Microbiological, immunological, and histological changes in the gut of *Salmonella* Enteritidis-challenged rats fed goat cheese containing *Lactobacillus rhamnosus* EM1107

F. R. L. Rolim,¹ C. J. B. Oliveira,^{2,3} O. C. de Freitas Neto,⁴ K. M. O. Dos Santos,⁵ G. C. B. Guerra,⁶ R. V. Rodrigues,⁷ P. O. A. de Assis,⁸ D. F. de S. Araújo,⁹ V. A. G. de Carvalho,¹⁰ M. L. P. Lemos,² N. M. V. da Silva,² J. K. B. Soares,⁷ H. E. M. Garcia,¹⁰ E. L. de Souza,⁸ F. de A. L. Souza,¹¹ M. E. G. de Barros,¹¹ M. E. G. de Oliveira,⁸ and R. C. R. E. Queiroga^{8*}

¹Post-Graduate Program in Food Science and Technology, Department of Food Engineering, Federal University of Paraíba, 58051-900, João Pessoa, Brazil

²Department of Animal Science, Center for Agricultural Sciences, Federal University of Paraíba, 58397-000, Areia, Brazil

³Global One Health initiative (GOHi), The Ohio State University, Columbus 43210

⁴Department of Preventive Veterinary Medicine, Veterinary School, Federal University of Minas Gerais, 31270-901, Belo Horizonte, Brazil

⁵Brazilian Agricultural Research Corporation (EMBRAPA), 23020-470, Rio de Janeiro, Brazil

⁶Department of Biophysics and Pharmacology, Biosciences Center, Federal University of Rio Grande do Norte, 59064-741, Natal, Brazil

⁷Department of Nutrition, Center for Education and Health, Federal University of Campina Grande, 58175-000, Cuité, Brazil

⁸Department of Nutrition, Health Sciences Center, Federal University of Paraíba, 58051-900, João Pessoa, Brazil

⁹Faculty of Health Sciences of Trairi, Federal University of Rio Grande do Norte, 59200-000, Santa Cruz, Brazil

¹⁰Department of Morphology, Health Sciences Center, Federal University of Paraíba, 58051-900, João Pessoa, Brazil

¹¹Department of Animal Morphology and Physiology, Federal Rural University of Pernambuco, 52171-900, Recife, Brazil

ABSTRACT

Cheeses are able to serve as suitable matrices for supplying probiotics to consumers, enabling appropriate conditions for bacteria to survive gastric transit and reach the gut, where they are assumed to promote beneficial processes. The present study aimed to evaluate the microbiological, immunological, and histological changes in the gut of *Salmonella* Enteritidis-challenged rats fed goat cheese supplemented with the probiotic strain *Lactobacillus rhamnosus* EM1107. Thirty male albino Wistar rats were randomly distributed into 5 experimental groups with 6 animals each: negative (NC) and positive (PtC) control groups, control goat cheese (CCh), goat cheese added with *L. rhamnosus* EM1107 (LrCh), and *L. rhamnosus* EM1107 only (EM1107). All animals, except NC group were challenged with *Salmonella* Enteritidis (10^9 cfu in 1 mL of saline through oral gavage). Microbial composition was assessed with high-throughput 16S rRNA sequencing by means of Illumina MiSeq (Illumina, San Diego, CA). Nuclear factor kappa B (NF- κ B) from the animal cecum tissue was determined by real-time PCR and interleukins (TNF- α , IL-1 β , IL-10, and IFN- γ) by means of ELISA. Myeloperoxidase and malondialdehyde levels were determined

biochemically. The administration of the *L. rhamnosus* EM1107 probiotic strain, either as a pure culture or added to a cheese matrix, was able to reduce *Salmonella* colonization in the intestinal lumen and lessen tissue damage compared with rats from PtC group. In addition, the use of cheese for the probiotic strain delivery (LrCh) was associated with a marked shift in the gut microbiota composition toward the increase of beneficial organisms such as *Blautia* and *Lactobacillus* and a reduction in NF- κ B expression. These findings support our hypothesis that cheeses might be explored as functional matrices for the efficacious delivery of probiotic strains to consumers.

Key words: goat cheese, gut microbiome, probiotic, *Salmonella* Enteritidis, *Lactobacillus rhamnosus*

INTRODUCTION

Probiotics are promising alternatives for promoting intestinal health and, consequently, reducing the use of antimicrobial drugs (Ma et al., 2018). Probiotics are defined as living microorganisms that provide benefits to the health of the host when administered in proper concentrations (Hill et al., 2014). These agents have been successfully used for the prevention and treatment of gastrointestinal diseases such as salmonellosis, as previously reported in an in vivo study (Acurcio et al., 2017).

Supplementation with probiotics can promote immunomodulatory effects that are characterized by

Received May 1, 2020.

Accepted August 1, 2020.

*Corresponding author: rcqueiroga@uol.com.br

increased innate and adaptive immune functions in response to the modulation of the gut microbiota (Ma et al., 2018). Some *Lactobacillus* spp. strains play an important role in the regulation of commensal microbiota by inhibiting the growth of opportunistic pathogens, such as *Yersinia enterocolitica* and *Escherichia coli*, and promoting the growth of beneficial bacteria (Kamdar et al., 2016; Zhang et al., 2017).

Given the increasing consumption of probiotics, it is necessary to understand the microbial shifts triggered by probiotic foods to elucidate their biological mechanisms on a solid scientific basis (Castillo et al., 2013; Ma et al., 2018). As microbiome plays a key role in many human health conditions (Weinberg et al., 2018), there is an increasing need to better understand the functions of microorganisms in foodstuff, as well as to identify the products derived from their metabolism, going beyond the benefits related to preservation and sensory attributes (Marco et al., 2017). Fermented foods such as cheese are able to serve as suitable matrices for supplying probiotics to consumers, providing appropriate conditions for bacteria to produce enzymes that promote beneficial biochemical processes. These enzymes act in the catabolism of proteins and lipids, supplying specific texture and flavor (Albenzio et al., 2013; Marco et al., 2017) and reducing protein oxidation by the formation of bioactive peptides (Mushtaq et al., 2016).

Coalho cheese is a semi-hard curd cheese commercialized throughout Brazil. This cheese can provide adequate conditions for the protection of probiotic strains during passage through the gastrointestinal tract, as it consists of a solid buffered matrix with high pH values and low oxygen concentrations (Oliveira et al., 2012; Rolim et al., 2015). Furthermore, probiotic goat cheese needs to be explored as a functional and value-added food (Dantas et al., 2016). Goat dairy products are considered a delicacy in many countries and have been reported to promote anti-inflammatory effects by reducing tissue damage (Rodrigues et al., 2018; Verruck et al., 2019).

Lactobacillus rhamnosus EM1107 is a probiotic strain that has been previously tested in vitro, showing adequate technological properties and survivability rates in food matrices and conditions simulating the gastrointestinal tract (Dos Santos et al., 2015; Rolim et al., 2015). The consumption of cheese with *L. rhamnosus* EM1107 has been shown to reduce injuries caused by ulcerative colitis in rats, with an overall improvement in immunity and a reduction of inflammation and oxidative stress (Rodrigues et al., 2018). These promising findings encourage further investigation on the putative protective effects of *L. rhamnosus* EM1107 in animals

challenged by enteric pathogens, such as *Salmonella enterica*, a leading foodborne pathogen worldwide. The mitigation of *Salmonella enterica* is difficult considering their wide distribution and complex epidemiology, high tolerance and adaptability to different environmental conditions, besides their increasing resistance to multiple antimicrobial drugs (Chen et al., 2013). The present study aimed to evaluate the microbiological, immunological, and histological changes in the gut of rats fed goat cheese supplemented with a probiotic *L. rhamnosus* EM1107 (probiotic cheese) and orally challenged with *Salmonella enterica* serovar Enteritidis (*Salmonella* Enteritidis).

MATERIALS AND METHODS

Bacteria and Inoculum Preparations

The *L. rhamnosus* EM1107 strain was provided by the Brazilian Agricultural Research Corporation (EMBRAPA, Rio de Janeiro, Brazil), and the inoculum was prepared according to Rodrigues et al. (2018). The nalidixic acid-resistant *Salmonella* Enteritidis (SE) P125109 strain used in this study was provided by Paul Barrow, University of Nottingham, UK.

