Probiotics and Antimicrobial Proteins (2023) 15:601–613
https://doi.org/10.1007/s12602-021-09878-1

Limosilactobacillus fermentum Strains with Claimed Probiotic Properties Exert Anti-oxidant and Anti-inflammatory Properties and Prevent Cardiometabolic Disorder in Female Rats Fed a High-Fat Diet

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Accepted: 15 November 2021 / Published online: 24 November 2021

Abstract

This study assessed the effects of a mixed formulation containing *Limosilactobacillus* (*L.*) *fermentum* 139, *L. fermentum* 263, and *L. fermentum* 296 on cardiometabolic parameters, inflammatory markers, short-chain fatty acid (SCFA) fecal contents, and oxidative stress in colon, liver, heart, and kidney tissues of female rats fed a high-fat diet (HFD). Female Wistar rats were allocated into control diet (CTL, $n=6$), HFD ($n=6$), and HFD receiving *L. fermentum* formulation (HFD-LF, $n=6$). *L. fermentum* formulation (1×10^9 CFU/mL of each strain) was administered two twice a day for 4 weeks. Administration of *L. fermentum* increased acetate and succinate fecal contents and reduced hyperlipidemia and hyperglycemia in rats fed a HFD ($p<0.05$). Administration of *L. fermentum* decreased low-grade inflammation and improved antioxidant capacity along the gut, liver, heart, and kidney tissues in female rats fed a HFD ($p<0.05$). Administration of *L. fermentum* prevented dyslipidemia, inflammation, and oxidative stress in colon, liver, heart, and kidney in female rats fed a HFD.

Keywords High-fat diet · Inflammation · Oxidative stress · *Limosilactobacillus* · Probiotic

Introduction

The high prevalence of cardiometabolic disorders, such as type 2 diabetes mellitus, dyslipidemias, arterial hypertension, and obesity, has been recognized as most important cardiovascular disease risk factors, being associated, in part, with poor food pattern, including a diet rich in calories, sugar, salt, saturated fatty acids, and cholesterol [1, 2]. Sex difference has been related to cardiometabolic disorder prevalence and evidence suggests that female hormones could have a protective effect [3].

High-fat diet (HFD) consumption, specifically diet rich in saturated and trans fats, increases the abundance of lipopolysaccharides (LPS)-expressing bacteria and provokes elevated levels of LPS in systemic circulation, characterizing a pro-inflammatory state, named metabolic endotoxemia [4, 5]. Additionally, prolonged HFD consumption increases oxidative stress and mitochondrial damage in several organs [6–8]. The findings suggest that interventions targeting gut microbiota and exerting anti-inflammatory and anti-oxidant properties could reduce the risk of cardiometabolic disorders provoked by HFD.

Previous investigations have suggested that gut microbiota differs between the sexes in rodents and humans. Women commonly harbor a higher ratio of Firmicutes/Bacteroidetes

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(F/B), enhanced lactobacilli population, and short-chain fatty acid producers in gut microbiota when compared with men [9]. The related sex differences can lead to sex-dependent changes in systemic immunity, low-grade inflammation, oxidative stress, and response to gut microbiota modulation [8, 10–12].

A broader range of studies carried out with male have demonstrated that administration of probiotics promoted beneficial effects on gut microbiota, inflammation, oxidative stress, and cardiometabolic functions [13–17]. However, the effects of probiotic administration on inflammation, oxidative stress, and cardiometabolic parameters in female have been little explored and remain to be elucidated.

An early investigation characterized three *Lactobacillus fermentum* (recently renamed as *Limosilactobacillus fermentum*) [18], namely, *L. fermentum* 139, *L. fermentum* 263, and *L. fermentum* 296, as potential candidates for use as probiotics in a set of functionality-related in vitro properties, such as performance regarding adhesion, aggregation, co-aggregation, antagonism, and survival to exposure to simulated gastrointestinal conditions, besides showing absence of hemolytic and mucinolytic activities and resistance to antibiotics [19]. In this study, we have evaluated the effects of a mixed formulation containing these three potentially probiotic *L. fermentum* strains on cardiometabolic variables, biomarkers of inflammation, caecum short-chain fatty acid production, and oxidative stress markers in gut, liver, heart, and kidney tissues of female rats fed a HFD diet.

Methods

Animals and Ethical Aspects

Female Wistar rats (*Rattus norvergicus*) in same estrus cycle were used in this study. Determination of the estrous cycle was carried according to previous study [20]. The animals received water and diet ad libitum and were maintained in collective polypropylene cages (03 animals/cage) under controlled temperature (22 ± 1 °C), humidity between 50 and 55% and a 12-h light–dark cycle. The experimental procedures were approved by Institutional Animal Care and Use Committee/Federal University of Paraíba (CEUA-UFPB protocol # 6,080,240,418, João Pessoa, Paraíba, Brazil) and followed the guidelines of National Council for the Control of Animal Experimentation (CONCEA) and International Principles for Biomedical Research.

Experimental Design

The rats were randomly assigned into control group (CTL, $n = 6$) receiving a control diet prepared according to the American Institute of Nutrition – AIN-93 M) [21]; HFD group receiving a high-fat diet (HFD, $n = 6$) purchased from Rhoster® Company (Araçoiaba da Serra, São Paulo, Brazil) and treated with placebo; and HFD group receiving a probiotic formulation containing a mix of *L. fermentum* 139, 263, and 296 (HFD-Lf, $n = 6$). Composition of CTL and HFD diets are shown in Supplemental Table 1.

In CTL and HFD groups, phosphate-buffered saline (PBS) solution was administered as placebo for 4 weeks. In HFD-Lf group, a mix formulation containing *L. fermentum* 139, 263, and 296 in a solution of approximately 3×10^9 CFU/mL of each strain were administered twice a day for 4 weeks. Administration of placebo or *L. fermentum* formulation was done by oral gavage (1 mL). Body weight were weekly measured using an appropriate scale (model AS-1000; Marte, Santa Rita, Minas Gerais, Brazil). After 4 weeks, rats were euthanized by decapitation and biochemical and cytokines were measured in serum, short-chain fatty acid (SCFA) were measured in caecum contents, and oxidative stress variables were assessed in gut, liver, heart, and kidney tissues.

Probiotic Strains and Cell Suspension Preparation

The *L. fermentum* 139, *L. fermentum* 263, and *L. fermentum* 296 strains were gently provided by Laboratory of Food Microbiology, Department of Nutrition, Federal University of Paraíba (João Pessoa, Paraíba, Brazil). Stocks were stored at − 20 °C in de Mann, Rogosa, and Sharpe (MRS) broth (HiMedia, Mumbai, India) containing glycerol 20% (Sigma-Aldrich, St. Louis, USA; 20 mL/100 mL).

The probiotic cell suspension was daily obtained from overnight cultures of each strain grown in MRS broth (HiMedia, Mumbai, India) anaerobically incubated (Anaerobic System Anaerogen, Oxoid Ltda., Wade Road, UK) at 37 °C, according to a previously described procedure [11, 14]. The cell suspension with viable counts of approximately 9 log CFU/mL were obtained by mixing the suspension of each probiotic strain in a ratio of 1:1:1.

