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RESEARCH

Bifidobacterium animalis subsp. lactis as adjunct to non-surgical periodontal treatment in periodontitis: a randomized controlled clinical trial

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Abstract

Objectives The purpose of this study is to investigate the clinical and microbiological effects of *Bifidobacterium animalis subsp. lactis* DN-173010 containing yogurt as an adjunct to non-surgical periodontal treatment in periodontitis patients.

Materials and methods This is a prospective randomized controlled clinical study registered with NCT05408364 under clinical trial registration. Thirty periodontitis patients were divided into 2 groups at random. As adjunctive to supra and subgingival instrumentation, the test group consumed *Bifidobacterium animalis subsp. lactis* DN-173010 containing yogurt while the control group consumed natural yogurt, once daily for 28 days. The plaque index (PI), gingival index (GI), bleeding on probing (BOP), probing depth (PD), and clinical attachment level (CAL) were recorded at baseline, 28th day, and 3rd month. Microbiological analysis was performed using culture method by obtaining subgingival plaque samples from 2 periodontal sites with 4≤PD≤6 mm at the same time points.

Results The inter-group comparisons of PI, GI, and BOP as well as the changes between the measurement time points were statistically significant in favor of the test group. There were no significant differences in terms of PD and CAL changes between the study groups at all times (Δbaseline–28 days, Δbaseline–3 months) ($p>0.05$). The number of patients presenting subgingival *Bifidobacterium* species was significantly greater in the test group than the control group at the 28th day ($p<0.05$).

Conclusions The administration of probiotics has shown beneficial effects, albeit limited, on clinical and microbiological outcomes in the management of periodontitis patients.

Clinical relevance Daily consumption of probiotic yogurt may be supportive for supra and subgingival instrumentation.

Keywords *Bifidobacterium* · Dental scaling · Periodontitis · Probiotics · Root planing

Introduction

Periodontitis is a prevalent and irreversible inflammatory disease affecting over 50% of the world's adult population representing a major public health problem in countries worldwide [1]. It is accepted that dental plaque microorganisms existing in the form of biofilm are the primary etiological agents of periodontitis of which the sequential basic steps of treatment are oral hygiene instructions, lifestyle management, and supra and subgingival instrumentation. This cause-related therapeutic approach aims to remove pathogenic biofilm, toxins, and calculus, and re-establishes a biologically acceptable root surface. However, non-surgical periodontal treatment might be insufficient to eliminate periodontal pathogens located within the soft tissues and/or in areas inaccessible to periodontal instrumentation, and to prevent frequent recolonization of treated areas with pathogenic microorganism. Although systemic or local delivery of antibiotic systems into periodontal pockets can be effective in disinfecting [2], repeated use of antibiotics increases the risk of resistant bacteria developing. Besides the development of resistance, problems associated with these adjunctive pharmacological regimens include disruption of the microflora of the oral cavity and gastrointestinal tract. As a result, the

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development of alternative antimicrobial strategies is still needed.

Probiotics, live microorganisms, provide a health benefit to the host when given in sufficient proportions. Mechanisms of probiotic microorganisms proposed are prevention of pathogenic species adhesion to host tissues, production of antimicrobial substances against periodontopathogens, and stimulation of host immune response [3]. Probiotic microorganisms mostly belong to genera *Lactobacillus* or *Bifidobacterium* are usually delivered through dairy products or food supplements in tablet form. Recently, their potential benefits on systemic health and some disorders have been described elaborately [4].

