ± 0.04	0.101
IL-1β concentration/pooled site (pg/mL)	125.21 ± 136.45	89.84 ± 105.49	0.152
IL-1β total amount/pooled site (pg)	0.05 ± 0.05	0.03 ± 0.04	0.080

the probiotic and control groups, no statistically significant differences were found between the groups on D0 and D28 for any of the examined GCF parameters. However, comparing both groups after the plaque accumulation period on D33, significantly more GCF volume and higher concentration and total amount of IL-1β could be detected in the control group. Furthermore, all intergroup comparisons of intragroup differences for D33 versus D0, D33 versus D28, and D28 versus D0 were significantly different in favor of the probiotic group.

DISCUSSION

This study in periodontally healthy individuals shows the effects of 28 days of consumption of probiotic yogurt containing ≥10^8 cfu/g of *B. animalis* subsp. *lactis* versus placebo yogurt, and a subsequent 5-day non-brushing period. After the 5-day non-brushing period (D33), clinical indices (PI, GI, and BOP) were elevated in both groups compared with D0 and D28. For GCF parameters, intragroup differences could only be found in the control group. Lower PI and GI scores, less BOP, lower GCF volume, and lower total amount and concentration of IL-1β were measured for the probiotic group compared with the control group. As a proinflammatory cytokine, IL-1β is released by macrophages after bacterial infection or tissue injury.[37] Higher IL-1β levels in GCF were detected in patients with periodontitis compared with healthy patients and those with gingivitis; the levels declined after mechanical periodontal therapy.[38] The finding that both concentration and total amount of GCF IL-1β were lower in the probiotic group than in the control group could be interpreted as a result of the anti-inflammatory effect of the probiotic. Therefore, this study shows a positive effect on inflammatory parameters when plaque regrowth is induced after consumption of probiotic yogurt.

Gingival inflammatory changes as well as GCF parameters are indicative of local host response. Bleeding is the most sensitive clinical indicator for gingival health and provides a reliable assessment for gingival inflammatory changes.[39] Gingival inflammation is also associated with increased levels of a variety of inflammatory mediators.[40] Increase in IL-1β release rates has been found in GCF after at least 3 days of plaque accumulation.[41] There is no doubt that after 5 days of refraining from mechanical plaque control, microbial dental plaque accumulates. It is important to discard the possible immediate effect of probiotics on plaque accumulation during the non-brushing period to evaluate differences between the test and control groups. Therefore, no probiotic yogurt was given during the non-brushing period.

To the authors' knowledge, this is the first study examining the influence of single-strain *Bifidobacterium* probiotics on gingival health in periodontally healthy dentate people. Positive effects of single-strain *Bifidobacterium* probiotics on mutans streptococci in young adults were already described.[24,26,42] Decreased PI and GI could be seen after a 4-week use

Table 2.