The SE inoculum was prepared on the day before the inoculation and contained 1×10^9 cfu/mL. Briefly, the strain was streaked onto brilliant green agar (Oxoid, Basingstoke, UK) and incubated at 37°C for 24 h. A loopful was transferred to brain-heart infusion (BHI) broth (Sigma-Aldrich, St. Louis, MO) and incubated aerobically at 37°C for 18 h. The culture was then centrifuged ($4,500 \times g$, 15 min, 4°C), washed twice, and resuspended in a sterile saline solution (NaCl at 0.85%). To enumerate the number of viable SE cells in the inoculum, the final bacterial solution was serially diluted (10^{-1} to 10^{-9}), plated onto BHI agar (Sigma-Aldrich) and incubated under aerobic conditions at 37°C for 24 h.

Two types of curd cheese were produced for this study: probiotic cheese, in which coagulation was performed with addition of the coagulant enzyme (rennet) and *L. rhamnosus* EM1107 (1×10^9 cfu/g), and cheese without probiotic bacteria (control cheese). Detailed cheese production process is described elsewhere (Rodrigues et al., 2018). The physicochemical properties of the curd cheese used in the present experiment have been reported (Dos Santos et al., 2012). The use of this type of cheese as a matrix enhanced the survivability of the *L. rhamnosus* EM1107 by improving buffer capacity and nutrient availability and providing more appropriate pH conditions compared with fermented dairy matrices (Rolim et al., 2015).

Figure 1. Experimental design showing the treatments performed through chronological order and the main procedures used in the study. NC = negative control; PtC = positive control; CCh = control cheese; LrCh = probiotic cheese; EM1107 = *Lactobacillus rhamnosus* EM1107.

Animals

The experimental protocol had been previously approved by the Animal Ethics Committee of the Federal University of Paraíba (CEUA/UFPB-Protocol no. 021/2016), according to regulations established by the National Council for the Control of Animal Experimentation (CONCEA, Brazil). We used male albino Wistar rats (*Rattus norvegicus*) at 6 weeks of age. They were kept in cages at room temperature ($\pm 22^{\circ}\text{C}$), with a 12-h light-dark cycle and ad libitum access to food (Presence-Purina, São Paulo, Brazil) and water.

Experimental Design

The animals ($n = 6/\text{group}$) were randomly distributed into 5 experimental groups. Negative control (NC) and positive control (PtC) animals were given saline solution (1 mL) as placebo; goat cheese (1 g) was administered either alone (CCh) or supplemented with *L. rhamnosus* EM1107 (LrCh); and EM1107 animals were given *L. rhamnosus* EM1107 only (10^9 cfu/mL of saline). Placebo and dietary treatments were administered daily for 24 d by means of intragastric gavage.

Inoculation and Sampling

Animals were deprived of water and fed 4 h before and 2 h after SE inoculation, as in a previous experimental protocol (Moreau et al., 2016). After this time, the animals had free access to water and food.

All animals, except those in NC treatment, were pretreated with 20 mg streptomycin (Sigma-Aldrich) administered intragastrically on d 15 of the experiment and inoculated with SE (10^9 cfu) 24 h later. Thirty minutes before inoculation, SE-inoculated animals were also given 100 μL of sodium bicarbonate (10% in saline). The NC group animals were given distilled water as pretreatment (Barthel et al., 2003), and saline was given on the day of inoculation (Tennant et al., 2008). The experimental design is presented in Figure 1.

The animals were euthanized at 1, 4, and 8 d post-infection (DPI; $n = 6/\text{group per day}$). Samples of tissues, feces, and cecal contents were collected for analyses.

Salmonella Enteritidis Counts

Cecal contents were aseptically collected from the euthanized animals at 1 DPI. Liver and spleen samples were collected at 1, 4, and 8 DPI for bacterial translocation assessment. Samples were serially diluted in sterile saline (0.85% NaCl) and aliquots transferred to brilliant green agar (Oxoid) plates supplemented with nalidixic acid (Sigma-Aldrich; 100 $\mu\text{g/mL}$), which were incubated under aerobic conditions at 37°C for 24 h. Logarithmically transformed ($\log_{10}$) SE counts were expressed in colony-forming units per gram (cfu/g). For samples showing no growth in the agar plates, a 1-mL aliquot of the first dilution was transferred to Rappaport Vassiliadis R10 broth (Acumedia, Lansing, MI) and incubated at 37°C for 24 h. Afterward, they

were plated onto brilliant green agar for the qualitative detection of SE.

Microbiota Composition by 16S rRNA Sequencing

Samples and DNA Extraction. Stool samples from animals were collected at 4 DPI (d 20 of experiment), just before euthanasia. These samples were collected individually, directly transferred to DNase/RNase free microtubes, and stored at -80°C . Genomic DNA was extracted using a commercial kit (DNeasy PowerSoil, Qiagen, Hilden, Germany) according to the manufacturer's recommendations. DNA concentration was determined by means of a micro-volume spectrophotometer (Colibri, Berthold Titertek, Germany), and its integrity assessed visually in 1% agarose gel.

Library Preparation and Sequencing. The DNA samples were amplified by PCR for the hypervariable regions V3 and V4 of the 16s rRNA microbial gene according to a standard protocol (Caporaso et al., 2011). The following primer sequences were used in PCR assays: Fw: 5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG; Rv: 5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATC.

The DNA libraries were prepared using the Nextera XT Index Kit (Illumina, San Diego, CA) with multiplex indexing according to the manufacturer's protocol. The PCR products were purified with Agencourt AMPure XP reagent (Beckman Coulter, Brea, CA) and quantified using a Qubit 3.0 fluorometer (Life Invitrogen, San Diego, CA). Paired-end sequencing was performed in Illumina MiSeq using a 2×250 -bp cycles V2 kit (Illumina). PhiX (Illumina) kit at 15% run concentration was used as sequencing control.

16S rRNA Amplicons Preprocessing and Microbiota Analyses. Demultiplexed paired-end fastq sequences were uploaded on QIIME 2 v.19.7 (Bolyen et al., 2019). They were merged, and short reads (<200 bp) were discarded. The remaining sequences were quality filtered using a minimum PHRED score of 20 and replicated using VSEARCH (Rognes et al., 2016). Chimeric sequences were filtered out using UCHIME (Edgar et al., 2011). Operational taxonomic unit (OTU) identification was performed by means of de novo clusterization method with 99% similarity between the centroid groups. Sequences were aligned using MAFFT (Katoh et al., 2002) to build the phylogenetic tree by means of Fasttree2 (Price et al., 2010). The number of sequences per sample was normalized to 14,900 reads.

Alpha diversity was estimated by Chao1 and Shannon indices, and β diversity was estimated by classical multidimensional scaling using Unweighted Unifrac

distance matrix (Lozupone and Knight, 2005), represented in a principal coordinate analysis. Relative OTU abundances were assessed using Phyloseq v.1.8.2 (McMurdie and Holmes, 2013). Taxonomic classification was assigned through the Naïve Bayes method using the reference database SILVA v. 132 cured for V3-V4 regions with 99% OTU similarities (Quast et al., 2013). The linear discriminant analysis effect size (LEfSe) was performed according to Segata et al. (2011) to detect significant differences between the treatment groups in terms of specific OTU abundances. The LEfSe refers to individual pairwise comparisons between the NC and the other groups.

Gene Expression by Real-Time PCR

The expression of nuclear factor kappa B (NF- κ B) from the animal cecum tissue was performed by means of real-time PCR analysis according to Rodrigues et al. (2018) using NF- κ B p65 primers (Fw: GAAGAAGC-GAGACCTGGAGCAA; Rv: GTTGATGGTGCTGAGGGATGCT). Glyceraldehyde-3-phosphate dehydrogenase was used as the reference gene, amplified by the primers Fw: AACTTGGCATCGTGGAAGG; Rv: GTGGATGCAGGGATGATGATGTTTC. Primers were designed using the software Primer express 3.0.1. (Thermo Fisher) and synthesized by Invitrogen Life Technologies (Thermo Fisher Scientific Inc., Waltham, MA).

Biochemical Analyses

Cecum samples were homogenized in ice-cold 10 mmol/L phosphate buffer pH 7.4 (1:5 wt/vol) and centrifuged at $9,000 \times g$ at 4°C for 10 min. The supernatant was used to determine the concentrations of tumor necrosis factor- α (TNF- α), IL-1 β , IL-10, and IFN- γ by enzyme-linked immunosorbent assay (ELISA) using commercial kits (R & D Systems, Minneapolis, MN), according to the manufacturer's recommendations. Results were expressed in nanograms per gram (ng/g) of tissue.

