



UNIVERSIDADE FEDERAL DA PARAÍBA
CENTRO DE CIÊNCIAS DA SAÚDE
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS DA NUTRIÇÃO
CURSO DE DOUTORADO



LUCIANA CAROLINE PAULINO DO NASCIMENTO

**EFEITOS DA ADMINISTRAÇÃO DE *Limosilactobacillus fermentum* SOBRE
PRESSÃO ARTERIAL E CAPACIDADE ANTIOXIDANTE NO EIXO ÊNTERO
RENAL NA PROLE DE RATAS ALIMENTADAS COM DIETA HIPERLIPÍDICA
DURANTE GESTAÇÃO E LACTAÇÃO**

João Pessoa

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Tese apresentada ao programa de Pós-graduação
em Ciência da Nutrição do Centro de Ciências
da Saúde, Universidade Federal da Paraíba,
como requisito para obtenção do título de
Doutor em Ciências da Nutrição.

Orientador: Profº Dr. José Luiz de Brito Alves

Coorientadora: Profª Dra. Claudia Jacques Lagranha

João Pessoa

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Universidade Federal da Paraíba

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Ata da 47ª (quadragésima sétima) Sessão Pública de Defesa de Tese para obtenção do grau de Doutor em Ciências da Nutrição, da discente Luciana Caroline Paulino do Nascimento.

Aos nove dias do mês de junho de dois mil e vinte e dois (09/06/2022) às 09:00 h, reuniram-se em cerimônia pública, no Auditório Anexo do Centro de Ciências da Saúde (Enfermagem e Nutrição), os membros da Banca Examinadora e a doutoranda Luciana Caroline Paulino do Nascimento, candidata ao grau de Doutor em Ciências da Nutrição. A Banca Examinadora foi constituída pelos seguintes Professores: Dr. José Luiz de Brito Alves (Presidente/Orientador), Drª Claudia Jacques Lagranha (Co-orientadora), Dr. Alexandre Sérgio Silva (Examinador Interno), Drª Jailane de Souza Aquino (Examinadora Interna), Dr. Evandro Leite de Souza (Examinador Interno Suplente), Dr. Lêucio Duarte Vieira Filho (Examinador Externo), Drª Mariana Pinheiro Fernandes (Examinadora Externa) e Drª Josiane de Campos Cruz (Examinadora Externa Suplente). Dando início a sessão pública, o Prof. José Luiz de Brito Alves, convidou a mim, Carlos Fernando da Silva para secretariá-los comunicando o fim específico da reunião. A seguir, foi cedida a palavra à examinada, para que, no tempo previsto no Regimento do PPGCN/CCS/UFPB, fizesse sua exposição de motivos e objetivos da sua tese, metodologia adotada, resultados encontrados, discussão e conclusão, trabalho esse sob o título “EFEITOS DA ADMINISTRAÇÃO DE *Limosilactobacillus fermentum* SOBRE PRESSÃO ARTERIAL E CAPACIDADE ANTIOXIDANTE NO EIXO ÊNTERO RENAL NA PROLE DE RATAS ALIMENTADAS COM DIETA HIPERLIPÍDICA DURANTE A GESTAÇÃO E A LACTAÇÃO”. Concluída a exposição, a candidata em seguida foi arguida sucessivamente por cada membro da banca examinadora. Dando continuidade, os membros examinadores foram inquiridos pelo Sr. Presidente se estavam aptos a proferir o julgamento e, recebendo respostas afirmativas, procederam com a deliberação da avaliação. Após certo espaço de tempo, o Sr. Presidente proclamou como “APROVADA” a tese de doutorado intitulada “EFEITOS DA ADMINISTRAÇÃO DE *Limosilactobacillus fermentum* SOBRE PRESSÃO ARTERIAL E CAPACIDADE ANTIOXIDANTE NO EIXO ÊNTERO RENAL NA PROLE DE RATAS ALIMENTADAS COM DIETA HIPERLIPÍDICA DURANTE A GESTAÇÃO E A LACTAÇÃO”. A seguir, preenchendo devidamente os mapas concernentes aos valores e conceitos, declarou que sua autora estava em condições de receber o grau de Doutor em Ciências da Nutrição, devendo a Universidade Federal da Paraíba, providenciar na forma da lei a expedição do respectivo Diploma. Nada mais havendo a ser tratado, eu, Carlos Fernando da Silva Secretário do Programa de Pós-Graduação em Ciências da Nutrição, lavrei á presente Ata que vai datada e assinada pelos membros da Banca Examinadora. João Pessoa, 09 de junho de 2022.



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UNIVERSIDADE FEDERAL DA PARAÍBA
PRÓ-REITORIA DE PÓS-GRADUAÇÃO E PESQUISA
COORDENAÇÃO GERAL DE PÓS-GRADUAÇÃO

EXAME DE DEFESA DE: () DISSERTAÇÃO (X) TESE

NOME DO PROGRAMA
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS DA NUTRIÇÃO

NOME DA ÁREA DE CONCENTRAÇÃO
CIÊNCIAS DA NUTRIÇÃO

TÍTULO DO TRABALHO FINAL
EFEITOS DA ADMINISTRAÇÃO DE *Limosilactobacillus fermentum* SOBRE PRESSÃO ARTERIAL E CAPACIDADE ANTIOXIDANTE NO EIXO ÊNTERO RENAL NA PROLE DE RATAS ALIMENTADOS COM DIETA HIPERLIPÍDICA DURANTE A GESTAÇÃO E A LACTAÇÃO
NOME DO (A) DISCENTE: Luciana Caroline Paulino do Nascimento

NOME (S) DO (S) ORIENTADOR (ES)
José Luiz de Brito Alves
Cláudia Jacques Lagrãha

OBSERVAÇÕES DA BANCA EXAMINADORA

A BANCA EXAMINADORA, TENDO EM VISTA A EXPOSIÇÃO ORAL APRESENTADA PELA ALUNA E PROCEDIDA A ARGUIÇÃO PERTINENTE AO TRABALHO FINAL CONSIDEROU O (A) CANDIDATO(A).	
(X) APROVADO(A) () INSUFICIENTE () REPROVADO(A)	
MEMBROS DA BANCA	ASSINATURA
Prof. Dr. José Luiz de Brito Alves (UFPB)	José Luiz de Brito Alves
Prof. Dr. Alexandre Sérgio Silva (UFPB)	Alexandre Sérgio Silva
Profª Drª Jailane de Souza Aquino (UFPB)	Jailane de Souza Aquino
Prof. Dr. Lécio Duarte Vieira Filho (UFPE)	Lécio Duarte Vieira Filho
Profª Drª Mariana Pinheiro Fernandes (UFPE)	Mariana Pinheiro Fernandes

LOCAL: Sessão pública presencial realizada no Auditório Anexo do CCS (Enfermagem e Nutrição) em 09/06/2022 as 09:00 horas.

INSTRUÇÕES PARA BANCA EXAMINADORA:

1 – O conceito da avaliação deverá ser expresso como: Aprovado; Insuficiente; Reprovado. 2 – A avaliação é feita logo após o encerramento da exposição oral e arguição do candidato. 3 – Caso seja sugerida reformulação do trabalho final, a Banca Examinadora deverá estabelecer um prazo disponível para o aluno procedê-la.

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**RELATÓRIO FINAL DO (A) ORIENTADOR (A)
DOUTORADO**

Eu, José Luiz de Brito Alves, orientador (a) de trabalho final do(a) aluno (a) Luciana Caroline Paulino do Nascimento, matrícula nº 20181017070, do Programa de Pós-graduação em Ciências da Nutrição e área de concentração em Ciências da Nutrição, da Universidade Federal da Paraíba, após exame de vida acadêmica do (a) mencionado (a) aluno (a), tenho a relatar:

A integralização de curso foi feita em 51 meses, portando no prazo estabelecido pela Legislação vigente da UFPB.

Quanto ao desempenho acadêmico, constata-se que o (a) doutorando (a) cursou 38 créditos da estrutura curricular a que está submetido (a) e foi aprovado (a) no exame de verificação da capacidade de leitura em Inglês e Espanhol no período letivo 2018.1.

Já na apresentação da Tese, intitulada “EFEITOS DA ADMINISTRAÇÃO DE *Limosilactobacillus fermentum* SOBRE PRESSÃO ARTERIAL E CAPACIDADE ANTIOXIDANTE NO EIXO ÊNTERO RENAL NA PROLE DE RATAS ALIMENTADAS COM DIETA HIPERLIPÍDICA DURANTE A GESTAÇÃO E A LACTAÇÃO”, realizada no dia 09 de junho de 2022, às 09h00 horas, no Auditório Anexo do CCS (Enfermagem e Nutrição), a discente obteve conceito APROVADA.

A Banca examinadora foi composta pelos (as) seguintes especialistas:

Prof. Dr. José Luiz de Brito Alves (UFPB)
Prof. Dr. Alexandre Sérgio Silva (UFPB)
Profª Drª Jailane de Souza Aquino (UFPB)
Prof. Dr. Lécio Duarte Vieira Filho (UFPE)
Profª Drª Mariana Pinheiro Fernandes (UFPE)

Diante do exposto, considerando que LUCIANA CAROLINE PAULINO DO NASCIMENTO, dentro do prazo regimental, satisfaz todas as exigências do Regimento Geral da UFPB, do Regulamento Geral dos Cursos e Programas de Pós-Graduação Stricto Sensu da UFPB e do Regulamento do Programa de Pós-graduação em Ciências da Nutrição/CCS/UFPB, está APTO (A) a obter o grau de DOUTOR EM CIÊNCIAS DA NUTRIÇÃO a ser conferido pela Universidade Federal da Paraíba.

João Pessoa, 09 de junho de 2022.

José Luiz de Brito Alves

Orientador (a) do Trabalho Final

Às minhas avós Ana e Maria (in memoriam), que mesmo diante das inúmeras dificuldades impostas pela vida sempre se mantiveram fortes, resilientes e perseverantes, dedico.

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A Deus, que me permitiu realizar este sonho. Minha fonte de força maior e que com sua providência sempre me acompanhou em todos os momentos;

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Ao meu amor, Dioner Gonçalves, que acompanhou a conclusão dessa tese nos últimos meses e foi um dos meus maiores pontos de apoio. Sua presença na minha vida é ânimo, força e esperança no amor;

Ao Professor José Luiz de Brito Alves, pela oportunidade que me foi dada de estar aqui concluindo o doutorado. Sua paciência, dedicação, fé na ciência mesmo em um país que pouco a valoriza, o faz um profissional exemplo para nós. Obrigada por tudo!

À Professora Claudia Jacques Lagranha, primeira que me apresentou o mundo científico e me proporcionou um universo de possibilidades. A ela toda admiração como profissional e pela relação de amizade duradoura construída por anos;

A todos que compõem o Laboratório de Pesquisa Translacional em Nutrição e Hipertensão Arterial Sistêmica, pelas discussões que ampliaram e instigaram meus conhecimentos, o que fizeram toda a diferença para o desenvolvimento deste trabalho;

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Ao Programa de Pós-Graduação em Ciências da Nutrição (PPGCN) - UFPB, e todos os professores que o compõem, pela oportunidade de cursar o doutorado e por todo o conhecimento compartilhado durante o curso, que são de extrema importância para nossa formação profissional;

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“Se fosse preciso, começaria tudo outra vez do mesmo jeito, andando pelo mesmo caminho de dificuldades, pois a fé, que nunca me abandona, me daria forças para ir sempre em frente. ”

Santa Dulce dos Pobres

RESUMO

O consumo de uma dieta com alto teor de gordura durante a gestação e lactação tem sido considerado como fator de risco para o desenvolvimento de hipertensão arterial na vida adulta da prole. A modulação da microbiota intestinal por sua vez, vem sendo associada a benefícios no controle da pressão arterial, apontando assim para a necessidade de investigação dos efeitos de cepas com capacidade probiótica na hipertensão induzida por dieta. Diante disso, o presente trabalho propôs-se investigar os efeitos da administração de *Limosilactobacillus fermentum* (*L. fermentum*) sobre função renal, pressão arterial e parâmetros oxidativos do eixo intestino-rim na prole de ratos expostos a dieta hiperlipídica materna. Para isso, ratas prenhas foram alimentadas com dieta controle (CTL) ou dieta hiperlipídica (HFHC) durante a gestação e lactação. Aos 90 dias, na prole dos grupos CTL e HFHC foram avaliadas a pressão arterial, por pletismografia da cauda e variáveis urinárias e bioquímicas. Além disso, no tecido intestinal e renal analisamos níveis de malondialdeído (MDA), defesa antioxidante enzimática (atividade da superóxido dismutase, SOD; catalase, CAT; e glutathione-S-transferase, GST) e não enzimática (teor de tióis totais). Em um segundo momento seguindo o mesmo protocolo inicial, aos 100 dias, um grupo de ratos HFHC foi suplementado com um mix de *L. fermentum* (grupo HFHC+Lf) até os 130 dias. Sendo assim, foram realizadas nos grupos CTL, HFHC e HFHC+Lf as mesmas análises da primeira etapa do trabalho. Nossos dados apontam que a prole de machos e fêmeas de mães alimentadas com dieta HFHC apresentaram PA aumentada quando comparados ao seu respectivo grupo CTL. Filhotes machos de mães HFHC mostraram redução da atividade de GST (30%, $p<0,05$) e conteúdo de tióis no cólon (28%, $p<0,05$), redução da atividade de SOD no córtex renal (73%, $p<0,05$) e diminuição da excreção de creatinina (69%, $p<0,001$) urinária quando comparado ao grupo CTL. Em relação à prole feminina, a atividade da CAT e o conteúdo de tióis foram reduzidos no cólon quando comparado ao grupo CTL (redução em 52%, $p<0,05$ e em 37%, $p<0,05$; respectivamente). Embora a peroxidação lipídica tenha aumentado no córtex renal da prole HFHC fêmeas (300%, $p<0,05$), as atividades da CAT e SOD aumentaram quando comparado com a prole masculina. Aos 130 dias a administração de cepas de *L. fermentum*, reduziu pressão arterial sistólica, diastólica e média nos filhotes machos. No mesmo grupo observamos ainda aumento da atividade da SOD (24%, $p<0,05$) no cólon, enquanto que aumento da SOD (31%, $p=0,05$) e CAT (97%, $p<0,05$) no tecido renal com restauração da função renal comparado ao grupo HFHC. Conclui-se que a dieta HFHC durante a gestação e lactação induz estresse oxidativo específico do sexo ao longo do eixo intestino rim na prole, que pode induzir disfunção renal e hipertensão arterial mais tarde. Porém, a

administração de *L. fermentum* promoveu melhora dos níveis de pressão arterial, capacidade antioxidante no eixo intestino rim e melhora na função renal na prole nos animais machos.

Palavras-chave: Dieta rica em gordura; eixo intestino-rim; estresse oxidativo; hipertensão arterial; *Limosilactobacillus fermentum*.

ABSTRACT

The consumption of a high-fat diet during pregnancy and lactation has been considered a risk factor for the development of arterial hypertension in the offspring's adult life. In turn, modulation of the intestinal microbiota has been associated with benefits in blood pressure control, thus pointing to the need to investigate the effects of strains with probiotic capacity on diet-induced hypertension. Therefore, the present work aimed to investigate the effects of administration of *Limosilactobacillus fermentum* (*L. fermentum*) on renal function, blood pressure and oxidative parameters of the gut-kidney axis in the offspring of rats exposed to a high-fat maternal diet. For this, pregnant rats were fed a control diet (CTL) or high fat diet (HFHC) during pregnancy and lactation. At 90 days, in the offspring of the CTL and HFHC groups, blood pressure was evaluated by tail plethysmography and urinary and biochemical variables. Furthermore, in intestinal and renal tissue we analyzed levels of malondialdehyde (MDA), enzymatic (superoxide dismutase activity, SOD; catalase, CAT; and glutathione-S-transferase, GST) and non-enzymatic antioxidant defense (total thiols). In a second moment, following the same initial protocol, at 100 days, a group of HFHC rats was supplemented with a mix of *L. fermentum* (HFHC+*Lf* group) until 130 days. Therefore, the same analyzes of the first stage of the work were performed in the CTL, HFHC and HFHC+*Lf* groups. Our data indicate that the offspring of males and females of mothers fed the HFHC diet presented increased BP when compared to their respective CTL group. Male pups from HFHC dams showed reduced GST activity (30%, $p<0.05$) and colonic thiol content (28%, $p<0.05$), reduced SOD activity in the renal cortex (73%, $p<0.05$) and decreased urinary creatinine excretion (69%, $p<0.001$) when compared to the CTL group. Regarding female offspring, CAT activity and thiol content were reduced in the colon when compared to the CTL group (52% reduction, $p<0.05$ and 37%, $p<0.05$, respectively). Although lipid peroxidation increased in the renal cortex of female HFHC offspring (300%, $p<0.05$), CAT and SOD activities increased when compared to male offspring. At 130 days, the administration of *L. fermentum* strains reduced systolic, diastolic and mean blood pressure in male offspring. In the same group, we also observed an increase in SOD activity (24%, $p<0.05$) in the colon, while an increase in SOD (31%, $p=0.05$) and CAT (97%, $p<0.05$) in renal tissue with restoration of renal function compared to the HFHC group. It is concluded that the HFHC diet during pregnancy and lactation induces sex-specific oxidative stress along the gut-kidney axis in the offspring, which can induce renal dysfunction and arterial hypertension later on. However, the administration of

L. fermentum promoted improvement in blood pressure levels, antioxidant capacity in the intestine-kidney axis and improvement in renal function in offspring in male rats.

Key words: High-fat diet; arterial hypertension; gut-kidney axis; oxidative stress; *Limosilactobacillus fermentum*.

LISTA DE FIGURAS

Figuras da tese

Figura 1: Microbiota intestinal e possíveis vias de indução de hipertensão. Os AGCCs podem estimular vias associadas aos receptores acoplados a proteína G (em inglês, *G-protein-coupled receptors (GPCRs)*): o Gpr41 (do inglês, *Free Fatty Acid Receptor 3*) e o Olfr78 (do inglês, *olfactory receptor 78*). Eles atuam aumentando a atividade nervosa simpática e a secreção de renina. Os AGCCs também induzem vasodilatação pela ativação dos receptores Gpr43 (do inglês, *Free Fatty Acid Receptor 2*) e Gpr109a (conhecido do inglês, *hydroxy-carboxylic acid (HCA) receptor 2*) prevenindo hipertensão. O desequilíbrio da microbiota intestinal eleva produção de LPS que atuam como padrões moleculares associados a patógenos (em inglês, *pathogen-associated molecular patterns (PAMPs)*) que ao se ligarem aos receptores *TRL4*, aumentam citocinas pró inflamatórias. A ativação do *TRL4* também aumenta radicais livres e desencadeiam estresse oxidativo. Estes oxidam o LDL (*low density lipoprotein*) circulante, promovendo inibição na produção de óxido nítrico (ON) e aumento de endotelina-1 (ET-1) favorecendo a hipertensão. AGCCs- ácidos graxos de cadeia curta; SNS- sistema nervoso simpático; ET-1 – endotelina; GPCRs- receptores acoplados a proteína G; LDL- lipoproteína de baixa densidade; ON –óxido nítrico; oxLDL: lipoproteína de baixa densidade oxidada; LPS – lipopolissacarídeos; PAMPs - padrões moleculares associados a patógenos. Adaptado de OYAMA; NODE, 2019..... 38

Figura 2. Associação entre microbiota intestinal, estresse oxidativo e dano tecidual. Alguns microorganismos apresentam enzimas como a NADPH oxidase (NOX). A NOX produz o H_2O_2 , que ativa Nrf2 que aumenta a atividade antioxidante mantendo a homeostase intestinal. A disbiose por sua vez ativa a DUOX) aumentando a síntese de EROs que induz processos inflamatórios e danos ao DNA, caracterizando inflamação crônica. DUOX- dupla oxidase; EROS- espécies reativas de oxigênio; H_2O_2 - peróxido de hidrogênio; NOX- NADPH oxidase; Nrf2- fator nuclear 2 relacionado ao eritroide 2. Adaptado de HU *et al.*, 2019..... 41

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Figura 4: Esquema representativo do segundo protocolo experimental formando os grupos CTL, HFHC e HFHC+Lf. O protocolo inicial é seguido até a prole completar 100 dias quando um grupo de animais HFHC iniciara a suplementação com o mix de *L fermentum*. A administração era realizada por gavagem, mantendo o início diário da administração às 12h. A administração do mix probiótico ou do placebo foram realizados por gavagem até os 130 dias de vida, idade que foram iniciadas as análises experimentais. 0= dia 0 de gestação; G= gestação; N= nascimento; L=lactação; D=desmame; M=machos e F=fêmeas..... 45

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LISTA DE ABREVIATURAS

AGCC	Ácido graxo de cadeia curta
AIN	<i>American Intitute of Nutrition</i>
Ang II	Angiotensina II
AVC	Acidente Vascular cerebral
CAT	Catalase
CCr	Clearance de creatinina
CONCEA	Conselho nacional de controle de experimentação animal
CTL	<i>Control</i>
DAC	Doença arterial coronariana
DCNT's	Doenças crônicas não transmissíveis
DCV's	Doenças cardiovasculares
DRC	Doença renal crônica
EROS	Espécies reativas de oxigênio
FO	<i>Female offspring</i>
GLP-1	<i>Glucagon-like peptide-1</i>
GST	Glutathione S transferase
HAS	Hipertensão arterial sistêmica
HF	<i>High fat</i>
HFHC	<i>High fat and high cholesterol</i>
HFHC+Lf	<i>High fat and high cholesterol + Limosilactobacillus fermentum</i>
IC	Insuficiência cardíaca
IgA	Imunoglobulina A
LPS	Lipopolissacarídeos
MDA	Malondaldeído
MO	<i>Male offspring</i>
NADP	<i>Nicotinamide adenine dinucleotide-phosphate</i>
NADPH	<i>Nicotinamide adenine dinucleotide-phosphate reduced</i>
PA	Pressão arterial
PAD	Pressão arterial diastólica
PAM	Pressão arterial média
PAS	Pressão arterial sistólica

PYY	<i>Peptide YY</i>
RNAr	<i>Ribonucleic acid ribossomal</i>
SOD	Superóxido dismutase
SRAA	Sistema renina angiotensina aldosterona
TBA	Thiobarbituric acids
TRL	<i>Toll like receptor</i>
TMAO	N-óxido de trimetilamina
UFC	Unidades formadoras de colônia
WHO	<i>World health organization</i>

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INTRODUÇÃO

Níveis elevados de pressão arterial afetam mais de um bilhão de pessoas em todo o mundo e a hipertensão arterial sistêmica (HAS) continua sendo o principal fator de risco para doenças cardiovasculares e doença renal crônica (MILLS *et al.*, 2016). A HAS é uma condição clínica de etiologia multifatorial, envolvendo fatores genéticos, fisiológicos, comportamentais e ambientais. Estudos apontam que, entre os fatores ambientais relacionados, as desordens nutricionais pré e/ou pós-natais são responsáveis por predispor a prole ao desenvolvimento de HAS, seja pela escassez de nutrientes (BARKER *et al.*, 1989; DE BRITO ALVES *et al.*, 2015; CHARLES *et al.*, 2016) ou pelo excesso (SPELIOTES *et al.*, 2010; ROBERTS *et al.*, 2015; GUIMARÃES *et al.*, 2017).

O consumo de uma dieta hiperlipídica durante a gestação e lactação tem sido considerado como fator de risco para o desenvolvimento de desordens metabólicas e HAS na vida adulta da prole (LONGO *et al.*, 2016; TAIN *et al.*, 2017). Recentemente, foi demonstrado que a HAS observada em ratos e ratas de mães alimentadas com uma dieta rica em gordura saturada, colesterol e gorduras trans durante a gestação e a lactação está associada com prejuízos no controle da pressão arterial em curto-prazo, uma vez que foi observado hipersensibilidade de quimiorreceptores periféricos e hiperatividade simpática (GUIMARÃES *et al.*, 2017; DE ARAÚJO *et al.*, 2019). Apesar dos achados relevantes, os mecanismos mediadores pelos quais a dieta materna com alto teor de gordura saturada e colesterol contribui para o desenvolvimento de hipertensão arterial ainda não estão elucidados completamente.

É sugerido que o consumo de uma dieta rica em ácidos graxos saturados e colesterol durante a gestação e/ou lactação poderia alterar a composição da microbiota intestinal materna e, através de um processo de transmissão vertical, a microbiota materna pode exercer impacto direto sobre o processo de colonização da microbiota intestinal da prole (MA *et al.*, 2014; CHU *et al.*, 2016). Alteração no processo de colonização microbiana pode influenciar a suscetibilidade da prole ao desenvolvimento de doenças em curto ou em longo prazo (INDRIO *et al.*, 2017). Estudos relatam que o processo de disbiose intestinal pode causar danos às vilosidades do intestino e levar a um aumento dos níveis circulantes de endotoxinas derivadas do intestino, a exemplo do lipopolissacarídeo (LPS). Essa toxina pode circular e chegar a vários órgãos, induzindo uma resposta inflamatória (PINDJAKOVA *et al.*, 2017; YOSHIOKA *et al.*, 2017). Tem sido descrito que o LPS poderia atingir os rins e induzir inflamação, estresse oxidativo e consequentemente efeitos deletérios sobre a função renal (WEBER *et al.*, 2016).

O estresse oxidativo é apontado como um dos mecanismos associados a fisiopatologia de DCVs. Este fenômeno é caracterizado pelo aumento na produção de espécies reativas e /ou

redução da capacidade antioxidante tecidual, acarretando em lesão a lipídeos de membrana, proteínas e DNA (PISOSCHI; POP, 2015). Entre elas podemos incluir principalmente o ânion superóxido, radical hidroxila e peróxido de hidrogênio que podem ser combatidos por um sistema de defesa antioxidante que incluem enzimas, a exemplo superóxido dismutase (SOD), catalase (CAT) e vários tipos de glutatona; e moléculas não enzimáticas, a exemplo da glutatona reduzida - GSH (WANG et al., 2017). A ingestão combinada de dieta rica em gordura e sacarose resultou em aumento de níveis circulantes de LPS, biomarcadores de estresse oxidativo e redução na atividade de enzimas antioxidantes no rim de ratos (ROSAS VILLEGAS et al., 2017).

De fato, evidências experimentais apontam que a exposição a LPS compromete a nefrogênese, aumentando os danos na estrutura renal com alteração na sua hemodinâmica, ao mesmo tempo em que aumenta os indicadores de estresse oxidativo renal em ratos (HAO et al., 2010; VIEIRA et al., 2018). A ligação entre o intestino e o rim é denominado de eixo intestino-rim, estabelecendo uma relação bidirecional onde um órgão pode influenciar na funcionalidade do outro de diversas formas (FORESTO-NETO; GHIROTTI; CÂMARA, 2021). Neste contexto, é razoável propor que alterações na resposta antioxidante no eixo intestino-rim poderia favorecer o desenvolvimento de hipertensão arterial.

Apoiado por diversos estudos, a administração do probiótico demonstra capacidade de promover melhora na dislipidemia, resistência à insulina e hipertensão arterial (YAMANO et al., 2006; BAGAROLLI et al., 2017; YADAV et al., 2019). Entre os diversos microorganismos estudados, o *Limosilactobacillus fermentum* é considerado um potencial probiótico uma vez que apresenta características como não patogenicidade, resposta benéfica ao sistema imune além de apresentar-se como natural ao ambiente interno do organismo humano (MIKELSAAR; ZILMER, 2009). Recentemente, ao testar a capacidade probiótica de cepas de *Lactobacillus* isoladas de subprodutos do processamento de frutas, foi demonstrado que o *L. fermentum* 296 promove uma melhora no perfil lipídico, redução no tônus vascular e pressão arterial sistólica de animais submetidos a dieta rica em gordura (CAVALCANTE et al., 2019). Quando utilizado uma formulação contendo três cepas de *L. fermentum* (139, 263 e 296) em animais com ingestão de dieta hiperlipídica na gestação e lactação, notou-se que além de melhora no perfil lipídico e na disfunção autonômica, houve redução sérica de malondialdeído, indicador de estresse oxidativo (DE OLIVEIRA et al., 2020). De maneira fatídica o uso concomitante de várias cepas pode potencializar os efeitos benéficos ao organismo humano e que este efeito pode ser explicado pela capacidade de cepas de *Limosilactobacillus* regularem a atividade de enzimas antioxidantes e promover efeito protetor contra o estresse oxidativo (FENG; WANG, 2020).

Diante dessas recentes descobertas, nosso interesse foi voltado para a investigação de alterações funcionais na comunicação intestino-rim, uma vez que podem estar envolvidas no desenvolvimento de HAS na prole de ratos expostos a uma dieta hiperlipídica na gestação e lactação. Além disso, demonstrar os possíveis efeitos da intervenção com uma formulação probiótica contendo três cepas de *L. fermentum* como proposta terapêutica frente os danos resultantes da dieta rica em gordura saturada e colesterol materna.

2.1 OBJETIVO GERAL

Avaliar os efeitos da administração de cepas de *L. fermentum*: 139, 263 e 296 sobre a capacidade antioxidante e funcionalidade do eixo intestino-rim da prole de mães submetidas a dieta hiperlipídica durante a gestação e lactação.

