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Probiotic milk drink as adjuvant therapy for the treatment of periodontitis: a randomized clinical trial with 180 days follow-up

Fabio Cascardo BILOURO[1], Ramon Silva ROCHA[2,3], Jonas Toledo GUIMARÃES[3], Tatiana Colombo PIMENTEL[4], Marciane MAGNANI[5], Erick Almeida ESMERINO[3], Monica Queiroz de FREITAS[3], Marcia Cristina SILVA[2], Adriano Gomes da CRUZ[2], Antonio CANABARRO[1,6]*

Abstract

The effect of probiotic milk drinks associated with non-surgical periodontal therapy (NSPT) in treating periodontitis was evaluated. A double-blind placebo-controlled randomized clinical trial with patients with periodontitis was conducted. Two groups were used: TG (n=15, test group, probiotic milk drink, *Lacticaseibacillus casei* 01, 8-9 log CFU/mL) and CG (n=15, control group, conventional milk drink). The milk drinks (100 mL) were consumed once a day at breakfast for 15 days. Both groups also received NSPT, supra and subgingival scaling and root planning per quadrant, and oral hygiene instruction. All patients were clinically evaluated after 30, 90, and 180 days of the last NSPT session. Reductions in clinical attachment loss and probing pocket depth were observed at 30 days in both groups, maintained throughout the follow-up. Decreases in visible plaque index (VPI) and bleeding on probing were observed after probiotic milk drink consumption for 30 days, suggesting an effect provided by probiotics in the control of biofilm and inflammation. However, the impact on VPI was not persistent for more than 30 days, demonstrating the need for regular consumption of the probiotic milk drink. In conclusion, probiotic milk drinks may be used as adjuvant therapy to treat periodontitis.

Keywords: probiotic milk drink; gingivitis; periodontitis; non-surgical periodontal debridement.

Practical Application: Probiotic milk drinks (*L. casei*) may be used as an adjuvant therapy to mechanical control to treat periodontitis.

1 Introduction

Periodontitis is defined as an inflammatory disease of the supporting tissues of the teeth, caused by groups of specific microorganisms, resulting in progressive destruction of the periodontal ligament and alveolar bone, with the formation of a periodontal pocket gingival recession, or both (Papapanou et al., 2018). The prevalence of this disease is high and can vary from 20 to 50% worldwide (Messora et al., 2021). This disease is a public health problem and can result in tooth disability and loss, impair aesthetics and chewing, and reduce the quality of life (Messora et al., 2021). Pathogenic bacteria in the microbial biofilm are considered the main etiological factor. In an attempt to increase the efficiency and longevity of conventional periodontal treatment (scaling and root planning, SRP), new adjunct approaches have been discussed, such as antibiotic therapy, phototherapy, laser therapy, homeopathy, and probiotics (Gruner et al., 2016; Jayaram et al., 2016; Morales et al., 2018).

The use of antibiotics has effectively altered the balance of the oral microbiota, eliminating undesirable and pathogenic microorganisms. However, it also eliminates the beneficial bacteria, promoting the growth of resistant microorganisms. Thus, there is an interest in other microbial replacement therapies that could recreate a healthy oral microenvironment (Gupta et al., 2017). Probiotics are live microorganisms that promote benefits to the host when administered in an adequate amount (Hill et al., 2014). Probiotic therapy may reduce the concentration of pathogens residing in the oral cavity (Bustamante et al., 2020) as probiotics compete for sites and nutrients with the pathogens (Alshareef et al., 2020; Invernici et al., 2018; Vives-Soler & Chimenos-Küstner, 2020). Furthermore, probiotics can alter the distribution of the bacteria that colonize the oral biofilm, functioning as a biological approach to microbial replacement (Gupta et al., 2017). In the last decade, there has been a growing interest in probiotics for improving oral health (Chugh et al., 2020; Nadelman et al., 2017, 2019). Probiotic administration (capsules/tablets) has been associated with beneficial effects on periodontal conditions and halitosis (Soares et al., 2019). Furthermore, a change in the subgingival microbiota after probiotic administration has been

