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Prato cheese containing *Lactobacillus casei* 01 fails to prevent dextran sodium sulphate-induced colitis

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ABSTRACT

The role of experimental probiotic Prato cheese containing *Lactobacillus casei* 01 in the prevention of dextran sodium sulphate (DSS)-induced ulcerative colitis in mice was evaluated. For the DSS *in vivo* model, mice were divided into six groups. Groups 1–3 represented noninflamed groups and groups 4–6 received a DSS (2%) solution. Mice from groups 1 and 4 intragastrically received 500 μL of phosphate-buffered saline (control groups), while mice from groups 2 and 5 intragastrically received 500 μL of conventional Prato cheese (control groups). Finally, mice from group 3 and 6 intragastrically received 500 μL of Prato probiotic cheese containing *L. casei* 01 (9.47 log cfu mL^{-1}). Groups treated with probiotic Prato cheese exhibited reduced weight loss caused by the consumption of DSS solution. However, the probiotic cheese failed to reduce the inflammation scores in DSS-induced colitis group. Overall, probiotic Prato cheese was not effective to ameliorate the symptoms of DSS-induced colitis.

1. Introduction

Inflammatory bowel diseases (IBD), including ulcerative colitis (UC) and Crohn's disease (CD), are disorders that severely affect the gastrointestinal tract (GIT), which can lead to irreversible impairment of its structure and function (Schreiber, Nikolaus, & Hampe, 1998). Although IBDs are marked by periods of clinical remission and relapse whose exact aetiology is still not well understood, scientific evidence suggests that they result from abnormal immune responses to the gut microbiota in individuals with genetic predisposition (Santos Rocha et al., 2014). Epidemiological studies have shown that the incidence of IBD has increased considerably throughout the world, thus becoming an important global public health problem (Ananthakrishnan, 2015). According to Kaplan (2015), the reported prevalence of Crohn's disease and ulcerative colitis in Northern America and Europe is approximately 300 per 100,000 inhabitants. However, in countries undergoing robust industrialisation, like South American countries, the incidence, and prevalence of IBDs has increased, mainly due to the drastic changes in eating habits together with the food production system (Ng et al., 2017). Moreover, these changes may alter the host's commensal microbiota and the immune response, in addition to the genetic predisposition of the individuals (Kaplan, 2015).

UC is the most common clinical form of IBD, and the main symptoms include abdominal pain, diarrhoea, rectal bleeding, malaise, and weight loss (Neurath, 2012). The inflammation during UC is limited to the colon and affects only the mucosa and submucosa layers of this segment, with the presence of oedema, goblet cell mucus depletion, changes in tissue architecture and ulcerations (Cho, 2008; Maloy & Powrie, 2011; Tontini, Vecchi, Pastorelli, Neurath, & Neumann, 2015). The treatments of UC are based on the administration of anti-inflammatories, immunosuppressive drugs, antibiotics or surgeries (Pithadia & Jain, 2011). Although such treatments reduce the inflammatory action of the disease and relieve symptoms, they are not curative and can lead to serious side effects in patients (Luerce et al., 2014). In this context, probiotic bacteria have been suggested as promising candidates for the UC

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treatment (Chibbar & Dieleman, 2015; Elsa, Chain, Sokol, Langella, & Bermudez-Humaran, 2017; Hosoya, Ogawa, Sakai, & Kadooka, 2012; Luerce et al., 2014; Santos Rocha et al., 2014).

Probiotics are live microorganisms that, when administered in adequate amounts, confer a health benefit on the host (Hill et al., 2014; WHO, 2002), and several strategies to improve their survival in food products have been reported (Champagne, Gomes da Cruz, & Daga, 2018). Probiotic bacteria have been extensively explored in inflammatory disease models with promising results. The therapeutic effects of probiotics are based on different mechanisms of action, which have been successfully demonstrated in experimental colitis animal models (Abraham & Quigley, 2017).

Among the probiotic microorganisms, *Lactobacillus casei* 01 stands out, a lactic acid bacteria strain that has been associated with health benefits, such as immune system stimulation, antioxidant activity, cholesterol lowering, anticarcinogenic activity and reduction in pathogen infection symptoms (Galdeano & Perdigo, 2006; Sperry et al., 2018). In addition, a clinical trial with rheumatoid arthritis (RA) patients showed an improved immune response in RA patients serum after the supplementation with a daily capsule of probiotic *L. casei* 01 (10^8 cfu), with an increase in anti-inflammatory cytokine IL-10 and, consequently, a reduction of the inflammation process caused by RA (Alipour et al., 2014); thus, *L. casei* 01 strain could be used as RA adjunctive therapy. Studies regarding probiotic lactic acid bacteria and previous finds concerning *L. casei* 01, reinforce the choice of this strain as a good candidate for a "2 in1" probiotic and dairy product.

A great number of potential health benefits have been associated with the regular intake of probiotic-containing products, especially fermented dairy products, including some types of cheese (Carmo et al., 2017). In addition, a single-bacteria cheese (*Propionibacterium freudenreichii*) was able to reduce experimental colitis in mice model (Plé et al., 2015). Strategies to improve the efficacy of the therapeutic effects through the addition of probiotics to food products have been studied, indicating the important role of bacterial viability factor (Champagne et al., 2018; Hill et al., 2014).

Cheese has been recognized as an adequate matrix for protection of probiotics, due to its high fat and protein contents, which can form complex coacervates that enable the microencapsulation of the probiotic bacteria for oral delivery (Chapeau et al., 2017; Cruz, Buriti, Souza, Faria, & Saad, 2009; Montassier et al., 2016), besides the benefits of regular ingestion confirmed by several authors in animal and human clinical models (Lollo et al., 2015; Sperry et al., 2018). Prato cheese is a ripened Brazilian cheese, accounting for 20% of all cheese produced in Brazil (Nepomuceno, Junior, & Costa, 2016). Previous studies have shown a good performance of Prato cheese as a functional food, i.e., a nutritional supplement with the ability to promote physiological benefits to the host, which is consumed as part of a regular diet. In fact, Prato cheese was shown to have the ability to protect the probiotic bacteria (*L. casei* 01) during ripening and storage in previous studies (Silva et al., 2017, 2018a,b), besides the good performance of *in vivo* studies on the prevention of development of renal calculi in rat model (Martins et al., 2018). It is important to perform different clinical trials using probiotics associated to foods which taken part of the normal diet of the a population to provide data that will help improve the understanding of the benefits caused by the probiotic strain as there is a consensus which they are related to the interaction among the probiotic strain and food matrix. In this context, the aim of this present study was to evaluate the role of probiotic Prato cheese made with *L. casei* 01 in the prevention of dextran sodium sulphate (DSS)-induced ulcerative colitis in mice.