2.1.1 OBJETIVOS ESPECÍFICOS

Nos grupos de ratos provenientes de mães submetidas a dieta controle, dieta hiperlipídica durante a gestação e lactação; e naqueles com administração de *L. fermentum*:

- Avaliar ganho ponderal desde o nascimento aos 130 dias de vida;
- Mensurar os níveis de pressão arterial sistólica, diastólica e média;
- Avaliar a função renal através da mensuração de marcadores urinários e plasmáticos e clearance de creatinina;
- Quantificar níveis de indicador de estresse oxidativo (malondialdeído) no cólon e no rim;
- Quantificar atividade de enzimas antioxidantes e antioxidante não enzimático no cólon e no rim.

3. REFERENCIAL TEÓRICO

3.1 HIPERTENSÃO ARTERIAL SISTÊMICA (HAS)

Entre as doenças crônicas não transmissíveis (DCNT's), a HAS destaca-se por ser considerada tanto causa direta de perda de saúde, como também um fator de risco independente de outras doenças cardiovasculares (DCV's) englobando a doença arterial coronariana (DAC), a insuficiência cardíaca (IC) e o acidente vascular cerebral (AVC) (SU; SUN; XU, 2018). Diversos estudos definem comumente a hipertensão em: I) Pré-hipertensão, caracterizada por pressão arterial sistólica (PAS) entre 121 e 139 e pressão arterial diastólica (PAD) entre 81 e 89mmHg; II) hipertensão estágio 1, quando o indivíduo apresenta PAS/PAD de 140/90 mmHg; III) estágio 2, quando esses valores estiverem entre 161-179 de PAS e 100-109mmHg de PAD; e IV) estágio 3, quando os valores de PAS/PAD atingirem $\geq 180/\geq 110$ mmHg (MANCIA *et al.*, 2013; JAMES *et al.*, 2014; MANCIA; GRASSI, 2014; MALACHIAS *et al.*, 2016). A HAS tem contribuído para o crescente aumento da taxa de mortalidade por doenças cardiovasculares, que lideram a lista de principais causas de morte mundial entre as doenças crônicas não transmissíveis (WHO, 2017).

A prevalência de HAS em adultos é em torno de 30 a 45% da população mundial. Em 2015, a prevalência de HAS era de 24 e 20% em homens e mulheres, respectivamente (ZHOU *et al.*, 2017). Estes dados são consistentes mundialmente e são independentes de renda. Sendo assim, tanto aqueles classificados com renda baixa, média ou renda mais alta apresentam-se com igual prevalência, tornando-se mais comum com o avanço da idade, aumentando para 60% a prevalência em pessoas com idade acima de 60 anos (CHOW *et al.*, 2013). Caso não haja mudança neste cenário, estima-se que até 2025, a prevalência de pessoas com HAS aumentará de 15 a 20% (KEARNEY *et al.*, 2005).

O aumento progressivo dos casos dessa doença muito se deve a sua etiologia multifatorial. Entre os fatores que tornam o indivíduo propenso à doença incluem-se, predisposição genética, sobrepeso e obesidade, alta ingestão de sódio e álcool, tabagismo, além de inatividade física (WHELTON *et al.*, 2017). Ademais, muito têm sido estudado sobre a relação do surgimento de HAS a partir de alterações na microbiota intestinal (TANG *et al.*, 2017), assim como também sobre a influência do ambiente perinatal (ARIMA *et al.*, 2018).

A PA é determinada por diversos parâmetros do sistema cardiovascular, que incluem de forma geral o volume sanguíneo, o débito cardíaco, bem como o tônus arterial. Estes elementos são afetados tanto pelo volume intravascular como também por sistemas neuro-hormonais

(OPARIL *et al.*, 2018). Uma variedade de sistemas fisiológicos interage de forma complexa influenciando na pressão sanguínea. Entre esses sistemas estão inclusos: I) o sistema barorreflexo, que detectam mudanças agudas de pressão nos vasos sanguíneos; II) os peptídeos natriuréticos, que são produzidos pelo cérebro e pelo coração como resposta ao aumento da pressão nesses órgãos; III) o sistema cinina-caliceína, afetando o tônus vascular, bem como o fluxo renal de sais; IV) o sistema receptor adrenérgico, influenciando a frequência e contração cardíaca, além do tônus vascular; V) moléculas produzidas pelos vasos sanguíneos, responsáveis pela vasodilatação (óxido nítrico) e contração (endotelina); por fim o sistema VI) renina-angiotensina-aldosterona (SRAA), que pode ser controlado pelos rins e pelo cérebro. Esse sistema renina angiotensina atua influenciando na homeostase do volume sanguíneo e no tônus vascular (LIFTON; GHARAVI; GELLER, 2001; NAKAGAWA *et al.*, 2020).

A predisposição genética aliada aos diversos fatores etiológicos anteriormente citados contribui para o desenvolvimento da HAS, com aumento dessa probabilidade com o envelhecimento (MIKAEL *et al.*, 2017). Isso se deve, principalmente, ao enrijecimento progressivo da vasculatura arterial, podendo ser causada, entre outros fatores, por alterações no colágeno vascular e aumento de aterosclerose (SINDLER *et al.*, 2011; STEPPAN *et al.*, 2011).

O funcionamento incorreto ou interrupção de algum dos sistemas envolvidos no controle da PA pode direta ou indiretamente levar ao aumento da PA média ou à variabilidade desta PA (OPARIL *et al.*, 2018). Mais especificamente, a ativação crônica do SRAA relaciona-se com a promoção e perpetuação de insuficiência cardíaca, doença renal crônica e principalmente com a HAS. O aumento dos níveis circulantes de angiotensina II (Ang II) e aldosterona nos tecidos acarretam um ambiente pró fibrótico, inflamatório e hipertrófico, provocando nos tecidos cardiovasculares remodelamento e consequente disfunção (AMES; ATKINS; PITT, 2019).

A renina é liberada pelas células justaglomerulares renais em situações de baixa pressão arterial sistêmica, hipovolemia, privação de sódio e estimulação simpática. Uma vez na circulação, a renina metaboliza o angiotensinogênio formando a angiotensina I (Ang I). A enzima conversora de angiotensina (ECA) converte Ang I em Ang II. A ligação da Ang II no receptor AT1 leva ao aumento da retenção de sódio, vasoconstrição, estímulo da sede, aumento da atividade do sistema nervoso simpático e liberação de aldosterona pela glândula adrenal (AMES; ATKINS; PITT, 2019). Este último hormônio é responsável pela retenção hídrica, de sódio e potássio (SEELIGER *et al.*, 2005).

Conforme anteriormente citado, o aumento dos níveis de aldosterona e principalmente de Ang II são relacionados a desfechos cardiovasculares negativos. É demonstrado que a ligação de Ang II a seus receptores, seja o AT1 ou AT2 são responsáveis por ativar fatores de

transcrição nucleares que regulam a transcrição de genes específicos (*matrix metalloproteinase* (MMP), *plaminogen activator inhibitor-1* (PAI-1), *connective tissue growth factor* (CTGF) e *transforming growth factor-beta* (TGF β)) (TE RIET *et al.*, 2015). Essa ativação desponta processos de produção e proliferação de metaloproteínas, fibrose, diferenciação e inflamação (MOLTZER *et al.*, 2010; XU *et al.*, 2014). Além disso, a ligação de Ang II a seu receptor está relacionada a ativação da NADP oxidase, aumentando produção de espécies reativas de oxigênio (MONTEZANO; TOUYZ, 2014). Esses achados demonstram que tanto a inflamação como o estresse oxidativo fazem parte da fisiopatologia da HAS (TE RIET *et al.*, 2015).

3.2 AMBIENTE NUTRICIONAL MATERNO E DESORDENS CARDIOVASCULARES

O período de gestação e lactação, também conhecido como um dos períodos críticos de desenvolvimento, é uma fase de susceptibilidade em que ocorre grande multiplicação e diferenciação celular, além de maturação de órgãos e sistemas, o que torna o organismo passível de sofrer influências de fatores externos e vir a apresentar modificações tanto bioquímicas como estruturais (MORGANE *et al.*, 2002). Durante este período, órgãos e sistemas definem para longo prazo adaptações que sofrem influências do ambiente materno, caracterizando o que conhecemos por plasticidade do desenvolvimento. Sendo assim, alterações no genoma condicionam o organismo ao desenvolvimento de doenças na vida adulta como obesidade, diabetes tipo 2 e HAS (SIMEONI *et al.*, 2018).

Os estudos sobre a epigenética tentam explicar a relação entre o meio ambiente materno e modificações celulares na prole por meio do estudo de alterações de DNA. Destaca-se que processos de metilação e acetilação de DNA, ligação e dobramento de cromatina, acondicionamento de DNA em torno de nucleossomos e modificações de proteínas histonas podem alterar expressão de genes da prole, modificando função de células e órgãos levando a doenças (LANGLEY-EVANS, 2009; DESAI; JELLYMAN; ROSS, 2015).

Condições ambientais saudáveis nas fases iniciais da vida apresentam um papel substancial na redução de mortalidade, morbidade e custos com a saúde (DESAI; JELLYMAN; ROSS, 2015). Por outro lado, uma diversidade de condições durante o desenvolvimento pode influenciar na predisposição a doenças, tais como tabagismo, infecção materna e agentes farmacológicos como glicocorticoides (SELCKL; MEANEY, 2004; SALSAMI *et al.*, 2010; SCHMIEGELOW *et al.*, 2013). Além disso, o ambiente nutricional perinatal também tem sido alvo de estudos por ser capaz de levar a alterações epigenéticas comprometendo o metabolismo do organismo (CANANI *et al.*, 2011). É observado que um desequilíbrio nutricional por

carência ou excesso, em períodos críticos do desenvolvimento, está associado com a gênese de doenças crônicas, principalmente as doenças cardiovasculares (REMACLE *et al.*, 2011; ZOHD *et al.*, 2014).

Tem sido discutido que o feto se adapta e prediz o ambiente pós-natal no qual ele irá se desenvolver, caracterizando um processo chamado de resposta adaptativa preditiva (GLUCKMAN *et al.*, 2009). Estas adaptações, de acordo com a condição ao qual é submetido, podem ser benéficas em curto prazo por garantir a sobrevivência do organismo. No entanto, os custos dessa adaptação, pode ser expressa, em longo prazo, por alterações fenotípicas que contribuem para o aparecimento de desordens metabólicas como diabetes tipo 2, doença arterial coronariana e HAS (HALES; BARKER, 2001; GLUCKMAN *et al.*, 2009).

Neste contexto, estudos utilizando modelos de restrição de nutrientes no período de gestação e lactação observaram alterações moleculares, com modificação na morfologia e funcionamento das mitocôndrias de tecidos como fígado e músculo (WEI *et al.*, 2006; MAGWERE *et al.*, 2006). Tais achados podem contribuir para o desenvolvimento de resistência insulínica na vida adulta da prole de mães que receberam dieta com redução de proteína (RAMADAN; ALSHIRAIHI; AL-KARIM; 2013). No tecido cardíaco, a restrição proteica além de induzir menor atividade mitocondrial, culminou em estresse oxidativo, fatores esses que aumentam o risco de DCVs (NASCIMENTO *et al.*, 2014). Ademais, a dieta materna com restrição de proteínas causou aumento da atividade simpática e dos níveis de pressão arterial na prole (DE BRITO ALVES *et al.*, 2014; DE BRITO ALVES *et al.*, 2015).

Em uma revisão, Lee *et al* (2019) aponta para os efeitos da restrição proteico-calórica durante o período perinatal na indução de HAS na prole. Entre os efeitos renais indutores são observadas alterações em número e tamanho de néfrons, bem como modificações funcionais incluindo proteinúria, microalbuminúria, clearance de creatinina e taxa de filtração glomerular. Mais recentemente, foi demonstrado que tais alterações podem permanecer por gerações (TORREGGIANI *et al.*, 2020).

Por outro lado, estudos de coorte indicam que o excesso de nutrientes, a exemplo da obesidade materna em humanos, também está associada ao aumento do risco de doenças cardiometabólicas na prole (GADEMAN *et al.*, 2013; REYNOLDS *et al.*, 2013). Corroborando com os estudos conduzidos em humanos, um estudo com ratos demonstrou que consumo de uma dieta hiperlipídica durante a gestação induziu hipertrofia cardíaca com aumento significativo da espessura da parede ventricular, aumento da área dos cardiomiócitos e diminuição da densidade dos vasos coronarianos (MOREIRA *et al.*, 2009). Evidências mostram que de maneira mais específica a obesidade materna pode aumentar o risco de hipertensão uma

vez que causa à prole inflamação de baixo grau, aumento de atividade simpática, estresse oxidativo (HALL *et al.*, 2012). Mais recentemente, foi demonstrado que a supernutrição pós-natal (lactação) contribuiu para disfunção mitocondrial e desbalanço oxidativo, caracterizando estresse oxidativo no tecido cardíaco na prole pós injúria por isquemia/reperfusão (DE MOURA FREITAS *et al.*, 2018).

Em conjunto, os achados demonstram que desordens nutricionais no início da vida podem ter efeitos duradouros na saúde de um indivíduo podendo resultar em aumento da adiposidade, alteração no metabolismo da glicose, disfunção renal e aumento de risco de HAS na vida adulta (ALEXANDER; DASINGER; INTAPAD, 2011).

3.1.1 Dieta materna hiperlipídica e repercussões sobre o controle da pressão arterial

Além desses modelos de insultos nutricionais anteriormente destacados, estudos utilizando dietas maternas com teor de gordura elevado também têm sido relatadas como sendo capazes de induzir, entre outras desordens, obesidade e hipertensão arterial na prole (BUETTNER; SCHÖLMERICH; BOLLHEIMER, 2007; WILLIAMS *et al.*, 2014). Em particular, o maior risco cardiovascular refere-se à exposição fetal ou durante o período lactacional, a uma dieta com alto teor de gordura saturada proveniente de fonte de gordura animal (CERF, 2018). Elahi *et al* (2009), demonstrou que houve aumento nos níveis da pressão arterial sistólica na prole de mães que consumiram uma dieta hiperlipídica durante o período de gestação e lactação.

Buscando compreender possíveis associações entre a ingestão materna de dieta hiperlipídica e efeitos pressão arterial na prole, Gray *et al* (2015) observaram que a prole de mães que receberam dieta hiperlipídica, apresentou resistência vascular periférica em idade jovem que progrediu até a idade adulta com repercussão no aumento da pressão arterial. Outro estudo previamente publicado demonstrou que a dislipidemia induzida por dieta hiperlipídica rica em gordura saturada e colesterol durante a gestação e a lactação, promoveu aumento da sensibilidade de quimiorreceptores respiratórios, a hiperatividade simpática e o surgimento da HAS na prole de ratos em idade adulta jovem (GUIMARÃES *et al.*, 2017).

A regulação da PA é realizada, entre outros processos, pelos rins. Curiosamente, esse órgão é bastante vulnerável aos efeitos do ambiente nutricional perinatal (TAIN; JOLIS, 2016). É demonstrado que a ingestão direta de uma dieta hiperlipídica provoca acúmulo de lipídeos em células tubulares proximal e aumento da transição epitelial através da ativação local de componentes do sistema renina angiotensina (CHUNG *et al.*, 2010). Adicionalmente, um

trabalho utilizando uma dieta hiperlipídica na gestação e lactação por sua vez, concluiu que a prole em idade adulta apresentou hiperatividade do sistema nervoso autonômico, com aumento de expressão de componentes do sistema renina angiotensina e de NADPH oxidase, elevando estresse oxidativo e por fim aumento de citocinas inflamatórias. Dessa forma, houve um aumento da susceptibilidade a elevação dos níveis pressóricos (ZHANG *et al.*, 2018). Os efeitos da dieta rica em gordura materna vêm sendo propostos como capazes de aumentar estresse oxidativo, ativar inadequadamente as enzimas do sistema renina angiotensina e prejudicar o mecanismo de reabsorção de sódio tubular, especialmente na prole em machos, acontecimentos que predis põem a HAS (TAIN *et al.*, 2017). Ademais, lesões renais também são relatadas na prole do sexo masculino com aumento de indicadores de estresse oxidativo e albuminúria (YAMADA-OBARA *et al.*, 2016).

Buscando entender melhor a relação entre dieta hiperlipídica e desfechos metabólicos, em uma revisão sistemática, Wolters *et al.* (2018) indicam que estudos observacionais e de intervenção demonstram que a dieta hiperlipídica com maior percentual de gordura saturada promove menor riqueza e diversidade da microbiota intestinal, estando associada a desfechos metabólicos desfavoráveis comparado a dietas ricas em gorduras mono e poli-insaturadas. Estudos reforçam ainda que alterações na microbiota intestinal seria o mecanismo mediador do disfunção renal e hipertensão em ratos expostos a dieta hiperlipídica materna (JOSE; RAJ, 2015; HSU *et al.*, 2019). Isso se torna consistente uma vez que trabalhos conduzidos em humanos ou em roedores sugerem que o consumo de uma dieta rica em ácidos graxos saturados e colesterol durante a gestação e/ou lactação poderiam alterar a composição e a diversidade da microbiota intestinal em mães e filhos (MA *et al.*, 2014; CHU *et al.*, 2016; VAL-LAILLET *et al.*, 2017).

Sabe-se que quando a composição da microbiota é alterada, pode haver o favorecimento da passagem de metabólitos intestinais para a corrente sanguínea estimulando estresse oxidativo e produção de citocinas inflamatórias em órgãos alvo como vasos e rins, desencadeando doenças como a hipertensão (WEBER *et al.*, 2016). Os estudos avaliando mecanismos que relacionem as alterações pressóricas em animais expostos à diferentes insultos nutricionais perinatais, incluindo a dieta rica hiperlipídica, é de extrema importância dado a etiologia multifatorial da doença e dos seus diferentes mecanismos fisiológicos de regulação.

3.3 MODULAÇÃO DA MICROBIOTA POR PROBIÓTICOS NAS DOENÇAS CARDIOVASCULARES

A diversidade de microrganismos que povoam o nosso corpo caracteriza a microbiota humana. Embora diversas regiões do corpo possuam sua microbiota específica, o cólon é um órgão diversificado quando se trata de presença de microrganismos e, principalmente, por estes desempenharem funções fisiológicas benéficas ao hospedeiro (TOJO *et al.*, 2014). Na sua composição predominam na idade adulta os filos *Bacteroidetes* e *Firmicutes*, enquanto que em menor proporção, encontram-se as *Actinobacteria*, *Proteobacteria* e *Verrucomicrobia* (ECKBURG *et al.*, 2005; KIM *et al.*, 2013). Embora exista diversidade, normalmente, a maioria das pessoas apresentam as espécies *Faecalibacterium prausnitzii* (*F. prausnitzii*), *Roseburia intestinalis*, *Bacteroides uniformis* e gêneros de bifidobactérias e lactobacilos (LOZUPONE *et al.*, 2012). Considera-se que uma maior diversidade bacteriana intestinal está associada positivamente na manutenção de funções fisiológicas conferindo menor risco de doenças (YANG *et al.*, 2018).

É bem conhecido que a microbiota intestinal humana atua fortalecendo o mecanismo de defesa do trato gastrointestinal do hospedeiro. Em parte, os efeitos benéficos atribuídos às populações de microrganismos decorrem da capacidade de cepas bacterianas metabolizarem carboidratos complexos em ácidos graxos de cadeia curta (AGCC) como acetato, propionato e butirato. Especialmente o butirato é utilizado como fonte de energia para os colonócitos e assim garante a manutenção da função de barreira do epitélio intestinal (RAMEZANI *et al.*, 2016). Os AGCC promovem ainda a liberação de peptídeos intestinais como o GLP 1 (*glucagon-like peptide-1*), GIP (*glucose-dependent insulintropic polypeptide*) e o PYY (*peptide YY*) que se relacionam a homeostase da glicose, gasto energético e saciedade (CANI *et al.*, 2013). Como previamente demonstrado, houve melhoria da tolerância à glicose e redução dos níveis de insulina e leptina durante o jejum quando administrado butirato e propionato, fato que promoveu melhora à sensibilidade à insulina (LIN *et al.*, 2012). Além disso, o aumento do gasto energético em animais obesos foi observado com a administração do butirato (GAO *et al.*, 2009).

A garantia de uma barreira intestinal íntegra com total proteção fisiológica e imunológica é capaz de promover as funções tanto digestivas como absorptivas intestinais. Além disso, também garante a restrição de patógenos e metabólitos tóxicos circulantes, sendo processos dependentes de uma microbiota equilibrada (ULLUWISHEWA *et al.*, 2011; YANG *et al.*, 2018). De modo geral, a microbiota intestinal exerce atuação primordial no metabolismo e imunidade sistêmica e seu equilíbrio é de fundamental importância para o desempenho das funções vitais do organismo (NEISH, 2009). Além disso, entre os benéficos que a microbiota intestinal pode exercer, os microrganismos e seus metabólitos ainda atuam de forma a garantir

a homeostase do sistema nervoso simpático e em vasos renais regulando a PA (PLUZNICK *et al.*, 2013; YANG *et al.*, 2018).

As mudanças na composição e função da microbiota intestinal humana é comumente conhecida como disbiose. Esta condição pode predispor indivíduos ao desenvolvimento de doenças, a exemplo das doenças crônicas não transmissíveis (CARDING *et al.*, 2015). Previamente, o microbioma humano já havia sido caracterizado e devido sua diversidade de funções está estreitamente associado a regulação de reações que conferem benefícios à saúde humana (HUTTENHOWER *et al.*, 2012). No entanto, análises desvendaram que o microbioma intestinal significativamente alterado está associado com obesidade e doença metabólica (SHANAHAN *et al.*, 2017). Além disso, evidências apontam que as alterações na composição da microbiota são cada vez mais relacionadas ao desenvolvimento da hipertensão arterial sistêmica (AL KHODOR; REICHERT; SHATAT, 2017).

Uma vez que a homeostase microbiana intestinal é interrompida, por diversos fatores, tanto microorganismos intestinais, assim como seus componentes estruturais, lipopolissacarídeos, peptidoglicanos, nucleotídeos, proteínas e lipoproteínas podem atravessar a barreira intestinal. Especificamente os LPS, quando atravessam essa barreira intestinal, são reconhecidos pelos receptores de reconhecimento padrão de células imunes do hospedeiro, os receptores *Toll-like 4* (TLR4) e esta ligação LPS-receptor resulta em estimulação das respostas imunes do hospedeiro (IWASAKI; MEDZHITOW, 2004). Esse estímulo pode desencadear a produção de citocinas inflamatórias e maior formação de espécies reativas de oxigênio (YU *et al.*, 2012; CHEN *et al.*, 2018).

O epitélio intestinal apresenta função que garante a absorção de nutrientes, além de ser uma eficiente barreira prevenindo ou impedindo a translocação sistêmica de patógenos ou de metabólitos. Essa característica é conferida pela presença de um complexo proteico, do inglês “*tight junctions*”, que garante uma permeabilidade seletiva (ODENWALD; TURNER, 2017). Durante a disbiose há aumento dessa permeabilidade, permitindo que endotoxinas como os LPS atinjam órgãos específicos favorecendo seus efeitos negativos em nível sistêmico (LU; YEH; OHASHI, 2008).

A ingestão alimentar é considerada um importante modificador da microbiota intestinal. Para exemplificar, polissacarídeos e oligossacarídeos dietéticos, incluindo fibras, são conhecidos como bifidogênicos, ou seja, capazes de aumentar o crescimento de bifidobactérias benéficas. Por outro lado, efeitos negativos na composição microbiana intestinal foram associados a níveis elevados de gordura ingeridos na dieta (BIBBÒ *et al.*, 2016). É mostrado

que a disbiose intestinal pode ocorrer secundário a ingestão de dietas com teor de açúcar e gordura elevado, implicando na patogênese de doenças como diabetes melitos, esteatose hepática e DCV's (BROWN; HAZEN, 2015). Em um estudo com ratos a disbiose intestinal contribuiu para a disfunção vascular quando utilizaram dieta ocidentalizada, sendo caracterizada por alto teor de carboidratos e gordura saturada (BATTSON *et al.*, 2017). É importante destacar que a qualidade da gordura interfere diretamente na disbiose intestinal uma vez que é observado que dietas ricas em gordura saturadas reduzem riqueza e diversidade de microbiota intestinal comparadas a dietas ricas em gorduras mono e poliinsaturadas (WOLTERS *et al.*, 2019; COELHO; CÂNDIDO; ALFENAS, 2019).

É evidenciado que a disbiose intestinal pode contribuir no desenvolvimento de processos inflamatórios, estresse oxidativo e prejuízos na função vascular. Como exemplo, vários metabólitos microbianos e componentes bacterianos estruturais são capazes de migrar do ambiente intestinal e provocar inflamação e estresse oxidativo (CANI *et al.*, 2008; BATTSON *et al.*, 2017). Ademais, as alterações que ocorrem na microbiota intestinal podem afetar a pressão arterial. Na condição de hipertensão arterial, a abundância e diversidade da microbiota encontra-se reduzida, impactando em uma redução na produção de AGCC's, que apresentam papel fisiológico fundamental na regulação dos níveis pressóricos (YANG *et al.*, 2015; YANG *et al.*, 2020). Tal relação será detalhada posteriormente ainda nesta revisão.

O tratamento da disbiose pode ser usado como uma terapia adicional de distúrbios associados a microbiota intestinal, especialmente a hipertensão (VALLIANOU; GELADARI; KOUNATIDIS, 2020). Os estudos utilizando prebióticos, probióticos e simbióticos na modulação intestinal tem aumentado sua área de interesse uma vez que impactam em diversas doenças, não sendo diferente na DRC e HAS (YANG *et al.*, 2018). Os prebióticos são ingredientes alimentares não digeríveis que estimulam o crescimento de espécies de bactérias intestinais específicas, enquanto que probióticos são definidos “microorganismos vivos que quando consumidos em quantidades adequadas conferem benefícios a saúde do indivíduo”. Quando prebióticos e probióticos são combinados demonstrando efeito benéfico sinérgico, são por sua vez denominados de simbióticos (BRASIL, 2018; MARTYNIK *et al.*, 2021).

Entre os estudos com prebióticos, duas metanálises que incluíram ensaios clínicos randomizados com pacientes hipertensos, mostraram que a ingestão aumentada de fibras dietéticas reduziu níveis de PA sistólica e diastólica (WHELTON *et al.*, 2005; ALJURAIBAN *et al.*, 2015). Similarmente, a suplementação por 4 semanas com mirtilo fermentado por *L. fermentum* levou a redução significativa da PA sistólica e diastólica de animais com hipertensão induzida (AHRÉN *et al.*, 2015). Efeitos hipotensores também foram observados em humanos

que consumiram leite fermentado com *Limosilactobacillus* por um período de 21 semanas (SEPPO *et al.*, 2003).

Particularmente, os probióticos podem prevenir desordens metabólicas e disfunção endotelial por redução de endotoxemia no sistema circulatório. A suplementação com esses microorganismos pode regular a abundância colônica total e de seus metabólitos, que inibem a proliferação de bactérias patogênicas (FENG *et al.*, 2019). Os estudos atuais na sua maioria utilizam cepas de bactérias ácido lácticas como *Bifidobacterias* e *Limosilactobacillus* (DE BRITO ALVES *et al.*, 2019).

Não se conhece ao certo a relação de causa e efeito entre a composição colônica e a hipertensão. Porém, Santisteban *et al* (2017) demonstrou que animais espontaneamente hipertensos apresentaram redução do conteúdo de *Limosilactobacillus* e que esteve associado ao aumento de permeabilidade intestinal, disbiose e inflamação. Por outro lado, uma metanálise sugere, que em estudos com humanos, o consumo de probióticos reduziu significativamente pressão sistólica e diastólica quando comparados a seus controles (KHALESI *et al.*, 2014). Diversas cepas de *Lactobacillus*, com recente atualização de nomenclatura para *Limosilactobacillus* (EFSA, 2020), suplementadas durante 5 semanas, tiveram-se associadas a efeitos hipotensores e redução da hipertrofia cardíaca e renal (GÓMEZ-GUZMÁN *et al.*, 2015). Efeitos semelhantes foram encontrados quando foi suplementado *L. casei* por 8 semanas, posto que atenuou disfunção vascular e reduziu PA em ratos espontaneamente hipertensos (YAP *et al.*, 2016).

Uma das possíveis explicações se deve ao fato amplamente discutido de que processos de fermentação dessas bactérias resultam na geração de peptídeos bioativos inibidores da enzima conversora de angiotensina (ECA) (YAMAMOTO; TAKANO, 1999; KORHONEN, 2009). Cepas de *Limosilactobacillus fermentum* (*L. fermentum* 139, *L. fermentum* 263 ou *L. fermentum* 296) foram extraídas a partir do bioprocessamento de frutas em estudo pioneiro, em uma instituição no nordeste do Brasil. Tais cepas estão sendo estudadas por suas potenciais características probióticas (GARCIA *et al.*, 2016; DE ALBUQUERQUE *et al.*, 2018). De fato, um dos primeiros estudos demonstraram efeito cardiometabólico protetor da cepa *L. fermentum* 296, uma vez que a cepa reduziu atividade simpática e níveis de pressão arterial sistólica em ratos após ingestão de dieta hiperlipídica (CAVALCANTE *et al.*, 2019).