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[1] *Departamento de Periodontologia, Universidade Veiga de Almeida – UVA, Rio de Janeiro, RJ, Brasil*
[2] *Departamento de Alimentos, Instituto Federal de Educação, Ciência e Tecnologia do Rio de Janeiro – IFRJ, Rio de Janeiro, RJ, Brasil* [3] *Faculdade de Veterinária, Universidade Federal Fluminense – UFF, Niterói, RJ, Brasil*
[4] *Instituto Federal de Educação, Ciência e Tecnologia do Paraná – IFPR, Paranavaí, PR, Brasil*
[5] *Departamento de Engenharia de Alimentos, Universidade Federal da Paraíba – UFPB, João Pessoa, PB, Brasil*
[6] *Departamento de Periodontologia, Universidade Estadual do Rio de Janeiro – UERJ, Rio de Janeiro, RJ, Brasil*
*Corresponding author: andradejr13@gmail.com

reported (Vives-Soler & Chimenos-Küstner, 2020). Probiotic therapy has been demonstrated to be safe and effective as an adjunct to conventional mechanical periodontal treatment, reducing the need for antibiotics (Matsubara et al., 2016).

In the last years, milk drink consumption has increased due to the consumer demand for products with high nutritional value, such as high-quality protein, calcium, and bioactive compounds (Balthazar et al., 2018, 2019; Monteiro et al., 2020). Furthermore, they have a lower cost than yogurts due to whey's utilization as an ingredient, which is the main by-product of the cheese industry (Coutinho et al., 2021). Previous studies have evaluated the potential of probiotic yogurt (Ghasemi et al., 2017; Kuru et al., 2017; Staab et al., 2009), probiotic fermented milk (Nadelman et al., 2019), probiotic ice cream (Nadelman et al., 2017), and kefir (Ghasempour et al., 2014) in improving oral health. However, the results showed high heterogeneity, and they were dependent on the probiotic characteristics (strain and dose), food product, the clinical status of the population, among others (Magno et al., 2019). Furthermore, no randomized clinical trials have been performed using probiotic milk drinks and patients with periodontitis.

Probiotic liquid matrices, such as milk drinks, may be more effective in improving oral health than capsules (Nadelman et al., 2018). Therefore, the objective of this study was to verify the efficacy of probiotic milk drink (*Lacticaceseibacillus casei* 01) as an adjunct treatment to conventional mechanical therapy in the clinical parameters of periodontitis.

2 Methods

2.1 Probiotic milk drink processing

Two formulations of milk drinks were prepared: probiotic milk drink and conventional milk drink. First, whole pasteurized milk (3% w/v fat, 70%) and powdered cheese whey (Alibra, São Paulo, Brazil, 30%) were mixed, added with xylitol sweetener (2% w/v) and strawberry flavored fruit (5% w/v, Duas Rodas, Jaraguá do Sul, Santa Catarina, Brazil), pasteurized at 72-75 °C, and cooled to 5 °C. Then, the probiotic milk drink was added with 2% w/v of *Lacticaseibacillus casei* 01 (8-9 log CFU/mL) (Chr Hansen, Campinas, Brazil). Both milk drinks were stored under refrigeration (5 ± 1 °C). The final pH of the products was 5.2 (Coutinho et al., 2019; Silveira et al., 2019).

2.2 Clinical trial

This study followed the CONSORT recommendations for randomized clinical trials (CONSORT, 2021). A double-blind, placebo-controlled randomized clinical trial was conducted at the Veiga de Almeida University (UVA) after being approved by the Research Ethics Committee (REC) of UVA (Brazilian protocol number 70594017.0. 0000.5291, REC register number: 2.152.328). The project was also registered in the Brazilian Registry of Clinical Trials (ID: RBR-2sdy28). The experimental design is shown in Figure 1. Thirty volunteers of both sexes, with Periodontitis, were selected from the population referred to the Periodontal clinic at the School of Dentistry – UVA and were randomly allocated into 2 groups: Test Group (TG, n = 15, consumed probiotic milk drink) and Control Group (CG, n = 15, consumed conventional milk drink). Both groups received the milk drinks in a plastic bottle containing 1500 mL at the end of consultation 3 (detailed below). The patients were requested to consume the milk drinks once a day (100 mL) in the morning (together with the typical breakfast) for 15 days. After ingesting the product, patients were instructed to rinse their mouths under running water and, after 30 minutes, perform complete oral hygiene to minimize the possible effects of acidic pH on enamel. The consumption of other milk drinks was not supervised.