The therapeutic effects of probiotics in periodontology have been examined in a variety of in vitro [5–8] and in vivo [9–23] studies. Recent studies have been conducted to determine the effect of probiotics as adjunctive to supra and subgingival instrumentation in the management of periodontal disease [14, 16–19]. However, the findings are somewhat controversial. Although improvement in periodontal clinical parameters [14, 16–20], reduction in periodontopathogen levels [16], and decrease of pro-inflammatory cytokines levels [17] were observed, no additional clinical [19, 20] and/or microbiological [19] effects were reported. Most of the probiotic studies focused mainly on the effects of *Lactobacillus* species, so *Bifidobacterium* as a potential probiotic microorganism with immunomodulatory and antimicrobial properties has caught little attention in the management of periodontal diseases. *Bifidobacterium* occur naturally in the oral cavity and are among the most predominant anaerobic bacteria within the intestinal lumen. Hojo et al. [24] reported that *Bifidobacterium* counts might be linked to periodontal health due to inhibition in several black-pigmented anaerobes by competing for a crucial growth factor vitamin K. However, there are limited studies on the impact of *Bifidobacterium* on PD and its treatment [23]. Recently, three in vivo studies investigating the effects of *Bifidobacterium animalis* subsp. *lactis* (*B. lactis*) on periodontal diseases were published. While Oliveira et al. [8] demonstrated the microbiologic and immunoinflammatory effects of *B. lactis* HN019 on the experimental gingivitis model in rats, Ricoldi et al. [7] suggested a role for the same probiotic bacteria in the management of experimental periodontitis model, as an adjunct to non-surgical periodontal treatment. Kuru et al. [25] reported that *B. lactis* DN-173010 has a positive impact on plaque accumulation and gingival inflammation parameters in a non-brushing model in humans. So far, only one clinical trial [23] using *B. lactis* HN019 in addition to supra and subgingival instrumentation concluded that it provides additional microbiological, immunological, and clinical improvements in the management of chronic periodontitis.

Due to the beneficial effects of probiotics on human health, probiotic bacteria have been added to various types of foods, which are considered vehicles for administration. A systematic review and meta-analysis [26] reported that consumption of probiotic-containing dairy products appears to be an adjunctive modality that positively influences the microbial ecology of oral biofilm and caries development. In fact, liquid matrices such as milk and yogurt appear to be more effective at lowering *Streptococcus mutans* and *Lactobacillus* levels. To the best of our knowledge, there is no study evaluating the effectiveness of dairy products containing *Bifidobacterium* with supra and subgingival instrumentation in the treatment of periodontal diseases. Thus, the purpose of the present study is to investigate the adjuvant effects of 28-day consumption of *B. lactis* DN-173010 containing yogurt with supra and subgingival instrumentation on periodontal clinical parameters and microbiological outcomes of chronic periodontitis patients.

Materials and methods

The present double-blinded, randomized, and placebo-controlled clinical study with two parallel arms involved 30 chronic periodontitis [27] patients that are considered under stage III grade B according to today's categorization [28]. They were recruited from patients referred for periodontal therapy to the Periodontology Department, Dental Faculty, Marmara University, from April 2011 to April 2013. The study protocol was approved by the Marmara University Health Sciences Ethical Committee (30.03.2011-24) and registered at NCT05408364 (identification number NCT, clinicaltrials.gov). According to the Helsinki Declaration, each participant in the study signed an informed consent form prior to participation.

Inclusion criteria for entry were as follows: systemically healthy previously untreated chronic periodontitis patients, the presence of at least 2 teeth with one approximal site having a probing depth (PD) of 4–6 mm and a gingival index (GI) of ≥2 in each quadrant. Exclusion criteria were as follows: pregnancy, smoking (current and past), presence of systemic diseases such as immunologic diseases, diabetes, kidney or liver disease and neurologic deficiencies, rheumatic fever, use of any medicine that has effect on the periodontal tissues (cyclosporine, phenytoin, nifedipine, etc.), antibiotics administered for any reason within the previous 6 months, use of probiotic supplements, and allergic reactions to fermented milk products or lactose.

Patients who met the study's eligibility requirements were invited to participate. After a detailed description of the study's purpose and treatment regimen, a written informed consent form was signed by all individuals.

