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INFLUENCE OF A PROBIOTIC BEVERAGE CONSUMPTION (ENTEROCOCCUS FAECIUM CRL 183 AND LACTOBACILLUS HELVETICUS 416 WITH ADDITION OF BIFIDOBACTERIUM LONGUM ATCC 15707) IN THE ACUTE PHASE OF CHEMICALLY INDUCED COLITIS IN MICE

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Abstract: Background: The ingestion of probiotic microorganisms as a non-pharmacological alternative to treating and preventing inflammatory bowel disease (IBDs), including ulcerative colitis (UC), seems to be promising, and their beneficial effects depends on the strain and the disease stage. **Objective:** The aim of this study was to investigate the effect of a probiotic soy product fermented with *Enterococcus faecium* and *Lactobacillus helveticus* CRL 183 416 with addition of *Bifidobacterium longum* ATCC 15707 in the acute phase of UC chemically induced in mice. **Methods:** Colitis was inducted by the administration of dextran sodium sulphate 3% in the drinking water (7 days). During the trial period (14 days), the animals were allowed in four groups (n=10): C - healthy animals; CL - animals with chemically induced colitis; CLF - animals with chemically induced colitis and receiving the probiotic fermented product; Group CLP - animals with chemically induced colitis and receiving the unfermented product (placebo). The primary end point was the disease index activity (DAI) improvement by ≥25% during the induction period. Secondary end points included changes histology and in the fecal microbiota composition (≥0.5 log CFU/g). **Results:** The animals treated with probiotic soy product (CLF group) showed a reduction of 30.9% in the DAI compared to CL group ($p<0.05$), while the animals receiving the placebo showed a non-significant reduction of 7.1%. Only the CLF group exhibited a lower degree of inflammation and ulceration, maintaining the crypt architecture. The animal of CLF group also showed a positive microbiota modulation with higher increase in the *Lactobacillus* spp. (1.5 log UFC/g) and less increase of enterobacteria (1.34 log UFC/g) population during the experimental protocol. **Conclusions:** The regular intake of probiotic fermented soy product can help in the reduction of the symptoms and preservation of colon integrity during the acute UC induced in mice.

Keywords: inflammatory bowel disease, ulcerative colitis, probiotics, microbiota, fermented soy product, *Enterococcus faecium*, *Bifidobacterium longum*.

INTRODUCTION

Inflammatory bowel disease (IBD) is a generic term used to indicate a group of idiopathic diseases, including Crohn's disease (CD) and ulcerative colitis (UC). CD usually affects the whole gastrointestinal tract, but it is most common in the terminal ileum and colon whereas ulcerative colitis (UC) affects mainly the colon (1,2). CD and UC are characterized by chronic inflammation of the intestinal mucosa, and although IBD pathogenesis has not been completely understood studies suggest that the impairment of the epithelial barrier leads to an exaggerated immune response to the commensal microbiota and/or food components (3,4).

The role of the intestinal microbiota in the pathogenesis of UC is justified as individuals with this disease usually exhibit dysbiosis, where the microbial diversity and composition are altered as compared to healthy individuals. Several studies had shown that IBD patients have decreased microbial diversity with reduction of *Firmicutes* (*Clostridium* ssp.) and increased of *Proteobacteria* (Enterobacteriaceae) (6–8). In this line, probiotics have been used as an alternative or adjuvant therapy to assist in maintaining the balance of the intestinal microbiota and reduce the risk of diseases associated with alterations in the gut environment, such as UC and CD (9). Probiotics – "live micro-organisms that, when administered in adequate amounts, confer a health benefit on the host" (10,11) mainly represented by lactobacilli and bifidobacteria, have the potential to improve

the host inflammatory profile and increase the production of beneficial metabolites such as butyrate and other short chain fatty acids (SCFA).

The mechanisms proposed to explain the beneficial effect of probiotics in IBD treatment include: reduction of pathogens by adhesion or nutrients competition and production of antimicrobial substances (lactic acid, acetic acid, hydrogen peroxide and bacteriocins); positive modulation of the immune system, with production of anti-inflammatory interleukins (mainly IL-10); maintenance and improvement of the intestinal barrier function by mucin secretion and enhancement of tight-junctions proteins; as well as production of SCFA and polyamines (12,13).