All eligible patients were informed of the nature and potential risks and benefits of their participation in the study. The research was conducted in full accordance with ethical principles, including the Declaration of Helsinki and additional requirements.

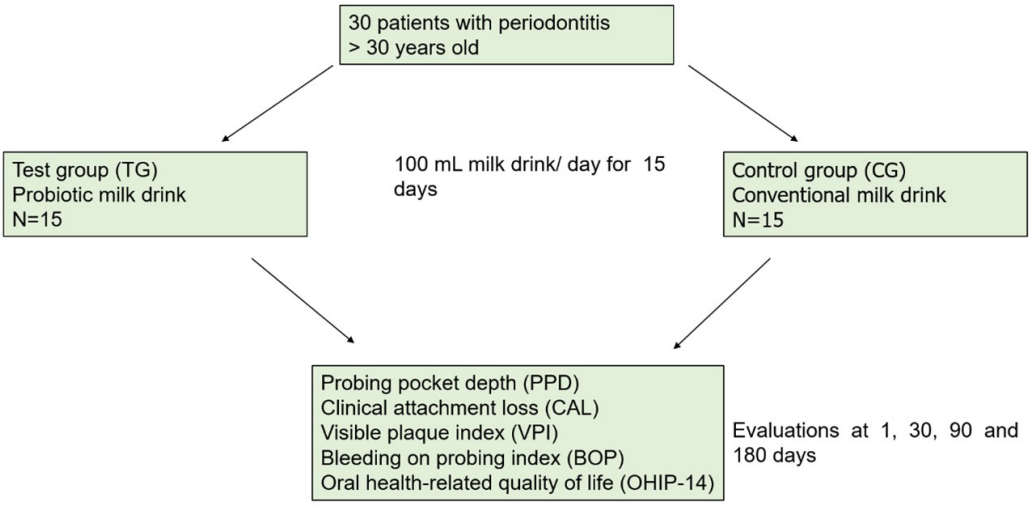

Figure 1. Experimental design.

2.3 Inclusion and exclusion criteria

For the diagnosis of periodontitis, the following criteria were used: clinical attachment loss (CAL) detected in 2 or more non-adjacent interproximal sites; or probing pocket depth (PPD) of 3 mm or more in the buccal or lingual / palatine in at least 2 teeth. Exceptions: 1) gingival recession of traumatic origin; 2) dental caries extending to the cervical area of the tooth; 3) presence of CAL on the distal surface of a second molar and associated with poor positioning or extraction of the third molar; 4) endodontic-periodontal lesion draining through the marginal periodontium, or 5) occurrence of vertical root fracture (Papapanou et al., 2018). The inclusion criteria were patients with at least 20 teeth and older than 30 years old with untreated Periodontitis.

The exclusion criteria were patients that used antibiotics in the 6 months before treatment, pregnancy, presence of acute or necrotizing lesions, diabetes, neurological deficiencies, immunological diseases, use of medications that affect periodontal tissues (phenytoin, cyclosporine, nifedipine, and anti-inflammatory drugs) and smokers.

All patients included in this study fulfilled a questionnaire that contained basic questions, such as age and gender. After it, they signed the Informed Consent Form.

2.4 Outcome variables

PPD was evaluated at 4 sites per tooth, with a manual probe (North Carolina-15 periodontal probe, Hu-Friedy), corresponding to the measurement in mm from the free gingival margin to the bottom of the periodontal pocket / gingival sulcus (Morales et al., 2018). CAL was evaluated at 4 sites per tooth, with a manual probe (North Carolina-15 periodontal probe, Hu-Friedy), corresponding to the measurement in mm from the cementoenamel junction to the bottom of the periodontal pocket (Morales et al., 2018). Visible plaque index (VPI) corresponds to the percentage of faces with visible biofilm in all teeth, 4 faces per tooth (Morales et al., 2018). Bleeding on probing (BOP) corresponds to the percentage of faces with bleeding up to 20 seconds after probing all teeth, 4 faces per tooth. VPI and BOP were scored as plaque and bleeding absent or present (0 or 1, respectively) (Morales et al., 2018). The oral health impact profile (OHIP)-14 questionnaire contained 14 questions structured with answers (never, rarely, sometimes, often, and always) and weighted 0, 1, 2, 3, and 4, respectively, composing a score of 0 to 56 points for the scale. The higher the number of OHIP-14, the lower the oral health-related quality of life (Oliveira & Nadanovsky, 2005).