Biochemical Analysis

Serum samples were analyzed to determine the levels of glucose, total cholesterol, high-density lipoprotein cholesterol (HDL-c), triglycerides, creatinine, urea, alanine aminotransferase (ALT), and aspartate aminotransferase (AST) using commercial kits and semi-automatic photometer HumaLyzer 3500 (HUMAN Gesellschaft für Biochemica und Diagnostica mbH, Wiesbaden, Germany). Low-density lipoprotein cholesterol (LDL-c) levels were calculated according to Friedewald formula: LDL-c (mg/dL) = [TC − HDL-c − TG]/5 [22].

Quantification of Organic Acids in Caecum Contents

Caecum sample contents were collected at the end of experiments and stored under − 80 °C. Organic acids were quantified by high-performance liquid chromatography (HPLC) using an LC 1260 Infinity system (Agilent Technologies, Santa Clara, CA, USA) coupled to a PDA detector (G1315D; Agilent Technologies) as previously described [23].

Cytokine Measurement

Cytokine levels (IL-6, IL-10, IL1β, and TNF-α) were determined using Millipore 7-plex kit (Millipore Corp., Billerica, MA, USA). Assays were performed on a 96-well plate containing a filter membrane following the manufacturer's instructions. The concentrations of cytokines in samples were estimated from a standard curve using a third-order polynomial equation and expressed as pg/mL. Samples with values below the limit of detection were recorded as zero, while for samples with values above the quantification upper limit of standard curves were assigned the highest curve value.

Oxidative Stress Measurement in Tissues

The colon, liver, heart, and renal tissues were homogenized in a cold buffer solution with 50 mM TRIS and 1 mM EDTA, pH 7.4, 1 mM sodium orthogonadate, and 200 µg/mL phenylmethanesulfonylfluoride using an IKA RW 20 digital homogenizer, a pestle of potter–Elvehjem, and glass tubes on ice. The homogenates were centrifuged ($1.180 \times g$, 10 min, 4 °C) [24] and protein levels were measured with Bradford protocol [25].

Assessment of Lipid Peroxidation

An aliquot (0.3 mg/mL) of homogenate of tissues was used to quantify the production of malondialdehyde (MDA) in reaction with thiobarbituric acid (TBA, 100 °C). Sequential addition of 30% (v/v) of trichloroacetic acid and Tris–HCl (3 mM) were done to the sample, followed by centrifugation ($2500 \times g$, 10 min, 4 °C). TBA (0.8%, v/v) was added to resulting supernatant, mixed, boiled for 15 min, and after cooling, the reaction was read at 535 nm on a spectrophotometer.

Assessment of Superoxide Dismutase (SOD) Activity

Total superoxide dismutase (SOD) enzyme activity was determined according to Misra and Fridovich method. The tissue samples (0.3 mg/mL) were mixed with sodium carbonate buffer (0.05%, pH 10.2, 0.1 mmol/L EDTA, 37 °C), added of 30 mM/L of epinephrine (in 0.05% acetic acid), and SOD activity was measured by kinetics of epinephrine auto-oxidation inhibition for 1.5 min at 480 nm read on a spectrophotometer [26].

Assessment of Catalase (CAT) Activity

Catalase activity was determined by decomposition of H_2O_2 into O_2 and H_2O. A sample of tissues homogenate (0.3 mg/mL) in 50 mM phosphate buffer (pH 7.0) was added of 0.3 M H_2O_2. Absorbance was measured at 240 nm for 1.5 min on a spectrophotometer [27].

Assessment of Glutathione S-Transferase (GST) Activity

A sample of tissue homogenate (0.3 mg/mL) was used to quantify GST activity, as previously described [28]. Phosphate buffer (0.1 M, pH 6.5 containing 1 mM EDTA), 1 mM 1-chloro-2,4-dinitrobenzene (CDNB), and 1 mM reduced glutathione (GSH) was added to tissue homogenate samples. Absorbance was measured at 340 nm for 1.5 min on a spectrophotometer.

Assessment of Total Thiol Groups

Tissue homogenate samples (0.3 mg/mL) were incubated in extraction buffer (previously described) with 10 mM of 5,5′-dithiobis (2-nitrobenzoic acid) in a dark environment for 30 min. The absorbance of the reaction was measured at 412 nm on a spectrophotometer [29].

Statistical Analysis

Values were reported as mean ± standard deviation for parametric data or median (maximum − minimum) for non-parametric data. Kolmogorov–Smirnov and Shapiro–Wilk tests were used to assess the normality of data. Most of variables required the one-way ANOVA parametric test and Tukey post-hoc test. Non-parametric variables were compared using Kruskal–Wallis with Dunn's post-hoc test. A Pearson's or Spearman correlation coefficient (r) was used to evaluate the relationships among oxidative stress parameters in colon and liver, heart, and kidney tissues. The correlations were classified as bad ($r \leq 0.20$), weak (0.21–0.40), moderate (0.41–0.60), good (0.61–0.80), and excellent (0.81–1.00). Statistical analysis was done with software Prism 9 (GraphPad Software, San Diego, CA, USA). The difference was considered significant when p was < 0.05.

Results

Body Weight and Biochemical Parameters

The percentage of weight gain at the end of the protocol was similar among groups (Table 1; $p > 0.05$). Female rats fed a HFD had increased serum levels of glucose, triglycerides, cholesterol, LDL-cholesterol, urea, ALT, and AST when compared to CTL group (Table 1; $p < 0.05$). Administration of *L. fermentum* formulation reduced serum levels of glucose (140.7 ± 14.0 vs. 207.5 ± 18.3, $p < 0.05$), triglycerides (94.0 ± 11.2 vs. 151.5 ± 13.1, $p < 0.05$), cholesterol (147.4 ± 12.1 vs. 399.5 ± 22.0, $p < 0.05$), LDL-cholesterol (104.9 ± 19.2 vs. 240.7 ± 27.9, $p < 0.05$), urea (24.4 ± 2.5 vs. 47.1 ± 7.8, $p < 0.05$), ALT (64.8 ± 12.0 vs. 106.3 ± 21.5, $p < 0.05$), and AST (122.5 ± 12.2 vs. 189.0 ± 36.7, $p < 0.05$) when compared to HFD group (Table 1). In addition, *L. fermentum* formulation increased serum levels of HDL-c (67.8 ± 6.2 vs. 45.7 ± 8.7, $p < 0.05$) when compared to HFD and CTL groups (Table 1).

Table 1 Assessment of body weight gain and biochemical variables

Variables	CTL	HFD	HFD-Lf
% weight gain	4.5 ± 3.0	6.7 ± 3.2	3.7 ± 2.6
Glucose (mg/dL)	112.7 ± 9.7	207.5 ± 18.3*	140.7 ± 14.0*,#
Triglycerides (mg/dL)	86.5 ± 5.7	151.5 ± 13.1*	94.0 ± 11.2#
Cholesterol (mg/dL)	134.2 ± 11.4	399.5 ± 22.0*	147.4 ± 12.1#
LDL-cholesterol (mg/dL)	66.9 ± 5.3	240.7 ± 27.9*	104.9 ± 19.2*,#
HDL-cholesterol (mg/dL)	53.1 ± 3.9	45.7 ± 8.7	67.8 ± 6.2*,#
Creatinine (mg/dL)	0.41 ± 0.09	0.39 ± 0.23	0.44 ± 0.06
Urea (mg/dL)	14.4 ± 0.7	47.1 ± 7.8*	24.4 ± 2.5*,#
ALT (U/L)	38.0 ± 4.1	106.3 ± 21.5*	64.8 ± 12.0*,#
AST (U/L)	117.3 ± 15.9	189.0 ± 36.7*	122.5 ± 12.2#