Previous studies conducted by our research group showed that regular ingestion of a soy-based probiotic product, fermented with *Enterococcus faecium* CRL 183 and *Lactobacillus helveticus* 416 and with addition of *Bifidobacterium longum* ATCC 15707, was able to reduce the severity of DSS-induced colitis in mice, during the recovery stage of the disease. The animals treated with the probiotic beverage exhibited a decrease in the disease activity index (DAI) during the induction phase, reduction of colonic epithelium damage at the end of the protocol and a positive modulation of the fecal microbiota composition (12,14,16). However, a more in-depth study, considering different stages of the disease, is necessary for a better understanding of the real beneficial effect of the product. Therefore, the present study aimed to investigate the effect of the same probiotic soy-based beverage in the acute phase of DSS-induced colitis in mice.

MATERIAL AND METHODS

PROBIOTIC AND PLACEBO PRODUCTS

Soy milk (UNIVERSOJA, UNESP, Araraquara - Brazil) fermented with *E. faecium* CRL 183 (CERELA – Reference Center for Lactobacilos, San Miguel de Tucumán - Argentina) and *L. helveticus* 416 (ITAL – Institute of Food Technology, Campinas, SP - Brazil), with addiction of *B. longum* ATCC 15707 (American Type Culture Collection, EUA; imported by the cells bank of Rio de Janeiro).

The soy-based probiotic product was processed according to Rossi et al. (13). The bacterial inoculum consisted of an equal mixture (1:1) of *E. faecium* CRL 183 (probiotic strain) and *L. helveticus* 416 (fermentation adjuvant). Fermentation was carried out at 37˚C until the product reached pH 4.5. After fermentation, a suspension of *B. longum* ATCC 15707 was added to yield 8 log CFU/g of product. These cells were inoculated in a milk medium (10% skimmed milk powder, 1% glucose, 0.5% yeast extract), and incubated overnight at 37 ˚C, before being added to the product. The unfermented soy product (placebo) had the same composition, without the bacterial cultures and was chemically acidified with food-grade lactic acid (Purac, Sao Paulo, Brazil) to reach the equivalent pH of the probiotic product. The products were freshly produced weekly and stored at 5 ºC ± 1ºC.

ANIMALS AND EXPERIMENTAL PROTOCOL

Forty female C57bl/6 mice (8-week-old), Specific Pathogen Free (SPF), were purchased from Central Animal Facility Unicamp (CEMIB, Campinas, Brazil). Animals were group-housed in a ventilated rack (Alesco,

Brazil) that delivered HEPA filtered air to all cages, with controlled humidity (60%) and temperature conditions (22ºC ± 2ºC). Mice were exposed to 12-12h light-dark cycles, and had free access to drink water and standard diet (3.39 kcal/g, Nuvilab CR-1, NUVITAL QUIMTIA, Brazil). The experiment followed the guidelines of the Brazilian College of Animal Experimentation (COBEA) and it was approved by the Research Ethics Committee of the School of Pharmaceutical Sciences – UNESP - Araraquara, Brazil (protocol number: 04/2015).

Animals were randomly assigned into four experimental groups (n=10): **Group C** (negative control) - healthy animals that did not receive the products under study; **Group CL** (positive control – dextran sodium sulfate (DSS)-induced colitis) - animals with chemically induced colitis that did not receive the products under study; **Group CLF** (colitis probiotic/fermented product) - animals with chemically induced colitis that received the probiotic product; **Group CLP:** (colitis placebo product) - animals with chemically induced colitis that received the non-fermented product (placebo). The experimental protocol lasted 14 days (T0 – T14), with 10 days of adaptation period.

A maximum of 0.5 ml (sufficient to ensure the intake of 8 log CFU/day) of fermented probiotic (CLF group) or placebo products (CLP group) were daily administered to the animals by oral gavage. The administration of products had started out 7 days prior to the induction of colitis (T0) and lasted for the 7 days of DSS induction (T7-T14) (Figure 1). Animals from negative control (C) and colitis (CL) groups received the same amount of sterile water by oral gavage daily.

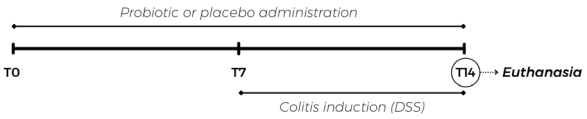

Figure 1. Protocol scheme. T0 - baseline; T7 – 3% DSS-colitis started, T14 - end of the protocol and euthanasia. Probiotic or placebo products were administrated during the entire experiment.