Sample size calculation and randomization

Based on the study of Vivekananda et al. [14], the sample size was determined for the primary outcome variable, PD. It was determined that each group would require 12 patients to provide 90% power with a p level of 0.05. In case of any dropouts, it was decided that each group would have 15 participants.

Thirty participants were randomly assigned in two treatment groups by a computer-assisted randomization program. Each patient was assigned a number (1–30) and a code. The test group patients received supra and subgingival instrumentation +110 g probiotic (*B. lactis* DN-173010, $\geq 10^8$ colony-forming units (CFUs)/g) containing plain yogurt daily (Danone Activia® Plain, Luleburgaz, Türkiye), while the control group patients received supra and subgingival instrumentation +110 g of plain yogurt (Danone® yogurt, Luleburgaz, Türkiye) without probiotic bacteria. The coordinator of the study (BEK) distributed the coded yogurt container to the patients at baseline. Treatment, measurements, and sampling were performed by HOO who was unaware of the yogurt type. The study personnel and participants were unaware of the study group assignment, except for the study coordinator. Prior to statistical analysis, the coordinator cracked the code to allocate the patients to the proper groups.

Study plan

Participants were informed of oral hygiene instructions, 1 week before the start of the study. At the start of the study, intra-oral photographs were taken, microbiological samples were collected, and periodontal clinical parameters were measured. A total of 3 sessions and supra and subgingival instrumentation was performed with an ultrasonic device (BOBCAT® Pro, DENTSPLY International, USA) and hand instruments (Gracey curettes (curettes), Hu-Friedy Co., Chicago, IL, USA) at 1-week intervals. The participants were asked to consume either 110 g probiotic plain yogurt or 110 g plain yogurt at the onset of supra and subgingival instrumentation and then once a day for 28 days [23, 29]. All individuals were instructed to consume the yogurt in the morning after brushing their teeth and to not brush their teeth or eat for at least 1 h than yogurt consumption. No probiotic-containing products were allowed during the study period. At baseline, 28 days, and 3 months, clinical periodontal parameters and microbiological examinations were repeated.

Clinical examination and outcome variables

At baseline, day 28, and month 3, clinical parameters were measured by the same periodontist (HOO) with a periodontal probe (PCP 15 UNC, Hu-Friedy, Chicago, IL, USA). Full-mouth plaque index (PI) [30] and gingival index (GI) [31] were evaluated at 4 sites, whereas bleeding on probing (BOP), PD, and clinical attachment level (CAL) at 6 sites per tooth, excluding third molars.

The primary outcome was PD whereas secondary outcome variables were PI, GI, CAL, BOP, and microbial parameters.

Site selection and microbiological sampling

Subgingival plaque samples were obtained from one single-rooted tooth in the mandible and one multi-rooted tooth in the contralateral quadrant with $4 \leq PD \leq 6$ mm and $GI \geq 2$, with horizontal bone loss. For each tooth type, the representative pockets were selected as appropriate for periodontitis. Subgingival samples were collected with sterile paper points (#30, DiaDent, Almere, the Netherlands), following the isolation of selected teeth with cotton rolls and gentle drying with compressed air at baseline, 28 days, and 3 months from the same sites. Per site, only one paper point was placed into the pocket's bottom and left for 10 s.

Microbiological culturing

Subgingival samples were aseptically transferred immediately after collection to 4.5 ml of phosphate-buffered saline (Oxoid LTD, England)–containing tubes. In order to achieve immediate homogeneous distribution, the tubes were mixed in a vortex mixer for 30 s, and then serially diluted. Two portions of 0.1 ml each, from every dilution (10^{-1}, 10^{-2},…….10^{-6}), were plated separately onto trypticase soy agar medium (Merck, 64271 Darmstdat, Germany) supplemented with 5% defibrinated sheep blood, 0.0005% hemin (Sigma Chemical Co, USA), and 0.00005% menadione (Libavit K, Meta İlaç Sanayi AŞ, Türkiye). The first trypticase soy agar plate was incubated in Gas Pak Jars (AnaeroGen kit, Oxoid Ltd, Basingstoke, Hampshire, England) at 37°C for 7–10 days. The other plate was incubated in 10% CO_2 at 37°C for 5 days. The total viable count (TVC) was calculated as the total number of bacterial colonies on the anaerobically incubated plates. All of the microbiological data was converted to colony-forming units/milliliter (CFU/ml) of transport medium. TVC minus total colony counts on plates incubated in 10% CO_2 was used to the amount of obligate anaerobic bacteria, which was expressed as a percentage of TVC.