2. Material and methods

2.1. Cheese processing

Two types of cheese were produced, as follows: a conventional cheese i. e. Prato cheese (starter culture consisting of *Lactococcus lactis* ssp. *lactis* and *Lc. lactis* ssp. *cremoris* R-704) and a probiotic cheese containing *L. casei* 01 (Chr. Hansen, Valinhos, Brazil), as described by Silva et al. (2018c). The bacteria viability during cheese processing and storage (30 days) was evaluated in a previous study (Silva et al., 2017). The experiment was conducted at the Advanced Centre in Food Technology (NATA), using 120 L of full-fat pasteurised milk (65 °C, 30 min). Milk was cooled until 37-35 °C, and the lactic acid bacteria starter (*Lc. lactis* ssp. *lactis* and *Lc. lactis* ssp. *cremoris* R-704) was added directly to the milk (1% w/v, 7–8 log cfu g^{-1}) and allowed to stand for 40 min. For the manufacture of probiotic cheese, the *L. casei* 01 were added together with the starter culture directly to the milk (2% w/v, about 7–8 log cfu g^{-1}) and allowed to stand for 40 min. Then, calcium chloride (80 mL per 120 L milk), annatto dye (36 mL per 120 L milk) and coagulant (Ha La 1175, Chr Hansen Industria e Comercio, São Paulo, Brazil) were added for milk coagulation within 35–50 min. The optimal curd set point was determined, and the curd was cut into 1 cm cubes and submitted to slow mixing for 15 min. Then, part (30%) of whey was removed, and further heating was carried out by progressively adding hot water at 80 °C (25 L) to increase the temperature to 42 °C (0.33 °C min^{-1}), until reaching the typical consistency of Prato cheese. Then, the curd was placed in rectangular plastic moulds (2 kg) and pressed (0.1 MPa for 15 min; 0.24 MPa for 30 min; and 0.31 MPa for 90 min). Cheeses were kept for 5 h at room temperature and then dried at 12 °C for 72 h, vacuum-packed, and stored at 12 °C for 25 days.

2.2. Probiotic bacteria and lactic acid bacteria counts

M17 agar (Oxoid Brasil LTDA, São Paulo, Brazil) was used to enumerate *Lc. lactis*, incubated at 37 °C for 72 h under aerobic conditions. The *L. casei* 01 counts were performed in duplicate using MRS agar (Oxoid Brasil LTDA, São Paulo, Brazil) containing vancomycin 0.1% (w/v), and incubated at 37 °C for 72 h under anaerobic conditions (Silva et al., 2018a,b). Anaerobic jars (Anaerobac Probac Ltd.®) were used to generate an anaerobic atmosphere, thus ensuring anaerobic conditions.

2.3. Proximate composition, calcium and sodium levels

The proximate composition (moisture, protein, and fat; g 100 g^{-1}) and the mineral contents (Ca and Na) were determined according to the conventional methods (Silva et al., 2018a). Moisture was determined by oven-drying 5 g sample at 100–105 °C for 24 h. Protein was determined by the Kjeldahl method, and fat was determined by the Gerber method. Ca and Na levels were determined by atomic absorption spectrometry in an air-acetylene flame using the iCE 3000 series atomic absorption spectrometer (Thermo-Scientific, Hemel Hempstead, Hertfordshire, UK).

2.4. Animal model for ulcerative colitis

Female C57/BL6 inbred mice strain of 8 weeks of age were obtained at Federal University of Minas Gerais (UFMG–Belo Horizonte, Brazil). Mice were kept in a temperature-controlled room with access to water and standard laboratory chow diet ad libitum. The study was approved by the Ethics Committee on Animal Experimentation of the Federal University of Minas Gerais (CEUA-UFMG, Brazil, protocol 340/2017).

2.5. Manufacture of probiotic cheese and conventional Prato cheese for in vivo model

Both cheeses were suspended in phosphate buffered saline (PBS; NaCl 8 g L^{-1}; KCl 0.2 g L^{-1}; NaH_2PO_4 1.44 g L^{-1}; K_2HPO_4 0.24 g L^{-1}; pH 7.4). Briefly, the cheeses were weighed and 250 mg of Prato cheese or probiotic cheese were resuspended in 250 μL PBS buffer pH 7.4 and homogenised with the aid of the IKA T 10 Basic Ultra Turrax homogeniser probe for 2 min. Samples were prepared daily according to this procedure, prior to intragastric gavage. Bacterial viability in cheese solution (Prato cheese or probiotic Prato cheese) was determined by cfu counts, which were performed according to the previously described protocol (section 2.2). Each mouse received 500 μL of cheese solution (Prato cheese or probiotic Prato cheese) by intragastric administration.

2.6. Pretreatment and DSS-induced colitis

The pretreatment with cheese (Prato cheese or probiotic Prato cheese) and colitis induction was performed according to schematic workflow (Fig. 1). The animals had free access to food before and during the DSS colitis induction phase. The intragastric administration of probiotic cheese and conventional Prato cheese solution was performed for 7 days before the beginning of the DSS colitis induction. At day 8, the colitis was chemically induced by administration of 2% (w/v) DSS aqueous solution (36–50 kDa, MP Biomedicals, CAT 260110, LOT Q5756), for 7 days (day 15). For the experimental *in vivo* study, mice were divided into six groups, each containing 5–6 animals per group. Animals from group 1–3 represented the noninflamed group that drank DSS-free water, and consisted of: group 1 receiving intragastrically 500 μL PBS (group PBS); group 2 receiving intragastrically 500 μL conventional Prato cheese suspension (group Prato cheese) and group 3 gavage with 500 μL probiotic Prato cheese suspension containing *L. casei* 01 (group Probiotic cheese). Mice from group 4–6 received DSS (2%) solution as the only source of drinking water to cause colon inflammation, and consisted of: group 4 receiving 500 μL PBS (group PBS + DSS); group 5 receiving 500 μL conventional Prato cheese suspension (group Prato cheese + DSS) and finally, group 6 receiving 500 μL probiotic cheese suspension (group Probiotic cheese + DSS). All mice were euthanized at day 15 (last day of the experimental). Xylazine (8 mg kg^{-1}) and ketamine (100 mg kg^{-1}) were administered by intraperitoneal route and mice were subsequently euthanised by cervical dislocation.

2.7. Weight monitoring, food intake, and liquid intake

Mice weight was individually measured every day until the end of the experiment (day 15). Weight change was expressed as the percentage of change in weight in relation to the initial body weight. Food intake and liquid intake was carefully measured throughout the experiment.