INDUCTION AND MEASURING OF COLITIS

Colitis was chemically induced by adding 3% of DSS - MP Biomedicals, EUA; PM = 36.000–50.000) in the drinking water for a period of seven days (T7-T14). Individual body weight, total food and water intake, diarrhea and rectal bleeding were recorded daily. During the DSS administration, clinical signs were investigated such as pronounced weight loss (>20%), severe diarrhea, dehydration, general activity, and any other symptoms indicating the animal was at or near endpoint. DSS-colitis severity was also determined daily using the disease activity index (DAI), according to Murthy et al. (17), that considers weight loss, stool consistency, and occult blood in the stool. To determine the presence of fecal occult blood, the commercial kit Hemoplus (Newprov, Brazil) was used according to the manufacturer's instructions.

TISSUE COLLECTION AND HISTOPATHOLOGICAL ANALYSIS

After 14 days of protocol, the animals were euthanized by CO_2 inhalation and colonic tissues were removed for further histopathological analysis. Samples were measured and weighted for inflammation assessment.

Laparotomy was performed, and colon sections were removed and opened longitudinally. Samples were fixed in 10% buffered formalin, washed in tap water for

24h and then stored in 70ºGL ethanol. The samples were routinely processed, paraffin-embedded and distal colon tissues sections (5 µm) were stained with hematoxylin-eosin for further examination under a light microscope (Olympus BX51-Olympus Optical, Tokyo, Japan) (18).

MICROBIOLOGIC ANALYSIS

Fresh stool pellets were collected at the beginning of the protocol (T0 – baseline); one week after products administration (T7), after the colitis induction (T14 - end of experiment) and stored at -80°C (Indrel, Brazil – IULT 335D model) for further analysis. Fecal microbial analyses performed by media-dependent assay were based on the determination of the following bacterial groups: *Enterococcus* spp., *Lactobacillus* spp., *Clostridium* spp., *Bacteroides* spp., *Bifidobacterium* spp., and enterobacteria. Fecal pellets were diluted in sterile peptone water and the suspensions were used to inoculate selective culture media: *Enterococcus* spp. in KF Streptococcus agar (Acumedia – Neogen, United States); 37ºC/ 48h (19); *Lactobacillus* spp. in Man Rogosa Sharpe agar (Acumedia– Neogen, United States); 37ºC/ 48h, under anaerobic conditions (20); *Bifidobacterium* spp. in Bifidobacterium iodoacetate medium 25 (BIM-25) (Acumedia– Neogen, United States); 37ºC/ 72h, anaerobiose (21); enterobacteria in MacConkey agar (Acumedia– Neogen, United States); 37ºC/4 8h, under anaerobic conditions (22); *Bacteroides* spp. in Bacteroides Bile Esculin agar (Acumedia– Neogen, United States); 37ºC/ 48h, under anaerobic conditions (23)Lactobacillus paracasei A, to survive gastrointestinal (GI; *Clostridium* spp. in Reinforced Clostridium agar (Acumedia– Neogen, United States); 37ºC/ 48h, under anaerobic conditions (24).

STATISTICAL ANALYSIS

The data were expressed as the mean ± standard deviation (SD) for each group. One-way analysis of variance (ANOVA) and Tukey's multiple-comparison tests were used to analyze the results. Statistical analyses were performed using GraphPad Prism Software Version 9.0 (GraphPad Software, San Diego, California) and significance was declared when $p<0.05$.

RESULTS AND DISCUSSION

PROBIOTIC PRODUCT REDUCES DSS-INDUCED COLITIS SYMPTOMS

Several studies have demonstrated that *E. faecium* exhibit functional properties (25–30). The probiotic strain of *B. longum* ATCC 15707 was incorporated due to its immunomodulatory effect (31), whereas *L. helveticus* provides technological benefits associated with a faster fermentation process. Microbiological analyses revealed that the average population of *E. faecium* CRL 183, *L. helveticus* 416 and *B. longum* was higher than 10 log CFU/g throughout the experimental period (data not shown), which is considered appropriate for them to exert their probiotic effects (32,33)underlining the need of microbial viability and the requirement of a suitable dose to obtain a health benefit. The dose and the administration regimen are critical issues for probiotics either ingested as foods claiming health benefits or used as drugs in clinics. In fact, regulatory authorities demand to guarantee consumers that a probiotic is effective in the recommended conditions of use and responds to its specific claims. Thus, a proper identification of probiotic strain