From the previously diluted solutions, another sample was obtained to cultivate *Bifidobacterium* species and was plated on *Bifidobacterium* selective agar (Sigma-Aldrich, Germany). The culture plates were incubated for 72 h at 37°C, anaerobically (AnaeroGen, Oxoid, Sollentuna,

Sweden). Later, the number of *Bifidobacterium* species was calculated as the colony counts on plates.

Compliance and adverse reactions

The compliance of the participants and adverse effects of *Bifidobacterium*-containing yogurt during the study were checked and confirmed verbally. The participants were asked whether they presented any symptoms such as diarrhea, stomach gas, signs of infection (chills, fever), allergic reactions (swelling of face/mouth/tongue/lips, hives, itching, rash, difficulty in breathing), and dizziness for checking the adverse effects.

Statistical analysis

The participant was used as the unit of measurement in all statistical analyses. Data analysis was done by a statistical package (IBM-SPSS Statistics, SPSS v.15.0, IBM, Chicago, IL). The data distribution was evaluated by the Kolmogorov-Smirnov test. The gender distributions were determined by the chi-square test between the study groups. The Mann-Whitney *U* or Student's *t*-tests were performed to analyze the inter-group comparisons of the mean based on the normality of distribution. Multiple intra-group comparisons of repeated measures at different time points of the clinical and microbiological analysis were performed using the Friedman test within groups and the Wilcoxon signed-rank test for pairwise comparison. Statistical significance was set at $p < 0.05$.

Results

No statistical differences were detected between the groups in terms of baseline clinical and microbiological parameters ($p>0.05$) (Table 1). Throughout the trial, no adverse effects were identified. All of the individuals completed the 3-month study period and complied according to the requirements of the study.

Clinical data

Clinical parameters are presented in Table 2. Both periodontal treatment modalities resulted in significant reductions in PI, GI, and BOP at 28 days and 3 months in comparison to their respective baseline values ($p<0.05$). When compared to the control group, PI, GI, and BOP scores were significantly lower in the test group at the 28th day and 3rd month after treatment ($p<0.05$). When the reductions (Δ) of these parameters were analyzed, there were significant differences between the baseline and 28-day values (all, $p<0.05$) and between the baseline and 3 months values (all, $p<0.05$, except BOP).

Table 1 Baseline data of the patients

Characteristic	Test group Mean±SD (*N*=15)	Control group Mean±SD (*N*=15)	*p*
Age (years) (range)	41.40±6.8 35–57	42.27±8.8 35–57	0.835*
Gender (F/M)	7/8	8/7	0.715†
PI	1.81±0.23	1.79±0.36	0.548*
GI	1.46±0.34	1.44±0.30	0.874‡
BOP (%)	52.70±21.98	49.16±15.09	0.611‡
PD (mm)	2.76±0.38	2.59±0.43	0.256‡
CAL (mm)	2.93±0.37	2.67±0.46	0.104‡
TVC ($\times10^5$ CFU/ml)	93.95±76.18	75.64±42.72	0.424‡
OA (%TVC)	24.53±25.34	23.67±19.37	0.918‡

*Mann-Whitney's *U* test, †chi-square test, ‡Student's *t* test, $p<0.05$

PI plaque ındex, *GI* gingival ındex, *BOP* bleeding on probing, *PD* probing depth, *CAL* clinical attachment level, *TVC* total viable count, *OA* obligate anaerobes

As shown in Table 2, both the control group and the test group showed significant CAL gain and reduction in PD at the 28th day and 3rd month in comparison to their baseline value ($p<0.05$). There were no significant differences between the study groups in all assessment time points as well as in the changes of clinical parameters (Δbaseline–28 days, Δbaseline–3 months) ($p>0.05$).