Myeloperoxidase (MPO) was determined by the method described by Krawisz et al. (1984) and the results were expressed as units per gram (U/g) of tissue. Each MPO unit is defined as the amount of the enzyme that catalyzes 1 nmol/min of hydrogen peroxide at 25°C . The absorbance was interpolated by means of a standard curve using human leukocyte myeloperoxidase (M6908, Sigma-Aldrich LTDA, São Paulo, Brazil). Malondialdehyde levels (MDA) were determined as described by Esterbauer and Cheeseman (1990), and results were calculated by interpolation in a standard

curve of malonaldehyde bis (diethyl acetal; T9889, Sigma-Aldrich LTDA) and expressed in nanomoles per gram of tissue. The absorbance was measured in a microplate reader (Polaris, Celer Biotechnology S/A, Belo Horizonte, Minas Gerais, Brazil) at 450 nm for cytokines and MPO, and at 586 nm for MDA.

Histopathological Analysis

Fragments of the cecum were fixed in 10% formalin for 24 h and then processed for histological analysis under routine standard procedures. Slides were mounted with 5-µm sections and Entellan (Merck, Kenilworth, NJ) before staining with hematoxylin and eosin.

Statistical Analyses

Conventional microbiological analyses of cecal contents, biochemical determinations, and gene expression by real-time PCR were expressed as treatment mean $\pm$ standard error. Differences among treatment means were tested by ANOVA, followed by Tukey test at 5% significance level ($P < 0.05$). Statistical analyses were performed using Graph Pad Prism 5.0 (GraphPad Software Inc., San Diego, CA).

For the microbiota analysis using NGS sequencing, α diversity index differences were evaluated by nonparametric Kruskal-Wallis pairwise test ($P < 0.05$). Beta diversity dissimilarities among treatments were evaluated by permutational multivariate analysis (Anderson, 2001).

RESULTS

Intestinal Colonization and Systemic Salmonella Infection

The SE counts were obtained from the cecal contents of all inoculated rats at 1 DPI. No SE was detected in the animals in the uninoculated control group. A significant difference ($P < 0.05$) in mean SE counts was observed across the treatments (Table 1). Rats receiving EM1107 or LrCh showed lower SE counts in the

cecal contents compared with the PtC and CCh groups. Translocation of SE to the liver and spleen of inoculated animals was assessed by microbial culture at 1, 4, and 8 DPI (data not shown). The presence of SE in the liver and spleen was detected from the beginning of the infection, indicating early penetration through the intestinal barrier and bacterial translocation. However, no statistical difference among the treatment groups was observed.

Microbiota Composition by 16S rRNA Sequencing

Reads ranged from 200 to 490 base pairs, averaging 447.13 base pairs. A total of 36,600 different OTU were obtained. Simpson and Shannon's index values were higher ($P < 0.05$) in NC compared with LrCh group, indicating a reduced gut microbial evenness associated with the use of the probiotic cheese. On the other hand, rats from LrCh group showed highest values for microbial richness (Chao1 and ACE plots), probably due to the increase of lactic acid bacteria (**LAB**) in the gut (Figure 2A and Supplemental Figure S1, <https://doi.org/10.3168/jds.2019-18820>). According to the beta diversity analysis (Figure 2B), NC and LrCh were the most dissimilar groups in terms of microbial composition.

Firmicutes and *Bacteroidetes* were the most abundant phyla across the treatments (Figure 3). Animals fed LrCh had marked increase in *Firmicutes* (69.82%) compared with NC (49.43%). The CCh group was the only group showing higher *Bacteroidetes* abundance (42.88%) compared with the control NC (39.82%), whereas all other groups had lower abundances (PtC = 21.58%; LrCh = 22.54%; EM1107 = 32.15%). *Spirochaetes* were mostly detected in NC group. The microbial abundances related to class and order levels are shown in Supplemental Figures S2 and S3 (<https://doi.org/10.3168/jds.2019-18820>), respectively.

At the family level (Figure 4), the most marked shifts in the microbiota were observed in rats from PtC and LrCh groups compared with NC. Both PtC and LrCh showed increased abundances of *Erysipelotrichaceae* (8.41 and 7.29%, respectively) compared with NC (1.55%), CCh (3.4%), and EM1107 (3.55%). Higher abundances of *Lachnospiraceae* were observed in LrCh (40.36%), CCh (37.66%), and EMM1107 (36%) compared with PtC (30.49%) and NC (27.46%). *Prevotellaceae* abundances were similar between CCh (37.66%) and NC (36.89%) groups, which were higher than those observed in EM1107 (26.58%), LrCh (18.48%), and PtC (17.21%). All groups showed reduced *Ruminococcaceae* abundances compared with NC (23.51%): PtC (19.38%), EM1107 (16.98%), LrCh (16.09%), and CCh (14.92%).

Table 1. *Salmonella* Enteritidis counts (mean $\pm$ SEM) in the cecal contents of rats 24 h after the challenge

Group	Count ¹ (log ₁₀ cfu/g)
Positive control	4.93 $\pm$ 0.39 ^a
Control cheese	4.75 $\pm$ 0.28 ^a
Probiotic cheese	4.22 $\pm$ 0.31 ^b
<i>Lactobacillus rhamnosus</i> EM1107	4.32 $\pm$ 0.23 ^b

^{a,b}Means in a same column with different letters differ significantly ($P < 0.05$).

Figure 2. Microbial diversity in the cecum of rats under different dietary treatments: NC = negative control; PtC = positive control; CCh = control cheese; LrCh = probiotic cheese; EM1107 = probiotic alone. (A) Plots showing α diversity determined by different indices: Chao1, ACE, Shannon, Simpson, InvSimpson, and Fisher. (B) Relative relatedness of the microbial community members according to the principal coordinate analysis with Bray-Curtis distance.

In terms of genera (Figure 5), we highlight the marked increase in *Blautia* spp. in the feces of rats fed LrCh (28.43%) and EM1107 (23.47%) compared with CCh (13.5%), PtC (11.26%), and NC (6.75%) groups. Variations in the abundance of *Prevotella* across the groups were also observed; NC and CCh groups showed similar abundances (32.69 and 31.43%, respectively) compared with EM1107 (17.89%), PtC (14.21%), and LrCh (10.74%).

Figure 6 presents the relative abundance of *Lactobacillus* spp among the experimental groups. Interestingly, rats in the LrCh group presented the highest *Lactobacillus* spp. abundance compared with the others. The results of the LEfSe analysis are shown in Figure 7, which presents OTU at different taxonomic levels that are significantly different between the NC and the other groups ($P < 0.05$).

NF- κ B Determinations

At 1 DPI, the expression of NF- κ B was higher ($P < 0.05$) in PtC compared with other groups. This difference was observed up to 4 DPI when compared with

CCh and EM1107 groups. Lower ($P < 0.05$) NF- κ B levels were seen in rats fed LrCh compared with PtC at 8 DPI (Figure 8A).

Cytokine Level

The IL-1 β and IFN- γ levels were lower ($P < 0.05$) in EM1107 at 1 DPI compared with PtC. At 4 DPI, IFN- γ was reduced ($P < 0.05$) in LrCh and EM1107 compared with PtC. On the last sampling day (8 DPI), TNF- α was lower in CCh and EM107 groups, whereas the IFN- γ decreased in both of the experimental groups in which rats were fed cheese (CCh and LrCh) compared with PtC. No statistical difference was observed in IL-10 levels among SE-inoculated groups (Figure 8B–E).

MPO and MDA Determinations

There was a reduction ($P < 0.05$) in MPO activity in EM1107 compared with PtC at 4 DPI. Higher levels ($P < 0.05$) of MPO were observed in rats fed cheese (LrCh and CCh treatment groups) compared with PtC group. No difference was observed in MDA levels among the

Figure 3. Effect of group treatments on the top 7 most abundant taxa at the phylum level. NC = negative control; PtC = positive control; CCh = control cheese; LrCh = probiotic cheese; EM1107 = probiotic only.

Figure 4. Effect of group treatments on the top 10 most abundant taxa at the family level. NC = negative control; PtC = positive control; CCh = control cheese; LrCh = probiotic cheese; EM1107 = probiotic only.

treatment groups, except for a reduction ($P < 0.05$) in EM1107 compared with PtC group at 4 and 8 DPI (Figure 9).

Histopathology

At the beginning of the inoculation (1 DPI), there was a reduction of neutrophilic inflammation in the cecum of rats from EM1107 group, with similar findings observed in healthy animals (NC). In the LrCh group, discrete lymphohistiocytic inflammation was observed in the cecal mucosa accompanied by discrete edema of the submucosa. The PtC animals presented diffuse lymphohistiocytic inflammation in the cecal mucosa accompanied by severe hemorrhage and submucosal edema (Figure 10). At 4 DPI, a slight multifocal lymphohistiocytic inflammation in the mucosa was found in EM1107 animals, whereas the PtC group presented diffuse lymphohistiocytic inflammation in the cecal mucosa accompanied by edema of submucosa. Similar to EM1107, the LrCh group presented a reduction in

tissue inflammation, with discrete lymphohistiocytic inflammation in the cecal mucosa accompanied by discrete submucosal edema, in contrast to PtC (Figure 11). Finally, there was an apparent improvement in lymphohistiocytic inflammation in the EM1107 group at 8 DPI, whereas there was a discrete improvement at the submucosa level in the LrCh group (Figure 12).