2.8. Colitis macroscopy analysis

The disease activity index (DAI) was determined as described by Murthy et al. (1993) by scoring three major colitis clinical signs: weight loss, diarrhoea, and rectal bleeding. All mice were sacrificed at day 15, and longitudinal abdominal incision was performed to remove the intestine and colon for further analyses. The colon length was measured from the cecum to the final portion of the rectum. The values obtained for each animal were used to calculate the mean of each group.

2.9. Colitis histomorphological analysis

For histomorphological analysis, the distal portion of the mice colon was collected after the euthanasia and washed with PBS. Afterward, tissue samples were immersed in formaldehyde solution (4%) for tissue fixation. The material was embedded in paraffin, and a 4 μm section was placed on a glass slide and stained with haematoxylin and eosin (HE). Slides of each experimental group were photographed (20 × magnification objective) using a digital camera (Spot Insight Color) coupled to an optical microscope (Olympus, BX-41, Japan). The histological inflammation score was determined as described by McCafferty et al. (2000), considering the following features: extent of destruction of normal mucosal architecture (0: normal; 1: mild; 2: moderate; and 3: extensive damage), presence and degree of cellular infiltration (0: normal; 1: mild; 2: moderate; and 3: transmural infiltration), extent of muscle thickening (0: normal; 1: mild; 2: moderate; and 3: extensive thickening), presence or absence of crypt abscesses (0: absent; 1: present) and the presence or absence of goblet cell depletion (0: absent; 1: present). The histopathological score was measured by a

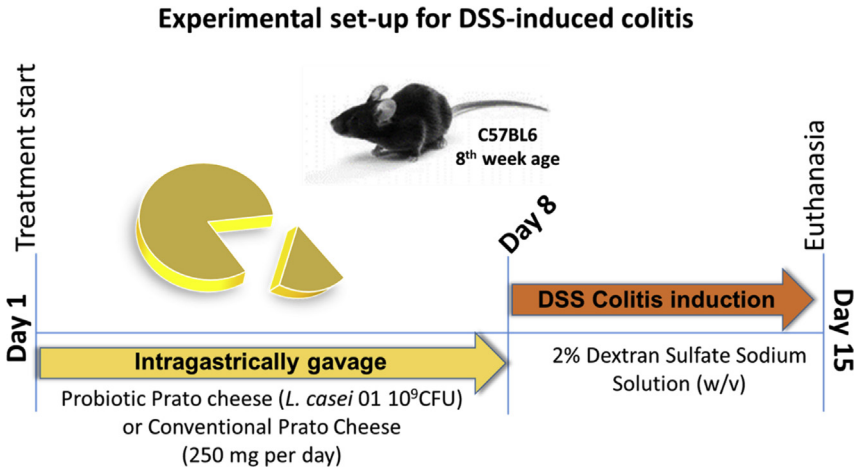

Fig. 1. Experimental protocol of colitis induced in a murine model. C57BL6 mice (n = 6 animals per group) were pre-treated with Prato cheese or probiotic cheese for 7 days.

pathologist, through the evaluation of the histological colon sections.

2.10. Measurement of secretory IgA

To determine the secretory IgA (sIgA), the small bowel intestinal content of mice was collected using PBS, and the sIgA concentration was determined as previously described by Cordeiro et al. (2018). Briefly, intestinal fluid samples from the small bowel were vortexed and centrifuged for 30 min at 850×*g* at 4 °C. Then, the supernatant was transferred to NuncMaxiSorp 96 well ELISA plates and used for determining sIgA concentration. The results were expressed as the sIgA concentration (μg) per mL of intestinal fluid, according to the standard curve.

2.11. Relative expression of cytokines in colon

The quantitative gene expression in colon tissue was determined according to Oliveira et al. (2018). After mice euthanasia, small fragments (1 cm) of colon were collected and total RNA was isolated using RNeasy mini kit (Qiagen; Hilden, Germany) according to the manufacturer's protocol. Samples were treated using DNase I to digest residual genomic DNA (Invitrogen; Waltham, MA, USA) and then Turbo DNA-free Kit® (Ambion; Austin, TX, USA) was used for DNA removal according to manufacturer's instructions. Reverse transcription was performed to obtain cDNA of the samples, using High Capacity cDNA Reverse Transcription kit (Applied Biosystems; Foster City, CA, USA). The quantitative PCR (qPCR) was determined using iTaq universal SYBR green supermix (Biorad; Hercules, CA, USA) and gene specific-primers for IL-10, IL-6, *muc2*, Claudin-1 (Cld1), ZO-1, ZO-2 and Occludin, and housekeeping genes for β-actin and GAPDH 23. The amplification cycle consisted of the following steps: 95 °C for 30 s, and 40 cycles of 95 °C for 15 s and 60 °C for 30 s on an ABI PRISM 7900HT Sequence Detection System (Applied Biosystems). The results of gene expression of the control group (with no treatment) were used as calibration data. Results were expressed as a fold-change of expression levels, using the mean and standard deviations of target cytokine expression ($2^{-\Delta\Delta Ct}$).

Colonic Barrier Integrity Index was determined according to Zaylaa et al. (2018), which indicates the strain potential to restore the epithelial barrier function. The index was calculated by combining the % of mRNA relative gene expression of four tight junction proteins (Occludin, Claudin-1, ZO-1, and ZO-2) when compared with the PBS control group, which was considered as 100%.

2.12. Statistical analysis

All analyses were performed in triplicate, and the results were expressed as mean ± standard deviation. Parametric data were analysed using one-way ANOVA followed by Tukey post-test. Statistical analyses were performed in GraphPad Prism version 7.00 for Windows (GraphPad Software, San Diego, CA, USA.). Asterisks represent significant differences between the strains, and were indicated as follows: $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$; $^{****}p < 0.0001$.