During the experimental protocol there was no significant difference ($p<0.05$) in water and feed ingestion among groups (data not shown). As expected, we observed no

alteration in animals' stool consistency and body weight on the negative control group (C). The increased DAI scores in the CL, CLP and CLF groups indicate that DSS was able to induce experimental colitis as expected. This result resembles the ones from other authors (34,35), who used the same induction method and observed that the symptoms started between the second and the third day of DSS administration, reaching maximum severity on the sixth and seventh day. The groups CL and CLP presented higher weight loss (Figure 2B), changes in stool consistence (soft or diarrhea) and apparent blood in the stool on the third day of DSS administration. The beneficial effects of the probiotic drink in the CLF group were evidenced by the change of the analyzed parameters during the induction period, such as stool with regular consistency up to the sixth day and presence of visible rectal blood only on the fourth day, expressed as lower DAI score in comparison with the other DSS groups (Figure 2A). Animals in the CLF group showed weight loss from the third day of induction, but this effect may not be associated with the progression of colitis, as the same probiotic product was able to reduce weight gain in a previous study of obesity induced by diet (36).

Celiberto et al. (37) observed similar results to the present study when evaluated the effect of the same probiotic product (*E. faecium* CRL 183 and *B. longum* ATCC 15707) in the recovery phase of DSS-colitis in rats. The probiotic product was able to reduce the DAI score as compared to the placebo and the control groups, characterized by lessweight loss, normal stool consistency up to the fourth day of DSS, and the presence of either occult or visible blood in stool only on the last two induction days (days 6th and 7th).

The severity of UC symptoms vary significantly among patients thus impairing their quality of life. Probiotics have been used as an adjuvant therapy to reduce UC symptoms and induce or maintain the remission phase of the disease. Chen et al. (38) analyzed the effect of four probiotic strains separately –*Lactobacillus acidophilus, Clostridium butyricum, Bifidobacterium adolescents* and *Enterococcus faecalis*- in mice with DSS-colitis and observed that the DAI score was higher in all groups that did not receive probiotics. A study performed with rats with DSS-colitis showed that the administration of the probiotic mixture VSL#3 significantly improves the DAI score, starting at day 4 of induction, as compared to the placebo group (39). Cui et al (40) analyzed groups of mice with DSS-colitis in treatment with 3 probiotic strains separately and observed that the administration of *Lactobacillus fermentum* proved to be effective in attenuating DSS-induced colitis, whereas the other two strains (*Lactobacillus plantarum* or *Lactobacillus crispatus*) failed to reduce the colitis symptoms as compared to the control group (DSS + saline). A study performed by Pan et al. (41) investigated the effect of *Lactobacillus paracasei* (LC-01) in Balb/c mice with chemically induced colitis by 2.5% DSS. The DAI score in the groups treated with average ($1x10^8$ CFU/ml) and high ($1x10^{10}$ CFU/ml) doses of LC-01 was meaningfully lower in comparison with the groups treated with a low dose of probiotic ($1x10^6$ CFU/ml) or with no treatment ($p<0.05$). Tamaki et al. (42) studied the possible effects of the probiotics ingestion in UC patients originating from nine hospitals in Japan. It was observed that the patients who received the probiotic formulation (*Bifidobacterium longum* 536) have displayed meaningfully reduction in the clinical DAI score starting at eight weeks of treatment (3.8±0.4 to 2.6±0.4; $p<0.01$) when compared to the patients who received placebo (4.5±0.5 to 3.2±0.6; $p=0.88$). The results of the endoscopic evaluations and the remission rates were also higher in the