Groups: control group (CTL, $n = 6$), high-fat diet (HFD, $n = 6$), and HFD receiving a mixed *Limosilactobacillus fermentum* 139, 263, and 269 (HFD-Lf, $n = 6$). Data are presented as mean ± standard deviation and analyzed by ANOVA one-way test with Tukey as post-hoc test. *$p < 0.05$ indicates significant difference between HFD or HFD-Lf and CTL group; #$p < 0.05$ indicates significant difference between HFD-Lf and HFD group

Short-Chain Acid Fatty in Caecum Contents

Fecal contents of acetate, propionate, and succinate were similar between HFD and CTL groups ($p > 0.05$; Fig. 1A–C). Administration of *L. fermentum* formulation increased fecal contents of acetate (0.1 ± 0.01 vs. 0.03 ± 0.005 vs. 0.03 ± 0.02 g/L, $p < 0.05$) and succinate (1.38 ± 0.53 vs. 0.46 ± 0.30 vs. 0.78 ± 0.18 g/L, $p < 0.05$) when compared to CTL and HFD groups, respectively (Fig. 1A–C), but it did not change fecal contents of propionate ($p > 0.05$; Fig. 3B). Butyric acid contents were below the limit of detection.

Cytokine Serum Levels

Female rats fed a HFD had increased serum levels of proinflammatory cytokines TNFα (152.8 ± 6.3 vs. 78.4 ± 6.0 pg/mL, $p < 0.05$) and IL-1β (159.8 ± 13.2 vs. 54.8 ± 2.1 pg/mL, $p < 0.05$) and decreased levels of IL-6 (38.2 ± 5.3 vs. 64.6 ± 2.9 pg/mL, $p < 0.05$) and IL-10 (28.1 ± 4.0 vs. 66.8 ± 4.6 pg/mL, $p < 0.05$) when compared with CTL group (Fig. 2A–D). HFD group receiving *L. fermentum* formulation had decreased serum levels of TNF-α (107.6 ± 19.5 vs. 152.8 ± 6.3 pg/mL, $p < 0.05$) and IL1β (107.2 ± 10.9 vs. 159.8 ± 13.2 pg/mL, $p < 0.05$), as well as higher IL-6 (56.1 ± 5.8 vs. 38.2 ± 5.3 pg/mL, $p < 0.05$) and IL-10 (41.9 ± 5.6 vs. 28.1 ± 4.0 pg/mL, $p < 0.05$) when compared to HFD receiving placebo treatment (Fig. 2A–D).

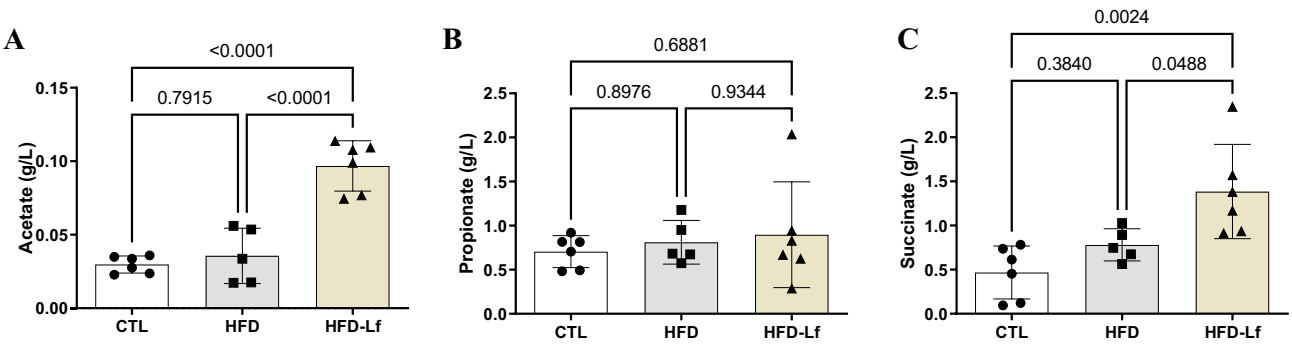

Fig. 1 Effects of a mixed formulation with *Limosilactobacillus fermentum* 139, 263, and 269 on short-chain fatty acid concentration in fecal samples in female rats fed a high-fat diet. Assessment of acetate (**A**), propionate (**B**), and succinate (**C**) in the fecal samples. Groups: control group (CTL, $n = 6$), high-fat diet (HFD, $n = 6$), and HFD receiving a mixed *L. fermentum* formulation (HFD-Lf, $n = 6$). Data are presented as mean ± standard deviation and analyzed by ANOVA one-way test with Tukey as post-hoc test

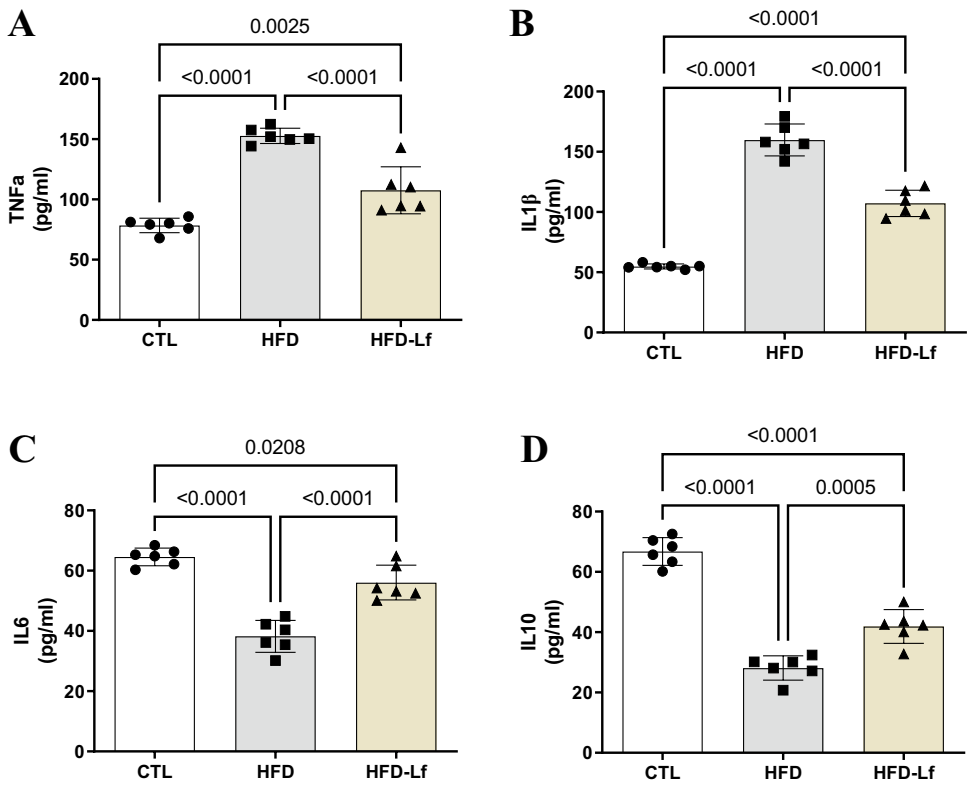

Fig. 2 Effects of a mixed formulation with *Limosilactobacillus fermentum* 139, 263, and 269 on cytokines serum levels in female rats fed a high-fat diet. Assessment of tumoral necrosis factor alpha (TNF-α) (**A**), interleukin 1 beta (IL-1β) (**B**), interleukin 6 (IL-6) (**C**), and interleukin 10 (IL-10) (**D**). Groups: control group (CTL, $n=6$), high-fat diet (HFD, $n=6$), and HFD receiving a mixed *L. fermentum* formulation (HFD-Lf, $n=6$). Data are presented as mean ± standard deviation and analyzed by ANOVA one-way test with Tukey as post-hoc test