Microbiological data

The microbiological data for the TVC ($\times10^4$ CFU/ml) and percentages of obligate anaerobes (%) are presented in Tables 2 and 3. The treatments applied to both groups led to significant decreases in TVC ($\times10^4$ CFU/ml) and percentages of obligate anaerobes (%) at day 28 and month 3 ($p<0.05$), without any inter-group differences ($p>0.05$). When inter-group comparisons were carried out for the changes (Δ) in the amount of TVC ($\times10^4$ CFU/ml) and the percentages of obligate anaerobes, the differences were not statistically significant ($p>0.05$).

As shown in Table 3, throughout the study period, the *Bifidobacterium* species were determined in both groups. At baseline, the number of patients presenting *Bifidobacterium* species was 8 in the control group and 7 in the test group ($p>0.05$). After periodontal treatment, *Bifidobacterium* species were detected in 7 patients in the test group and in 2 patients in the control group at day 28 with a statistically significant difference ($p<0.05$). The 3-month evaluation revealed the number of patients with *Bifidobacterium* species was 6 in the control group and 4 in the test group ($p>0.05$).

Table 2 Overview of all examined parameters

		Test group		Control group		Inter-group *p* value	
Clinical parameters	Time point	Mean±SD	Delta Baseline±SD	Mean±SD	Delta Baseline±SD	For mean	For delta Baseline
PI	Baseline	1.81±0.23		1.79±0.36		0.548*	
	28th day	0.17±0.08[c]	−1.64±0.22	0.34±0.21[c]	−1.44±0.17	0.025*	0.011†
	3rd month	0.18±0.08[c]	−1.63±0.23	0.35±022[c]	−1.43±0.16	0.028*	0.010*
Intra-group $P^{\ddagger}$ value		<0.001		<0.001			
GI	Baseline	1.46±0.34		1.44±0.30		0.874†	
	28th day	0.11±0.04[c]	−1.35±0.36	0.34±0.04[c]	−1.11±0.28	<0.001*	0.048†
	3rd month	0.12±0.05[c]	−1.34±0.35	0.37±0.05[b]	−1.08±0.27	<0.001*	0.029†
Intra-group $P^{\ddagger}$ value		<0.001		<0.001			
BOP (%)	Baseline	52.70±21.98		49.16±15.09		0.611†	
	28th day	6.42±1.80[c]	−46.28±21.95	17.48±3.58[c]	−31.36±14.23	<0.001*	0.039†
	3rd month	10.58±3.16[c]	−42.12±22.30	22.34±5.23[c]	−26.82±11.63	<0.001†	0.028*
Intra-group $P^{\ddagger}$ value		<0.001		<0.001			
PD (mm)	Baseline	2.76±0.38		2.59±0.43		0.256†	
	28th day	1.92±0.34[c]	−0.85±0.31	1.95±0.34[c]	−0.65±0.23	0.828†	0.056†
	3rd month	2.05±0.36[c]	−0.71±0.29	2.06±0.35[c]	−0.53±0.23	0.956†	0.071†
Intra-group $P^{\ddagger}$ value		<0.001		<0.001			
CAL (mm)	Baseline	2.93±0.37		2.67±0.46		0.104†	
	28th day	2.12±0.45[c]	−0.81±0.35	2.05±0.42[c]	−0.62±0.20	0.657†	0.152*
	3rd month	2.26±0.45[c]	−0.67±0.33	2.16±0.43[c]	−0.52±0.21	0.539†	0.130†
Intra-group $P^{\ddagger}$ value		<0.001		<0.001			
Microbiologic Parameters							
TVC ($\times 10^5$ CFU/ml)	Baseline	93.95±76.18		75.64±42.72		0.424†	
	28th day	25.99±19.64[b]	−67.96±67.39	23.90±25.52[b]	−51.74±34.70	0.803†	0.414†
	3rd month	31.95±39.62[b]	−62.00±81.03	24.90±28.31[b]	−50.75±47.68	0.917*	0.646*
Intra-group $P^{\ddagger}$ value		0.002		<0.001			
OA (TVC%)	Baseline	24.53±25.34		23.67±19.37		0.918*	
	28th day	7.12±16.62[a]	−17.41±29.67	8.64±15.37[b]	−15.03±17.03	0.756*	0.790†
	3rd month	7.54±15.33[a]	−16.99±26.55	8.99±16.06[a]	−14.69±26.92	0.833*	0.815*
Intra-group $P^{\ddagger}$ value		0.031		0.015			