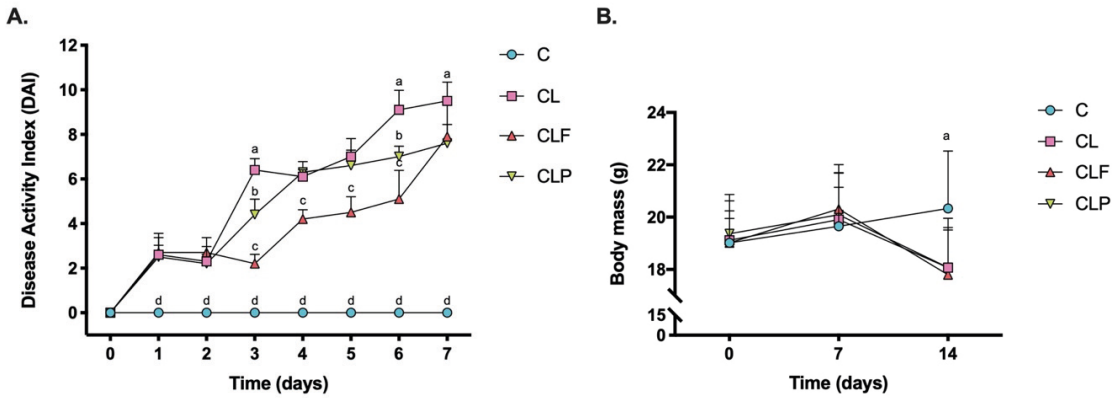

Figure 2 – Disease Activity Index (DAI) and Body mass

Data presented as mean ± SD. Means with the same letter in the same graphic do not differ ($p<0.05$). **C group:** Healthy animals that have not received the products under study. **CL group**: animals with chemically induced colitis and who have not received the products under study. **CLF group**: animals with chemically induced colitis who received the probiotic product**. CLP group**: animals with chemically induced colitis who received unfermented product (placebo).

***Enterococcus* ssp.**	**T0**	**T7**	**T14**
C	5.84±0.02^{b}	6.74±0.05^{a}	7.29±0.39^{a}
CL	6.32±0.02^{b}	6.41±0.02^{b}	9.08±0.29^{a}
CLF	6.05±0.01^{c}	8.67±0.10^{b}	9.27±0.06^{a}
CLP	6.30±0.02^{b}	6.44±0.05^{b}	8.36±0.00^{a}
***Lactobacillus* ssp.**			
C	7.83±0.01^{a}	6.89±0.04^{b}	8.12±0.25^{a}
CL	7.80±0.14^{b}	7.41±0.02^{c}	8.45±0.03^{a}
CLF	7.55±0.07^{c}	8.49±0.09^{b}	9.05±0.01^{a}
CLP	7.70±0.02^{b}	7.56±0.02^{b}	8.76±0.12^{a}
***Bifidobacterium* ssp.**			
C	6.19±0.00^{c}	6.82±0.05^{b}	7.45±0.13^{a}
CL	6.81±0.05^{b}	6.45±0.01^{c}	8,41±0.01^{a}
CLF	6.71±0.03^{c}	8.60±0.02^{b}	8.82±0.04^{a}
CLP	6.25±0.01^{b}	6.42±0.01^{b}	8.60±0.15^{a}
***Clostridium* ssp.**			
C	8.59±0.02^{a}	6.83±0.04^{c}	8.22±0.16^{b}
CL	8.70±0.02^{a}	6.87±0.08^{c}	8.14±0.03^{b}
CLF	6.85±0.03^{c}	8.10±0.04^{b}	8.96±0.04^{a}
CLP	6.60±0.03^{c}	6.80±0.06^{b}	8.73±0.10^{a}
Enterobacteria.			
C	3.86±0.05^{c}	4.82±0.09^{b}	6.58±0.09^{a}
CL	4.24±0.14^{c}	4.97±0.13^{b}	6.44±0.01^{a}
CLF	4.12±0.07^{c}	5.29±0.02^{b}	5,46±0.06^{a}
CLP	6.60±0.03^{c}	6.80±0.06^{b}	8.73±0.10^{a}

Bacteroides **spp.**			
C	3.65±0.03[a]	3.65±0.03[a]	3.73±0.10[a]
CL	3.72±0.07[a]	3.79±0.10[a]	3.86±0.11[a]
CLF	2.77±0.01[a]	2.53±0.08[a]	2.65±0.08[a]
CLP	3.57±0.03[c]	3.77±0.06[b]	4.26±0.05[a]

Table 1 – Population of the strains (log CFU/g)

Data are presented as mean ± SD. Means with the same letter in the same line do not differ statistically by Tukey test ($p < 0.05$). **C group:** Healthy animals that have not received the products under study. **CL group**: animals with chemically induced colitis and who have not received the products under study. **CLF group**: animals with chemically induced colitis who received the probiotic product**. CLP group**: animals with chemically induced colitis who received unfermented product (placebo). **TO=** before product administration, **T7=** after a week of product ingestion, **T14=** induction period/end of the experiment.

probiotic treated group as compared to the placebo group.

The results of different studies were inconsistent as the dosage, strains and protocols adopted are distinct. In the present study, the probiotic product protected mice from DSS-colitis by reducing colonic damage and the severity of inflammation, thus showing the beneficial effect of these particular strains when used in the concentrations adopted.