DISCUSSION

The findings of the present study indicate that *L. rhamnosus* EM1107 led to anti-inflammatory effects associated with the modulation of the immune system, reduced intestinal tissue damage, ameliorating the oxidative stress response induced by *Salmonella* infection in rats challenged with *Salmonella* Enteritidis. The administration of *L. rhamnosus* EM1107, either in a cheese matrix or alone, was able to reduce the intestinal SE colonization. A previous in vitro study showed that a probiotic *Lactobacillus* spp. strain (KSBT56) led to an inhibitory effect over *Salmonella* Enteritidis

P125109 by decreasing its multiplication, adhesion, and invasion in colon epithelial cells, probably due to the production of lactic acid (Das et al., 2013). *Lactobacillus* spp. metabolize carbohydrates to produce lactic acid that decreases fecal pH. It is possible the lower pH contributed to the reduced SE counts in the feces of rats receiving the probiotic strain (EM1107 and LrCh). Hydrogen peroxide, carbon dioxide, and antibacterial compounds, including bacteriocins and nonbacteriocins and nonlactic acid molecules can also be produced by LAB and could be detrimental to intestinal *Salmonella* strains (Marianelli et al., 2010; Zhang et al., 2018).

In addition, the presence of SE in the liver and spleen at the beginning of the infection in the animals

of all SE-challenged groups confirms the invasiveness of the SE strain used in the present experiment. According to Finlay and Brumell (2000), SE can cross the lining of intestinal epithelial cells and translocate to extraintestinal organs such as the spleen and liver. *Salmonella*'s capacity to cross the gut barrier, spread to other organs, and survive in host defense cells, such as macrophages and dendritic cells, is attributed to the *Salmonella* pathogenicity island I and II, respectively, which encode for genes that allow enterocyte invasion and bacterial proliferation in extraintestinal tissue and therefore cause systemic infections (Silva et al., 2012; Foley et al., 2013). According to our model, the treatment with *L. rhamnosus* EM1107 had no effect on the

Figure 5. Effect of group treatments on the top 15 most abundant taxa at the genus level. NC = negative control; PtC = positive control; CCh = control cheese; LrCh = probiotic cheese; EM1107 = probiotic only.

Figure 6. Effect of administration of treatments on the relative operational taxonomic unit (OUT) counts related to *Lactobacillus* spp. across the different treatment groups. NC = negative control; PtC = positive control; CCh = control cheese; LrCh = probiotic cheese; EM1107 = probiotic alone. Data are expressed as mean $\pm$ SEM.

systemic survival of SE promoted by *Salmonella* pathogenicity island II.

In terms of gut microbial diversity, the 16S rRNA sequencing analysis revealed that *L. rhamnosus* EM1107 led to significant changes in the gut microbiota of rats toward a reduced evenness and increased richness compared with the sham treatment (NC; Figure 2). This finding is probably related to the antimicrobial effects of the probiotic strain, which plays a role as a rare specimen in the colonization of the gut. The LAB-supplemented feed is associated with significant shifts in gut microbiome in different species, such as shrimp (Sha et al., 2016) and chicken (Wang et al., 2017). In terms of β -diversity, the dissimilarities between NC and the other treatments, mostly with the LrCh group, indicate that the insertion of an alien bacterium strain into the gut can cause a switchover into the microbial composition to the point of decreasing the microbial relatedness in each community (Figure 2). According to our data, the NC group presented a higher diversity of OTU compared with the other treatments (Figure 6).

The *L. rhamnosus* strain EM1107 favored the growth of some commensal bacteria, such as those belonging to the family *Lachnospiraceae* and the genus *Blautia* (Figures 4 and 5). *Lachnospiraceae* are abundant gut commensal organisms and are consistently depleted

in people with intestinal diseases, suggesting that these organisms are important to maintain intestinal homeostasis (Suchodolski, 2013). Reeves et al. (2012) demonstrated that *Lachnospiraceae* strains were able to partially restore eubiosis in response to *Clostridium difficile* postinfection and improve clinical status in germfree mice. According to Jenq et al. (2015), *Blautia* spp. are associated with reduced lethal graft-versus-host disease and negatively correlated with poor health conditions, such as malnutrition (Million et al., 2016), irritable bowel syndrome (Weinberg et al., 2018), and early-stage breast cancer patients (Luu et al., 2017).

We observed that cheese probably served as a *Prevotella* source for mice gut colonization (Figure 5). Milk is considered an important source of *Prevotella* organisms in consumers, as has been reported in several species (Brink et al., 2019; Petrullo et al., 2019). The higher abundance of *Lactobacillus* in rats fed cheese (Figure 6) corroborates our hypothesis that cheese serves as a protective matrix for *L. rhamnosus* EM1107. Compared with other dairy products, cheese represents a better matrix favoring LAB, attributed to its chemical and physical properties such as less acidic environment, high buffering capacity and low oxygen contents due to the solid matrix composed both of high fat and protein contents (Cichosz et al., 2014; Haman and Ahmed, 2019).

Previous studies have shown that the consumption of probiotic-added dairy products leads to beneficial microbial effects in hosts affected by several clinical disorders. Nonfermented dairy products with added *Lactobacillus* spp. may halt dysbiosis associated with penicillin after administration, and therefore prevent *Clostridium* spp. overgrowth (Korpela et al., 2016). Moreover, dairy products supplemented with probiotics may favor the growth of butyrate-producing strains, which lead to amelioration of autoimmune illnesses such as atopic dermatitis (Jong-Hwa et al., 2019).

The reduced expression of NF- κ B in EM1107 animals at 1 and 4 DPI (Figure 8) was probably associated with the decrease in TNF- α , IL-1 β , and IFN- γ . Similarly, the reduction of the levels of NF- κ B in the LrCh group, which occurred on 1 and 8 DPI, can explain lower cytokine levels at 1 (TNF- α), 4 (IL-1 β and IFN- γ), and 8 DPI (IFN- γ). Minor differences in the cytokine expression patterns between EM1107 and LrCh groups may be attributed to the effects of the lactic matrix. Interestingly, lower levels of NF- κ B expression were observed in the CCh group from 1 to 4 DPI, with consequent reduction in TNF- α levels at 1 and 8 DPI, IL-1 β at 4 DPI, and IFN- γ at 8 DPI. Our results corroborate a previous study using the same probiotic strain (Rodrigues et al., 2018), which reported a reduction in the expression of NF- κ B, TNF- α , and IL-1 β in

rats receiving the probiotic strain alone or added to a cheese matrix.

Invasive pathogens, such as *Salmonella*, stimulate the induction of the innate host immune response. The intestinal endothelium detects the presence of the invader through receptors and begins activation of mitogen-activated protein kinase that induces gene expression of transcription factors such as activating protein 1 and NF- κ B. This results in the production of pro-inflammatory cytokines IL-18 and IL-12, which stimulate the activation of type 1 helper cells that in turn synthesize IFN- γ , increasing phagocyte activity and destruction of internalized bacteria. Interleukin-1 β , TNF- α , and IL-23 recruit neutrophils that are responsible for the defense of extracellular bacteria (Santos et al., 2009; Thiennimitr et al., 2011). In contrast, the use of probiotics can modulate different pro-inflammatory cytokine profiles, such as TNF- α , IFN- γ , and IL-1 β , and anti-inflammatory cytokines, such as IL-10 and IL-4, which may promote improved host immune response against invasion by pathogens such as *Salmonella* (Castillo et al., 2012; Alvim et al., 2016).

In the present study, there was a reduction in MPO activity during infection (4 DPI) in the EM1107 group compared with PtC (Figure 9). Therefore, EM1107

could reduce the effects of inflammatory stress. On the other hand, MPO activity was higher both in the CCh and LrCh groups (Figure 9). As a consequence of TNF- α induction by SE, neutrophil infiltration into the epithelium generates the release of MPO, an enzyme responsible for the overproduction of reactive oxygen species that causes a decrease in intestinal antioxidant defenses, such as glutathione (de Moreno de LeBlanc and Perdigon, 2010). Furthermore, it triggers an increase in the production of MDA, a secondary product of lipid peroxidation.

Although the histopathological findings revealed a reduction in the gut inflammation observed in CCh and EM1107 groups compared with the PtC (Figures 10, 11, and 12), there was no direct correlation with MPO activity, either at the beginning or the end of evaluated periods (Figure 9). Similar results have been reported by de Moreno de LeBlanc and Perdigon (2010). In addition, the amelioration of tissue inflammation in rats fed cheese could be also associated with the presence of glutathione peroxidase in goat milk, which is part of a defense system against pathogenic microorganisms (Slaćanac et al., 2010).