*Mann-Whitney's *U* test, †Student's *t* test, ‡Friedman's test, $p<0.05$, [a,b,c]Wilcoxon's sign test, [a]significant difference compared to baseline, $p<0.05$, [b]significant difference compared to baseline, $p<0.01$, [c]significant difference compared to baseline, $p<0.001$

PI plaque ındex, *GI* gingival ındex, *BOP* bleeding on probing, *PD* probing depth, *CAL* clinical attachment level, *TVC* total viable count, *OA* obligate anaerobes

Discussion

This prospective controlled randomized study examined the clinical and microbiological effects of a 28-day adjunctive usage of yogurt containing *B. lactis* DN-173010 with supra and subgingival instrumentation versus supra and subgingival instrumentation alone. The present study showed that clinical improvements in gingival

Table 3 The number of patients presenting *Bifidobacterium* species in groups at baseline, day 28, and month 3

Time point	Test group			Control group			*p*	
	Total *N* (%)	$<10^5$ *N*	$\geq 10^5$ *N*	Total *N* (%)	$<10^5$ *N*	$\geq 10^5$ *N*	Total	$<10^5/\geq 10^5$
Baseline	7 (46.7)	2	5	8 (53.3)	3	5	0.715†	0.573
28th day	7 (46.7)	4	3	2 (13.3)	1	1	0.046†	0.722
3rd month	4 (26.7)	4	4	6 (40.0)	2	4	0.439†	0.076

Chi-square test, $p<0.05$

inflammation and plaque accumulation for the patients using the *B. lactis* DN-173010 containing yogurt presented along with elevated levels of *Bifidobacterium* species.

