UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"

Faculdade de Ciências Farmacêuticas

Efeito da proteína do soro de leite submetida à radiação UV-C na colite induzida em modelo animal

Maria Jara Montibeller

Tese apresentada ao Programa de Pós-graduação em Alimentos e Nutrição para obtenção do título de Doutor em Alimentos e Nutrição.

Área de concentração: Ciência de Alimentos
Orientador: Profª. Drª Daniela Cardoso Umbelino Cavallini
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Atestamos ainda que a obtenção do título dependerá de homologação pelo Órgão Colegiado competente.

Araraquara, 24 de fevereiro de 2023

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RESUMO

A colite ulcerativa é uma doença inflamatória intestinal crônica e redicivante, que acomete parte do cólon, impactando a qualidade de vida do paciente. Diversas estratégias não farmacológicas têm sido utilizadas para auxiliar na modulação da microbiota intestinal e reduzir os sintomas dessa doença, com destaque para a ingestão de alimentos com potencial anti-inflamatório. Entre as estratégias dietéticas, a ingestão de proteína do soro de leite foi associada à modulação da microbiota e do sistema imune, tendo remissão dos sintomas da colite. Além disso, entre as tecnologias não térmicas que podem ser utilizadas para a produção de soro de leite, a luz ultravioleta pode aumentar a digestibilidade proteica, auxiliando na absorção de diversos aminoácidos essenciais. O objetivo do estudo foi avaliar o efeito do consumo de proteína de soro de leite (WP) submetida ao tratamento de luz na região do ultravioleta de 200-280 nm (UV-C) na colite aguda induzida em camundongos. Para isso os animais foram divididos em quatro grupos (n=10): (H) animais sadios sem consumo de WP, (C) animais com colite e sem consumo de WP, (W) animais com colite e que receberam WP não submetido à UV-C e (WU) animais com colite que receberam WP submetido à UV-C. O protocolo teve duração total de 14 dias, sendo que o WP foi ingerido durante todo o período experimental e a indução da colite pela ingestão de dextran sulfato de sódio ocorreu a partir do sétimo dia. A evolução da colite foi acompanhada pela determinação do índice de atividade da doença (IAD), variação da relação peso e comprimento e análise histopatológica do cólon, determinação da concentração de citocinas pró e anti-inflamatórias, análise da composição da microbiota fecal ao longo do experimento e análises metabolômicas nos rins e plasma após eutanásia. A determinação de metabólitos foi feita por Espectroscopia de Ressonância Magnética Nuclear de Prótons (RMN de 1H) e a determinação da composição da microbiota fecal foi realizada em sequenciador de nova geração (IlluminaMiseq). O nível de 5% de significância ($p \leq 0,05$) foi adotado para todas as tomadas de decisão. Considerando a análise do conjunto de sintomas do IAD, o grupo WU resultou em menor progressão da doença. Os diferentes grupos experimentais não apresentaram diferenças significativas em relação às concentrações de IL-10 e TNF-α. Contudo, os níveis das citocinas inflamatórias IL-2 e IL-6 foram superiores no grupo induzido à colite e sem tratamento ($p < 0,05$). Não houve alteração na diversidade e riqueza da microbiota antes e após indução da colite nos grupos pertencentes às dietas WP (número de observações). Os resultados revelaram que cada um dos suplementos de WP modula a microbiota intestinal durante a inflamação de maneiras diferentes, com destaque para WP tratados com UV-C que apresentaram menor regressão da progressão nos parâmetros clínicos e conservação na abundância relativa dos gêneros. Além disso, a ingestão de WP tratado com UV-C resultou em modulação positiva da MI, com destaque para aumento de Turicibacter spp. e recuperação da família Ruminococcaceae, Lachnospiraceae_UCG_001 e Blautia, o que provavelmente contribuiu para o controle da colite. As análises de metabólitos nos rins não evidenciaram uma sobrecarga das funções renais devido

consumo de WP, e foram encontradas alterações em rotas metabólicas, que podem ter sido uma forma de reduzir a progressão da doença. A maior parte dos componentes encontrados nos rins foram estatisticamente diferentes em suas concentrações, quando comparados os grupos controle H e C ($p < 0,05$). O grupo W não apresentou diferenças estatísticas com grupo saudável em relação à asparagina, alanina, dimetilamina, isoleucina, fenilalanina e valina. Os resultados da análise de metabólitos no plasma sugeriram que este não é um biomarcador ideal para a colite, devido à semelhança entre os grupos que receberam os tratamentos e o grupo controle. Por fim, pode-se concluir que o consumo de W e WU reduziram a progressão da doença, porém com diferentes respostas nas vias metabólicas e na microbiota intestinal, constituindo uma opção de suplemento alimentar para auxiliar no controle dos sintomas da colite.

Palavras–chave: Colite ulcerativa. Soro de leite. Metabolômica. Microbiota intestinal. Tratamento por ultravioleta.

ABSTRACT

Ulcerative colitis is a chronic and relapsing inflammatory bowel disease that affects part of the colon, impacting the patient's quality of life. Several non-pharmacological strategies have been used to help modulate the intestinal microbiota and reduce the symptoms of this disease, with emphasis on the intake of foods with anti-inflammatory agent. Among dietary strategies, whey protein intake was associated with modulation of the microbiota and the immune system, with remission of colitis symptoms. Also, among the non-thermal technologies that can be used for the production of whey, ultraviolet light can increase protein digestibility, aiding in the absorption of several essential amino acids. The aim of the study was to evaluate the effect of consumption of whey protein (WP) subjected to light treatment in the ultraviolet region of 200-280 nm (UV-C) on acute colitis induced in mice. In this regard, the animals were divided into four groups (n=10): (H) healthy animals without WP consumption, (C) animals with colitis and without WP consumption, (W) animals with colitis and that received WP not submitted to UV-C and (WU) animals with colitis that received WP subjected to UV-C. The protocol had a total duration of 14 days. The WP was ingested throughout the experimental period and the induction of colitis by ingestion of sodium dextran sulfate occurred from the seventh day. The evolution of the colitis was followed by the determination of the disease activity index (DAI), variation in the weight and length ratio and histopathological analysis of the colon, determination of the concentration of pro and anti-inflammatory cytokines, analysis of the composition of the fecal microbiota throughout the experiment and metabolomic analysis of kidneys and plasma after euthanasia. The determination of metabolites was performed by Proton Nuclear Magnetic Resonance (1H NMR) and the determination of fecal microbiota composition was performed in a new generation sequencer (IlluminaMiseq). The 5% significance level ($p \leq 0.05$) was adopted for all tests hypothesis. Considering the analysis of the set of DAI symptoms, the WU group resulted in less disease progression. The different experimental groups did not show significant difference in relation to the concentrations of IL-10 and TNF-α. However, the levels of the inflammatory cytokines IL-2 and IL-6 were higher in the colitis-induced and untreated groups ($p < 0.05$). There was no change in the diversity and richness of the microbiota before and after colitis induction in the groups belonging to the WP diets (number of observations). The results revealed that each of the WP supplements modulates the gut microbiota during inflammation in different ways, but UV-C-treated WP was more effective, considering less progression in clinical parameters and conservation in the relative abundance of the genera. In addition, the ingestion of WP treated with UV-C resulted in a positive modulation of gut microbiota, with emphasis on the increase of Turicibacter spp. and abundance recovery in the families Ruminococcaceae , Lachnospiraceae_UCG_001 and Blautia, which probably contributed to the control of colitis. Metabolites analysis in the kidneys did not show an overload of renal functions due to WP consumption, and alterations in metabolic pathways were found, which may have been a way to reduce the progression of the disease. Most of the components found in the kidneys were statistically

different in their concentrations when comparing the control groups ($p < 0.05$). The W group did not show statistical differences with the healthy group in relation to asparagine, alanine, dimethylamine, isoleucine, phenylalanine and valine. Results of plasma metabolite analysis suggested that this is not an ideal biomarker for colitis. Finally, it can be concluded that the consumption of W and WU reduced the progression of the disease, but with different responses in the metabolic pathways and in the intestinal microbiota, being an option for a food supplement to help control the symptoms of colitis.

Keywords: Ulcerative colitis; Whey protein; Metabolomics; Gut microbiota; Ultraviolet treatment.

LISTA DE ABREVIATURAS E SIGLAS

16S rRNA	RNA ribossomal 16S
CLA	*cis-9,trans-11 conjugated linoleic acid*
CU	Colite ulcerativa
Cys	Cisteína
DC	Doença de Crohn
DII	Doenças Inflamatórias Intestinais
DNA	Ácido desoxirribonucleico
DNBS	Ácido dinitrobenzeno sulfônico.
DSS	Dextran sulfato de sódio
ESPE	*European Society for Clinical Nutrition and Metabolism*
HTST	High Temperature, Short Time
IAD	Índice de Atividade da Doença
IL-10	Interleucina 10
IL-6	Interleucina 6
LTLT	*Low Temperature, Long Time*
MALDI-TOF/TOF	*Matrix Assisted Laser Desorption Ionization Time Of Flight Mass Spectrometry*
MUC2	Mucina 2
NFK	N-formilquinurenina
nLC-ESI-MS	*Nano-scale liquid chromatographic tandem mass spectrometry*
NOD2	*Nucleotide-Binding Oligomerization Domain* 2
OTU	*Operational taxonomic units*
REG3γ	*Regenerating islet-derived protein 3*
RMN de H^1	Espectroscopia de ressonância magnética nuclear de prótons
RNA	Ácido ribonucleico
RSSR•-	Ânion radical
-SH	Pontes de dissulfeto
Th1	*T helper* 1
Th17	*T helper* 17
TNBS	Ácido 2,4,6 trinitrobenzeno sulfônico
Treg	Células T reguladoras
Trp	Triptofano
UHT	*Ultra High Temperature*
UV	Luz ultravioleta
UV-C	Luz na região do ultravioleta de 200-280 nm
UV-vis	Luz ultravioleta visível
α-La	α-lactalbumina
B-Lg	β-lactoglobulina

LISTA DE SÍMBOLOS

kg	Quilograma
s	Segundo
g	Grama
W / m^2	Potência por área em metros
W / cm^3	Potência por área em centímetros
g / L	Gramas por litro
kDa	Quilo Dalton
nm	Nanômetro

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1 INTRODUÇÃO EXPANDIDA

1.1 Soro de leite: métodos de produção

O leite é uma importante fonte de proteína (30 a 36 g/L) com alto valor nutricional, sendo composta aproximadamente por 80% de caseína e 20% de proteínas presentes em seu soro [1,2].

A porção aquosa do leite, conhecida como soro de leite, possui em sua composição proximal cerca de 94% de água, 5% de lactose e 1% de proteínas. Além desses, há presença de traços de minerais, ácidos cítrico e lático [3].

A fração proteica do soro de leite é utilizada das mais diversas formas na indústria de alimentos e é composta por β-lactoglobulina (β-Lg), α-lactalbumina (α-La) e pequenas concentrações de soroalbumina, peptonas e imunoglobulinas. Essas proteínas são formadas principalmente por lisina, triptofano, isoleucina e treonina, o que torna o soro uma fonte de aminoácidos essenciais [2,4].

A β-Lg possui um peso molecular de aproximadamente 18,4 kDa, que proporciona uma resistência às enzimas proteolíticas presentes no estômago, sendo absorvida apenas no intestino. É composta por 16 tipos de aminoácidos, totalizando 178 aminoácidos, com a presença de duas pontes de dissulfeto (-SH). Essa proteína globular, com sua formação principal em β-

barril, corresponde a cerca de 50% do total de proteínas presentes no soro de leite bovino, e sua desnaturação completa ocorre a partir de 65°C [4,5,6].

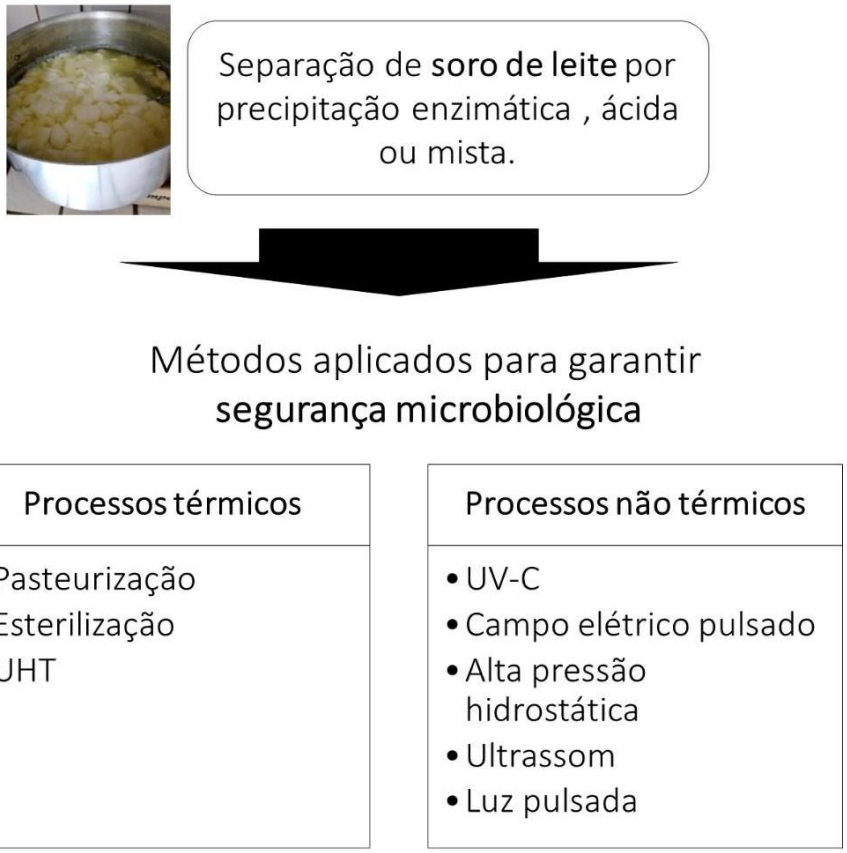
A α-La é a segunda proteína mais encontrada no soro de leite bovino, representando cerca 20% do total de proteínas desse produto. Com peso molecular de aproximadamente 14,2 kDa, é definida como uma metaloproteína, pois contém íons metálicos que se ligam a átomos de cálcio. Sua composição conta com um total de 142 aminoácidos, constituída por 19 diferentes aminoácidos e possui a maior quantidade de triptofano dentre todas as fontes proteicas (aproximadamente 6%). A estrutura terciária dessa proteína inclui um domínio helicoidal e um pequeno domínio β-folha. Por fim, apresenta instabilidade frente ao tratamento térmico, sendo totalmente desnaturada quando exposta a temperaturas iguais ou superiores a 90°C por mais de 30 minutos [4,5,7].

O soro de leite é considerado um ingrediente fundamental na indústria de alimentos e exibe propriedades funcionais como gelificação, emulsificação e capacidade de formação de espuma. Esse pode ser submetido a operações unitárias para obtenção de diferentes produtos, como *spray drying* (proteína de soro concentrada), microfiltração (proteína de soro isolada) e aplicação de hidrólise enzimática (hidrolisados de proteína de soro de leite)[8]. As concentrações de proteínas obtidas por essas operações variam de 35% a 80% em soro de leite concentrado, enquanto o soro de leite isolado pode conter até 90% [8]. Essas variações nas concentrações de proteínas afetam diretamente as propriedades físico-químicas dos produtos. Por exemplo, o soro de leite isolado tem características de gel sob condições de baixa

temperatura, o que altera as interações hidrofóbicas e as funcionalidades da proteína [8]. Com isso, para otimizar a funcionalidade do produto, é fundamental selecionar matéria-prima e processamento adequados, incluindo os métodos de obtenção e conservação aplicados para fornecer componentes biodisponíveis e segurança alimentar [5].

A primeira etapa para obtenção das proteínas do soro de leite envolve a precipitação ácida e/ou enzimática da caseína, separando-a da fração solúvel (soro de leite) [5]. Antes de ser concentrado ou desidratado, o soro de leite deve ser submetido a tratamentos capazes de garantir a sua segurança microbiológica [9]. O resumo sobre os principais métodos utilizados na produção de soro de leite pode ser visualizado na Figura 1. Além desses, outros processos como precipitação, complexação e separação por cromatografia podem ser utilizados [10].

Figura 1 – Principais processamentos aplicados ao soro de leite para garantir a sua segurança microbiológica.



Fonte: Elaborado pelo autor Adaptado de TUNICK, 2008 [11] apud MARWAHA; KENNEDY, 1988 [12]; SISO, 1996 [13]; ABD EL-SALAM; EL-SHIBINY; SALEM, 2009 [8]; ORDÓÑEZ et al., 2005 [5]. Notas: UHT –*Ultra High Temperature*; UV-C – Aplicação de luz na região do ultravioleta de 200 - 280 nm.

As principais tecnologias utilizadas para garantir a segurança do soro de leite para o consumo humano envolvem o uso de temperaturas elevadas (esterilização e *Ultra High Temperature* - UHT), ou mais brandas

(pasteurização). A pasteurização é subdividida em "*Low Temperature, Long Time*" (LTLT - 63°C, 30 min) e "*High Temperature, Short Time*" (HTST - 72°C – 15 s), conferindo ao produto um prazo de validade de três a seis dias, sob refrigeração. Os processos que envolvem o uso de temperaturas mais altas, como a esterilização (120°C – 20 min) e UHT (130-150°C – 3 a 5 s) aumentam a estabilidade microbiológica do produto, que permanece em condições ideais de consumo por quatro meses, sem a necessidade de refrigeração [5].

Apesar de sua eficácia para controlar a contaminação e prolongar a vida útil dos alimentos, os processos térmicos podem afetar a digestibilidade gastrointestinal e a alergenicidade das proteínas do soro de leite [14,15]. As principais alterações físico-químicas observadas são a desnaturação, agregação, reação de Maillard, redução de lisinas, interações proteína-lipídeo ou proteína-proteína, redução de compostos bioativos, além de promoverem alterações sensoriais no produto final [16].

Com isso, métodos de conservação não térmicos têm sido propostos como alternativa para garantir segurança microbiológica, sem alterar negativamente as propriedades tecnológicas, sensoriais e funcionais do soro de leite [17,18]. Os métodos não térmicos com maior destaque atualmente são: alta pressão hidrostática [19], exposição ao campo elétrico pulsado [20], ultrassom, luz pulsada e a luz ultravioleta (UV) [21].

A alta pressão hidrostática utiliza pressão em um sistema fechado por redução mecânica de volume e gera um aumento moderado na temperatura (geralmente adicionado por aquecimento adiabático), o que lhe confere vantagens nos aspectos sensoriais dos produtos alimentícios [22]. Zeece,

Huppertz e Kelly (2008) estudaram o efeito desse tratamento na digestibilidade *in vitro* da β-lactoglobulina e demonstraram um aumento na digestibilidade e acessibilidade dos grupos tióis [23].

Na exposição aos campos elétricos pulsados, a inativação microbiológica também ocorre em temperaturas mais baixas. Essa tecnologia é baseada na eletroporação para permeabilizar as membranas celulares. Os principais parâmetros a serem controlados são a intensidade do campo elétrico, tempo, formato e largura do pulso [24]. Em relação a sua ação no soro de leite, quando comparadas concentrações de 5% e 3% de soro de leite isolado, a porção mais rica em proteínas (5%) causou menor intensidade de fluorescência intrínseca relativa [25]. Além disso, esse processo apresentou a capacidade de inativar a *Listeria innocua*, um dos indicadores de inativação microbiológica eficiente [26].

O tratamento com ultrassom é dividido em duas categorias: uma que utiliza ondas sonoras mais altas (>3 W/cm^2) e outra com uso de ondas sonoras baixas (<3 W/cm^2). Ambas possuem capacidade de alterar aspectos físico-químicos do material e podem ser aplicadas em temperatura ambiente ou combinadas com altas temperaturas [27]. Jambrak et al. (2008) compararam o efeito dos dois tipos de ondas sonoras e concluíram que o ultrassom com alta potência melhora as propriedades físicas, como solubilidade e capacidade de formação de espuma da proteína do soro de leite [28].

No tratamento com luz pulsada, o equipamento utiliza uma lâmpada xenon e pulsos de curta duração [29]. Esse processo foi associado à melhora de muitas propriedades físico-químicas do soro de leite, incluindo solubilidade

e propriedades emulsificantes [30]. Além disso, Siddique, Maresca, Pataro e Ferrari (2017), após analisar as mudanças estruturais e demonstrar o aumento imediato do conteúdo de tióis livres em soro de leite isolado, levantou a hipótese que a desnaturação parcial pode melhorar as propriedades funcionais e tecnológicas desse produto [31].

Por fim, no Quadro 1 são apresentadas algumas vantagens associadas à utilização desses métodos. O tratamento por luz ultravioleta será tratado em seção a seguir.

Quadro 1 – Resumo dos efeitos físicos e na bioatividade de soro de leite obtido por diferentes processos não-térmicos.

Processo	**Efeitos**	**Referências**
Alta pressão hidrostática	Aumento da digestibilidade de β-Lg (em condições gástricas *in vitro*).Retenção de sabor e coloração características do produto.	22,23
Exposição ao campo elétrico pulsado	Aumento da solubilidade e poder de emulsificação do produto.Sem ação negativa em compostos bioativos presentes no soro.	25, 26, 30
Ultrassom	Aumento da solubilidade da proteína. Diminuição da turbidez.Alterações físicas que promovem a bioatividade.	28, 32,33
Luz Pulsada	Melhoria na solubilidade, na agregação de monômeros desdobrados por meio de interações hidrofóbicas e na formação de espuma. Essas mudanças podem melhorar as propriedades funcionais.	31,34,35
Exposição à luz ultravioleta	Perdas sensoriais e nutricionais menores que em processos térmicos. Ausência de formação de compostos tóxicos.Aumento da hidrólise pela ação da pepsina.Redução na desnaturação, quando comparada com UHT.	36,37

Fonte: Elaborado pelo autor.

1.1.1 Aplicação de Luz na região do ultravioleta de 200-280 nm (UV-C) em soro de leite

Dentre as tecnologias não térmicas que podem ser utilizadas para a produção de soro de leite, a luz ultravioleta (UV) destaca-se por sua comprovada ação no alimento, garantindo a segurança do alimento [36]. O tratamento por UV pode ocorrer em diversos comprimentos de ondas: ondas curtas (UV-C) - 200 a 280 nm, ondas médias (UV-B) - 280 a 320 nm e ondas longas (UV-A) - 320 a 400 nm. O mecanismo de ação da luz UV envolve absorção da radiação pelo material genético da célula do microrganismo e a formação de fotoprodutos (dímeros de ciclobutano pirimidina e (6-4)-pirimidina-pirimidona). Os fotoprodutos formados inibem a transcrição e replicação do DNA, além de poder causar mutagênese e morte celular [38,39].

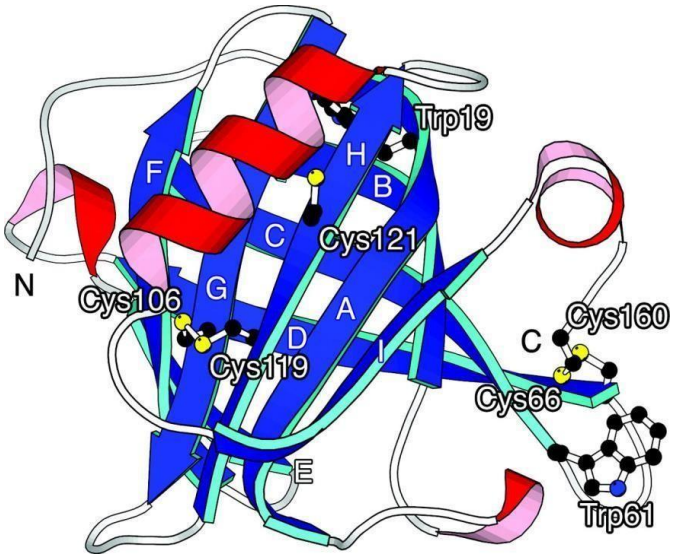
A região UV-C é considerada a mais eficiente na conservação de alimentos, pois corresponde ao comprimento de onda mais absorvido pelo DNA [36]. Entretanto, esse processo é mais recomendado para alimentos líquidos ou com pequenos sólidos em suspensão, pois possui baixo poder de penetração e necessita de equipamentos operados sob turbulência, para facilitar a ação no alimento [40].

O tratamento por UV-C é qualitativamente comparável aos processos de conservação térmicos. Cappozzo, Koutchma e Barnes (2015) compararam o efeito de UHT, HTST, UV-C (254 nm, 1,045 e 2,090 J/L) e diferentes combinações entre HTST e UV-C na conservação e composição do leite [41]. Os autores observaram que o UV-C, sozinho ou em associação com HTST,

não alterou a composição proximal, a oxidação de lipídios e o perfil de ácidos graxos e de proteínas ao longo dos 14 dias de armazenamento do produto. Nas condições do estudo, a tecnologia não térmica apresentou potencial para ser usada como alternativa para aumentar a vida útil do leite. Outro estudo conduzido por Buhler et al. (2019) analisou a eficiência do uso do tratamento UV-C (253,7 nm) na qualidade e no valor nutricional de soro de leite desnatado e concentrado [42]. O estudo concluiu que a segurança microbiológica e a qualidade nutricional do soro de leite desnatado foram preservadas, inclusive exibindo maior concentração de proteínas solúveis em comparação com a amostra submetida ao tratamento térmico. Para a amostra de soro de leite concentrado, os autores sugerem uma otimização na configuração do reator para melhorar a segurança do produto final.

Além disso, o processo UV-C gera redução de -SH nas pontes de tióis pela transferência direta de elétrons da cadeia lateral e de triptofanos excitados próximo às pontes. Com isso, o estado triplete de um triptofano transfere elétron diretamente para a ligação de dissulfeto, formando um ânion radical (RSSR•-) e ocasionando a quebra da ponte de dissulfeto, o que expõe mais facilmente a β-Lg a reações enzimáticas (quebra parcial de enovelamento) (Figura 2) [43].

Figura 2 – Estrutura da β- Lg. As ligações das duas pontes de dissulfeto (Cys-66 / Cys-160, Cys-106 / Cys-119), um resíduo de cisteína livre (Cys121), dois resíduos de triptofano (Trp-19, Trp-61) e cadeias presentes na estrutura (A até I).



Fonte: YAGI et al., 2003 [44]. O diagrama foi criado com uso do programa Molscript (KRAULIS, 1991) [45] e possui código 1BEB (3) do Protein Data Bank.

Em estudo realizado por Cardoso et al. (no prelo), constatou-se que o tratamento por UV-C (266 nm, 4 W/m2) resulta em mudança conformacional das proteínas majoritárias do soro do leite em solução aquosa (especialmente da β-Lg), com aumento de 127% na sua digestibilidade e mudança no perfil de aminoácidos [46]. O tratamento não térmico provocou alterações na estrutura secundária da β-Lg, com redução significativa na contribuição da estrutura em folha e volta β. Tais alterações foram induzidas pela clivagem de -SH de β-Lg (Cys160 - Cys66 e Cys106 - Cys119), com um rendimento quântico de 0,0015 ±0,0003. Os resultados de espectroscopia de luz ultravioleta visível (UV-Vis)

e de emissão molecular de fluorescência sugerem ainda a formação de N-formilquinurenina (NFK), como principal produto de fotooxidação por triptofano-61. Apesar de não existir consenso entre os pesquisadores, dados da literatura apontam um efeito positivo de metabólitos do triptofano, em especial da quinurenina, na colite induzida em modelo animal, via inibição da resposta inflamatória [47,48]. O perfil de peptídeos liberados durante a digestão *in vitro* da proteína modificada por UV-C foi determinado por MALDI-TOF/TOF e nLC-ESI-MS. Os resultados evidenciaram que o perfil de aminoácidos da proteína irradiada é significativamente diferente da amostra não irradiada, com maior abundância de peptídeos com bioatividade (por homologia) anti-hipertensiva, antioxidante, imunomoduladora e anti-inflamatória [49].

1.2 Doenças Inflamatórias Intestinais (DII): Colite Ulcerativa

As doenças inflamatórias intestinais (DII) são caracterizadas por inflamações crônicas e recidivantes, que podem acometer parte (colite ulcerativa - CU) ou todo o trato intestinal (doença de Crohn - DC)[50].

Uma revisão sistemática conduzida por Alatab et al. (2020) avaliou a evolução global das DII entre os anos de 1990 e 2017, envolvendo 195 países [51]. Os resultados evidenciaram um aumento de cerca de 85% na prevalência dessa doença inflamatória comparado ao período inicial do estudo, totalizando cerca de 6 milhões de pessoas acometidas por DII no mundo.