HISTOLOGICAL EVALUATION

The results of the histological analysis (Figure 3) indicate that the colon of the animals in the control group (Fig 3a) (with no colitis induction by DSS) has presented itself with no structural changes and no focus of inflammation. This result was expected due to the control group not being exposed to the inductor (DSS). The CL group, in turn, has displayed great structural changes characterized by the crypt's disruption, presence of inflammatory cells, as well as submucosal edema (Fig 3b). The intestine of the animals that consumed the placebo product (CLP) has shown great quantity of infiltration of cells characterizing the inflammation (Fig 3d). These results have highlighted a seeming destruction of the crypts, suggesting that the placebo has no beneficial effect in the acute phase of colitis. The samples of CLF animals' tissues show cell infiltrates, with less edema, and crypts with more preserved morphology, thus indicating that the probiotic product reduced the colonic damage in the colitis acute phase, under the conditions of this study (Fig 3c). Similar protector effect was verified with this probiotic product in another study that evaluated the recovery phase of the colitis (37).

A study performed by Hegazy et al. (43) has evaluated 30 UC patients (mild to moderate) that were divided in two treatment groups: G1 - sulfasalazine (2400 mg/d) and G2 - sulfasalazine (2400 mg/d) plus the *Lactobacillus delbrueckii* and *Lactobacillus fermentum* probiotic strains ($1x10^{10}$ CFU/ml). After eight weeks of study, it was observed that the treatment which associated sulfasalazine and probiotics has reduced the extent of the inflammation, prevented mucosa injury and reduced colitis symptoms, thus assisting in the maintenance of the remission and preventing the recurrence of the disease. Chen et al. (44) have performed a study which evaluated the effect of a drink fermented with *Lactobacillus paracasei* 01 strain over the intestinal barrier, using *in vitro* tests. The results have shown that the drink can protect the intestine, promoting the proliferation of the intestinal epithelial cells, improving the epithelium integrity and modulating the production of inflammatory

cytokines in the presence of DSS.

In vitro tests have shown that certain *Lactobacillus* spp. have the ability to increase the expression of e-cadherin, an important protein in cell adherence, thus positively reinforcing the barrier function of the intestinal mucosa (16). A study performed by Srutkova et al. (45) has analyzed the effect of two *Bifidobacterium longum* ssp. *longum* CCM 7952 and CCDM 372 strains in the development of the acute colitis in mice, induced by DSS. The *Bifidobacterium longum* ssp. *longum* CCDM 372 strain had no protective effect in colitis development, highlighting the strain-specific nature of the *Bifidobacterium* genus.

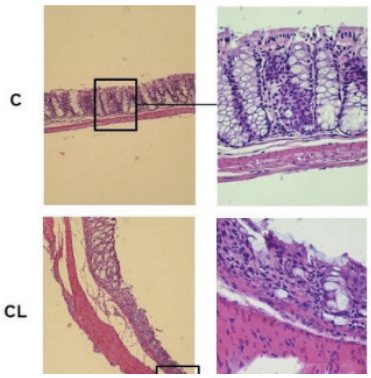

Figure 3 – Representative photomicrographs of colonic tissue stained with hematoxylin/eosin in the different groups.

a. C group: Healthy animals that have not received the products under study. Unchanged epithelium. **b. CL group**: animals with chemically induced colitis and who have not received the products under study. Regions with inflammatory cells infiltrate, changes in the crypts and edema. **c. CLF group**: animals with chemically induced colitis who received the probiotic product**.** Regions with an inflammatory cell infiltrate, but no change in the crypt. **d. CLP group**: animals with chemically induced colitis who received unfermented product (placebo). Regions with inflammatory cells infiltrate and change in the crypts. All group images are presented at 10x, 20x and 40x magnification.

MICROBIOLOGICAL EVALUATION

It is required, among others, studies that investigate this microbiota composition to best clarify the UC etiology, that is yet unclear (47,48). - Despite this knowledge gap in the pathogenesis of the disease, studies have indicated that IBD patients display an exacerbated response to commensal microbes leading to an overactivation of the immune response (49–51)recently weaned mice were either orally administered ferrous (Fe2+. Even though there are plenty of evidences showing how intestinal microorganisms are required to trigger inflammation in IBD the question is whether it is really an abnormal immune response to the dysbiosis of the intestinal microbiota that plays the most important role in, or it is a specific microorganism/microbial group which share distinguishing characteristics responsible for this pathogenesis (49,52)a chronic nonspecific intestinal inflammatory disease, is comprised of Ulcerative Colitis (UC.