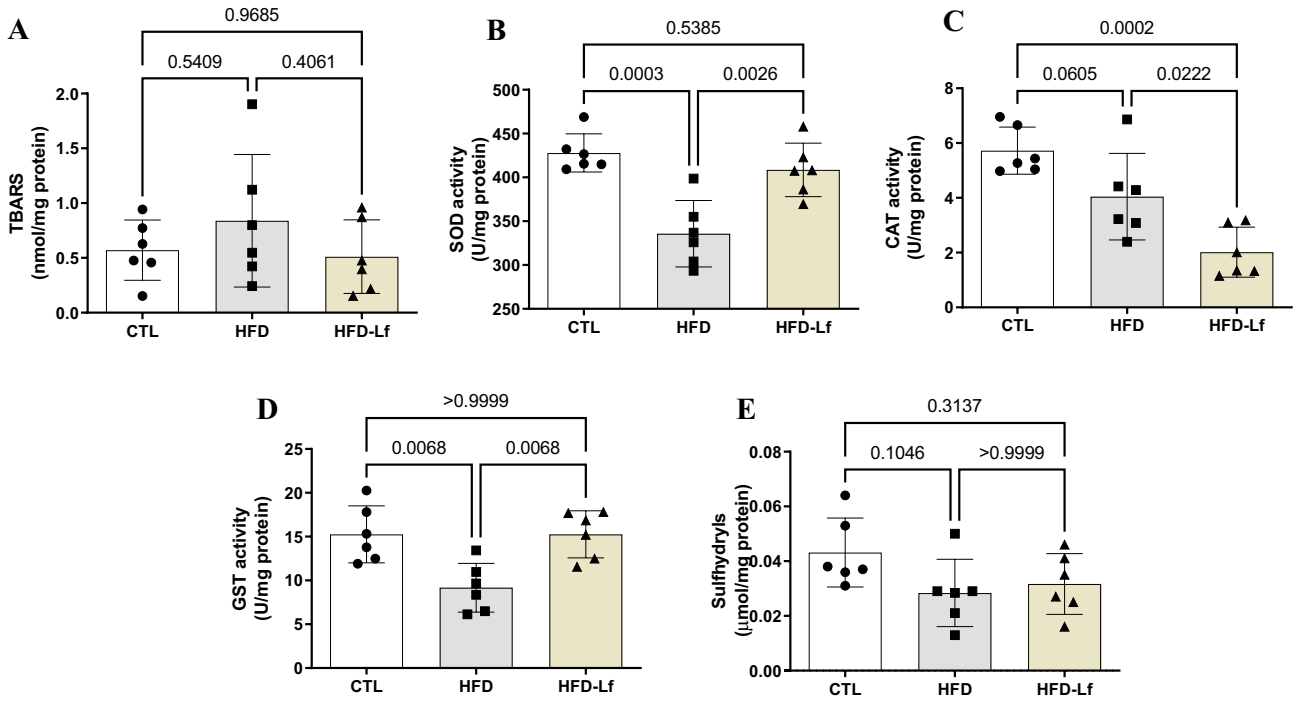

Fig. 3 Effects of a mixed formulation with *Limosilactobacillus fermentum* 139, 263, and 269 on oxidative stress parameters in colon mucosa of female rats fed a high-fat diet. Assessment of malondialdehyde levels (MDA) (**A**), superoxide dismutase activity (SOD) (**B**), catalase activity (CAT) (**C**), glutathione S-transferase activity (GST) (**D**), and total sulfhydryl content (**E**) in the colon mucosa. Groups: control group (CTL, $n=6$), high-fat group (HFD, $n=6$), and HFD receiving *L. fermentum* formulation (HFD-Lf, $n=6$). Data are presented as mean ± standard deviation and analyzed by ANOVA one-way test with Tukey as post-hoc test

Oxidative Stress Biomarkers in Colon Tissues

The MDA levels and sulfhydryl content in colonic mucosa were similar among groups ($p > 0.05$; Fig. 3A, E). Female rats fed a HFD had decreased SOD (335.7 ± 38.0 vs. 427.9 ± 21.8 U/mg protein, $p < 0.05$) and GST (9.2 ± 2.7 vs. 15.3 ± 3.2 U/mg protein, $p < 0.05$) activities in colonic mucosa when compared to CTL group (Fig. 3B, D). Administration of *L. fermentum* formulation although had decreased CAT activity in colonic mucosa of rats fed a HFD ($p < 0.05$; Fig. 3C), it caused increased SOD (408.7 ± 34.1 vs. 335.7 ± 38.0 U/mg protein, $p < 0.05$) and GST (15.2 ± 2.7 vs. 9.2 ± 2.7 U/mg protein, $p < 0.05$) activities when compared to HFD placebo treated (Fig. 3B, D).

Oxidative Stress Biomarkers in Liver Tissues

The MDA level was enhanced in liver tissues of rats fed a HFD when compared to CTL group ($p < 0.05$; Fig. 4A). Female rats fed a HFD had decreased SOD (195.7 ± 37.1 vs. 331.0 ± 44.2 U/mg protein, $p < 0.05$) and CAT (7.6 ± 4.1 vs. 16.6 ± 2.2 U/mg protein, $p < 0.05$) activities in liver tissues when compared to CTL group (Fig. 4B, C). Administration of *L. fermentum* formulation although did not change CAT activity in liver of rats fed a HFD ($p > 0.05$; Fig. 4C), it caused increased SOD (301.8 ± 56.0 vs. 195.7 ± 37.1 U/mg protein, $p < 0.05$) and GST (340.8 ± 37.2 vs. 215.8 ± 20.1 U/mg protein, $p < 0.05$) activities and sulfhydryl content (0.10 ± 0.01 vs. 0.07 ± 0.01 µmol/mg protein, $p < 0.05$) when compared to HFD placebo treated (Fig. 4B, D, and E).

Oxidative Stress Biomarkers in Heart Tissues

The SOD and CAT activities and sulfhydryl content in heart tissues were similar between CTL and HFD groups ($p > 0.05$; Fig. 5B, C, and E). Female rats fed a HFD had reduced GST activity (28.6 ± 4.1 vs. 38.5 ± 4.2 U/mg protein, $p < 0.05$) and enhanced MDA levels (0.22 ± 0.10 vs. 0.06 ± 0.02 nmol/mg protein, $p < 0.05$) in heart tissues when compared to CTL group (Fig. 5C, D). Administration of *L. fermentum* formulation increased SOD activity (622.4 ± 55.4 vs. 374.9 ± 21.4 vs. 385.5 ± 42.6 U/mg protein, $p < 0.05$) and sulfhydryl content (0.35 ± 0.05 vs. 0.13 ± 0.01 vs. 0.16 ± 0.02 mmol/mg protein, $p < 0.05$) in heart tissues when compared to HFD and CTL group, respectively (Fig. 5B, E).