Os sintomas da CU e DC são semelhantes e incluem diarreia, dor abdominal, presença de sangue nas fezes e perda de peso [50]. Os fatores

envolvidos na iniciação e progressão das DII envolvem uma associação entre predisposição genética, fatores ambientais (dieta, tabagismo, região geográfica, situação econômica e social e uso de drogas), resposta imune desregulada e composição do microbioma intestinal [52,53,54].

Entre os fatores de predisposição genética, destacam-se as mutações em alguns genes, como domínio de oligomerização de ligação a nucleotídeos (*Nucleotide-Binding Oligomerization Domain* 2 - NOD2) e genes relacionados à autofagia (ATG16L1, IRGM, XBP1 e LRRK2), que estão associados ao desenvolvimento de DII. Essas alterações, juntamente com os fatores ambientais (dieta, uso de antibióticos e tabagismo), podem favorecer a disbiose intestinal (desequilíbrio da composição e funcionalidade da microbiota intestinal), a penetração e a translocação de microrganismos que irão participar do processo inflamatório associado à colite [55]. No processo inflamatório observa-se hiperativação dos linfócitos T CD4 de padrão das células *T helper* 1 (Th1) e *T helper* 17 (Th17), alteração nas proteínas de junção e oclusão, aumento da permeabilidade intestinal, redução de células Treguladoras (Treg), de proteína 3 (peptídeo antimicrobiano) derivada de ilhotas em regeneração (*Regenerating islet-derived protein 3 - REG3γ*) e da citocina anti-inflamatória IL-10 [56].

Por ser considerada uma doença multifatorial, de difícil diagnóstico e cuja patogênese não é completamente conhecida, a colite tem sido alvo de estudos que avaliaram diferentes terapias (tanto medicamentosas como alimentares), que possam auxiliar na melhora da qualidade de vida do paciente [52,53,54].

Os tratamentos convencionais para DII são baseados na gravidade dos sintomas e incluem anti-inflamatórios, antibióticos, imunossupressores ou agentes biológicos (como a terapia monoclonal, como uso de anti-TNF). No entanto, não existe uma terapia universalmente eficaz ou aceita, e muitos pacientes apresentam efeitos adversos que afetam a continuidade do tratamento [56,57]. Por exemplo, o tratamento com imunossupressores, pode favorecer infecções e até levar a óbito, caso não ocorra um acompanhamento correto [59]. O uso de sulfassalazina pode causar erupção cutânea, pancreatite, pneumonite e agranulocitose, enquanto o tratamento prolongado com corticosteróides pode favorecer o desenvolvimento de obesidade, hipertensão, diabetes, catarata, glaucoma, depressão, ansiedade, insônia, irritabilidade e necrose avascular [60]. Além disso, muitos dos tratamentos clínicos perdem eficácia ao longo do tempo, sendo que pesquisadores da área recomendam a procura de terapias individualizadas com auxílio de uma equipe médica multidisciplinar [61].

As pesquisas iniciais para avaliação da ação das terapias alternativas são realizadas em modelos animais, envolvendo majoritariamente o uso de ratos ou camundongos. Esses animais podem ser manipulados geneticamente, imunodeficientes ou induzidos à doença pela ingestão de compostos químicos ou microrganismos capazes de promover a inflamação intestinal [58]. Na indução química, as cobaias recebem uma dose específica de determinado substrato (oxazolona, ácido acético, carragenina, peptidoglicano, anti-inflamatórios não-esteroidais, dextran sulfato de sódio - DSS ou ácido 2,4,6 trinitrobenzeno sulfônico – TNBS), que promove a inflamação [62]. TBNS e DSS são os compostos mais recomendados na indução da colite em modelo animal e amplamente aplicados em estudos de DII [63,64,65,66,67]. Entre esses, o uso de DSS destaca-se como o mais popular, devido a sua simplicidade e reprodutibilidade [68].

A verificação de eficácia do tratamento ou suplementação alimentar em animais induzidos à colite envolvem a determinação do índice de atividade da doença (IAD), perfil de citocinas pró e anti-inflamatórias, análise histológica de porções do cólon e composição da microbiota intestinal [69].

1.2.1. Microbiota intestinal de DII

Pacientes acometidos por DII apresentam modificações na composição da microbiota intestinal e inibição na produção ou degradação de mucinas intestinais (MUC 2) – proteínas glicosiladas que protegem a superfície da mucosa [70,71].

A microbiota intestinal e os seus metabólitos influenciam inúmeras funções vitais e estão envolvidos na manutenção da integridade da mucosa intestinal e da homeostase do sistema imune [72,73]. Possíveis alterações em sua composição podem auxiliar no entendimento da colite e no estabelecimento de tratamentos ou abordagens dietéticas adequadas [74].

O termo microbiota está relacionado a uma comunidade de microrganismos, comumente quantificada por métodos independentes de cultivo, baseados em seu material genético [75]. A microbiota intestinal humana é composta por centenas de espécies, que conferem ao hospedeiro uma identidade própria. Estudo metagenômico de Arumugam et al. (2011) concluiu que a mesma pode ser dividida em três “*Clusters*” ou "entererótipos" identificados pela variação na população de *Bacteroides* spp. (*Enterotype* 1), *Prevotella* spp. (*Enterotype* 2) e *Ruminococcus* spp. (*Enterotype* 3)[75]. Essa

divisão tem sido questionada por sua incapacidade de representar uma comunidade dinâmica e outras classificações, baseadas em combinações ou gradientes de filos e gêneros, foram propostas [76,77]. Apesar de ser criticada por alguns pesquisadores, essa segmentação em “enterótipos” explicaria, ao menos parcialmente, as diferenças observadas em estudos clínicos com intervenções dietéticas ou medicamentosas [75]. Novas técnicas de bioinformática para identificar essas particularidades e, principalmente compreendê-las, ainda estão em seus passos iniciais [78,79,80].

Pacientes com DII exibem modificação na diversidade e predominância na microbiota intestinal, sendo comum a redução de *Firmicutes* e aumento de *Enterobacteriaceae* [71,81,82]. Apesar da participação da microbiota na etiologia da colite ser inegável, não está completamente estabelecido se tais alterações são causa ou consequência da colite, sendo necessários maiores estudos acerca do tema [72].

Com isso, destaca-se a importância de coletar dados para buscar saber quais são as modificações na microbiota intestinal que ocorrem durante a progressão da doença e quais são os diversos gêneros bacterianos envolvidos nesse mecanismo [83].

Heenan et al., (2020), em sua revisão sobre o papel da microbiota intestinal nas DII, destacaram três tópicos que devem ser priorizados: as diferenças na composição da microbiota entre os diversos subtipos de DII e modelos saudáveis; como a proporção de “más” e “boas” bactérias influencia a progressão da doença; e aprimoramento de técnicas de sequenciamento genético (como 16S rRNA), pois as técnicas dependentes de cultura

convencionais não são apropriadas para estudar a complexidade da microbiota intestinal [74].

1.2.2.Tecnologias ômicas nos estudos das DII

As tecnologias ômicas, que englobam a genômica (metataxonômica e metagenômica), transcriptômica, proteômica e metabolômica, oferecem instrumentos para estudar a estrutura e função de um sistema biológico, de forma integrada. O sequenciamento de DNA e RNA é muito utilizado para a análise do genoma, enquanto técnicas de espectrometria de massa e espectroscopia de ressonância magnética nuclear de prótons (RMN de ^{1}H) são escolhidas para avaliar o proteoma e o metaboloma [84].

As principais estratégias de sequenciamento de alto rendimento (*High throughput sequencing*) utilizadas para a análise de comunidades microbianas, usando plataformas de sequenciamento de nova geração (*Next-generation sequencing*) compreendem a metataxonômica (sequenciamento de amplicon de genes alvo) e a metagenômica (sequenciamento do DNA isolado de toda a comunidade microbiana). A metataxonômica é a técnica mais utilizada para o estudo da composição da microbiota, pois permite a execução eficiente de muitas amostras a custos relativamente baixos em comparação com as técnicas de metagenômica [85]. Esta consiste no agrupamento de sequências similares com limiar de circunscrição definido (entre 97 a 99%), em unidades taxonômicas operacionais (*Operational taxonomic units* - OTU), baseado (*open-reference*) ou não (*closed-reference*)

em bancos de dados de sequências referenciais. As OTU são comparadas com esses bancos de dados taxonômicos, como SILVA ou *Greengenes*, onde recebem a atribuição taxonômica à qual representam [85].

Além disso, essa análise permite a compreensão da diversidade alfa e beta. De forma resumida, a alfa diversidade pode ser definida como a diversidade dentro de um ecossistema específico e geralmente é expressa pelo número de espécies no mesmo (ou em um grupo do experimento). Enquanto a beta diversidade examina a mudança na diversidade de espécies entre esses ecossistemas (comparação de diferentes grupos do experimento) [86].

As análises metagenômicas e metataxonômicas, juntamente com análises metabolômicas abrem novas possibilidades de compreensão da etiologia das DII. O avanço nessas técnicas possibilita o entendimento global sobre a relação das rotas metabólicas, suas funções e estruturas em comparação com os achados na microbiota [87,88].

Nese contexto, a RMN de ^{1}H é uma técnica espectroscópica com objetivo de verificar campos magnéticos locais ao redor de núcleos atômicos e tem como principais vantagens a precisão e exatidão na medição de macro e micromoléculas, além de ser uma análise não destrutiva e de fácil preparação das amostras [89].

A RMN de ^{1}H é considerada uma potente técnica para a análise de metabólitos (metabôlomica), e utilizada para auxiliar no entendimento das vias metabólicas associadas a iniciação e progressão das DII. Esta mostrou-se aplicável na separação de grupos saudáveis e entre grupos de modelo animal

empregados a diferentes subtipos da doença, além de fornecer importantes informações sobre o desenvolvimento da mesma [90]. Nesse aspecto, essa análise tem demostrado grandes avanços na identificação de alterações metabólicas e há uma tendência em torná-la uma ferramenta preditiva pois, estas assinaturas metabólicas podem auxiliar no diagnóstico precoce de doenças [91].

1.3 Suplementação com proteína do soro de leite e seu impacto nas DII

Apesar de não existir consenso sobre o perfil da microbiota associado à colite, diferentes estratégias têm sido utilizadas para auxiliar na modulação desse ecossistema, objetivando a redução dos sintomas da doença. Entre as estratégias não farmacológicas utilizadas para modular a microbiota destacam-se a restrição calórica, a ingestão de microrganismos probióticos e de substâncias prebióticas e a suplementação com vitamina D [72]. Alguns estudos também evidenciam um efeito positivo da ingestão de dietas contendo proteínas específicas, como β-caseínas, proteínas do soro de leite e peptídeos hidrolisados presentes em produto lácteos, como adjuvante no controle da colite [67,92].

O soro de leite bovino possui aminoácidos essenciais com diversos benefícios na regressão de DII. Entre eles estão a histidina (associado à remissão de danos ocorridos na parede do cólon e diminuição da citocina IL-6), a metionina (envolvido na produção de glutationa, um potente antioxidante)

e a treonina (essencial para a manutenção da integridade da mucosa intestinal) [93,94]. Outros aminoácidos como leucina, isoleucina e valina também possuem propriedades anti-inflamatórias e podem reduzir alguns dos sintomas da DII [95,96]. Martínez-Maqueda et al. (2013) concluíram que a ingestão regular de um concentrado de soro de leite hidrolisado e rico em β-Lg estimula a secreção de mucina e a expressão gênica de mucina 5AC *in vitro*, mostrando potencial para melhora na saúde gastrointestinal [97]. Além disso a β-Lg e a α-La demostraram conter sequências de peptídeos bioativos com funções imunomoduladoras [98].

O mecanismo de ação relacionado ao efeito protetor das proteínas do soro de leite envolve a modulação da microbiota e do sistema imune, bem como a produção de metabólitos. Algumas bactérias anaeróbias presentes no cólon são capazes de fermentar aminoácidos, produzindo ácidos graxos de cadeia curta (AGCC), como acetato, butirato e propionato. Tais metabólitos estão envolvidos em diferentes processos fisiológicos, incluindo a modulação do metabolismo energético, da resposta inflamatória e a produção de neurotransmissores [99]. Ademais, esses estão também envolvidos na manutenção da fisiologia intestinal, do fígado e de tecidos periféricos [100]. Entretanto, considerando que o principal substrato para a produção de AGCC são os carboidratos complexos não digeríveis, a sua produção a partir de aminoácidos está relacionada à quantidade e qualidade da proteína ingerida na dieta e a sua composição de aminoácidos [99,100]. A contribuição de cada um desses fatores na produção de AGCC não é completamente conhecida, sendo recomendada a realização de estudos que avaliem o efeito da fonte proteica

e dos processamentos aplicados no perfil de aminoácidos, bem como a sua relação com os metabólitos gerados [100].

Entretanto, os benefícios obtidos pelo consumo dos peptídeos bioativos presentes no soro são influenciados por diversos aspectos, como protocolo de indução da DII, quantidade ingerida e método de produção [101,102]. Com isso, o efeito das proteínas do soro do leite no controle de sintomas e auxílio da remissão da CU ainda é controverso. No Quadro 2 são apresentados resultados de estudos publicados entre 2016-2022, que avaliaram o uso de proteína de soro de leite ou derivados no controle de DII.

Quadro 2 – Efeitos da intervenção com componentes do leite e seus derivados em modelos animais de DII.

Estratégia não farmacológica	**Concentração do ingrediente**	**Modelo *in vivo*, duração do estudo e forma de consumo**	**Modelo da indução da DII**	**Principais resultados**	**Principais conclusões**	**Referência**
Lactoferrina saturada de ferro derivada de leite bovino	1,25% da ração	Camundongos machos BALB/c (7–12 semanas de vida). Duração: 24 dias. Consumo *ad libitum*. Adição dos compostos	4% DSS (adicionado em água potável com 10% sacarose) adicionado em água potável do quinto a	O colostro de soro de leite foi o mais eficaz na redução da atividade de mieloperoxidase (enzima derivada de leucócitos que catalisa a formação	Primeiro artigo publicado com objetivo de avaliar estes componentes do leite em DII. Cada um dos componentes	66
Angiogenina	0,3% da ração					
Osteopontina	0,3% da ração					
Colostro de soro de leite	1,25% da ração					

Extrato de caseína em pó (Nestlé®)	4,2% da ração	na ração levando em conta volume final da ração e peso do composto (peso/volume).	sétimo dia de protocolo.	de numerosas espécies reativas oxidantes) e redução do IAD.	apresentou benefícios diferenciados no modelo de estudo.	
Gordura do leite enriquecida com *cis-9,trans-11 conjugated linoleic acid* (CLA)	2% da ração					
Soro de leite de cabra obtido após coagulação mista (enzimática e ácida) de leite de cabra	1 g/kg/dia, dissolvido em 1 mL de água potável	Ratos *Wistar* fêmeas (peso médio de 220 ± 20 g)	CU induzida por por enema retal de solução contendo	Os tratamentos com soro de leite de cabra e SAZ causaram efeitos benéficos (parâmetros	Os autores sugerem que o soro de leite de cabra pode trazer benefícios durante o	64
	2 g/kg/dia, dissolvido em 1					

pasteurizada, seguida de desidratação por *spray drying*	mL de água potável	Duração: 17 dias. Consumo por gavagem oral.	ácido acético a 10%	histológicos preservados, diminuição do IAD e atividades enzimáticas).	tratamento da inflamação intestinal. Destaque para o efeito nas doses de 2 e 4 g/kg.	
	4 g/kg/dia, dissolvido em 1 mL de água potável					
Sulfasalazina (SAZ)	250 mg/kg/dia, dissolvido em 1 mL de água potável					
Mesalamina	4800 mg/dia					
Soro de leite de cabra obtido após coagulação mista (enzimática e	4 g/kg/dia, dissolvido em 100 µL de água potável	Camundongos machos CD1 (peso médio de 3 ± 2 g).	CU induzida por DNBS (2,5 mg dissolvido em 100 µl com	O soro de leite de cabra inibiu a secreção de citocinas pró-	Recomenda o uso de soro de leite de cabra	65

ácida) de leite de cabra pasteurizada, seguida de *spray drying*		Duração: 17 dias. Consumo por gavagem oral.	50% de etanol) utilizando cateter intracolônico no 13º dia.	inflamatórias e preservou parâmetros histológicos.	para tratamento de DII.	
Proteína de leite 14% (P14)	140 g/kg de ração	Camundongos machos C57BL/6 (7 semanas de vida) Duração: 28 dias. Consumo *ad libitum.*	CU induzida por 3,5% DSS adicionado em água potável do primeiro a sétimo dia de protocolo.	P30 apresentou os melhores resultados em relação à restauração da barreira do cólon e outras funções para controle da doença.	Recomenda analisar um valor limite de ingestão de proteína para evitar efeitos negativos.	67
Proteína de leite 30% (P30)	300 g/kg de ração					
Proteína de leite 53% (P53)	530 g/kg de ração					

Fonte: Elaborado pelo autor. Notas: [1]mistura de proteínas do leite contendo soro de leite e caseína na porção 20:80, DSS: Dextran sulfato de sódio, DNBS: ácido dinitrobenzeno sulfônico.

Os resultados obtidos nos estudos do Quadro 2 foram inconsistentes entre si, provavelmente devido aos protocolos adotados, que variaram em relação à concentração do indutor (DSS - 3,5% e 4%) [66,67] e ao tempo de indução (entre quatro e cinco dias) [65,66,67].

A concentração de proteínas do soro de leite administradas diariamente também é um fator discrepante. Araújo et al., (2016)[65] restringiram a administração diária por gavagem oral entre 1 e 4 g/Kg de peso corpóreo, enquanto Vidal-lletjós et al. (2019)[67] e Kanwar et al. (2016)[66] optaram pela ingestão *ad libitum*, o que certamente influenciou os resultados obtidos.

Diretrizes de ingestão diária de proteínas para humanos, como as propostas pela *European Society for Clinical Nutrition and Metabolism* (ESPE), afirmam que não há evidências de que pacientes com DII precisem de mais proteína diária do que pessoas saudáveis (sendo essa em torno de 1g/Kg)[103]. No entanto, a ESPE destacou que aumento no consumo de proteína pode ser necessário devido à perda de apetite e ingestão dietética restrita dos pacientes, justificando um aumento na oferta para 1,2 ou 1,5 g/kg de peso corporal [103].

Dietas contendo proteína do soro de leite têm sido associadas ao controle dos sintomas e à redução do risco da colite. No primeiro caso, a ingestão da proteína do soro de leite foi feita de forma concomitante ou somente após a indução da doença [66,67] e no segundo, o consumo é iniciado antes do estabelecimento da colite [64,65].

Outra questão a ser discutida é como os diferentes produtos derivados do soro de leite influenciam os efeitos observados em modelos animais

induzidos à DII. Kanwar, et al. (2016) relataram como sendo o primeiro estudo que comparou o efeito de diferentes componentes do leite na colite induzida por DSS em camundongos. Cada produto lácteo mostrou respostas positivas diferentes no modelo de estudo [66]. Os melhores resultados foram obtidos com a gordura do leite enriquecida com *cis-9,trans-11 conjugated linoleic acid* (CLA) – que ajudou a restaurar o peso e inibiu as citocinas inflamatórias e os danos no epitélio – e o extrato de caseína em pó (Nestlé®) que diminuiu os efeitos clínicos e o escore inflamatório. Os autores enfatizaram a necessidade de mais estudos para entender como os suplementos podem funcionar em sinergia para tratar a DII.

Araújo et al. (2016) e (2017) publicaram outras descobertas sobre esse tema. O primeiro estudo comparou o efeito de fármacos anti-inflamatórios comumente usados no tratamento de DII (sulfasalazina e mesalamina) e do soro de leite de cabra em diferentes concentrações em modelo com ratos e agente indutor por enema retal [64,65]. O soro de leite de cabra trouxe benefícios na remissão da colite induzida em animais. Já em 2017, esse grupo de pesquisa aplicou a concentração de soro de leite que obteve melhores respostas (4 g/kg/dia) em modelo com camundongos machos CD1 e também obteve respostas positivas no seu auxílio na regressão de colite ulcerativa. Nesse caso o agente indutor foi químico por ácido dinitrobenzeno sulfônico (DNBS).

Por fim, em 2019, Vidal-Lletjós e colaboradores também verificaram o efeito do consumo de diferentes porcentagens de produtos lácteos (sendo nesse caso, com porção 20:80 de proteínas do soro do leite e caseína,

respectivamente) na remissão da colite [67]. Os autores optaram por ingestão *ad libitum*, o que correspondeu a presença de 140 g a 530 gramas do produto por quilo de ração oferecida aos animais. Os principais resultados relacionados ao consumo desses suplementos foram a manutenção da barreira do cólon e modulação da microbiota intestinal.

Apesar das discrepâncias relatadas, em geral os resultados obtidos nos diferentes protocolos foram promissores, com sinais de restauração da barreira intestinal, e redução de escores clínicos, evidenciando a importância da continuação dos estudos utilizando esta abordagem.

Todos os estudos apresentados no Quadro 2 avaliaram produtos obtidos por processo térmico tradicional. No entanto, alguns processos não térmicos, como UV-C e outros tratamentos, podem induzir modificações físicas e a produção de compostos bioativos em soro de leite e gerar um impacto positivo na função corporal [104].

Até o presente momento não foram encontradas publicações acerca do uso de soro de leite bovino tratado por UV-C em DII. O presente estudo faz parte do projeto temático "*Novel Aging - Technologies and solutions to manufacture novel dairy products for healthy aging*" (Acordos de Cooperação / IFD - *Innovation Fundation Denmark*) (FAPESP 2017/01189-0), sediado no IQSC - USP - São Carlos, em parceria com a EMBRAPA - São Carlos, University of Copenhagen - Dinamarca, e FCF - UNESP – Araraquara, que avaliou os efeitos do tratamento UV-C na qualidade da proteína do soro de leite e o uso desse suplemento para manutenção da saúde. A partir dos resultados iniciais, que evidenciaram que o tratamento da proteína do soro de

leite com UV-C gerou peptídeos bioativos e melhorou a sua digestão, nossa hipótese é que esse tratamento não-térmico possibilitará a obtenção de um suplemento alimentar com capacidade de auxiliar no controle da colite [105].

2. OBJETIVOS GERAIS

O objetivo principal do estudo foi avaliar o efeito do consumo de proteína de soro de leite submetida ao tratamento de UV-C na colite aguda induzida em camundongos.

Objetivos específicos:

Comparar grupos de estudo por meio de avaliação de:

1. Índice de Atividade da Doença (IAD);
2. Determinação da concentração de citocinas pró e anti-inflamatórias.
3. Determinação dos metabólitos no plasma e rins por Espectroscopia de Ressonância Magnética Nuclear de Prótons (RMN de 1H);
4. Variação da relação peso e comprimento (da junção ileocecal à margem anal);
5. Histomorfologia do cólon dos animais;
6. Determinação da composição da microbiota fecal ao longo do protocolo experimental.

Destaca-se que durante doutorado foi realizado um estágio no exterior pelo programa Capes PDSE (Processo 88881.624770/2021-01), sob a supervisão do Dr Ilario Ferrocino (*Microbiology and Food Science laboratory - University of Turin* – DISAFA - Itália), de novembro de 2021 a abril de 2022. Neste estágio foram realizadas análises dos dados obtidos no sequenciamento realizado no Brasil, utilizando técnicas avançadas de bioinformática.

2.1. Organização dos capítulos

Os capítulos 1 e 2 visaram apresentar e discutir os objetivos específicos definidos nos tópicos anteriores de 1 a 3 e de 4 a 6, respectivamente.

3. REFERÊNCIAS

1 SWAISGOOD, H. E. Características do Leite. *In*: DAMODARAN, S.; PARKIN, K.. **Química de Alimentos de Fennema**. 4. ed. Porto Alegre: Artmed, 2010. p. 689–717.

2 GUETOUACHE, M.; GUESSAS, B.; MEDJEKAL, S. Composition and nutritional value of raw milk. **Issues in Biological Sciences and Pharmaceutical Research**, v. 2, n. 10, p. 115-122, 2014.

3 LO, Y. M.; ARGIN-SOYSAL, S.; HSU, C. Bioconversion of whey lactose into microbial exopolysaccharides. **Bioprocessing for value-added products from renewable resources**, p. 559-583, 2007.

4 FARRELL JR, H. M. *et al*. Nomenclature of the proteins of cows' milk—Sixth revision. **Journal of dairy science**, v. 87, n. 6, p. 1641-1674, 2004.

5 ORDÓÑEZ, J. A. *et al.* Características gerais do leite e componentes fundamentais. *In:* ORDÓÑEZ, J. A**. Tecnologia de Alimentos: Volume 2 - Alimentos de Origem Animal**. 1. ed. Porto Alegre: Artmed, 2005. p. 279.

6 BOLAND M., 3 - Whey proteins. *In:* Phillips G.O., Williams P.A. , **Handbook of Food Proteins**, 1. Ed. Woodhead Publishing, 2011. p. 30-35.

7 BREW, K. α-Lactalbumin. *In:* FOX, P.F., MCSWEENEY, P.L.H. **Advanced Dairy Chemistry—1 Proteins**. Boston: Springer, 2003. p 387-419.

8 ABD EL-SALAM, M. H.; EL-SHIBINY, S.; SALEM, A. Factors affecting the functional properties of whey protein products: A review. **Food Reviews International**, v. 25, n. 3, p. 251–270, 2009.

9 BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. **Instrução Normativa n. 94, de 18 de setembro de 2020.** Diário Oficial da União: seção 1, edição 182, página 5, 2020.

10 SCHMIDT, R. H.; PACKARD, V. S.; MORRIS, H. A. Effect of processing on whey protein functionality. **Journal of Dairy Science**, v. 67, n. 11, p. 2723-2733, 1984.

11 TUNICK, M. H. Whey protein production and utilization: a brief history. In: ONWULATA, C.I.; HUTH P.J. **Whey processing, functionality and health benefits**, 1 ed. Iowa: Wiley-Blackwell, 2008. p. 1-13.

12 MARWAHA, S. S.; KENNEDY, J. F. Whey—pollution problem and potential utilization. **International journal of food science & technology**, v. 23, n. 4, p. 323-336, 1988.

13 SISO, MI González. The biotechnological utilization of cheese whey: a review. **Bioresource technology**, v. 57, n. 1, p. 1-11, 1996.

14 RAHAMAN, T.; VASILJEVIC, T.; RAMCHANDRAN, L. Effect of processing on conformational changes of food proteins related to allergenicity. **Trends in Food Science & Technology**, v. 49, p. 24–34, 2016.

15 RANKIN, S. A. et al. The application of alkaline phosphatase assays for the validation of milk product pasteurization. **Journal of dairy science**, United States, v. 93, n. 12, p. 5538–5551, 2010.

16 RUDLOFF, S.; LONNERDAL, B. Solubility and digestibility of milk proteins in infant formulas exposed to different heat treatments. **Journal of pediatric gastroenterology and nutrition**, United States, v. 15, n. 1, p. 25–33, 1992.

17 DE TOLEDO GUIMARÃES, J. *et al.* Non-thermal emerging technologies and their effects on the functional properties of dairy products. **Current Opinion in Food Science**, v. 22, p. 62-66, 2018.

18 RIBEIRO, Nathalia G. *et al.* Dairy foods and novel thermal and non-thermal processing: A bibliometric analysis. **Innovative Food Science & Emerging Technologies**, v. 76, p. 102934, 2022.

19 DUMAY, E. *et al.* Technological aspects and potential applications of (ultra) high-pressure homogenisation. **Trends in Food Science & Technology**, v. 31, n. 1, p. 13–26, 2013.

20 YU, L. J.; NGADI, M.; RAGHAVAN, G. S. V. Effect of temperature and pulsed electric field treatment on rennet coagulation properties of milk. **Journal of Food Engineering**, v. 95, n. 1, p. 115–118, 2009.

21 DE CASTRO, R. J. S. *et al.* Whey protein as a key component in food systems: Physicochemical properties, production technologies and applications. **Food Structure**, v. 14, p. 17– 29, 2017.

22 NAIK, L.; SHARMA, R.; MANJU, G. Application of High Pressure Processing Technology for Dairy Food Preservation - Future Perspective: A Review. **Journal of Animal Production Advances**, v. 3, n. 8, p. 232, 2013.

23 ZEECE, M.; HUPPERTZ, T.; KELLY, A. Effect of high-pressure treatment on in-vitro digestibility of β-lactoglobulin. **Innovative food science & emerging technologies**, v. 9, n. 1, p. 62–69, 2008.

24 ARSHAD, R. N. *et al*. Electrical systems for pulsed electric field applications in the food industry: An engineering perspective. **Trends in Food Science and Technology**, v. 104, p. 1–13, 2020.