3. Results

3.1. Probiotic and lactic acid bacteria counts, proximate composition, and calcium and sodium contents of probiotic and conventional Prato cheese

Both the starter and probiotic counts remained above 8 log cfu g^{-1} for all cheeses after 60 days of refrigerated storage, with values of 8.12–9.02 and 8.75 log cfu g^{-1} for *Lc. lactis* and *L. casei* 01,

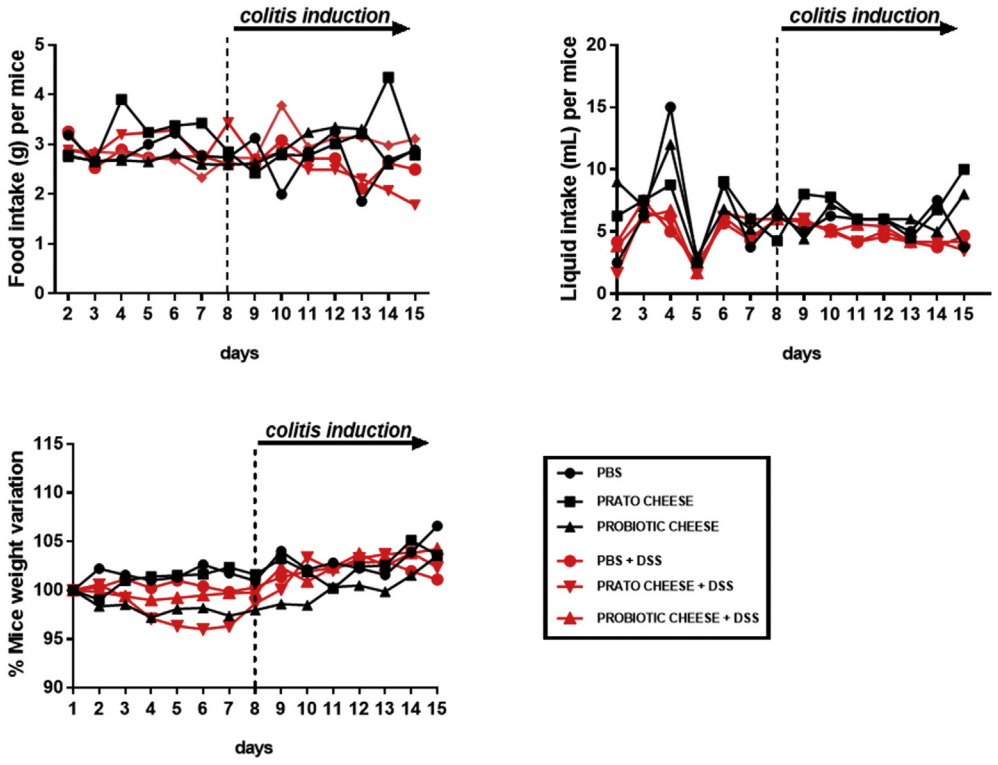

Fig. 2. Food consumption (A), liquid consumption (B) and weight variation (C) observed during experimental procedure: ●, phosphate buffered saline (PBS); ■, Prato cheese; ▲, probiotic cheese; ●, PBS + DSS; ▼, Prato cheese + DSS; ▲, probiotic cheese + DSS.

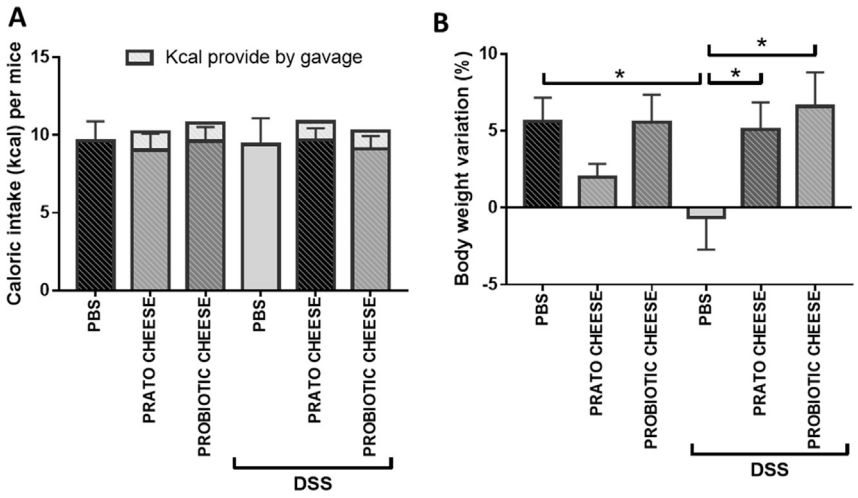

Fig. 3. Caloric intake in kcal per mice (A) during experimental procedure and weight loss (B) observed after beginning colitis induction.