Oxidative Stress Biomarkers in Renal Cortex

MDA levels, SOD and CAT activities, and sulfhydryl content in renal cortex were similar between CTL and HFD

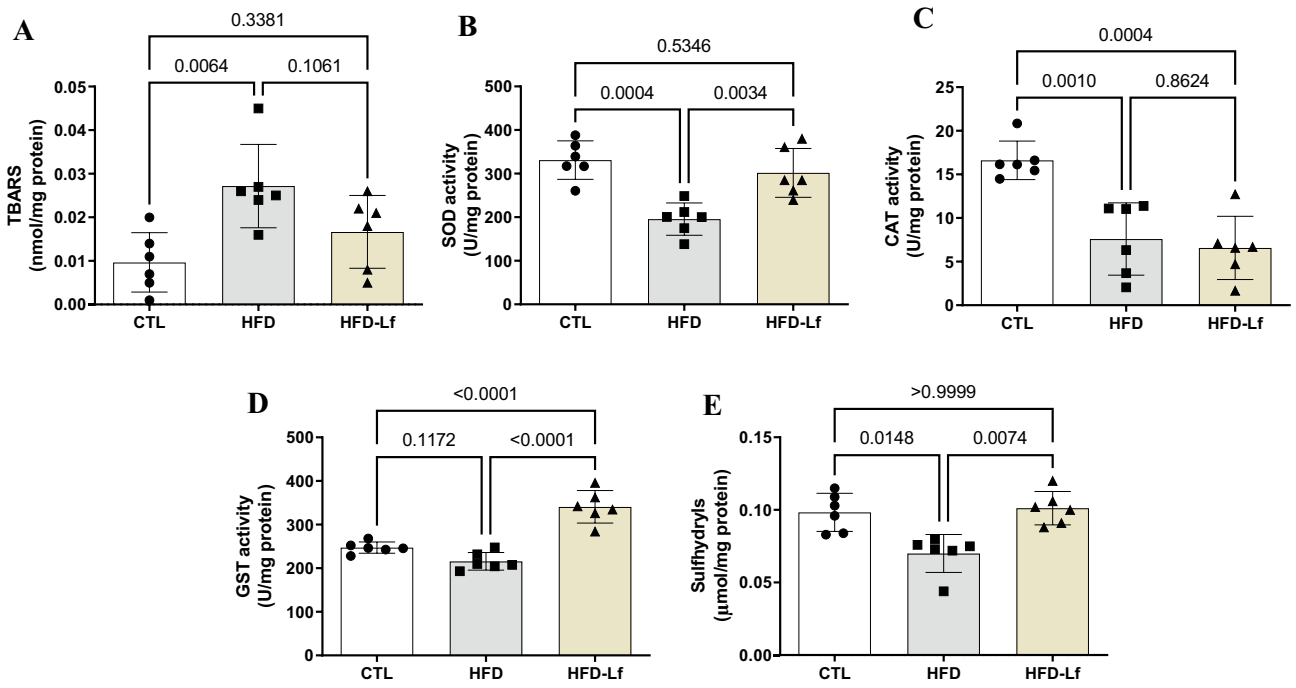

Fig. 4 Effects of a mixed formulation with *Limosilactobacillus fermentum* 139, 263, and 269 on oxidative stress parameters in liver of female rats fed a high-fat diet. Assessment of malondialdehyde levels (MDA (**A**), superoxide dismutase activity (SOD) (**B**), catalase activity (CAT) (**C**), glutathione S-transferase activity (GST) (**D**), and total sulfhydryl content (**E**) in the liver. Groups: control group (CTL, $n = 6$), high-fat group (HFD, $n = 6$), and HFD receiving *L. fermentum* formulation (HFD-Lf, $n = 6$). Data are presented as mean ± standard deviation and analyzed by ANOVA one-way test with Tukey as post-hoc test

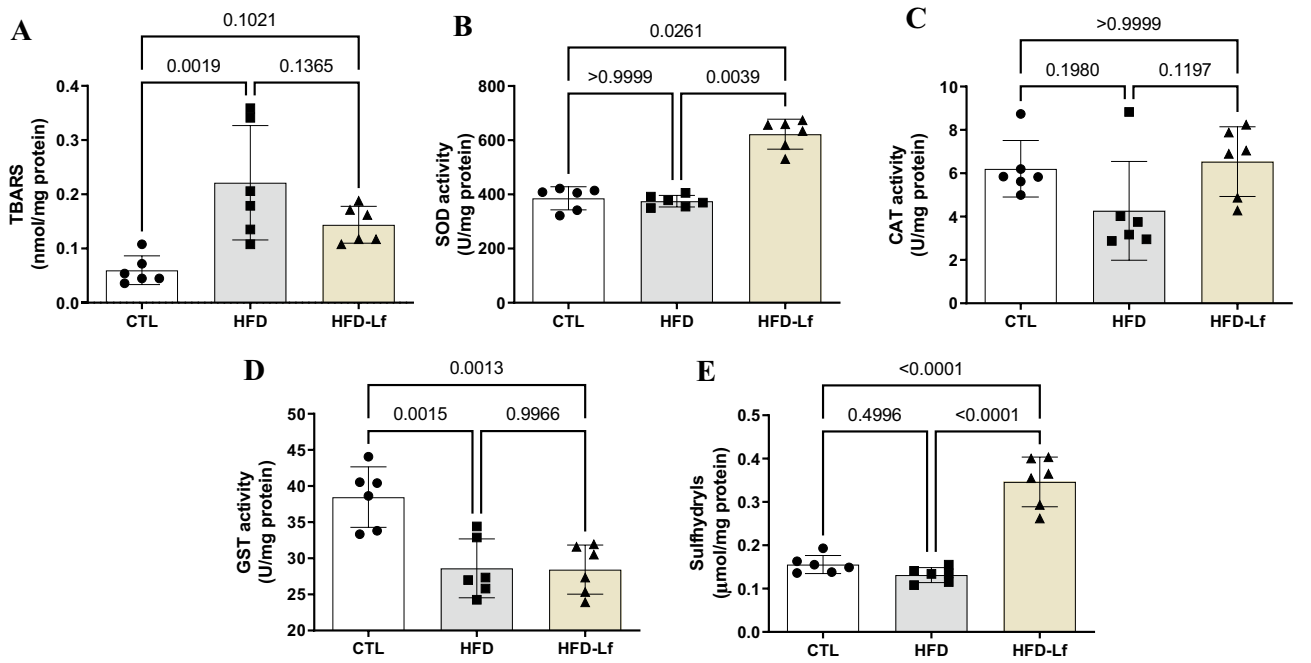

Fig. 5 Effects of a mixed formulation with *Limosilactobacillus fermentum* 139, 263, and 269 on oxidative stress parameters in heart tissue of female rats fed a high-fat diet. Assessment of malondialdehyde levels (MDA) (**A**), superoxide dismutase activity (SOD) (**B**), catalase activity (CAT) (**C**), glutathione S-transferase activity (GST) (**D**), and total sulfhydryl content (**E**) in the heart tissue. Groups: control group (CTL, $n=6$), high-fat group (HFD, $n=6$), and HFD receiving *L. fermentum* formulation (HFD-Lf, $n=6$). Data are presented as mean ± standard deviation and analyzed by ANOVA one-way test with Tukey as post-hoc test

groups ($p>0.05$; Fig. 6A, B, C, and E). Female rats fed a HFD had reduced GST activity (30.0 ± 9.1 vs. 49.2 ± 6.8 U/mg protein, $p<0.05$) in renal cortex when compared to CTL group (Fig. 6C, D). Administration of *L. fermentum* formulation increased SOD (380.4 ± 116.5 vs. 211.1 ± 35.4 U/mg protein, $p<0.05$) and GST activities (53.6 ± 17.8 vs. 30.0 ± 9.1 U/mg protein, $p<0.05$) in renal cortex when compared to HFD group (Fig. 6B, D), but did not change MDA levels, CAT activity, and sulfhydryl content ($p>0.05$; Fig. 6A, C, and E).