25 XIANG, B. Y. *et al.* Pulsed electric field-induced structural modification of whey protein isolate. **Food and Bioprocess Technology**, v. 4, n. 8, p. 1341–1348, 2011.

26 SCHOTTROFF, F. *et al.* Pulsed electric field preservation of liquid whey protein formulations–Influence of process parameters, pH, and protein content on the inactivation of Listeria innocua and the retention of bioactive ingredients. **Journal of food engineering**, v. 243, p. 142–152, 2019.

27 CHÁVEZ-MARTÍNEZ, A. *et al.* Low and High-Intensity Ultrasound in Dairy Products: Applications and Effects on Physicochemical and Microbiological Quality. **Foods**, v. 9, n. 11, p. 1-27, 2020.

28 JAMBRAK, A. R. *et al.* Effect of ultrasound treatment on solubility and foaming properties of whey protein suspensions. **Journal of Food Engineering**, v. 86, n. 2, p. 281–287, 2008.

29 POLLOCK, A. M. et al. Pulsed light destruction kinetics of L. monocytogenes. **LWT - Food Science and Technology**, v. 84, p. 114–121, 2017.

30 SUN, W.-W. *et al*. Properties of whey protein isolate–dextran conjugate prepared using pulsed electric field. **Food Research International**, v. 44, n. 4, p. 1052–1058, 2011.

31 SIDDIQUE, M. A. B.; MARESCA, P.; PATARO, G.; FERRARI, G. Influence of pulsed light treatment on the aggregation of whey protein isolate. **Food Research International**, v. 99, p. 419–425, 2017.

32 MARTINI, S.; POTTER, R.; WALSH, M. K. Optimizing the use ofpower ultrasound to decrease turbidity in whey protein suspensions. **Food Research International**, v. 43, n. 10, p. 2444–2451, 2010.

33 SHEN, X.; SHAO, S.; GUO, M. Ultrasound-induced changes in physical and functional properties of whey proteins. **International Journal of Food Science and Technology**, v. 52, n. 2, p. 381–388, 2017.

34 FERNÁNDEZ, E. et al. Effect of pulsed-light processing on the surface and foaming properties of β-lactoglobulin. **Food Hydrocolloids**, v. 27, n. 1, p. 154–160, 2012.

35 SIDDIQUE, M. A. B.; MARESCA, P.; PATARO, G.; FERRARI, G. Effect of pulsed light treatment on structural and functional properties of whey protein isolate. **Food Research International**, v. 87, p. 189–196, 2016.

36 GAYÁN, E.; CONDÓN, S.; ÁLVAREZ, I. Biological Aspects in Food Preservation by Ultraviolet Light: a Review. **Food and Bioprocess Technology**, v. 7, n. 1, p. 1–20, 2014.

37 KRISTO, E.; HAZIZAJ, A.; CORREDIG, M. Structural changes imposed on whey proteins by UV irradiation in a continuous UV light reactor. **Journal of agricultural and food chemistry**, United States, v. 60, n. 24, p. 6204–6209, 2012.

38 LÓPEZ-MALO, A.; PALOU, E. Ultraviolet Light and Food Preservation. *In:* BARBOSA-CANOVAS G.V., TAPIA M.S., CANO M. P. **Novel food processing technologies**. Madrid: CRC, 2004. p. 408–409.

39 ADAMS, M. R.; MOSS, M. O. **Food Microbiology.** Cambridge:Royal Society of chemistry, 1995.

40 KOUTCHMA, T. N.; FORNEY, L. J.; MORARU, C. I. Principles and Applications of UV Technology. *In:* SUN, D.-W. **Ultraviolet Light in**

Food Technology: Principles and Applications. 2. ed. New York: Taylor & Francis Group, 2009. p. 300.

41 CAPPOZZO, J. C.; KOUTCHMA, T.; BARNES, G. Chemical characterization of milk after treatment with thermal (HTST and UHT) and nonthermal (turbulent flow ultraviolet) processing technologies. **Journal of Dairy Science**, v. 98, n. 8, p. 5068–5079, 2015.

42 BUHLER, S. *et al.* UV irradiation as a comparable method to thermal treatment for producing high quality stabilized milk whey. **LWT**, v. 105, p. 127–134. 2019.

43 NEVES-PETERSEN, Maria Teresa; GAJULA, Gnana Prakash; PETERSEN, Steffen B. UV light effects on proteins: from photochemistry to nanomedicine. **Molecular photochemistry-various aspects**, p. 125-158, 2012.

44 YAGI, M. *et al*. Reversible Unfolding of Bovine β-Lactoglobulin Mutants without a Free Thiol Group. **Journal of Biological Chemistry**, v. 278, n. 47, p. 47009–47015, 2003.

45 KRAULIS, P. J. MOLSCRIPT. A program to produce both detailed and schematic plots of protein structures. **Journal of Applied Crystallography**, v. 24, n. 5, p. 947–950, 1991.

46 CARDOSO, Daniel R. *et al.* UV-C Light Promotes Reductive Cleavage of Disulfide Bonds in B-Lactoglobulin Improving Digestibility and Release of Bioactive Peptides. **Available at SSRN 4189832**. No prelo.

47 TAKAMATSU, M. *et al.* IDO1 plays an immunosuppressive role in 2,4,6-trinitrobenzene sulfate-induced colitis in mice. **Journal of immunology**, United States, v. 191, n. 6, p. 3057–3064, 2013.

48 TASHITA, C. *et al.* Kynurenine plays an immunosuppressive role in 2,4,6- trinitrobenzene sulfate-induced colitis in mice. **World journal of gastroenterology**, United States, v. 26, n. 9, p. 918–932, 2020.

49 DA SILVA, J. F. **Processamento UV-C de proteínas do soro do leite: Efeitos na estrutura e digestibilidade**. Dissertação (Mestre em Ciências), Universidade de São Paulo, Instituto de Química de São Carlos, São Carlos, 2019. Disponível em: Acesso em: https://doi.org/10.11606/D.75.2019.tde-03102019-090331. Acesso em: 18 jan. 2023.

50 YOUNIS, N.; ZARIF, R.; MAHFOUZ, R. Inflammatory bowel disease: between genetics and microbiota. **Molecular Biology Reports**, v. 47, n. 4, p. 3053–3063, 2020.

51 ALATAB, Sudabeh *et al.* The global, regional, and national burden of inflammatory bowel disease in 195 countries and territories, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017. **The Lancet gastroenterology & hepatology**, v. 5, n. 1, p.17-30, 2020.

52 KOBAYASHI, T. *et al.* Ulcerative colitis (Primer). **Nature Reviews: Disease Primers**, v. 6, n. 1, 2020.

53 GAJENDRAN, Mahesh *et al.* A comprehensive review and updateon ulcerative colitis. **Disease-a-month**, v. 65, n. 12, p. 100851, 2019.

54 ARMUZZI, A.; LIGUORI, G. Quality of life in patients with moderate to severe ulcerative colitis and the impact of treatment: A narrative review. **Digestive and Liver Disease**, v. 53, n. 7, p. 803-808,2021.

55 CARUSO, R.; LO, Bernard C.; NÚÑEZ, G. Host–microbiota interactions in inflammatory bowel disease. **Nature Reviews Immunology**, v. 20, n. 7, p. 411-426, 2020.

56 ZHANG, Ming *et al*. Interactions between intestinal microbiota and host immune response in inflammatory bowel disease. **Frontiers in Immunology**, v. 8, p. 942, 2017.

57 BÜRGER, M.; SCHMIDT, C.; STALLMACCH, A. Medical Therapy of Active Ulcerative Colitis. Viszeralmedizin: **Gastrointestinal Medicine and Surgery**, [s. l.], v. 31, n. 4, p. 236–245, 2015.

58 HENDRICKSON, B. A.; GOKHALE, R.; CHO, J. H. Clinical aspects and pathophysiology of inflammatory bowel disease. **Clinical microbiology reviews**, United States, v. 15, n. 1, p. 79–94, 2002.

59 ZABANA, Yamile et al. Relevant infections in inflammatory bowel disease, and their relationship with immunosuppressive therapy and their effects on disease mortality. **Journal of Crohn's and Colitis**, v. 13, n. 7, p. 828-837, 2019.

60 LICHTENSTEIN, Gary R. et al. American Gastroenterological Association Institute medical position statement on corticosteroids, immunomodulators, and infliximab in inflammatory bowel disease. **Gastroenterology**, v. 130, n. 3, p. 935-939, 2006.

61 CAI, Zhaobei; WANG, Shu; LI, Jiannan. Treatment of inflammatory bowel disease: a comprehensive review. **Frontiers inMedicine**, v. 8, p. 765474, 2021.

62 RANDHAWA, P. K.; SINGH, K.; SINGH, N.; JAGGI, A. S. A review on chemical-induced inflammatory bowel disease models in rodents. **The Korean journal of physiology & pharmacology : official journal of**

the Korean Physiological Society and the Korean Society of Pharmacology, Korea (South), v. 18, n. 4, p. 279–288, 2014.

63 AL-OMARI, M. M. *et al.* Camel milk whey inhibits inflammatory colorectal cancer development via down regulation of pro-inflammatory cytokines in induced AOM/DSS mouse model. **Emirates Journal of Food and Agriculture**, v. 31, n. 4, p. 256–262, 2019.

64 ARAÚJO, D. F. S. *et al.* Goat whey ameliorates intestinal inflammation on acetic acid-induced colitis in rats. **Journal of Dairy Science**, v. 99, n. 12, p. 9383–9394, 2016.

65 ARAÚJO, D. F. S. *et al*. Intestinal anti-inflammatory effects of goatwhey on DNBS-induced colitis in mice. **PloS one**, v. 12, n. 9, p. e0185382–e0185382, 2017.

66 KANWAR, J. R. *et al.* Comparative activities of milk components in reversing chronic colitis. **Journal of Dairy Science**, v. 99, n. 4, p. 2488–2501, 2016.

67 VIDAL-LLETJÓS, S. *et al*. Dietary Protein Intake Level Modulates Mucosal Healing and Mucosa-Adherent Microbiota in Mouse Model of Colitis. **Nutrients**, v. 11, n. 3, p. 514, 2019.

68 EICHELE, D. D.; KHARBANDA, K. K. Dextran sodium sulfate colitis murine model: An indispensable tool for advancing our understanding of inflammatory bowel diseases pathogenesis. **World journal of gastroenterology**, v. 23, n. 33, p. 6016–6029, 2017.

69 KIM, J. J.; SHAJIB, M. S.; MANOCHA M. M.; KHAN, W. I. Investigating Intestinal Inflammation in DSS-induced Model of IBD. JoVE, n. 60, p. e3678, 2012.

70 CORNICK, S.; TAWIAH, A.; CHADEE, K. Roles and regulation of the mucus barrier in the gut. **Tissue barriers**, United States, v. 3, n. 1–2, p. e982426, 2015.

71 MUKHOPADHYA, I.; HANSEN, R.; EL-OMAR, E. M.; HOLD, G. L. IBD-what role do Proteobacteria play? **Nature reviews. Gastroenterology & hepatology**, England, v. 9, n. 4, p. 219–230, 2012.

72 CELIBERTO, L. S. *et al.* Inflammatory bowel disease and immunonutrition: novel therapeutic approaches through modulation of diet and the gut microbiome. **Immunology**, England, v. 155, n. 1, p. 36–52, 2018.

73 VALDES, A. M.; WALTER, J.; SEGAL, E.; SPECTOR, T. D. Role of the gut microbiota in nutrition and health. **BMJ**, v. 361, 2018.

74 HEENAN, *et al.* Irritable bowel syndrome and the gut microbiota. **Journal of the Royal Society of New Zealand**, v. 6758, 2020.

75 ARUMUGAM, M. *et al.* Enterotypes of the human gut microbiome. **Nature**, England, v. 473, n. 7346, p. 174–180, 2011.

76 COSTEA, P. I. *et al.* Enterotypes in the landscape of gut microbial community composition. **Nature Microbiology**, v. 3, n. 1, p. 8–16, 2018.

77 KNIGHTS, D. *et al.* Rethinking “enterotypes.” **Cell Host &Microbe**, v. 16, n. 4, p. 433–437, 2014.

78 ELZINGA, J.; OOST J. V. D.; VOS W. M.; SMIDT H. The use of defined microbial communities to model host-microbe interactions in the human gut. **Microbiology and Molecular Biology Reviews**, v. 83, n. 2, p. e00054-18, 2019.

79 MORAN, N. A.; OCHMAN, H.; HAMMER, T. J. Evolutionary and ecological consequences of gut microbial communities. **Annual Review of Ecology, Evolution, and Systematics**, v. 50, n. 1, p. 451, 2019.

80 MALLOTT, E. K.; AMATO, K. R. Host specificity of the gutmicrobiome. **Nature Reviews Microbiology**, v. 19, n. 10, p. 639-653, 2021.

81 HARRIS, K. G.; CHANG, E. B. The intestinal microbiota in the pathogenesis of inflammatory bowel diseases: new insights into complex disease. **Clinical science**, England, v. 132, n. 18, p. 2013–2028, 2018.

82 KOSTIC, Aleksandar D.; XAVIER, Ramnik J.; GEVERS, Dirk. The microbiome in inflammatory bowel disease: current status and the future ahead. **Gastroenterology**, v. 146, n. 6, p. 1489-1499, 2014.

83 DISTRUTTI, E.; MONALDI, L.; FIORUCCI S. Gut microbiota role in irritable bowel syndrome: New therapeutic strategies. **World journal of gastroenterology**, v. 22, n. 7, p. 2219, 2016.

84 DAI, X.; SHEN, L.; LU, P. Advances and trends in omicstechnology development. **Frontiers in Medicine**, p. 1546, 2022.

85 QUAST, M. *et al.* Comparative Analysis of Metagenomics and Metataxonomics for the Characterization of Vermicompost Microbiomes. **Frontiers in microbiology**, v. 13, 2022.

86 WHITTAKER, Robert H. Evolution and measurement of species diversity. **Taxon**, v. 21, n. 2-3, p. 213-251, 1972.

87 JI, B.; NIELSEN, J. New insight into the gut microbiome through metagenomics. **Adv Genomics Genet**, v. 5, p. 77–91, 2015.

88 JOVEL, J. *et al.* Characterization of the gut microbiome using 16S or shotgun metagenomics. **Frontiers in microbiology**, v. 7, p. 459, 2016.

89 BHARTI, S. K.; ROY, R. Quantitative 1H NMR spectroscopy. **TrAC Trends in Analytical Chemistry**, v. 35, p. 5-26, 2012.

90 STORR, M.; VOGEL, Hans J.; SCHICHO, R. Metabolomics: is it useful for IBD?. **Current opinion in gastroenterology**, v. 29, n. 4, p. 378, 2013.

91 ALDARS-GARCÍA, L.; GISBERT, J. P.; CHAPARRO, M. Metabolomics Insights into Inflammatory Bowel Disease: A Comprehensive Review. **Pharmaceuticals**, v. 14, n. 11, p. 1190, 2021.

92 SANTIAGO-LOPEZ, L.; GONZALEZ-CORDOVA, A. F.; HERNANDEZ-MENDOZA, A.; VALLEJO-CORDOBA, B. Potential Use of Food Protein- Derived Peptides in the Treatment of Inflammatory Diseases. **Protein and peptide letters**, Netherlands, v. 24, n. 2, p. 137–145, 2017.

93 ALMEIDA, C. C.; ALVARES, T. S.; COSTA, M. P.; CONTE-JUNIOR, C. A. Protein and Amino Acid Profiles of Different Whey Protein Supplements. **Journal of Dietary Supplements**, v. 13, n. 3, p. 313–323, 2016.

94 LIU, Y.; WANG, X.; HU, C. A. A. Therapeutic potential of aminoacids in inflammatory bowel disease. **Nutrients**, v. 9, n. 9, p. 1–18, 2017.

95 LAGRANGE, V.; CLARK, D. C. Nutritive and Therapeutic Aspects of Whey Proteins. *In*: DEETH, H. C.; BANSAL, N. **Whey Proteins: From Milk to Medicine**. 1 ed. Elsevier Inc., 2019. p 549-577.

96 SMITHERS, G. W. Whey and whey proteins—From 'gutter-to-gold'. **International dairy journal**, v. 18, n. 7, p. 695-704, 2008.

97 MARTÍNEZ-MAQUEDA, D. *et al.* Effect of β-lactoglobulin hydrolysate and β-lactorphin on intestinal mucin secretion and gene expression in human goblet cells. **Food Research International,** v. 54,n. 1, p. 1287-1291, 2013.

98 URISTA, M. C. et al. Production and functionality of active peptides from milk. **Food Science and Technology International**, v. 17, n. 4, p. 293-317, 2011.

99 SILVA, Y. P.; BERNARDI, A.; FROZZA, R. L. The Role of Short-Chain Fatty Acids From Gut Microbiota in Gut-Brain Communication. **Frontiers in Endocrinology**, v. 11, p. 1–14, 2020.

100 PORTUNE, K. J. *et al.* Gut microbiota role in dietary protein metabolism and health- related outcomes: The two sides of the coin. **Trends in Food Science & Technology**, v. 57, 2016.

101 DULLIUS, A.; GOETTERT, M. I.; DE SOUZA, C. F. V. Whey protein hydrolysates as a source of bioactive peptides for functional foods Biotechnological facilitation of industrial scale-up. **Journal of Functional Foods**, v. 42, p. 58–74, 2018.

102 YADAV, J. S. S. *et al.* Cheese whey: A potential resource to transform into bioprotein, functional/nutritional proteins and bioactive peptides. **Biotechnology Advances**, v. 33, n. 6, p. 756–774, 2015.

103 FORBES, A*. et al.* ESPEN guideline: Clinical nutrition in inflammatory bowel disease. Clinical Nutrition, v. 36, n. 2, p. 321–347, 2017.

104 ESTEGHLAL, S. *et al.* Bridging the knowledge gap for the impact of non-thermal processing on proteins and amino acids. **Foods,** v. 8, n.7, 2019.

105 SILVA,J. F.; MORAIS, A. T. B.; SANTOS, W. G.; CARDOSO, D. R. UV-light induced conformational changes in β-lactoglobulin improves digestibility and generation of bioactive peptides. *In:* LA FACULTAD DE CIENCIAS EXACTAS DE LA UNIVERSIDAD ANDRÉS BELLO- UNAB, 2019, Viña del Mar. **Anais** [...] Viña del Mar: La Facultad de Ciencias Exactas de la Universidad Andrés Bello- UNAB, 2019. Disponível em: http://14elafot.ciq.uchile.cl/Documentos/LIBRO%20DEFINITIVO%20sp .pdf. Acesso em: 18 jan. 2023.

Capítulo 1.

Effect of whey and UV-C processed whey protein supplementation on plasma and kidney metabolomic profiles in a DSS-induced colitis mouse model

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Effect of whey and UV-C processed whey protein supplementation on plasma and kidney metabolomic profiles in a DSS-induced colitis mouse model

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Alternatives to help with the management of ulcerative colitis (UC) are of major interest. We focused on the effect of an emerging non-thermal processing technology named UV-C, which we applied to modify whey protein (WP). Thus, we have compared the recovery of the mouse model after ingestion of UV-C-treated and non-treated WP by accessing the plasma and kidney metabolomic profiles. Analysis of UC activity during the experiment showed a lower rate of disease development in mice who received UV-C-treated WP compared to animals receiving WP without UV-C treatment ($p < 0.05$). Plasma results revealed similarities in cluster biomarkers between the healthy and colitis groups, implying that plasma metabolomic signatures do not demonstrate good performance for discriminating between healthy mice and mice with acute colitis. However, the kidneys of the mouse model of UC have not shown an overload of kidney functions, and some metabolic pathways were reprogrammed and alleviated disease progression. The results revealed that UV-C-treated milk generated different responses in the metabolomic pathways and could attenuate the severity of UC compared with non-UVC-treated WP. This is the first report on the effect of UV-C on a whey protein supplement in an ulcerative colitis mouse model.

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Introduction

Inflammatory bowel diseases (IBD), including ulcerative colitis (UC) and Crohn's disease (CD), are characterized by chronic relapsing intestinal inflammation. Although CD affects any region of the gastrointestinal tract and UC is limited to the colon, there are some similarities between them, such as symptoms and etiology [1]. The etiology of these diseases is very complex and is yet unknown, but strong evidence points to a complex interaction between genetic predisposition, environmental factors, immune system activity, intestinal microbiome composition and metabolic changes [2,3].

The current medical treatment of IBD is based on the severity of the symptoms and includes anti-inflammatory (aminosalicylate and corticosteroids) and immunosuppressive or biological therapies (such as infusion of chimeric monoclonal antibodies). However, there is no universally effective or accepted therapy, and many patients have important adverse effects that affect the continuity of treatment [1,4].

An alternative to assist in the management of IBD is the use of combined strategies, including nutritional therapy. Dietetic approaches to UC recovery involve ingestion of bioactive peptides from milk protein, mainly whey protein, due to their positive properties on intestinal microbiota modulation and in the restoration of the colonic mucosa [7,8]. Previous studies with whey protein components have described changes in immunology, histopathology, and other parameters of disease activity to reverse chronic colitis in mice [8,12-14].

Many efforts have been made to offer dairy products, achieving the goal of being a nutritional therapy. In addition, these efforts include modification in whey protein products. Ultraviolet-visible (UV-C) light treatment is a non-thermal emerging technology that can impact the structure of milk proteins by changing its techno-functionalities and functional properties. In the case of milk whey proteins, UV-C light treatment has shown to induce reductive cleavage of disulfide bonds in β–lactoglobulin , promoting changes in the protein secondary structure and significantly reducing its resistance to pepsin digestion [9]. UV-C treatment of β–lactoglobulin has also shown to increase the number and quantity of bioactive peptides released during *in vitro* digestion [9]. Furthermore, this process is secure for inactivating microorganisms, generating safe and stable minimally processed foods, if critical parameters such as irradiation dose limits are observed. [10,11].

The effectiveness of a nutritional therapy in biomedicine is evaluated primary in dietary interventional studies in animal models. Regarding chronic colitis, rats or mice are usually used as animal models to study IBD. These animals can be genetically manipulated, immunodeficient, or chemically treated to promote inflammation [1]. In chemical-induced methods, animals receive a specific dose of a chemical substance (oxazolone, acetic acid, carrageenan, peptidoglycan-polysaccharide, nonsteroidal anti-inflammatory drugs, dextran sodium sulfate - DSS and 2,4,6 trinitrobenzene sulfonic acid - TNBS), which promote colonic inflammation [8,12-15]. TNBS and DSS are the most recommended substances to induce colitis in animal models [16]. Among these, DSS-induced colitis is more popular due to its simplicity and repeatability [17]. A tool for accessing the metabolome in animals is the metabolomics of biofluids, tissues, or cells, which refers to an analytical profile of low-molecular-weight compounds in biological material with respect to the system under investigation [18].

Metabolomic analysis can be useful to find potential biomarkers for the diagnosis of IBD and subtypes of the disease [18]. The ^{1}H RMN has the advantages of being reproducible and highly sensitive to the detection of small molecules [18,19]. In addition, molecules related to energy metabolism (such as pyruvate, succinate, citrate, lactate, creatine, and creatinine) are normally involved in the energy supply of mammalian cells and are naturally present in the blood [20]. Finally, we emphasize that the analysis of metabolites present in the kidneys is a way to verify whether the consumption of whey protein affected this organ. There are still few studies with this approach; however, it appears to be extremely promising and can show if there was no association between the consumption of a protein diet and the appearance of kidney problems [21].

In this paper, we explore the possible benefits of ultraviolet-visible (UV-C) light treatment, can bring to dairy ingredients, such as whey protein. Therefore, we propose to evaluate the recovery of the mouse with acute DSS-induced colitis after ingestion of whey protein, pre-irradiated with UV-C light, by accessing the blood and kidney metabolomics. Through this, we hope to provide new knowledge to the scientific community and help find new solutions to prevent ulcerative colitis, focusing on innovations for patient care.

Experimental

Production of whey protein and treatment by UV-C treatment

Pasteurized homogenized milk (Fazenda Bella Vista, skim milk, Tapiratiba, Brazil) was purchased from a local store. First, the milk was heated at 38 °C ± 2 °C, enzymatic coagulant was added (Estrella; Chr-Hansen A/S, Valinhos, Brazil), and then rested for 40 min. The firmness of the gel and the cut of the curd was previously tested with a restless time longer than 5 min. Finally, the milk containing the coagulant was heated at 50 ± 2 °C for 5 min and the cheese in addition to the serum to obtain the whey protein (WP). WP was lyophilized and solubilized in sterile deionized water in a proportion of 66 mg / mL. The WP solution was exposed to UV-C treatment using a UV-C LED that emits at 254 nm. The quartz cuvette model allocates 15 ml of sample, and the light intensity was 3.1 mW/cm^2. All equipment was kept at 5 ± 0.1 °C and the solution was stirred throughout the exposition time to UV-C treatment with a 500 mm magnetic stirrer bar at 500 rpm.

Experimental protocol

C57BL/6J male mice (Specific Pathogen Free) were purchased from the central animal facility (CEMIB) at the State University of Campinas (Campinas, SP, Brazil). The animals were exposed to 12-12 h of light-dark cycles and fed a standard diet (Labina Presence®) that included drinking water *ad libitum*. The protocol carried out followed the guidelines written by the Brazilian College of Animal Experimentation (COBEA) and was approved by the Research Ethics

Committee of the UNESP School of Pharmaceutical Sciences in Araraquara, Brazil (protocol number: 08/2020). The animals were randomly assigned to four groups (n = 10):

Group H: healthy animals that did not receive the products under study;

Group C: animals with chemically induced colitis that did not receive the products under study;

Group W: animals with chemically induced colitis that received whey protein (not UVC treated);

Group WU: animals with chemically induced colitis that received UV-C treated whey protein.

Dextran sulfate sodium-induced colitis

Colitis was chemically induced by dissolving 3% w/w DSS (MP Biomedicals, EUA; PM = 36.000–50.000) in sterilized drinking water that was ingested daily for a period of seven days. During DSS administration, the disease activity index (DAI) was determined daily. This follows the methodology that takes into account three parameters: weight loss, stool consistency, and occult or apparent blood in the stool [22]. To determine the presence of occult blood, the commercial kit Hemoplus (Newprov, Brazil) was used.

Diet administration

The whey protein, with or without UV-C treatment were administered to the animals daily for 14 days by oral gavage. The administration of the diet began seven days before the induction of colitis and lasted 7 days during exposure to DSS, a total of 14 days of treatment. Animals belonging to the control (H) and colitis (C) groups received water by oral gavage daily. Each animal consumed the amount of product according to its daily weight (1 g/Kg body weight). After 14 days of experimentation (**Figure 1**).

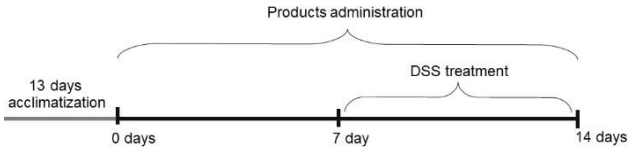

Figure 1 Induction of colitis and administration of products during the experimental protocol.

At the end of the nutritional intervention, the mice were placed under deep anesthesia with pentobarbital and blood was collected by cardiac puncture in tubes with heparin. The animals were euthanized by cervical dislocation. Blood was allowed to clot at room temperature for 30 min, centrifuged (1800 rcf for 10 min at 25 °C) and serum was aliquoted and stored at -80 ° C until use. Kidneys were excised, snap frozen in liquid nitrogen, and stored at -80°C until use.

Determination of IL-10, IL-6, IL-12-p40, and TNF-α by ELISA

Colon samples were weighed and stored in a phosphate buffer and protease inhibitor at -80 °C until analysis. Interleukin (IL-10) and tumor necrosis factor (TNF-) levels are measured using ELISA kits (BD Biosciences, San Jose, CA, USA; and R&D SystemsInc., Minneapolis, MN, USA). For quantification, a standard curve was constructed in the following concentration ranges: 15.6–1000 pg/mL for IL-6, IL-12-p40 and TNF, and 3.3–3000 pg/mL for IL-10.

Metabolites Extraction

Metabolites from plasma (200 ul) and kidney tissue (200 ug) were extracted by adding methanol in a 1:4 (plasma:methanol, v/v) proportion using a commercial cell disruptor (FastPrep®, MP Biomedicals) for 1 min, then centrifuged at 15,000 g for 15 minutes at 4 °C, and the supernatants were dried in speed-vac concentrator (model PD131DDA-115, ThermoFischer), and stored at -80 ° C until analysis [23]. Liquid nitrogen was added to the kidney samples to facilitate homogenization prior to adding methanol.