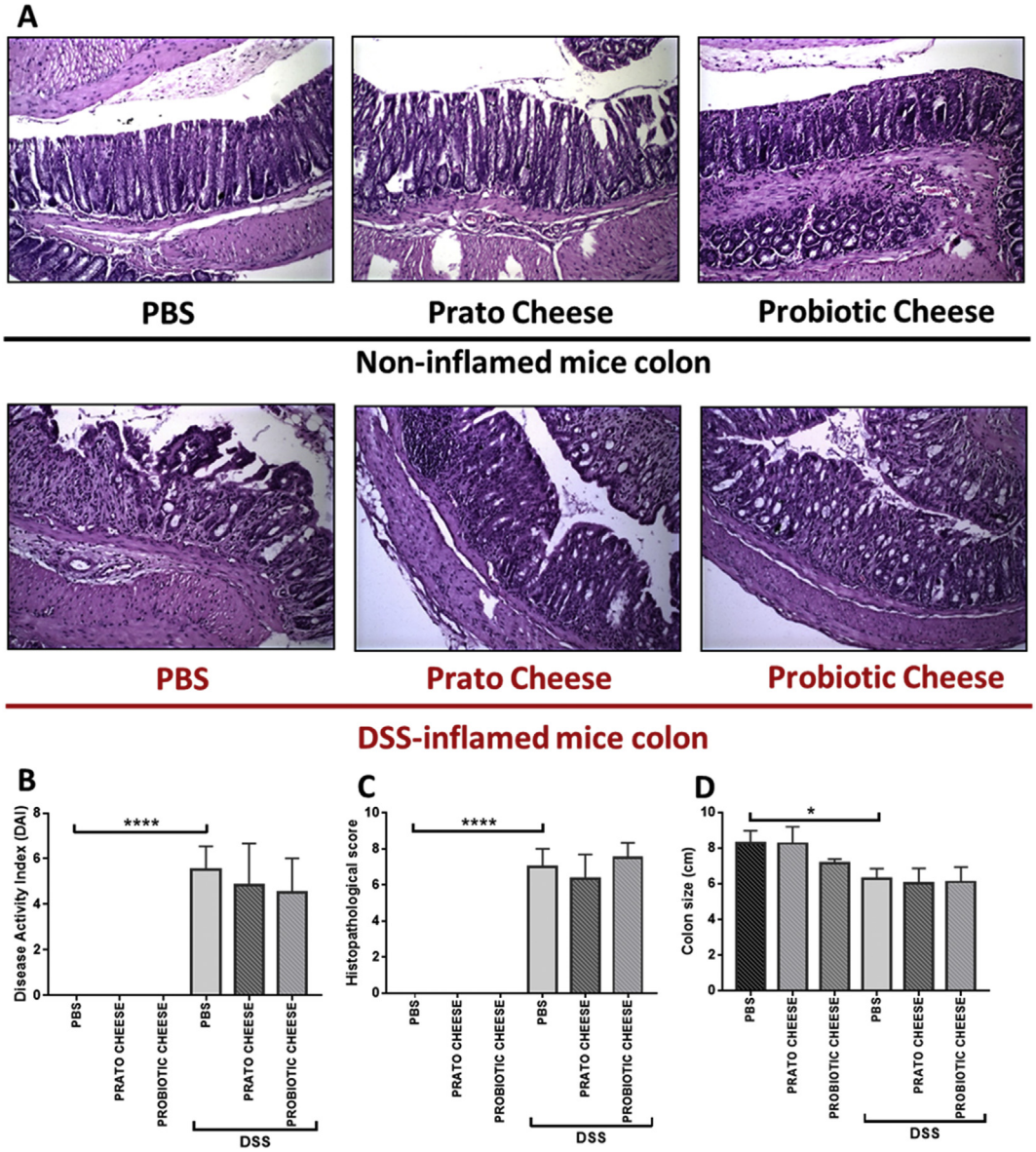

Fig. 4. Representative haematoxylin and eosin-stained images from colon mucosal histopathology (A), (B) disease activity index (DAI) for assessing DSS colitis severity and (C) histopathological score obtained in mice and (D) colon shortening (E).

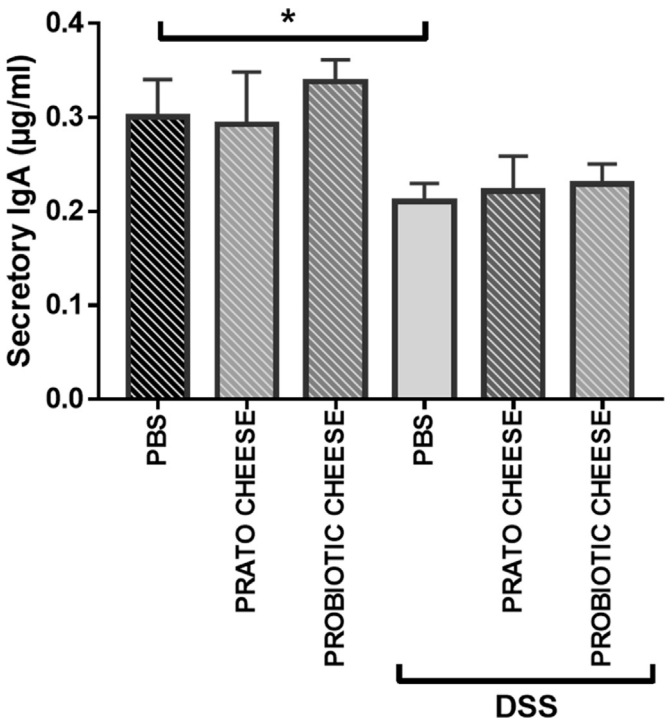

Fig. 5. Secretory immunoglobulin A (sIgA) in the small intestine content of healthy or inflamed mice.

respectively ($p > 0.05$). For intragastric gavage used in clinical trial, the cheese solution was resuspended in PBS pH 7.4 and presented 8.45 log cfu mL^{-1} *Lc. lactis* in Prato cheese and 8.32 log cfu mL^{-1} *Lc. lactis* and 9.47 log cfu mL^{-1} *L. casei* 01 in probiotic cheese.

Regarding the proximate composition, both the Prato cheese and probiotic Prato cheeses presented moisture, fat, and protein levels from 51.5 to 52.6, 36.9 to 38.5, and 29.3 and 27.5% (w/w) respectively ($p > 0.05$). Concerning Total solids, a range from 47.4 to 48.5% (w/w) was observed for Prato cheese and probiotic Prato cheese, respectively. Regarding the mineral content, the calcium and sodium levels ranged from 902.3 to 954.31 mg 100 g^{-1} and 666.7 to 621.9 mg 100 g^{-1} for Prato cheese and probiotic Prato cheese, respectively ($p > 0.05$).

3.2. Food intake, liquid intake, and body weight

The amount of Prato cheese or probiotic cheese administered by intragastric gavage (250 mg) for the pretreatment of DSS-induced colitis corresponds to approximately 8.7% of the daily food intake considering 2.87 ± 0.19 g per mouse. Fig. 2 shows the food intake (Fig. 2A) during experimental procedure, which includes the administration of DSS solution, the liquid intake (Fig. 2B) and the percentage variation in body weight (Fig. 2C) of all animals. No significant differences ($p < 0.05$) were observed for food intake, liquid intake and body weight variation between the groups during 15 days of the experiment. Caloric intake was calculated (Fig. 3A) according to food consumption. Gavage with Prato cheese (Prato cheese or probiotic) accounted for an extra 1.6 kcal to the daily intake, which was 9.40 ± 0.71 kcal per animal per day during the experimental procedure. However, the intragastric gavage did not alter the daily caloric content in each animal. No significant difference was found between groups, including the inflamed or non-inflamed groups. Although the administration of DSS solution led to a weight loss in mice (0.6%), the treatment with cheese containing or not the probiotic bacteria prevented the weight loss of the animals (Fig. 3B), with a weight gain similar to that of the healthy animals (6.5%).

3.3. Disease activity index and microscopic evaluation of inflamed colon

The results showed that the administration of DSS in drinking water induced an acute inflammation in the mice colon (Fig. 4), which was evidenced through the analysis of the major colitis clinical signs (weight loss, diarrhoea, and rectal bleeding), yielding a combined score (DAI) (Fig. 4B). However, the treatment with probiotic cheese was not sufficient to change this clinical condition, as the DAI score was not significantly different ($p > 0.05$) for the mice treated with the cheese made with *L. casei* 01 (4.5 DAI score) when compared with the untreated mice (5.5 DAI score). In addition, the administration of 2% DSS in water was able to alter the morphological structure of the mice colon, with a decrease in colon size (8.3 cm in the control and 6.2 in PBS inflamed mice) (Fig. 4C) and an increase in the histopathological parameters (Fig. 4A, B). This behaviour led to changes in the mucosal architecture of the colon and extent of muscle thickening, as well as inflammatory cell infiltration. However, the pretreatment with the probiotic cheese was not able to prevent the destruction of the intestinal mucosa, as