Malondialdehyde is a parameter for assessing oxidative stress caused by tissue inflammation. This is

Figure 7. Discriminative operational taxonomic units at different taxonomic levels between treatment groups, according to linear discriminant analysis effect size. NC = negative control; PtC = positive control; CCh = control cheese; LrCh = probiotic cheese; EM1107 = probiotic alone; f_ = family; g_ = genus; p_ = phylum; c_ = class; o_ = order.

Figure 8. Effect of administration of treatments on levels of (A) nuclear factor kappa B (NF-κB), (B) IL-1β, (C) tumor necrosis factor-α (TNF-α), (D) IL-10, and (E) INF-γ of cecal tissues in euthanized animals 1, 4, and 8 d after salmonellosis infection. Data are expressed as mean ± SEM. Different letters (a–c) indicate significant differences ($P < 0.05$). NC = negative control; PtC = positive control; CCh = control cheese; LrCh = probiotic cheese; EM1107 = *Lactobacillus rhamnosus* EM1107.

because increased free radicals cause a higher concentration of MDA, a secondary product of lipid peroxidation, which can cause DNA damage, resulting in lysis and cell death (Rodrigues et al., 2018). In the present study, there was a reduction in MDA levels throughout the course of infection (4 and 8 DPI) in the EM1107 group compared with PtC. These results demonstrate that the probiotic strain administered alone was able to reduce intestinal oxidative stress. Our results support previous findings showing MDA reduction in rats receiving EM1107 and submitted to the ulcerative colitis (Rodrigues et al., 2018).

The inflammatory features observed in the cecum of the PtC group animals were typical SE responses described in various studies. These responses were characterized by enterocolitis with infiltration of polymorphonuclear leukocytes, and presence of edema, hemorrhages, and epithelial erosion (Haraga et al., 2008; Santos et al., 2009). A general reduction in inflammation was observed in rats from EM1107, LrCh, and CCh groups compared with PtC group throughout the experimental period (Figure 8). Moreover, the histological findings in the animals from EM1107 and LrCh groups were similar to those observed in NC

group at 1, and 4 DPI, respectively (Figures 10, 11, and 12). De Moreno de LeBlanc et al. (2010) reported no inflammation but only increased lymphohistiocytic infiltration in rats receiving *Lactobacillus casei* 431 for 7 d after they had been challenged with *Salmonella* Typhimurium. Similarly, Kemgang et al. (2016) observed a significant improvement in villi structure of animals fed *L. rhamnosus* S1K3, 5 to 20 DPI. The protective action of probiotics in minimizing tissue damage caused by *Salmonella* has also been reported (Asahara et al., 2011; Castillo et al., 2013; Noto Llana et al., 2013).

According to similar study using a chemically induced colitis model (Rodrigues et al., 2018), EM1107 treatment increased the expression of mucin 2, indicating mucus as a protective factor for the gut epithelium against SE colonization and invasiveness. Although goat cheese reduced the tissue damage caused by SE, no synergic effect on inflammation status was observed by adding EM1107 in the form of a probiotic cheese.

CONCLUSIONS

Cheese supplemented with *L. rhamnosus* EM1107 strain was able to reduce *Salmonella* colonization, de-

Figure 9. Effect of administration of treatments on (A) myeloperoxidase (MPO) activity and (B) malondialdehyde (MDA) content in cecal tissues of euthanized animals 1, 4, and 8 d after salmonellosis infection. Data are expressed as mean $\pm$ SEM. Different letters (a–d) indicate significant differences ($P < 0.05$). NC = negative control; PtC = positive control; CCh = control cheese; LrCh = probiotic cheese; EM1107 = *Lactobacillus rhamnosus* EM1107.

crease the production of pro-inflammatory cytokines, and reduce tissue damage in the intestinal lumen of rats at early stages of infection. Although the administration of *L. rhamnosus* EM1107 alone provided protec-

tive effects against SE, the use of cheese as a vehicle for *L. rhamnosus* EM1107 resulted in a more pronounced shift in the microbial composition, favoring beneficial organisms such as *Blautia* and *Lactobacillus*.

Figure 10. Histopathological analysis of Wistar rat ceca on the first day after challenge with *Salmonella* Enteritidis P125109 with different degrees of typhlitis. (A) Mild neutrophilic inflammation of the cecum, in addition to submucosal edema in animals of the negative control group. (B) Diffuse lymphohistiocytic inflammation in the cecal mucosa accompanied by severe hemorrhage and submucosal edema in positive control group animals. (C) Focal and discrete neutrophilic and lymphohistiocytic inflammation in the cecal mucosa with edema in the submucosa of the control cheese group. (D) Focal and discrete neutrophilic and lymphohistiocytic inflammation in the cecal mucosa with marked hemorrhage and edema in the submucosa of the control cheese group. (E) Discrete multifocal neutrophilic inflammation in the cecum mucosa with slight degeneration of smooth muscle fibers from the *Lactobacillus rhamnosus* EM1107 group. (F) Mild lymphohistiocytic inflammation in the cecal mucosa accompanied by mild submucosal edema of the animals of the probiotic cheese group. L = cecal lumen; Lp = lamina propria; g = goblet cells; sa = submucosa; TM = muscular tunic; h = hemorrhage; sae = submucosa edema; MN = lymphohistiocytic or lymphohistioplasmicocyte infiltrate; fd = degeneration of smooth muscle fibers. * indicates neutrophilic infiltrate. H&E coloring (Merck, Darmstadt, Germany). Magnification = 100×.

ACKNOWLEDGMENTS

The authors thank Maria Lúcia da Conceição for support in the microbiological analysis in the Laboratory of Microbiology of Food (Department of Nutrition,

Center of Sciences of the Health, Federal University of Paraíba, João Pessoa, Brazil) and graduate students Karol Sampaio, Luana Martiniano, Camila Neves, Eduarda Marinho, Pedro Jusselino and Matheus Estrela (Federal University of Paraíba, João Pessoa, Brazil).

Figure 11. Histopathological analysis of Wistar rat ceca on d 4 after challenge with *Salmonella* Enteritidis P125109 with different degrees of typhlitis. (A) Focal lymphohistiocytic inflammation in the cecum mucosa of animals of the negative control group. Magnification = 100×. (B) Diffuse lymphohistiocytic inflammation in the cecal mucosa accompanied by submucosal edema in positive control group animals. Magnification = 100×. (C) Lymphohistiocytic inflammation and slight erosion of the epithelium in the cecal mucosa with slight edema in the submucosa of the animals of the control cheese group. Magnification = 100×. (D) Discrete multifocal lymphohistiocytic inflammation in the cecum mucosa of animals of the *Lactobacillus rhamnosus* EM1107 group. Magnification = 100×. (E) Discrete lymphohistiocytic inflammation in the cecal mucosa accompanied by slight edema of the submucosa of the animals of the probiotic cheese group. (F) Inflammatory lymphohistiocytic infiltrate in the lamina propria of the animals of the probiotic cheese group evidenced at greater magnification (400×). L = cecal lumen; g = goblet cells; sa = submucosa; TM = muscular tunic; h = hemorrhage; sae = submucosa edema; MN = lymphohistiocytic or lymphohistioplasmicocyte infiltrate; e = erosion of the lining epithelium; fd = degeneration of smooth muscle fibers. H&E coloring (Merck, Darmstadt, Germany).

Figure 12. Histopathological analysis of Wistar rat ceca on d 8 after challenge with *Salmonella* Enteritidis P125109 with different degrees of typhlitis. (A) Discrete multifocal neutrophilic inflammation in the cecal mucosa and hyperemia in the submucosa of animals in the negative control group. Magnification: 100×. (B) Moderate diffuse neutrophilic and lymphohistiocytic inflammation in the cecal mucosa, as well as multifocal erosion of the epithelium and lymphohistioplasmic cell inflammation with diffuse edema in the submucosa of positive control group animals. Magnification: 100×. (C) Multifocal neutrophilic and lymphohistiocytic inflammation in the cecum mucosa with diffuse edema in the submucosa of the control cheese group animals. Magnification = 100×. (D) Discrete multifocal lymphohistiocytic inflammation in the cecum mucosa and lymphohistiocytic inflammation, in addition to diffuse edema with focal hyperemia in the submucosa of animals of the *Lactobacillus rhamnosus* EM1107 group. Magnification: 100×. (E) Diffuse neutrophilic and lymphohistiocytic inflammation, as well as erosion of the cecal mucosal epithelium accompanied by mild lymphohistioplasmicocyte infiltrate and edema in the submucosa of the animals of the probiotic cheese group; probiotic cheese group evidenced at higher magnification (400×). L = cecal lumen; g = goblet cells; sa = submucosa; TM = muscular tunic; hp = hyperemia; sae = submucosa edema; MN = lymphohistiocytic or lymphohistioplasmicocyte infiltrate; e = erosion of the lining epithelium. * indicates neutrophilic infiltrate. H&E coloring (Merck, Darmstadt, Germany).