The polar metabolite-containing dried extracts were resuspended in 750 l of deuterium oxide phosphate buffer (0.10 mol/l; pD = 7.1) containing 0.23 mM deuterated sodium trimethylsilylpropanesulfonate (DSS-d6) and 0.01 % sodium azide. The samples were filtered through a 0.22 µm syringe filter and 550 µl of the filtrate was transferred to a 5-mm NMR tube for analysis.

Quantitative ^{1}H NMR analysis

^{1}H NMR spectra were obtained at 298 K on a 11.7T Agilent DD2 spectrometer equipped with a 5 mm ONEPROBE probe head with gradients. The spectra were acquired for proton NMR using the presaturation pulse sequence (Agilent PRESAT pulse sequence) for the residual water suppression, 32 K data points, with a spectral width of 16.0306 ppm, an acquisition time of 4.089 s, were acquired with a fixed receiver gain of 56, a recycle delay 5*T1 (27 s), dummy scans of 2, and an accumulation of 128 transients.

The 1D spectra were assigned using Chenomix software as a database supported by the literature, and the 2D NMR spectra (gCOSY, gHSQC) in which were previously obtained in selected samples. The FIDs processing were performed within the Chenomx software in which Fids were multiplied by a 0.3-Hz exponential multiplication function prior to applying the Fourier transform, and phase and baseline corrections were applied. For quantification, the metabolite concentrations were acquired relative to the reference standard ([DSS] = 0.23 mM).

Statistical data analysis

Metabolite concentrations were analyzed on the MetaboAnalyst 5.0 platform (http://www.metaboanalyst.ca/faces/home.xhtml), using Partial Least Squares Discriminant Analysis (PLS-DA). Data pre-processing included no data filtering, sample normalization by sum, no data transformation, and autoscaling. Five main components were used for the discrimination of metabolite samples. Specifically, for PLS-DA, we applied LOOCV as the cross-validation method. The precision and importance of the variable in projection (VIP) were also assessed to measure the performance and importance characteristics of the analysis, respectively.

One-way analysis of variance (ANOVA one-way) was applied to homoscedastic metabolomic results, and Welch's ANOVA was applied to heteroscedastic metabolomic results. Tukey's honestly significant difference (Tukey's HSD) was used as a post hoc homoscedastic analysis and the Games-Howell post hoc Multiple Comparisons Test (Games-Howell PHMC) as post hoc heteroscedastic analysis. The two-way of variance (ANOVA two-way) was applied to homoscedastic DAI results and used a Sidak correction as a post hoc test. An adjusted p-value cut-off point (FDR) of 0.05 was applied for all analyzes. Nonparametric Kruskal-Wallis analysis and a post-Dunn test were applied for cytokine analysis. Statistical analyzes were performed using the R software version 4.1.2 [24].

Metabolic pathway analysis was performed applying the differential metabolites cross -listed with the pathways in the Kyoto Encyclopedia of Genes and Genomes (KEGG), using the pathways previously identified in the Kyoto Encyclopedia of Genes and Genomes (KEGG) of Mus musculus, and the top altered pathways were identified and built according to the potential functional analysis. For this purpose, the MetaboAnalyst 5.0 platform was also used.

Results and Discussions

DAI information and ELISA results

The sickness behavior of all animal groups was compared to evaluate disease development during the 7 days of DSS induction (Figure 2, Table S1). Figure 2 highlights the WU group that had fewer disease progression; for more information on the statistics, see Table S1.

Taking into account all symptoms related to DAI (weight loss, consistency of stool and bleeding in stool), the WU group showed less disease progression compared to the colitis group up to day 4. However, it should be noted that from the fifth day of induction there was no further difference between treatments in relation to DAI activity ($p > 0.05$).

The DSS-induced colitis protocol is the most widely used model that provides a simple and reproducible model to study various aspects, such as changes in metabolomic compounds. However, DSS can cause an aggressive disruption of intestinal barrier function [25]. DSS-induced colitis protocols have variations in relation to inductor concentration (1.5 % to 3.0 % w/w) and induction times (between 5 and 10 days) [26]. In this sense, the protocol chosen may have generated irreversible damage after the fifth day of induction, which explains the increase in the DAI result.

Along with changes in clinical aspects, cytokines have a crucial influence on the progression of colitis [27]. In this study, all groups did not show statistical differences in IL-10 and TNF-α concentrations ($p<0.05$). However, the colitis-induced and untreated group (C) differed significantly from the healthy group (H) in terms of IL-12 and IL-6 cytokines ($p < 0.05$). Furthermore, it should be noted that animals that received treatment with both types of whey protein (W and WU) likely showed a lower progression of the inflammatory process. This

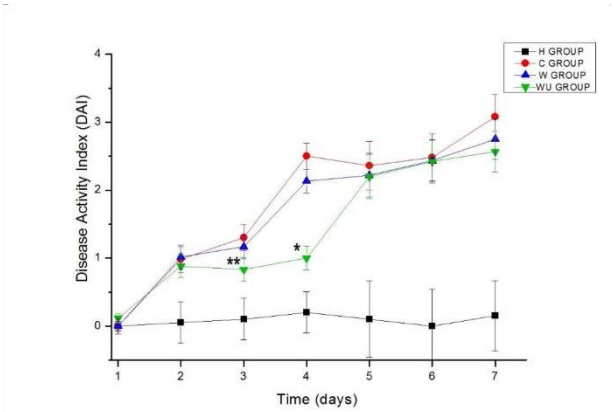

Figure 2 Disease Activity Index (DAI) of each group during DSS induction. Group H: healthy animals that did not receive the products under study; Group C: animals with chemically induced colitis that did not receive the products under study; Group W: animals with chemically induced colitis that received the whey protein without UV-C treatment; Group WU: animals with chemically induced colitis that received the whey protein with UV-C treatment. (**) no statistical difference with day 1; (*) no statistical difference with the healthy group. The Sidak p-value cut-off was: $p < 0.05$ (significant). Values are expressed as mean ± standard deviation.

is due to the fact that the median concentrations of the inflammatory cytokines IL-6 and IL-12 have intermediate values compared to those of the control groups (H and C) (Figure 3).

IL-6 is the main cytokine secreted by the lamina propria in the large intestine, having both local and systemic action. In light of current knowledge, there is controversy about its pro- or anti-inflammatory action in different diseases, including IBD [28,29]. However, this cytokine stands out for its role in intestinal homeostasis, acting on stimulation and proliferation of the intestinal lining epithelium [30]. Elevated levels of IL-6 are found in the blood and colonic mucosa of patients with IBD compared to healthy controls and are positively related to the DAI seen in UC [31]. These interrelationships between the DAI and IL-6 results can also be seen in our study.

The cytokines IL-12 and IL-23 belong to the same cytokine family (IL-12 family) and share the same p-40 domain. In the present study, the IL-12 p40 kit was used, which allows the determination of the two cytokines. These cytokines can exacerbate intestinal inflammation and are involved in the pathogenesis of CD and CU [30]. Uchiyama et al. (2021) found an increase in IL-12 and IL-23 in patients in the relapse phase of ulcerative colitis, which reinforces the importance of such cytokines in the development of IBD [32]. This may suggest that despite the progression of the disease after the fifth day of DSS-induced colitis (Figure 2), this cytokine does not show a significant relapse in the inflammation parameters, as it did not show significant differences with both control groups.

Plasma and kidney metabolic profile

Plasma and kidney tissue metabolome profiles were a total of 30 and 36 metabolites, respectively (Table 1; Figures S1 and S2). A total of 14 compounds were found in both types of

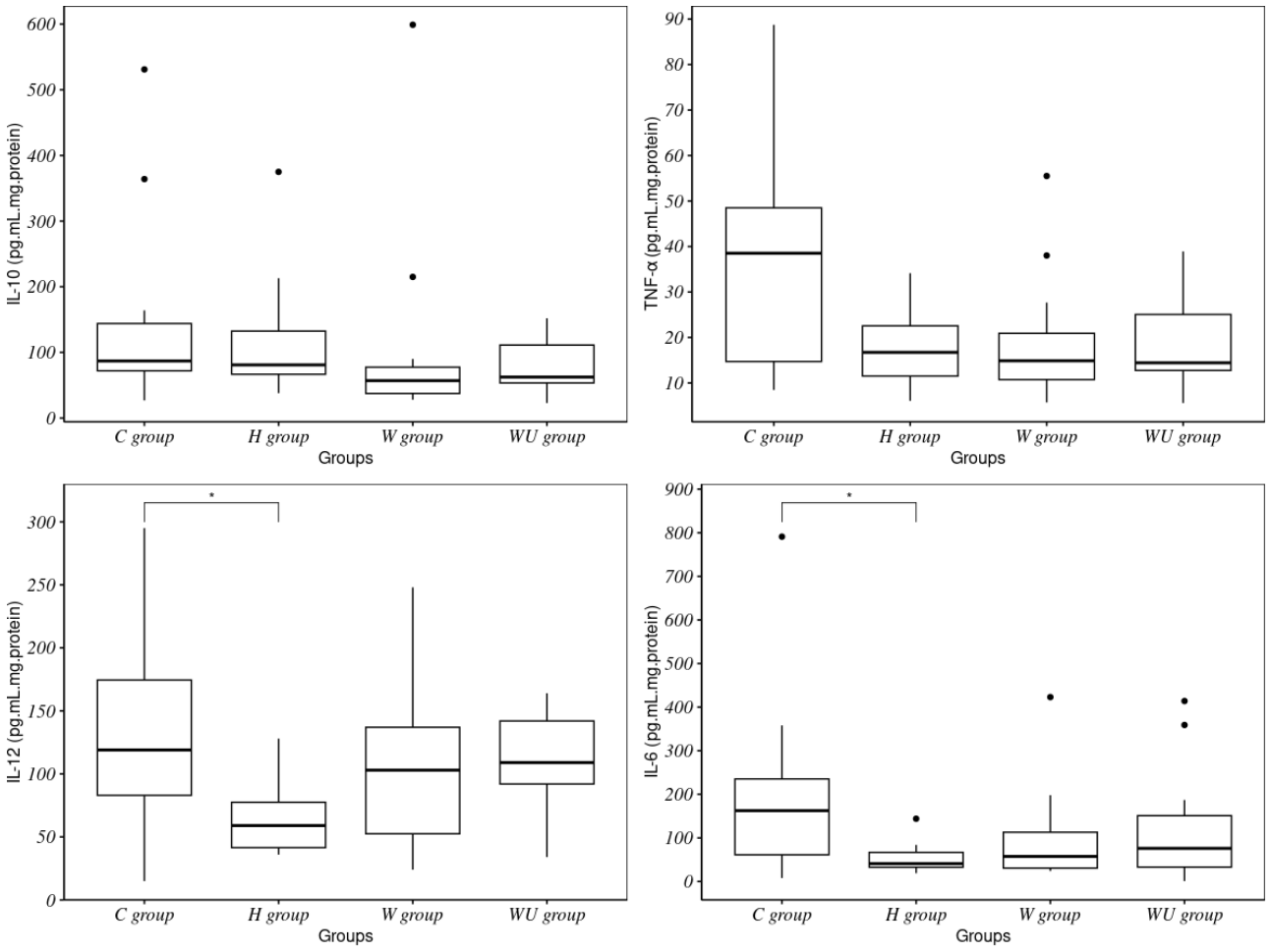

Figure 3. interleukins (IL-10, IL-6, and IL-12) and Tumor Necrosis factor alpha (TNF-α) of the different experimental groups. Non-parametric Kruskal-Wallis analysis and post Dunn's test was used to analyse compounds. p-value cut off was p < 0.05 (significant). Different small letters indicate statistically significant differences. G post Dunn's test group H: healthy animals that did not receive the products under study; Group C: animals with chemically induced colitis that did not receive the products under study; Group W: animals with chemically induced colitis that received the whey protein without UV-C treatment; Group WU: animals with chemically induced colitis that received the whey protein with UV-C treatment.

samples. The assignment is described in Table 1. The annotated compounds and the determined concentration range in our plasma and kidney tissue samples are in agreement with the literature [6,2].

The most abundant metabolites were glucose and lactate for plasma samples and glutamate, myo-inositol, and lactate for kidney tissue, as shown in Table S2. However, the metabolites that showed statistically significant differences between nutritional interventions and the colitis control group were asparagine, alanine, dimethylamine, isoleucine, phenylalanine, tyrosine and valine, as presented in Figures 4 for plasma and 5 for kidney tissue.

Table 1 ^{1}H NMR Assignments of the main metabolic metabolites from plasma and kidney samples

Annotated Compound	NMR Peak Assignment	
	Plasma	Kidney
2 -Hydroxyisobutyrate	1.34 (s, 6H)	-
2-Oxoglutarate	2.43 (t; 2H), 3.01 (t; 2H)	-
Acetate	1.89 (s; 3H)	1.91 (s; 3H)
Acetoacetate	2.26(s; 3H), 3.44 (s; 2H)	-
Acetone	2.21 (s; 3H)	-
Adenine	-	8.19 (s; 1H), 8.21 (s; 1H)
Alanine	1.45 (d; 3H), 3.75 (q; 1H)	1.47 (d; 3H), 3.81(q; 1H)
AMP	-	3.99 (m; 1H), 4.02 (m; 1H), 4.35 (m; 1H), 5.00 (t; 1H), 4.79 (t; 1H), 6.13 (d; 1H), 8.24 (s; 1H), 8.58 (s; 1H)
Arginine	-	1.64 (m; 1H), 1.72 (M; 1H), 1.92 (td ; 2H), 3.23 (t; 2H), 3.76 (t; 1H)
Asparagine	-	2.87(dd; 2H); 2.95 (dd; 2H), 4.00 (dd; 1H)
Aspartate	-	2.69 (dd; 1H), 2.80 (dd; 1H), 3.89 (t; 1H)
Carnitine	-	2.41 (1H; dd); 3.21 (9H; s); 3.38 (2H; dd); 4.56 (21H; m)
Betaine	3.25 (s; 9H) ,3.89 (s; 2H)	-
Choline	3.18 (s; 9H) , 3.51 (dd; 2H), 4.08 (m; 2H)	3.20 (s; 9H) , 3.52 (dd; 2H), 4.06 (m; 2H)
Citrate	2.48 (d; 2H), 2.63 (d; 2H)	-
Creatine	3.01 (s; 3H), 3.91 (s; 2H)	-
Creatine phosphate	-	3.03 (s; 4H), 3.94 (s; 1H)
Creatinine	3.04 (s;3H), 4.05(s;2H)	-
Dimethylamine	-	2.72 (s; 3H)
Ethanolamine	-	3.14 (t; 2H), 3.81 (t; 2H)
Formate	8.43 (s; 1H)	8.44 (s; 1H)
Fumarate	6.49 (s; 2H)	6.50 (s; 2H)
Glucose	3.22 (dd; 1H), 3.52 (dd; 1H), 3.70 (m,2H), 3.81 (dt; 1H), 3.87(dd, 1H), 5.21(d, 1H)	3.24 (dd; 1H), 3.49 (dd; 1H), 3.71 (m; 2H), 3.81 (dt; 1H), 3.89(dd; 1H), 5.23 (d; 1H)
Glutamate	-	2.11 (m; 2H), 2.31 (dt; 1H), 2.35 (dt; 1H), 3.76 (dd; 1H)
Glutamine	2.09 (td;2H), 2.41 (dt;1H) , 2.45 (dt;1H), 3.76 (td;1H)	-
Glycerol	3.52 (m; 4H), 3.63 (m; 4H), 3.78 (tt; 1H)	-
Glycine	3.55 (s;2H)	3.56 (s;2H)
Hippurate	3.93 (d; 2H), 7.53 (m; 2H) , 7.62 (tt;1H), 8.52 (dd, 2H)	3.96 (d; 2H), 7.45 (m; 2H) , 7.63 (tt;1H), 8.52 (dd, 2H)
IMP	-	4.03 (m; 1H), 4.36 (m; 1H), 4.50 (dd; 1H), 6.13 (d; 1H), 8.21 (s; 1H), 8.58 (s; 1H)
Inosine	-	3.83 (dd; 2H), 3.91 (dd;2H), 4.27 (dd; 2H), 4.77 (t; 1H), 6.10 (d; 1H), 8.24 (s; 1H), 8.33 (s; 1H)

Isoleucine	0.96 (t; 3H), 1.02 (d;2H), 1.25 (d;1H), 1.46 (m;2H), 2.31 (m;1H), 3.65 (d;1H)	0.91 (t; 3H), 1.00 (d;2H), 1.25 (d;1H), 1.46 (m;2H), 1.97 (m;1H), 3.66 (d;1H)
Isopropanol	1.17 (d;4H), 4.00 (m;3H)	-
Lactate	1.30 (d; 3H), 4.09 (q; 1H)	1.32 (d; 3H), 4.10 (q; 1H)
Leucine	-	0.94 (m; 3H), 0.96 (m; 3H), 1.68 (m; 1H);1.70(ddd; 1H), 1.77 (ddd;1H) 3.73 (t; 1H)
myo-Inositol	-	3.28 (t; 1H), 3.54 (dd; 2H), 3.61 (t; 2H), 4.06 (t; 1H)
Niaciamide	-	7.42 (dd; 1H), 7.59 (ddd; 1H), 8.71 (dd; 1H), 8.93 (dd; 1H)
Methanol	3.33 (s;3H)	-
Phenylalanine	3.12 (dd; 1H), 3.28 (m; 1H), 3.99 (dd; 2H), 7.31 (dd; 1H), 7.35 (m; 1H), 7.41 (m; 1H)	3.12 (dd; 1H), 3.28 (m; 1H), 3.99 (dd; 2H), 7.32 (dd; 1H), 7.37 (m; 1H), 7.42 (m; 1H)
Pyroglutamate	-	2.02 (m; 1H), 2.34 (ddd; 1H), 2.41 (ddd; 1H), 2.50 (m;1H), 4.17 (dd; 1H)
Pyruvate	2.35 (s; 3H)	-
Sarcosine	2.71 (s;3H), 3.58 (s;2H)	-
sn-Glycero-3-phospholine	-	3.22 (s; 9H), 3.61 (m; 4H), 3.91 (m; 3H), 4.32 (m;2H)
Succinate	2.40 (s;H4)	2.39 (s;H4)
Taurine	-	3.26 (t; 2H), 3.42 (t; H2)
Threonine	1.30 (d; 3H), 3.58 (d; 1H), 4.25 (m; 1H)	1.32 (d; 3H), 3.56 (d; 1H), 4.27 (m; 1H)
Trimethylamine	-	2.90 (s; 9H)
Trimethylamine N-oxide	3.26 (s;9H)	-
Tyrosine	3.03(dd, 1H), 3.19(dd, 1H), 3.94(t;1H), 6.88 (ddd; 1H), 7.17 (ddd; 1H)	3.04 (dd, 1H), 3.19 (dd, 1H), 3.93 (t;1H), 6.89 (ddd; 1H), 7.19 (ddd; 1H)
Uridine	-	3.80 (dd; 1H), 3.90 (dd; 1H), 4.12 (m; 1H), 4.23 (t; 1H), 4.35 (t; 1H), 5.60 (d; 1H), 6.10 (d; 1H), 7.86 (d; 1H)
Valine	0.98 (d; 3H), 1.04 (d; 3H), 2.26 (m; 1H), 3.59 (d; 1H)	0.98 (d; 3H), 1.03 (d; 3H), 2.26 (m; 1H), 3.60 (d; 1H)
Xanthine	-	7.92 (s; 1H)

One-way ANOVA analysis showed that isoleucine, glycerol, isopropanol, and glycine were not statistically different in plasma from WU compared to healthy control ($p > 0.05$), which means that WU was similar to healthy control and also similar to control samples ($p > 0.05$). The W intervention group revealed that the promissory group was different from the colitis group and similar to the healthy group, but similarity was observed between the healthy and colitis control groups (Figure 4). The kidney results obtained presented healthy controls and colitis statistically different from each other in most of the metabolite concentrations ($p < 0.05$). Furthermore, asparagine, alanine, dimethylamine, isoleucine, leucine, phenylalanine, tyrosine, and valine presented WU different from healthy control and similar to colitis control and also similar to the W intervention group (Figure 5).

IBD is currently associated with high levels of amino acids in plasma, including isoleucine [34,35]. Furthermore, a Korean epidemiological follow-up study showed that the main biomarkers positively associated with progressive to chronic kidney disease are related to amino acid metabolism (including tyrosine, phenylalanine, asparagine, alanine, isoleucine, and leucine compounds)[36]. However, only one of the amino acids found in plasma samples (isoleucine) had high concentrations in the colitis groups ($p < 0.05$) compared to a healthy control, suggesting a low action in the evolution of possible

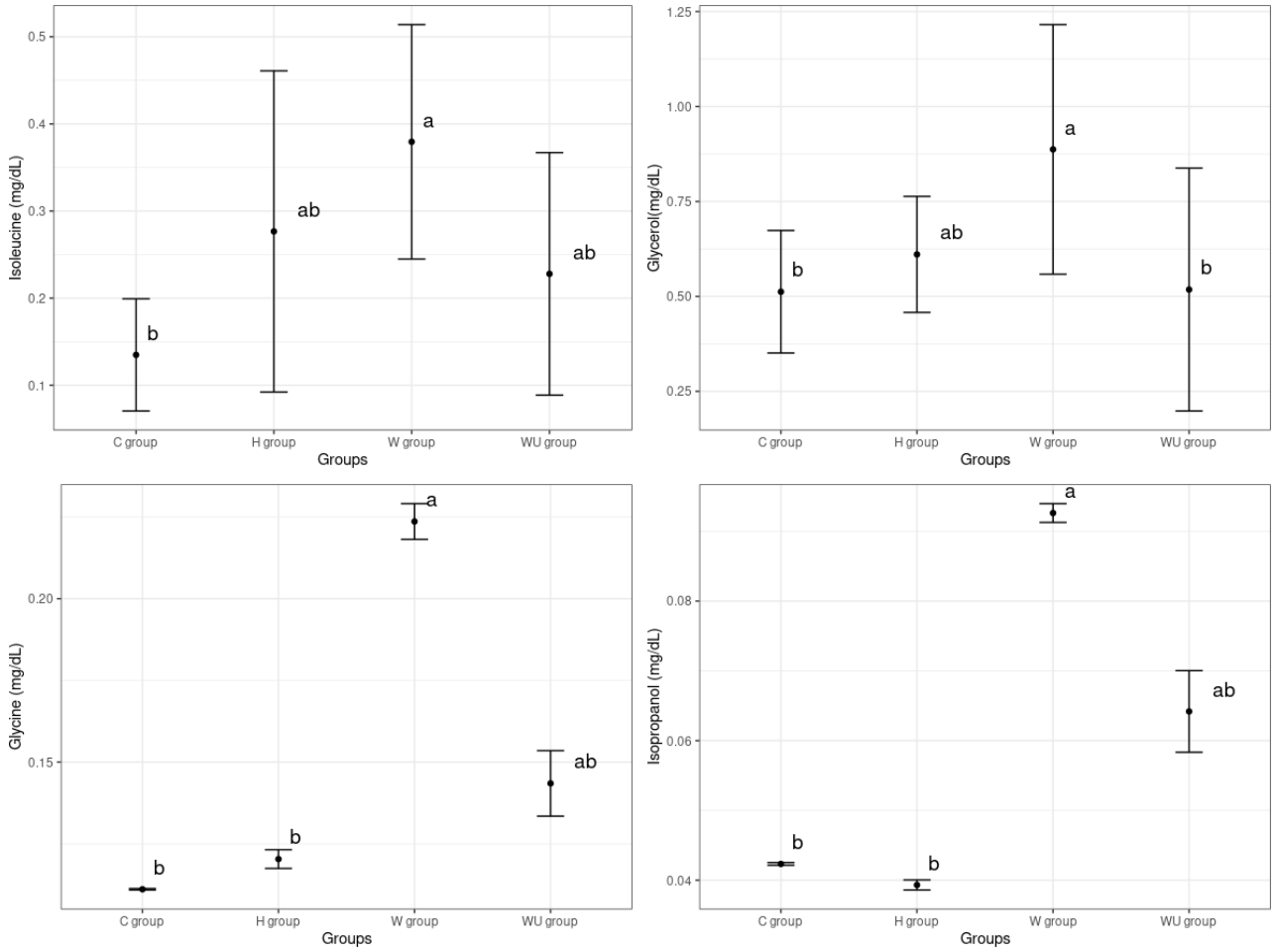

Figure 4 . Concentration in mg dL-1 (Mean ± SEM) of metabolites from plasma samples between groups. Tukey's HSD was used to analyze Isoleucine and Glycerol compounds. The Sidak comparison was used to analyze isopropyl alcohol and Glycine compounds. The cut-off point for the p-value was p < 0.05 (significant). Different small letters indicate statistically significant differences. SEM: standard error of the mean. Group H: healthy animals that did not receive the products under study; Group C: animals with chemically induced colitis that did not receive the products under study; Group W: animals with chemically induced colitis that received the whey protein without UV-C treatment; Group WU: animals with chemically induced colitis that received the whey protein with UV-C treatment.

kidney diseases in the groups that consumed whey protein. Moreover, we did not find statistical differences ($p < 0.05$) between the study groups in creatinine results (Table S1).

According to the National Kidney Foundation, the serum creatinine test can help check kidney health [37]. Wey et al. (2014) analyzed changes in serum creatinine profiles after renal ischemia / recaperfusion injury in C57BL/6 mice. Mice in a sham operation underwent a similar procedure except renal ischemia obtained serum creatinine values less than 1 mg / dl [38]. All groups in our study had values between 0.05 and 0.08 mg / dl, which may indicate that there were no renal injuries related to whey protein supplementation and progression of colitis.

Figure 6A shows the hierarchical cluster analysis for the plasma metabolite profile, resulting in colitis and healthy animal groups as the primary similarity group. This finding leads us to hypothesize that annotated metabolites might not be ideal as plasma biomarkers of the disease; they could also suggest lower concentrations of metabolites concentrations found in plasma compared to the kidney (Table S2). Blood biomarkers for UC could be other types of compounds, a more specific class of lipoproteins, which can be analyzed by NMR [39], but this was not our analytical goal, as systemic lipid metabolism could be essential to describe chronic intestinal inflammation [40].