The relationship between antioxidant enzyme activities in colon, liver, heart, and kidney tissues was demonstrated as a hierarchical heat map (Fig. 7), which showed distinct clusters of associations based on antioxidant enzyme activities found in CTL, HFD, and HFD-LF groups (Fig. 7). In addition, we have carried correlation analysis between oxidative stress biomarkers in colon with oxidative stress biomarkers in liver, heart, and renal cortex (Table 2). SOD activity in colon correlated positively with SOD activity in liver ($r=0.708$, $p=0.001$), but not with SOD activity in heart ($r=0.234$, $p=0.349$) and renal cortex ($r=0.199$, $p=0.428$). Similarly, CAT activity in colon correlated positively with CAT activity in liver ($r=0.641$, $p=0.004$), but not with CAT activity in heart and renal cortex ($p>0.05$; Table 2). GST activity in colon also correlated positively with GST activity in liver ($r=0.0.566$, $p=0.014$), but not with GST activity in heart and renal cortex ($p>0.05$; Table 2). TBARS and sulfhydryl contents in colon had not correlation with liver, heart, and renal cortex ($p>0.05$; Table 2).

Discussion

In recent years, our research group has isolated and characterized potentially probiotic fruit-derived strains. The strains of *L. fermentum* 139, *L. fermentum* 296, and L. *fermentum* 263 were recovered from Brazilian fruit by-products [19, 30]. *L. fermentum* 139 was isolated from *Mangifera indica* L. (mango), *L. fermentum* 263 was isolated from *Ananas comosus* (pineapple), and *L. fermentum* 296 was isolated from *Fragaria vesca* L. (strawberry). All the three strains displayed potential for use as probiotics in terms of a set of functionalities related in vitro properties, such as auto-aggregation, co-aggregation, survival during exposure to simulated gastrointestinal conditions, and pathogen antagonism, in addition to showing the absence of hemolytic and mucolytic activities and resistance to antibiotics [19]. Such findings indicated that these *L. fermentum* fruit-derived strains could be potential candidates for use as novel probiotics. Using those strains, we have demonstrated for the first time that administration of a mixed formulation containing three potentially probiotic *L. fermentum* strains, twice a day

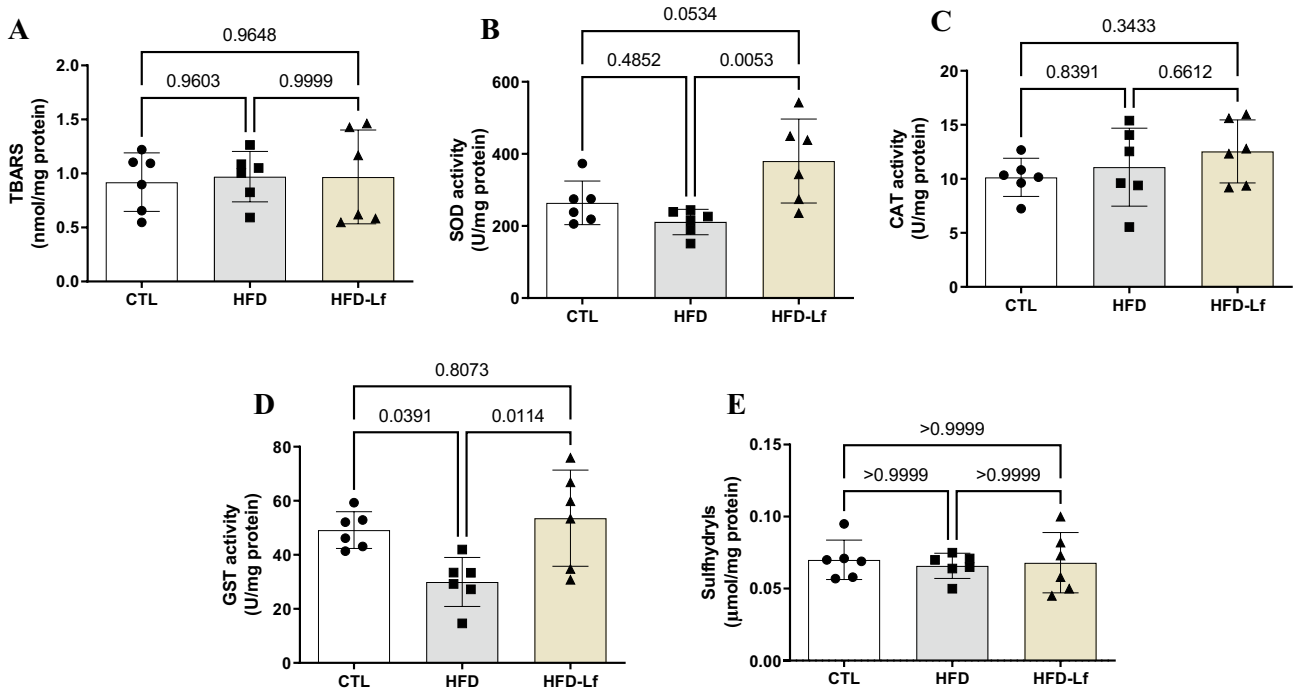

Fig. 6 Effects of a mixed formulation with *Limosilactobacillus fermentum* 139, 263, and 269 on oxidative stress parameters in renal cortex of female rats fed a high-fat diet. Assessment of malondialdehyde levels (MDA) (**A**), superoxide dismutase activity (SOD) (**B**), catalase activity (CAT) (**C**), glutathione S-transferase activity (GST) (**D**), and total sulfhydryl content (**E**) in the renal cortex. Groups: control group (CTL, $n=6$), high-fat group (HFD, $n=6$), and HFD receiving *L. fermentum* formulation (HFD-Lf, $n=6$). Data are presented as mean ± standard deviation and analyzed by ANOVA one-way test with Tukey as post-hoc test

for 4 weeks, increased fecal acetate and succinate contents, reduced glycemia, dyslipidemia, systemic low-grade inflammation, and oxidative stress in colonic mucosa, liver, heart, and kidney tissues of female rats fed a HFD.