2.5 Randomization and generation sequence

Simple randomization was used as a strategy. The patients were initially examined by the study coordinator (AC), who defined a random numeric code for each of them. When the desired total number of patients was reached (n = 30), a free online tool was (https://www.4devs.com.br/sorteador) for the allocation of patients in the two different groups with the same number of components. Codes were only revealed after the statistical analysis.

2.6 Allocation: concealment mechanism

The products were also coded by a blinded author who only revealed them after the statistical analysis. This author was responsible for the initial assessment, randomization, and allocation in the TG and CG groups. One professional processed the milk drinks and distributed them according to the randomization performed. This professional was unaware of the product used by the different groups. Finally, patients received the encoded products and did not know which group they belonged to.

2.7 Calibration

Clinical evaluations were performed by a single calibrated periodontist (FCB). He served many evaluations until a satisfactory level of agreement was reached. Ten periodontitis patients (not included in the study) with at least two teeth with PPD and CAL ≥ 4 mm were selected. The intra-examiner Kappa index should be greater than 0.80 for all indexes evaluated to begin the study.

2.8 Interventions

Periodontal treatment was performed by only 1 professional (FCB). In Phase 1 (Pre-treatment), a consultation of 30 min was performed with the objective of diagnosis. Then, clinical examinations (PPD, CAL, VPI, and BOP), the questionnaire's completion, and the informed consent form were performed. Next, the patients were requested for toothbrushes and dental floss. Then, the second consultation of 30 min was performed to reduce supragingival biofilm's presence and prevent cross-contamination between treatment sessions (Preus et al., 2013). For that, a supragingival scaling of all teeth was performed.

Furthermore, patients were instructed for oral hygiene (brush and dental floss) in front of the mirror. After training, the recommendation for all patients was to brush with a soft brush for 2 minutes (at least) and floss twice a day throughout the study period. In Phase 2 (Treatment of periodontitis), 4 consultations were performed for 30 min each. Scaling and root planning (SRP) of quadrant I, II, III, and IV with anesthesia and ultrasonic instruments (Dabi Atlante, Rio de Janeiro, RJ, Brazil) associated with hand instruments (Gracey curettes, Hu-Friedy, Chicago, IL, USA) was performed. Note: if there was no need for SRP in one quadrant, it was automatically moved to the next. This served all other areas. Daily hygiene was not supervised.

2.9 Follow-up

After the initial phase, patients returned at 30, 90, and 180 days for reassessment. Clinical examinations were carried out in all follow-up consultations, and a new OHIP questionnaire was completed. In the maintenance consultations, performed at 90 and 180 days, the patients also received reinforcement in oral hygiene and SRP instruction.

2.10 Statistical analysis

For statistical analysis, the program SPSS 17.0 (IBM) was used. The data were organized in tables, and parametric

tests, ANOVA followed by the Duncan test, were used after confirming the normality of the data (Kolgomorov-Smirnov test). The sample size was calculated based on previous work (Teughels et al., 2013). Considering the primary endpoint, the reduction in PPD, the following values were used as parameters for the sample calculation: standard deviation of 0.47 mm and difference between the PPD of the TG and the CG at the end of the treatment 0.56 mm. Thus, 15 patients per group were needed for a study power of 90% and a significance level of 5%.

3 Results

Thirty patients started the study, but only 24 patients completed it. The participants alleged particular reasons to give up the study, with no correlation with the consumption of the milk drinks. The TG group consisted of 12 participants (7 women and 5 men) who were 55.67 ± 10.87 years old. The CG consisted of 12 participants (7 women and 5 men) who were 49.83 ± 9.84 years old.

The consumption of probiotic (TG) or conventional (CG) milk drinks associated with non-surgical periodontal therapy (NSPT) resulted in a significant reduction in PPD and CAL at 30 days ($p < 0.05$). This reduction remained throughout the follow-up evaluation (90 and 180 days, $p < 0.05$). Therefore, both treatments effectively reduced PPD and gained periodontal attachment for 6 months (Table 1). The consumption of probiotic milk drink (TG) also resulted in a significant reduction in VPI and BOP at 30 days ($p < 0.05$). However, the decrease in VPI was not verified in the other periods ($p > 0.05$), indicating a return to the initial values with the interruption of probiotic use. In addition, the reduction in BOP remained throughout the study ($p < 0.05$), indicating an additional effect of the probiotic through the 6 months follow-up. Regarding OHIP, no differences were found in intra or inter-group comparisons ($p > 0.05$).