Another thought is that relevant plasma metabolites are related to the inflammation process, which would be accessed during the chemical induction of the disease rather than at the end of the experimental protocol. However, because the amount of sample required for analysis is incompatible with the size of the mice, blood can only be collected after euthanasia. What usually occurs in plasma analysis is the comparison of different IBD subtypes that mimic this disease progression [18]. Furthermore, in our study the worsening of symptoms started on day 4, suggesting that the greatest activation of the immune system and production of characteristic metabolites may have occurred mainly at that time. So, at the end of the protocol, the disease had spread vigorously. These results confirm the findings of Dong et al. (2013) [41]. In this study, the researchers aimed to provide a response to metabolic alterations of biological matrices such as plasma, using 1H RMN of a mouse model with DSS-induced colitis. Applying the same protocol as ours (3% DSS for 7

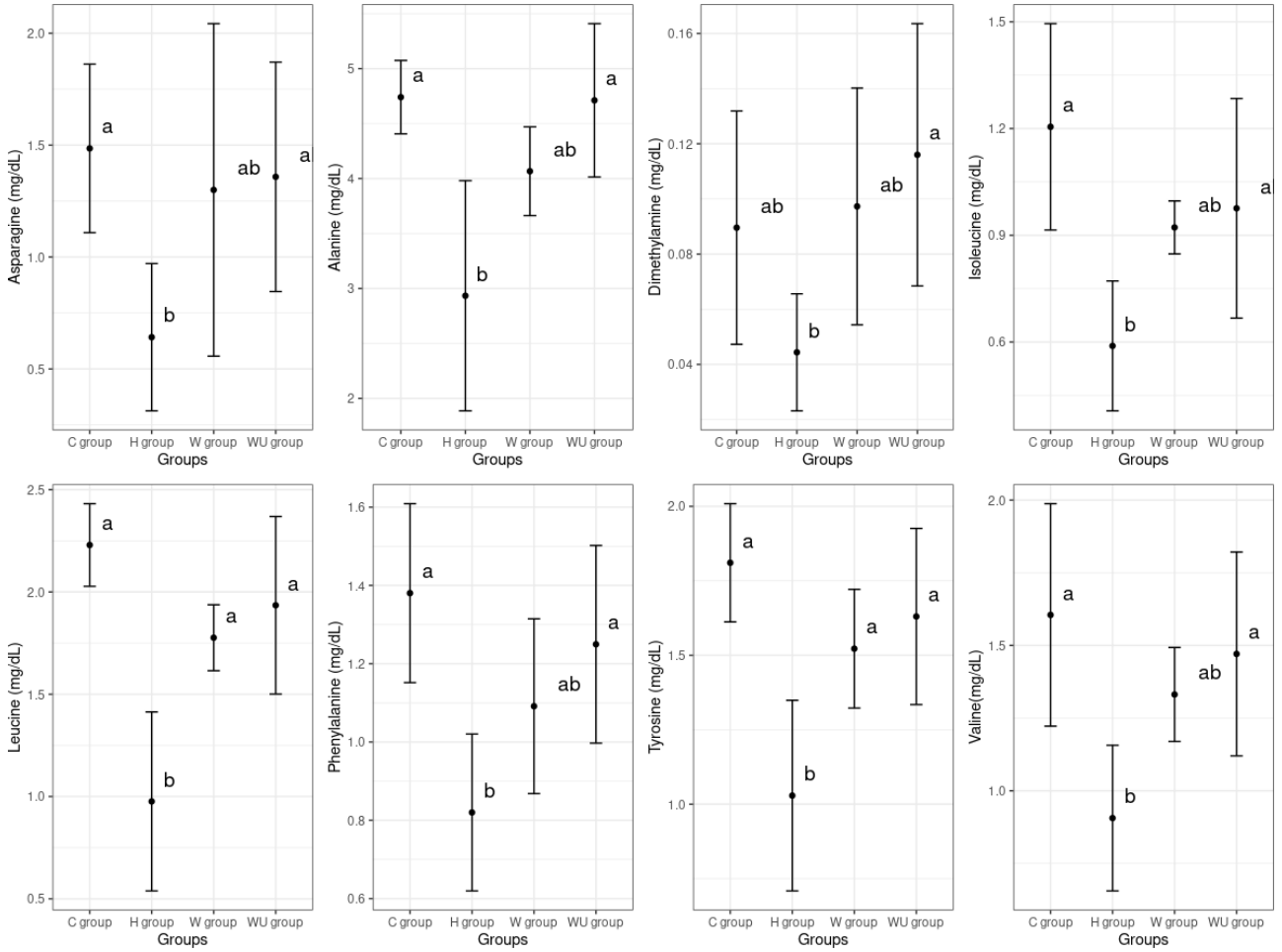

Figure 5 Concentration in mg dL-1 (Mean ± SEM) of metabolites from kidney samples between groups. Tukey's HSD was used to analyze the compounds. p-value cutoff was p < 0.05 (significant). Different small letters indicate statistically significant differences. SEM: standard error of the mean. Group H: healthy animals that did not receive the products under study; Group C: animals with chemically induced colitis that did not receive the products under study; Group W: animals with chemically induced colitis that received the whey protein without UV-C treatment; Group WU: animals with chemically induced colitis that received the whey protein with UV-C treatment.

days), they presented clinical parameters extremely affected by DSS induction after euthanasia. Furthermore, temporal analysis of urine samples showed that DSS treatment induces a rapid change in metabolic profiles on the first day of DSS ingestion. In our case, the intervention group did not have concentrations similar to a healthy control in kidney samples (Figure 6B), and this is related to the fact that the chemically induced colitis protocol was shown to be an aggressive method that could have caused irreversible damage explaining the results of DAI.

However, we can mention that the WU and W interventions clearly showed a regression of the disease, as shown by the decreases in the concentrations in kidney samples (Figures 1 and 6B). Therefore, the kidney may be an important defining point in establishing whether intervention dietary treatment with whey protein is effective in recovering from colitis disease with the information that there is no kidney damage, as some authors [24,35,42]. They mention that although there is little analysis of kidney metabolite profile and their relationship with the progression of colitis, this may be an efficient organ for a better understanding of the entire inflammation process. Furthermore, metabolomics analyzes can be a contribution to understanding the intestinal-kidney axis in different pathologies, for example, kidney disease and autoimmune disorders [43].

In fact, analysis of the metabolic pathway of the concentrations found in the kidneys revealed alterations in seven metabolic pathways when the colitis group is compared to the healthy group, as follows by the order of the highest impact value: (1) Alanine, aspartate, and glutamate metabolism; (2) Tyrosine metabolism; (3) Glyoxylate and dicarboxylate metabolism; (4) Phenylalanine, tyrosine, and tryptophan metabolism; (5) Arginine biosynthesis; (6) Glutathione metabolism; and (7) Phenylalanine metabolism (Figure 6, Table S3).

All colitis groups (colitis and colitis with Whey and Whey UVC diet interventions) showed differences in glyoxylate and dicarboxylate metabolism (metabolic pathway 3) compared to the healthy group. Previous literature shows similar results [20,25], which could suggest a relationship between amino acids and IBD. Furthermore, glycolate and dicarboxylate are a type of energy supply, and up-regulation can

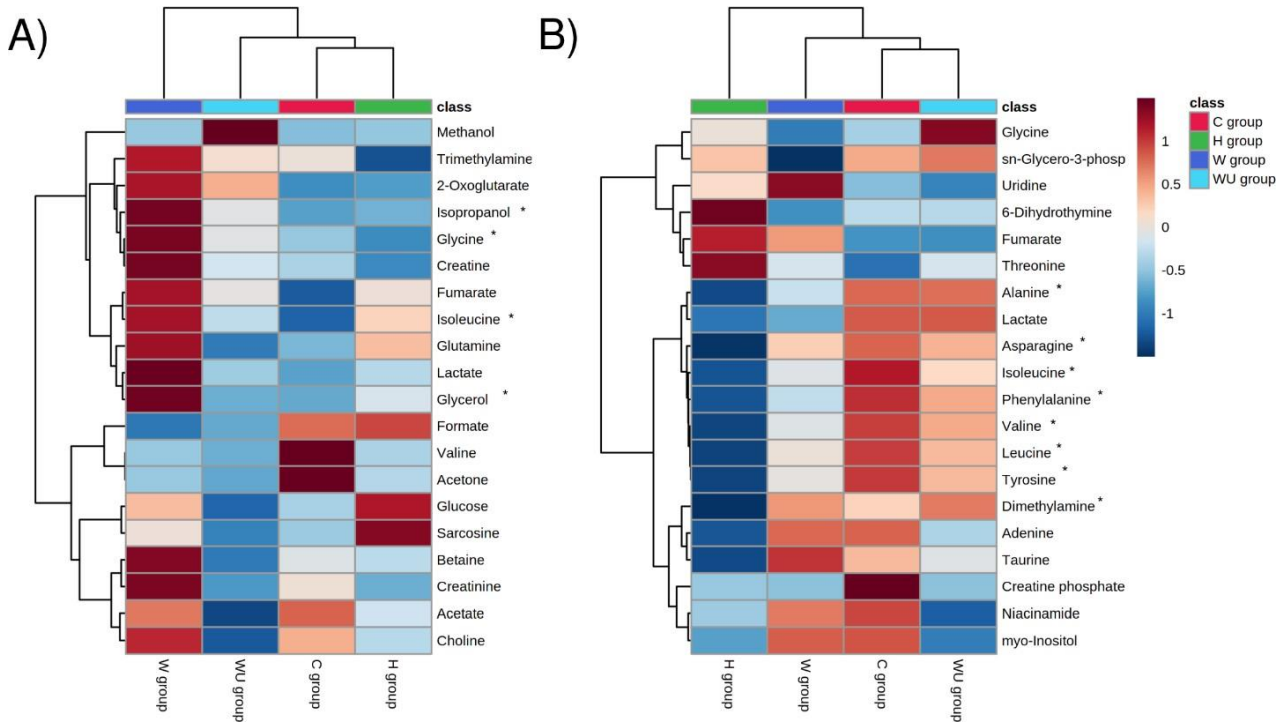

*Figure 6. Heatmaps hierarchical clustering (distance measure using Euclidean and ANOVA, clustering algorithm using ward method). A) Plasma samples; B) Kidney samples. * p < 0.05 (significant) by Tukey's HSD. Group H: healthy animals that did not receive the products under study; Group C: animals with chemically induced colitis that did not receive the products under study; Group W: animals with chemically induced colitis that received the whey protein without UV-C treatment; Group WU: animals with chemically induced colitis that received the whey protein without UV-C treatment. Group H: healthy animals that did not receive the products under study; Group C: animals with chemically induced colitis that did not receive the products under study; Group W: animals with chemically induced colitis that received the whey protein without UV-C treatment; Group WU: animals with chemically induced colitis that received the whey protein with UV-C treatment.*

be related to oxidative stress and infection in the animal [26]. Along with this metabolism, alanine, aspartate, and glutamate metabolism can be associated with an injury such as hypoxia and ischemia reperfusion injury [44]. The 1 and 3 metabolisms that were not altered during the whey interventions suggested a non-maintenance of energetic stress and no oxidative stress by the additional treatments applied (Figure 7 B-C).

In contrast to the high metabolic alteration found in the control groups, when the W group and the H group were compared, the metabolic pathways (2), (4), (5), (6), (7) were not altered (Figure 7). Our data suggest that the consumption of whey protein may benefit the maintenance of healthy metabolism in the kidneys. Furthermore, the dietary intervention with UV-C pretreated whey protein shows alterations in metabolic pathways (1), (3), (2) and (4) compared to the healthy group. Thus, three metabolic pathways could be linked to the recovery of healthy individuals with the disease: arginine biosynthesis, glutathione metabolism, and phenylalanine metabolism.

Arginine biosynthesis (pathway 5, Figure 7) is part of the urea cycle, and arginase activity is correlated with endothelial dysfunction in human inflammatory bowel disease, and the presence of L-arginine attenuates intestinal dysbiosis in murine colitis models [45,46]. Furthermore, the gut-kidney axis for arginine synthesis is connected to the flow of amino acids through the kidney in normal subjects [47]. The metabolism of glutathione (pathway 6, Figure 7) and phenylalanine (pathway 7, Figure 7) has been associated with ulcerative colitis. Glutathione has an important protection against oxidative stress, and phenylalanine (found in the high-dose results in our colitis groups (Figure 5) could be a substrate for Firmicutes and create a typical dysbiosis characteristic found in patients with IBD [48-51].

The difference between whey protein treated or not with UV-C was suggested as the goal of this study, as UV-C treatment can improve protein digestion and the release of bioactive peptides. However, diets that contain proteins as a source of nitrogen can contribute to host metabolism. Considering the protein source, digestibility, and amino acid composition, it is assumed that it can influence health effects in the course of colitis in a mouse model [52]. The UV-C light, can induce physical modifications and the production of bioactive peptides related to beneficial health effects [53]. These effects can intensify the ability of these compounds to interact with the surface of enteroendocrine cells and stimulate enteric hormone secretion [54]. However, no studies have evaluated the effect of the whey protein submitted to UV-C treatment on the evolution of IBD, such as the model *in vivo* of colitis. Even considering the complexity of IBD, we can infer that some amino acids present in conventional whey protein that are still in the protein structure but later released as free amino acids are useful for the regression of this disease.

The whey protein is known to have a high content of essential amino acids, which are necessary for body homeostasis at different stages of life [55-56]. These amino acids are the main constituents of mucin, a protein related to the protection and maintenance of intestinal mucosa integrity. Martínez-Maqueda et al. (2013) concluded that a hydrolysate from a whey protein concentrate rich

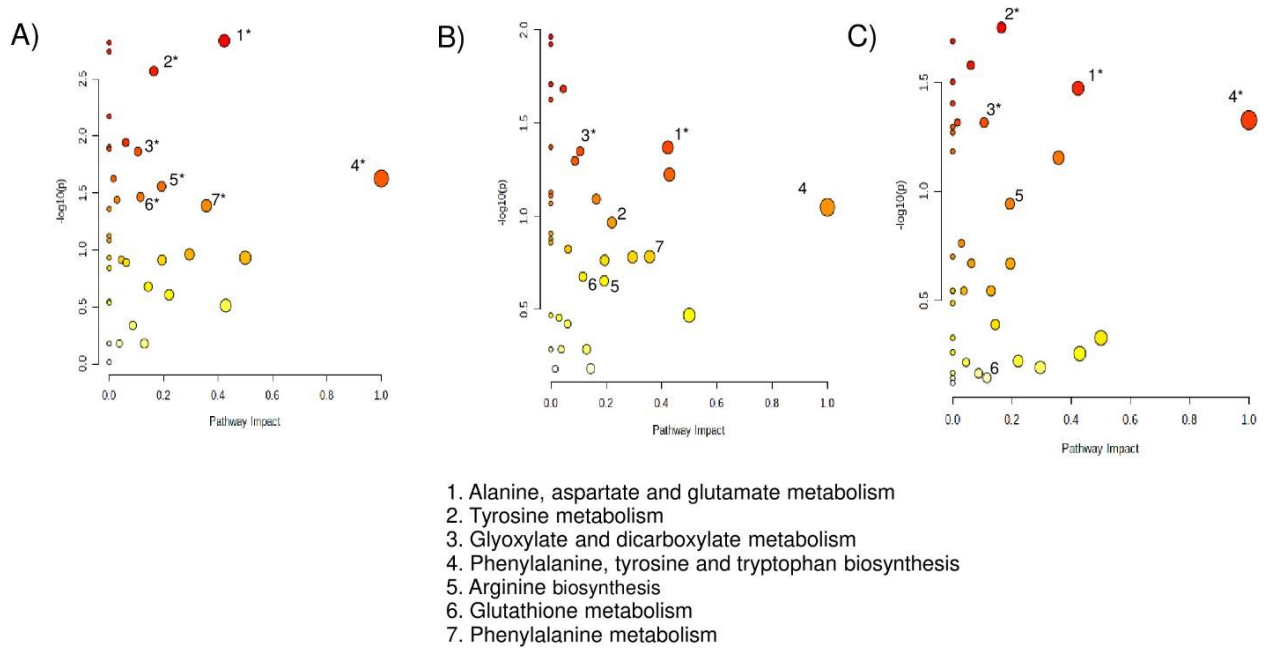

*Figure 7. Metabolic Pathway analysis from different comparisons of kidney samples. A) Group H vs. Group C; B) Group H vs. Group W; C) Group H vs. Group WU. Higher symbols indicate a higher number of metabolites, in the corresponding pathway, detected in our analysis. Red color ind icates high impact of the respective metabolic pathway. See Tables S2 for complementary information. * Indicate p < 0.05 (significant) and impact score > 0.1. Gro up H: healthy animals that did not receive the products under study; Group C: animals with chemically induced colitis that did not receive the products under st udy; Group W: animals with chemically induced colitis that received the whey protein without UV-C treatment; Group WU: animals with chemically induced colitis that received the whey protein with UV-C treatment.*

in β-lactoglobulin stimulates mucin secretion and mucin 5AC gene expression *in vitro*, showing potential to improve gastrointestinal health[57]. Furthermore, leucine, isoleucine, and valine are considered amino acids with anti-inflammatory properties and may reduce symptoms of inflammatory bowel disease [58,59].

Therefore, kidney tissue from mouse model UC has shown that relevant metabolic pathways related to infection syndrome are reprogramming and alleviate the progressive progression of disease when conventional and irradiated whey was administered as a supplementary diet, comparing their pathway analysis with healthy groups according to metabolic changes observed in positive controls (UC) versus negative controls (healthy). Comparison of the metabolic pathway between the colitis group and the treatment groups did not show differences ($p < 0.05$ or impact < 0.1) (data not shown), as we expect due to the fact that treatment was applied in colitis mice and treatment is not a cure, but seeks regression and/or reduction in the severity of the disease.

Conclusions

We show that consuming a UV-C-treated whey protein supplement on a regular basis reduced the symptoms of DSS-induced colitis and positively affected kidney metabolism. This is the first study to demonstrate that the recently developed UV-C processing technology may be utilized to increase the efficacy of whey protein supplementation on colitis control. We also confirm that the kidney metabolic profile is a promising tool for discovering biomarkers for colitis diagnosis. More research is required before UV-C-processed whey protein can be marketed as a functional food for UC patients.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

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Notes and references

1 Hendrickson BA, Gokhale R, Cho JH, Clinical Aspects and Pathophysiology of Inflammatory Bowel Disease. *Clinical Microbiology Reviews*, 2002, **15**(1), 79–94.

2 Ananthakrishnan AN., Epidemiology and risk factors for IBD. *Nature Reviews Gastroenterology & Hepatology*, 2015, **12**(4), 205–17.

3 Celiberto LS, Graef FA, Healey GR, Bosman ES, Jacobson K, Sly LM, et al., Inflammatory bowel disease and

immunonutrition: novel therapeutic approaches through modulation of diet and the gut microbiome, *Immunology*, 2018, **155**(1), 36–52.
4 Bürger M, Schmidt C, Teich N, Stallmach A., Medical Therapy of Active Ulcerative Colitis, *Visceral Medicine*, 2015, **31**(4), 236–45.
5 Ng SC, Shi HY, Hamidi N, Underwood FE, Tang W, Benchimol EI, et al., Worldwide incidence and prevalence of inflammatory bowel disease in the 21st century: a systematic review of population-based studies, The *Lancet*, 2017, **390**(10114), 2769–78.
6 Schicho R, Nazyrova A, Shaykhutdinov R, Duggan G, Vogel HJ, Storr M, Quantitative Metabolomic Profiling of Serum and Urine in DSS-Induced Ulcerative Colitis of Mice by 1H NMR Spectroscopy, *Journal of Proteome Research*, 2010, **28;9**(12), 6265–73.
7 Santiago-Lopez L, F. Gonzalez-Cordova A, Hernandez-Mendoza A, Vallejo-Cordoba B, Potential Use of Food Protein-Derived Peptides in the Treatment of Inflammatory Diseases*, Protein & Peptide Letters*, 2017, **24**(2), 137–45.
8 Vidal-Lletjós S, Andriamihaja M, Blais A, Grauso M, Lepage P, Davila A-M, et al., Dietary Protein Intake Level Modulates Mucosal Healing and Mucosa-Adherent Microbiota in Mouse Model of Colitis, *Nutrients*, 2019, **28;11**(3), 514.
9 Kristo E, Hazizaj A, Corredig M., Structural Changes Imposed on Whey Proteins by UV Irradiation in a Continuous UV Light Reactor*, Journal of Agricultural and Food Chemistry*, 2012, **60**(24), 6204–9.
10 Täufel A. M. R. , Adams M. O. Moss, *Food microbiology*, The Royal Society of Chemistry,Seiten, zahlr. Abbildungen und Tabellen. The Royal Society of Chemistry, Cambridge, 1995.
11 Gayán E, Condón S, Álvarez I. Biological Aspects in Food Preservation by Ultraviolet Light: a Review, *Food and Bioprocess Technology*, 2013, **7**(1), 1–20.
12 Araújo DF de S, Guerra GCB, Júnior RF de A, Antunes de Araújo A, Antonino de Assis PO, Nunes de Medeiros A, et al., Goat whey ameliorates intestinal inflammation on acetic acid-induced colitis in rats, *Journal of Dairy Science,* 2016, **99**(12), 9383–94.
13 Araújo DFS, Guerra GCB, Pintado MME, Sousa YRF, Algieri F, Rodriguez-Nogales A, et al., Intestinal anti-inflammatory effects of goat whey on DNBS-induced colitis in mice, *PLOS ONE*, 2017, **12**(9), DOI: 10.1371/journal.pone.0185382
14 Kanwar JR, Kanwar RK, Stathopoulos S, Haggarty NW, MacGibbon AKH, Palmano KP, et al., Comparative activities of milk components in reversing chronic colitis. *Journal of Dairy Science*, 2016, **99**(4), 2488–501.
15 Randhawa PK, Singh K, Singh N, Jaggi AS., A Review on Chemical-Induced Inflammatory Bowel Disease Models in Rodents, *The Korean Journal of Physiology & Pharmacology*, 2014, **18**(4), 279.
16 Al-Omari MM, Abed Alkarem Abu Alhaija RBA-G, Zoubi HA-, Al-Qaoud KM, Camel milk Whey Inhibits Inflammatory Colorectal Cancer Development Via Down regulation of Pro-inflammatory Cytokines in Induced AOM/DSS Mouse Model, *Emirates Journal of Food and Agriculture*, 2019,**31** (4), 256-262.
17 Eichele DD, Kharbanda KK, Dextran sodium sulfate colitis murine model: An indispensable tool for advancing our understanding of inflammatory bowel diseases pathogenesis, *World Journal of Gastroenterology*, 2017, **23**(33), 6016–29.
18 Storr M, Vogel HJ, Schicho R., Metabolomics: Is it useful for inflammatory bowel diseases? *Current Opinion in Gastroenterology,* 2013, **29**(4), 378–83.
19 Tudoroniu EE, Costache MA, Stancu M-A, Pop AL, New Metabolic and Genetic Biomarkers for Diagnosis of Ulcerative Colitis, *Preprints*, 2021, DOI: 10.20944/preprints202108.0048.v1
20 Schicho R, Haykhutdinov R, Ngo J, Nazyrova A, Schneider C, Panaccione R, et al., Quantitative Metabolomic Profiling of Serum, Plasma, and Urine by 1H NMR Spectroscopy Discriminates between Patients with Inflammatory Bowel Disease and Healthy Individuals, Journal *of Proteome Research*, 2012, **11**(6), DOI: 10.1021/pr300139q.
21 Vasconcelos QDJS, Bachur TPR, Aragão GF, Whey protein supplementation and its potentially adverse effects on health: a systematic review, *Applied Physiology, Nutrition, and Metabolism*, 2021, **46**(1), 27–33.
22 Murthy S, Murthy NS, Coppola D, Wood DL, The efficacy of BAY y 1015 in dextran sulfate model of mouse colitis, *Inflammation Research*, 1997, **46**(6), 224–33.
23 Nagana Gowda GA, Raftery D. Analysis of Plasma, Serum, and Whole Blood Metabolites Using 1H NMR Spectroscopy, *Methods in molecular bioogy, 20*19, **2037**, 17–34.
24 R Core Team. R: The R Project for Statistical Computing. 2021. Available from: https://www.R-project.org/
25 Hong Z. C., Cai Q., Wu H.Z., Yang Y.F., Fan H., Duan X.Y., Compound Sophorae Decoction: treating ulcerative colitis by affecting multiple metabolic pathways. *Chinese Journal of Natural Medicines*, 2021, **19**(4), 267–83.
26 Sun M, Du B, Shi Y, Lu Y, Zhou Y, Liu B., Combined Signature of the Fecal Microbiome and Plasma Metabolome in Patients with Ulcerative Colitis, *Medical Science Monitor*, 2019, **25**, 3303–15.
27 Neurath, M. Cytokines in inflammatory bowel disease. *Nature Reviews Immunology*, 2014, **14**, 329–342.
28 Ahmed, S. T., Ivashkiv, L. B. Inhibition of IL-6 and IL-10 Signaling and Stat Activation by Inflammatory and Stress Pathways. *The Journal of Immunology*, 2000, **165**(9), 5227–5237.
29 Andres-Hernando A, Okamura, K, Bhargava R, Kiekhaefer C M, Soranno D, Kirkbride-Romeo L A, Gil H, Altamann C, Faubel S. Circulating IL-6 upregulates IL-10 production in splenic CD4+ T cells and limits acute kidney injury–induced lung inflammation. *Kidney International*, 2017, **91**(5), 1057–1069.
30 Guan Q, Zhang J. Recent Advances: The Imbalance of Cytokines in the Pathogenesis of Inflammatory Bowel Disease. *Mediators of Inflammation*, 2017, DOI: 10.1155/2017/4810258
31 Schreiber S, Aden K, Bernardes J P, Conrad C, Tran F, Höper H, Volk V, Mishra N, Blase J I, Nikolaus S, Bethge J, Kühbacher T, Röcken C, Chen M, Cottingham I, Petri N, Rasmussen B B, Lokau J, Lenk L, Garbers C, Feuerhake F, Rose-John S, Waetzig G H, Rosenstiel P. Therapeutic Interleukin-6 Trans-signaling Inhibition by Olamkicept (sgp130Fc) in Patients With Active Inflammatory Bowel Disease. *Gastroenterology*, 2021, **160**(7), 2354- 2366.
32 Uchiyama K, Takagi T, Mizushima K, Kajiwara-Kubota M, Kashiwagi S, Toyokawa Y, Tanaka M, Hotta Y, Kamada K, Ishikawa T, Konishi H, Kishimoto M, Naito Y, Itoh Y. Increased mucosal IL-12 expression is associated with relapse of ulcerative colitis. *BMC Gastroenterology*, 2021, **21**(1), p. 1-9.

33 Gallagher K, Catesson A, Griffin JL, Holmes E, Williams HRT, Metabolomic Analysis in Inflammatory Bowel Disease: A Systematic Review, *Journal of Crohn's and Colitis*, 2020, **15**(5), 813–26.

34 Probert F, Walsh A, Jagielowicz M, Yeo T, Claridge TDW, Simmons A, et al., Plasma Nuclear Magnetic Resonance Metabolomics Discriminates Between High and Low Endoscopic Activity and Predicts Progression in a Prospective Cohort of Patients with Ulcerative Colitis, *Journal of Crohn's and Colitis*, 2018, **12**(11), 1326–37.

35 Xie D, Li F, Pang D, Zhao S, Zhang M, Ren Z, et al., Systematic Metabolic Profiling of Mice with Dextran Sulfate Sodium-Induced Colitis, Journal *of Inflammation Research*, 2021, **14**, 2941–53.

36 Lee H, Jang HB, Yoo M-G, Park SI, Lee HJ, Amino Acid Metabolites Associated with Chronic Kidney Disease: An Eight-Year Follow-Up Korean Epidemiology Study, *Biomedicines*, 2020, **8**(7), DOI: 10.3390/biomedicines8070222.

37 National Kidney Foundation, https://www.kidney.org/atoz/content/what-creatinine#what-normal-level-creatinine (accessed October 2022).

38 Wei Q, Xiao X, Fogle P, Dong Z, Changes in Metabolic Profiles during Acute Kidney Injury and Recovery following Ischemia/Reperfusion, *PLoS ONE*, 2014, **9**, DOI: 10.1371/journal.pone.0106647.

39 Stevens VL, Hoover E, Wang Y, Zanetti KA, Pre-Analytical Factors that Affect Metabolite Stability in Human Urine, Plasma, and Serum: A Review, *Metabolites*, 2019, **9**(8), DOI: 10.3390/metabo9080156.

40 Williams HRT, Willsmore JD, Cox J, Walker DG, Cobbold JFL, Taylor-Robinson SD, et al., Serum Metabolic Profiling in Inflammatory Bowel Disease, *Digestive Diseases and ScienceS,* 2012, **57**(8), DOI: 10.1007/s10620-012-2127-2.

41 Dong F, Zhang L, Hao F, Tang H, Wang Y, Systemic Responses of Mice to Dextran Sulfate Sodium-Induced Acute Ulcerative Colitis Using 1H NMR Spectroscopy, *Journal of Proteome Research*, 2013,**12**(6), 2958–66.

42 Ahn S, Jung J, Jang I-A, Madsen EL, Park W, Role of Glyoxylate Shunt in Oxidative Stress Response, *Journal of Biological Chemistry*, 2016, **291**(22),11928–38.

43 Stavropoulou E, Kantartzi K, Tsigalou C, Konstantinidis T, Romanidou G, Voidarou C, et al., Focus on the Gut–Kidney Axis in Health and Disease, *Frontiers in Medicine*, 2021,**7**, DOI: 10.3389/fmed.2020.620102.

44 Fox BM, Gil H-W, Kirkbride-Romeo L, Bagchi RA, Wennersten SA, Haefner KR, et al., Metabolomics assessment reveals oxidative stress and altered energy production in the heart after ischemic acute kidney injury in mice, *Kidney International*, 2019, **95**(3), 590–610.

45 Horowitz S, Binion DG, Nelson VM, Kanaa Y, Javadi P, Lazarova Z, et al., Increased arginase activity and endothelial dysfunction in human inflammatory bowel disease, *American Journal of Physiology-Gastrointestinal and Liver Physiology*, 2007, **292**(5), 1323–36.

46 Coburn LA, Gong X, Singh K, Asim M, Scull BP, Allaman MM, et al., L-arginine Supplementation Improves Responses to Injury and Inflammation in Dextran Sulfate Sodium Colitis, *PLoS ONE*, 2012, **7**(3), DOI: doi.org/10.1371/journal.pone.0033546.

47 Brosnan ME, Brosnan JT, Renal Arginine Metabolism, *The Journal of Nutrition*, 2004, **134**(10), 2791S-2795S.

48 Jagt JZ, Verburgt CM, de Vries R, de Boer NKH, Benninga MA, de Jonge WJ, et al., Faecal Metabolomics in Paediatric Inflammatory Bowel Disease: A Systematic Review. Journal of Crohn's and Coliti, 2022, DOI: 10.1093/ecco-jcc/jjw158.

49 Dolan KT, Chang EB. Diet, gut microbes, and the pathogenesis of inflammatory bowel diseases, *Molecular Nutrition & Food Research*, 2016, 61(1), DOI: 10.1002/mnfr.201600129.