In agreement with previous studies, HFD consumption provoked hyperglycemia and hyperlipemia [14, 31, 32]. Here, administration of mixed *L. fermentum* formulation prevented increases of glucose, triglycerides, urea, ALT, AST, and cholesterol serum levels, as well increased serum levels of HDL-cholesterol in female rats, indicating to occur relevant hypoglycemic and hypolipidemic effects and reduced serum indicators of hepatic injury. The use of different *L. fermentum* strain types have also caused hypolipidemic and hypoglycemic findings, as well reduced hepatic injury indicators in rodents and humans in early investigations [13, 33–36]. For example, in female rats, administration of *L.*

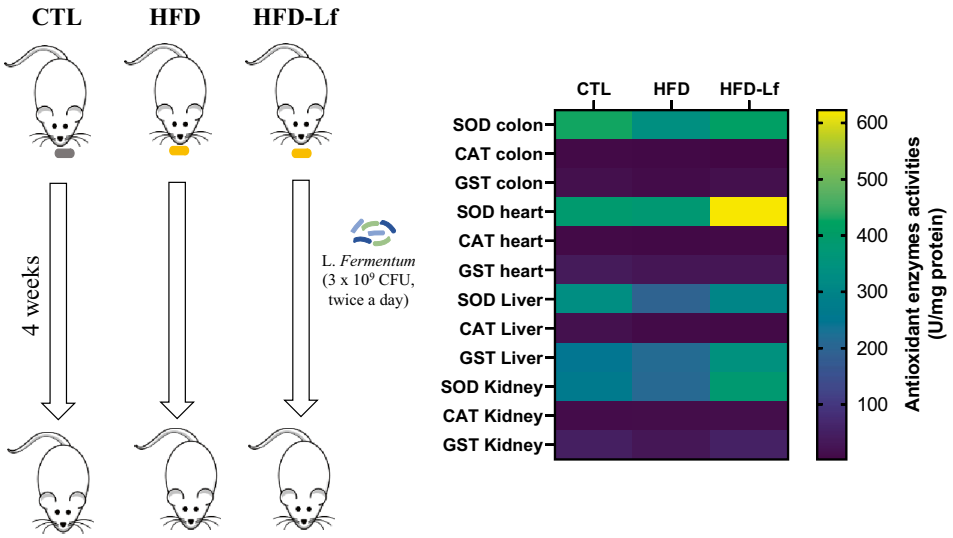

Fig. 7 Effects of a mixed formulation with *Limosilactobacillus fermentum* 139, 263, and 269 on antioxidant enzyme activities. Heatmap showing antioxidant enzyme activities in colon, liver, heart, and kidney tissues in female rats. Groups: control group (CTL, $n=6$), high-fat group (HFD, $n=6$), and HFD receiving *L. fermentum* formulation (HFD-LF, $n=6$)

Table 2 Correlation coefficients among oxidative stress biomarkers in colon with oxidative stress biomarkers in liver, heart, and kidney

Parameters	Colon × liver		Colon × heart		Colon × kidney	
	r	*p*-value	*r*	*p*-value	*r*	*p*-value
TBARS (nmol/mg protein)	0.418	0.084	0.030	0.905	−0.272	0.274
SOD (U/mg protein)	0.708	0.001	0.234	0.349	0.199	0.428
CAT (U/mg protein)	0.641	0.004	−0.221	0.378	0.004	0.985
GST (U/mg protein)	0.566	0.014	0.199	0.427	0.450	0.060
Sulfhydryls (μmol/mg protein)	0.225	0.369	−0.012	0.959	−0.163	0.515

TBARS thiobarbituric acid reaction, *SOD* superoxide dismutase, *CAT* catalase, and *GST* glutathione S-transferase

fermentum MCC2759 and MCC2760 alleviated inflammation and improved intestinal function in high-fat diet-fed and streptozotocin-induced diabetic [36].

For us, the results of the present study reinforce that *L. fermentum* 139, *L. fermentum* 263, and *L. fermentum* 296 strains has great potential to act as novel anti-dyslipidemia and anti-diabetes biotherapeutic products due to their ability to attenuate lipid-glucose metabolism disorders and further translational studies should be carried out [11, 14].

The SCFAs, primarily are acetate, propionate, and butyrate, can be generated in the colon as end products of bacterial fermentation [37]. In the present study, butyrate contents were below the analytical detection limit. Administration of mixed *L. fermentum* formulation although had no significant effect on propionate fecal contents, it effectively increased acetate and succinate fecal contents in female rats fed a HFD, indicating heterofermentative properties of *L. fermentum* strains used in this formulation and a stimulatory effect on gut microbiota. In the colon, SCFAs play a key role in maintenance of epithelial integrity, energy source of colonocytes, fluid absorption, and important anti-inflammatory effect [37]. Acetate has been found as prevalent SCFA concentration in colon and early studies have demonstrated acetate as a relevant suppressor of colonic inflammation via G-protein coupled receptor 43 (GPR43) signaling [37, 38]. If *L. fermentum* exert a suppressor effect of colonic inflammation remain to be elucidated.

Gut microbiota can also produce important amounts of succinate, an intermediary microbial product especially derived from fermentation of fibers and oligosaccharides [39, 40]. Early investigations have reported enhanced succinate cecal contents and improved glycemic control in mice fed a fiber-rich diet [40, 41]. The role and tolerance level of succinate gut-derived is still unclear, but it has been suggested that succinate acting in GPR91 exert an important function in modulation of intestinal inflammation [39] and against colonization and growth of exogenous pathogens [42].

In agreement to results of early studies, HFD consumption provoked diabetic dyslipidemia phenotype [43] and a low-grade inflammation condition in female rats [44, 45], which was linked to increased serum levels of pro-inflammatory cytokines TNFα and IL1β and reduced anti-inflammatory cytokine IL-10. Pro- and anti-inflammatory properties have been reported as potential functions of cytokine IL-6 [46]. Although reduced serum levels of IL6 have been found in female rats fed a HFD, the result set of measured cytokines could indicate a metabolic endotoxemia condition.

Strain- and sex-specific immunological effects have been found in probiotic bacteria [47]. Regarding anti-inflammatory properties of *L. fermentum* CECT5716, preceding studies have reported a significant reduction in pro-inflammatory cytokines TNFα and IL-1β in inflamed tissue of rats displaying colitis [48, 49]. Additionally, it has been reported that *L. fermentum* CECT5716 [50] and *L. fermentum* UCO-979C [51] can modulate the host immune system by increasing regulatory T cells (Treg) leading to enhanced IL-10 production in serum and intestine of female mice. The results of this study indicate for first time that administration of potentially probiotic *L. fermentum* promoted an important immune modulation in female rats fed a HFD, as indicated by induction of increased levels of IL-10 and IL-6 and decreased levels of IL-1β and TNF-α.

In physiological condition, reactive oxygen species (ROS) are found at low concentration into normal cells due to an efficient enzymatic and non-enzymatic anti-oxidant system [52]. However, it has been broadly reported that during nutritional stress (e.g., HFD consumption) excessive ROS production or down-regulation of anti-oxidant response can provoke oxidative stress, damage cells, and development of chronic diseases [7, 53]. Available evidence has suggested that powerful oxidative stress inducers may be associated with gut dysbiosis and pro-inflammatory processes [54–56]. In the present study, HFD consumption increased systemic low-grade inflammation and damaged the anti-oxidant system in colon mucosa, liver, heart, and kidney tissues of female rats.

Many beneficial effects of probiotics on oxidative stress tolerance and antioxidant capacity have been reported [13, 16, 35]. In the present study, a mixed *L. fermentum* formulation promoted increased antioxidant enzyme activities in colonic mucosa, liver, heart, and kidney tissues of rats fed a HFD, suggesting a direct and broad antioxidant effect.

Probiotic strains with antioxidant properties have been growing explored [16] and studies have reported a relevant anti-oxidant capacity in other *L. fermentum* strains. Administration of *L. fermentum* CECT5716 [57] and *L. fermentum* MTCC: 5898 [13] has shown to reduce oxidative stress in cardiometabolic disorders.

Although underlying mechanism by which mixed *L. fermentum* formulation increased SOD and GST activities in colonic mucosa, liver, heart, and kidney tissues were not explored, MnSODs enzyme activity has been reported for some lactic acid bacteria [16]. Additionally, it has been demonstrated that *L. fermentum* strains could have a fully functional GSH system composed of GSH peroxidase and GSH reductase capable of protecting cells against oxidative stress [58].