In the CLF group, the *Enterococcus* spp. population showed the highest increase after the intake of the probiotic product fermented with *E. faecium* CRL 183 (T7), maintaining it high up to the end of the experiment (T14) (CLF: 2.62 log CFU/g, $p<0.05$). The increase of the *Enterococcus* spp. population in the other groups was not expected. *Enterococcus* spp. is composed of different species of Gram-positive bacteria, with *E. faecalis* and *E. faecium* being the most found in human microbiota. Even though this genus has species with proved probiotic activity, several others are associated with pathogenic effects, thus possibly causing opportunistic infections (27). Therefore, the observed changes do not denote, necessarily, the increase of the probiotic species. A study performed by Bedani et al. (53) verified an increase in *Enterococcus* spp. population in mice that ingested a soy-based product. However, the group fed with the sterilized

soybean probiotic product has also shown an increase in the genus of microorganisms in study, suggesting that, possibly, there are fermentation metabolites that may influence *Enterococcus* spp. population. An increase in the *Enterococcus* spp. population was also observed in a study conducted by Cavallini et al. (12) that evaluated the relationship between fecal microbiota and risk factors of cardiovascular diseases in rabbits fed with a similar probiotic product, without *B. longum* addition.

Some bacterial genera are considered beneficial to the host, such as *Lactobacillus* spp. and *Bifidobacterium* spp., presenting immunomodulating and anticarcinogenic properties, and with protection potential against IBD (54–58). The results indicated a variation in the *Lactobacillus* spp. population in all groups throughout the experimental protocol. In the T7 (one week after starting the interventions), only the CLF group presented an increase in the *Lactobacillus* spp. population (0.94 log CFU/g). At the end of the experiment (T14) it was observed an increase in the *Lactobacillus* spp. population when compared to the earlier period (T7) in the CL, CLF and CLP groups, suggesting that the DSS has no negative effect on the viability of this microbial genus (1.04; 0.56 and 1.2 log CFU/g, respectively). In the T14 the CLF group showed the highest increase in the *Lactobacillus* spp. population when compared to the beginning of the protocol (T0) (CLF: 1.50 log CFU/g). The raise in the *Lactobacillus* spp. population of the CLP group at the end of the protocol suggests a possible modulator effect of the basic mixture used in the production of the product, once the placebo had no probiotic microorganisms (CLP: 1.06 log CFU/g). The C group showed no change in the *Lactobacillus* spp. population and the CL group presented an increase lower than a log (C: 0.29 log CFU/g; CL: 0.65 log CFU/g) ($p<0.05$) after the 14 days of treatment. Celiberto (37) observed similar results in a study with Wistar rats, where the same product led to an increase (0.86 log CFU/g) of the *Lactobacillus* spp population. A study conducted by Cavallini (12) showed that the regular intake of soy-based probiotic product, only fermented with *E. faecium* CRL183 and *L. helveticus* 416, also led to an increase in the *Lactobacillus* spp. (1.27 log CFU/g) on the fecal population of rabbits with induced hypercholesterolemia.

Bullock et al. (57) reported a decrease in *Lactobacillus* spp. population in patients with active ulcerative colitis or in remission. Peran et al (58) verified that consumption of *Lactobacillus fermentum* 5716 (isolated strain from breast milk) increased *Lactobacillus* spp. population when compared to the group of animals with colitis induced by the sulfonic trinitrobenzene acid without treatment. No significant changes were observed in *Bifidobacterium* spp. population and the ones from potentially pathogenic bacteria such as the ones belonging to the coliform and Enterobacteriaceae groups. Literature data indicate that the elevation of the *Bifidobacterium* spp. population in the colon is beneficial to the host, leading to an increase in the SCFA production, modulation of the immune system, reduction of the intestinal pH and decrease of pathogenic microorganisms (59,60)diagnosis and management of C. difficile infection (CDI.

According to Table 1, all groups displayed an increase of the *Bifidobacterium* spp. after the induction period (T14), suggesting that DSS has no negative influence in the intestinal population of this bacterial genus. The CLF and CLP groups have presented the highest increases in the *Bifidobacterium* spp. population, when compared to the previous period (T0) (2.11 and 2.35 log CFU/g, respectively). This result shows, once again,

the possible beneficial effects of placebo and probiotic product in the microbiota modulation of the animals in study. It is relevant to highlight that in the T7 only the CLF group has shown an increase in the *Bifidobacterium* spp. population superior to 1 log (1.89 log CFU/g). This was expected since the probiotic product had the *B. longum* ATCC 15707 in its composition.