50 Filimoniuk A, Daniluk U, Samczuk P, Wasilewska N, Jakimiec P, Kucharska M, et al., Metabolomic profiling in children with inflammatory bowel disease, *Advances in Medical Sciences,* 2020,**65**(1), 65–70.

51 Anderson ME, Glutathione: an overview of biosynthesis and modulation, *Chemico-Biological Interactions*, 1998, **111–112**, 1–14.

52 Lan A, Blais A, Coelho D, Capron J, Maarouf M, Benamouzig R, et al., Dual effects of a high-protein diet on DSS-treated mice during colitis resolution phase, *American Journal of Physiology-Gastrointestinal and Liver Physiology*, 2016,**311**(4), G624–33.

53 Esteghlal S, Gahruie HH, Niakousari M, Barba FJ, Bekhit AE-D, Mallikarjunan K, et al., Bridging the Knowledge Gap for the Impact of Non-Thermal Processing on Proteins and Amino Acids, *Foods*, 2019, **8**(7), 262, DOI: doi.org/10.3390/foods8070262.

54 Tulipano G, Role of Bioactive Peptide Sequences in the Potential Impact of Dairy Protein Intake on Metabolic Health, *International Journal of Molecular Sciences*, 2020, 21(22), 8881, DOI: 10.3390/ijms21228881.

55 Krissansen GW, Emerging Health Properties of Whey Proteins and Their Clinical Implications, *Journal of the American College of Nutrition*, 2007, **26**(6), 713S-723S.

56 Layman DK, Lönnerdal B, Fernstrom JD, Applications for α-lactalbumin in human nutrition, *Nutrition Reviews*, 2018, **76**(6), 444–60.

57 Martínez-Maqueda D, Miralles B, Ramos M, Recio I, Effect of β-lactoglobulin hydrolysate and β-lactorphin on intestinal mucin secretion and gene expression in human goblet cells, *Food Research International*, 2013, **54**(1), 1287–91.

58 Lagrange V, Clark DC. In *Whey proteins*, ed. Deeth H C, Bansal N, first edition, 2019, Chapter 15 - Nutritive and Therapeutic Aspects of Whey Proteins, 549–77.

59 Smithers GW, Whey and whey proteins—From 'gutter-to-gold.', *International Dairy Journal*, 2008, **18**(7), 695–704.

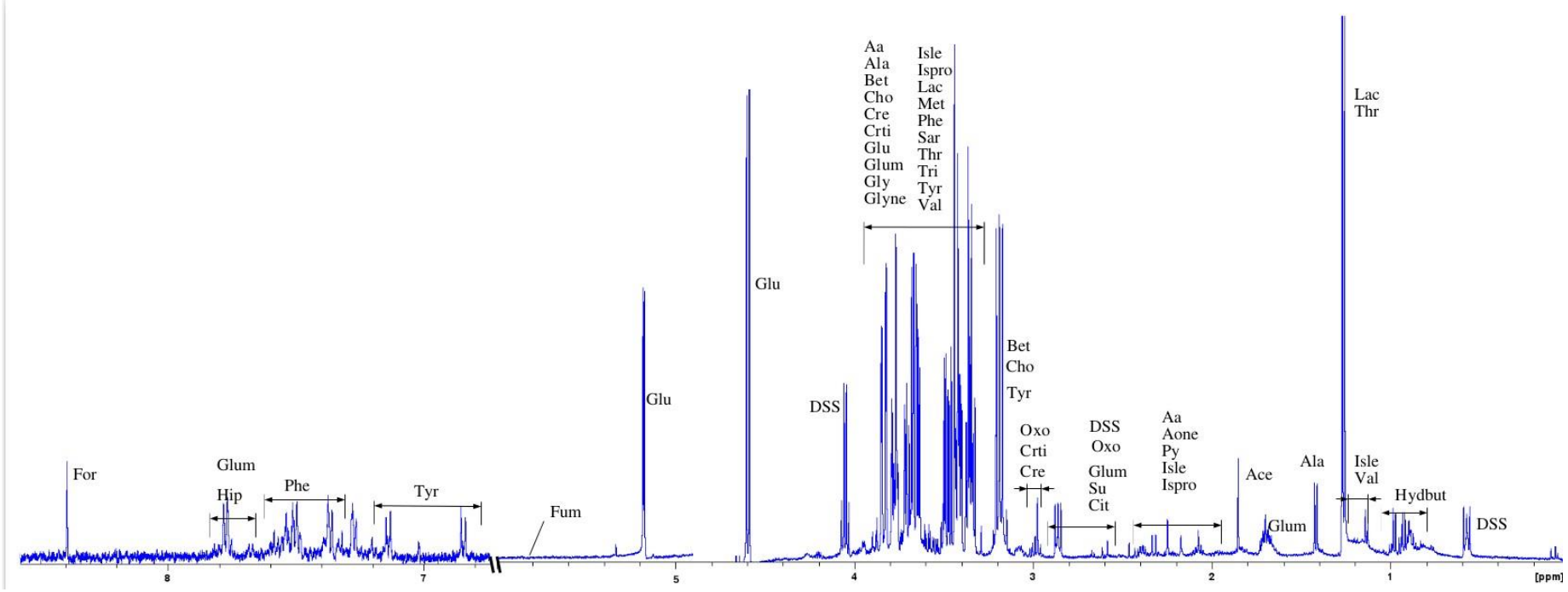
Support Information

Table S1. Disease Activity Index (DAI) of each group during the DSS colitis induction period.

Groups	day 1	day 2	day 3	day 4	day 5	day 6	day 7	Total
H	0.00 ± 0.11 ^{Aa}	0.05 ± 0.30 ^{Aa}	0.10 ± 0.31 ^{Aa}	0.20 ± 0.30 ^{Aa}	0.10 ± 0.56 ^{Aa}	0.00 ± 0.54 ^{Aa}	0.15 ± 0.52 ^{Aa}	0.09 ± 0.26 ^{a}
C	0.00 ± 0.07 ^{Aa}	0.98 ± 0.19^{Ab}	1.30 ± 0.19 ^{Ab}	2.50 ± 0.19 ^{Bb}	2.36 ± 0.35 ^{Bb}	2.48 ± 0.34 ^{Bb}	3.08 ± 0.33 ^{Bb}	1.81 ± 0.17 ^{b}
W	0.00 ± 0.07 ^{Aa}	1.02 ± 0.17 ^{Bb}	1.17 ± 0.17 ^{Bb}	2.13 ± 0.17 ^{Bb}	2.22 ± 0.30 ^{Bb}	2.43 ± 0.31 ^{Bb}	2.75 ± 0.30 ^{Bb}	1.68 ± 0.15 ^{Bb}
WU	0.11 ± 0.07 ^{Aa}	0.88 ± 0.17 ^{BCab}	0.84 ± 0.17 ^{ABb}	1.00 ± 0.17 ^{BCa}	2.20 ± 0.32 ^{BCb}	2.42 ± 0.31 ^{Cb}	2.57 ± 0.30 ^{Cb}	1.43 + 0.15 ^{b}

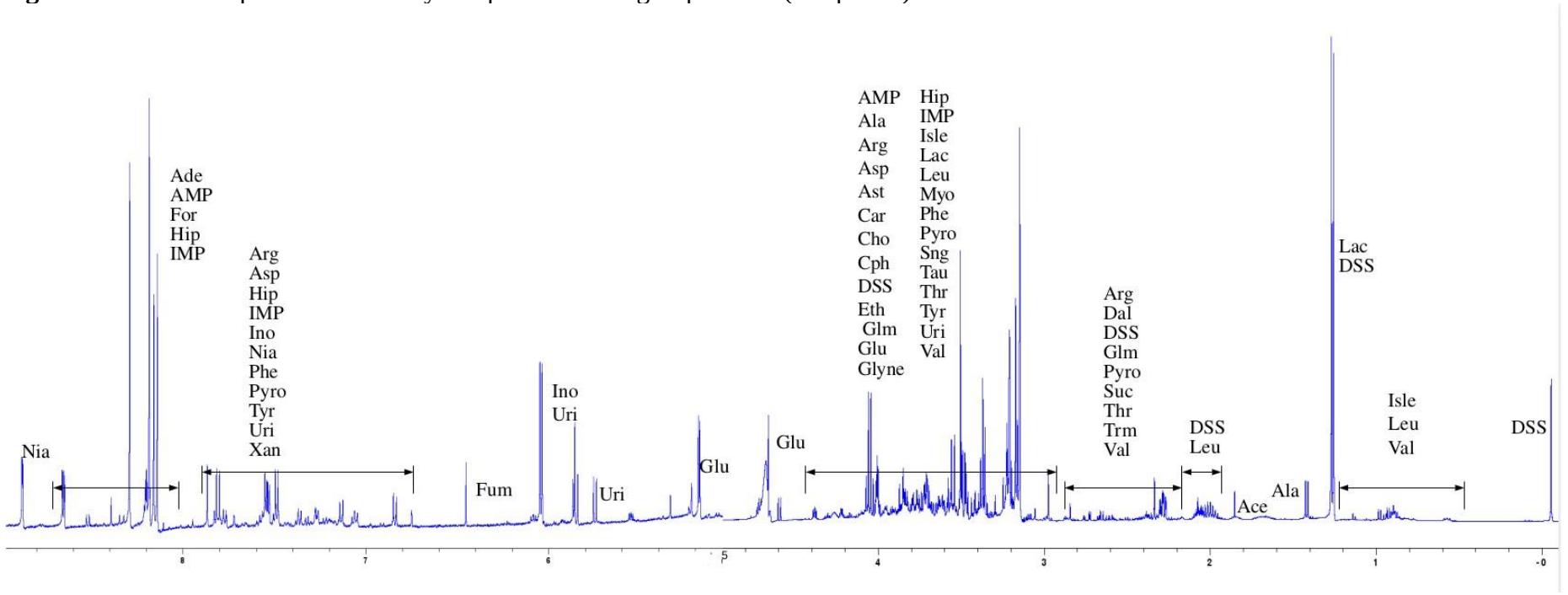
Legend: Different capital letters indicate statistically significant differences between days in the group and different small letters indicate statistically significant differences between groups on the same day. Group H: healthy animals that did not receive the products under study; Group C: animals with chemically induced colitis that did not receive the products under study; Group W: animals with chemically induced colitis that received the whey protein without UV-C treatment; Group WU: animals with chemically induced colitis that received the whey protein without UV-C treatment. Results present the mean ± standard error of the mean (Mixed ANOVA . Sidak post test . $\alpha = 0.05$).

Figure S1. ^{1}H NMR spectrum of blood plasma sample from a W group mouse (sample 18).



Legend: Hydbut: 2 -Hydroxyisobutyrate. Oxo: 2-Oxoglutarate. Ace: Acetate. Aa: Acetoacetate Aone: Acetone. Ala: Alanine. Bet: Betaine.Cho: Choline.Cit: Citrate. Cre: Creatine. Crti: Creatinine. DSS: Chemical Shape Indicator.For: Formate. Fum: Fumarate.Glum: Glutamine.Glu: Glucose. Gly: Glycerol. Glyne: Glycine.Hip: Hippurate.Isle: Isoleucine.Ispro: Isopropanol.Lac: Lactate. Met: Methanol. Phe: Phenylalanine. Py: Pyruvate. Sa: Sarcosine.Su: Succinate.Thr: Threonine. Tri: Trimethylamine N-oxide.Tyr: Tyrosine. Val: Valine.

Figure S2. ^{1}H NMR spectrum of kidney sample from a W group mouse (sample 18).



Legend: Ace: Acetate. Ade: Adenine.AMP: 5'-Adenylic acid.Ala: Alanine. Arg:Arginine. Asp:Asparagine.Ast:Aspartate. Car: Carnitine. Cho: Choline. Cph: Creatine phosphate. Dal:Dimethylamine. Eth: Ethanolamine. DSS: Chemical Shape Indicator. For: Formate. Fum: Fumarate. Glu: Glucose. Glm: Glutamate. Glyne: Glycine. Hip: Hippurate. IMP: 5'-Inosinic acid. Ino: Inosine Isle: Isoleucine. Lac: Lactate. Leu: Leucine.Myo: myo-Inositol. Nia: Niacinamide. Phe: Phenylalanine. Pyro: Pyroglutamate. Sng: sn-Glycero-3-phosphocholine. Su: Succinate. Tau: Taurine. Thr: Threonine. Trm: Trimethylamine. Tyr: Tyrosine. Uri: Uridine. Val: Valine. Xan: Xanthine.

Table S2. Metabolites concentration in plasma and kidney samples (mg/dL).

Samples	Plasma				Kidney			
Metabolites	**C**	**H**	**W**	**WU**	**C**	**H**	**W**	**WU**
2 -Hydroxyisobutyrate	0.01 ± 0.01 [a]	0.01 ± 0.01 [a]	0.01 ± 0.01 [a]	0.01 ± 0.01 [a]	-	-	-	-
2-Oxoglutarate	0.30 ± 0.20 [a]	0.30 ± 0.20 [a]	0.48 ± 0.29 [a]	0.41 ± 0.29 [a]	-	-	-	-
Acetate	0.25 ± 0.14 [a]	0.23 ± 0.09 [a]	0.25 ± 0.09 [a]	0.21 ± 0.13 [a]	0.74 ± 0.11 [a]	0.76 ± 0.09 [a]	0.72 ± 0.09 [a]	0.73 ± 0.15 [a]
Acetoacetate	0.02 ± 0.01 [a]	0.05 ± 0.05 [a]	0.04 ± 0.02 [a]	0.03 ± 0.03 [a]	-	-	-	-
Acetone	0.03 ± 0.03 [a]	0.22 ± 0.01 [a]	0.02 ± 0.00 [a]	0.02 ± 0.01 [a]	-	-	-	-
Adenine	-	-	-	-	10.94 ± 1.13 [a]	8.86 ± 1.11 [a]	10.80 ± 0.50 [a]	9.77 ± 1.88 [a]
Alanine	0.42 ± 0.22 [a]	0.64 ± 0.32 [a]	0.72 ± 0.33 [a]	0.41 ± 0.28 [a]	4.74 ± 0.33 [a]	2.93 ± 1.05 [b]	4.17 ± 0.44 [ab]	4.71 ± 0.70 [a]
AMP	-	-	-	-	0.52 ± 0.19 [a]	0.43 ± 0.34 [a]	0.44 ± 0.24 [a]	0.39 ± 0.06 [a]
Arginine	-	-	-	-	3.56 ± 0.99 [a]	2.20 ± 1.11 [a]	3.38 ± 0.91 [a]	4.32 ± 0.70 [a]
Asparagine	-	-	-	-	1.48 ± 0.38 [a]	0.64 ± 0.33 [b]	1.28 ± 0.67 [ab]	1.36 ± 0.51 [a]
Aspartate	-	-	-	-	4.90 ± 0.61 [a]	4.96 ±0.28 [a]	4.52 ± 0.56 [a]	4.67 ± 1.30 [a]

Carnitine	-	-	-	-	4.29 ± 0.53 [a]	3.23 ± 0.43 [a]	3.78 ± 0.65 [a]	3.90 ± 0.77 [a]
Betaine	0.19 ± 0.11 [a]	0.18 ± 0.10 [a]	0.26 ± 0.07 [a]	0.14 ± 0.09 [a]	-	-	-	-
Choline	0.08 ± 0.05 [a]	0.07 ± 0.03 [a]	0.08 ± 0.03 [a]	0.06 ± 0.04 [a]	12.55 ± 1.26 [a]	10.57 ± 1.20 [a]	11.94 ± 1.18 [a]	12.03 ± 1.70 [a]
Citrate	0.41 ± 0.18 [a]	0.56 ± 0.20 [a]	0.61 ± 0.1 [a]	0.38 ± 0.23 [a]	-	-	-	-
Creatine	0.24 ± 0.14 [a]	0.21 ± 0.16 [a]	0.35 ± 0.11 [a]	0.26 ± 0.17 [a]	-	-	-	-
Creatine phosphate	-	-	-	-	7.31 ± 2.00 [a]	4.60 ± 2.25 [a]	4.28 ± 0.46 [a]	5.31 ± 0.41 [a]
Creatinine	0.06 ± 0.04 [a]	0.05 ± 0.03 [a]	0.08 ± 0.04 [a]	0.05 ± 0.04 [a]	-	-	-	-
Dimethylamine	-	-	-	-	0.09 ± 0.04 [ab]	0.04 ± 0.02 [b]	0.10 ± 0.04 [ab]	0.10 ± 0.05 [a]
Ethanolamine	-	-	-	-	1.13 ± 0.26 [a]	0.78 ± 0.42 [a]	0.91 ± 0.04 [a]	1.19 ± 0.34 [a]
Formate	0.16 ± 0.07 [a]	0.17 ± 0.404 [a]	0.16 ± 0.01 [a]	0.13 ± 0.08 [a]	0.22 ± 0.02 [a]	0.23 ± 0.42 [a]	0.21 ± 0.03 [a]	0.18 ± 0.06 [a]
Fumarate	0.01 ± 0.01 [a]	0.02 ± 0.03 [a]	0.04 ± 0.04 [a]	0.02 ± 0.02 [a]	0.31 ± 0.09 [a]	0.34 ± 0.17 [a]	0.38 ±0.06 [a]	0.31 ± 0.08 [a]
Glucose	37.75 ± 18.69 [a]	55.24 ± 16.44 [a]	46.09 ± 14.91 [a]	29.06 ± 23.22 [a]	12.17 ± 8.15 [a]	15.90 ± 5.28 [a]	14.08 ±2.44 [a]	15.05 ± 5.11 [a]
Glutamate	-	-	-	-	18.06 ± 4.37 [a]	18.29 ± 1.84 [a]	17.41 ± 2.44 [a]	18.99 ± 4.15 [a]
Glutamine	0.72 ± 0.16 [a]	0.87 ± 0.30 [a]	1.02 ± 0.22 [a]	0.65 ± 0.47 [a]	-	-	-	-
Glycerol	0.51 ± 0.15 [b]	0.61 ± 0.15 [ab]	0.89 ± 0.33 [a]	0.52 ± 0.32 [b]	-	-	-	-

Glycine	0.12 ± 0.05 [b]	0.13 ± 0.04 [b]	0.22 ± 0.0 [a]	0.14 ± 0.10 [ab]	11.43 ± 2.00 [a]	11.43 ± 2.00 [a]	11.46 ± 0.90 [a]	12.07 ± 0.72 [a]
Hippurate	0.15 ± 0.15 [a]	0.21 ± 0.16 [a]	0.27 ± 0.18 [a]	0.15 ± 0.24 [a]	3.37 ± 0.98 [a]	2.32 ± 2.50 [a]	3.62 ±2.23 [a]	5.16 ± 1.91 [a]
IMP	-	-	-	-	0.70 ± 0.23 [a]	0.51 ± 2.50 [a]	0.70 ± 0.39 [a]	0.88 ± 0.76 [a]
Inosine	-	-	-	-	14.55 ± 1.14 [a]	11.43 ± 5.78 [a]	14.40 ± 1.07 [a]	13.85 ± 0.91 [a]
Isoleucine	0.13 ± 0.06 [b]	0.28 ± 0.18 [ab]	0.38 ± 0.13 [a]	0.23 ± 0.14 [ab]	1.20 ± 0.29 [a]	0.59 ± 0.18 [b]	0.92 ±0.07 [ab]	0.98 ± 0.31 [a]
Isopropanol	0.04 ± 0.03 [b]	0.04 ± 0.01 [b]	0.09 ± 0.04 [a]	0.11 ± 0.16 [ab]	-	-	-	-
Lactate	3.79 ± 1.58 [a]	4.94 ± 0.01 [a]	9.75 ± 6.65 [a]	4.62 ± 3.47 [a]	39.75 ± 8.06 [a]	28.64 ± 10.19 [a]	35.81 ± 6.00 [a]	39.82 ± 4.40 [a]
Leucine	-	-	-	-	2.23 ± 0.20 [a]	0.98 ± 0.44 [b]	1.76 ± 0.15 [a]	1.93 ± 0.43 [a]
myo-Inositol	-	-	-	-	24.79 ± 3.82 [a]	17.63 ± 9.01 [a]	23.20 5.69 [a]	20.44 ± 3.91 [a]
Niaciamide	-	-	-	-	4.23 ± 0.41 [a]	3.35 ± 1.62 [a]	4.12 ± 0.34 [a]	3.87 ± 0.85 [a]
Methanol	0.12 ± 0.04 [a]	0.19 ± 0.18 [a]	0.12 ± 0.08 [a]	0.18 ± 0.17 [a]	-	-	-	-
Phenylalanine	0.24 ± 0.11 [a]	0.29 ±0.03 [a]	0.33 ± 0.18 [a]	0.21 ± 0.11 [a]	1.38 ± 0.23 [a]	0.82 ± 0.20 [a]	1.09 ± 0.20 [a]	1.25 ± 0.25 [a]
Pyroglutamate	-	-	-	-	2.47 ± 0.78 [a]	2.33 ± 1.06 [a]	2.56 ± 0.64 [a]	2.87 ± 0.94 [a]
Pyruvate	0.06 ± 0.03 [a]	0.10 ± 0.07 [a]	0.13 ± 0.11 [a]	0.06 ± 0.06 [a]	-	-	-	-
Sarcosine	0.03 ± 0.01 [a]	0.04 ± 0.02 [a]	0.13 ± 0.11 [a]	0.03 ± 0.01 [a]	-	-	-	-

sn-Glycero-3-phospholine	-	-	-	-	16.77 ± 5.22 [a]	14.03 ± 6.20 [a]	13.01 ± 3.70 [a]	17.31 ± 6.51 [a]
Succinate	0.03 ± 0.01 [a]	0.02 ± 0.01 [a]	0.03 ± 0.01 [a]	0.02 ± 0.02 [a]	1.66 ± 0.46 [a]	1.24 ± 0.58 [a]	1.42 ± 0.24 [a]	1.65 ± 0.05 [a]
Taurine	-	-	-	-	37.14 ± 2.82 [a]	26.94 ± 10.82 [a]	38.59 ± 5.67 [a]	35.46 ± 3.54 [a]
Threonine	0.37 ± 0.15 [a]	0.37 ± 0.09 [a]	0.49 ± 0.27 [a]	0.28 ±0.14 [a]	1.53 ± 0.91 [a]	1.16 ± 0.17 [a]	1.22 ± 0.22 [a]	1.78 ± 0.80 [a]
Trimethylamine	-	-	-	-	0.12 ± 0.07 [a]	0.09 ± 0.06 [a]	0.12 ± 0.07 [a]	0.10 ± 0.05 [a]
Trimethylamine N-oxide	0.03 ± 0.02 [a]	0.02 ± 0.01 [a]	0.03 ± 0.01 [a]	0.03 ± 0.01 [a]	-	-	-	-
Tyrosine	0.19 ± 0.11 [a]	0.27 ± 0.15 [a]	0.26 ± 0.15 [a]	0.16 ± 0.09 [a]	1.81 ± 0.20 [a]	1.03 ± 0.32 [b]	1.52 ± 0.18 [a]	1.63 ± 0.30 [a]
Uridine	-	-	-	-	3.55 ± 0.70 [a]	3.08 ± 1.45 [a]	3.80 ± 0.21 [a]	3.48 ± 0.63 [a]
Valine	0.28 ± 0.28 [a]	0.10 ± 0.04 [a]	0.09 ± 0.02 [a]	0.07 ± 0.05 [a]	1.60 ± 0.38 [a]	0.91 ± 0.25 [b]	1.33 ± 0.14 [ab]	1.47 ± 0.35 [a]
Xanthine	-	-	-	-	2.93 ± 1.27 [a]	1.38 ± 1.19 [a]	2.81 ± 0.68 [a]	2.48 ± 0.82 [a]

Legend: Concentration in mg dL^{-1} (Mean ± SEM) of metabolites from kidney and samples groups. Tukey's HSD was used to analyze the compounds. p-value cutoff was $p < 0.05$ (significant). Different small letters indicate statistically significant differences between groups in the same type of sample. SEM: standard error of the mean. Group H: healthy animals that did not receive the products under study; Group C: animals with chemically induced colitis that did not receive the products under study; Group W: animals with chemically induced colitis that received the whey protein without UV-C treatment; Group WU: animals with chemically induced colitis that received the whey protein without UV-C treatment.

***Table* S3.** Metabolic pathways highlighted for the metabolomics profile from kidney samples (Figure 7).

Number	Via	Comparison	Total	Hits	Raw p	LOG10 (p)	Holm adjust	FDR	Impact
1	Alanine. aspartate and glutamate metabolism	Group H vs. Group C	28	6	0	2.84	0.05	0.02	0.42
		Group H vs. Group W	28	6	0.04	1.37	1	0.18	0.42
		Group H vs. Group WU	28	6	0.03	1.47	1	0.18	0.42
2	Tyrosine metabolism	Group H vs. Group C	42	2	0	2.57	0.09	0.02	0.16
		Group H vs. Group WU	42	2	0.02	1.75	0.64	0.18	0.16
3	Glyoxylate and dicarboxylate metabolism	Group H vs. Group C	32	4	0.01	1.87	0.38	0.05	0.11
		Group H vs. Group W	32	4	0.04	1.35	1	0.18	0.11
		Group H vs. Group WU	32	4	0.05	1.32	1	0.18	0.11
4	Phenylalanine. tyrosine and tryptophan biosynthesis	Group H vs. Group C	4	2	0.02	1.63	0.64	0.08	1
		Group H vs. Group WU	4	2	0.05	1.33	1	0.18	1
5	Arginine biosynthesis	Group H vs. Group C	14	4	0.03	1.56	0.69	0.08	0.19
6	Glutathione metabolism	Group H vs. Group C	28	3	0.03	1.46	0.82	0.09	0.12
7	Phenylalanine metabolism	Group H vs. Group C	12	3	0.04	1.39	0.9	0.1	0.36

Capítulo 2.

Longitudinal fecal microbiota profile in a colitis mouse model: effects of UV-C treated whey protein ingestion

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Longitudinal fecal microbiota profile in a colitis mouse model: effects of UV-C treated whey protein ingestion

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ABSTRACT

One of the most common inflammatory bowel diseases is the ulcerative colitis. Currently, several studies to control this disease are focused on understanding the relationship between gut microbiota composition and the evolution of inflammatory process in the gut. This study focuses on using short-wave ultraviolet (UV-C) to produce a whey protein with better nutritional profile and understanding the complex interplay between this supplement and microbiota composition. Colitis was induced by dextran sulfate sodium and two types of supplementations were evaluated in mice (n=10): whey protein without UV-C treatment (W) and whey protein subjected to this process (WU). The composition of the fecal microbiota of the animals during the

experiment was evaluated by sequencing the 16S rRNA region. Both treated groups showed a difference in community composition (beta-diversity) and most phyla and genera was recovered or maintained healthy bacterial abundance during colitis induction. Although both supplements reduced the progression of the disease, the WU treatment was more effective since it showed less progression in the clinical parameters of and the intake of UV-C treated WP resulted in a positive modulation of GM, with emphasis the abundance increase in *Turicibacter* spp., recovery in *Ruminococcaceae* family and maintaining of *Lachnospiraceae_UCG_001* and *Blautia* spp which certainly contributed to the control of colitis.

keywords: ulcerative colitis, non-thermal process, milk proteins.

Introduction

Ulcerative colitis (UC) is one of the most common subtypes of inflammatory bowel disease (IBD), affecting the colon with a mucosal inflammation extending from the rectum (GAJENDRAN et al., 2019). The high incidence of IBD in the western world and its continued increase in newly industrialized countries emphasize the need to find new solutions to control this disease and care for patients (NG et al., 2017).

The initiation and progression of IBD involve an association of genetic predisposition, environmental factors (diet, smoking, geographic region, economic and social situation, drug use), abnormal immune response and gut microbiome composition (KOBAYASHI et al. 2020, GAJENDRAN et al., 2019; ARMUZZI; LIGUORU, 2021).

Human gut microbiota (GM) contributes to intestinal barrier integrity and homeostasis and have millions of no-redundant genes that generate a unique microbiology identity (ARUMUGAM et al., 2011; TIERNEY et al., 2019; VALDES; WALTER; SEGAL; SPECTOR, 2018). Patients with IBD exhibit modification on diversity and dominance of GM, like reduction of *Firmicutes* and increase of *Enterobacteriaceae* (HARRIS; CHANG, 2018; KOSTIC; XAVIER; GEVERS, 2014; MUKHOPADHYA; HANSEN; EL-OMAR; HOLD, et al., 2012). However, there is not just one specific microbial profile that has been exactly associated with IBD, and although we know that GM composition affects the etiology of IBD, whether this is cause or effect remains elusive (PEYRIN-BIROULET et al, 2016). Therefore, further

studies are needed to assess the effect of different therapies on microbiota composition and colitis development (DISTRUTTI et al., 2016).