In relation to the PD as the primary outcome, the findings of this study demonstrated that PD reductions at day 28 and month 3 tended to be higher in the test group (0.85±0.31, 0.71±0.29 mm, respectively) than in the control group (0.65±0.23, 0.53±0.23 mm, respectively); however, no significant difference was detected. The study by Invernici et al. [23], conducted similarly with our research, is the only study using a probiotic lozenge with 1×10^9 CFUs of *Bifidobacterium animalis subsp. lactis* HN019 as a supplement to supra and subgingival instrumentation in chronic periodontitis patients. Administration of probiotics with supra and subgingival instrumentation was reported to reveal the difference between the groups in PD reduction on 30 days (0.48±0.30 mm in the probiotic group versus 0.32±0.27 mm in the placebo group); however, PD reduction was significant in favor of the test group on 90 days (0.52±0.32 mm in probiotic group versus 0.25±0.22 mm in the placebo group). So far, the duration of probiotic bacteria usage such as *Streptococcus* and *Lactobacillus* species in addition to supra and subgingival instrumentation varied considerably. Vivekananda et al. [14] demonstrated significantly higher PD reduction in the group using probiotic lozenge with *L. reuteri* (1×10^8 CFUs) for 21 days in addition to supra and subgingival instrumentation compared to supra and subgingival instrumentation alone. Teughels et al. [16] reported that there were significantly better outcomes for PD reduction in moderate and deep periodontal pockets in the group using lozenges with *L. reuteri* (1×10^8 CFUs) at 12-week follow-up after supra and subgingival instrumentation compared with the group using placebo lozenge, although there was a nonsignificant reduction in full-mouth PD. İnce et al. [17] and Tekçe et al. [18] demonstrated significantly better PD reduction at 3-month and 1-year follow-ups in chronic periodontitis patients using *L. reuteri* (1×10^8 CFUs) for 3 weeks as an adjunct to supra and subgingival instrumentation versus the placebo. Contrary to the above studies, Laleman et al. [19] showed nonsignificant PD reduction between the group receiving a S*treptococci* (1×10^8 CFUs) containing probiotic tablet and the control group receiving a placebo tablet in full-mouth, moderate, and deep pocket analyses at 12 and 24 weeks. Morales et al. [20] compared *L. rhamnosus* SP1 (2×10^7 CFUs) with supra and subgingival instrumentation to supra and subgingival instrumentation alone and no statistically significant intergroup difference was observed in full-mouth PD analysis at 12 months. In a recent study, Vohra et al. [22] reported similar findings in periodontal clinical parameters at 3- and 6-month follow-ups in patients who applied supra and subgingival instrumentation with and without adjunct *L. reuteri* (1×10^8 CFUs) probiotic lozenge for 21 days. A number of different species of *Lactobacillus*, *Streptococci*, and *Bifidobacterium* have been chosen as probiotic bacteria used with supra and subgingival instrumentation for varying time periods and varying initial pocket depths in the abovementioned studies. As in our study, the common result of studies using probiotic bacteria (1×10^8 CFUs of *B. lactis* DN-173010) as an adjunct to supra and subgingival instrumentation and the statistically significant decreases in PD in all of the groups after supra and subgingival instrumentation demonstrate the clinical importance of periodontal treatment with or without probiotics.

Regarding the attachment gain, there was no statistically significant difference between the treatment groups in our study at all time intervals, similar to studies by Laleman et al. [19], Morales et al. [20], and Vohra et al. [22]. On the other hand, some studies reported significant differences in the attachment gain between the study groups in favor of probiotic groups [14, 16–18, 23].

BOP and GI have been used to characterize clinically the degree of gingival inflammation. Our finding that the mean values and reductions from baseline to 1st and 3rd months of PI, GI, and BOP were statistically significant in favor of the test group supported the results by Invernici et al. [23]. It is well known that *B. lactis* is a part of the human microbiota and possesses both immunomodulatory and antimicrobial properties with anti-inflammatory effects. Additional improvement in gingival inflammation reported by Invernici et al. and also in our study could result from the anti-inflammatory effect of *B. lactis*. Moreover, studies using *Lactobacillus* or *Streptococcus* species adjunctive to supra and subgingival instrumentation reported significantly superior changes in PI, GI, and BOP indices as a result of treatment in the probiotic group at all of the assessment time points [14, 17, 18]. However, a contradictory report is also present showing no clinical benefits of probiotics in plaque accumulation and GI [20] or only in a few evaluation time points [16, 19].