A study conducted by Celiberto (37) has observed a significant increase in the *Bifidobacterium* spp. (1.35 log CFU/g) population in the group of animals that ingested the same soy-based probiotic product. However, the ingestion of the placebo product caused no change in the population of this genus in the recovery phase of colitis. Other studies, conducted in different animal models have also shown an increase in the *Bifidobacterium* spp. population after the regular ingestion of the same probiotic product, without *B. longum* addition, suggesting that the product is capable of beneficially modulating the fecal microbiota (54,57,61).

Literature data indicate that *Clostridium* spp. genus might present a pathogenic character and unwanted metabolic activities related to intestinal inflammatory processes, as well as the *Bacteroides* spp. strains (55,62,63). In the present study *Clostridium* spp. population presented a reduction in the C and CL groups, at the end of the T7. It is important to notice that these animals received only water and chow up to this moment of the protocol. Regarding the initial population of this microbial genus (T0), there was an increase of *Clostridium* spp. in the CLF and CLP groups and the remaining groups (C and CL) demonstrated no changes. It is important to highlight the increase of the *Clostridium* spp. population in the CLF group after the ingestion of probiotic product (T7). These data were discordant to the previously observed by our research group using different models of animal. Celiberto (37) concluded that the administration of the same probiotic product had no influence in the clostridia population. Cavallini et al. (12) also verified no variation higher than half log in the *Clostridium* spp. population in the animals that received the probiotic drink. On the other hand, Bedani et al. (51) verified that rats with a diet based on meat and supplemented with a suspension of the *E. faecium* CRL 183 pure strain, showed a slight increase in the *Clostridium* spp. (0.55 log CFU/g) population, after 30 days of treatment. Even though the increase is lower than a log, the result suggests a possible effect of the probiotic strain on the *Clostridium* spp. population present in the intestinal microbiota.

The *Enterobacteriaceae* family is the largest group of Gram-negative bacilli (*Escherichia coli, Salmonella* spp., *Shigella* spp., *Yersinia enterocolitica, Klebsiella* spp., *Proteus* spp. and *Citrobacter* spp.), besides being the most heterogeneous, it presents clinical importance for being associated to a range of pathologies that affect the human being (64). Previous studies have determined higher prevalence of enterobacteria in feces of IBD patients when compared to healthy patients (65,66). It is possible to see that all groups have presented significant increase in the enterobacteria population in the experimental period. However, comparing the induction period (T14) to the previous period (T7) this increase was not relevant in the in the CLF group. Such result suggests a possible protector effect of the probiotic product under study, since in the CL and CLP groups, the increase in the population of enterobacteria was higher than one log in the same period (between T7 and T14) (1.47 and 1.48 log CFU/g respectively). On the other hand, Cavallini et al (12) observed a significant reduction in enterobacteria population (more than three log) with the administration of the

same probiotic product, with no addition of *B. longum* in induced hypercholesterolemic rabbits.

The *Bacteroides* spp. population kept itself low (average of 2.53 and 4.26 log CFU/g) and with no significant changes during the entire experiment. These results are similar to the ones previously obtained by our research group, in a study that used Wistar rats as animal model (37). Studies indicate that the *Bacteroides* spp. presents pro-inflammatory properties, being associated with higher risk of colon cancer, since these bacteria can produce harmful metabolites (56,67,68). In a study conducted by Ruseler et al. (67)the population of *Bacteroides* spp. was similar between healthy patients and patients with CD. However, the population of *Bacteroides fragilis* group was higher in patients with CD. Bloom et al. (68) found that commensal species of the *Bacteroides* genera may be related to the development of IBDs under predisposed genetic and environmental conditions.

The duration of the treatment, the phase and the type of disease, as well as the animal model used in the experiments, might explain, partially, the differences observed between the present study and the ones that were previously performed with the same soy-based probiotic product. The results of the fecal microbiota composition obtained are important for the understanding of the effects of the probiotic product in the development of colitis. However, they must be interpreted with caution, since it is known that only a small portion of the microorganisms present in the intestinal microbiota are cultivable through traditional methods. The most advanced techniques, the ones using molecular microbiology, despite being expensive, are the most suitable to study the variation in gut microbiota composition (69).

CONCLUSION

The results of the present study indicate that regular ingestion of the soy-based probiotic product reduces the symptoms and histological alterations in the acute phase of DSS-induced colitis in mice. This effect probably involves a positive modulation of the intestinal microbiota, since animals of CLF group showed an increase in the *Lactobacillus* spp. population and a lower increase of enterobacteria after the period of the induction of the disease.

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