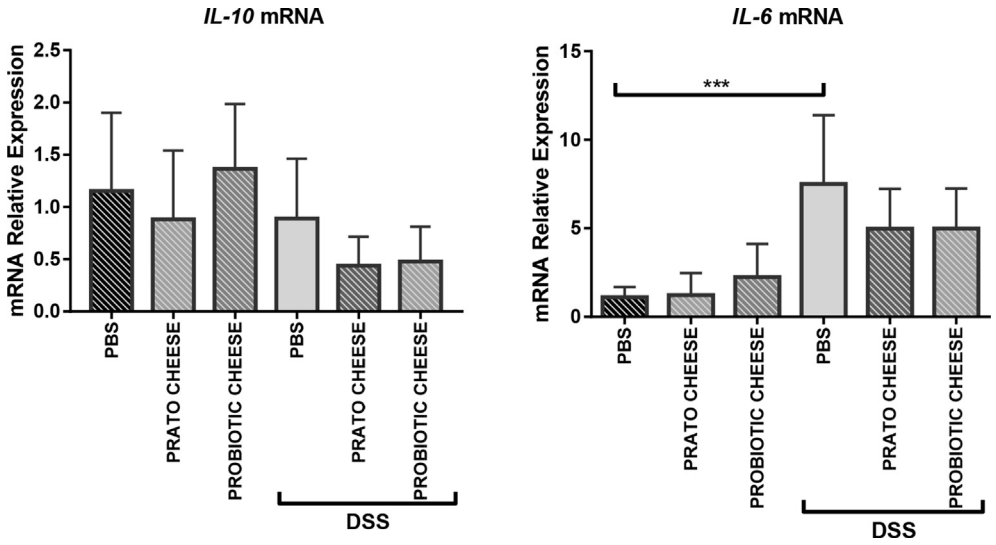

Fig. 6. Relative expression of mRNA of the anti-inflammatory IL-10 and pro-inflammatory IL-6 genes in mice colon.

expected. Therefore, there were no significant differences in the histopathological score and colon size for all inflamed groups.

3.4. Secretory IgA levels in mice small intestine

Fig. 5 represents sIgA levels in the small bowel intestinal fluid, at day 15. The results showed that the administration of probiotic cheese in healthy mice led to an increase in sIgA when compared with both the PBS control and mice treated with Prato cheese, which was not observed in DSS-induced colitis mice with no significant differences ($p > 0.05$) between the inflamed groups.

3.5. IL-10 and IL-6 gene expression in mice colon

To investigate the potential mechanisms of probiotic cheese containing *L. casei* 01 in mice, the anti-inflammatory IL-10 and pro-inflammatory IL-6 gene expression was evaluated. In both healthy

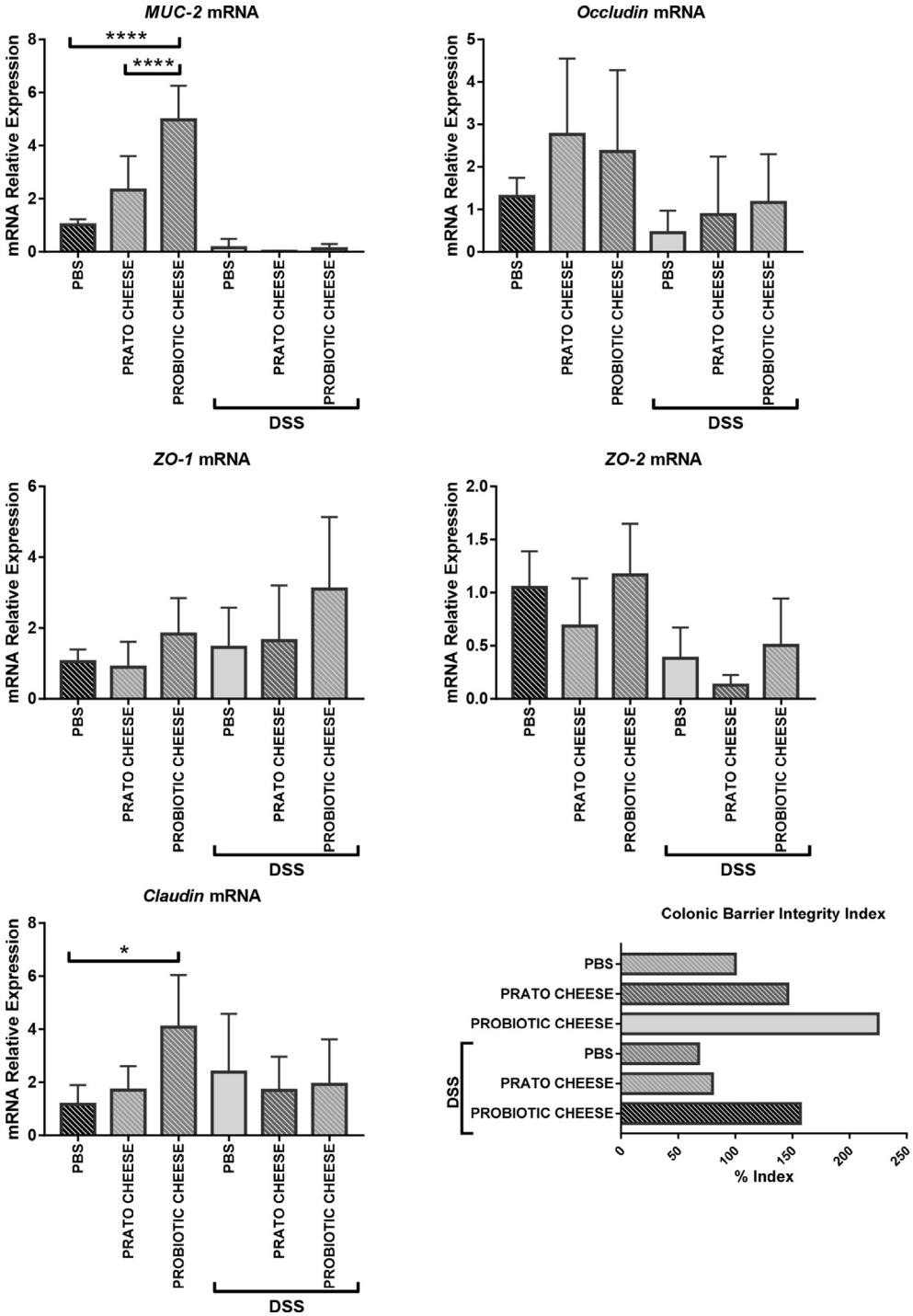

Fig. 7. Relative expression of mRNA of the (A) MUC-2, (B) Occludin (C) ZO-1, (D) ZO-2, (E) Claudin-1, genes in mice colon. Expression levels was monitored by RT-PCR (F) Colonic Barrier Integrity Index was calculated from the combination of mRNA gene expression of tight junction (TJ) proteins (Occludin, Claudin-1, ZO-1 and ZO-2).

mice and mice receiving DSS, no significant difference was observed for the IL-10 gene expression (Fig. 6A), while the IL-6 gene expression was increased in inflamed mice (Fig. 6B). Nevertheless, as previously observed in our other results, the probiotic cheese was not able to alter the inflammatory scenario in mice.