Overview of All Examined Parameters

Variable		Treatment Group						Intergroup *P* Value		
		Probiotic			Control					
		Mean ± SD	δ Versus D0 ± SD	δ Versus D28 ± SD	Mean ± SD	Δ Versus D0 ± SD	Δ Versus D28 ± SD	For Mean	For Δ Versus DO	For Δ Versus D28
PI										
Overall	D0	0.28 ± 0.14			0.37 ± 0.15			NS		
	D28	0.27 ± 0.13	0.01 ± 0.10		0.38 ± 0.15	0.01 ± 0.18		NS	NS	
	D33	0.80 ± 0.30*†	0.52 ± 0.35	0.53 ± 0.34	1.80 ± 0.42*†	1.43 ± 0.44	1.42 ± 0.45	<0.001	<0.001	<0.001
Intragroup *P* value		<0.001			<0.001					
GI										
Overall	D0	0.19 ± 0.08			0.22 ± 0.07			NS		
	D28	0.19 ± 0.07	0.00 ± 0.05		0.22 ± 0.05	0.00 ± 0.08		NS	NS	
	D33	0.80 ± 0.33*†	0.61 ± 0.34	0.61 ± 0.35	1.52 ± 0.44*†	1.30 ± 0.44	1.30 ± 0.45	<0.001	<0.001	<0.001
Intragroup *P* value		<0.001			<0.001					
BOP (%)										
Overall	D0	1.42 ± 0.92			1.36 ± 0.65			NS		
	D28	1.42 ± 0.66	0.01 ± 1.22		1.58 ± 0.91	0.21 ± 1.05		NS	NS	
	D33	11.87 ± 4.12*†	10.46 ± 4.21	10.45 ± 4.22	22.81 ± 6.12*†	21.44 ± 6.20	21.23 ± 6.32	<0.001	<0.001	<0.001
Intragroup *P* value		<0.001			<0.001					
PD (mm)										
Overall	D0	1.49 ± 0.20			1.53 ± 0.13			NS		
	D28	1.47 ± 0.17	0.02 ± 0.21		1.52 ± 0.13	0.00 ± 0.25		NS	NS	
	D33	1.44 ± 0.15	0.05 ± 0.24	0.03 ± 0.20	1.58 ± 0.12*	0.06 ± 0.10	0.06 ± 0.14	0.002	NS	NS
Intragroup *P* value		0.881			0.003					
GCF volume/pooled site (μL)										
Overall	D0	0.19 ± 0.04			0.14 ± 0.04			NS		
	D28	0.21 ± 0.09	0.02 ± 0.11		0.19 ± 0.09*	0.05 ± 0.09		NS	0.03	
	D33	0.19 ± 0.07	0.01 ± 0.08	0.01 ± 0.11	0.33 ± 0.12*†	0.18 ± 0.12	0.13 ± 0.14	<0.001	<0.001	<0.001
Intragroup *P* value		0.448			<0.001					
IL-1β concentration/pooled site (pg/mL)										
Overall	D0	125.21 ± 136.45			89.84 ± 105.49			NS		
	D28	132.36 ± 184.35	7.14 ± 136.34		154.99 ± 179.56*	65.15 ± 131.75		NS	0.01	
	D33	144.72 ± 97.21	19.50 ± 96.67	12.36 ± 130.74	1,267.05 ± 848.31*†	1,177.21 ± 832.41	1,112.06 ± 777.78	<0.001	<0.001	<0.001
Intragroup *P* value		0.151			<0.001					
IL-1β total amount/pooled site (pg)										
Overall	D0	0.05 ± 0.05			0.03 ± 0.04			NS		
	D28	0.05 ± 0.07	0.00 ± 0.05		0.06 ± 0.06*	0.02 ± 0.04		NS	0.01	
	D33	0.09 ± 0.07	0.04 ± 0.10	0.04 ± 0.12	0.63 ± 0.44*†	0.60 ± 0.44	0.57 ± 0.42	<0.001	<0.001	<0.001
Intragroup *P* value		0.028			<0.001					

NS = not significantly different.
* Intragroup significant difference from D0.
† Intragroup significant difference from D28.

Table 3.

Intragroup Comparisons (*P* values) in Pairs

	Probiotic			Control		
Variable	D28 Versus D0	D33 Versus D0	D33 Versus D28	D28 Versus D0	D33 Versus D0	D33 Versus D28
PI	NS	<0.001	<0.001	NS	<0.001	<0.001
GI	NS	<0.001	<0.001	NS	<0.001	<0.001
BOP (%)	NS	<0.001	<0.001	NS	<0.001	<0.001
PD (mm)	NS	NS	NS	NS	NS	0.01
GCF volume/pooled site (μL)	NS	NS	NS	0.01	<0.001	<0.001
IL-1β concentration/pooled site (pg/mL)	NS	NS	NS	<0.001	<0.001	<0.001
IL-1β total amount/pooled site (pg)	NS	NS	NS	<0.001	<0.001	<0.001

NS = not significantly different.