É conhecido que o uso de diversas cepas probióticas apresentam maior eficiência terapêutica comparado ao uso de maneira isolada (YOO *et al.*, 2013). Isso se deve principalmente pelo fato de que os probióticos não apresentam capacidade de desempenhar efeitos iguais entre eles (WIEËRS *et al.*, 2020). Diante disso, foi avaliado o efeito da

combinação de *L. fermentum* (139, 263 e 296) em ratos expostos à dieta hiperlipídica materna. Após intervenção por 8 semanas, foi observado melhora no perfil lipídico, redução de pressão arterial e menores níveis de marcador sérico de estresse oxidativo (DE OLIVEIRA *et al.*, 2020). Todos esses fatores justificam nosso interesse em experimentar os afeitos sinérgicos utilizando concomitantemente as 3 cepas no desenvolvimento desta tese.

Nossos estudos experimentais servirão de base para posteriores estudos clínicos. Para isso, essas cepas precisam antes de tudo apresentar comprovada segurança de uso através dos critérios estabelecidos pela ANVISA (Agência Nacional de Vigilância Sanitária). Na RDC 241/2018, a comprovação de segurança deve ser por meio de estudos que comprovem entre outros critérios, ausência de efeitos adversos, ausência de resistência potencialmente transferível a certos antibióticos, grau de toxicidade e genotoxicidade (BRASIL, 2018b).

3.4 EIXO INTESTINO-RIM

O eixo intestino-rim é entendido como uma ligação onde estes órgãos onde um pode afetar a funcionalidade do outro estabelecendo uma relação bidirecional (FORESTO-NETO; GHIROTTI; CÂMARA, 2021). O equilíbrio na composição da microbiota intestinal garante a homeostase do epitélio do intestino, desempenhando diversas funções, como síntese de vitaminas, motilidade, absorção de nutrientes, metabolismo e barreira de proteção contra patógenos (DOMINGUEZ-BELLO *et al.*, 2011). Alterações na composição da microbiota intestinal têm sido associadas ao estado de saúde do indivíduo. De fato, tais alterações vêm sendo estudadas por desencadear doenças como a disfunção renal (ANDERS; ANDERSEN; STECHER, 2013; RAMEZANI; DOMINIC, 2014). A produção de AGCC's pela microbiota intestinal reduz danos renais uma vez que atua na proteção de células tubulares renais contra o estresse oxidativo (FELIZARDO *et al.*, 2016). Além disso, também é relatado que o tratamento utilizando o acetato, por exemplo, diminuiu a inflamação, produção de espécies reativas de oxigênio (EROS) e infiltração de células imunes e apoptóticas renais, contribuindo para uma redução expressiva da lesão renal induzida por isquemia/reperfusão (ANDRADE-OLIVEIRA *et al.*, 2015).

As vias de regulação da PA através da microbiota intestinal está demonstrado na Figura 1. A regulação da PA pelos AGCCs acontece uma vez que estes podem estimular vias associadas aos receptores acoplados a proteína G (em inglês, *G-protein-coupled receptors* (GPCRs)). Entre esses, o Gpr41 (do inglês, *Free Fatty Acid Receptor 3*) e o Olfr78 (do inglês, *olfactory receptor 78*) atuam respectivamente aumentando a atividade nervosa simpática e a

secreção de renina. Por outro lado, os AGCC induzem vasodilatação por meio da ativação dos receptores Gpr43 (do inglês, *Free Fatty Acid Receptor 2*) e Gpr109a (conhecido do inglês, *hydroxy-carboxylic acid (HCA) receptor 2*) (PLUZNICK, 2013; 2017), fator que previne hipertensão. Ainda na Figura 1, é possível observar que, na condição de desequilíbrio microbiano intestinal pode haver elevação na produção de LPS. Nesse caso, moléculas de LPS poderiam atuar como padrões moleculares associados a patógenos (em inglês, *pathogen-associated molecular patterns (PAMPs)*) e, ao se ligarem aos receptores *TRL4*, promovem a produção de citocinas pró inflamatórias. Além disso, tanto a ativação do *TRL4* por LPS como o aumento de citocinas inflamatórias podem promover a produção de radicais livres e desencadear estresse oxidativo. Os radicais livres podem oxidar o LDL (*low density lipoprotein*) circulante. Esse LDL oxidado (oxLDL) promove inibição na produção de óxido nítrico (ON) e aumento de endotelina-1 (ET-1), mecanismos que favorecem a hipertensão (OYAMA; NODE, 2019).

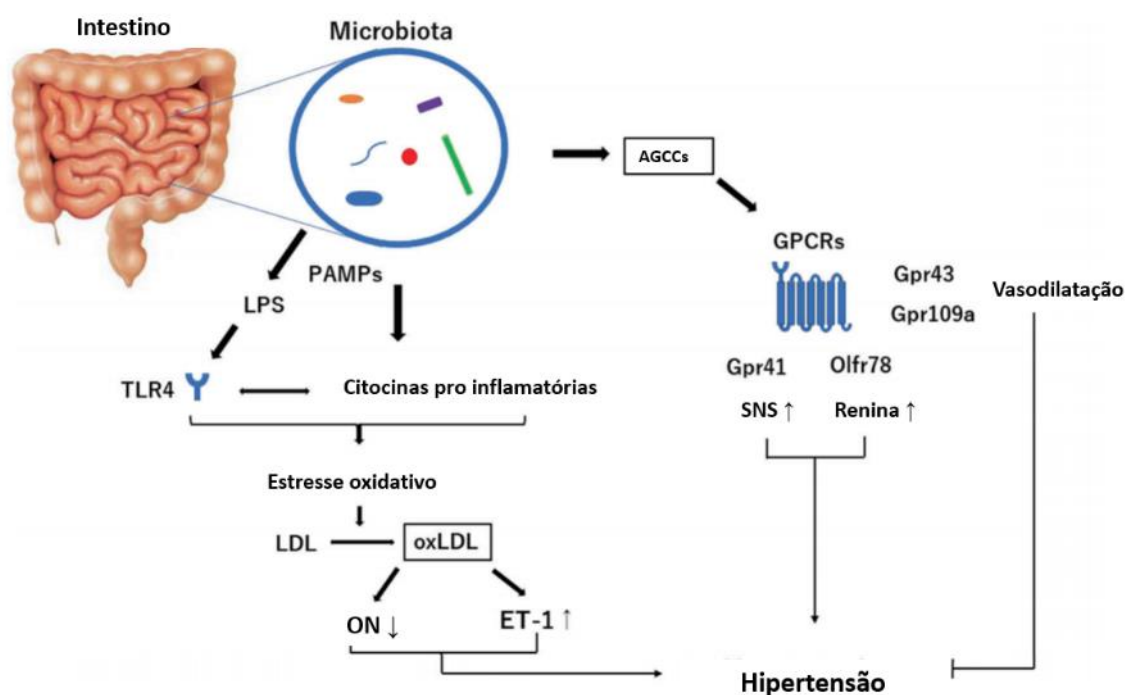


Figura 1: Microbiota intestinal e possíveis vias de indução de hipertensão. Os AGCCs podem estimular vias associadas aos receptores acoplados a proteína G (em inglês, *G-protein-coupled receptors (GPCRs)*): o Gpr41 (do inglês, *Free Fatty Acid Receptor 3*) e o Olfr78 (do inglês, *olfactory receptor 78*). Eles atuam aumentando a atividade nervosa simpática e a secreção de renina. Os AGCCs também induzem vasodilatação pela ativação dos receptores Gpr43 (do inglês, *Free Fatty Acid Receptor 2*) e Gpr109a (conhecido do inglês, *hydroxy-carboxylic acid (HCA) receptor 2*) prevenindo hipertensão. O desequilíbrio da microbiota intestinal eleva produção de LPS que atuam como padrões moleculares associados a patógenos (em inglês, *pathogen-associated molecular patterns (PAMPs)*) que ao se ligarem aos receptores *TRL4*, aumentam citocinas pró inflamatórias. A ativação do *TRL4* também aumenta radicais livres e desencadeiam estresse oxidativo. Estes oxidam o LDL (*low density lipoprotein*) circulante, promovendo inibição na produção de óxido nítrico (ON) e aumento de endotelina-1 (ET-1)

favorecendo a hipertensão. AGCCs- ácidos graxos de cadeia curta; SNS- sistema nervoso simpático; ET-1 – endotelina; GPCRs- receptores acoplados a proteína G; LDL- lipoproteína de baixa densidade; ON –óxido nítrico; oxLDL: lipoproteína de baixa densidade oxidada; LPS – lipopolissacarídeos; PAMPs - padrões moleculares associados a patógenos. Adaptado de OYAMA; NODE, 2019.

Em associação com o aumento da permeabilidade intestinal, consequências sistêmicas podem ser observadas pela presença da LPS na circulação sanguínea (TURNBAUGH *et al.*, 2007). Estudos têm demonstrado que a exposição perinatal ao LPS está associada a comprometimento na nefrogênese, dano à estrutura renal com redução do clearance de creatinina na idade adulta (HAO *et al.*, 2010). Os LPS também ativam a via de sinalização do NF- κ B (do inglês, *nuclear factor- κ B*), que hiperativa o sistema renina angiotensina renal e o aumento da produção de EROS (HAO *et al.*, 2010; RUBATTU *et al.*, 2013). Ainda no rim, o aumento circulante de LPS ocasionou mudanças em estruturas renais e aumento de EROS, comprometendo a excreção de sódio (WANG *et al.*, 2014) e risco elevado de HAS. Existe uma relação bidirecional entre microbiota intestinal e hospedeiro na doença renal crônica (DRC) (MAHMOODPOOR *et al.*, 2017). Barrios *et al.* (2015) reforçam que os metabólitos derivados da microbiota podem servir como marcadores primários de declínio de função renal. Por outro lado, quando a DRC já está instalada, mudanças na microbiota com excessiva produção de diversos metabólitos pode implicar na progressão da doença (RAMEZANI; RAJ, 2014). A disbiose provocada por diversos fatores etiológicos, com redução de metabólitos renoprotetores e produção excessiva de toxinas urêmicas como indoxil sulfato, p-cresil sulfato, e N-óxido de trimetilamina (TMAO), implica em estresse oxidativo, uremia, inflamação e deterioração da função renal. Esses efeitos interferem aumentando a progressão da doença, risco cardiovascular e de morte em pacientes com DRC (BARRETO *et al.*, 2009; MEIJERS *et al.*, 2010; TANG *et al.*, 2015).

A disbiose ainda está relacionada a Nefropatia IgA (HAN *et al.*, 2016) e a formação de cálculos renais (SURYAVANSHI *et al.*, 2016). A já estabelecida relação entre a microbiota intestinal e o sistema reno-cardiovascular é considerada uma via que pode ser importante tanto na patogênese como no tratamento da hipertensão arterial (PLUZNICK *et al.*, 2013).

3.4.1 Estresse oxidativo e eixo intestino-rim

O aumento na produção de espécies reativas (ER's) de maneira geral, seja as espécies reativas de oxigênio (EROs) ou de nitrogênio (ERNs), favorece o desenvolvimento de estresse oxidativo (EO). Esse EO surge quando há excessiva produção de ER's e/ou redução da

capacidade antioxidante endógena em combater tais moléculas. Esta condição está diretamente relacionada a diversos processos patológicos, incluindo doenças neurodegenerativas, cardiovasculares, doenças intestinais e renais (PISOSCHI; POP, 2015). A formação de ER's ocorre de forma fisiológica nas mitocôndrias e na NADPH oxidase. Ademais, as enzimas xantina oxidase, óxido nítrico sintase, ciclooxigenases, citocromo P450 e lipoxigenases; e por fim organelas como retículo endoplasmático e peroxissomo também contribuem para a formação de tais compostos (HOLMSTRÖM; FINKEL, 2014).

O excesso de ER's lesiona moléculas celulares como lipídeos, proteínas e DNA, assim dando origem a processos patológicos (HALLIWELL; GUTTERIDGE, 2007). Por outro lado, no nosso organismo existe um sistema de defesa antioxidante enzimático e não enzimático, que age combatendo esses compostos reativos. De característica não enzimática podemos citar tocoferol, beta caroteno, ascorbato, nicotinamida e glutatona (GSH); enquanto que os enzimáticos abrangem a superóxido dismutase (SOD), catalase (CAT) e glutatonas como a glutatona peroxidase (GPx) e glutatona-S-tranferase (GST) (HALLIWELL; GUTTERIDGE, 2007; SENONER; DICHTIL, 2019).

No intestino, algumas formas de SOD e GPx podem ser localizadas na superfície celular externa. Elas agem detoxificando ER's provenientes dos alimentos como por exemplo a GPx, que remove o excesso de peróxidos presentes nos lipídeos absorvidos e a GST que pode metabolizar peróxidos e aldeídos citotóxicos (AW, 2005; WARK *et al.*, 2004). Além disso, Li *et al* (2017) publicou que em condições de equilíbrio da microbiota intestinal, ou seja, quando há grande quantidade e diversidade bacteriana, existe produção constante de AGCC e esses quando absorvidos na corrente sanguínea podem reduzir estresse oxidativo renal. Em parte, tem sido sugerido que níveis plasmáticos elevados de AGCC poderiam aumentar a atividade da SOD, CAT e níveis de GSH, diminuindo óxido nítrico e EROs com consequente redução de fibrose e dano renal (LI *et al.*, 2017). Do contrário, em condições de disbiose, os LPS provocam estresse oxidativo e este aumenta risco de HAS (Figura 1).

De fato, é reconhecido que a estreita ligação entre a disbiose e o estresse oxidativo contribui para a progressão de doenças (HU *et al.*, 2019). Diante disso, evidências sugerem que algumas cepas de bactérias ácido lácticas (a exemplo de *Limosilactobacillus*) podem aumentar a atividade de enzimas antioxidantes ou reduzir níveis circulatórios de estresse oxidativo (DOWARAH *et al.*, 2018). Sugere-se que esses microorganismos apresentam enzimas consumidoras de O₂ como a NADPH oxidase (NOX). A NOX produz o H₂O₂, que apesar de ser uma espécie reativa não causa um desequilíbrio oxidativo significativo. Ao contrário, ele ativa Nrf2, um regulador da resposta antioxidante celular, que aumenta a atividade

antioxidante mantendo a homeostase intestinal (FENG; WANG, 2020; HU *et al.*, 2019) (Figura 2a). Em condições de disbiose, a ativação da DUOX (dupla oxidase: enzima da família da NOX oxidase que pode ser ativada por uracila derivada de bactérias patogênicas intestinais) associado a redução da atividade antioxidante, pode aumentar a síntese de EROs (LEE *et al.*, 2013) e, consequentemente, cooperar no desenvolvimento de processos inflamatórios e danos ao DNA (HU *et al.*, 2019) (Figura 2b).

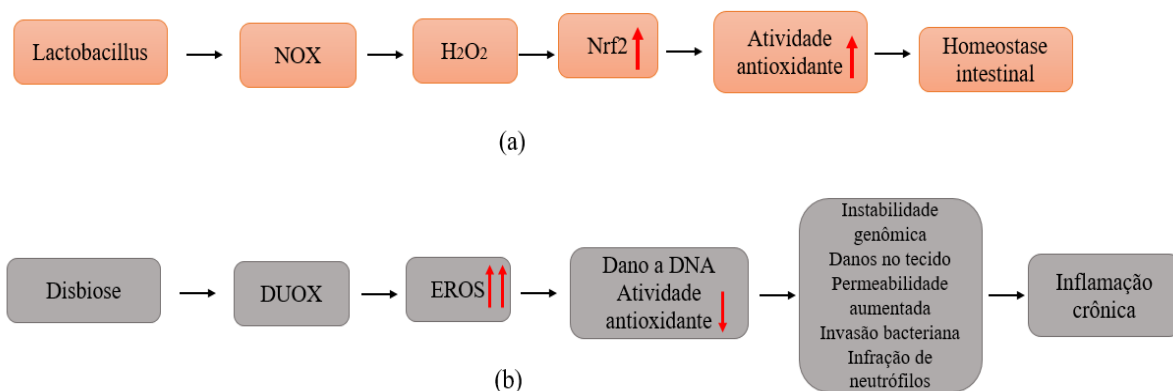


Figura 2. Associação entre microbiota intestinal, estresse oxidativo e dano tecidual. Alguns microorganismos apresentam enzimas como a NADPH oxidase (NOX). A NOX produz o H_2O_2 , que ativa Nrf2 que aumenta a atividade antioxidante mantendo a homeostase intestinal. A disbiose por sua vez ativa a DUOX aumentando a síntese de EROs que induz processos inflamatórios e danos ao DNA, caracterizando inflamação crônica. DUOX- dupla oxidase; EROS- espécies reativas de oxigênio; H_2O_2 - peróxido de hidrogênio; NOX- NADPH oxidase; Nrf2- fator nuclear 2 relacionado ao eritroide 2. Adaptado de HU *et al.*, 2019.

Conforme previamente descrito o excesso de ER's e/ou a redução de capacidade antioxidante, expõe o tecido a processos patológicos, a exemplo da hipertensão arterial (ROMERO; RECKELHOFF, 1999). Entre os estudos que mostram essa correlação, o grupo de Chung *et al* (2010) confirmou que existe ativação intra-renal do sistema renina angiotensina aldosterona na presença de estresse oxidativo no rim com agravamento da hipertensão arterial. A microbiota intestinal, bem como a redução de estresse oxidativo se torna alvo para tratamento de desordens renais e hipertensão arterial (YANG *et al.*, 2018; YANG *et al.*, 2020). É conhecido que diversas cepas de *Limosilactobacillus* apresentam perfil antioxidante uma vez que nelas são encontradas um tipo de SOD, bem como de CAT em várias espécies (FENG; WANG, 2020). Adicionalmente foi relatado que a terapia com AGCCs promoveu melhora em disfunção renal associada com redução de estresse oxidativo (JANG *et al.*, 2009).

Este referencial teórico demonstrou que estudos em animais experimentais e observações epidemiológicas mostram que o ambiente nutricional durante o início da vida promove susceptibilidade a HAS na vida adulta. Além disso, alterações na microbiota intestinal

e o estresse oxidativo no eixo intestino rim parecem mediar o desenvolvimento de HAS na prole. Sabendo disso, estudos com suplementação probiótica avaliando a regulação na capacidade antioxidante no eixo entero-renal tornam-se imprescindíveis uma vez que a utilização de cepas probióticas podem tornar-se estratégias de reprogramação prevenindo a HAS causada pela exposição a dieta hiperlipídica materna.

4. MATERIAIS E MÉTODOS

4.1 ANIMAIS E ASPECTOS ÉTICOS

Foram utilizados ratos Wistar (*Rattus norvegicus*) provenientes do biotério do Instituto de Pesquisas em Fármacos e Medicamentos (IPeFarM). Os animais foram mantidos no biotério do Laboratório de Nutrição experimental (departamento de nutrição, UFPB), em gaiolas de polipropileno, com água filtrada e ração *ad libitum*, mantidos em ciclo claro escuro de 12h (início as 8h da manhã) e com temperatura ($22 \pm 2^\circ\text{C}$) e umidade ($55 \pm 5\%$) controladas. Este projeto foi aprovado pelo comitê de ética em experimentação animal da Universidade Federal da Paraíba (protocolo nº/CEUA Nº 9635020519) e seguiu as orientações do Conselho Nacional para o Controle de Experimentação Animal (CONCEA) e os Princípios Internacionais para Pesquisas Biomédicas Envolvendo Animais (ANEXO A).

4.2 DIETAS EXPERIMENTAIS

A dieta controle foi elaborada de acordo com o Instituto Americano de Nutrição - (AIN-93) (REEVES et al., 1993). A dieta rica em gordura saturada e colesterol foi confeccionada pela empresa Rhoster (Araçoiaba da Serra, SP, Brasil). Os teores de proteína e carboidratos são semelhantes entre as dietas, no entanto os níveis de lipídios, especialmente gorduras saturadas e colesterol estão elevados na dieta considerada dislipidêmica (Tabela 1) (GUIMARÃES et al., 2017).

Tabela 1. Composição nutricional dietética (g/100g)

Nutrientes	Dieta Controle	Dieta hiperlipídica
Caseína (85%)	20	19.86
Amido dextrinizado	13	15.5
Celulose	5	5
Sacarose	10	6
Amido de milho	39.5	33.6
Óleo de soja	7	3
Banha de porco	0	6
Gordura vegetal hidrogenada	0	5
Ácido cólico (Sigma)	0	0.5
Colesterol (Sigma)	0	0.5
Colina	0.25	0.25
Metionina	0.3	0.3
Mix de vitaminas	1	1
Mix de minerais	3.5	3.5
Hidroxitolueno terc-butilato	0.014	0.014
Calorias (kcal/g)	3.96	4.34
Carboidratos (kcal %)	63.62	50.32
Proteínas (kcal %)	20.47	18.58
Lipídeos (kcal %)	15.92	31.11

*A caseína apresenta 85% de pureza (sendo 85g de caseína por 100g de dieta).

4.3 OBTENÇÃO DA FORMULAÇÃO PROBIÓTICA

As cepas probióticas utilizadas foram *Limosilactobacillus fermentum* (*L. fermentum* 139, *L. fermentum* 263 e *L. fermentum* 296), anteriormente identificadas usando análise de RNAr 16S (GARCIA et al., 2016) e fornecidas pelo Laboratório de Microbiologia de Alimentos, do Departamento de nutrição, da Universidade Federal da Paraíba. Para a obtenção da suspensão das células probióticas, foram cultivadas em caldo MRS (abreviação para Man, Rogosa e Sharpe) e incubadas anaerobicamente a 37°C. Posteriormente as células foram coletadas por centrifugação (8000g, 10min a 4°C), lavadas duas vezes com solução salina estéril, ressuspensas e homogeneizadas por 30s. Em seguida, para fornecimento de contagem de células viáveis, foi realizada uma leitura de densidade óptica a 660nm, fornecendo assim aproximadamente 9 log UFC/mL. A suspensão do mix de células foi obtida por suspensão de cada cepa na proporção de 1:1:1.

4.4 DESENHO EXPERIMENTAL DO ESTUDO

As figuras 3 e 4 representam os desenhos experimentais utilizados neste estudo. Para ambos os desenhos uma semana antes dos experimentos, foi ofertada a dieta dislipidêmica ou controle para adaptação dos grupos experimentais. Foram utilizadas para o acasalamento 12 ratas *Wistar* (200-230g) e 8 machos (250-300g). O acasalamento foi realizado na proporção de 1 macho para 2 fêmeas (1:2). A presença de espermatozoides no esfregaço vaginal foi considerada um marcador de dia 0 de gestação. Logo após as ratas prenhes foram então transferidas para gaiolas individuais e receberam dietas controle (CTL) ou dieta rica em colesterol e gordura saturada (HFHC) e água *ad libitum* durante o período de gestação e lactação.

Após 24h do nascimento, o número de filhotes na prole foi ajustado para 8 filhotes por ninhada para garantir a nutrição padronizada até o desmame. Após o período de lactação (21º dia pós-natal), as proles de fêmeas e machos foram alocadas separadamente (quatro por gaiola) e receberam dieta padrão de laboratório *ad libitum* até os 90 dias de vida para posteriores análises fisiológicas e bioquímicas (Figura 3).

Em uma segunda etapa desse estudo, os animais após completarem 100 dias iniciaram a suplementação com o mix de *L. fermentum* contendo as três cepas anteriormente citadas. A administração era realizada por gavagem, mantendo o início diário da administração às 12h. Sendo assim, obtivemos três grupos: o grupo CTL, prole de mães alimentadas com dieta controle; um grupo HFHC, prole de mães alimentadas com dieta rica em colesterol e gordura saturada; e um grupo HFHC+Lf, prole de mães alimentadas com dieta rica em colesterol e

gordura saturada e que receberam a suplementação com o mix probiótico. Os grupos CTL e HFHC receberam PBS como placebo durante o mesmo tempo. A administração do mix probiótico ou do placebo foram realizados por gavagem e o peso dos ratos foram acompanhados até os 130 dias de vida, idade de realização das análises experimentais (Figura 4).

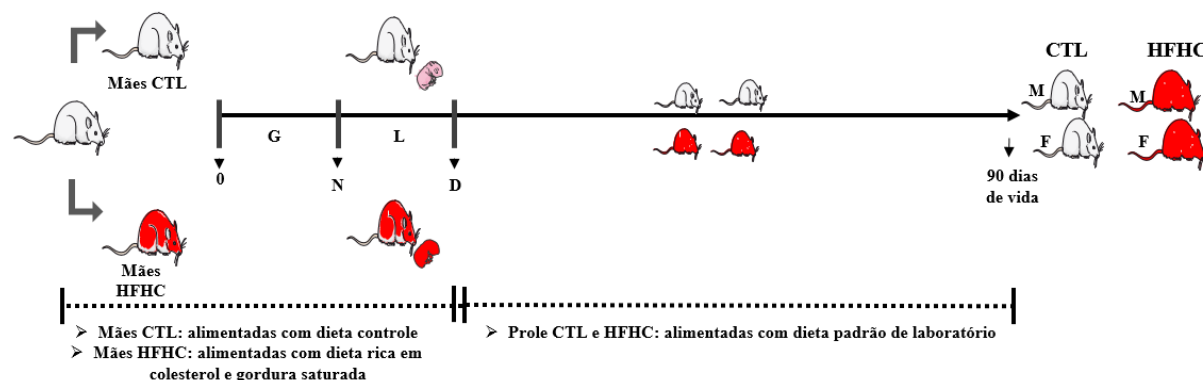


Figura 3: Esquema representativo do protocolo utilizado para a formação dos grupos experimentais CTL e HFHC. Uma semana antes dos experimentos, foi ofertada a dieta dislipidêmica ou controle para fêmea. O acasalamento foi realizado e logo após as ratas prenhes foram então transferidas para gaiolas individuais e receberam dietas controle (CTL) ou dieta rica em colesterol e gordura saturada (HFHC) durante o período de gestação e lactação. Aos 21 dias de vida foi realizado o desmame e as proles de fêmeas e machos foram separadas receberam dieta padrão de laboratório até os 90 dias de vida para realização das análises. 0= dia 0 de gestação; G= gestação; N= nascimento; L=lactação; D=desmame; M=machos e F=fêmeas.

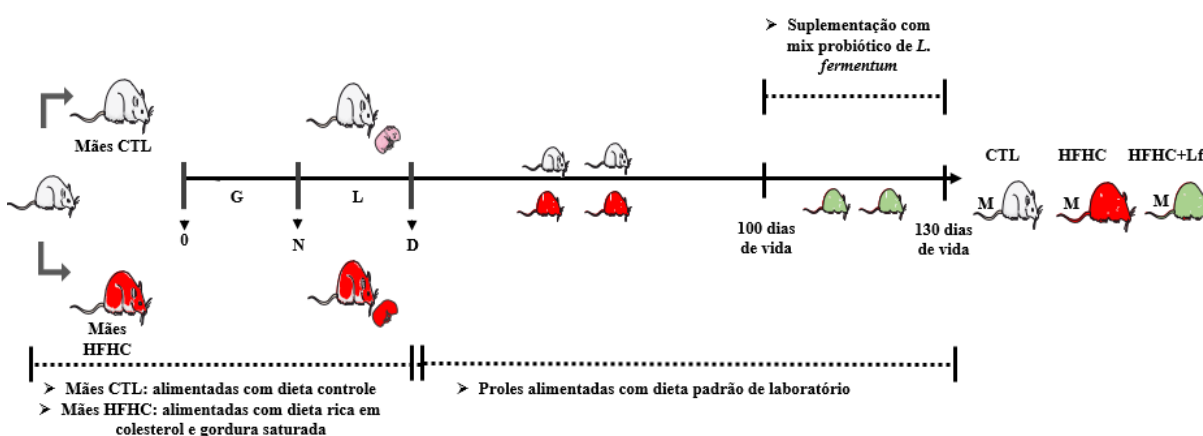


Figura 4: Esquema representativo do segundo protocolo experimental formando os grupos CTL, HFHC e HFHC+Lf. O protocolo inicial é seguido até a prole completar 100 dias quando um grupo de animais HFHC iniciara a suplementação com o mix de *L. fermentum*. A administração era realizada por gavagem, mantendo o início diário da administração às 12h. A administração do mix probiótico ou do placebo foram realizados por gavagem até os 130 dias de vida, idade que foram iniciadas as análises experimentais. 0= dia 0 de gestação; G= gestação; N= nascimento; L=lactação; D=desmame; M=machos e F=fêmeas.

4.5 AVALIAÇÃO DE GANHO PONDERAL

A avaliação do peso corporal dos ratos foi realizada mensalmente desde o desmame até os 100 dias de vida e semanalmente durante a administração do probiótico até os 130 dias de vida. O peso foi registrado utilizando uma balança eletrônica (modelo AS-1000; Marte, Santa Rita -MG, Brasil).

4.6 COLETA DE URINA E SANGUE PARA ANÁLISES BIOQUÍMICAS

A coleta da urina foi realizada após período de adaptação dos animais em gaiolas metabólicas individuais e após 4 dias de adaptação foi coletada a urina de 24h. Em seguida foi estocado em refrigeração a -20°C para posteriores análises de função renal.

O sangue foi coletado após eutanásia dos animais e em seguida centrifugado a 3500rpm por 15 min e armazenado à -20°C até o início das análises bioquímicas. As dosagens realizadas no sangue abrangem colesterol total, triglicerídeo, LDL-colesterol, HDL-colesterol e creatinina plasmática. As avaliações foram realizadas por técnica colorimétrica utilizando respectivos kits comerciais (Bioclin, Belo Horizonte - Minas Gerais - Brasil) seguindo os protocolos propostos pelo fabricante.