We have postulated that tissue oxidative damage provoked by HFD consumption, in part, could be due to increased oxidative stress in colon. Here, we have reported that antioxidant enzyme activities in colon were correlated positively with antioxidant enzyme activities in liver, but not in heart and renal cortex. Our findings suggest that *L. fermentum* formulation might modulate oxidative stress biomarkers through gut-liver axis [59], but the modulation of oxidative stress biomarkers in gut-heart axis and gut-kidney axis was not demonstrated.

Early investigation has suggested that gut-heart axis and gut-kidney axis could be considered as novel areas for therapeutic research to prevent and reduce the risk of cardiovascular disease [60, 61] and renal disease [8, 62]. For us, further studies using probiotic therapy are needed to investigate the potentiality of gut-heart axis and gut-kidney axis as therapeutic strategy.

The lack of gut microbiota composition analysis could be described as a main limitation of this study, although we have previously documented enhanced *Lactobacillus* counts in feces from rats treated with *L. fermentum* strains with claimed probiotic properties [11, 14].

In conclusion, administration of a mixed formulation containing three potentially probiotic *L. fermentum* strains

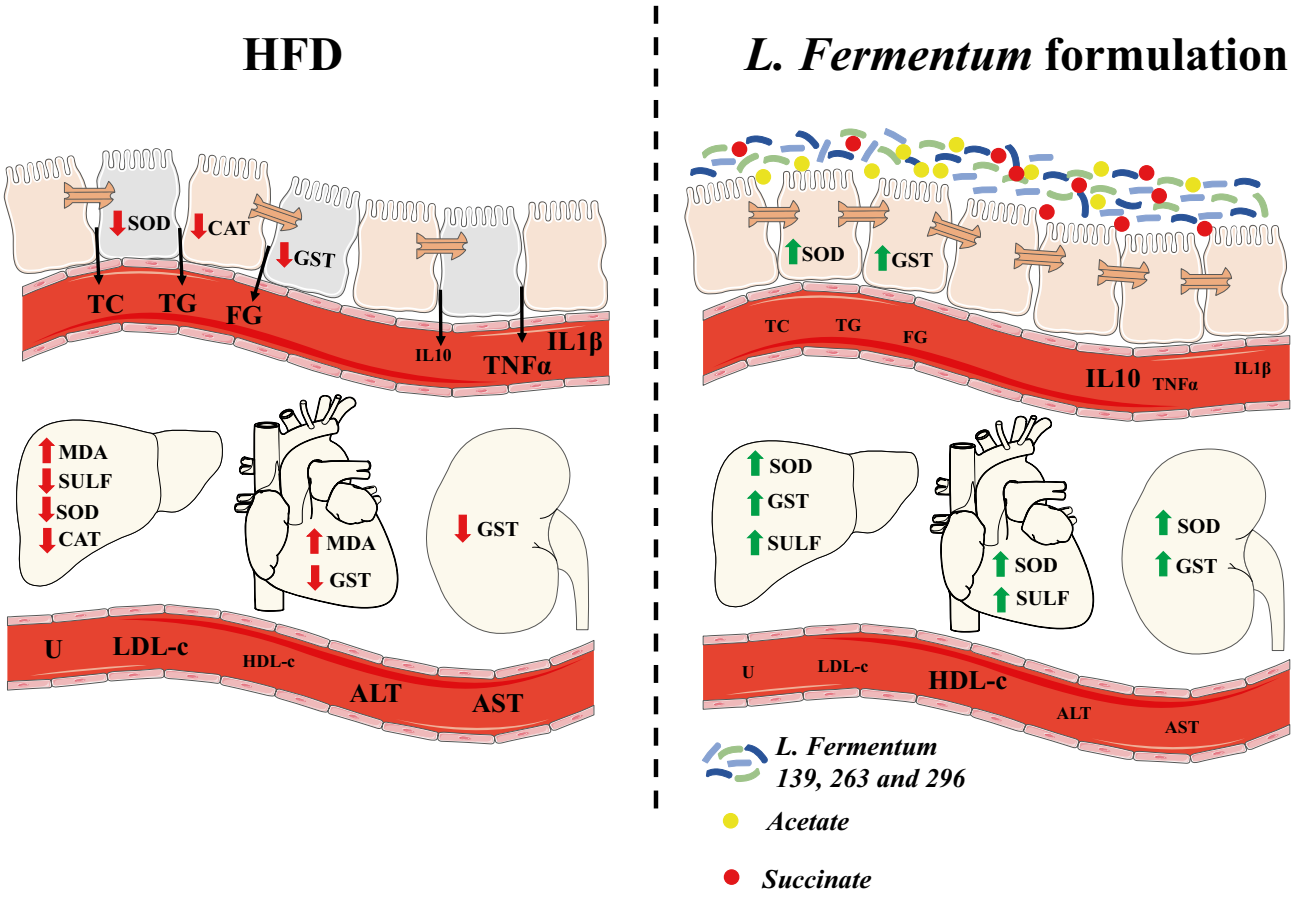

Fig. 8 Schematic drawing showing the impact of the high-fat diet receiving placebo (HFD) or a mixed of *Limosilactobacillus fermentum* 139, 263, and 296 (HFD-Lf, 3×10^9 CFU, twice a day, 4 weeks) on short-chain fatty acid, biochemical, inflammatory variables, and oxidative stress along the gut, liver, heart, and kidney tissues in female rats. *ALT* alanine aminotransferase, *AST* aspartate aminotransferase, *CAT* catalase, *CTL* control group, *FG* fasting glucose, *GST* glutathione S-transferase, *HFD* high-fat diet, *IL* interleukins, *MDA* malondialdehyde, *SOD* superoxide dismutase, *SULF* sulfhydryl content, *TNF-α* tumoral necrosis factor alpha, *TC* total cholesterol, *TG* triglycerides, *U* urea

prevented diabetic dyslipidemia, low-grade inflammation, and oxidative stress along the gut, liver, heart, and kidney tissues in female rats fed a HFD (Fig. 8). Additionally, the mixed *L. fermentum* formulation was effective to increase acetate and succinate fecal contents. These results encourage the development of future clinical trials to assess the effects of examined *L. fermentum* formulation in subjects with dyslipidemias.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s12602-021-09878-1.

Acknowledgements The authors are grateful to the Coordenação de Aperfeiçoamento de Pessoal de nível Superior (CAPES, Brazil—Finance code 001) for the scholarships awarded to M.O. de Luna Freire (MSc student). The authors are also grateful to the CAPES for the scholarships awarded to K.Á.R. de Oliveira (post-doctoral). The authors are grateful to the Fundação de Apoio à Pesquisa do Estado da Paraíba (FAPESQ, Brazil) for the scholarships awarded to L.C.P. do Nascimento (PhD student). Additionally, the authors give thanks for the research productivity fellowship granted by the Brazilian National Council for Scientific and Technological Development (CNPq) to J.L. de Brito Alves.

Author Contribution J.L. de Brito Alves designed the study. M.O. de Luna Freire, L.C.P. Nascimento, K.A.R. de Oliveira, A.M. Oliveira, and M.S. Lima conducted the experiments. M.O. de Luna Freire, L.C.P. Nascimento, and J.L. de Brito Alves analyzed the data. J.L. de Brito Alves prepared the manuscript. C.J. Lagranha, J.H. da Costa-Silva, and E.L. de Souza reviewed critically the manuscript.

Data Availability The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Declarations

Conflict of Interest The authors declare no competing interests.

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