of multistrain probiotics (*Lactobacillus rhamnosus* GG and *B. animalis* subsp. *lactis* BB-12) in healthy adults.[23] It was also suggested that periodontal health might be associated with high bifidobacterial counts.[28] Moreover, bifidobacteria, isolated from probiotic yogurt, can – at least in vitro – inhibit growth of periodontopathogens, such as *P. gingivalis*, *Fusobacterium nucleatum*, and *Aggregatibacter actinomycetemcomitans*,[43] when the bifidobacteria were inoculated before the periodontal pathogens. van Essche et al.[44] confirmed these results using *Bifidobacterium dentium* strains isolated from healthy individuals. From all the isolated inhibitory bacteria in their study, bifidobacteria were the strongest inhibitors of *P. gingivalis*. Additionally, in an experimental periodontitis model in rats, it was shown that topical use of *B. animalis* subsp. *lactis* HN019 promotes a protective effect against alveolar bone and connective tissue AL.[29]

The present study uses a similar set up as Staab et al.[12] and Slawik et al.[32] Staab et al.[12] investigated the effect of the consumption of a milk drink containing *Lactobacillus casei Shirota* for 8 weeks, followed by a 4-day experimental gingivitis period. Clinically, no statistically significant differences were found between patients using the probiotic milk drink for 8 weeks and control patients, although polymorphonuclear elastase activity was significantly lower in the test group after this 8-week period. Additionally, at the end of the 4-day experimental gingivitis period, the test group had significantly lower myeloperoxidase activity than the control group. Slawik et al.[32] used the same probiotic milk drink in periodontally healthy patients. Patients were instructed to use the product for 2 weeks prior to starting the 2-week experimental gingivitis period.[32] After the experimental gingivitis period, BOP and GCF volume were significantly lower in the test group compared with the control group. This experiment also revealed a positive effect on clinical parameters in the probiotic group.

The reason why this yogurt containing *B. lactis* showed this effect is highly speculative. Besides the above-mentioned antimicrobial properties of *Bifidobacteria* toward periodontopathogens, it was shown that *Bifidobacteria* could survive in saliva and bind to *F. nucleatum*–covered hydroxyapatite in vitro.[45] Although a microbiologic analysis was not performed, these properties could have influenced the biofilm composition by inhibiting periodontopathogens during the non-brushing period. Consequently, this may have had an impact on the inflammatory response and, in turn, because inflammation increases plaque growth, resulted in reduced PI.[46]

The findings must be interpreted considering the following points. First, although a well-established non-brushing model was used,[12] despite similar amounts of plaque accumulation, patients may respond differently to experimentally induced gingival inflammation.[47-50] Second, although this study was primarily designed to evaluate clinical parameters, an analysis of the microbiota could have given more information about the observed effect. Because data are still sparse to explain molecular and biologic mechanisms of probiotics on oral health, microbiologic analysis could have given, for example, more information about (temporary) colonization by probiotic microorganisms. Furthermore, participants could possibly deduce the type of yogurt if the specific green color and the text on the upper seal of

the probiotic yogurt were taken into account. This could have affected compliance and retention of the trial participants.[51] However, all patients declared to have never missed one yogurt, and the examiner who did the clinical measurements was masked to treatment allocation.

CONCLUSIONS

This study demonstrates reduced clinical and immunologic signs of inflammation in a non-brushing model after 28 days of consumption of yogurt containing $\geq 10^8$ cfu/g of *B. animalis* subsp. *lactis*. This effect was seen at the clinical level (PI, GI, BOP, and PD) and for GCF markers (GCF volume, IL-1β concentration, and IL-1β total amount). To the best of the authors' knowledge, this is the first study describing *Bifidobacteria* as a potential probiotic to combat gingival inflammation. The effect of *B. animalis* on patients with "real" gingivitis should be investigated together with its effects on microbiologic parameters, ideal concentration, method of administration, and duration of the positive effect after product use.

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