4.7 AVALIAÇÃO DA FUNÇÃO RENAL

As excreções urinárias de creatinina, ureia e proteínas totais foram obtidas através do produto da concentração urinária e o volume urinário de 24h. Além dessas medidas foi obtido o clearance de creatinina (CCr) que é um importante método de investigação da função renal. Para realização do cálculo do CCr foi utilizada a fórmula: $CCr = Cr \text{ Urinária} \times V / Cr \text{ plasmática}$, onde V é o volume urinário de 24hs (OGUNDIPE et al., 2017).

4.8 ANÁLISE DE PRESSÃO ARTERIAL

Os níveis de pressão arterial foram avaliados através da técnica de pletismografia de cauda (WANG; THATCHER; CASSIS, 2017). Os valores são apresentados em pressão sistólica (PAS), pressão diastólica (PAD), pressão arterial média (PAM) e frequência cardíaca (FC). Após três dias de adaptação os valores foram coletados após 15 ciclos de inflação e deflação para cada animal, sendo utilizados 10 valores para obtenção da média para cada dado coletado.

4.9 AVALIAÇÃO DE INDICADORES DE ESTRESSE OXIDATIVO NO TECIDOS INTESTINAL E RENAL

4.9.1 Coleta dos tecidos e preparo das amostras

A eutanásia dos ratos foi realizada por decapitação o rim direito e uma porção do cólon foram removidos e rapidamente estocados sob refrigeração a -20°C para posteriores análises. Para preparo das amostras os tecidos foram homogeneizados em uma solução tampão de extração, contendo TRIS (50 mM) e EDTA (1 mM), ajustado para o pH 7,4, ainda foi adicionado ortovanadato de sódio (1 mM) e fluoreto de fenilmetanossulfonila (200 µg/mL). Em seguida, os homogenatos foram centrifugados a 4.000rpm por 10min a 4°C (PEDROZA et al., 2018). Os níveis de proteína foram quantificados pelo método de Bradford (1976).

4.9.2 Quantificação dos níveis de peroxidação lipídica

Para a dosagem da peroxidação lipídica foi utilizada a técnica colorimétrica de Buege e Aust (1978). Utilizamos 0,500 mg/L de homogenato do córtex renal e cólon para quantificação da produção de malondialdeído (MDA) em reação com ácido tiobarbitúrico (TBA) a 100° C. Em resumo, foram realizadas adições sequenciais de 30% do ácido tricloroacético e Tris-HCl (3mM, pH) à amostra e foram seguidos de uma mistura completa por meio de centrifugação a 6.500rpm por 5min. Em seguida, o sobrenadante resultante foi transferido para outro tubo e acrescentado TBA a 0,8% (v/v) antes de misturar e ferver por 15 minutos. Por fim, após o resfriamento, foi lida a absorbância a 535nm por espectrofotometria (espectrofotômetro modelo IL-592 UV/VIS, marca Kasuaki) e o resultado apresentado em nmol por miligrama de proteína.

4.9.3 Avaliação da atividade da Superóxido dismutase (SOD)

A atividade da SOD foi avaliada através do método de inibição auto oxidação da adrenalina, medido em espectrofotômetro a 480nm. Em uma cubeta, adiciona-se tampão carbonato (0,1M, pH= 10,2), amostra (300µg de proteína) e adrenalina (150mM). A absorbância foi registrada por um período de aproximadamente 3 minutos. Os resultados foram expressos em unidades por mg proteína utilizando o coeficiente de extinção da adrenalina 4020 M⁻¹ cm⁻¹ (MISRA; FRIDOVICH, 1972).

4.9.4 Avaliação da atividade da Catalase (CAT)

A atividade da catalase foi medida através da avaliação do consumo de peróxido de hidrogênio pelo decréscimo na absorção a 240 nm ([] máx do H_2O_2) de um meio de reação, contendo tampão fosfato (50mM, pH=7,4 e temperatura a 20°), peróxido de hidrogênio (H_2O_2) 0,3mM e amostra (300µg de proteína). O coeficiente de extinção usado foi 2,3 $\text{microM}^{-2} \text{cm}^{-1}$ e os resultados expressos em unidade por mg de proteína (AEBI, 1984).

4.9.5 Avaliação da atividade da Glutathione S transferase (GST)

O princípio do ensaio da atividade da GST baseia-se na determinação da absorbância a 340nm da conjugação de 1-cloro, 2,4-dinitrobenzeno (CDNB), na presença de glutathione reduzida (GSH, 1mM) em tampão fosfato de potássio (0,1M, pH= 6,5, a 30° de temperatura) e amostra na concentração de 300µg de proteína. A medida da absorbância é realizada pela captação do produto da reação, o 2,4-dinitrofenil-s-glutathione (DNP-SG), utilizando o coeficiente de extinção 9,6 $\text{Mm}^{-1} \text{cm}^{-1}$, sendo os dados expressos em unidade por mg de proteína (HAGIB; PABST; JAKOBY, 1974).

4.9.6 Quantificação de grupamentos tiois totais

As amostras de tecido (0,3 mg/mL) foram incubadas em tampão de extração (previamente descrito) adicionado 5,5'-ditiobis (ácido 2-nitrobenzóico, 10mM) em um ambiente escuro por 30 min. A absorbância da reação foi medida a 412 nm e os dados expressos em µmol por miligramas de proteínas (ELMAN, 1959).

4.10 ANÁLISES ESTATÍSTICAS

Os dados foram expressos em média $\pm$ erro padrão de média. Os valores passaram pelo teste de normalidade de Kolmogorov Smirnov. O teste t de Student não pareado foi utilizado para avaliação entre dois grupos, enquanto que o teste de variância ANOVA *two way* foi utilizado para avaliar os fatores de dieta e sexo seguido do pós teste de Bonferroni. A análise de variância ANOVA *one way* seguida do pós teste de Tukey foi aplicada quando o teste envolvia uma variável independente, nesse caso o tratamento. As diferenças foram consideradas

significativas quando o valor de p foi $\leq 0,05$. As análises estatísticas foram realizadas usando o software Prisma versão 8.0 (GraphPad Software, San Diego, CA, EUA).

5. RESULTADOS

Os resultados desta tese estão apresentados na forma de três artigos, sendo dois originais e um de revisão conforme descritos:

O artigo apresentado no apêndice A (*Maternal exposure to high-fat and high-cholesterol diet induces arterial hypertension and oxidative stress along the gut-kidney axis in rat offspring*) aborda os efeitos da dieta materna rica em colesterol e gordura saturada, com capacidade dislipidêmica, sobre os níveis de pressão arterial, indicadores de estresse oxidativo e função renal. Nossos achados apontam para hipertensão arterial aos 90 dias de vida tanto na prole macho como fêmea. Além disso, a prole macho de mães alimentadas com dieta HFHC mostraram redução da atividade de GST e de grupamentos tióis no cólon, redução da atividade de SOD no córtex renal e diminuição da excreção de creatinina urinária. Nas fêmeas, a atividade da CAT e o conteúdo de grupamentos tióis foram reduzidos no cólon. De maneira geral, a dieta HFHC materna induz estresse oxidativo específico do sexo ao longo do eixo intestino rim na prole, propiciando disfunção renal e hipertensão arterial.

No apêndice B apresentamos o artigo de revisão intitulado “*Limosilactobacillus fermentum, current evidence on the antioxidant properties and opportunities to be exploited as a probiotic microorganism.*” Alguns estudos experimentais e clínicos demonstram que cepas probióticas de *L. fermentum* podem apresentar capacidade antioxidante beneficiando o combate a desordens metabólicas. Diante disso, foi reunido neste trabalho bases científicas que demonstram todas as cepas de *L. fermentum* com propriedades antioxidantes e habilidades em modular estresse oxidativo. Entre essas elencadas destaca-se as cepas *L. fermentum* 139, *L. fermentum* 263, *L. fermentum* 296, genuinamente brasileiras, com potencial para serem estudadas como proposta terapêutica e preventiva de doenças não comunicáveis por combate ao estresse oxidativo.

A investigação dos efeitos das cepas de *L. fermentum* 139, 263 e 296 originou o artigo apresentado no apêndice C: “*Limosilactobacillus fermentum prevent gut-kidney oxidative damage and the rise in blood pressure in male rat offspring exposed to a maternal high fat diet*”. Nos propomos avaliar os efeitos da terapia com um mix contendo cepas de *L. fermentum* em indicadores de estresse oxidativo, função renal e pressão arterial em filhos de mães alimentadas com dieta HFHC. Os resultados obtidos nos permitem observar que a administração das cepas probióticas reduziu pressão arterial sistólica, diastólica e média. Apesar de não observarmos alterações quanto aos indicadores de estresse oxidativo no cólon, a atividade da SOD encontrou-se aumentada. No tecido renal, o estresse oxidativo foi mitigado uma vez que houve aumento na atividade da SOD e CAT. Associado a esses dados, os

resultados demonstram melhora da função renal com a suplementação probiótica. Em resumo, a administração com as cepas de *L. fermentum* reduziu pressão arterial, melhorou atividade antioxidante e função renal na prole masculina de mães alimentadas com dieta HFHC.

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APÊNDICES

APÊNCIDE A - ARTIGO 1

Maternal exposure to high-fat and high-cholesterol diet induces arterial hypertension and oxidative stress along the gut-kidney axis in rat offspring

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Maternal exposure to high-fat and high-cholesterol diet induces arterial hypertension and oxidative stress along the gut-kidney axis in rat offspring

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ABSTRACT

Aims: Evaluate the effects of maternal high fat and high cholesterol (HFHC) diet consumption on blood pressure (BP), renal function and oxidative stress along the gut-kidney axis in male and female rat offspring.

Materials and methods: Pregnant rats were fed with a control (CTL) or HFHC diet during pregnancy and lactation. At 90 days, BP was assessed by tail-cuff plethysmography, and urinary and biochemical variables were measured. Biomarkers for oxidative stress, enzymatic antioxidant defense (activity of superoxide dismutase-SOD, catalase, and glutathione-S-transferase-GST) and nonenzymatic antioxidant defense (thiols content) were evaluated in the colon and renal cortex.

Key findings: Male and female offspring from dams fed with a HFHC diet presented increased BP when compared to their respective CTL group. Male offspring from dams fed with HFHC diet showed reduced GST activity and thiols content in the colon, reduced SOD activity in the renal cortex and decreased urinary creatinine excretion when compared to the CTL group. Regarding female offspring, catalase activity and thiols content were reduced in the colon when compared to CTL group. Although lipid peroxidation had been increased in the renal cortex of HFHC female offspring, the CAT and SOD enzymatic antioxidant activities (CAT and SOD) were increased in the renal cortex of female offspring when compared with male offspring; and the renal function was not impaired by maternal HFHC diet consumption.

Significance: HFHC diet during pregnancy and lactation induces sex-specific oxidative stress along the gut-kidney axis in offspring, which might induce renal dysfunction and arterial hypertension in later life.

1. Introduction

Maternal high-fat and high-cholesterol (HFHC) diet consumption during pregnancy and lactation is a risk factor for the development of arterial hypertension in offspring in later life [1–3]. The arterial hypertension programmed by maternal HFHC diet consumption is complex and has been linked to sympathetic overactivity, impaired baroreflex function and augmented peripheral chemoreceptor sensitivity [1,2]. In addition, early studies have demonstrated that kidney oxidative stress, hyper activation of the renin-angiotensin system and impaired tubular sodium handling are altogether considered to play an important role in the development of arterial hypertension in the offspring from dams fed with a high-fat diet [4,5].

One important question to be answered is: why several BP control mechanisms are impaired in hypertensive offspring from dams fed with

HFHC diet during pregnancy and lactation? Recent studies have reported that high-fat diet intake during pregnancy and/or lactation shifts the composition of maternal-fetal gut microbiota facilitating lipopolysaccharide (LPS) translocation from intestinal barriers to the systemic circulation and contributes to the low-grade inflammation and developmental programming of arterial hypertension [6–8].

The increase of circulating LPS due to loss of intestinal permeability can reach to the kidneys and bind to Toll-like receptors leading to inflammation. This process may enhance the production of reactive oxygen species, such as superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2), down-regulate the renal antioxidant system and to promote the progression of the kidney disease [9].

Oxidative stress, characterized by an imbalance between the pro-oxidants and antioxidants, in the colon mucosa increases colonic epithelial barrier permeability and drives colonic mucosa inflammation

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[10]. In the kidney, oxidative stress has been linked to the developmental programming of arterial hypertension [5]. However, whether intestinal and renal oxidative stress occurs simultaneously in hypertensive offspring from dams fed with an HFHC diet remains to be elucidated.

Another important aspect is that arterial hypertension evidenced in offspring who have been exposed to nutritional insults during early life may be sex-dependent [11,12]. Of note, males seems to be more susceptible to tissue oxidative damage than females [13–15]. Here we evaluated the effects of maternal HFHC diet consumption on blood pressure, renal function and oxidative stress along the gut-kidney axis in male and female rat offspring.

2. Material and methods

2.1. Animals and ethical aspects

Wistar rats (*Rattus norvegicus*) were used in this study. The animals received water and diet *ad libitum* and were maintained in polypropylene cages under controlled temperature ($22 \pm 1^\circ\text{C}$), humidity between 50 and 55%, and a 12 h light-dark cycle. The experimental procedures were approved by the Institutional Animal Care and Use Committee of the Federal University of Paraíba (CEUA-UFPA protocol # 9635020519) and followed the guidelines of the National Council for the Control of Animal Experimentation (CONCEA) and the International Principles for Biomedical Research.

2.2. Experimental design

Female Wistar rats (*Rattus norvegicus*) were maintained in polypropylene cages receiving filtered water and were fed *ad libitum* (Presence Purina®, Paulínea, Brazil) in a 12-hour dark-light cycle with temperature ($22 \pm 2^\circ\text{C}$) and controlled humidity until 90 days of age. Then, female rats were adapted for one week to control diet prepared according to the American Institute of Nutrition – AIN-93G) or to a HFHC diet (Table 1) purchased from Rhoster® Company (Araçoiaba da Serra, São Paulo, Brazil).

After the adaptation period, primiparous Wistar rats ($n = 10$) were mated to 1:2 fertile male rats. The presence of sperm in the vaginal smear was considered a marker for day 0 of pregnancy. The pregnant rats fed with a control (CTL, $n = 5$) or a HFHC diet (HFHC, $n = 5$) were placed on individual cages and continued receiving their diets

throughout the pregnancy and lactation period (Fig. 1).

At 24 h after birth, offspring were adjusted to eight 8 pups per litter (4 males and 4 females) to ensure standardized nutrition. The experimental groups were formed with one or two male or female offsprings from each litter. At weaning (postnatal day 21), the females (CTL, $n = 6$ and HFHC, $n = 6$) and males (CTL, $n = 6$ and HFHC, $n = 6$) rats offspring were housed separately (3 per cage) and had free access to a commercial diet (Presence Purina®, Paulínea, Brazil) and water *ad libitum* up to 90 days of age.

2.3. Urinary analysis

Rats were individually acclimatized in metabolic cages for three days and on the fourth day, 24-h urine collection was performed. The urinary volume was measured and stored under refrigeration at -20°C for further analysis. Urinary measurements of urea, creatinine, and total proteins were performed using commercial kits following the protocols according to the manufacturer (Bioclin, Belo Horizonte - Minas Gerais - Brazil).

2.4. Blood pressure and heart rate recordings

Blood pressure was recorded by tail-cuff plethysmography (V2.11 Plethysmography, Insight, Ribeirão Preto, São Paulo, Brazil). The BP measurements were carried in a designated quiet area (22°C). For three days, rats were encouraged to walk into the restraint tubes and acclimatized for an approximately 1-hour period before experiments began. After the adaptation period, BP and heart rate were measured using the tail plethysmography method [16]. For each record, 15 cycles of inflation and deflation were carried out. The first values were discarded, while the last 10 were used to perform the average.

2.5. Collection of blood and intestinal and renal tissues

After urine collection and BP measurements, the rats were euthanized by decapitation. Blood was collected and the right kidney, as well as a portion of the colon, were quickly removed. The right kidney and a portion of the colon were packaged under refrigeration at -20°C for further oxidative analysis. To obtain the serum, the blood was centrifuged ($58.136g$ for 15 min at 4°C). Serum measurements for total cholesterol, triglycerides, glucose and creatinine concentration were performed using appropriate enzymatic colorimetric kits according to the manufacturer instructions (Bioclin, Belo Horizonte - Minas Gerais - Brazil).

2.6. Preparation of samples for evaluation of oxidative stress biomarkers

The renal cortex and colon tissues were homogenized in a cold buffer solution, containing 50 mM TRIS and 1 mM EDTA, pH 7.4, 1 mM sodium orthogonadate and 200 $\mu\text{g/mL}$ phenylmethanesulfonyl fluoride using an IKA RW 20 digital homogenizer, a pestle of potter-Elvehjem and glass tubes on ice. Then, the homogenates were centrifuged at $1.180g$ for 10 min at 4°C [17]. Protein levels were determined by the Bradford protocol [18].

2.7. Assessment of lipid peroxidation

We used 0.3 mg/mL of homogenate of the renal cortex and colon tissue to quantify the production of malondialdehyde (MDA) in reaction with thiobarbituric acid (TBA) at 100°C . Briefly, sequential additions of 30% of trichloroacetic acid and Tris-HCl (3 mM) were performed to the sample followed by centrifugation at $2500g$ for 10 min. To the resulting supernatant, 0.8% (v/v) TBA was added, mixed and boiled for 15 min. Finally, after cooling, the reaction was read at 535 nm on a spectrophotometer.

Table 1
Nutritional composition of the control diet (CTL diet) and high-fat and high-cholesterol diet (HFHC diet). Values are presented as g/100 g diet.

Nutrient	CTL diet	HFHC diet
Casein (85%) ^a	20	19.86
Dextrin maize starch	13	15.5
Cellulose	5	5
Sucrose	10	6
Maize starch	39.7	33.6
Soyabean oil	7	3
Animal fat (lard)	0	6
Non-hydrolysed vegetable fat	0	5
Cholic acid	0	0.5
Cholesterol	0	0.5
Choline	0.25	0.25
Methionine	0.3	0.3
Vitamin mix	1	1
Mineral mix	3.5	3.5
Tert-butylated hydroxytoluene	0.014	0.014
Calories (kcal/g)	3.96	4.34
Carbohydrates (kcal %)	63.62	50.32
Proteins (kcal %)	20.47	18.58
Lipids (kcal %)	15.92	31.11

^a Casein showed 85% purity (85 g protein for each 100 g casein).

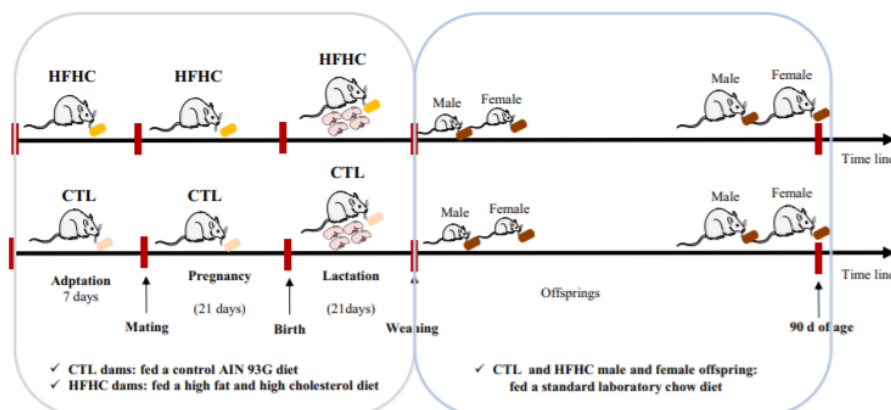


Fig. 1. Experimental desing performed in the study to evaluate the effects of maternal high-fat and high-cholesterol diet consumption during pregnancy and lactation on oxidative stress along the gut-kidney axis in rat offspring.

2.8. Assessment of superoxide dismutase (SOD) activity

Total superoxide dismutase (SOD) enzyme activity was determined according to the method of Misra and Fridovich. The tissues samples (0.3 mg/mL) were incubated with sodium carbonate buffer (0.05%, pH 10.2, 0.1 mmol/L EDTA) at 37 °C. In addition, 30 mM/L of epinephrine (in 0.05% acetic acid) were added and the SOD activity was measured by the kinetics of epinephrine auto-oxidation inhibition for 1.5 min at 480 nm [19].

2.9. Assessment of catalase (CAT) activity

Catalase activity was determined by the decomposition of H_2O_2 into O_2 and H_2O . We used 0.3 mg/mL of tissue homogenates in 50 mM phosphate buffer (pH 7.0) and addition of 0.3 M H_2O_2 . Absorbance was measured at 240 nm for 1.5 min [20].

2.10. Assessment of glutathione S-transferase (GST) activity

We used 0.3 mg/mL of tissue homogenates to quantify GST activity, as previously described [21]. We added phosphate buffer (0.1 M, pH 6.5 containing 1 mM EDTA) to the samples, 1 mM 1-chloro-2,4-dinitrobenzene (CDNB) and 1 mM reduced glutathione (GSH). Absorbance was measured at 340 nm for 1.5 min.

2.11. Assessment of total thiols groups

Samples of tissue homogenates (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 [22].

2.12. Statistical analysis

Values were reported as mean $\pm$ standard error mean. All data were checked for normal distribution using Kolmogorov Smirnov test. For blood pressure, biochemical and urinary measurements, the comparisons between HFHC and CTL groups were tested using unpaired Student's *t*-test. For oxidative stress measurements, statistical significance were evaluated using analysis of variance ANOVA two-way test with maternal diet (CTL/HFHC) and sex (males and females) as factors. Bonferroni's *post hoc* test was used. Differences were considered significant when *p* value was ≤ 0.05 . Statistical analyses were performed using the computational software Prism 5.0 (GraphPad Software, San Diego, CA, USA).

3. Results

3.1. Blood pressure and heart rate

Male and female offspring from dams fed with a HFHC diet during pregnancy and lactation had increased systolic and diastolic and mean BP at 90 days of age when compared to the respective CTL groups, but not alteration in heart rate (Table 2).

3.2. Oxidative stress biomarkers in colon tissues

The MDA levels were not sex- ($F = 0.96$, $p = 0.33$) and diet-dependent [($F = 0.45$, $p = 0.50$), Fig. 2A). Regarding antioxidant enzyme activities, SOD activity was similar between CTL and HFHC groups for both male and female offspring ($p > 0.05$, Fig. 2B). Changes in SOD activities were sex-dependent ($F = 11.9$, $p = 0.002$, Fig. 2B). In contrast, CAT ($F = 0.08$, $p = 0.76$, Fig. 2C) and GST ($F = 0.33$, $p = 0.57$, Fig. 2D) activities were not sex-dependent.

The CAT activity was reduced in the colon of HFHC female offspring

Table 2

Systolic arterial pressure (SAP), diastolic arterial pressure (DAP), mean arterial pressure (MAP) and heart rate (HR) in male and female offsprings at 90 d of age from dams fed with control (CTL) or high-fat and high-cholesterol diet (HFHC) during pregnancy and lactation.

Variables	Males			Females		
	CTL	HFHC	<i>p</i> -Value	CTL	HFHC	<i>p</i> -Value
SAP (mmHg)	141 $\pm$ 4.3	152 $\pm$ 1.5	0.018	118 $\pm$ 2.7	137 $\pm$ 2.5	0.0003
DAP (mmHg)	92 $\pm$ 3.5	104 $\pm$ 2.0	0.009	79 $\pm$ 2.9	95 $\pm$ 3.9	0.009
MAP (mmHg)	109 $\pm$ 2.9	120 $\pm$ 1.6	0.004	92 $\pm$ 2.7	108 $\pm$ 3.2	0.002
HR (bpm)	361 $\pm$ 12.4	350 $\pm$ 17.8	0.624	360 $\pm$ 4.2	374 $\pm$ 9.0	0.213

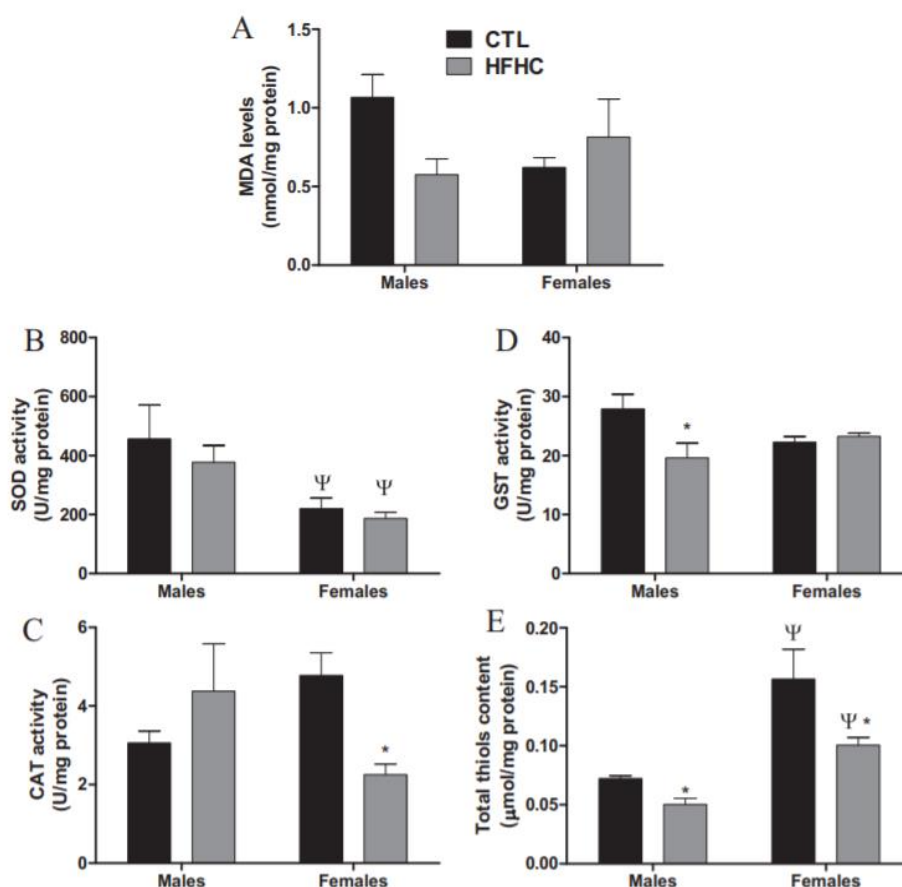


Fig. 2. Effects of high-fat and high-cholesterol diet consumption during pregnancy and lactation on malondialdehyde levels (MDA, A), superoxide dismutase activity (SOD, B), catalase activity (CAT, C), glutathione S-transferase activity (GST, D), and total thiols content (E) in the colon mucosa in male and female rat offspring. Groups: control group (CTL, $n = 6$), high-fat and high-cholesterol group (HFHC, $n = 6$). Data are presented as mean $\pm$ standard error mean and analyzed by ANOVA two-way test with maternal diet (CTL/HFHC) and sex (males and females) as factors and Bonferroni's as post hoc test. * $p < 0.05$ indicates significant difference between HFHC and CTL group; $\Psi p < 0.05$ indicates significant difference between female and male offspring.

when compared to respective CTL female offspring (CTL: 4.77 ± 0.57 vs. HFHC: 2.25 ± 0.27 , $p < 0.05$, Fig. 2C); and male offspring from dams fed with a HFHC diet displayed decreases in GST activity when compared to respective CTL male offspring (CTL: 27.9 ± 2.4 vs. HFHC: 19.6 ± 2.5 , $p < 0.05$, Fig. 2D).

Assessing non-enzymatic antioxidant compounds, we found that the total thiols content were sex- ($F = 32.8$, $p < 0.0001$) and diet-dependent ($F = 11.0$, $p = 0.003$). In addition, total thiols content was reduced in the colon of HFHC male (CTL: 0.07 ± 0.002 vs. HFHC: 0.05 ± 0.005 , $p < 0.05$, Fig. 2E) and female offspring (CTL: 0.16 ± 0.02 vs. HFHC: 0.10 ± 0.006 , $p < 0.05$, Fig. 2E) when compared to their respective CTL groups.

3.3. Oxidative stress biomarkers in renal cortex

The MDA levels were diet-dependent ($F = 9.21$, $p = 0.006$), but not sex-dependent ($F = 2.27$, $p = 0.14$, Fig. 3A). In female offspring, the MDA levels were augmented in the renal cortex of HFHC group when compared to their respective CTL group (CTL: 0.10 ± 0.04 vs. HFHC: 0.40 ± 0.09 , $p < 0.05$, Fig. 3A). Changes in SOD activities were sex- ($F = 22.1$, $p = 0.0002$) and diet-dependent ($F = 11.1$, $p = 0.004$). Although SOD activity had been similar between female offspring ($p > 0.05$), the SOD activity was significantly reduced in the renal

cortex of HFHC male offspring when compared to respective CTL male offspring (CTL: 197.6 ± 38.6 vs. HFHC: 51.9 ± 17.6 , $p < 0.05$, Fig. 3B).