Bifidobacterium might live in saliva and attach to *Fusobacterium nucleatum*–covered hydroxyapatite. Co-aggregation may reduce the quantity of periodontopathogens in the oral biofilm, which could be a crucial step in a successful periodontal treatment. The capacity of *F. nucleatum* to facilitate co-aggregation with other periodontopathogens such as *Tannerella forsythia*, *Porphyromonas gingivalis*, and *Aggregatibacter actinomycetemcomitans* appears to be its most important function in subgingival bacterial biofilm [32]. *Bifidobacterium animalis subsp. lactis* was first used in rats in addition to supra and subgingival instrumentation in 2017 and has been shown to promote a higher ratio between aerobic and anaerobic bacteria in biofilm samples [7]. To our knowledge, this is the first study detecting *Bifidobacterium* species in subgingival samples by a culture-based technique. While

the number of patients presenting *Bifidobacterium* species was similar in both groups at baseline, *Bifidobacterium* species were determined higher in the test group patients than the control group after consuming yogurt containing probiotics, as expected. This microbiological improvement was concomitant with significantly lower plaque accumulation and gingival inflammation in the test group on 28 days. Similarly, Invercini et al. [23] demonstrated that DNA copies of *B. lactis* HN019 were present in both groups at baseline and were also higher in subgingival biofilm specimens in the test group at day 30 than in the control group. Unlike our study, *B. lactis* HN019 was detected in subgingival biofilm on 90 days in the study by Invercini et al. [23] suggesting the longer-term effectiveness of the lozenge administration of probiotics adjunct to supra and subgingival instrumentation. Probiotics can be applied in many different forms. Dairy products are crucial in the delivery of probiotic bacteria to humans because they provide a favorable environment for probiotic bacteria growth and survival [33, 34]. In an in vitro study [6], when yogurt *Bifidobacterium* was inoculated first, periodontopathogens were inhibited. The antimicrobial activity of bio-yogurt in vitro could explain the findings of clinical trials that found that regular consumption of probiotic-rich foods has a beneficial effect on periodontal disease [35].

In the present study, the TVC and the percentages of obligate anaerobes decreased significantly in both treatment groups at day 28 and month 3 compared to baseline. However, inter-group analyses of values and changes in the TVC and proportions of obligate anaerobes revealed no differences at any assessment time point. Among the studies using probiotic bacteria with supra and subgingival instrumentation, there was only one study evaluating TVC and the percentages of obligate anaerobes and reporting that the percentages of obligate anaerobes and TVC demonstrated significant differences in favor of the *L. lactis* containing lozenge as adjunctive to supra and subgingival instrumentation on 21, 90, and 180 days [18]. Although our investigation did not attempt to identify specific obligatory anaerobic bacteria, it is thought that the finding of no difference in TVC and the percentages of obligate anaerobes between the treatment groups may be related to the obligatory anaerobic characteristic of *Bifidobacterium*. The main limitation could be the lack of specific bacterial identification, besides the lack of long-term follow-up and comparison with different probiotic administration techniques and durations. However, considering that there is only one study evaluating the effect of *Bifidobacterium animalis* subsp. *lactis* as a probiotic together with subgingival microbiota when lozenges including *Bifidobacterium animalis* subsp. *lactis* were used as an adjunct to non-surgical periodontal treatment of periodontitis, our well-designed clinical study is believed to contribute to the literature related to the usage of probiotics in periodontology.

Conclusions

According to the results of this study, the routine intake of *Bifidobacterium animalis subsp. lactis* DN-173010 containing yogurt with supra and subgingival instrumentation may have a better but limited beneficial impact on periodontal treatment. However, further clinical studies are required with specific analysis of bacteria at different periods of time comparing other dairy probiotic products to evaluate the relationship between the intake of probiotic food and periodontal therapy.

Authors' contributions Hafize Öztürk Özener has been involved in patient selection, sample collection, treatment procedures, data interpretation, and manuscript writing. Leyla Kuru has been involved in manuscript writing and critical reading. Tanju Kadir has been involved in microbiological analysis. Bahar Kuru has been involved in the conception and design of the study, data interpretation, manuscript writing, and critical reading. All authors have read and approved the final version of the manuscript.

Data availability The data presented in this study are available on request from the corresponding author.

Declarations

Ethics approval and consent to participate Written informed consent was obtained from all the participants before enrollment into the study.

Conflict of interest The authors declare no competing interests.

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