This work was supported by the EMBRAPA (Cod 02.09.01.024.00.00), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brazil (CAPES), Brazil (Finance Code 001) and Financiadora de estudos e

Projetos (FINEP). All procedures were approved by the Animal Ethics Committee of the Federal University of Paraíba (CEUA/UFPB- protocol no. 021/2016) following the National Institutes of Health (Bethesda,

MD) guide for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978). The authors declare no conflict of interest.

REFERENCES

- Acurcio, L. B., R. W. Bastos, S. H. C. Sandes, A. C. C. Guimarães, C. G. Alves, D. C. dos Reis, S. Wuyts, A. C. Nunes, G. D. Cassali, S. Lebeer, M. R. de Souza, and J. R. Nicoli. 2017. Protective effects of milk fermented by *Lactobacillus plantarum* B7 from Brazilian artisanal cheese on a *Salmonella enterica* serovar Typhimurium infection in BALB/c mice. *J. Funct. Foods* 33:436–445. <https://doi.org/10.1016/j.jff.2017.04.010>.
- Albenzio, M., A. Santillo, M. Caroprese, D. Ruggieri, F. Napolitano, and A. Sevi. 2013. Physicochemical properties of Scamorza ewe milk cheese manufactured with different probiotic cultures. *J. Dairy Sci.* 96:2781–2791. <https://doi.org/10.3168/jds.2012-6218>.
- Alvim, L. B., S. H. C. Sandes, B. C. Silva, R. S. Steinberg, M. H. A. Campos, L. B. Acurcio, R. M. Arantes, J. R. Nicoli, E. Neumann, and Á. C. Nunes. 2016. *Weissella paramesenteroides* WpK4 reduces gene expression of intestinal cytokines, and hepatic and splenic injuries in a murine model of typhoid fever. *Benef. Microbes* 7:61–73. <https://doi.org/10.3920/BM2015.0093>.
- Anderson, M. J. 2001. A new method for non-parametric multivariate analysis of variance. *Austral Ecol.* 26:32–46.
- Asahara, T., K. Shimizu, T. Takada, S. Kado, N. Yuki, M. Morotomi, R. Tanaka, and K. Nomoto. 2011. Protective effect of *Lactobacillus casei* strain Shiota against lethal infection with multi-drug resistant *Salmonella enterica* serovar Typhimurium DT104 in mice. *J. Appl. Microbiol.* 110:163–173. <https://doi.org/10.1111/j.1365-2672.2010.04884.x>.
- Barthel, M., S. Hapfelmeier, L. Quintanilla-Martínez, M. Kremer, M. Rohde, M. Hogardt, K. Pfeffer, H. Rüssmann, and W. D. Hardt. 2003. Pretreatment of mice with streptomycin provides a *Salmonella enterica* serovar Typhimurium colitis model that allows analysis of both pathogen and host. *Infect. Immun.* 71:2839–2858. <https://doi.org/10.1128/IAI.71.5.2839-2858.2003>.
- Bolyen, E., J. R. Rideout, M. R. Dillon, N. A. Bokulich, C. Abnet, G. A. Al-Ghalith, H. Alexander, E. J. Alm, M. Arumugam, F. Asnicar, Y. Bai, J. E. Bisanz, K. Bittinger, A. Brejnrod, C. J. Brislawn, C. T. Brown, B. J. Callahan, A. M. Caraballo-Rodríguez, J. Chase, E. Cope, R. Da Silva, P. C. Dorrestein, G. M. Douglas, D. M. Durall, C. Duvallet, C. F. Edwards, M. Ernst, M. Eustaki, J. Fouquier, J. M. Gauglitz, D. L. Gibson, A. Gonzalez, K. Gorlick, J. Guo, B. Hillmann, S. Holmes, H. Holste, C. Huttenhower, G. Huttley, S. Janssen, A. K. Jarmusch, L. Jiang, B. Kaehler, K. B. Kang, C. R. Keefe, P. Keim, S. T. Kelley, D. Knights, I. Koester, T. Kosciulek, J. Kreps, M. G. I. Langille, J. Lee, R. Ley, Y.-X. Liu, E. Loftfield, C. Lozupone, M. Maher, C. Marotz, B. D. Martin, D. McDonald, L. J. McIver, A. V. Melnik, J. L. Metcalf, S. C. Morgan, J. Morton, A. T. Naimy, J. A. Navas-Molina, L. F. Nothias, S. B. Orchanian, T. Pearson, S. L. Peoples, D. Petras, M. L. Preuss, E. Priesse, L. B. Rasmussen, A. Rivers, M. S. Robeson, P. Rosenthal, N. Segata, M. Shaffer, A. Shiffer, R. Sinha, S. J. Song, J. R. Spear, A. D. Swofford, L. R. Thompson, P. J. Torres, P. Trinh, A. Tripathi, P. J. Turnbaugh, S. Ul-Hasan, J. J. J. van der Hooft, F. Vargas, Y. Vázquez-Baeza, E. Vogtmann, M. von Hippel, W. Walters, Y. Wan, M. Wang, J. Warren, K. C. Weber, C. H. D. Williamson, A. D. Willis, Z. Z. Xu, J. R. Zaneveld, Y. Zhang, Q. Zhu, R. Knight, and J. G. Caporaso. 2019. QIIME 2: Reproducible, interactive, scalable, and extensible microbiome data science. *Nature Biotechnology* 37:852–857. <https://doi.org/10.1038/s41587-019-0209-9>.
- Brink, L. R., K. Matazel, B. D. Piccolo, A. K. Bowlin, S. V. Chintapalli, K. Shankar, and L. Yeruva. 2019. Neonatal diet impacts bioregional microbiota composition in piglets fed human breast milk or infant formula. *J. Nutr.* 149:2236–2246. <https://doi.org/10.1093/jn/nxz170>.
- Caporaso, J. G., C. L. Lauber, W. A. Walters, D. Berg-Lyons, C. A. Lozupone, P. J. Turnbaugh, N. Fierer, and R. Knight. 2011. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. *Proc. Natl. Acad. Sci. USA* 108(Supplement 1):4516–4522. <https://doi.org/10.1073/pnas.1000080107>.
- Castillo, N. A., A. M. de Moreno de LeBlanc, C. M. Galdeano, and G. Perdigon. 2012. Probiotics: An alternative strategy for combating salmonellosis: Immune mechanisms involved. *Food Res. Int.* 45:831–841. <https://doi.org/10.1016/j.foodres.2011.04.031>.
- Castillo, N. A., A. M. de Moreno de LeBlanc, C. M. Galdeano, and G. Perdigon. 2013. Comparative study of the protective capacity against *Salmonella* infection between probiotic and nonprobiotic lactobacilli. *J. Appl. Microbiol.* 114:861–876. <https://doi.org/10.1111/jam.12074>.
- Chen, H.-M., Y. Wang, L.-H. Su, and C.-H. Chiu. 2013. Nontyphoid *Salmonella* infection: Microbiology, clinical features, and antimicrobial therapy. *Pediatr. Neonatol.* 54:147–152. <https://doi.org/10.1016/j.pedneo.2013.01.010>.
- Cichosz, G., M. Aljewicz, and B. Nalepa. 2014. Viability of the *Lactobacillus rhamnosus* HN001 probiotic strain in Swiss- and Dutch-type cheese and cheese-like products. *J. Food Sci.* 79:M1181–M1188. <https://doi.org/10.1111/1750-3841.12458>.
- Dantas, A. B., V. F. Jesus, R. Silva, C. N. Almada, E. A. Esmerino, L. P. Cappato, M. C. Silva, R. S. L. Raices, R. N. Cavalcanti, C. C. Carvalho, A. S. Sant'Ana, H. M. A. Bolini, M. Q. Freitas, and A. G. Cruz. 2016. Manufacture of probiotic Minas Frescal cheese with *Lactobacillus casei* Zhang. *J. Dairy Sci.* 99:18–30. <https://doi.org/10.3168/jds.2015-9880>.
- Das, J. K., D. Mishra, P. Ray, P. Tripathy, T. K. Beuria, N. Singh, and M. Suar. 2013. In vitro evaluation of anti-infective activity of a *Lactobacillus plantarum* strain against *Salmonella enterica* serovar Enteritidis. *Gut Pathog.* 5:11. <https://doi.org/10.1186/1757-4749-5-11>.
- de Moreno de LeBlanc, A., and G. Perdigon. 2010. The application of probiotic fermented milks in cancer and intestinal inflammation. *Proc. Nutr. Soc.* 69:421–428. <https://doi.org/10.1017/S002966511000159X>.
- dos Santos, K. M. O., M. A. D. Bomfim, A. D. S. Vieira, S. D. Benevides, S. M. I. Saad, F. C. A. Buriti, and A. S. Egitto. 2012. Probiotic caprine Coalho cheese naturally enriched in conjugated linoleic acid as a vehicle for *Lactobacillus acidophilus* and beneficial fatty acids. *Int. Dairy J.* 24:107–112. <https://doi.org/10.1016/j.idairyj.2011.12.001>.
- dos Santos, K. M. O., A. D. S. Vieira, F. C. A. Buriti, J. C. F. do Nascimento, M. E. S. de Melo, L. M. Bruno, M. de Fátima Borges, C. R. C. Rocha, A. C. de Souza Lopes, B. D. G. de Melo Franco, and S. D. Todorov. 2015. Artisanal Coalho cheeses as source of beneficial *Lactobacillus plantarum* and *Lactobacillus rhamnosus* strains. *Dairy Sci. Technol.* 95:209–230. <https://doi.org/10.1007/s13594-014-0201-6>.
- Edgar, R. C., B. J. Haas, J. C. Clemente, C. Quince, and R. Knight. 2011. UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics* 27:2194–2200. <https://doi.org/10.1093/bioinformatics/btr381>.
- Esterbauer, H., and K. H. Cheeseman. 1990. Determination of aldehydic lipid peroxidation products: Malonaldehyde and 4-hydroxynonenal. *Methods Enzymol.* 186:407–421. [https://doi.org/10.1016/0076-6879\(90\)86134-H](https://doi.org/10.1016/0076-6879(90)86134-H).
- Finlay, B. B., and J. H. Brumell. 2000. *Salmonella* interactions with host cells: In vitro to in vivo. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 355:623–631. <https://doi.org/10.1098/rstb.2000.0603>.
- Foley, S. L., T. J. Johnson, S. C. Rieke, R. Nayak, and J. Danzeisen. 2013. *Salmonella* pathogenicity and host adaptation in chicken-associated serovars. *Microbiol. Mol. Biol. Rev.* 77:582–607. <https://doi.org/10.1128/MMBR.00015-13>.
- Hammam, A. R. A., and M. S. I. Ahmed. 2019. Technological aspects, health benefits, and sensory properties of probiotic cheese. *SN Appl. Sci.* 1:1113. <https://doi.org/10.1007/s42452-019-1154-4>.
- Haraga, A., M. B. Ohlson, and S. I. Miller. 2008. *Salmonellae* interplay with host cells. *Nat. Rev. Microbiol.* 6:53–66. <https://doi.org/10.1038/nrmicro1788>.
- Hill, C., F. Guarner, G. Reid, G. R. Gibson, D. J. Merenstein, B. Pot, L. Morelli, R. B. Canani, H. J. Flint, S. Salminen, P. C. Calder,