3.6. Intestinal barrier genes relative expression in mice colon

The potential of Prato probiotic cheese to modulate genes involved in epithelial barrier integrity in healthy and DSS-induced colitis mice was evaluated by the relative expression of genes encoding tight junction (TJ)-associated proteins (Occludin, ZO-1, ZO-2, and Claudin-1), and the MUC-2 gene expression. No significant differences for the relative expression of the genes Occludin (Fig. 7B), ZO-1 (Fig. 7C), ZO-2 (Fig. 7D) was observed. However, a significant increase was observed in the mRNAs for MUC-2 ($p < 0.0001$) and Claudin-1 ($p < 0.05$), as shown in Fig. 7A, E, respectively, for the healthy mice treated with probiotic Prato cheese when compared with the untreated animals (PBS). Interestingly, the colonic barrier integrity (CBI) index was calculated, and the pretreatment with probiotic Prato cheese showed the highest CBI index in both the healthy mice and the DSS-induced colitis mice (Fig. 7F).

4. Discussion

The incidence of IBDs has considerably increased throughout the world, thus becoming an important global public health problem (Ananthakrishnan, 2015). This increase has been associated with a change in lifestyle that includes the intake of processed foods, usually rich in fat and sugar and poor in fiber, in addition to the intensified and uncontrolled use of antibiotics, especially during childhood (Ananthakrishnan, 2015; Vangay, Ward, Gerber, & Knights, 2015). Current treatments, including anti-inflammatory drugs such as aminosalicylates and corticosteroids, immunosuppressive agents can produce significant side effects and low efficacy of the treatment (Bernstein, 2015; Pithadia & Jain, 2011). Studies have focused on alternative therapies to alleviate the symptoms caused by the disease, including the use of probiotics, which exerts anti-inflammatory effects and has been proposed for the treatment of ulcerative colitis (Carvalho et al., 2017; Rabah, Rosa do Carmo, & Jan, 2017).

Probiotics can act in the host by enhancing the intestinal epithelial barrier, or by modulating the immune response system through their interaction with intestinal cells and regulation of anti and pro-inflammatory cytokines. Moreover, probiotics can adhere to the intestinal mucosa and decrease pathogen adhesion to the host epithelial cells and competitively exclude pathogenic microorganisms. In addition, some probiotics species produce antimicrobial compounds which inhibit pathogenic microorganism proliferation.

These mechanisms are the basis for determining whether the species has a probiotic potential to be used in a disease model (Bermudez-Brito, Plaza-Díaz, Muñoz-Quezada, Gómez-Llorente, & Gil, 2012). The probiotics potential has been studied in IBD murine models (Fujiya, Ueno, & Kohgo, 2014; Santos Rocha et al., 2014), such as the DSS-induced colitis model, which mimics the mucosal injury, ulceration, diarrhoea, impaired mucus epithelial barrier function, and inflammatory cytokine production features routinely observed in human UC (Laroui et al., 2012). The functional foods, including the probiotic cheese, may be an attractive alternative to attenuate the symptoms of IBD, besides presenting an excellent market potential (Al Mijan & Lim, 2018; Silva et al., 2018c).

Colonization of probiotic bacteria in the human gut is associated with beneficial effects on the host, such as by modulating intestinal microbiota (Carmo et al., 2017). Thus, the pretreatment using probiotic inserted in protective matrices shows a good choice as to deliver the anti-inflammatory effects and thereby reduce inflammation caused by IBDs. It is worth emphasising that the therapeutic effects of probiotics foods depend on the ability of the bacteria to survive the industrial process and the storage period (Carmo et al., 2017), as these environments impose a series of bad growth conditions, which can severely affect bacterial viability (Cordeiro et al., 2018). Thus, a food matrix, for example, cheese can be used to maximize the tolerance of bacteria to the stressful environments and to increase their probiotic ability. In this sense, Prato cheese of this study has proven to be a good probiotic protection matrix as it presented the minimum requirements of 8 log cfu g^{-1} counts after storage, being in accordance with the regulatory recommendations for probiotic concentrations to promote beneficial effects (9 log cfu g^{-1} or cfu mL^{-1}) (Brasil, 2007). Concerning Prato cheese, the Brazilian legislation has established moisture and fat in dry matter (FDM) as high fat (>60%) and high moisture (46–54.9%). Moreover, Prato cheese (30 g) should provide approximately 15% of the daily protein intake recommended by law (50 g protein per day). For mineral content, calcium and sodium levels should range from 163 to 226 mg and 60–206 mg per serving (30 g), respectively (Matera et al., 2018). Indeed, the proximate composition of all Prato cheese of this study including moisture, fat, protein, calcium and sodium levels was in accordance with the Brazilian regulation (Brasil, 1997).

Whereas Prato cheese is a good food matrix for bacteria survival, this study investigated the ability of Prato cheese containing *L. casei* 01 strain to prevent the symptoms of chemically induced colitis by DSS in mice. The experimental procedure was based on previous studies (Plé et al., 2016, 2015) which showed that the 5-day pretreatment with probiotic strains in cheese matrices succeeded in alleviating the symptoms of colitis. Weight loss is one of the clinical parameters observed in colitis (Luerce et al., 2014). The present results showed that the administration of DSS caused a weight loss in the control group (PBS), as expected; however, the pretreatment with the Prato cheese and probiotic cheese was able to interfere with weight loss. This result suggests that the Prato cheese, as well as the probiotic Prato cheese, possess nutraceutical compounds, such as vitamins, amino acids, and fatty acids, which may aid in the prevention of DSS-induced weight loss (Larussa, Imeneo, & Luzza, 2017).