Table 1. Mean and SD of clinical parameters was evaluated at baseline and after adjusting probiotics (GT) or placebo (GC) for 30, 90, and 180 days.

Variable	Timepoint	TG mean (SD)	CG mean (SD)	p-value
PPD (mm)	Baseline	4.61 (0.34)^{A}	4.60 (0.37)^{A}	0.967
	30 days	3.39 (0.72)^{B}	3.47 (0.74)^{B}	0.785
	90 days	3.39 (0.71)^{B}	3.64 (0.47)^{B}	0.335
	180 days	3.43 (0.84)^{B}	3.24 (0.58)^{B}	0.529
CAL (mm)	Baseline	4.75 (0.31)^{A}	5.01 (0.83)^{A}	0.325
	30 days	3.60 (0.75)^{B}	4.11 (1.16)^{B}	0.213
	90 days	3.63 (0.78)^{B}	3.81 (0.86)^{B}	0.590
	180 days	3.41 (0.88)^{B}	4.00 (1.09)^{B}	0.159
VPI (%)	Baseline	38.31 (18.75)^{A}	45.33 (28.72)^{A}	0.251
	30 days	16.45 (16.25)^{B}	34.07 (15.82)^{A}	**0.013***
	90 days	28.63 (20.50)^{A}	33.99 (23.81)^{A}	0.876
	180 days	29.29 (16.33)^{A}	36.21 (20.10)^{A}	0.830
BOP (%)	Baseline	30.83 (16.42)^{A}	38.12 (33.29)^{A}	0.503
	30 days	14.02 (13.52)^{B}	30.99 (18.24)^{A}	**0.011***
	90 days	12.61 (14.63)^{B}	21.05 (15.88)^{A}	0.189
	180 days	14.60 (15.16)^{B}	23.90 (24.84)^{A}	0.280
OHIP	Baseline	19.10 (14,20)^{A}	17.60 (6.06)^{A}	0.729
	30 days	11.17 (12.87)^{A}	15.20 (7.41)^{A}	0.340
	90 days	8.33 (9.25)^{A}	12.60 (2.80)^{A}	0.071
	180 days	8.83 (8.10)^{A}	12.00 (1.89)^{A}	0.095

*Intergroup analysis (TG vs. CG, lines): ^{AB}Significant; Intragroup analysis (columns): different letters indicate statistical differences ($p<0.05$). PPD: probing pocket depth; CAL: clinical attachment loss; VPI: visible plaque index; BOP: bleeding on probing; OHIP: oral health impact profile.

4 Discussion

In the present study, the effectiveness of probiotics when associated with NSPT in the treatment of periodontitis was evaluated. It was found that the consumption of probiotic or conventional milk drinks associated with NSPT effectively reduced the pocket depth and gained periodontal attachment. The effectiveness of NSPT in reducing clinical parameters of periodontitis has already been established (Canabarro et al., 2015; Heitz-Mayfield et al., 2002) and is associated with the initial reduction in the number of pathogenic bacteria and the interference in the bacterial composition of the biofilm (Morales et al., 2018). However, pathogen reduction appears to be transient, even when the therapy is combined with antiseptics or antibiotics (Teughels et al., 2013). Furthermore, probiotics can benefit many infectious diseases, including those of the oral cavity (Bustamante et al., 2020). Possible mechanisms are reducing the cultivable microbiota, direct interaction with the pathogenic microbiota, modulation of the immune response, and synthesizing antimicrobial compounds (Vives-Soler & Chimenos-Küstner, 2020). Besides, they can reduce the periodontal pathogens of the red and orange complexes (Invernici et al., 2018) and the levels of pro-inflammatory cytokines compared to the control group (Alshareef et al., 2020; Invernici et al., 2018). However, the mechanisms of action are dependent on the probiotic strain and probiotic product (Ho et al., 2020).