Thus, studies are carried out to elucidate the exact mechanism involved in the development of IBD and how diets or specific food components can modulate the composition of the GM and help in choosing the most appropriate nutritional therapy (AL-OMARI et al., 2019; ARAÚJO et al., 2016; KANWAR et al., 2016). One of dietetic approach to IBD recovery is ingestion of whey protein (WP), that seems it can modify the composition of the GM, while fermentation of amino acid by gut bacteria produces metabolites with positives physiological effects (PORTUNE et al., 2016).

Whey protein is a source of essential amino acids such as histidine (associated with the remission of damage occurring in the colon and a decrease in the cytokine IL-6), methionine (involved in the production of glutathione, a potent antioxidant) and threonine (essential for maintaining the integrity of the intestinal mucosa) (ALMEIDA; ALVARES; COSTA; CONTE-JUNIOR, 2016; LIU; WANG; HU, 2017). Other amino acids like leucine, isoleucine and valine also have anti-inflammatory properties and may reduce some of the symptoms of IBD (LAGRANGE; CLARK, 2019; SMITHERS, 2008). In addition, WP intake was related to the expression and secretion of mucin, helping to maintain the integrity of the intestinal barrier (SPRONG; SCHONEWILLE; VAN DER MEER, 2010).

Kanwar et al. (2016) reported the first study to compare the effect of different milk components (bovine milk derived iron-saturated lactoferrin, angiogenin, osteopontin, colostrum WP, powdered casein and milk fat enriched with cis-9, trans-11 conjugated linoleic acid (CLA) on dextran sulfate sodium (DSS)-induced colitis in mice. Each dairy product showed some different positive responses in the study model. Best results were obtained with CLA (reversed weight loss and inhibited inflammatory cytokines and epithelial damage) and casein powder (decreased clinical and inflammatory score). Concerning colostrum WP, it also demonstrated effectiveness in reducing the inflammatory score (neutrophil infiltration was significantly lower) and decreased clinical score when compared to untreated animals. The authors emphasized the necessity of more studies to understand how WP supplements may help treat or prevent IBD.

However, is important highlight that the bacterial metabolites derived from the digestion of peptides from milk protein can cause a negative or positive impact on the mucosal immune system, depending on the origin and structure (TRIANTAFILLIDIS; TZOUVALA; TRIANTAFYLLIDI, 2020).

The thermal processes are widely used in industry for processing and maintaining food quality. The most common thermal processes are pasteurization, sterilization and ultra-high temperature (UHT). Despite their efficacy to control microbiological contamination and extend shelf-life of foods, thermal processes can affect gastrointestinal digestibility and allergenicity of proteins (RAHAMAN; VASILJEVIC; RAMCHANDRAN, 2016; RANKIN et al., 2010). When food is submitted to high temperatures, some significant changes occur in the structure of proteins, such as denaturation, Maillard reactions, decrease in lysine, protein-protein and protein-lipid interaction, which impair the sensory and nutritional quality of products (BU; LUO; ZHENG; ZHENG, 2013, RUDLOFF; LONNERDAL, 1992). WP fraction is more sensible to industrial processes than casein fraction, since it is thermolabile (VERHOECKX et al., 2015). Maillard reactions induce aggregation of free amino and amino acids groups and can change the natural digestion process of β-Lg, stimulating its absorption in the stomach, along with α-La (SAH; MCAINCH; VASILJEVIC, 2016). Bu, Luo, Zheng, & Zheng (2009) demonstrated temperatures above 90°C lead to an increase in allergenicity of β-Lg and α-La in WP. This is because the exposure to higher temperatures affects the sulfhydryl (-SH) / disulfide (S-S) exchange reactions and the β-lactoglobulin conformation.

The use of alternative non-thermal methods (as short-wave ultraviolet - UV-C) can modify protein digestibility and increase levels of bioactive peptides (ESTEGHLAL et al., 2019, TULIPANO, 2020). Cardoso et al. (in press) showed that UV-C treatment (266 nm, 4 W/m^2) leads to a conformational change in the WP in aqueous solution, altering the secondary structure of β-Lg, with a significant reduction in the contribution of the β sheet and the loop structure. This non-thermal treatment resulted in a 127% increase in β-Lg digestibility and a change in the protein's amino acid profile.

Thus, we hypothesize that UV-C treatment may improve WP digestibility and amino acid profile, assisting in the IBD control and maintain healthy gut microbiota. This is the first study that applies UV-C in WP and analyze its effect on the microbiota

of animals with induced colitis, aiming to understand the complex interaction between the regular intake of this supplement and the development of the disease.

Material and methods

Production of whey protein and treatment by UV-C irradiation

Pasteurized homogenized skin milk (Fazenda Bella Vista, Tapiratiba, Brazil) was purchased from a local store. First, the milk was heated to 38 °C ± 2 °C, the enzymatic coagulant was added (Estrella; Chr-Hansen A/S, Valinhos, Brazil) and then left to rest for 40 minutes. Finally, the milk containing the coagulant was heated at 50 ± 2 °C for 5 min to precipitate casein and obtain whey fraction. Whey was concentrated by lyophilization and solubilized in sterile deionized water at the rate of 66 mg/mL (ORDÓÑEZ et al., 2007). The resulting WP solution was exposed to UV-C treatment using a UV-C LED that emits at 254 nm. The quartz cuvette model allocates 15 mL of sample, and the light intensity was 3.1 mW/cm^2. All equipment was kept at 5 ± 0.1 °C and the solution was stirred throughout the exposition time to UV-C treatment with a 500 mm magnetic stirrer bar at 500 rpm (See Article).

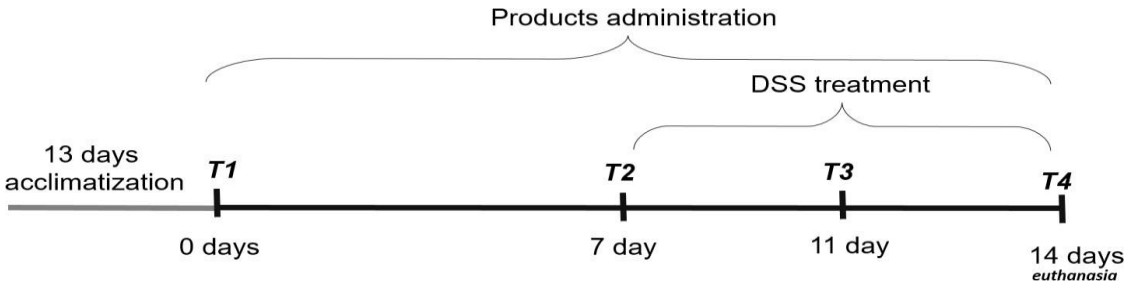
Protocol induction and clinical parameters of DSS-induced colitis mouse

Male C57BL/6J mice (*Specific Pathogen Free*) were purchased from the Multidisciplinary Center for Biological Research (Centro Multidisciplinar para Investigação Biológica - CEMIB) at the State University of Campinas (Campinas, SP, Brazil). The animals were exposed to 12-12 h of light-dark cycles and fed a standard diet (Labina Presence®) that included drinking water *ad libitum*. The protocol was carried out followed the guidelines written by the Brazilian College of Animal Experimentation (COBEA) and was approved by the Research Ethics Committee of the UNESP School of Pharmaceutical Sciences in Araraquara, Brazil (protocol number: 08/2020).

The animals were randomly assigned to four groups (n = 10): Group H: healthy animals without intervention; Group C: animals induced to colitis and without intervention; Group W: animals induced to colitis that received WP; Group WU: animals induced to colitis that received UV-C treated WP.

Colitis was chemically induced by dissolving 3% w/w DSS (MP Biomedicals, EUA; PM = 36.000–50.000) in sterilized drinking water that was ingested daily (*ad libitum*) during seven days. WP, with or without UV-C treatment (1 g/Kg body weight), was administered daily for 14 days via oral gavage. Treatment began seven days before colitis induction and lasted for 7 days during DSS exposure. The animals belonging to the control groups (C and H) received the same volume of sterile water by oral gavage daily to simulate the stress of the procedure. Mice fecal samples were collected on days 0, 7, 11 and 14 of the experimental protocol to determine the microbiota composition (Figure 1) and kept at -80°C until evaluation.

Figure 1 - Induction of colitis, administration of products and collection of fecal samples (T1, T2, T3, T4) during the experimental protocol.



At the end of the experiment (day 14), mice were placed under deep anesthesia with pentobarbital and euthanized by cervical dislocation. After colon removal, the weight and length of the ileocecal junction at the anal margin were measured with an analog caliper (precision of 0.05 mm), to calculate the weight/length ratio (mg/mm). Then, the colon tissues were opened longitudinally, fixed in 10% buffered formalin, washed in running water for 24 hours and stored in 70˚GL ethanol. Subsequently, the samples were routinely processed and sections of approximately 5 µm were mounted on slides and stained with hematoxylin-eosin. The colitis severity was evaluated by a professional pathologist in a blinded manner (Olympus BX51—Olympus Optical, Tokyo, Japan). Each sample was scored for histomorphological damage according to methodology adapted from Erben et al. (2014) (Table 1).

Table 1 - Definition of the histologic scoring system.

Category	Definition	Score Values
Inflammation	Inflammatory cell infiltrate: severity and extension	1 to 4
Hyperplasia	Increase in epithelial cell numbers in longitudinal crypts relative to baseline epithelial cell number per crypt	1 to 5
Globet cell loss	Reduction of globet cell numbers relative to baseline globlet cell numbers per crypt	1 to 4
Irregular crypts*	Non-parallel crypts, variable crypt diameters and/or bifurcation and branched crypts	4 or 5
Crypt loss*	Mucosa devoid of crypts	4 or 5

Notes: *Score inserted only in samples that present this anomaly in the mucosal architecture.

DNA extraction and quantification

DNA extraction was performed using the FastDNA® Spin Kit for Soil (MP Biomedicals, Catalog Number 6560200). The DNA was isolated according to the manufacturer's protocol with the following modifications: samples were incubated in a dry bath at 60 °C for 5 minutes and after that, for another 4 minutes at room temperature. The quality and quantity of DNA were assessed by agarose gel electrophoresis and fluorometric quantification with Qubit® (Thermo Fisher Scientific, Waltham, MA), respectively. The samples were stored at -80 °C until the moment of sequencing.

Library preparation and sequencing

To access the bacterial communities, the V3-V4 region of the 16S ribosomal gene (16S rRNA) was sequenced using the 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') primers in a two-step PCR, following the methodology proposed by de Souza et. al. (2016). The first step (PCR 1) was designed to amplify the targeted ribosomal gene regions (V3-V4, 16S rRNA) and to add the Illumina Nextera transposase sequences to the amplicons. The second step

(PCR 2) used the overhangs of the Illumina adapter to attach the dual indexes of the Illumina Index PCR Kit, thus enabling the multiplexing of up to 96 samples. Both forward (515F) and reverse (806R) primers for PCR 1 were amended with 4 to 6 frame shift (FS) sequences in its 5' termination to improve sequence diversity and overall read quality (FADROSH et. al., 2014). All primers were obtained from Sigma-Aldrich (Merck KGaA, Darmstadt, Germany) that used HPLC purification and adjusted to 10 µM for use.

The sequencing libraries were denatured by mixing 5µL of the 2nM pooled libraries with 5µL of NaOH 0.2 N and incubated for 5min at room temperature. Following denaturation, 980µl of chilled Illumina HT1 buffer was added to adjust the library at 8 pM. The suitable library concentration was sequenced on the MiSeq Illumina Sequencer.

After sequencing, fastq files were imported in QIIME2 program (version 2020.6.0). Sequence adapters and primers were removed and sequences shorter than 300 bp were joined using the DADA2 denoise paired plug-in of QIIME2. DADA2 algorithm was used to trim low quality reads and remove chimeric sequences. Amplicon sequence variants (ASVs) obtained by DADA2 were used for taxonomic assignment using the QIIME feature-classifier and the database was constructed using SILVA database and from NCBI.

Statistical analysis

QIIME2 diversity script was used to perform the alpha (number of observations, Chao1 and Shannon index) and beta diversity (Bray-Curtis index) analysis. The statistics were calculated and adjusted using Bonferroni's method in the R environment (version 4.1.2) (R CORE TEAM, 2021). Phylum actual abundance graphic was performed in the Microbiome Analyst platform (XU et al, 2020).

Data from histopathological scores were evaluated by Kruskal Wallis test with post hoc Dunn's test (non-parametric data). The weight/length of the ileocecal junction at the anal margin were evaluated by one-way analysis of variance (ANOVA one-way) and Tukey's honestly significant difference (Tukey's HSD). Statistical and graphical analysis were performed using the R software (version 4.1.2) (R CORE TEAM, 2021).

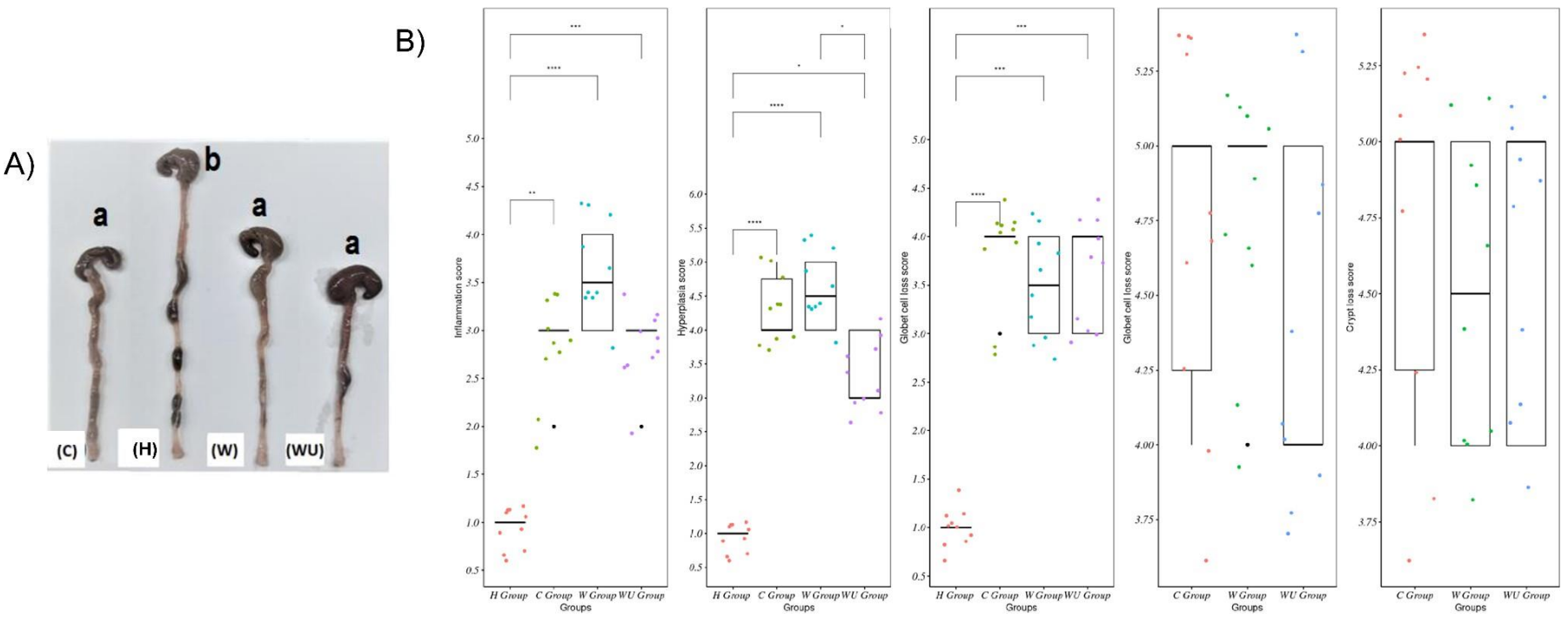
Results and Discussion

Effects of WP on colon weight/length ratio and histopathology in DSS-induced colitis in mice

The weight/length of the ileocecal junction at the anal margin of H group was statistically higher compared to the other groups ($p < 0.05$) (Supplementary Table 1). Although there are no statistical differences between the colitis-induced groups ($p < 0.05$), a trend towards less colonic shortening is observed in group W (Figure 2A). The colon weight/length ratio plays an important role in understanding the intestinal inflammatory process, as inflammation causes shortening, thickening and edema in the intestinal walls (ARAÚJO et al., 2017). Also, in animal's protocols colon shorting are correlated to IBD and intestinal infections (QIN et al, 2019).

The intestinal mucus barrier is the first protection that the body has against bacterial infections and the analysis of mucosal architecture is commonly used to confirm the diagnosis of UC and the severity of this disease in patients (GAJENDRAN et al., 2019; FANG et al., 2021). Regarding the histopathological scores, there was less hyperplasia in the group treated with WP plus UV-C compared the WP without UV-C ($p > 0.05$), suggesting a protective effect of this supplement (Figure 2 B). Colonic hyperplasia is correlated with a disturbance of immune cell homeostasis and an imbalance of cytokines present in colitis (ERBEN et al., 2014).

Figure 2 - Colon weight/length ratio and histopathologic score in DSS-induced



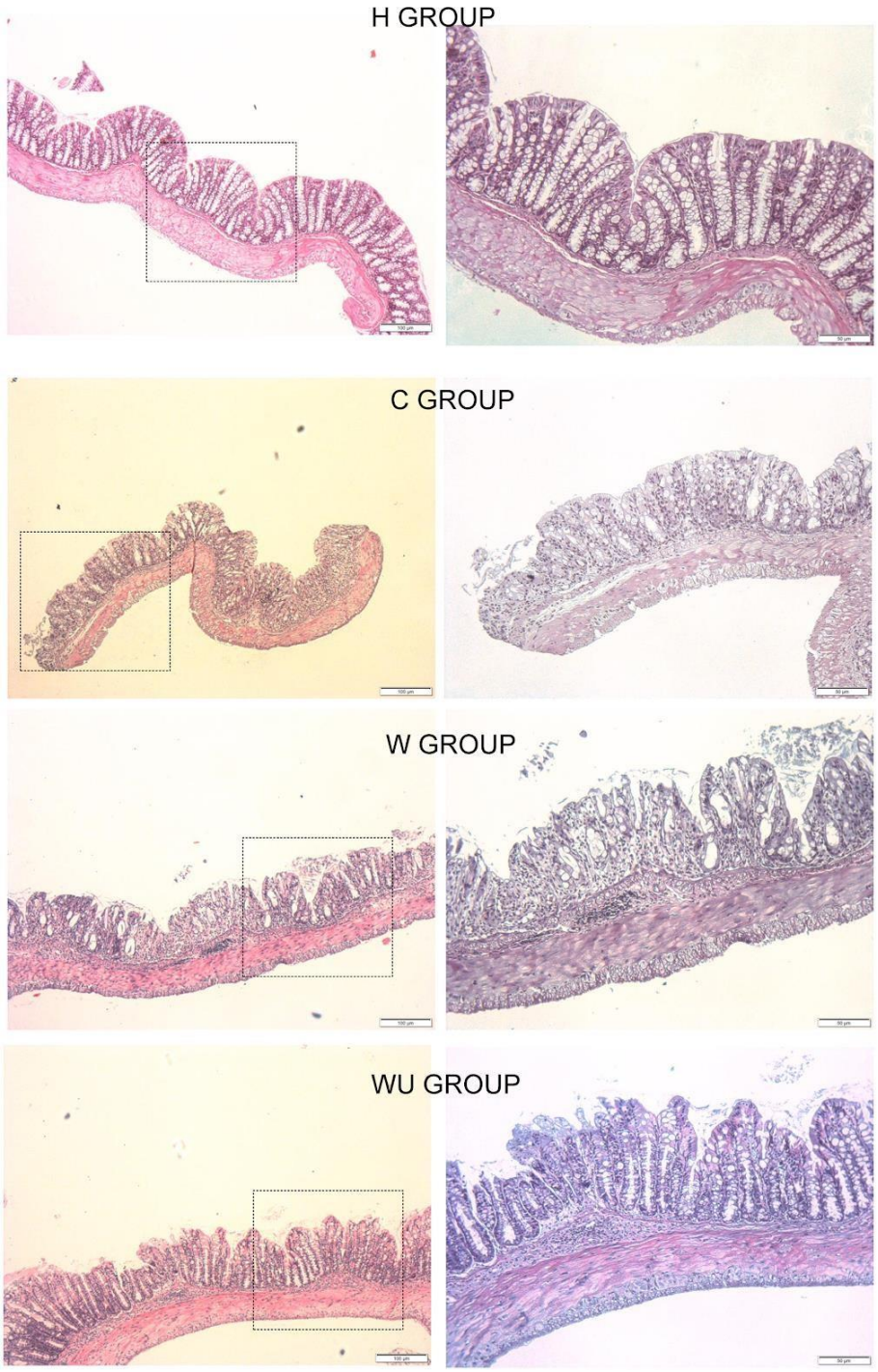
Notes: A) Representative photo of the colons of animals from different experimental groups B) Histopathologic scores. Values are expressed as median ± IQR. (*) $p < 0.05$;(**) $p < 0.01$; (***) $p < 0.001$ and (****) $p < 0.0001$ by Kruskal–Wallis tests and post hoc Dunn test. Group H: healthy animals that did not receive the products under study; Group C: animals with chemically induced colitis

that did not receive the products under study; Group W: animals with chemically induced colitis that received the WP without UV-C treatment; Group WU: animals with chemically induced colitis that received the WP with UV-C treatment.

Despite the few differences in histopathological scores, overall, the animals that received WP with UV-C treatment exhibited more preserved intestinal architecture when compared to the C and W groups. Also, it was possible to observe areas of preservation of the crypts, interspersed with areas of poorly preserved mucosa and a decrease in goblet cells. In some cases, a decrease in the inflammatory infiltrate was observed (Figure 3). These results are consistent with our academic group obtained in another study, where the ingestion of WP treated with UV-C led to a reduction in the disease activity index and modulation of inflammatory cytokines (IL-6 and IL-12) (See Article).

The extensive ulceration caused by the DSS-induced colitis protocol sometimes causes an irreparable disruption of the intestinal barrier (HONG et al., 2021), which would explain the few statistical differences observed in the histopathological scores of W and WU groups. This highlights the importance of carrying out analyzes throughout the study period as a way of understanding the action of this supplementation in the evolution of the disease.

Figure 3 - Representative H&E staining of animal colon (scale bar: 100 µm and 50 µm).



Notes:Scale bar: 100 µm and 50 µm. Group H: healthy animals that did not receive the products under study; Group C: animals with chemically induced colitis that did not receive the products under study; Group W: animals with chemically induced colitis that received the WP without UV-C treatment; Group WU: animals with chemically induced colitis that received the WP without UV-C treatment.

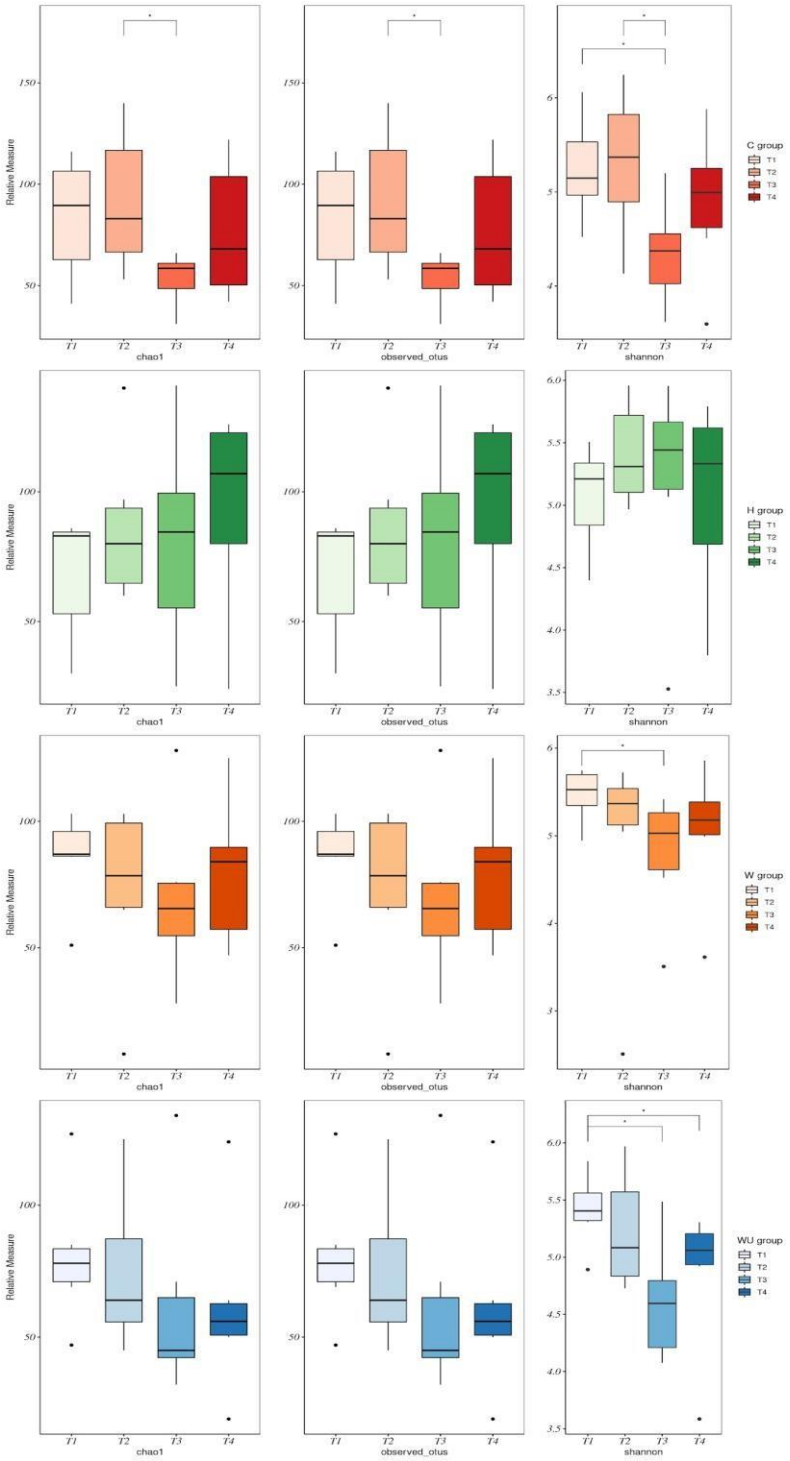
Alpha and beta diversity

After sequencing, 9,508,794 (2 x 300 bp) raw reads were obtained and the sequence merging and quality control result at 7,740,202 reads of 455 bp were left, with an average of 80,637 ± 790,910 per sample. Finally, a rarefaction was performed considering the library with the lowest read count (1565 reads/sample). The Chao1 and number of observations (alpha diversity) were equal (Supplementary Table 2), that show the results did not show singletons in the samples (CHIU; CHAO, 2016).

As expected, our results revealed that in healthy animals there was no significant variation in alpha-diversity values ($p > 0.05$) at the evaluated times (Figure 4). However, it was observed a reduction in the diversity of the microbial community of animals treated with DSS on the fourth day of colitis induction (T2 *vs* T3, $p < 0.05$). This is consistent with results obtained in other studies that reported a reduction in microbial richness after colitis induction which suggested that DSS disrupted the microbiology structure (GUO ET AL., 2021, LIU ET AL., 2021, SUN ET AL., 2020). Munyaka et al., (2016) also found a remarkable decrease in the alpha-diversity of bacterial species in fecal samples from male C57BL/6 mice induced to colitis (5% DSS) for five days.

W and C groups recover the initial diversity of the bacterial species community at the end of the study (T1 *vs* T4, $p > 0.05$, Shannon index). Also, number of observations, related the bacterial richness, was similar during the experiment in both groups under WP supplementation ($p > 0.05$), while the C group showed a significant decrease in this parameter (T1 vs T3, $p < 0.05$) (Supplementary Table 2 and Figure 4). Finally, the consumption of WP for seven days (T1 to T2) did not change any of the microbiological parameters ($p > 0.05$), which may show that supplementation did not negatively affect the GM of the animals.

Figure 4 - Alfa-diversity measures of fecal microbiota during the timeline of experiment.



Notes: (*) $p < 0.05$ by Bonferroni's method (comparison between experiment times). Group H: healthy animals that did not receive the products under study; Group C: animals with chemically induced colitis that did not receive the products under study;

Group W: animals with chemically induced colitis that received the whey protein without UV-C treatment; Group WU: animals with chemically induced colitis that received the whey protein without UV-C treatment. T1: first of protocol. T2: 7 days of protocol. T3: 11 days of protocol. T4: last day of protocol.