Regarding CAT activities, we found that CAT activities were sex-dependent ($F = 15.9$, $p = 0.001$), but not diet-dependent ($F = 0.88$, $p = 0.35$, Fig. 3C) with CAT activity being similar between CTL and HFHC groups for both sexes ($p > 0.05$, Fig. 3C). The GST activities were not sex- ($F = 0.88$, $p = 0.36$) and diet-dependent ($F = 0.0009$, $p = 0.92$) with GST activity being similar between CTL and HFHC groups for both sexes ($p > 0.05$, Fig. 3D).

Lastly, in Fig. 3E the total thiols content were sex- ($F = 20.1$, $p = 0.0002$), but not diet-dependent ($F = 0.95$, $p = 0.34$) with total thiols being similar between CTL and HFHC groups for both sexes.

3.4. Urinary and biochemical variables

Male and female offsprings had similar urinary volume, urea and total proteins were similar (Table 3). While urinary creatinine excretion was similar between female offspring, male offspring from dams fed with a HFHC diet had reduced urinary creatinine excretion when compared to their respective CTL group (Table 3). Male and female offsprings had similar serum creatinine, triglycerides and glucose (Table 3). Although serum cholesterol had been similar between female

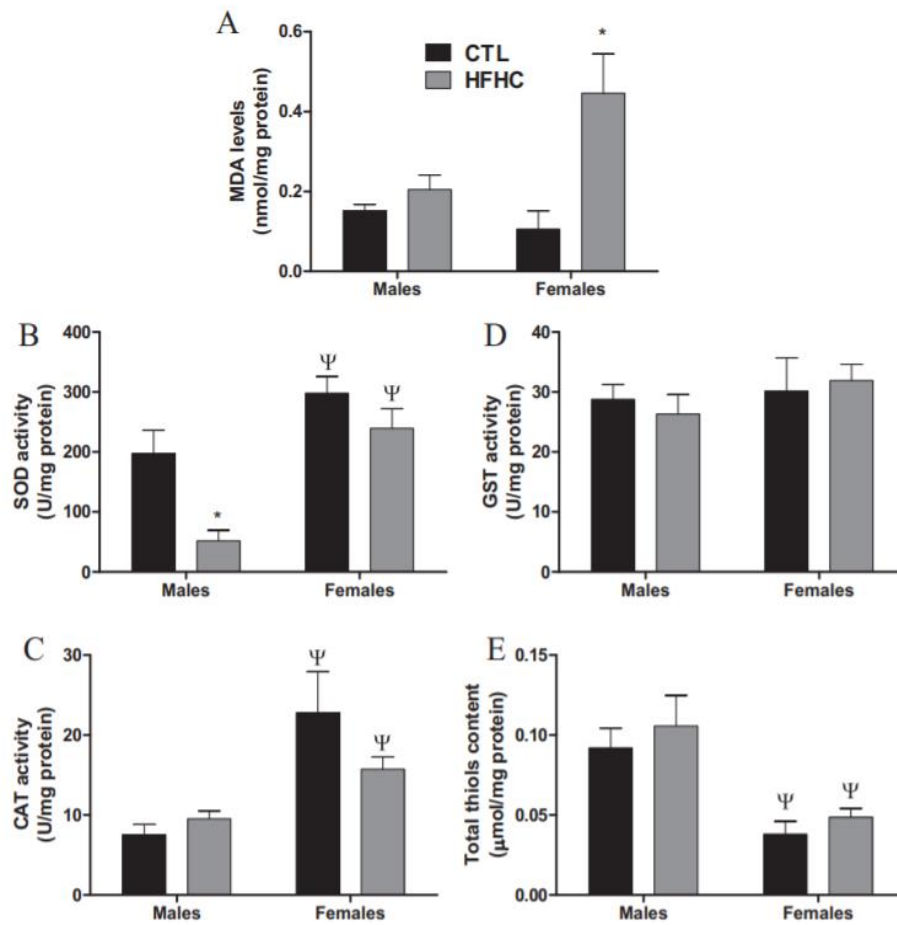


Fig. 3. Effects of high-fat and high-cholesterol diet consumption during pregnancy and lactation on malondialdehyde levels (MDA, A), superoxide dismutase activity (SOD, B), catalase activity (CAT, C), glutathione S-transferase activity (GST, D), and total thiols content (E) in the renal cortex in male and female rat offspring. Groups: control group (CTL, $n = 6$), high-fat and high-cholesterol group (HFHC, $n = 6$). Data are presented as mean $\pm$ standard error mean and analyzed by ANOVA two-way test with maternal diet (CTL/HFHC) and sex (males and females) as factors and Bonferroni's *post hoc* test. * $p < 0.05$ indicate significant difference between HFHC and CTL group; $\Psi p < 0.05$ indicate significant difference between female and male offspring.

Table 3

Urinary and serum variables in male and female offsprings at 90 d of age from dams fed with control (CTL) or high-fat and high-cholesterol diet (HFHC) during pregnancy and lactation.

	Males			Females		
	CTL	HFHC	<i>p</i> -Value	CTL	HFHC	<i>p</i> -Value
<i>Urinary variables</i>						
UV (mL/24 h)	5.5 $\pm$ 0.6	5.5 $\pm$ 0.9	0.960	4.6 $\pm$ 1.0	4.1 $\pm$ 1.0	0.780
Urea (g/24 h)	314.1 $\pm$ 33	297.9 $\pm$ 62	0.815	284 $\pm$ 78	304 $\pm$ 66	0.858
tPTN (mg/24 h)	6.0 $\pm$ 0.6	6.9 $\pm$ 0.8	0.382	2.1 $\pm$ 0.3	3.6 $\pm$ 1.3	0.345
Creatinine (mg/dL)	3.74 $\pm$ 0.60	1.16 $\pm$ 0.15	0.001	0.62 $\pm$ 0.22	0.47 $\pm$ 0.11	0.545
<i>Serum variables</i>						
Creatinine (mg/dL)	0.147 $\pm$ 0.01	0.234 $\pm$ 0.06	0.195	0.228 $\pm$ 0.04	0.281 $\pm$ 0.04	0.392
Cholesterol (mg/dL)	63.9 $\pm$ 3.9	78.1 $\pm$ 4.9	0.04	78.2 $\pm$ 6.8	69.4 $\pm$ 3.3	0.236
Triglycerides (mg/dL)	92.6 $\pm$ 15.6	95.2 $\pm$ 18.0	0.902	115 $\pm$ 14.9	78.9 $\pm$ 6.4	0.06
Glucose (mg/dL)	158.5 $\pm$ 5.7	162.4 $\pm$ 12.1	0.775	151.2 $\pm$ 7.8	159.8 $\pm$ 3.2	0.263

UV (urinary volume); tPTN (total proteins).

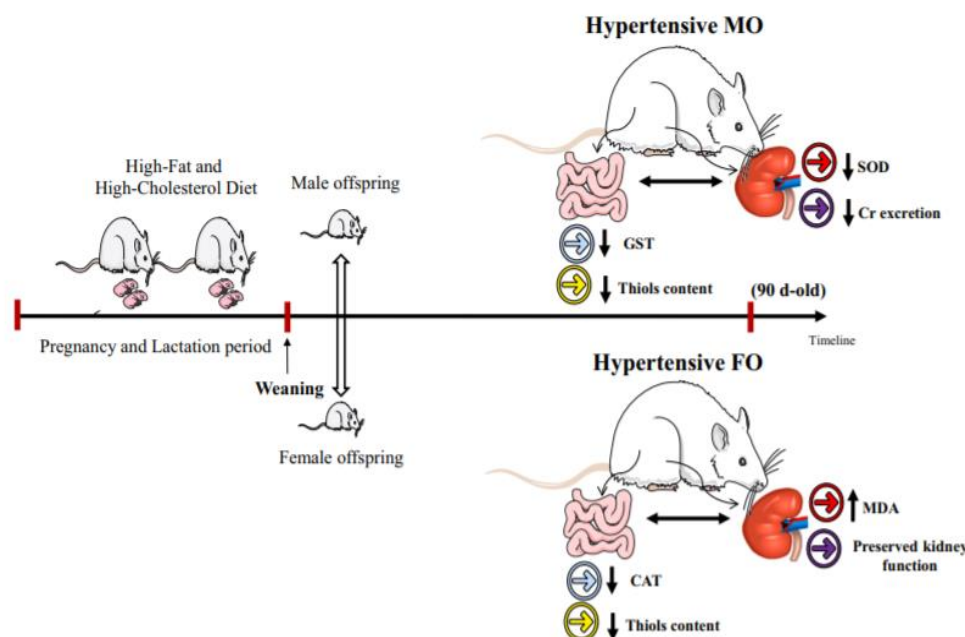


Fig. 4. Schematic drawing showing the impact of the maternal high-fat and high-cholesterol diet on oxidative stress along the gut-kidney axis of male and female offsprings. CAT (catalase); Cr (creatinine); FO (female offspring); GST (glutathione S-transferase); MDA (malondialdehyde); MO (male offspring); SOD (superoxide dismutase).

offspring, male offspring from dams fed with a HFHC diet had increased serum cholesterol when compared to their respective CTL group (Table 3).

4. Discussion

In the present study, we demonstrated for the first time that the offspring from dams fed with a HFHC diet during pregnancy and lactation displayed important alterations on oxidative status along the gut-kidney axis in a sex- and organ-dependent manner. In addition, in agreement with early findings from our research group, male and female offsprings from dams fed with HFHC diet during pregnancy and lactation had increased blood pressure at 90 days of age [2,11,23], confirming a reliable model of the development of arterial hypertension programmed by maternal HFHC diet consumption.

A recent study demonstrated that high-fat diet consumption induces oxidative stress in the colonic mucosa contributing to augmented colonic epithelial barrier permeability and colonic mucosal inflammation [10]. In addition, has been demonstrated that increased oxidative stress, low levels of protein thiols and antioxidant disbalance in colon mucosa provoke disruption of intestinal epithelial tight junction and barrier dysfunction [24,25], as well as exert a key role in the development of colon cancer and colorectal carcinoma [26,27]. A reduction in systemic free thiols, a crucial biological target for other reactive species and a transducing element that link the sensing and adaptation machinery has also been described in inflammatory bowel disease [28].

Our findings show that CAT and GST antioxidant enzymes activities were reduced in the colon mucosa of female and male offsprings from dams fed with HFHC diet during pregnancy and lactation, respectively. In addition, total thiols content were reduced in the colon mucosa of HFHC male and female offsprings when compared with their respective CTL groups. Taken together, our findings suggest that the colon mucosa of offsprings from dams fed with HFHC diet during pregnancy and lactation display an impaired antioxidant system that, in part, could be associated with relevant barrier dysfunction [29].

In present study, we demonstrated for first time that female offspring displayed decreased SOD activity and increased thiols content in colon mucosa when compared to male offspring. Although oxidative stress in colon seem be sex-dependent, a early study using a histological analysis found epithelial villus atrophy for both male and female offspring from dams fed with a HFHC diet [29].

It is widely recognized that antioxidant enzymes, such as SOD, CAT and GST, exert a natural and key defense against oxidative impairment. In renal tissue, oxidative stress has been associated with renal injury, renal function decline and arterial hypertension in a sex-dependent manner [30–33]. In the present study, we found that, although the thiols content had been reduced in renal tissue of female when compared with male offspring, the antioxidant enzymes SOD and CAT were significantly increased in renal tissue of female when compared with male offspring. Similarly, early studies have demonstrated that female offspring showed elevated renal CAT activity than male offspring [32,34]. In contrast, there is study demonstrating that the impact of high cholesterol diet consumption on renal injury is higher in female than in male rats [35].

Male offspring from dams fed with HFHC diet during pregnancy and lactation displayed a reduction in SOD activity in renal cortex in comparison to respective CTL male offspring, indicating a greater susceptibility to oxidative impairment and renal dysfunction. Although biochemical markers to assess renal function have been similar between CTL and HFHC male offspring, the urinary creatinine was significantly reduced in HFCH male offspring when compared with their respective CTL group. Interestingly, it has been reported that a decrease in 24-hour urinary creatinine excretion occurs in the early stages of chronic kidney disease and has been associated with increased risk for mortality [36,37]. Considering that HFHC male offspring displayed increased blood pressure and serum cholesterol in comparison to CTL group, it is reasonable to infer that male offspring from dams fed with a HFHC diet during pregnancy and lactation exhibit an increased cardio-renal risk at 90 days of age. However, an early study demonstrated that the renal dysfunction in male offspring from dams fed with a high-fat and high-

fructose diet is more pronounced at the sixteenth week of life [38].

Regarding female offspring, it is possible to perceive that although MDA levels have been augmented in renal cortex, the renal antioxidant system and renal function were not impaired by the maternal HFHC diet consumption, suggesting that lipid peroxidation is not the underlying cause of renal dysfunction and arterial hypertension in HFHC female offspring. Previously, we demonstrated that altered breathing linked to enhanced central and peripheral chemosensitivity, impaired baroreflex, and augmented sympathetic tone may be predisposing factors for increased blood pressure in female offspring from dams fed with a HFHC diet during pregnancy and lactation [11].

In fact, females from dams fed with high-fat diet during pregnancy and/or lactation may be less susceptible to the renal oxidative stress and renal dysfunction [5,39]. The mechanism involving in this phenomenon is still under extensive investigation, but it has been proposed that the presence of estrogen hormone could exert a key role for the protective effect on female renal tissue against the deleterious effects of the high fat diet [40].

Taking into account the major findings presented here, it is reasonable to infer that altering the oxidative stress in the colon and kidney could prevent the development of arterial hypertension and metabolic disorders in offspring from dams fed with a HFHC diet during pregnancy and lactation. It has been demonstrated that *Lactobacilli* with superoxide dismutase-like or catalase activity might alleviate colon oxidative stress and inflammation [41] and prevent colon cancer in mice [42]. In addition, has been demonstrated that SOD mimetic therapy plays an important role in reducing the oxidative stress in renal injury [43]. An early study carried out by our research group demonstrated that the post-weaning intervention with probiotics containing *Lactobacillus fermentum* for 8 weeks improved lipid profile, blood pressure and serum MDA levels in male and female rats offspring from dams fed with HFHC diet during pregnancy and lactation [7]. Considering these important findings, our next step will be to investigate if the therapy with probiotic *Lactobacillus fermentum* could improve antioxidant system in colon mucosa and renal cortex in offspring from dams fed with a HFHC diet.

In conclusion, our study suggests that exposure to HFHC diet during pregnancy and lactation induces organ- and sex-specific oxidative stress on gut-kidney axis in offspring, which might cause renal dysfunction and arterial hypertension later in life (Fig. 4).

Declaration of competing interest

The authors declare no conflict of interest.

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APÊNDICE B – ANTIGO 2

***Limosilactobacillus fermentum*, current evidence on the antioxidant properties and opportunities to be exploited as a probiotic microorganism**

Artigo publicado no periódico *Probiotics and Antimicrobial Proteins*, fator de impacto: 4.609



Limosilactobacillus fermentum, Current Evidence on the Antioxidant Properties and Opportunities to be Exploited as a Probiotic Microorganism

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Abstract

The unbalance in the production and removal of oxygen-reactive species in the human organism leads to oxidative stress, a physiological condition commonly linked to the occurrence of cancer, neurodegenerative, inflammatory, and metabolic disorders. The implications of oxidative stress in the gut have been associated with gut microbiota impairments and gut dysbiosis. Some lactobacilli strains have shown an efficient antioxidant system capable of protecting against oxidative stress and related-chronic diseases. Recently, in vitro and experimental studies and some clinical trials have demonstrated the efficacy of the administration of various *Limosilactobacillus fermentum* strains to modulate beneficially the host antioxidant system resulting in the amelioration of a variety of systemic diseases phenotypes. This review presents and discusses the currently available studies on identifying *L. fermentum* strains with anti-oxidant properties, their sources, range of the administered doses, and duration of the intervention in experiments with animals and clinical trials. This review strives to serve as a relevant and well-cataloged reference of *L. fermentum* strains with capabilities of inducing anti-oxidant effects and health-promoting benefits to the host, envisaging their broad applicability to disease control.

Keywords Probiotic · *Limosilactobacillus fermentum* · Oxidative stress · Antioxidant capacity

Abbreviations

ARE	Antioxidant response element
CAT	Catalase
Cys	Cysteine
FFAR	Free fatty acid receptor
GSH	Glutathione reduced
GPR	G protein-coupled receptor
GST	Glutathione-S-transferases
Glu	Glutamate
HO-1	Heme oxygenase-1
H ₂ O ₂	Hydrogen peroxide
Keap-1	Kelch-like ECH-associated protein 1
MAPK	Mitogen-activated protein kinase

NF-κB	Nuclear factor kappa beta
Nrf2	Nuclear factor-erythroid 2 related factor 2
ROS	Reactive oxygen species
SOD	Superoxide dismutase
STAT3	Signal transducer and activator of transcription 3

Introduction

Lactobacilli are a bacterial genus of the normal gastrointestinal microbiota of humans and other animals. Many lactic acid bacteria, such as lactobacilli, have received the status of generally recognized as safe (GRAS) or presumably qualified as safe (QPS) and recognized as candidates for use as probiotics. According to the International Scientific Association for Probiotics and Prebiotics (ISAPP), probiotics are described as live nonpathogenic microorganisms that confer a health benefit to the host when administered in adequate amounts [1].

Recently, lactobacilli were reclassified into 25 distinct genera [2]. In this taxonomic reclassification, *Lactobacillus fermentum* was renamed as *Limosilactobacillus fermentum*

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and described as Gram-positive, rod- or coccoid-shaped, heterofermentative, and anaerobic or aerotolerant. *L. fermentum* has been commonly recovered from fermented cereals and other fermenting plant materials, dairy products, manure, sewage, feces, and human vagina [2].

The reported biological properties of probiotics have been strain-specific, and the success or failure of one probiotic strain in modulating the gut microbiota or improving specific physio-pathological conditions cannot be extrapolated to another strain. Some effects such as colonization resistance, short-chain fatty acid production, regulation of intestinal transit, normalization of perturbed microbiota, increased turnover of enterocytes, and competitive exclusion of pathogens are widely reported among distinct probiotic strains. Characteristics such as vitamin synthesis, direct pathogen antagonism, gut barrier reinforcement, bile salt metabolism, enzymatic activity, and neutralization of carcinogens are frequent and species-specific among studied probiotics, while neuro-immune-endocrinological effects are rare and strain-specific [1].

Oxidative stress is characterized by increased production of reactive oxygen species (ROS) and reactive nitrogen species (RNS), and reduced tissue antioxidant capacity leading to oxidative damage in DNA, RNA, carbohydrates, proteins, and lipids, with a key role in the development of various diseases [3]. Otherwise, the identification of antioxidant products should be an important strategy to combat oxidative stress and support the treatment of various chronic diseases. Early studies have demonstrated that some lactic acid bacteria display effective antioxidant properties and oxidative stress tolerance [4, 5]. However, an in-depth approach focusing on *L. fermentum* strains as potential anti-oxidant has not been found in the available literature.

This review aimed to identify all published studies on *L. fermentum* strains with anti-oxidant properties, their source, and the variety of dose and time intervention, carried out in vitro, with animal models, and clinical trials. Potential underlying mechanisms and gaps that need to be addressed in future studies approaching the anti-oxidant capacity and oxidative stress tolerance of *L. fermentum* strains are also discussed. This review strives to serve as a relevant, up-to-date, and well-cataloged reference of *L. fermentum* strains related to their anti-oxidant capacities and health-promoting benefits to a wide range of diseases.

Oxidative Stress, Gut Microbiota, and Metabolic Diseases

The evolution of living organisms has accompanied the changes in the atmospheric environment, where the increase of O₂ levels in the air due to photosynthetic reactions has required the ability of organisms to deal with oxygen-related

toxicity. What initially was only protection has evolved to the capacity to use O₂ as a final acceptor to produce energy through the mitochondrial electron transport chain (ETC) [6]. Part of the O₂ used in ETC during cellular respiration via monovalent reduction (superoxide anion – O₂^{•−}) produces oxidants related to oxygen called “reactive oxygen species” (ROS) [7].

Despite several other sources of ROS, the mitochondria represent the major producer of ROS in the human organism, whose levels must be controlled/regulated to avoid toxic effects. Humans have developed a variety of antioxidant defenses to deal with a variety of oxidant elements, where enzymatic and non-enzymatic systems “detoxify” the oxidant agent by removing or transforming it into a less reactive substance [8].

The fight against ROS begins with the superoxide dismutase (SOD) family comprising three specific enzyme isoforms: (i) the cytoplasmatic (Cu/ZnSOD – SOD1); (ii) the mitochondrial (MnSOD – SOD2); and (iii) the extracellular (ECSOD – SOD3) [9]. Despite having distinct characteristics, the isoforms dismutate the O₂^{•−} to hydrogen peroxide (H₂O₂), which can also be formed by specific oxidases. Under physiological conditions, most of H₂O₂ is enzymatically converted to H₂O and O₂ by catalase (CAT) via a double reaction between the hydrogen peroxide and enzyme catalytic center, as well as by peroxidases, which require a variety of reduced agents (e.g., ascorbate, thioredoxin, NAD(P)H, and glutathione), resulting in H₂O and an oxidized agent [10, 11].

Among the peroxidases, the glutathione-related peroxidase (GPx) is conspicuous due to its ability to deal with alkyl-hydroperoxides, especially those derived from the oxidation of polyunsaturated lipids, with an important role in higher animals [12]. The glutathione-related metabolism relies on selenocysteine residue present in the glutathione/reduced glutathione (GSH), a tripeptide produced into the cell cytoplasm. Since GSH has an active redox compound, it can act forming antioxidant defense directly by reducing oxidant agents (e.g., hydroxyl radical, peroxynitrite, and carbon radical) or indirectly as an enzymatic cofactor to GPx and glutathione-S-transferases (GST), which comprise a family of enzymes located in the cytosol and membranes involved in the removal of xenobiotic and electrophilic compounds [13–15].

Human beings possess a non-enzymatic antioxidant “system” combining endogenous (e.g., metal-binding proteins, coenzyme Q10, GSH, uric acid, melatonin, bilirubin, and polyamines) and exogenous/dietary elements (e.g., vitamins, carotenoids, phenolic compounds, and flavonoids) to prevent, repair, and inactivate the harmful effects of the reactive species and their derivatives [16–19]. In 1985, oxidative stress was conceptualized as “an imbalance between oxidants and antioxidants in favor of the oxidants, leading to

a redox signaling and molecular damage” [3, 8]. The disturbance in the production and removal of the reactive species has correlated to the occurrence of cancer, neurodegenerative, inflammatory, and metabolic diseases [20, 21].

In recent decades, studies have highlighted the role of the gut microbiota in inflammatory signaling where gut metabolites (mainly acetate, propionate, and butyrate) interact directly or indirectly with the cellular immune system of the host [22]. Gut dysbiosis, a phenomenon characterized by the decrease in the diversity and stability of the microbial population, reduction of beneficial microorganisms, and expansion of pathobionts, increases the risk of cardiometabolic disturbances such as obesity, diabetes, and cardiovascular diseases [23]. Furthermore, it has been reported that intestinal dysbiosis could favor the colonocytes for the Warburg effect [24]. This is a term described in cancer studies over the past 90 years and represents the metabolic tissue switch to anaerobic glycolysis even in the presence of O_2 , providing high production and large rate-induced ATP amounts to favor the required environment for uncontrolled cell proliferation [25].

In this context, gut oxidative stress should amplify microbiome changes by increasing the abundance of specific bacterial groups (e.g., Enterobacteriaceae), reinforcing inflammatory signals via its pyrogenic characteristics, and promoting the occurrence of chronic diseases [23]. Therapeutic strategies that protect against oxidative stress should represent a window of opportunity to prevent and treat non-communicable diseases [26].

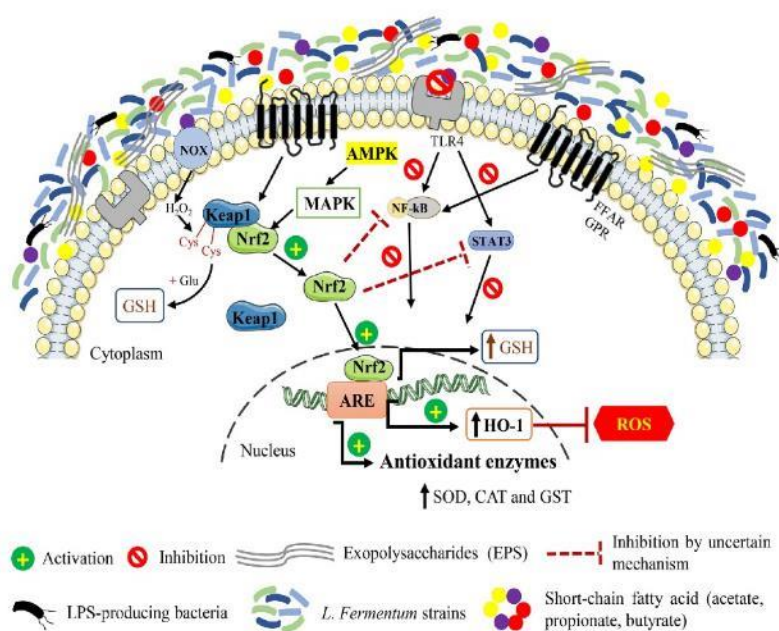
Oxidative Stress Tolerance and Anti-Oxidant Capacity in Lactobacilli Strain

The significant antioxidant activities of lactic acid bacterial strains, especially belonging to the lactobacilli, have been studied since they act by regulating signaling pathways involved in the production of enzymes and producing molecules with antioxidant capacity [4]. These antioxidant strains have been considered a potential bioresource to enhance antioxidant protection and oxidative stress tolerance [27, 28].

Available evidence suggests that some lactobacilli strains can increase the activity of antioxidant enzymes or reduce the circulatory levels of oxidative stress [29]. Among the proposed mechanisms, *Lactobacillus* strains could increase antioxidant capacity by upregulation in Keap1/Nrf2/ARE pathways and downregulation on NF- κ B/STAT3 signaling pathways, as shown in Fig. 1 [30–33].

These microorganisms should have O_2 consuming enzymes such as NADPH oxidase (NOX) to produce H_2O_2 , which despite being a reactive species, it does not promote a significant oxidative imbalance compared to other molecules. Still, this hydrogen peroxide activates the transcription factor Nrf2 (nuclear erythroid factor 2-related factor 2) that regulates the cellular antioxidant response [34]. Corroborating this mechanistic hypothesis, GSH is a compound acting to adjust the cellular redox state and is positively regulated via Nrf2 [35]. The activation of Nrf2

Fig. 1 Schematic illustrating the potential intracellular signaling pathways involved in *Limosilactobacillus fermentum* antioxidant properties



should be one of the pathways to reach the antioxidant effects induced by *L. fermentum* to provide cytoprotection against oxidative damage [32].

The antioxidant action of lactobacilli strains could occur by producing various molecules such as exopolysaccharides (EPS), carotenoids, ferulic acid, and histamine [4]. Similar to the effects reported to EPS, lactobacilli strains could reduce oxidative stress biomarkers (e.g., malondialdehyde) and increase enzyme activity (e.g., catalase and superoxide dismutase), conferring antioxidant effects in different cell lines [36, 37].

The oxidative stress tolerance capacity of some lactobacilli strains has been partially related to their capability of producing antioxidant enzymes. Superoxide dismutase has several metallic ions (e.g., Zn, Cu, and Mn) acting as cofactors in O₂-detoxification reactions. It is important to highlight that even in SOD-deficient bacteria (e.g., *Lactiplantibacillus plantarum*), Mn can eliminate cellular superoxide anions [38]. In addition, Mn-containing pseudo-catalases and heme-dependent catalases [39, 40], as well as high tolerance to oxidative stress [41, 42] have been found in lactobacilli strains.

***L. fermentum*: an Effective Bioresource Against Oxidative Stress**

L. fermentum strains have been isolated from several sources such as human milk, human feces, milk products, vegetables, and fruits in various parts of the world. Approximately 39 strains have been isolated and characterized as having antioxidant or oxidative stress tolerance in vitro and in vivo (Table 1). The following paragraphs will present and discuss *L. fermentum* strains isolated and characterized as potential antioxidants.

***L. fermentum* 139, *L. fermentum* 263, and *L. fermentum* 296**

The strains *L. fermentum* 139, *L. fermentum* 263, and *L. fermentum* 296 were isolated from Brazilian fruit pulp processing by-products [43, 44]. Specifically, the strain *L. fermentum* 139 was isolated from *Mangifera indica* L. (mango) by-product, *L. fermentum* 263 was isolated from *Ananas comosus* (pineapple) by-product, and *L. fermentum* 296 was isolated from *Fragaria vesca* L. (strawberry) by-product [43, 44]. These three strains showed aptitudes compatible with the use as potential probiotics in a set of functionality-related in vitro experiments [43]. Such results indicated that *L. fermentum* fruit-derived strains could be candidates for inclusion in studies in vivo to evaluate their health-promoting effects.