In this study, no significant improvement on PPD and CAL after probiotic milk drink consumption was observed compared to the consumption of conventional milk drinks. Studies have shown high heterogeneity regarding the advantages of using probiotics over these parameters (Ikram et al., 2018; Pelekos et al., 2019; Vives-Soler & Chimenos-Küstner, 2020), and reductions in PPD and clinical attachment gain have been reported in some cases (Tekce et al. 2015; İnce et al., 2015). However, these studies are related to severe periodontitis (PPD > 5 mm at baseline) and deep periodontal pockets, which may favor the probiotic effect (Ho et al., 2020; Vives-Soler & Chimenos-Küstner, 2020). Probiotic therapy may show better results in clinical symptoms of periodontitis in high-risk patients (Messora et al., 2021). Furthermore, improvements in CAL are commonly observed with prolonged administration of probiotic products and extended follow-up (Gruner et al., 2016), with significant impact after 3 months and more pronounced effects after 12 months (Ho et al., 2020). Therefore, the consumption of probiotic milk drinks for 14 days did not improve PPD and CAL in patients with non-severe periodontitis (PPD < 5 mm at baseline).

Probiotic milk drink consumption resulted in reductions in VPI and BOP at 30 days, which has already been reported in previous studies with *L. reuteri* as probiotic culture (İnce et al., 2015; Tekce et al., 2015; Vivekananda et al., 2010), which is a microorganism that is effective in controlling periodontitis (Martin-Cabezas et al., 2016). However, this is the first study

to observe improvements in periodontitis's clinical symptoms after consuming a probiotic milk drink using *L. casei* as probiotic culture. The biofilm was reduced only in the first month of the study. With the stoppage of probiotic consumption, the values returned to the initial parameters, which suggest a temporary effect and the need for long-term consumption of probiotic milk drinks. Probiotic cultures may incorporate into the subgingival biofilm, resulting in persistence in the oral cavity after discontinuing the probiotic therapy (Tekce et al., 2015). Furthermore, they can inhibit the adhesion and colonization of pathogens to hard and soft tissues when presented in adequate concentrations (Alanzi et al., 2018). The regular consumption of probiotics has been recommended by several authors (Jayaram et al., 2016; Matsubara et al., 2016; Vives-Soler & Chimenos-Küstner, 2020). Therefore, the regular consumption of probiotic milk drinks may control biofilm.

BOP was reduced in the first month with probiotic milk drinks, and the reduction was maintained throughout the study. Therefore, the probiotic product promoted a residual effect on inflammation. Probiotic cultures may decrease gingival inflammation, and this effect has been associated with reductions in the levels of pro-inflammatory cytokines, such as interleukin-1 β, TNF-α, and IL-17 (Alshareef et al., 2020). In this way, the probiotic effect may be on host responses and biofilm composition, not decreasing periodontal pathogens' counts (Ho et al., 2020).

In this study, probiotics did not offer side effects or adverse reactions. Furthermore, the six participants that gave up the study indicated personal issues, such as change of address, new job, lack of time, among others, which were unrelated to the consumption of the products. Thus, it can be said that the product is safe, well-tolerated, and does not affect people's quality of life. Furthermore, it can be interesting to use concomitantly with mechanical control if it is desired to reduce biofilm and gingival inflammation.

The present study has some limitations. The number of patients evaluated (n = 30) and the relatively short period of consumption (15 days) may not be sufficient to formulate clinical recommendations (Ho et al., 2020). However, the number of individuals was higher than needed for a study power of 90% and a significance level of 5%. Furthermore, as it was the first study comprising probiotic milk drinks, comparisons with previous studies are difficult, as different designs and methodologies have been applied (Jayaram et al., 2016). Future studies should homogenize primary and secondary outcomes, increase volunteers, and prolong the consumption time (Bustamante et al., 2020). Finally, the milk drinks were sweetened with xylitol (2%), and this dose was considered insufficient to alter the oral microbiota (Alanzi et al., 2018). Therefore, the effects may be attributed to the probiotic milk drink consumption.

5 Conclusion

This is the first study to evaluate the efficacy of probiotic milk drink (*L. casei* 01) on the clinical parameters of periodontitis. Probiotic milk drinks (*L. casei*) may be used as an adjuvant therapy to mechanical control for the treatment of periodontitis, with improvements in biofilm control and inflammation. The daily consumption of 100 mL of the product is advisable for the persistence of the benefits. The results are essential for dairy industries, which can use functional claims associated with oral health in their products. Furthermore, consumers may benefit from probiotics by consuming dairy products instead of capsules or tablets.

Conflict of interest

The authors declare no conflict of interest.

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