In the present study, no reduction on the macroscopic inflammatory disease score and the histological score of colitis was observed for the probiotic-treated mice, with no changes in the colon shortening of the treated groups. Thus, the pretreatment with probiotic Prato cheese had no effect on colonic inflammation in DSS-induced colitis mice model. In accordance with our results, Kennedy and collaborators reported that the probiotic therapy with *Lactobacillus plantarum* species 299 (LP299) failed to alleviate symptoms of colitis (Kennedy, Hoper, Deodhar, Kirk, & Gardiner, 2000). Our findings reinforce that the viability of the strain in a protective matrix, such as cheese, is not the decisive factor for the therapeutic effects of probiotics, thus the probiotic strain must act through multiple mechanisms to attenuate inflammatory processes in the host. Indeed, the therapeutic effect of functional food is dependent on the adequate selection of a probiotic strain that will act effectively in the proposed disease model. However, the delivery matrix (Prato cheese) of the probiotic candidate may enhance the probiotic effect of the strain, as observed in *L. casei* BL23, which is dependent on a dairy matrix to have a significant effect on the DSS-induced mice model (Lee, Yin, Griffey, & Marco, 2015). In this case, the choice of another probiotic strain might yield different results for future studies.

The role of mucus layer and sIgA in gut homeostasis is clear. The intestinal mucus layer provides a barrier limiting bacterial contact with the underlying epithelium, and sIgA reduces the penetration of commensal bacteria by preventing their adhesion to the epithelium (Pabst, Cerovic, & Hornef, 2016). Several studies have shown that the consumption of probiotics is associated with increased sIgA levels since this antibody can limit the penetration of pathogenic bacteria into host tissues through the neutralization of antigens (Malin, Suomalainen, Saxelin, & Isolauri, 1996; O'Sullivan, 2001). Although the immune exclusion or neutralization has been recognized as a key function of SIgA and often attributed as an important component of protective immunity, little is known about the specific details of the process (Stokes, Soothill, & Turner, 1975). This fact is very relevant in DSS-induced colitis models, once DSS is toxic to the intestinal epithelium, promoting increased bacterial translocation (Laroui et al., 2012; Okayasu et al., 1990). In our study, as also observed by Zurita-Turk et al. (2014), there was no significant difference in sIgA levels between the healthy control group and the DSS-induced control group. Interestingly, the treatment with *L. casei* 01 led to an increase in IgA levels in the small intestine of healthy mice, while *L. casei* 01 was not effective to alter the concentrations of this immunoglobulin during the colitis disease.

Furthermore, our findings showed that Prato probiotic cheese was able to stimulate *muc2* gene expression only in healthy animals. One of the components of the epithelial barrier is mucin 2, which is secreted by goblet cells. The *muc2* gene is responsible for encoding the main mucin that composes the intestinal mucus layer, which is important to prevent the direct adhesion of microorganisms to the epithelium and lubricate the intestinal walls (Cordeiro et al., 2018; Niv, 2016). Although Prato cheese containing *L. casei* 01 was able to increase the stimulation of production of mucus in healthy animals, it was not able to alter the production profile of *muc2* in animals with severe colitis. In accordance with our results, Duary, Bhausaheb, Batish, and Grover (2012) also showed that despite the *muc2* gene was overexpressed in healthy animal treated with *L. plantarum* Lp91, it was not able to alter the gene expression in colitis inflammation scenario. It is known that *muc2* knockout mice have a propensity to spontaneously develop colitis (Burger-van Paassen et al., 2011; Johansson et al., 2008; Van der Sluis et al., 2006; Wenzel et al., 2014). Moreover, a decrease in *muc2* expression has been observed in patients with ulcerative colitis (Tytgat, van der Wal, Einerhand, Büller, & Dekker, 1996; Van Klinken, Van der Wal, Einerhand, Buller, & Dekker, 1999).

Similarly, although no significant differences were observed in the TJP genes of the present study, except for Claudin-1, the results of the qualitative index that measures the colonic barrier integrity showed that the pretreatment with probiotic cheese was more effective to alter the barrier integrity when compared with the colitis-induced mice (PBS inflamed group).

The capacity of probiotic strains to improve the intestinal barrier in healthy animals has already been reported by other studies (Bruewer, Samarin, & Nusrat, 2010; Wang et al., 2018). However, further studies should be performed to verify the effect of *L. casei* 01 on the improvement of the mucosal barrier, since it failed to strengthen the gut epithelial integrity against the DSS. In addition, it was observed that the probiotic Prato cheese did not increase *il10* mRNA levels in the colon of DSS-induced colitis mice.

A previous study has shown that the protective effect of probiotic *L. lactis* NCDO2118 against ulcerative colitis in mice was related to increased IL-10 levels in the colon (Luerce et al., 2014). Furthermore, *L. lactis* NCDO2118 was ineffective in the reduction of *IL6* mRNA levels, as was *Lactobacillus fermentum* CECT5716 in another ulcerative colitis model (Mañé et al., 2009). The parameters analysed in this study, secretory IgA, *IL10* and *IL6* mRNA levels, and CBI index are directly connected to the probiotic mechanisms responsible for attenuating the inflammatory process caused by DSS. It is noteworthy that probiotic cocktail VSL# 3 (*L. plantarum* 299v, *Lactobacillus salivarius*, or *Bifidobacterium infantis* 35624), when used in a murine model of DSS-induced colitis, was able to change the composition of the cecal microbiota by increasing the *Bifidobacterium* spp. concentration (Gaudier, Michel, Segain, Cherbut, & Hoebler, 2005). However, VSL # 3 was ineffective in alleviating the inflammatory process caused by the effect of the chemical agent, probably due to the fact it does not enhance the epithelial barrier or increase mucin production (Gaudier et al., 2005). Therefore, the *L. casei* 01 strain delivered in a cheese matrix does not show the ability to change these parameters and failed to alleviate the inflammatory process.

5. Conclusion

Prato cheese has proven to be a good protective matrix to ensure the viability of the *L. casei* 01 strain during storage. Moreover, the continuous consumption of the experimental probiotic Prato cheese was able to interfere with the weight loss in DSS-induced mice. However, the pretreatment with probiotic Prato cheese to stimulate some parameters in healthy mice was not able to alter the parameters in DSS-inflamed mice. It is likely that the pretreatment period was not sufficient to control the disease parameters, as well as the probiotic potential of the *L. casei* 01 strain. Probably, the lack of probiotic potential observed in the present study, based on the property of alleviating the inflammatory process caused by experimental colitis, is due to several factors including the inability of the strain to modulate the immune response to control the pro-inflammatory environment induced by DSS, to improve the epithelial barrier or to modulate the intestinal commensal microbiota communities to withstand intestinal environmental changes caused by DSS. Therefore, further studies are required on ulcerative colitis model aimed to explore the use of cheese as a protective matrix associated with other selected probiotic strains with high anti-inflammatory potential.

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