Additional investigations showed that *L. fermentum* 296 improved, altered biochemical and cardiovascular parameters in rats fed a high-fat diet, but did not improve the concentration of malondialdehyde (MDA) and end products of lipid peroxidation [45]. Considering that multi-strains probiotic formulations can exert a more significant host health benefit when compared to a single probiotic strain [1], the effects of a mixed formulation containing the strains *L. fermentum* 139, *L. fermentum* 263, and *L. fermentum* 296 on the oxidative stress, gut microbiota, and cardiometabolic disorders in rats fed a high-fat diet were evaluated. The results demonstrated that administration of the mixed probiotic formulations (1×10^9 CFU of each strain) twice a day for 4 weeks improved gut-microbiota composition, increased content of short-chain fatty acid in the colon, reduced blood pressure, dyslipidemia, and increased antioxidant defense in the colon, liver, heart, and kidney in male and female rats fed a high-fat diet [46–48]. These results indicate that this mixed formulation containing distinct *L. fermentum* strains has a high potential to act as a novel anti-dyslipidemia and anti-oxidant product. Future studies will be needed to analyze *L. fermentum* species with a comparative reference. Comparing the effectiveness of different probiotic strains will allow identifying which strain may induce more antioxidant responses and which *Lactobacillus* species should be further explored, especially in clinical studies.

***L. fermentum* ATCC 11976**

The strain *L. fermentum* ATCC 11976 (or *L. fermentum* 11976) was obtained from American Type Culture Collection (VA, USA) [49]. Administration of *L. fermentum* ATCC 11976 to hamsters displaying non-alcoholic fatty liver disease reduced the concentrations of hepatic free cholesterol, esterified cholesterol, triglycerides, and phospholipids [50].

***L. fermentum* BR11**

The strain *L. fermentum* BR11 was isolated from the vaginal tract of sexually mature guinea pigs [51]. The administration of *L. fermentum* BR11 (1×10^{10} CFU) for 2 weeks did not reduce the myeloperoxidase (MPO) activity in colon tissues and prevented the dextran sulfate sodium (DSS)-induced colitis in rats [52]. However, a subsequent study showed the efficacy of the treatment with *L. fermentum* BR11 at a low (1×10^6 CFU) or high dose (1×10^9 CFU) twice a day for 1 week reduced the MPO activity and inflammation in the jejunum of rats with intestinal mucositis [53].

Table 1 Summary of in vitro, experimental and clinical studies investigating the antioxidant properties of *Limosilactobacillus fermentum* strains worldwide

Strain	Type of study	Dosage	TI	Disorder's condition or model	Primary endpoint	References
<i>L. fermentum</i> 139, <i>L. fermentum</i> 263, <i>L. fermentum</i> 296; strains isolated of Brazilian fruit pulp processing by-products	Experimental (rat)	1 × 10 ⁹ CFU, twice a day	4 wk	High-fat diet	Improved gut-microbiota composition, increased SCFA, and antioxidant defense in colon, liver, heart, and kidney	PMID: 34574313 PMID: 35066820 PMID: 34817804
	Experimental (rat)	1 × 10 ⁹ CFU, twice a day	4 wk	High-fat diet	Improved gut-microbiota composition, increased SCFA, and antioxidant defense in colon, liver, heart, and kidney	PMID: 34574313 PMID: 35066820 PMID: 34817804
<i>L. fermentum</i> ATCC 11976; strain obtained from American Type Culture Collection from Manassas (Virginia, USA)	Experimental (Syrian hamster)	11.51 log CFU/mL, twice daily	12 wk	Non-alcoholic fatty liver disease	Reduced hepatic oxidative stress	PMID: 23554890
	Experimental (rat)	1 × 10 ¹⁰ CFU, twice daily	2 wk	Colitis model	No effect on MPO activity	PMID: 17150273
<i>L. fermentum</i> BR11; supplied by the Faculty of Science, Queensland University, Australia	Experimental (rat)	Low dose: 1 × 10 ⁶ CFU twice daily, and high dose: 1 × 10 ⁹ CFU, twice daily	1 wk	Model of intestinal mucositis	Reduced MPO activity	PMID: 19005975
	Experimental (rat)	5 × 10 ⁸ CFU, once a day	3 wk	Colitis model	Increased GSH contents, reduced MPO activity and proinflammatory cytokines in colon	PMID: 16052308
<i>L. fermentum</i> CECT 5716; Isolated of human milk (Spain)	Experimental (rat)	5 × 10 ⁸ CFU, once a day	3 wk	Colitis model	Reduced MPO activity and TNFa levels in colonic tissues	PMID: 17217564
	Experimental (mice)	1 × 10 ⁸ CFU, once a day	2 wk	Experimental model of septic shock	Increased hepatic GSH contents, reduced MPO activity, and inflammation in the lung	PMID: 18445307
Experimental (mice)	Experimental (mice)	1 × 10 ⁹ CFU, once a day	2 wk	Colitis model	Reduced MPO activity and IL-6 levels	PMID: 19266568
	Experimental (mice)	1 × 10 ¹⁰ CFU, once a day	4 wk	Healthy animals	Stress oxidative markers were not different between control and probiotic-treated mice	PMID: 19281631
Experimental (rat)	Experimental (rat)	5 × 10 ⁸ CFU, once a day	2 wk	Colitis model	Reduced MPO activity, IL-8, and TNFa levels in colon	PMID: 25700085
	Experimental (rat)	3.3 × 10 ¹⁰ CFU, once a day	5 wk	SHR	Increased superoxide levels and reduced NADPH oxidase in aorta segments	PMID: 26255877
Experimental (rat)	Experimental (rat)	1 × 10 ⁹ CFU, once a day	2 wk	Newborn rats	Enhanced IFN γ secretion, and prevented IL-4 secretion in activated splenocytes	PMID: 28370715
	Experimental (mice)	5 × 10 ⁸ CFU, once a day	2 wk	Tacrolimus-induced hypertension model	Decreased NADPH activity and ROS production in aortic segments	PMID: 29851248
Experimental (rat)	Experimental (rat)	1 × 10 ⁹ CFU, once a day	4 wk	L-NAME induced hypertension	Decreased NADPH activity, ROS production, and pro-inflammatory cytokines in aortic segments	PMID: 30028078

Table 1 (continued)

Strain	Type of study	Dosage	TI	Disorder's condition or model	Primary endpoint	References
	Experimental (mice)	5×10^8 CFU, once a day	15 wk	Systemic lupus erythematosus model	Decreased NADPH activity, ROS production, and proinflammatory cytokines in aortic segments	PMID: 31173526
	Experimental (rat)	1×10^9 CFU, once a day	13 wk	SHR	Reduced NADPH oxidase activity, endotoxemia, and endothelial dysfunction	PMID: 31953983
	Experimental (mice)	5×10^8 CFU, once a day	13 wk	Systemic lupus erythematosus model	Decreased NADPH activity, ROS production, and pro-inflammatory cytokines in the renal cortex	PMID: 32458936
	Experimental (mice)	5×10^9 CFU, once a day	11 wk	High-fat diet-induced obesity	Decreased NADPH activity, ROS production, and proinflammatory cytokines in aortic segments	PMID: 33529749
<i>L. fermentum</i> CQPC04: Isolated from traditional fermented pickled in Chongqing (China)						
	Experimental (mice)	Low dose: 1.5×10^9 CFU, once a day, and high dose: 5×10^{10} CFU, once a day	5 wk	Dextran sulfate sodium-induced colitis	Reduced MPO activity and increased SOD2 expression in the colon. Increased SOD and CAT activities in serum, and reduced NF- κ B signaling pathway	PMID: 31477303
	Experimental (mice)	Low dose: 1×10^9 CFU, once a day, and high dose: 1×10^{10} CFU, once a day	8 wk	Non-alcoholic fatty liver disease	Reduced hepatic ROS-production, systemic inflammation, and liver damage	PMID: 32945305
<i>L. fermentum</i> CQPC06: Isolated from traditional fermented pickled in Chongqing (China)						
	Experimental (mice)	1×10^9 CFU, once a day	16 wk	4-Nitroquinoline-1-Oxide Induced Tongue Cancer	Reduced MDA contents and increased SOD and GSH activities in Tongue tissue levels. Upregulated the mRNA expression of Nrf2, HO-1, and GST- π	PMID: 30861992
	Experimental (rat)	1×10^9 CFU, once a day	12 wk	Experimental model of lead-induced oxidative damage	Reduced systemic low-grade inflammation and decreased oxidative damage in the liver, kidney, and brain tissues by up-regulation of antioxidant enzymes and Keap1/Nrf2/ARE signaling pathway	PMID: 34037025
<i>L. fermentum</i> CRL1446: Isolated from an Argentinean goat milk cheese						
	Experimental (mice)	1×10^7 CFU, once a day, and 1×10^9 CFU, once a day	2, 5, 7, and 10 days	Healthy animals	Increased intestinal feruloyl esterase activity, decreased TBARS serum levels, and increased in glutathione reductase activity	PMID: 22017704

Table 1 (continued)

Strain	Type of study	Dosage	TI	Disorder's condition or model	Primary endpoint	References
	Experimental (mice)	1×10^8 CFU, once a day	7 days	Healthy animals	Increased intestinal feruloyl esterase activity	PMID: 27090053
	Experimental (mice)	1×10^8 CFU, once a day	45 days	Caloric-restricted model	Increased intestinal feruloyl esterase activity, decreased lipid peroxidation, and increased glutathione reductase activity in plasma	PMID: 27399766
	Experimental (mice)	1×10^7 CFU, once a day	7 wk	High fat diet-induced obesity	Increased intestinal feruloyl esterase activity and improved hepatic glutathione reductase activity	PMID: 30525955
	Experimental (mice)	1×10^8 CFU, once a day	14 wk	High fat diet-induced metabolic syndrome	Increased intestinal feruloyl esterase activity	PMID: 32421119
<i>L. fermentum</i> DR9: isolated from fresh cow's milk in Penang (Malaysia)	Experimental (rat)	1×10^{10} CFU, once a day	12 wk	d-galactose-induced aging rats	Promoted antioxidative effect, and reduced proinflammatory cytokines	PMID: 30592688
<i>L. fermentum</i> L5007: isolated from the gastrointestinal mucosa of piglets (China)	Experimental (piglet)	1×10^8 CFU, once a day	2 wk	Weanling piglets	Decreased the expression of proteins related to apoptosis and stress response in the gastrointestinal tract	PMID: 22113866
	Experimental (piglet)	1×10^8 CFU, once a day	3 wk	Diquat-induced oxidative stress	Reduced MDA contents and increased SOD, GPx activities in liver, skeletal muscles, and plasma	PMID: 23480628
	Experimental (piglet)	6×10^9 CFU, once a day	2 wk	Neonates piglets	Decreased mRNA expression of IL-1p in the ileum	PMID: 24404892
<i>L. fermentum</i> IM12: Isolated from human fecal microbiota (South Korea)	Experimental (mice)	0.2×10^9 , 1×10^9 , or 5×10^9 CFU, once a day	3 days	Colitis model	Reduced MPO activity, expression of iNOS and COX-2, and inhibited NF- κ B-STAT3 signaling pathway	PMID: 28504577
<i>L. fermentum</i> J20 and <i>L. fermentum</i> J28: Isolated from Mexican artisanal cheese	Experimental (mice)	J20: 9×10^8 , J28: 1×10^9 CFU, once a day	2 wk	Indomethacin-induced inflammation	Decreased serum and intestinal levels of IL-17	PMID: 31315186
<i>L. fermentum</i> JDFM216: Isolated from Korean infant feces	Experimental (mice)	6×10^8 CFU, once a day	48 wk	Aged mice	Increased leukocyte respiratory burst activity	PMID: 33303803

Table 1 (continued)

Strain	Type of study	Dosage	TI	Disorder's condition or model	Primary endpoint	References
<i>L. fermentum</i> JX306	Experimental (mice)	Isolated from Chinese sauerkraut in Jiangxi Province 4 × 10 ⁸ CFU, once a day	8 wk	D-galactose-induced oxidative stress	Decreased MDA in serum, kidney, and liver; Increased GSH-px and T-AOC in serum, kidney, and liver	PMID: 32548989
<i>L. fermentum</i> KBL374 and <i>L. fermentum</i> KBL375	Experimental (mice)	Isolated from feces of healthy Koreans 1 × 10 ⁹ CFU, once a day	8 days	Dextran sulfate sodium-induced colitis model	Reduced MPO activity, decreased Th1-, Th2-, and Th17-related cytokine levels, and increased IL-10	PMID: 30939976
<i>L. fermentum</i> KFRI 164	Experimental (rat)	Isolated from traditional Korean herbal medicine formula Did not inform	-	Carbon tetrachloride (CCl ₄)-induced liver injury	Decreased release of serum transaminases (ALT, AST, and LDH)	PMID: 22468011
<i>L. fermentum</i> KU2000	In vitro study	Isolated from Korean Fermented Foods 1 × 10 ⁹ CFU	-	MPP + -induced cytotoxicity in SH-SY5Y cells	Increased antioxidant activity	PMID: 32059401
<i>L. fermentum</i> LA12	Experimental (rat)	Isolated from Korean infant feces 1 × 10 ⁹ CFU, once a day	4 wk	Alcohol-induced alcoholic steatohepatitis model	Decreased MPO and TNF-α in liver	PMID: 29725216
<i>L. fermentum</i> LC5	Experimental (mice)	Isolated from Curd Samples of Indigenous Cows (India) 1 × 10 ⁸ CFU, once a day	3 wk	Hepatotoxicity induced by Aflatoxin B1	No change in the antioxidant markers (AST, ALT, GST, and SOD). Decreased levels of pro-inflammatory cytokines (TNF-α, IL-12, IL-6)	PMID: 32941932
<i>L. fermentum</i> Lf1	Experimental (mice)	Isolated from Indian gut 1 × 10 ⁹ CFU, once a day	12 days	Colitis model	Increased expression of antioxidant enzymes (SOD2, TxR-1), and decreased MDA level	PMID: 25061603
<i>L. fermentum</i> LF31	In vitro study	Probiotic supplement available in Italian pharmacies	-	Effects on human colon adenocarcinoma HT-29 cell line	Increase of the antioxidant activity, as evaluated by ORAC values	PMID: 24985014
<i>L. fermentum</i> ME-3	Clinical	Isolated of healthy children (Estonia) 3 × 10 ¹¹ CFU, once a day	3 wk	Healthy volunteers aged 35–65 years	Lowered levels of peroxidized lipoproteins, oxidized LDL, 8-isoprostanes, and glutathione redox ratio, and enhanced total antioxidative activity	PMID: 12908907

Table 1 (continued)

Strain	Type of study	Dosage	TI	Disorder's condition or model	Primary endpoint	References
	Clinical	11.8 log CFU (fermented goat milk) once a day, or capsules with 9.2 CFU, twice daily	3 wk	Healthy volunteers aged 35–60 years	Improvement of blood TAA (total antioxidant activity) and TAS (total antioxidative status) indices	PMID: 16080791
	Experimental (mice)	1×10^8 CFU, once a day	5 or 10 days	Salmonella Typhimurium murine model	Reduced TNF- α levels and increased IL-10 levels in the small intestine and liver. Reduced ratio of glutathione GSSG/GSH in the small intestine	PMID: 20955459
<i>L. fermentum</i> MTCC 5898: Isolated from the feces of a 10-month-old infant (India)						
	Experimental (mice)	2×10^9 CFU, once a day	8 wk	Effects of aging	Increased levels of antioxidant enzymes (SOD, CAT, and GPx)	PMID: 25311611
	Experimental (rat)	2×10^9 CFU, once a day	12 wk	High-fat diet	Reduced MDA contents and increased SOD, CAT, and GPx activities in liver and kidney tissues	PMID: 29754388
	Experimental (rat)	1×10^9 CFU/mL, once a day	6 wk	Streptozotocin-induced diabetic rats	Reduced MDA contents and increases SOD, CAT, and GPx in liver and kidney tissues	PMID: 30316007
	Experimental (mice)	1×10^9 CFU/mL, once a day	2 wk	Colitis model	Decreased MPO activity	PMID: 30484143
	Experimental (mice)	1×10^7 , 1×10^9 , and 1×10^{11} CFU, once a day	4 wk	Weanling mice	Increased TGF- β , and decreased IFN- γ	PMID: 30847835
<i>L. fermentum</i> NCIMB 5221: Purchased from NCIMB (Aberdeen, Scotland, UK)						
	Experimental (rat)	1×10^{10} CFU, once a day	8 wk	Model of hyperlipidemia and hyperglycemia	Increased production of fecal acid reduced atherogenic index	PMID: 24121931
<i>L. fermentum</i> NS9: Isolated from fermented dairy product collected from grasslands in Inner Mongolia, China						
	Experimental (rat)	1×10^9 CFU/mL, once a day	6 wk	Microbiota disturbance using ampicillin	Decreased MPO activity	PMID: 25869281
<i>L. fermentum</i> PTCC 1638: Obtained from the culture collection at Iran Institute of Industrial and Scientific Research						
	Experimental (Cyprinus carpio)	1×10^8 to 1×10^{10} CFU, three times daily	8 wk	Immunological parameters and resistance against <i>Aeromonas hydrophila</i>	Increased leukocyte respiratory burst and lysozyme activity. Increased serum antioxidant enzymes (CAT, GPx, and SOD)	PMID: 31604151
<i>L. fermentum</i> PUM: Obtained by an Indian laboratory (MRS, Hi-Media Pvt. Ltd Laboratories Mumbai, India)						
	Experimental (rat)	1×10^9 CFU/mL, once a day	12 wk	High-fat diet-induced metabolic syndrome	Increased antioxidant markers (SOD, GSH) in the colon, liver, and adipose tissue	PMID: 32632629

Table 1 (continued)

Strain	Type of study	Dosage	TI	Disorder's condition or model	Primary endpoint	References
<i>L. fermentum</i> S1: Isolated from Fuyuan pickle (China)						
Experimental (nematodes)	Did not inform	4 days	-	-	Enhanced SOD activity, and reduced MDA levels	PMID: 31374277
In vitro study	Did not inform	-	-	-	Elicited free radical scavenging capacity	PMID: 31471140
<i>L. fermentum</i> SRK414: Obtained from the Division of Food Bioscience and Technology, Korea University						
In vitro study	1 × 10 ¹⁰ CFU	-	-	Lipopolysaccharide stimulated inflammation and dexamethasone-induced osteoporosis	Increased antioxidant markers (DPPH, ABTS). Downregulated the mRNA expression of the pro-inflammatory cytokines (TNF-α, IL-1β, and IL-8)	PMID: 31372901
<i>L. fermentum</i> Suo: isolated from yak yoghurt of Hongyuan grassland houseland (China)						
Experimental (mice)	0.5 and 1.0 × 10 ⁹ CFU	2 wk	-	HCl/Ethanol-induced gastric injury	Increased glutathione concentration and GPx activity in liver tissues. Increased mRNA and protein expression of Mn-SOD, CuZn-SOD, CAT in gastric tissues. Decreased serum levels of IL-6, IL-10, TNF-α, and IFN-γ	PMID: 26978395
<i>L. fermentum</i> TSF331: isolated from the intestines of healthy individuals (Taiwan)						
Experimental (mice)	8.2 × 10 ⁹ /kg, once a day	4 wk	-	Ethanol-induced liver oxidative stress (alcoholic steatohepatitis model)	Increased glutathione concentration and GPx activity in liver tissues, and downregulated proinflammatory cytokines (TNF-α and IL-6)	PMID: 33488797
<i>L. fermentum</i> U-21: Isolated of fecal microbiota of healthy adults (Russia)						
Experimental (mice)	1 × 10 ⁸ CFU, twice a day	3 wk	-	Paraquat-induced oxidative stress	Reduced death rate	PMID: 32632560
<i>L. fermentum</i> UCO-979C: Isolated from a human stomach (Chile)						
Experimental (mice)	1 × 10 ⁸ CFU, once a day	2 days	-	Gastric immune response against <i>H. pylori</i> infection	Increased the intestinal production of IFN-γ and IL-6	PMID: 31263467
<i>L. fermentum</i> XY18: Isolated from yogurt in Xinyuan County (China)						
Experimental (mice)	1 × 10 ⁸ CFU, once a day, and 1 × 10 ⁹ CFU, once a day	2 wk	-	HCl/Ethanol-induced gastric injury	Increased SOD and GPx activities, and glutathione in the gastric tissue. Reduced the serum levels of IL-6, IL-12, TNF-α, and IFN-γ	PMID: 33408461
<i>L. fermentum</i> ZYL0401: Obtained from Laboratory for Diagnosis and Treatment of Infectious Disease (Zhejiang University, Hangzhou, China)						
Experimental (mice)	2 × 10 ¹⁰ CFU, once a day	10 or 21 days	-	Lipopolysaccharide-induced hepatic injury	Increased levels of COX2 and IL-10 in the ileum. Reduced levels of TNF-α in the liver	PMID: 25978374

***L. fermentum* CECT 5716**

The strain *L. fermentum* CECT 5716 was isolated from human breast milk from healthy Spanish women on the 4th day postpartum [54]. *L. fermentum* CECT 5716 passed for safety assessment tests [55] and displayed a well-characterized and reproducible antioxidant effect. Daily administration of *L. fermentum* CECT5716 for 3 weeks increased short-chain fatty acid (SCFA) production and facilitated the recovery of inflammatory bowel disease by reducing MPO activity and TNF α in the colon in a trinitrobenzene sulphonic acid (TNBS) rat colitis model [56]. The same Spanish research group found that *L. fermentum* CECT5716 at a daily dose (1×10^8 CFU) for 2 weeks before the induction of an endotoxic shock by an intraperitoneal injection of lipopolysaccharide (LPS) increased antioxidant and anti-inflammatory effects in colon, lung, and hepatic tissues [57].

Antioxidant effects of *L. fermentum* CECT5716 were reported in animal models of arterial hypertension. Daily administration of *L. fermentum* CECT5716 during a time intervention varying from 2 to 13 weeks prevented gut dysbiosis by improving the richness, diversity, and gut microbiota composition, reduced blood pressure and endothelial dysfunction, decreased NADPH activity, ROS production, and pro-inflammatory cytokines in aortic segments in hypertensive rats [58–61].

Additionally, daily administration of *L. fermentum* CECT5716 for 13 or 15 weeks decreased the NADPH activity, ROS production, and pro-inflammatory cytokines in the renal cortex and aortic segments in a systemic lupus erythematosus mice model [62, 63]. More recently, a study on daily treatment with *L. fermentum* CECT5716 (5×10^9 CFU) for 11 weeks caused reduced weight gain, gut dysbiosis, NADPH activity, ROS production, and pro-inflammatory cytokines in aortic segments of high fat diet-induced obesity in mice [64].

Results of experimental studies have shown well-reproducible effects of *L. fermentum* CECT5716 on the improvement of gut dysbiosis and antioxidant and anti-inflammatory status in several disease models associated with oxidative stress. Despite this, the antioxidant and anti-inflammatory effects of *L. fermentum* CECT5716 have not yet been tested in a randomized, double-blind, placebo-controlled trial. Considering the safety of *L. fermentum* CECT5716 reported in previous clinical studies [65, 66], infants that received a milk formula supplemented with this probiotic, during the first year of life, showed a reduction in incidence and duration of diarrhea. However, it remains unclear the antioxidant, anti-inflammatory, and gut-modulating properties of *L. fermentum* CECT5716 in infants.

***L. fermentum* CQPC04, *L. fermentum* CQPC06, and *L. fermentum* CQPC08**

The strains *L. fermentum* CQPC04 [33], *L. fermentum* CQPC06 [67], and *L. fermentum* CQPC08 [32] were isolated from naturally fermented pickles in China. The daily administration of *L. fermentum* CQPC04 for 5 weeks reduced the MPO activity and increased the protein expression of SOD2 in the colon, increased the SOD and CAT enzyme activities in serum, and reduced the inflammation via down-regulation of NF- κ B signaling pathway in dextran sulfate sodium (DSS)-induced colitis in mice [33]. Similarly, the daily administration of *L. fermentum* CQPC06 for 8 weeks increased the gut tight-junction protein expression and reduced hepatic ROS-production, systemic inflammation, and liver damage in C57/BL6J in mice fed a high-fat diet [68]. The administration of *L. fermentum* CQPC08 for 12 weeks reduced the low-grade systemic inflammation and oxidative damage in the liver, kidney, and brain tissues by an upregulation of antioxidant enzymes and Keap1/Nrf2/ARE signaling pathway in an experimental model of lead-induced oxidative damage [32]. These results indicated that *L. fermentum* strains isolated from fermented pickles in China have remarkable antioxidant and anti-inflammatory properties.

***L. fermentum* CRL1446**

The *L. fermentum* CRL1446 strain was isolated from an Argentinean goat milk cheese [69]. Studies on the administration of *L. fermentum* CRL1446 reported enhanced antioxidant effects, improved gut-microbiota composition, and metabolic parameters in healthy mice [70], in caloric-restricted mice [69], and mice fed a high-fat diet [71, 72].

***L. fermentum* DR9**

The strain *L. fermentum* DR9 was isolated from fresh cow milk in Malaysia. The daily administrations of *L. fermentum* DR9 for 12 weeks improved the gut microbiota composition, reduced the proinflammatory cytokines, and promoted the antioxidative effects in D-galactose senescence-induced rats [73, 74].

***L. fermentum* I5007**

The strain *L. fermentum* I2007 was isolated from the gastrointestinal mucosa of piglets in China. Administration of *L. fermentum* I5007 for 2 weeks promoted beneficial effects on the weanling piglets primarily through affecting the proteome of the gastrointestinal mucosa [75]. Another study revealed the effects of early administration of *L. fermentum* I5007 on intestinal development and microbial composition

in the gastrointestinal tract of neonatal piglets [76]. Daily administration of *L. fermentum* I5007 for 2 weeks decreased the mRNA expression of IL-1 β in the ileum, although the treatment did not affect the MPO activity [76]. In addition, the effects of *L. fermentum* I2007 on the redox state were evaluated in piglets oxidatively stressed with diquat [77], a bipyridyl herbicide that utilizes molecular oxygen to produce super anion radicals and hydrogen peroxide. The results indicated that *L. fermentum* I2007 reduced the MDA contents and increased SOD and GPx activities in the liver, skeletal muscles, and plasma of piglets. Therefore, the dietary treatment with *L. fermentum* I2007 could improve the antioxidant status of oxidatively stressed piglets as indicated by increased activities of antioxidant enzymes.

***L. fermentum* IM12**

The strain *L. fermentum* IM12 was isolated from the fecal microbiota of healthy Koreans [30]. The administration of *L. fermentum* IM12 for 3 days caused the suppression of myeloperoxidase activity (MPO) and anti-inflammatory effects in the colon by inhibiting the NF- κ B-STAT3 signaling pathway [30].

***L. fermentum* J20 and *L. fermentum* J28**

The strains *L. fermentum* J20 and *L. fermentum* J28 were isolated from Mexican artisanal cheese. Studies have shown the anti-inflammatory and immunomodulatory effects of these strains in healthy and pro-inflammatory condition mice [78–80]. Once these strains were able to reduce intestinal and systemic acute inflammation, it is suggested that these probiotic strains may alleviate oxidative stress induced by indomethacin, highlighting the importance of performing additional studies to investigate the antioxidant properties of these strains. Moreover, future studies will be needed to elucidate the most effective probiotic strains, the appropriate dose, and the efficacy of probiotic mixtures versus a single strain.

***L. fermentum* JDFM216**

The strain *L. fermentum* JDFM216 was isolated from Korean infant feces [81]. The administration of *L. fermentum* JDFM216 for 48 weeks improved the gut microbiota composition and cognitive behavior besides modulating the immune response in aged mice [82]. In addition, the authors verified antioxidant effects of *L. fermentum* JDFM216 by increasing leukocyte burst activity in aged mice. However, the antioxidant properties of *L. fermentum* JDFM216 may be further explored by other oxidative stress markers, including key compounds, evaluation of antioxidant enzyme activities, and investigation of molecular mechanisms.

***L. fermentum* JX306**

The strain *L. fermentum* JX306 was isolated from a Chinese sauerkraut. This strain showed high antioxidant activity in vitro and effective inhibition of oxidative damage in the liver and kidney of D-galactose-induced aging mice [83]. Supplementation with *L. fermentum* JX306 triggered several antioxidant responses in aging mice, including decreased MDA levels, improved GSH-Px and TOC activity, and upregulating the transcriptional level of the antioxidant-related enzymes. To verify the role of this probiotic in achieving physiological aging, future studies could investigate the antioxidant properties of *L. fermentum* JX306 as a prophylactic and therapeutic agent in cardiometabolic disorders that commonly appear in older adults, such as hypertension, dyslipidemia, and type 2 diabetes mellitus.

***L. fermentum* KBL374 and *L. fermentum* KBL375**

The strains *L. fermentum* KBL374 and *L. fermentum* KBL375 were isolated from the feces of healthy Koreans [84]. A potential probiotic formulation with these strains was assessed in an experimental DSS-induced colitis model with measurements of several inflammatory and antioxidant parameters were analyzed in the colon tissue. Regarding the inflammatory parameters, the administration of *L. fermentum* KBL374 or *L. fermentum* KBL375 for 8 days decreased the Th1-, Th2-, and Th17-related cytokine levels and increased the IL-10 in the colon of mice subjected to the colitis model. In addition, both strains increased the CD4 + CD25 + Foxp3 + Treg cell population in mesenteric lymph nodes. Concerning the antioxidant parameters, the MPO concentrations were lower in animals subjected to DSS and treated with *L. fermentum* KBL375. These results indicated that *L. fermentum* KBL374 and *L. fermentum* KBL375 affected the immune cytokine expression and exerted antioxidant activity attenuating the damage linked to the inflammation induced by DSS.