- and M. E. Sanders. 2014. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nat. Rev. Gastroenterol. Hepatol.* 11:506–514. <https://doi.org/10.1038/nrgastro.2014.66>.
- Jenq, R. R., Y. Taur, S. M. Devlin, D. M. Ponce, J. D. Goldberg, K. F. Ahr, E. R. Littmann, L. Ling, A. C. Gobourne, L. C. Miller, M. D. Docampo, J. U. Peled, N. Arpaia, J. R. Cross, T. K. Peets, M. A. Lumish, Y. Shono, J. A. Dudakov, H. Poeck, A. M. Hanash, J. N. Barker, M. A. Perales, S. A. Giral, E. G. Pamer, and M. R. van den Brink. 2015. Intestinal *Blautia* is associated with reduced death from graft-versus-host disease. *Biol. Blood Marrow Transplant.* 21:1373–1383. <https://doi.org/10.1016/j.bbmt.2015.04.016>.
- Kim, J.-H., K. Kim, and W. Kim. 2019. Cream cheese-derived *Lactococcus chungangensis* CAU 28 modulates the gut microbiota and alleviates atopic dermatitis in BALB/c mice. *Sci. Rep.* 9:446.
- Kamdar, K., S. Khakpour, J. Chen, V. Leone, J. Brulc, T. Mangatu, D. A. Antonopoulos, E. B. Chang, S. A. Kahn, B. S. Kirschner, G. Young, and R. W. DePaolo. 2016. Genetic and metabolic signals during acute enteric bacterial infection alter the microbiota and drive progression to chronic inflammatory disease. *Cell Host Microbe* 19:21–31. <https://doi.org/10.1016/j.chom.2015.12.006>.
- Katoh, K., K. Misawa, K. Kuma, and T. Miyata. 2002. MAFFT: A novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Res.* 30:3059–3066. <https://doi.org/10.1093/nar/gkf436>.
- Kemgang, T. S., S. Kapila, V. P. Shanmugam, S. Reddi, and R. Kapila. 2016. Fermented milk with probiotic *Lactobacillus rhamnosus* S1K3 (MTCC5957) protects mice from *salmonella* by enhancing immune and nonimmune protection mechanisms at intestinal mucosal level. *J. Nutr. Biochem.* 30:62–73. <https://doi.org/10.1016/j.jnutbio.2015.11.018>.
- Korpela, K., A. Salonen, L. J. Virta, M. Kumpu, R. A. Kekkonen, and W. M. de Vos. 2016. *Lactobacillus rhamnosus* GG intake modifies preschool children's intestinal microbiota, alleviates penicillin-associated changes, and reduces antibiotic use. *PLoS One* 11:e0154012. <https://doi.org/10.1371/journal.pone.0154012>.
- Krawisz, J. E., P. Sharon, and W. F. Stenson. 1984. Quantitative assay for acute intestinal inflammation based on myeloperoxidase activity. Assessment of inflammation in rat and hamster models. *Gastroenterology* 87:1344–1350. [https://doi.org/10.1016/0016-5085\(84\)90202-6](https://doi.org/10.1016/0016-5085(84)90202-6).
- Lozupone, C., and R. Knight. 2005. UniFrac: A new phylogenetic method for comparing microbial communities. *Appl. Environ. Microbiol.* 71:8228–8235. <https://doi.org/10.1128/AEM.71.12.8228-8235.2005>.
- Luu, T. H., C. Michel, J. M. Bard, F. Dravet, H. Nazih, and C. Bobin-Dubigeon. 2017. Intestinal proportion of *Blautia* sp. is associated with clinical stage and histoprosthetic grade in patients with early-stage breast cancer. *Nutr. Cancer* 69:267–275. <https://doi.org/10.1080/01635581.2017.1263750>.
- Ma, T., Y. Suzuki, and L. L. Guan. 2018. Dissect the mode of action of probiotics in affecting host-microbial interactions and immunity in food producing animals. *Vet. Immunol. Immunopathol.* 205:35–48. <https://doi.org/10.1016/j.vetimm.2018.10.004>.
- Marco, M. L., D. Heeney, S. Binda, C. J. Cifelli, P. D. Cotter, B. Folligné, M. Gänzle, R. Kort, G. Pasin, A. Pihlanto, E. J. Smid, and R. Hutkins. 2017. Health benefits of fermented foods: Microbiota and beyond. *Curr. Opin. Biotechnol.* 44:94–102. <https://doi.org/10.1016/j.copbio.2016.11.010>.
- Marianelli, C., N. Cifani, and P. Pasquali. 2010. Evaluation of antimicrobial activity of probiotic bacteria against *Salmonella enterica* ssp. *enterica* serovar Typhimurium 1344 in a common medium under different environmental conditions. *Res. Microbiol.* 161:673–680. <https://doi.org/10.1016/j.resmic.2010.06.007>.
- McMurdie, P. J., and S. Holmes. 2013. phyloseq: An R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS One* 8:e61217. <https://doi.org/10.1371/journal.pone.0061217>.
- Million, M., M. T. Alou, S. Khelaifa, D. Bachar, J.-C. Lagier, N. Di-one, S. Brah, P. Hugon, V. Lombard, F. Armougom, J. Fromonot, C. Robert, C. Michelle, A. Diallo, A. Fabre, R. Guieu, C. Sokhna, B. Henrissat, P. Parola, and D. Raoult. 2016. Increased gut redox and depletion of anaerobic and methanogenic prokaryotes in severe acute malnutrition. *Sci. Rep.* 6:26051. <https://doi.org/10.1038/srep26051>.
- Moreau, M. R., D. S. S. Wijetunge, M. L. Bailey, S. R. Gongati, L. L. Goodfield, E. M. K. K. Hewage, M. J. Kennett, C. Fedorchuk, Y. V. Ivanov, J. E. Linder, B. M. Jayarao, and S. Kariyawasam. 2016. Growth in egg yolk enhances *Salmonella* Enteritidis colonization and virulence in a mouse model of human colitis. *PLoS One* 11:e0150258. <https://doi.org/10.1371/journal.pone.0150258>.
- Mushtaq, M., A. Gani, F. A. Masoodi, and M. Ahmad. 2016. Himalayan cheese (*Kalari/Kradi*) and effect of different probiotic strains on oxidative stability, microbiological, sensory and nutraceutical properties during storage. *LWT Food Sci Technol.* 67:74–81. <https://doi.org/10.1016/j.lwt.2015.11.039>.
- Noto Llana, M., S. H. Sarnacki, M. D. R. Aya Castañeda, M. I. Bernal, M. N. Giacomodonato, and M. C. Cerquetti. 2013. Consumption of *Lactobacillus casei* fermented milk prevents *Salmonella* reactive arthritis by modulating IL-23/IL-17 expression. *PLoS One* 8:e82588. <https://doi.org/10.1371/journal.pone.0082588>.
- Oliveira, M. E. G., E. F. Garcia, R. C. R. E. Queiroga, and E. L. de Souza. 2012. Technological, physicochemical and sensory characteristics of a Brazilian semi-hard goat cheese (coalho) with added probiotic lactic acid bacteria. *Sci. Agric.* 69:370–379. <https://doi.org/10.1590/S0103-90162012000600005>.
- Petrullo, L., M. J. Jorgensen, N. Snyder-Mackler, and A. Lu. 2019. Composition and stability of the vervet monkey milk microbiome. *Am. J. Primatol.* 81:e22982. <https://doi.org/10.1002/ajp.22982>.
- Price, M. N., P. S. Dehal, and A. P. Arkin. 2010. FastTree 2—Approximately maximum-likelihood trees for large alignments. *PLoS One* 5:e9490. <https://doi.org/10.1371/journal.pone.0009490>.
- Quast, C., E. Priesse, P. Yilmaz, J. Gerken, T. Schweer, P. Yarza, J. Peplies, and F. O. Glöckner. 2013. The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. *Nucleic Acids Res.* 41:D590–D596. <https://doi.org/10.1093/nar/gks1219>.
- Reeves, A. E., M. J. Koenigsnecht, I. L. Bergin, and V. B. Young. 2012. Suppression of *Clostridium difficile* in the gastrointestinal tracts of germfree mice inoculated with a murine isolate from the family *Lachnospiraceae*. *Infect. Immun.* 80:3786–3794. <https://doi.org/10.1128/IAI.00647-12>.
- Rodrigues, R., G. Guerra, J. Soares, K. Santos, F. Rolim, P. Assis, D. Araújo, R. F. Araújo, V. B. Garcia, A. A. de Araújo, and R. Queiroga. 2018. *Lactobacillus rhamnosus* EM1107 in goat milk matrix modulates intestinal inflammation involving NF- κ B p65 and SOCs-1 in an acid-induced colitis model. *J. Funct. Foods* 50:78–92. <https://doi.org/10.1016/j.jff.2018.09.013>.
- Rognes, T., T. Flouri, B. Nichols, C. Quince, and F. Mahé. 2016. VSEARCH: A versatile open source tool for metagenomics. *PeerJ* 4:e2584. <https://doi.org/10.7717/peerj.2584>.
- Rolim, F. R. L., K. M. O. dos Santos, S. C. de Barcelos, A. S. do Egito, T. S. Ribeiro, M. L. Conceição, M. Magnani, M. E. G. de Oliveira, and R. C. R. E. Queiroga. 2015. Survival of *Lactobacillus rhamnosus* EM1107 in simulated gastrointestinal conditions and its inhibitory effect against pathogenic bacteria in semi-hard goat cheese. *LWT Food Sci Technol.* 63:807–813. <https://doi.org/10.1016/j.lwt.2015.05.004>.
- Santos, R. L., M. Raffatellu, C. L. Bevins, L. G. Adams, C. Tukel, R. M. Tsolis, and A. J. Baumler. 2009. Life in the inflamed intestine, *Salmonella* style. *Trends Microbiol.* 17:498–506. <https://doi.org/10.1016/j.tim.2009.08.008>.
- Segata, N., J. Izard, L. Waldron, D. Gevers, L. Miropolsky, W. S. Garrett, and C. Huttenhower. 2011. Metagenomic biomarker discovery and explanation. *Genome Biol.* 12:R60. <https://doi.org/10.1186/gb-2011-12-6-r60>.
- Sha, Y., M. Liu, B. Wang, K. Jiang, C. Qi, and L. Wang. 2016. Bacterial population in intestines of *Litopenaeus vannamei* fed different probiotics or probiotic supernatant. *J. Microbiol. Biotechnol.* 26:1736–1745. <https://doi.org/10.4014/jmb.1603.03078>.
- Silva, C. A., C. J. Blondel, C. P. Quezada, S. Porwollik, H. L. Andrews-Polymenis, C. S. Toro, M. Zaldívar, I. Contreras, M. Mc-

- Clelland, and C. A. Santiviago. 2012. Infection of mice by *Salmonella enterica* serovar Enteritidis involves additional genes that are absent in the genome of serovar Typhimurium. *Infect. Immun.* 80:839–849. <https://doi.org/10.1128/IAI.05497-11>.
- Slačanac, V., R. Božanić, J. Hardi, J. R. Szabó, M. Lučan, and V. Krstanović. 2010. Nutritional and therapeutic value of fermented caprine milk. *Int. J. Dairy Technol.* 63:171–189. <https://doi.org/10.1111/j.1471-0307.2010.00575.x>.
- Suchodolski, J. S. 2013. Gastrointestinal microbiota. Canine and feline. *Gastroenterology* 2:32–41.
- Tennant, S. M., E. L. Hartland, T. Phumoonna, D. Lyras, J. I. Rood, R. N. Robins-Browne, and I. R. Van Driel. 2008. Influence of gastric acid on susceptibility to infection with ingested bacterial pathogens. *Infect. Immun.* 76:639–645. <https://doi.org/10.1128/IAI.01138-07>.
- Thiennimitr, P., S. E. Winter, M. G. Winter, M. N. Xavier, V. Tols-tikov, D. L. Huseby, T. Sterzenbach, R. M. Tsolis, J. R. Roth, and A. J. Bäumlér. 2011. Intestinal inflammation allows *Salmonella* to use ethanolamine to compete with the microbiota. *Proc. Natl. Acad. Sci. USA* 108:17480–17485. <https://doi.org/10.1073/pnas.1107857108>.
- Verruck, S., A. Dantas, and E. S. Prudencio. 2019. Functionality of the components from goat's milk, recent advances for functional dairy products development and its implications on human health. *J. Funct. Foods* 52:243–257. <https://doi.org/10.1016/j.jff.2018.11.017>.
- Wang, Y., J. Sun, H. Zhong, N. Li, H. Xu, Q. Zhu, and Y. Liu. 2017. Effect of probiotics on the meat flavour and gut microbiota of chicken. *Sci. Rep.* 7:6400. <https://doi.org/10.1038/s41598-017-06677-z>.
- Weinberg, J. D., M. Haschke, K. Kutz, J. Thebault, and A. Steven-son. 2018. Phase I randomized controlled study of a single-strain live biotherapeutic (*Blautia hydrogenotrophica*) in the treatment of patients with irritable bowel syndrome (IBS): Effects on the microbiome. *Gastroenterology* 154:S852–S853. [https://doi.org/10.1016/S0016-5085\(18\)32886-5](https://doi.org/10.1016/S0016-5085(18)32886-5).
- Zhang, W., Y. H. Zhu, D. Zhou, Q. Wu, D. Song, J. Dicksved, and J. F. Wang. 2017. Oral administration of a select mixture of *Bacillus* probiotics affects the gut microbiota and goblet cell function following *Escherichia coli* challenge in newly weaned pigs of genotype MUC4 that are supposed to be enterotoxigenic *E. coli* F4ab/ac receptor negative. *Appl. Environ. Microbiol.* 83:e02747–16. <https://doi.org/10.1128/AEM.02747-16>.
- Zhang, Z., J. Lv, L. Pan, and Y. Zhang. 2018. Roles and applications of probiotic *Lactobacillus* strains. *Appl. Microbiol. Biotechnol.* 102:8135–8143. <https://doi.org/10.1007/s00253-018-9217-9>.