***L. fermentum* KFRI 164**

The strain *L. fermentum* KFRI 164 was isolated from a Korean herbal medicine formula [85]. The efficacy of this strain to relieve the damage triggered by carbon tetrachloride (CCl₄) inducing liver injury was evaluated as an indicative of these probiotic properties. Ssanghwa-tang fermented with *L. fermentum* KFRI 164 decreased the serum levels of transaminases, including alanine transaminase (ALT), aspartate transaminase (AST), and lactate dehydrogenase (LDH). However, additional studies are needed to investigate other antioxidant properties and hepatoprotective activity of *L. fermentum* KFRI 164, including the level of lipid peroxidation, and the activity of liver antioxidant enzymes.

(CAT, SOD, GPx, GST), and other key compounds (MDA, FA, SCFA, GSH). The analysis of these biological markers could elucidate the antioxidant potential of this probiotic in relieving hepatic diseases.

***L. fermentum* KU2000**

The strain *L. fermentum* KU200 was isolated from Korean fermented foods [86]. The potential probiotic properties of this strain were investigated through 1-methyl-4-phenylpyridiniumMPP+ -induced cytotoxicity in SH-SY5Y cells. This model has been used to replicate chronic neural disturbances, such as Parkinson's disease. This study showed that *L. fermentum* KU200 has strong antioxidant properties, including reduction of DPPH radical scavenging activity and induced inhibition rate of β -carotene and linoleic acid oxidation in SH-SY5Y cells, indicating the use of this strain to prevent chronic neural disorders. However, further in vivo studies are needed to verify the efficacy of *L. fermentum* KU200 as an antioxidant agent in neural diseases.

***L. fermentum* LA12**

The strain *L. fermentum* LA12 was isolated from Korean infant feces and verified its antioxidant properties in an alcohol-induced alcoholic steatohepatitis model [87]. Alcoholic liver disease is a complex and multifaceted disorder having oxidative stress and inflammation as the key mechanisms. *L. fermentum* LA12 for 4 weeks was reported to protect against the injurious effects of alcohol on the intestine and liver by decreasing intestinal oxidative stress [87].

***L. fermentum* LC5**

A research group from India evaluated the antioxidant capacity of *L. fermentum* LC5 to attenuate hepatotoxicity induced by aflatoxin B₁ in mice [88]. The prolonged consumption of aflatoxin B₁ from food has been linked to the development of hepatocellular carcinoma and liver injury. Administration of *L. fermentum* LC5 for 3 weeks in mice receiving aflatoxin B₁ reduced the levels of pro-inflammatory cytokines, including TNF- α , IL-12, and IL-6. However, this strain did not change the levels of antioxidant markers such as GST and SOD.

***L. fermentum* Lf1**

The strain *L. fermentum* Lf1 was isolated from the feces of Indian subjects [89]. The antioxidant properties of *L. fermentum* Lf1 were evaluated using an experimental model of inflammatory bowel disease (colitis model) using DSS. Administration of *L. fermentum* Lf1 for 12 days led to an increase in key antioxidant enzymes such as SOD2 and TrxR-1. In addition, the

strain was able to decrease of MDA levels. The results indicated that *L. fermentum* Lf1 induced the activation of the antioxidant enzyme system in the mouse model, being a potential strategy for inflammatory bowel disease management. The researchers highlighted the necessity to investigate the antioxidant of *L. fermentum* Lf1 in well-designed double-blind controlled human trials to confirm its clinical efficacy.

***L. fermentum* LF31**

The strain *L. fermentum* LF31 was obtained from Italian pharmacies (Milonet, Bromatech s.r.l., Milan, Italy) [90]. The antioxidant activity of *L. fermentum* LF31 was evaluated using the oxygen radical absorbance capacity (ORAC) method in vitro on the human colon adenocarcinoma HT-29 cell line. The treatment with *L. fermentum* LF31 had in vitro antioxidant capacity and increased the total antioxidant potential evaluated by ORAC values. *L. fermentum* LF31 could be investigated using in vivo studies to confirm its antioxidant properties in colon neoplasm cells.

***L. fermentum* MTCC 5898**

The strain *L. fermentum* MTCC:5898 was isolated from the feces of a 10-month-old breast-fed Indian infant and characterized for standard probiotic attributes [91]. The assessment of the antioxidant capacity of *L. fermentum* MTCC:5898 in streptozotocin-induced diabetic rats demonstrated that its administration in fermented milk for 6 weeks reduced the inflammation and dyslipidemia in diabetic rats, as well as decreased lipid peroxidation and increased SOD and CAT activities in kidney and liver [92]. It was also demonstrated that the consumption of probiotic fermented milk with *L. fermentum* MTCC:5898 for 12 weeks attenuated dyslipidemia, inflammation, and oxidative stress in liver and kidney in male rats fed a cholesterol-enriched diet [93].

The results of another study showed the safety and non-toxicity of *L. fermentum* MTCC:5898 when administered to weaning mice [94], as well as its immunosuppressive effects in *E. coli* infection by modulating the expression of pro-inflammatory and anti-inflammatory cytokines [95]. These results demonstrate that *L. fermentum* MTCC:5898 could be in a transitional stage for clinical testing and functional food applications.

***L. fermentum* NCIMB 5221**

L. fermentum NCIMB 5221 was purchased from NCIMB (Aberdeen, Scotland, UK) [96] and investigated for its antioxidant properties in rats subjected to a model of hyperlipidemia and hyperglycemia. The results showed that administration of *L. fermentum* NCIMB 5221 for 8 weeks increased the production of FA and antioxidant markers [96].

***L. fermentum* NS9**

The strain *L. fermentum* NS9 was isolated from grasslands in China. The antioxidant capacity of *L. fermentum* NS9 was evaluated in rats treated with antibiotic ampicillin [97]. Administration of *L. fermentum* NS9 for 6 weeks alleviated gut microbiota disturbances and decreased MPO activity in the colon of rats treated with ampicillin, indicating the efficacy of *L. fermentum* NS9 in reducing the adverse effects induced by clinical antibiotic application.

***L. fermentum* PTCC 1638**

The strain *L. fermentum* PTCC 1638 was supplied by the culture collection from the Iran Institute of Industrial and Scientific Research [98]. Administration of *L. fermentum* PTCC 1638 for 8 weeks enhanced the serum respiratory burst, lysozyme activity, and serum levels of antioxidant enzymes such as CAT, GPx, and SOD in an experimental model using *Cyprinus carpio*. These results indicate that *L. fermentum* PTCC 1638 may be used as a beneficial feed additive to improve the immune response and disease resistance in the early stages of common carp culture.

***L. fermentum* PUM**

The strain *L. fermentum* PUM was isolated by an Indian research group [99]. This strain has antioxidant properties and can be used to treat high-fat-diet-induced metabolic syndrome. The treatment with *L. fermentum* PUM for 12 weeks increased antioxidant markers (SOD and GSH) in the colon, liver, and adipose tissue and reduced metabolic syndrome in rats. The researcher highlighted that these effects need to be investigated in clinical trials to confirm the potential clinical use of *L. fermentum* PUM against oxidative stress conditions in humans.

***L. fermentum* S1**

The strain *L. fermentum* SRK414 was isolated from Fuyuan pickle in China [100]. The probiotic properties of *L. fermentum* SRK414 were verified by enhancing T-AOC (total antioxidant capacity) and SOD activity and reduced MDA levels. The antioxidant activity of *L. fermentum* SRK414 was also evaluated in vivo, being reported remarkable scavenging activity including 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical, hydroxyl radical, ABTS radical cation (ABTS), and ferric reducing antioxidant power (FRAP) in nematodes [101]. These results indicate that *L. fermentum* SRK414 has antioxidant activities in vitro and in vivo and could be employed as a promising and effective antioxidant for future use in functional foods, dietary supplements, or drugs.

***L. fermentum* SRK414**

The strain *L. fermentum* SRK414 was obtained from the Food Microbiology Laboratory, Division of Food Bioscience and Technology, Korea University (Seoul, South Korea) and investigated in vitro for its probiotic, antioxidant, and anti-inflammatory properties [102]. The administration of 1×10^{10} CFU increased the scavenging activities of ABTS and DPPH in mammalian cell-free supernatants of cultured bacteria. Moreover, *L. fermentum* SRK414 induced downregulation of the mRNA expression of the pro-inflammatory cytokines TNF- α , IL-1 β , and IL-8. These results indicate the high antioxidant capacity of *L. fermentum* SRK414, as well as the necessity to perform studies in vivo to evaluate the effects of this strain against LPS and dexamethasone to confirm its efficacy to treat inflammation and osteoporosis.

***L. fermentum* Suo**

The strain of *L. fermentum* Suo was isolated from yak yoghurt from the Hongyuan grassland (China) [103]. The authors found that treatment with *L. fermentum* Suo for 2 weeks increased glutathione concentration and GPx activity in liver tissues in mice treated with hydrochloric acid and ethanol. In addition, mice treated with *L. fermentum* Suo had increased Mn-SOD, Cu/Zn-SOD, CAT (catalase) mRNA, or protein expressions and reduced COX-2, NF- κ B (nuclear factor kappaB), and iNOS (inducible nitric oxide synthase) expressions in gastric tissues. Lastly, *L. fermentum* Suo administration also reduced the serum levels of proinflammatory cytokines such as IL-6, IL-12, TNF- α (tumor necrosis factor- α), and IFN- γ , indicating that *L. fermentum* Suo has potential use as a probiotic due to its gastric injury treatment effects.

***L. fermentum* TSF331**

The strain *L. fermentum* TSF331 was isolated from the intestines of healthy individuals in Taiwan [104]. The therapeutic potential of *L. fermentum* TSF331 in attenuating the ethanol-induced liver oxidative stress was evaluated in an alcoholic steatohepatitis mice model. The administration of *L. fermentum* TSF331 for 4 weeks increased the glutathione concentration and GPx activity in mice liver tissues and downregulated proinflammatory cytokines such as TNF- α and IL-6. The treatment with *L. fermentum* TSF331 could prevent the development of alcoholic steatohepatitis and liver injury due to primarily the antioxidant properties of this strain.

***L. fermentum* U-21**

The strain *L. fermentum* U-21 was isolated from the gut of healthy Russian adults. The administration of *L. fermentum* U-21 for 3 weeks exerted protective effects against paraquat-induced oxidative stress, which is used to replicate Parkinson's disease in *Caenorhabditis elegans* and mouse models [105]. Main findings revealed that *L. fermentum* U-21 reduced the death rate and protected against anatomical changes in the liver, lung, and heart, typical of Parkinson's disease. Once paraquat triggers high ROS production, the researchers indicated the necessity of further molecular studies to confirm or refute the antioxidant capacity of *L. fermentum* U-21 through evaluation of critical compounds, regulation of antioxidant enzymes, and investigation of putative molecular mechanisms. The elucidation of the antioxidant potential of this *L. fermentum* U-21 will allow us to understand its capacity to control oxidative stress markers and combat Parkinson's disease symptoms.

***L. fermentum* UCO-979C**

The strain of *L. fermentum* UCO-979C was isolated from the stomach of human in Chile and characterized for its potential probiotic properties [106]. The administration *L. fermentum* UCO-979C for 2 days beneficially regulated the innate immune response triggered by *Helicobacter pylori* infection in gastric epithelial cells in mice. The administration of *L. fermentum* UCO-979C increased the production of intestinal IFN- γ , stimulated intestinal and peritoneal macrophages, and increased the number of Peyer's patches CD4+ T cells. These results indicate the antioxidant capacity of *L. fermentum* UCO-979C and its ability to improve the protection against *H. pylori* infection.

***L. fermentum* XY18**

The strain *L. fermentum* XY18 was isolated from yogurt in China and evaluated for its capability of attenuating the gastric injury induced by hydrochloric acid and ethanol in mice [100]. Administration of *L. fermentum* XY18 at a low and high dose for 2 weeks increased the levels of SOD and GPx in the gastric tissue of mice. The administration of *L. fermentum* XY18 reduced serum levels of proinflammatory cytokines such as TNF- α and interferon- γ (IFN- γ). The results showed overall a higher antioxidant capacity of *L. fermentum* XY18 when administered at a high dose, besides revealing the potential application of this strain to prevent gastric injury.

***L. fermentum* ZYL0401**

A research group from China isolated strain *L. fermentum* ZYL0401 and evaluated its efficacy to attenuate the hepatic and ileum injury caused by LPS administration in mice [107]. The oral pretreatment with *L. fermentum* ZYL041 for 10 days did not affect significantly the ileal MPO levels in mice. However, the pre-treatment with *L. fermentum* ZYL041 decreased the levels of TNF- α in the liver and increased the levels of COX2 and IL-10 in the ileum of mice. These results suggested that *L. fermentum* ZYL0401 could help to mitigate gastrointestinal and hepatic injury induced by LPS.

Future Perspectives

The rising oxygen levels detected during our evolutive process resulted in a redox upshift in microbial energetics. Based on the results found in studies with animals, the administration of various *L. fermentum* strains displayed antioxidant capacity and health-promoting benefits. A reasonable question is, "Why could *L. fermentum* strains have antioxidant effects from distinct sources?" Could *L. fermentum* strains have had an adaptive evolution and shifted their fermentative to oxidative metabolism, enabling their survival and oxidative stress tolerance? We hypothesize that the antioxidant capacity of *L. fermentum* strains could be an evolutionary defense mechanism allowing their survival in different matrices undergoing distinct complex environmental conditions, including a range of inhibitory and or stimulatory factors. However, how these specific *L. fermentum* strains could interact with other microorganisms coexisting in the same environment remains unclear.

Despite the broad body of studies examining the effects of various *L. fermentum* strains isolated from distinct sources on the oxidant and antioxidant control in a variety of animal disease models and clinical trials, the mechanisms underlying these effects have been poorly explored. In fact, the presence of probiotic bacteria and their metabolites in the gut can alter intracellular signaling pathways in host cells, favoring an antioxidant response through enzymatic and non-enzymatic activity. We emphasize that efforts should be directed towards the performance of future mechanistic studies to understand how the strains of *L. fermentum* examined could act in the reduction of oxidative stress in several non-communicable diseases. For example, whole-genome sequencing and comparative genome analysis could be used to investigate the mechanisms of these strains against oxidative stress environment and whether oxidative stress tolerance could be an adaptive evolution of these bacteria. Second, besides being central to cellular respiration and the

source of ATP, mitochondria exert a critical role in ROS production, generated due to electron transport chain activity. It is recognized that bacterial modification can alter epithelial mitochondrial function and that perturbation of the mitochondria can affect the bacteria-epithelial relationship. These emerging aspects in epithelial-microbe interactions have been early reviewed [108]. Considering that disturbed mitochondrial function can contribute to gut disease and that *L. fermentum* strains display antioxidant properties, it is reasonable to infer that a potential remodeling of the mitochondrial network could occur in the mutualistic relationship between the cells host and *L. fermentum* microbes. In fact, this has not yet been demonstrated. To solve this problem, electronic microscopy and molecular assays investigating biogenesis mitochondrial, ATP production, fusion, and fission mitochondrial could help to elucidate whether *L. fermentum* strains contribute to gut oxidative stress tolerance, at least in part, via affecting the epithelial mitochondrial network remodeling.

It is relevant to highlight that only one (*L. fermentum* ME-3) out of the 39 examined strains was evaluated concerning the antioxidant effects in a clinical trial, specifically in Estonian individuals aged 35–60 years old. Hence, the therapeutic potential of distinct *L. fermentum* strains to minimize oxidative stress needs to be investigated in randomized, double-blind, placebo-controlled trials to confirm their clinical effects. The additional gap is the variation of dose and time intervention used in the retrieved studies. A meta-analysis should be conducted using results from experimental and clinical studies to find reliable evidence of the effective dose and treatment time to reach an optimal antioxidant effect of *L. fermentum* strains against oxidative stress. Finally, considering the strain-specific effects reported in studies with *L. fermentum* strains, a well-cataloged identification of the probiotic *L. fermentum* strains for specific uses based on their performance should assist the successful applications and development of more effective products to combat oxidative stress and promote benefits to human health.

A decrease of LPS-producing bacteria promoted by the presence of *L. fermentum* strains and exopolysaccharides in the gut decreases the binding of LPS to TLR4. This reduces the translocation of NF- κ B and STAT3 to the nucleus and decreases pro-inflammatory stimuli. Additionally, *L. fermentum* and binding of short-chain fatty acid (SCFA: acetate, propionate, and butyrate) to GPR and FFAR can activate AMPK-MAPK, enhancing the Keap1/Nrf2/ARE pathway. The translocation of Nrf2 to the nucleus favors its binds to antioxidant response elements (ARE), thus increasing the transcription of antioxidant genes and alleviating ROS. *L. fermentum* can produce H₂O₂, oxidating cystine in Keap1, realizing Nrf2 to the nucleus.

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Data Availability Not applied. The study is a review.

Declarations

Conflict of Interest The authors declare no competing interests.

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APÊNDICE C - ARTIGO 3

***Limosilactobacillus fermentum* prevent gut-kidney oxidative damage and the rise in blood pressure in male rat offspring exposed to a maternal high-fat diet**

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Limosilactobacillus fermentum prevent gut-kidney oxidative damage and the rise in blood pressure in male rat offspring exposed to a maternal high-fat diet

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Abstract

Oxidative stress along the gut-kidney axis is a risk factor for developing arterial hypertension in offspring from dams fed a high-fat diet. Considering the antioxidant capacity of probiotic strains, this study evaluated the effects of a daily multistrain formulation with *Limosilactobacillus fermentum* 139, 263, and 296 on blood pressure (BP), renal function, and oxidative stress along the gut-kidney axis in male offspring from dams fed a high-fat high-cholesterol (HFHC) diet during pregnancy and lactation. Dams were fed a diet control or HFHC diet during pregnancy and lactation. At 100 days of age, part of the male offspring from dams fed a HFHC diet received *Limosilactobacillus fermentum* formulation for 4 weeks (HFHC + Lf) daily. After the 4-week intervention, BP (tail-cuff plethysmography) and urinary and biochemical variables were measured. In addition, malondialdehyde levels, enzymatic activities of superoxide dismutase, catalase, glutathione-S-transferase, and nonenzymatic antioxidant defense (thiols content) were measured in the colon and renal cortex. Male offspring from dams fed a HFHC had increased blood pressure, impaired renal function, and oxidative stress along the gut-kidney axis. Administration of *Limosilactobacillus fermentum* reduced systolic, diastolic, and mean blood pressure levels and alleviated renal function impairment and oxidative stress along the gut-kidney axis in male offspring from dams fed a HFHC diet. Administration of *Limosilactobacillus fermentum* formulation attenuated programmed hypertension in the HFHC group through oxidative stress modulation along the gut-kidney axis.

Introduction

Maternal high-fat diet (HFD) consumption is a risk factor for developing arterial hypertension in offspring through a complex mechanism involving sympathetic overactivity, enhanced peripheral chemosensitivity, baroreflex dysfunction, and impairment in kidney function.^{1–3} Additionally, HFD intake during pregnancy and/or lactation has been shown to shift the composition of maternal–fetal gut microbiota, which has been linked to impaired gut barrier integrity and developmental programming of arterial hypertension.⁴

Oxidative stress plays a significant role in developing many diseases, being characterized by increased production of oxygen/nitrogen/sulfur reactive species and reduced tissue antioxidant capacity.⁵ Increased renal oxidative stress and gut microbiota dysbiosis have been linked to the development of arterial hypertension and chronic kidney disease.^{6–8} It has been previously demonstrated that offspring from dams fed a high-fat high-cholesterol diet during pregnancy and lactation developed arterial hypertension linked to renal dysfunction and increased oxidative stress along the gut-kidney axis at 90 days of age,⁹ indicating that postweaning intervention targeting the gut microbiota could be a potential strategy to reprogramming arterial hypertension.

The administration of probiotics, an approach based on the administration of live nonpathogenic microorganisms that confer a health benefit to the host when administered in adequate amounts, has been considered a safe strategy capable of reducing oxidative stress.¹⁰ Growing evidence has demonstrated that interventions targeting gut microbiota with probiotics have emerged as an innovative strategy to treat arterial hypertension,^{11,12} and to protect cells from oxidative damage due to their antioxidant capacity.¹³

The administration of *Limosilactobacillus fermentum* 139, 263, and 296 has been shown to cause improvements in lipid profile and autonomic function in rat offspring from dams with

maternal dyslipidemia.¹⁴ However, whether postweaning probiotic therapy with a multistrain formulation containing these *Limosilactobacillus fermentum* strains effectively reduces blood pressure, kidney dysfunction, and oxidative stress along the gut-kidney axis in the offspring later in life remains to be elucidated. These *Limosilactobacillus fermentum* strains have been previously characterized as having probiotic aptitudes and qualities to be translated into nutritional approaches.^{14,15}

In the present study, the effects of a daily administration of a multistrain formulation with *Limosilactobacillus fermentum* 139, 263, and 296 on blood pressure, renal function, and oxidative stress along the gut-kidney axis in male offspring from dams fed with a high-fat high-cholesterol during pregnancy and lactation were evaluated.

Methods

Ethical aspects and experimental design

Wistar rats were used in this study. The animals were maintained in collective polypropylene cages under controlled temperature ($22 \pm 1^\circ\text{C}$), humidity between 50 and 55 % 12 h light-dark cycle, and received water and diet *ad libitum*. All animal procedures were approved by the Institutional Animal Care and Use Committee of the Federal University of Paraíba (CEUA-UFPA protocol #9635020519; #9492260418) and followed the guidelines of the National Council for the Control of Animal Experimentation (CONCEA) and International Principles for Biomedical Research.

The female Wistar rats ($n = 8$) were maintained in polypropylene cages up to 90 days of age. Posteriorly, pregnant rats were allocated to a control group ($n = 4$) and received a diet prepared according to the American Institute of Nutrition – AIN-93G; or a HFHC group ($n = 4$) that received a diet (Table 1) purchased from Rhosier Company (Araçoiaba da Serra, São Paulo, Brazil) during pregnancy and lactation period as previously described.^{1,16,17} After weaning (postnatal day 21), male rat offspring (CTL, $n = 16$ and HFHC, $n = 15$) were weighted, housed separately (3–4 per cage), and had free access to a commercial diet (Presence Purina, Paulínea, São Paulo, Brazil) and water *ad libitum* up to 100 days of age. At that age, the experimental groups were randomly formed with one to two rats from each litter. The CTL group was formed for six male offspring ($n = 6$) from the dams fed a CTL diet. The HFHC group was formed for five male offspring ($n = 5$) from the dams fed a HFHC diet. Similarly, HFHC + Lf group was formed for five male offspring ($n = 5$) from the dams fed a HFHC diet and received the multistrain *Limosilactobacillus fermentum* formulation (HFHC + Lf group) twice a day in a solution of approximately 3×10^9 CFU/mL by oral gavage for 4 weeks (Fig 1).

Limosilactobacillus fermentum strains

Limosilactobacillus fermentum 139, 263, and 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 Mann, Rogosa, and Sharpe (MRS) broth (HiMedia, Mumbai, India) containing glycerol (Sigma-Aldrich, St. Louis, USA; 20 mL/100 mL). The probiotic cell suspension was obtained from overnight cultures grown on MRS broth (HiMedia, Mumbai, India) under anaerobic conditions (Anaerobic System Anaerogen, Oxoid Ltda., Wade Road, UK) at 37°C .^{14,15} The

Table 1. Nutritional composition of control diet (CTL diet) and high fat and high cholesterol diet (HFHC diet)

Ingredients (for 100g)	Diets	
	CTL*	HFHC**
Corn starch	39.75	33.09
Dextrinized corn starch	13.20	15.50
Casein ^{oo}	20.00	19.86
Sucrose	10.00	6.00
Soybean oil	7.00	3.00
Animal fat (lard)	–	6.00
Sigma Cholesterol	–	1.00
Sigma Colic Acid	–	0.50
Cellulose	5.00	5.00
Mineral Mix 93M	3.50	3.50
Vitamin Mix	1.00	1.00
L-cystine	0.30	0.30
Choline bitartrate	0.25	0.25
t-BHQ [#]	0.014	0.014
Calories (kcal/g)	3.96	4.34
Carbohydrates (% kcal)	63.62	50.32
Proteins (% kcal)	20.47	18.58
Lipids (% kcal)	15.92	31.11

Composition values obtained from previously established protocols.

*CTL diet adapted from Reeves; Nielsen; Fahey, (1993).

**HFHC diet according to Rhosier - Industry and Trade Ltda.

^{oo}Casein had 85% purity (85 g protein for each 100 g casein).

[#]Tert-butylated hydroxytoluene.

mixed cell suspension with counts of approximately $9 \log$ CFU/mL of each strain was obtained with a mixture of each probiotic strain suspension in a ratio of 1:1:1.

Urinary analysis

After the supplementation period, the male rats were individually acclimatized in metabolic cages for 3 days and on the 4th day, 24-h urine collection was done. The urinary volume was measured and freeze-stored (-20°C). The urinary measurements of urea, creatinine, and total proteins were done with commercial kits following the protocols according to the manufacturer instructions (Bioclin, Belo Horizonte, Minas Gerais, Brazil). The creatinine clearance (CCr) was calculated with the formula: $\text{CCr} = \text{Urinary Creatinine} \times \text{Urinary volume of 24h/plasmatic creatinine}$.¹⁸

Blood pressure and heart rate measurement

Blood pressure (BP) was recorded by tail-cuff plethysmography (V2.11 Plethysmography, Insight, Ribeirão Preto, São Paulo, Brazil). For 3 days, rats were encouraged to walk into the restraint tubes and acclimatized for approximately 1 h before experiments began. After the adaptation period, BP and heart rate were measured.⁹ For each record, 15 cycles of inflation and deflation were done. Ten out of the 15 recorded values were used to calculate the average.

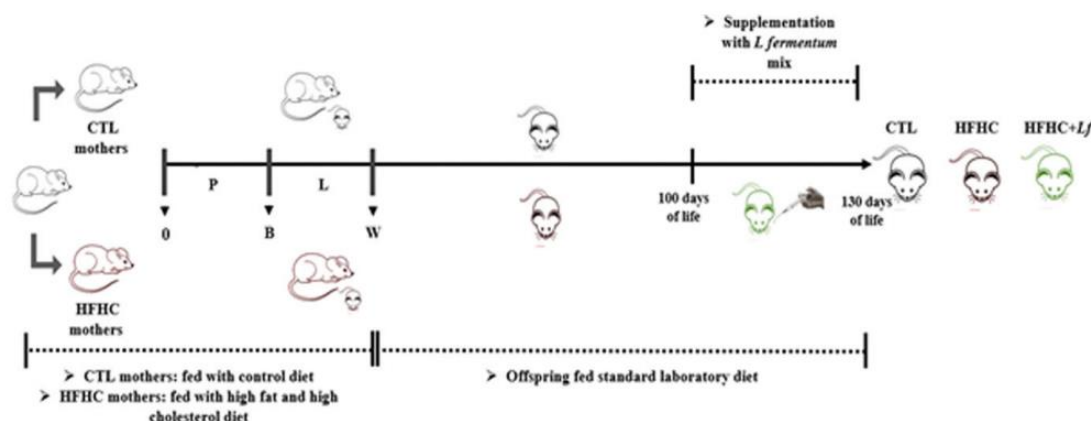


Fig. 1. Graphic design of the formation of experimental groups. B: Birth; CTL: Control; HFHC: High fat and high cholesterol; HFHC + Lf: High fat and high cholesterol diet and *Limosilactobacillus fermentum* formulation; L: Lactation; P: Pregnancy; W: weaning.

Euthanasia and blood collection

After urine collection and BP measurements, the rats were euthanized by decapitation, and blood was collected. To obtain the serum, the blood was centrifuged (58.136 g, 15 min, 4 °C). Serum measurements for creatinine concentration were done with enzymatic colorimetric kits (Bioclin).

Measurement of oxidative stress in colon and kidney

The right kidney and a portion of the colon were collected and freeze-stored (−80 °C) for further analysis. Renal cortex and colon tissues were homogenized in a cold buffer solution with 50 mm Tris and 1 mm EDTA (pH 7.4), 1 mm sodium orthovanadate, and 200 µg/mL phenylmethanesulfonylfluoride using an IKA RW 20 digital homogenizer, a pestle of potter-Elvehjem and glass tubes on ice. Homogenates were centrifuged (1,180 g, 10 min, 4 °C).¹⁹ Protein contents were determined with Bradford protocol.²⁰

Assessment of lipid peroxidation

An aliquot (0.3 mg/mL) of homogenate of tissues (renal cortex and colon) 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) was done to the sample, followed by centrifugation (2500xg, 10 min, 4 °C). TBA (0.8%, v/v) was added to the 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 (renal cortex and colon) 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). SOD activity was measured by the kinetics of epinephrine auto-oxidation inhibition for 1.5 min at 480 nm read on a spectrophotometer.²¹

Assessment of catalase (CAT) activity

Catalase activity was determined by the decomposition of H₂O₂ into O₂ and H₂O. A sample of tissue (renal cortex and colon) homogenate (0.3 mg/mL) in 50 mm phosphate buffer (pH 7.0) was added of 0.3M H₂O₂. Absorbance was measured at 240 nm for 1.5 min on a spectrophotometer.²²

Assessment of glutathione S-transferase (GST) activity

A sample of tissue (renal cortex and colon) homogenate (0.3 mg/mL) was used to quantify GST activity, as previously described.²³ 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) were added to tissue homogenate samples. Absorbance was measured at 340 nm for 1.5 min on a spectrophotometer.

Assessment of total thiols groups

Tissue (renal cortex and colon) homogenates 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.²⁴

Statistical analysis

Kolmogorov Smirnov test was used to assess the normality of data. The variables were reported as mean ± standard deviation and required the one-way analysis of variance (ANOVA) parametric test and Tukey post hoc test. For body weight measurements, statistical significance was evaluated with ANOVA (two-way test) with Bonferroni's post hoc test. Statistical analysis was done with the software Prism 5 (GraphPad Software, San Diego, CA, USA). The difference was considered significant when p was < 0.05.

Results

Body weight

Body weight at weaning up to 100 days of age was similar between groups ($p > 0.05$, Fig 2A). The body weight and weight gain at the

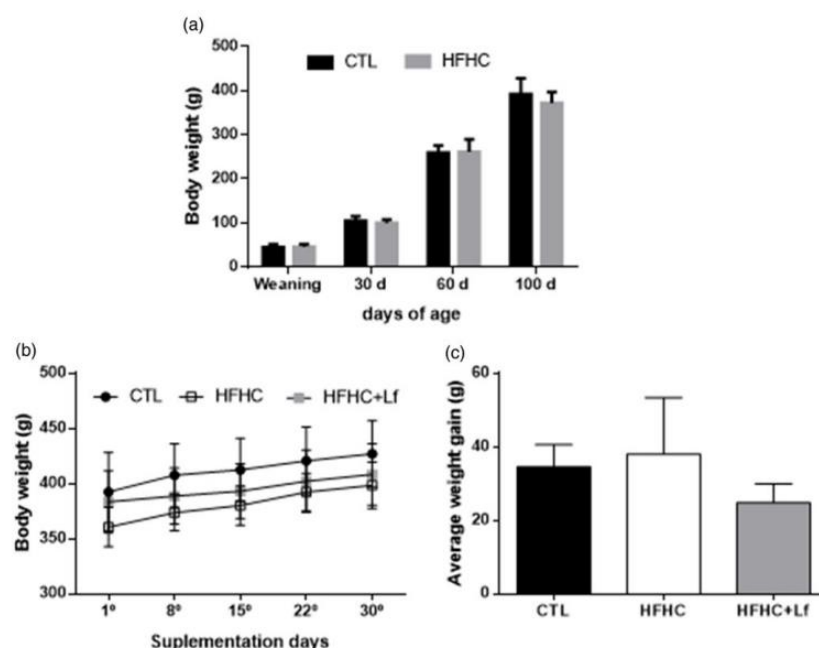


Fig. 2. Assessment of bodyweight at weaning up to 100 days of age and the effects of a multi-strain formulation with *Limosilactobacillus fermentum* 139, 263 and 296 on body weight in male offspring of dams fed a HFHC diet during pregnancy and lactation. Groups: control (CTL); high fat and high cholesterol (HFHC); high fat and high cholesterol receiving *Limosilactobacillus fermentum* 139, 263, and 296 (HFHC + Lf). Data are presented as mean $\pm$ standard deviation and analyzed by ANOVA one-way test with Tukey as post-hoc test or ANOVA two-way with Bonferroni post-hoc test.

end of the protocol were similar among groups ($p > 0.05$, Fig 2B and C).

Blood pressure

Male offspring from dams fed a HFHC diet had increased SAP (CTL: 115 ± 6.5 vs HFHC: 127 ± 1.1 mmHg, $p = 0.013$, Figure) and MAP (CTL: 89 ± 5.5 vs HFHC: 98 ± 3.8 mmHg, $p = 0.034$) when compared to the CTL group (Fig 3A and C). Administration of *Limosilactobacillus fermentum* formulation reduced SAP (HFHC: 127 ± 1.1 vs HFHC + Lf: 112 ± 4.2 mmHg, $p = 0.004$), DAP (HFHC: 83 ± 5.3 vs HFHC + Lf: 74 ± 3.7 mmHg, $p = 0.049$), and PAM (HFHC: 97 ± 1.9 vs HFHC + Lf: 87 ± 3.4 mmHg, $p = 0.015$) when compared to HFHC group (Fig 3A-C). HR was similar among groups ($p > 0.05$, Fig 3D).

Renal function

The HFHC group had a low urinary creatinine concentration compared to the CTL group ($p = 0.018$, Table 2). In contrast, the HFHC + Lf group had higher creatinine levels than the group exposed to HFHC diet ($p = 0.007$, Table 2). The urinary and serum creatinine values allowed to analyze creatinine clearance, an important indicator of renal function. There was a reduction in CCr in the HFHC group compared to the CTL group ($p = 0.048$, Table 2). On the other hand, administration of *Limosilactobacillus fermentum* formulation restored the renal function in male offspring from dams fed a HFHC diet ($p = 0.040$, Table 2). Serum levels of creatinine, urea, total

proteins, and urinary volume were similar among groups ($p > 0.05$, Table 2).

Indicators of oxidative stress in the colon

The MDA levels, CAT activity, and thiols content in colonic mucosa were similar among groups ($p > 0.05$, Fig 4A, C, and E). HFHC group displayed reduced GST enzyme activity (CTL: 28.9 ± 8.1 vs HFHC: 15.3 ± 2.8 U/mg protein, $p = 0.003$) compared to the CTL group (Fig 4D). Administration of a mixed *Limosilactobacillus fermentum* formulation increased antioxidant SOD activity (HFHC: 502 ± 75 vs HFHC + Lf: 626 ± 70 U/mg protein, $p = 0.040$) in colon mucosa of male offspring exposed to maternal HFHC diet (Fig 4B).

Indicators of oxidative stress in the renal cortex

The GST activity and thiols content in the renal cortex were similar among groups ($p > 0.05$, Fig 5D and E). Male offspring from dams fed a HFHC diet had increased MDA levels (CTL: 0.12 ± 0.05 vs HFHC: 0.25 ± 0.08 nmol/mg protein, $p = 0.023$) and reduced SOD (CTL: 399 ± 58 vs HFHC: 309 ± 38 U/mg protein, $p = 0.051$) and CAT activities (CTL: 18.2 ± 2.9 vs HFHC: 7.7 ± 2.7 U/mg protein, $p = 0.003$) in renal cortex when compared to CTL group (Fig 5A-C). Administration of *Limosilactobacillus fermentum* formulation restored the functional capacity of CAT activity in the renal cortex (HFHC: 7.7 ± 2.7 vs HFHC + Lf: 15.2 ± 2.1 U/mg protein, $p = 0.005$, Fig 5C), besides tending an increase SOD activity (HFHC: 309 ± 38 vs HFHC + Lf: 406 ± 75 U/mg protein, $p = 0.055$, Fig 5B) in male offspring from dams fed a HFHC diet.

Table 2. Biochemical parameters of male offspring of dams fed a control diet (CTL) or high-fat high cholesterol diet (HFHC) during pregnancy and lactation and supplemented with a multi-strain formulation with *Limosilactobacillus fermentum* 139, 263, and 296 (HFHC + Lf)

	CTL	HFHC	HFHC + Lf
Urinary creatinine (mg/dL)	8.7 ± 3.1	3.5 ± 1.0*	10.4 ± 1.8†
Serum creatinine (mg/dL)	0.78 ± 0.19	0.80 ± 0.16	0.78 ± 0.10
Urea (g/24h)	400 ± 145	232 ± 89	370 ± 149
Total proteins (mg/24h)	15.3 ± 4.0	9.1 ± 5.0	16.5 ± 6.6
Urinary volume (mL)	7.0 ± 2.3	5.5 ± 1.2	7.2 ± 1.6
CCr (mL/min)	86.4 ± 43.5	25.2 ± 9.5*	82.9 ± 20.5†

CCr, Creatinine clearance.

*Shows significant difference compared to CTL.

† Shows significant difference compared to HFHC

Discussion

The HFHC consumption during pregnancy has been shown to induce oxidative stress on the gut-kidney axis in rat offspring, renal dysfunction, and arterial hypertension later in life.⁹ The results of the present study have demonstrated for the first time that administration of a multistrain *Limosilactobacillus fermentum* formulation reduced systolic, diastolic, and mean blood pressure levels and alleviated renal dysfunction and oxidative stress along the gut-kidney axis in male offspring from dams fed a HFHC diet during pregnancy and lactation.

Programmed arterial hypertension provoked by HFD consumption during pregnancy and/or lactation is complex and

involves several impairments in key mechanisms related to blood pressure control.^{1,25} It has been reported that gut inflammation and oxidative stress can compromise gut barrier integrity, favor LPS-translocation, and promote low-grade inflammation.²⁶ At the central nervous system, inflammation can lead to sympathetic overactivity and increased blood pressure.²⁷ On the other hand, the activation of the sympathetic nervous system induces increased gut permeability, low-grade inflammation, and alterations of gut microbiota composition, which in turn contribute to neuronal activity by releasing pathogenic bacterial metabolites into circulation.^{28,29} Early studies have found increased gut damage, sympathetic hyperactivity, and enhanced blood pressure in offspring from dams fed a HFHC diet during pregnancy and lactation.^{1,16}

Therapeutic strategies to alleviate the deleterious effects of maternal HFD consumption on blood pressure and cardiovascular function in offspring later in life are under investigation. Recent studies have suggested that interventions targeting the gut microbiota during pre- and postnatal periods could be important to prevent or reduce the risk of cardiovascular disorders in offspring later in life.^{14,30,31} Dams fed a HFD and supplemented either with a prebiotic (long-chain inulin) or probiotic (*Lactobacillus casei*)³² or *Lactiplantibacillus plantarum* WJL³⁰ during pregnancy and lactation improved maternal gut microbiota diversity and protected male offspring against arterial hypertension and endothelial dysfunction.

Furthermore, it has been demonstrated that oral administration of *Limosilactobacillus fermentum* postweaning up to 90 days of age improved blood pressure and autonomic dysfunction in rat offspring exposed from dams fed a HFD.¹⁴ This study has shown that administration of *Limosilactobacillus fermentum* in adult rats also

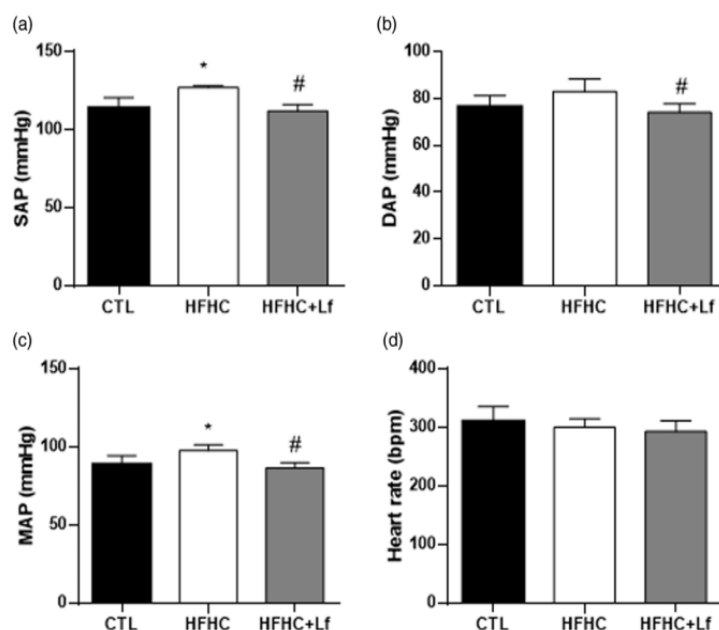


Fig. 3. Effects of a multi-strain formulation with *Limosilactobacillus fermentum* 139, 263, and 296 strains on blood pressure in male offspring of dams fed a HFHC diet during pregnancy and lactation. Assessment of systolic arterial pressure (SAP, A), diastolic arterial pressure (DAP, B), mean arterial pressure (MAP, C) and heart rate (D). Groups: control (CTL); high fat and high cholesterol (HFHC); high fat and high cholesterol receiving *Limosilactobacillus fermentum* 139, 263, and 296 (HFHC + Lf). 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 HFHC or HFHC + Lf and CTL group; # $p < 0.05$ indicates significant difference between HFHC + Lf and HFD group.

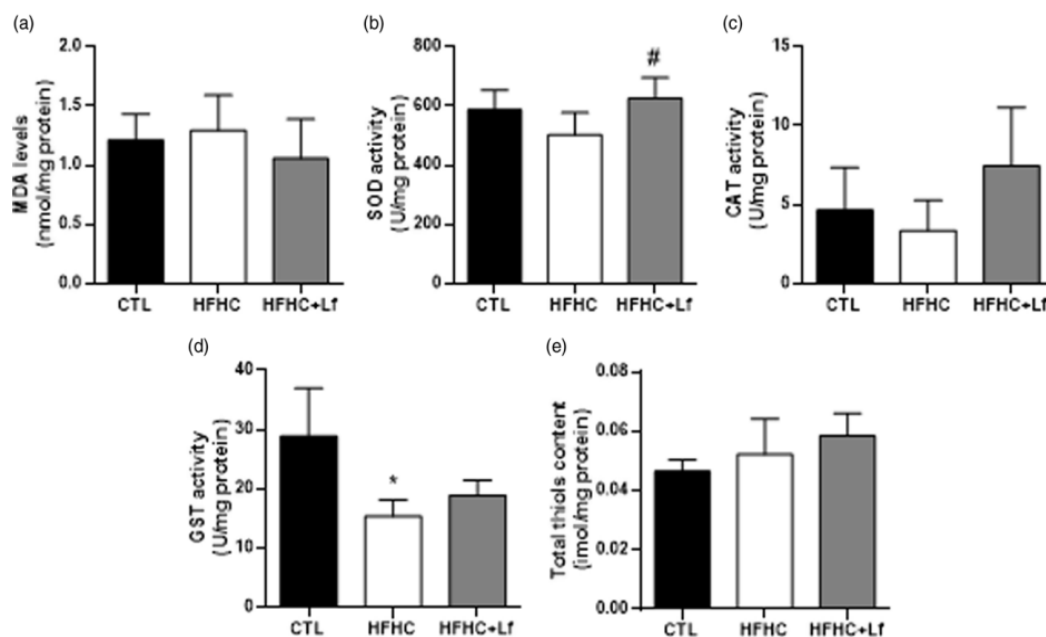


Fig. 4. Effects of a mixed formulation with *Limosilactobacillus fermentum* 139, 263, and 296 on oxidative stress parameters in colon mucosa in male offspring of dams fed a HFHC diet during pregnancy and lactation. Assessment of malondialdehyde levels (MDA, A), superoxide dismutase activity (SOD, B), catalase activity (CAT, C), glutathione S-transferase activity (GST, D), and total thiols content (E) in the colon mucosa. Groups: control (CTL); high fat and high cholesterol (HFHC); high fat and high cholesterol receiving *Limosilactobacillus fermentum* 139, 263, and 296 (HFHC + Lf). Data are presented as mean $\pm$ standard deviation and analyzed by ANOVA one-way test with Tukey as post-hoc test. * $p < 0.05$ indicates significant difference between HFHC or HFHC + Lf and CTL group; # $p < 0.05$ indicates significant difference between HFHC + Lf and HFD group.

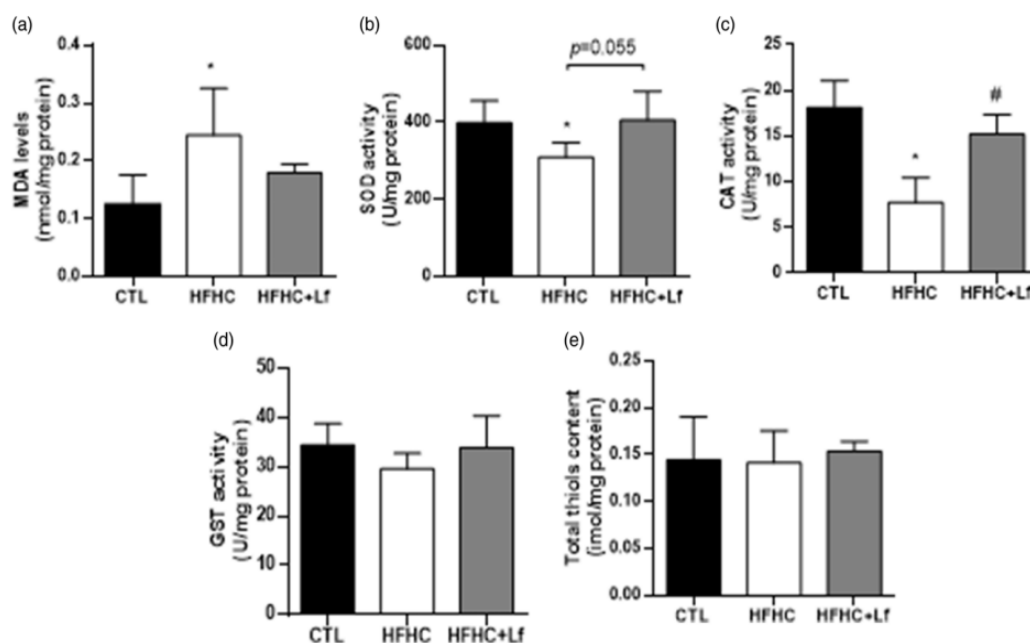


Fig. 5. Effects of a mixed formulation with *Limosilactobacillus fermentum* 139, 263, and 296 on oxidative stress parameters in renal cortex in male offspring of dams fed a HFHC diet during pregnancy and lactation. Assessment of malondialdehyde levels (MDA, A), superoxide dismutase activity (SOD, B), catalase activity (CAT, C), glutathione S-transferase activity (GST, D), and total thiols content (E) in the renal cortex. Groups: control (CTL); high fat and high cholesterol (HFHC); high fat and high cholesterol receiving *Limosilactobacillus fermentum* 139, 263, and 296 (HFHC + Lf). Data are presented as mean $\pm$ standard deviation and analyzed by ANOVA one-way test with Tukey as post-hoc test. * $p < 0.05$ indicates significant difference between HFHC or HFHC + Lf and CTL group; # $p < 0.05$ indicates significant difference between HFHC + Lf and HFD group.

had a hypotensive effect in male offspring from dams fed a HFHC diet during pregnancy and lactation. These results suggest that targeting the gut microbiota on different developmental windows can exert a reprogramming strategy against arterial hypertension induced by maternal HFD consumption.

Although the underlying mechanisms by which *Limosilactobacillus fermentum* reduced blood pressure have not been assessed in the present study, some possible pathways related to probiotic-induced-hypotensive effects have been proposed.³³ First, it has been demonstrated that gut barrier integrity can be rescued by probiotic therapy, reducing LPS-translocation, inflammation, sympathetic activity, endothelial dysfunction, and blood pressure.^{29,34} Second, short-chain fatty acids (SCFA), including acetate, propionate, and butyrate, are produced by specific gut microbes and can be enhanced during probiotic therapy.³⁵ An early study demonstrated that butyrate could lower arterial blood pressure via colon-vagus nerve signaling and GPR41/43 receptors.³⁶ The propionate effect on blood pressure showed that propionate at low concentrations could activate Gpr41 and decrease blood pressure. In contrast, high concentrations can activate Olfr78 and increase blood pressure via renin signaling in the renal juxtaglomerular apparatus.^{37,38} Third, the genus *Lactobacillus* has abundant gamma-aminobutyric acid (GABA)-producing species, including *Limosilactobacillus fermentum*.³⁹ Although the effects of luminal GABA in the gut on blood pressure control are still controversial, there is evidence demonstrating that GABA can bind GABA_B receptors and stimulate 5-HT release, attenuate sympathetic activity, and reduce blood pressure.⁴⁰

In agreement with early studies,^{9,41} maternal HFD diet consumption increased susceptibility to renal dysfunction and oxidative stress in kidney and colon mucosa in offspring later in life. Early investigations have demonstrated that administration of probiotic decrease renal dysfunction^{42,43} and alleviate oxidative stress^{13,44} in rats fed a HFD. For the first time, the results of this study have shown that the administration of a multistrain formulation with *Limosilactobacillus fermentum* with probiotic aptitudes^{14,15} improved renal function and antioxidant capacity along the gut-kidney axis in male offspring from dams fed a HFHD during pregnancy and lactation.

Although underlying mechanisms by which examined *Limosilactobacillus fermentum* formulation increased antioxidant capacity in colon and renal cortex were not explored in the present study, it has been reported MnSODs enzyme activity,¹³ pseudocatalases,⁴⁵ and heme-dependent catalase⁴⁶ for some lactic acid bacteria. This becomes important since SOD has a key role in catalyzes of dismutation of superoxide anion in hydrogen peroxide, while CAT has a fundamental role in cellular detoxification of hydrogen peroxide, promoting a critical oxidative stress tolerance and antioxidant effect.¹³ Second, the effects promoted by the administration of *Limosilactobacillus fermentum* formulation on renal function may be due to their ability to improve impermeability and immune function of the intestinal epithelium, preventing toxic compounds, such as lipopolysaccharides, from reaching the kidneys and causing their deleterious effects.⁴⁷ Lastly, short-chain fatty acid (acetate, propionate, and butyrate) generated from colonic bacterial fermentation can directly affect epigenome through histone posttranslational modifications.⁴⁸ Whether epigenetic pathways are involved in antioxidant capacity provoked by *Limosilactobacillus fermentum* formulation remains to be elucidated.

Although we have recently shown that *Limosilactobacillus fermentum* formulation alleviated gut microbiota impairment in male

rats fed a HFD,⁴⁹ the lack of gut microbiota composition after *Limosilactobacillus fermentum* administration could be considered a bias for the results in this preclinical study.

In conclusion, administration of a multistrain containing three potentially probiotic *Limosilactobacillus fermentum* strains alleviated programmed hypertension, renal dysfunction, and enhanced antioxidant capacity along the gut-kidney axis in male offspring from dams fed a HFHC diet during pregnancy and lactation. These results indicate that gut targeting interventions could be a safe strategy for developmental reprogramming of arterial hypertension.

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Conflicts of interest. The research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Ethical standards. The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national guides on the care and use of laboratory animals (National Council for the Control of Animal Experimentation) and has been approved by the institutional committee of Federal University of Paraíba.

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CONSIDERAÇÕES FINAIS

Neste estudo, a exposição materna a dieta hiperlipídica acarretou em estresse oxidativo no eixo entero-renal e aumentou níveis de pressão arterial na prole. Por sua vez, a administração das cepas de *L. fermentum* 139, 263 e 296 melhorou a capacidade antioxidante, função renal e reduziu níveis de pressão arterial na prole de mães que ingeriram dieta hiperlipídica na gestação e lactação. Nossos resultados reforçam a hipótese que alterações nutricionais em períodos iniciais da vida têm se tornado conhecidas pela capacidade em aumentar risco de doenças crônicas como a hipertensão arterial sistêmica. Concomitantemente, nossos resultados condizem com os estudos que demonstram que a modulação da microbiota pode minimizar os efeitos da inadequada nutrição materna através da ingestão dietética.

Adicionalmente, os efeitos benéficos da administração das cepas de *L. fermentum* apontam para a necessidade de explorá-las como potenciais probióticos na prevenção e no tratamento de doenças crônicas não transmissíveis. O uso das cepas *L. fermentum* 139, 263 e 296 torna possível o desenvolvimento de um produto nacional isolado a partir do reaproveitamento de frutas tropicais, sendo proposto assim que após avaliação de nível de segurança e estudos clínicos eficazes, tais cepas possam ser utilizadas na prática clínica.

ATIVIDADES COMPLEMENTARES DESENVOLVIDAS DURANTE O DOUTORADO

Durante o doutoramento foi possível o desenvolvimento de atividades simultâneas originando a publicação, em colaboração, de dois artigos científicos e três capítulos de livro conforme demonstrados:

- DE LUNA FREIRE, MICAELLE OLIVEIRA ; DO NASCIMENTO, LUCIANA CAROLINE PAULINO ; DE OLIVEIRA, KATARYNE ÁRABE RIMÁ ; DE OLIVEIRA, ALISSON MACÁRIO ; NAPOLEÃO, THIAGO HENRIQUE ; LIMA, MARCOS DOS SANTOS ; LAGRANHA, CLÁUDIA JACQUES ; DE SOUZA, EVANDRO LEITE ; DE BRITO ALVES, JOSÉ LUIZ . **Effects of a Mixed *Limosilactobacillus fermentum* Formulation with Claimed Probiotic Properties on Cardiometabolic Variables, Biomarkers of Inflammation and Oxidative Stress in Male Rats Fed a High-Fat Diet.** *Foods*, v. 10, p. 2202, 2021.
- DE LUNA FREIRE, MICAELLE OLIVEIRA ; DO NASCIMENTO, LUCIANA CAROLINE PAULINO ; DE OLIVEIRA, KATARYNE ÁRABE RIMÁ ; DE OLIVEIRA, ALISSON MACÁRIO ; DOS SANTOS LIMA, MARCOS ; NAPOLEÃO, THIAGO HENRIQUE ; DA COSTA SILVA, JOÃO HENRIQUE ; LAGRANHA, CLÁUDIA JACQUES ; DE SOUZA, EVANDRO LEITE ; DE BRITO ALVES, JOSÉ LUIZ . ***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.** *Probiotics and Antimicrobial Proteins*, v. 13, p. 00-00, 2021.
- DO NASCIMENTO, LUCIANA CAROLINE PAULINO DO; CRUZ NETO, JOSÉ PATROCÍNIO RIBEIRO; ALVES, JOSÉ LUIZ DE BRITO. **Probiotics for liver disease prevention and treatment.** In: SOUZA, E.L.; DE BRITO ALVES, J.L.; FUSCO, V. *Probiotics for Human Nutrition in Health and Disease*. Londres: Academic Press, 2022.
- ALVES, JOSÉ LUIZ DE BRITO; DA COSTA, PAULO CÉSAR TRINDADE; DO NASCIMENTO, LUCIANA CAROLINE PAULINO; DE SOUZA, EVANDRO LEITE. **Probiotics for dyslipidemia prevention and treatment.** In: SOUZA, E.L.; DE

BRITO ALVES, J.L.; FUSCO, V. *Probiotics for Human Nutrition in Health and Disease*. Londres: Academic Press, 2022.

- LACERDA, DIEGO CABRAL; DA COSTA, PAULO CÉSAR TRINDADE; DO NASCIMENTO, LUCIANA CAROLINE PAULINO; ALVES, JOSÉ LUIZ DE BRITO. **Probiotics for gastrointestinal health and disease treatment**. In: SOUZA, E.L.; DE BRITO ALVES, J.L.; FUSCO, V. *Probiotics for Human Nutrition in Health and Disease*. Londres: Academic Press, 2022.

ANEXOS

ANEXO A- Certificado de aprovação do comitê de ética no uso de animais da UFPB



Universidade
Federal da
Paraíba

Comissão de Ética no
Uso de Animais



CERTIFICADO

Certificamos que a proposta intitulada "EFEITOS DA DISLIPIDEMIA MATERNA SOBRE O EIXO ENTERO RENAL: UMA VIA POTENCIAL PARA ELUCIDAR O DESENVOLVIMENTO DA HIPERTENSÃO ARTERIAL NA PROLE DE RATOS", protocolada sob o CEUA nº 9635020519 (ID 000767), sob a responsabilidade de **José Luiz de Brito Alves** - que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica ou ensino - está de acordo com os preceitos da Lei 11.794 de 8 de outubro de 2008, com o Decreto 6.899 de 15 de julho de 2009, bem como com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi **aprovada** pela Comissão de Ética no Uso de Animais da Universidade Federal da Paraíba (CEUA/UFPB) na reunião de 09/08/2019.

We certify that the proposal "EFFECTS OF MATERNAL DISLIPIDEMIA ON THE GUT-KIDNEY AXIS: A POTENTIAL WAY TO ELUCIDATE THE DEVELOPMENT OF ARTERIAL HYPERTENSION IN RAT OFFSPRING", utilizing 128 Heterogenics rats (56 males and 72 females), protocol number CEUA 9635020519 (ID 000767), under the responsibility of **José Luiz de Brito Alves** - which involves the production, maintenance and/or use of animals belonging to the phylum Chordata, subphylum Vertebrata (except human beings), for scientific research purposes or teaching - is in accordance with Law 11.794 of October 8, 2008, Decree 6899 of July 15, 2009, as well as with the rules issued by the National Council for Control of Animal Experimentation (CONCEA), and was **approved** by the Ethic Committee on Animal Use of the Federal University of Paraíba (CEUA/UFPB) in the meeting of 08/09/2019.

Finalidade da Proposta: **Pesquisa (Acadêmica)**

Vigência da Proposta: de **06/2019** a **03/2022** Área: **Nutrição**

Origem:	Unidade de Produção Animal IPeFarM			
Espécie:	Ratos heterogênicos	sexo:	Machos	idade: 22 a 150 dias N: 56
Linhagem:	Rattus Norvegicus - Wistar			Peso: 40 a 400 g
Origem:	Unidade de Produção Animal IPeFarM			
Espécie:	Ratos heterogênicos	sexo:	Fêmeas	idade: 22 a 100 dias N: 72
Linhagem:	Rattus Norvegicus - Wistar			Peso: 40 a 240 g

Local do experimento: Laboratório de Nutrição Experimental (Departamento de Nutrição, Centro de Ciências da Saúde).

João Pessoa, 10 de agosto de 2019

Profa. Dra. Islania Gisela Albuquerque Gonçalves
Coordenadora da Comissão de Ética no Uso de Animais
Universidade Federal da Paraíba

Prof. Dr. Ricardo Romão Guerra
Vice-Coordenador da Comissão de Ética no Uso de Animais
Universidade Federal da Paraíba