Permutational multivariate analysis of variance (PERMANOVA) tests was used to analyze the Bray-Curtis dissimilarity (beta-diversity). The Bray-Curtis dissimilarity represents a distance metric, and the comparisons can be made between an arrangement of the bacterial communities to better understand the community structure (YOUNG; SCHMIDT, 2008). Comparing the microbiota into experimental groups, differences between healthy and all DSS-induced mice were observed ($p < 0.05$) (Supplementary Table 3), which is in line with that found in alpha-diversity. Additionally, at the end of the experiment only C and H groups showed statistically differences ($p < 0.05$). Furthermore, the comparison between times showed a statistical difference ($p < 0.05$) on C group (T1 *vs* T4) and WU group (T2 *vs* T4). (Supplementary Table 4). Therefore, both supplementations, especially WP without UV-C treatment, could have a positive effect on the richness and diversity of gut microbiota. These positive effects related to beta-diversity were also observed with the ingestion of other dietary supplements (polyphenols and probiotics) used to help control UC, which reinforces the potential use of WP in this disease (ZHAO; NAKATSU; JONES-HALL; JIANG, et al., 2022; WU et al., 2022).

Microbiota composition

As expected, *Bacteroidota* and *Firmicutes* were the most abundant phyla in the microbiota of animals under study (Figure 5A). The GM is mostly represented by *Firmicutes* and *Bacteroidota* (approximately 90% of total phyla present on gut), followed by *Proteobacteria*, *Fusobacteria*, *Verrucomicrobia*, and *Actinobacteria*. However, the GM composition presents interindividual variations and this delicate host-microbe relationship can be affected by age, diet and health status (RINNINELLA et al., 2019).

There was no variation in the phylum *Bacteroidota* in all groups during experimental protocol ($p > 0.05$) (data not shown). Considering the comparison between groups on the same days of the experiment, the WU group showed a

reduction in the *Firmicutes* phylum compared C group on the last day of experiment ($p < 0.05$), but no statistical difference to the other groups ($p > 0.05$) (Supplementary Figure 5).The *Firmicutes* phylum is composed by different bacteria that produce butyrate, a short-chain fatty acid (SCFA) involved in maintaining intestinal integrity. In general, patients with IBD (especially CD) exhibit a change in GM diversity and dominance, with a significant reduction in *Firmicutes* (HARRIS; CHANG, 2018; KOSTIC; XAVIER; RAMNIK; GEVERS., 2014).

About the families belonging to *Bacteroidota* phylum, *Bacteroidaceae, Rikenellaceae* and *Tannerellaceae* were found in the feces of the animals and some statistical differences were observed (Figure 5B). *Bacteroidaceae* is considering pathogenic and p-cresol productor, a substance accumulating in the body of patients with renal diseases (WONG et al., 2014). In our study, the increase in *Bacteroidaceae* occurred only with the ingestion of W without UV-C, which reinforces the idea that a higher ingestion can cause metabolomic alterations reported in a previous study (See Article), highlighting the benefit of treatments with UV-C. *Rikenellaceae* and *Tannerellaceae* families are commonly decrease their abundance in UC patients (ALAM et al., 2020). We observed decrease only in *Rikenellaceae* family in C and WU groups. However, *Tannerellaceae* increased the abundance in the last day of study in W group ($p < 0.05$) (Figure 5B).

Regarding *Firmicutes* phylum, a total to 13 families was found and 2 of them showed statistical differences (*Erysipelotrichaceae* and *Oscillospiraceae*, Figure 5B). There was a reduction in the Oscillospiraceae family in the WU group during colitis induction ($p < 0.05$, T1 vs T3), with recovery of abundance in T4 ($p > 0.05$, T1 *vs* T4). The *Oscillospiraceae* family are correlated to SCFA producing bacterial group, primordial components to maintain the intestinal homoeostasis and promote the host (MARTIN-GALLAUSIAUX et al., 2021).

Increase on *Erysipelotrichaceae* relative abundance were found in W and WU groups in the last day of protocol, with statical difference between T1 and T2 *vs* T4 ($p < 0.05$). Although *Erysipelotrichaceae* belongs to *Firmicutes* phylum, its presence in mouse models indicates gut inflammation, but opposite effect was observed in patients with IBD, thus, the effect of this family remains contradictory (KAAKOUSH et al., 2015). In our study, *Erysipelotrichaceae* family was represented by *Erysipelatoclostridium*, *Faecalibaculum*, *Holdemanella*, *Holdemania* and *Turicibacter* genera, and the last showed statistical differences (Supplementary Figure 1A). *Turicibacter* spp. showed

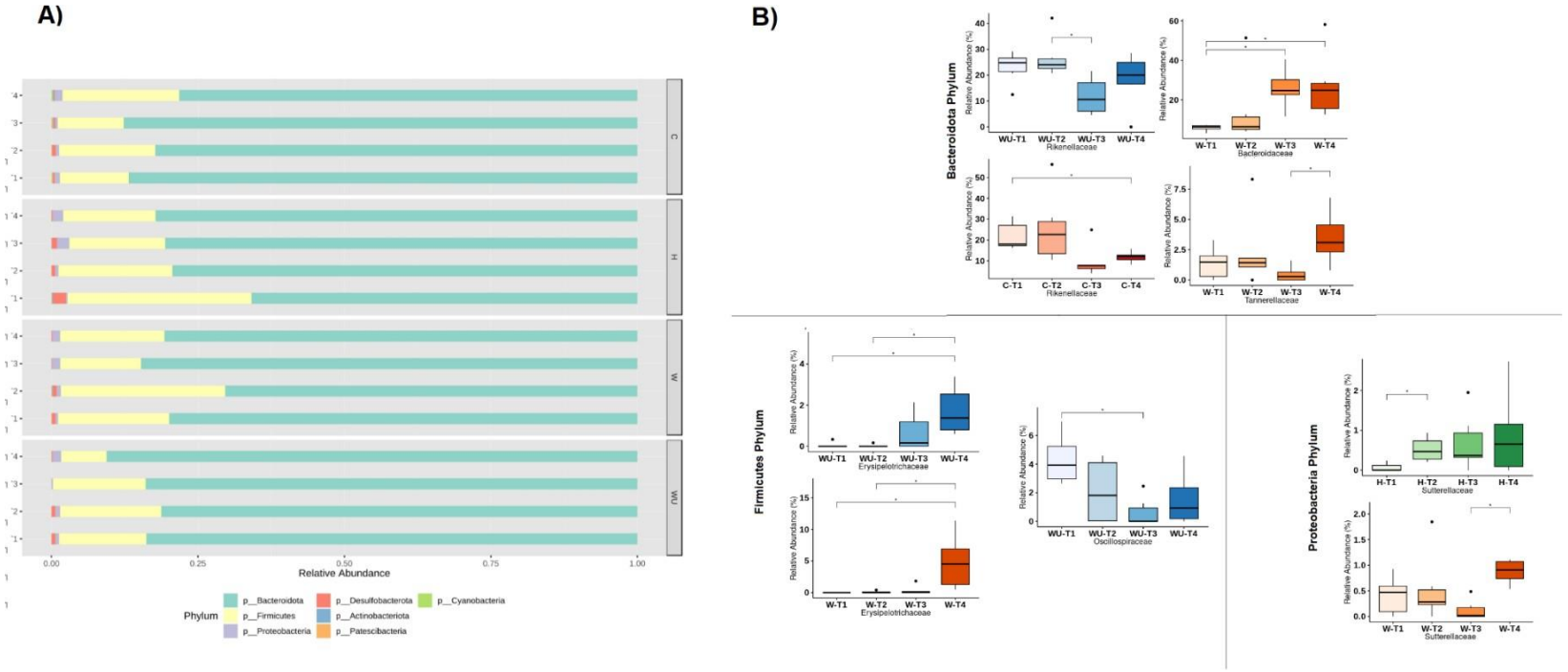
an abundance increase after DSS induction in WU group ($p < 0.05$, T1 and T2 *vs* T4; T2 *vs* T3). Hoffman and Margolins (2020) stated that *Turicibacter* spp. can contribute to intestinal homeostasis via lipid and steroid metabolism. Thus, increase in *Turicibacter* spp. could be a protection mechanism during the induction colitis in our experiment.

Despite the *Firmicutes* and *Bacteroidota* phyla, other statistical differences were observed in *Cyanobacteria*, *Desulfobacterota* and *Proteobacteria* phyla (Supplementary Table 5).

In our study, *Gastranaerophilales* spp. (belonging to the *Cyanobacteria* phylum) were increased only in animals with untreated colitis (group C) ($p < 0.05$, T3 vs T4) (Supplementary Table 6), and apparently, *Gastranaerophilales* spp. was mainly responsible for the increase in *Cyanobacteria* phylum in this study. This result was unexpected, as *Gastranaerophilales* spp. is a producer of butyrate, with anti-inflammatory properties and cancer-inhibiting effects, including colorectal adenoma (glandular tumor of the colon and rectum) (SHANG et al., 2022). It is suggested that the healthy and WP-fed groups did not need to activate this anti-inflammatory mechanism or that colitis control did not involve this pathway.

The *Proteobacteria* phylum showed an increase during colitis induction (Figure 5A). For groups C and WU, a statistical difference was observed when comparing the fourth and last day of colitis induction ($p < 0.05$, T3 vs T4) while in group W the difference was between the first and last day ($p < 0.05$, T1 vs T4) (Supplementary Table 5). *Proteobacteria* is composed of Gram-negative bacteria, which contain a lipopolysaccharide in their outer membrane, related to intestinal dysbiosis, inflammatory state and progression of IBD (VESTER-ANDERSEN et al, 2019, RIZZATTI et al., 2017). Focusing on families, *Sutterellaceae, Morganellaceae, Enterobacteriaceae* and a non-cultivated family (part of the order *Rhodospirillales*) were identified. H and W groups exhibited a elevation on relative abundance of *Sutterellaceae* (H: T1 vs T2; W: T3 vs T4). The relative abundance of *Parasutterella* spp. (*Sutterellaceae* family) also varied in all groups during the experiment ($p < 0.05$) (Supplementary Table 6), with no statistical difference between treatments at the end of the study ($p>0.05$). The role of intestinal ecology on this genus is not clear, but its high concentration may be positively associated with impaired functions of the cecal metabolome ($p < 0.05$) (JU; KONG; STOTHARD; WILLING, 2019).

Figure 5 - Relative abundance of bacteria on fecal microbiota of mice in different experimental groups.



Notes: A) Phylum relative abundance of fecal microbiota between groups during the timeline of experiment. Bar represents a group on a specific day, and the color of each cell indicates the abundance of the bacterial genus. B) Family relative abundance (%) of the groups with statistical difference over timeline of experiment. Values are expressed as median ± IQR. (*) $p < 0.05$;(**) $p < 0.01$; (***) $p < 0.001$ and (****) $p < 0.0001$ by Bonferroni method. Group H: healthy animals that did not receive the products under study; C: animals with chemically induced colitis that did not receive the products under study; Group W: animals with chemically induced colitis that received the whey protein without UV-C treatment; Group WU: animals with chemically induced colitis that received the whey protein without UV-C treatment.T1: first of protocol. T2: 7 days of protocol. T3: 11 days of protocol. T4: last day of protocol. Source: own authorship

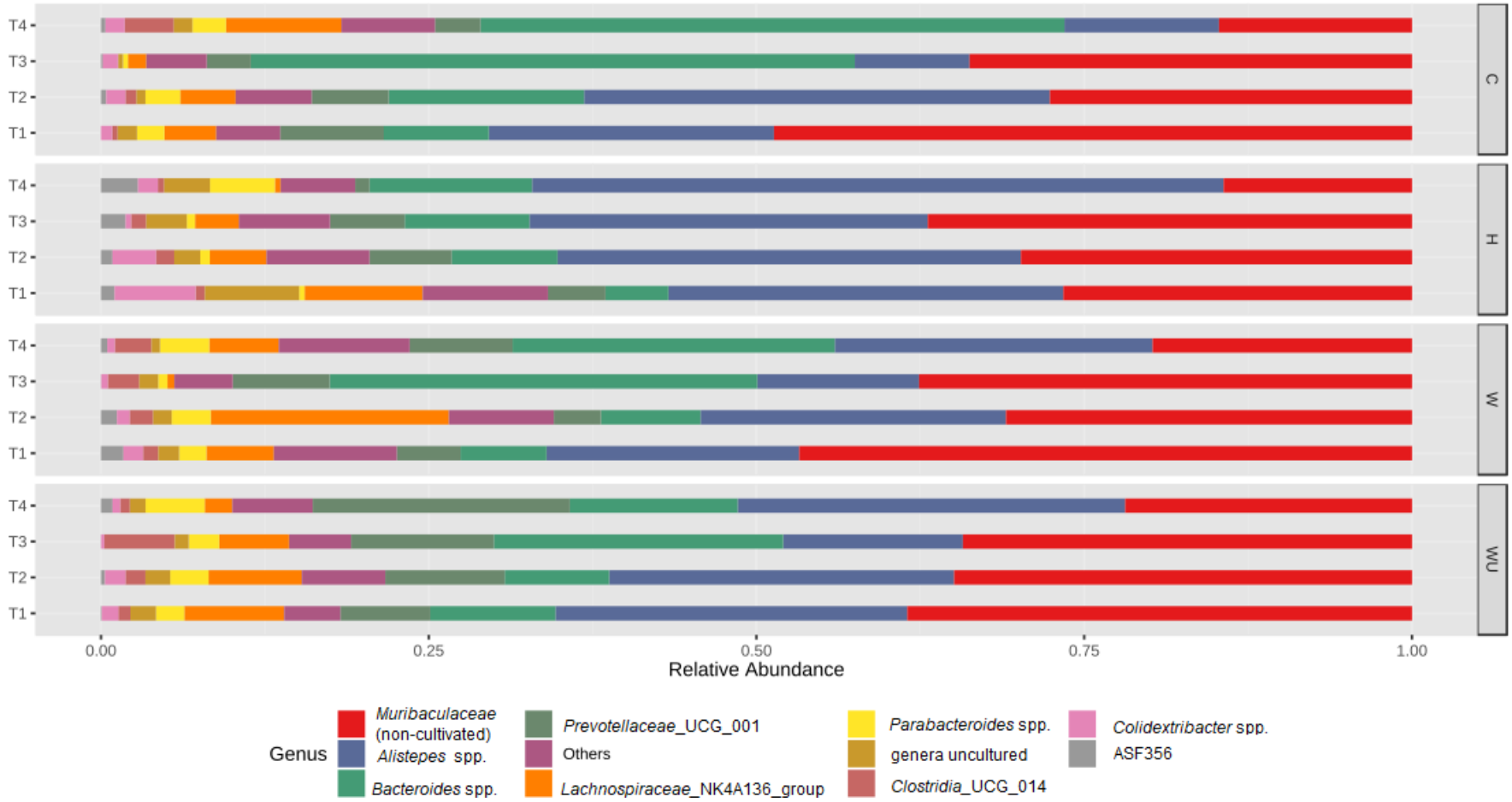
Reduction on *Desulfobacterota* phylum was observed in W and WU groups in the last day of protocol, with statistical reduction in abundace between T1 *vs* T4; T1 and T2 *vs* T4, respectively ($p < 0.05$) (Supplementary Table 5). No differences were observed for group C during the experiment and the abundance of this phylum was similar for all groups on the last day (T4) ($p > 0.05$) (Supplementary Table 5). The increase in abundance of *Desulfobacterota* is negatively correlated with Firmicutes, but when in smaller proportions it is considered a beneficial phylum (RAJPUT et al., 2023, SHIN; WHON; BAE, 2015), as found in our study. Also, our results did not show differences between the families of these phylum (data not shown). Nevertheless, we found that the main genus of this phylum in our study was *Desulfovibrio* (Supplementary Table 6). . Increase of the members of *Desulfovibrio* genus has been reported as a key factor to reduce the production of sulfide and acetate and is associated to a dysbiosis commonly found in IBD patients (KUSHKEVYCH; DORDEVIĆ; VÍTĚZOVÁ, 2021; KUSHKEVYCH; DORDEVIĆ; KOLLÁR; 2018). However, we only found a abundance decline at animals induced to colitis and supplemented with WP (T1 vs T4 , $p < 0.05$, Supplementary Table 5).

Going more deeply, the Figure 6 shown the top ten most abundant genera found in the study. The most of genera presented statistical differences between timeline of experiment and were part of *Firmicutes* and *Bacteroidota* phyla Supplementary Figure 1). The most abundant genera were *Muribaculaceae* (not-cultived), *Alistepes* spp. and *Bacteroides* spp., all related to Bacteroidota phylum.

Bacteroides spp. is known to expand inflammation in the gut (PARK; YEO; KANG; HUH, 2021), and showed a progressive increase in the W and WU groups during the development of colitis (Supplementary Table 6).

Alistipes spp., also is associated with inflammation diseases, including IBD (PARKER; WEARSCH; VELOO; RODRIGUEZ-PALACIOS, et al., 2020). The abundance of *Alistipes* spp. also exhibited a high decline for C group during colitis induction, while WU group showed some decrease (T3), but a recovery on the last day of study (Supplementary Figure 1B). However, it was not possible to evaluate the abundance of *Alistipes* spp. species, which would help in understanding the participation of this genus in the development of colitis.

Figure 6 - Genus relative abundance of fecal microbiota in different experimental groups.



Notes: Only the top ten most abundant genera were showed. The others genera were included in “Others” group. Bar represents a group on a specific day, and the color of each cell indicates the abundance of the bacterial genus. Group H: healthy animals that did not receive the products under study; C: animals with chemically induced colitis that did not receive the products under study; Group W: animals with chemically induced colitis that received the whey protein without UV-C treatment; Group WU: animals with chemically induced colitis that received the whey protein without UV-C treatment.T1: first of protocol. T2: 7 days of protocol. T3: 11 days of protocol. T4: last day of protocol.

Although no differences were found in their families, the genera *Muribaculaceae* (non-cultivated) and *Prevotellaceae*_UCG_001 showed statistical differences within the groups over the time of the experiment (Supplementary Figure 1B). The genus *Prevotellaceae*_UCG_001 showed a decrease only in group C and *Muribaculaceae* (uncultivated) showed a decline on the 11th day, with recovery of abundance at the end of the protocol (Supplementary Figure 1B). These genera are considered indicators of inflammation progression and have also been reported in patients with IBD and mouse models of colitis (FORBES; VAN DOMSELAAR; BERNSTEIN; 2016; SHANG et al, 2021; ZITOMERSKY et al., 2013).

Lachnospiraceae_UCG_001 and *Blautia* spp. (*Lachnospiraceae* family) are normally depleted on IBD patients (FRANK et al., 2007). Alterations in *Lachnospiraceae_UCG_001* are also founded in mouse induced to IBD and correlated to damage in mucosa mainly during inflammation (HUANGFU et al., 2021). In this study, *Lachnospiraceae_UCG_001* was the most abundant genus, with approximal 2% to 15% of the total abundance in *Firmicutes*. This genus decreased after induction of colitis in W group ($p < 0.05$, T3 *vs* T1, loss of approximal 13% of abundance), but its abundance the abundance has been recovered in the last day of study, ($p > 0.05$, T4 *vs* T1). On other way, W group did not recovery the abundance of *Blautia* spp. at the end of study ($p < 0.05$, T1 *vs* T4). *Blautia* spp. is considered a potential probiotic because can survive in the intestinal environment and improve the host health (LIU et al., 2021). In the WU group, the abundance of *Lachnospiraceae*_UCG_001 and *Blautia* spp. was maintained, highlighting the beneficial action of WP treated with UV-C in the modulation of the animal microbiota and its potential to help in the treatment of patients with colitis.

Oscilospiraceae spp. (associated with group H) increased during the experiment (T1 vs T4, $p < 0.05$) and *Ruminococcaceae* spp. (non-cultivated) decreased after colitis induction in animals challenged with DSS and treated with WP ($p < 0.05$, T1 vs T3), but on the last day of the study the relative abundance was recovered ($p > 0.05$, T3 vs T4). Members of

Ruminococcaceae family are precursors of energy and metabolites (like SCFA) because its capacity to break down a full range of plant-derived substrates indigestible by the host (BIDDLE; STEWART; BLANCHARD; LESCHINE, 2013).

Butyricicoccaceae_UCG_009 (*Butyricicoccaceae* family) decreased on the last day in W and WU groups (T1 *vs* T4, $p < 0.05$). This depletion is a sign of inflammation, as it indicates a lower production of butyrate, an important SCFA for intestinal homeostasis (GEIRNAERT et al., 2014). Furthermore, high abundance of *Butyricicoccaceae*_UCG_009 has been reported in other diseases such as obesity and insulin resistance (ZHAO et. al. 2022). Thus, the effect of reduction in *Butyricicoccaceae*_UCG_009 abundance at the end of induction is inconclusive.

Abundance of *Clostridia_vadinBB60_group* genus was higher in W and H group in the T3 ($p < 0.05$). Morever, both groups presented the same abundance when compare the first and the last day of experiment ($p > 0.05$, T1 *vs* T4). The regular ingestion of the probiotic *Lacticaseibacillus casei* was associated with higher abundance of *Clostridia_vadinBB60_group* and improve ou gut health in animal model (AINDELIS; YPSILANTIS; CHLICHLIA, 2021).

Parabacteroides spp., a member of the *Tannerellaceae* also present the same results plus alterations in C group (Supplementary Figure 1B). Although *Tannerellaceae* is commonly found in gut in minor abundance, this genus is a Gram negative with endotoxin that induces a strong pro-inflammatory reaction in the host (HIIPPALA et al., 2020).

Although some studies already conclude that different proteins, include WP, can contribute to the host's metabolism and colitis management (GUO et al., 2020; LAN et al., 2016, BOSCAINI, et al., 2023), the relationship between microbiota composition and colitis progress remains unclear (BARBERIO et al., 2022; PARK; YEO; KANG; HUH et al., 2021). This is the first research identified the positive effect of the supplementation with UV-C treated WP on colitis and its relationship with the modulation of the fecal microbiota.

Conclusion

Our study has provided better understanding of microbial shifts and subsequent alteration to the profile of GM from mice with inflammation of the colon. In particular, we have observed how the WP supplementation can help to reduce progression of UC disease.

The results revealed that each of the WP supplements can be modulate the GM during inflammation in different ways, but UV-C treated WP were most effective considering less progression on clinical parameters and conservation in genera relative abundance In addition, the intake of UV-C treated WP resulted in a positive modulation of GM, with emphasis on an increase in *Turicibacter* spp., recovery in *Ruminococcaceae* family and maintaining of *Lachnospiraceae_UCG_001* and *Blautia* spp which certainly contributed to the control of colitis. Despite the positive results, studies are recommended to evaluate the effect of supplementation with UV-C treated WP in other aumounts to establish the best doses and duration of treatment.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

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References

AINDELIS, G.; YPSILANTIS, P.; CHLICHLIA, K. Alterations in faecal microbiota and elevated levels of intestinal IgA following oral administration of Lacticaseibacillus casei in mice. **Probiotics and Antimicrobial Proteins**, p. 1-11, 2021.

ALAM, M. T. et al. Microbial imbalance in inflammatory bowel disease patients at different taxonomic levels. **Gut pathogens**, v. 12, n. 1, p. 1-8, 2020.

ALMEIDA, C. C.; ALVARES, T. S.; COSTA, M. P.; CONTE-JUNIOR, C. A. Protein and Amino Acid Profiles of Different Whey Protein Supplements. **Journal of Dietary Supplements**, v. 13, n. 3, p. 313–323, 2016.

AL-OMARI, M. M. *et al.* Camel milk whey inhibits inflammatory colorectal cancer development via down regulation of pro-inflammatory cytokines in induced AOM/DSS mouse model. **Emirates Journal of Foodand Agriculture**, v. 31, n. 4, p. 256–262, 2019.

ARAÚJO, D. F. S. *et al.* Goat whey ameliorates intestinal inflammationon acetic acid-induced colitis in rats. **Journal of Dairy Science**, v. 99, n. 12, p. 9383–9394, 2016.

ARAÚJO, D. F. S. *et al.* Goat whey ameliorates intestinal inflammationon acetic acid-induced colitis in rats. **Journal of Dairy Science**, v. 99, n. 12, p. 9383–9394, 2016.

ARAÚJO, D. F. S. *et al*. Intestinal anti-inflammatory effects of goat whey on DNBS-induced colitis in mice. **PloS one**, v. 12, n. 9, p. e0185382–e0185382, 2017.

ARMUZZI, A.; LIGUORI, G. Quality of life in patients with moderate tosevere ulcerative colitis and the impact of treatment: A narrative review. **Digestive and Liver Disease**, v. 53, n. 7, p. 803-808, 2021.

ARUMUGAM, M. *et al.* Enterotypes of the human gut microbiome. **Nature**, England, v. 473, n. 7346, p. 174–180, 2011.

BARBERIO, B. *et al*. A specific microbiota signature is associated to various degrees of ulcerative colitis as assessed by a machine learning approach. **Gut microbes**, v. 14, n. 1, p. 2028366, 2022.

BIDDLE, A.; STEWART, L.; BLANCHARD, J.; LESCHINE, S. Untangling the genetic basis of fibrolytic specialization by ; Lachnospiraceae and Ruminococcaceae in diverse gut communities. **Diversity**, v. 5, n. 3, p. 627-640, 2013.

BOSCAINI, S. *et al*. The ‘Whey’to good health: Whey protein and its beneficial effect on metabolism, gut microbiota and mental health. **Trends in Food Science & Technology**, 2023.

BU, G.; LUO, Y.; ZHENG, Z.; ZHENG, H. Effect of heat treatment onthe antigenicity of bovine α-lactalbumin and β-lactoglobulin in whey protein isolate. **Food and Agricultural Immunology**, v. 20, n. 3, p. 195-206, 2009.

CARDOSO, Daniel R. *et al.* UV-C Light Promotes Reductive Cleavageof Disulfide Bonds in B-Lactoglobulin Improving Digestibility and Release of Bioactive Peptides. **Available at SSRN 4189832**. In press.

CHIU, C.; CHAO, A. Estimating and comparing microbial diversity inthe presence of sequencing errors. **PeerJ**, v. 4, p. e1634, 2016.

DE SOUZA, R. S. C. *et al.* Unlocking the bacterial and fungal communities assemblages of sugarcane microbiome. **Scientific Reports**, v.6, n. 1, p. 1-15, 2016.

DISTRUTTI, E.; MONALDI, L.; FIORUCCI S. Gut microbiota role in irritable bowel syndrome: New therapeutic strategies. **World journal of gastroenterology**, v. 22, n. 7, p. 2219, 2016.

ERBEN, U. *et al.* A guide to histomorphological evaluation of intestinal inflammation in mouse models. **International journal of clinical and experimental pathology**, v. 7, n. 8, p. 4557, 2014.

ESTEGHLAL, S. *et al.* Bridging the knowledge gap for the impact of non-thermal processing on proteins and amino acids. **Foods,** v. 8, n. 7, 2019.

FADROSH, D. W. *et al.* An improved dual-indexing approach for multiplexed 16S rRNA gene sequencing on the Illumina MiSeq platform. **Microbiome,** v. 2, n. 1, p. 1-7, 2014.

FANG, J. *et al.* PPARγ: The Central Mucus Barrier Coordinator in Ulcerative Colitis. **Inflammatory Bowel Diseases**, v. 27, n. 5, p 732–741, 2021.

FORBES, J. D.; VAN DOMSELAAR, G.; BERNSTEIN, C. N. The gut microbiota in immune-mediated inflammatory diseases**. Frontiers in microbiology**, v. 7, p. 1081, 2016.

FRANK, D. N. *et al.* Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. **Proceedings of the national academy of sciences**, v. 104, n. 34, p. 13780-13785, 2007.

GAJENDRAN, Mahesh *et al.* A comprehensive review and update on ulcerative colitis. **Disease-a-month**, v. 65, n. 12, p. 100851, 2019.

GEIRNAERT, A. *et al.* Butyricicoccus pullicaecorum, a butyrate producer with probiotic potential, is intrinsically tolerant to stomach and smallintestine conditions. **Anaerobe**, v. 30, p. 70-74, 2014.

GUO, D. *et al*. Elemental diet enriched with amino acids alleviates mucosal inflammatory response and prevents colonic epithelial barrier dysfunction in mice with DSS-induced chronic colitis**. Journal of immunology research**, v. 2020, 2020.

GUO, S. *et al.* Ginger alleviates DSS-induced ulcerative colitis severityby improving the diversity and function of gut microbiota. **Frontiers in Pharmacology**, v. 12, p. 632569, 2021.

HARRIS, K. G.; CHANG, E. B. The intestinal microbiota in the pathogenesis of inflammatory bowel diseases: new insights into complexdisease. **Clinical science**, England, v. 132, n. 18, p. 2013–2028, 2018.

HIIPPALA, K. *et al.* Isolation of anti-inflammatory and epithelium reinforcing Bacteroides and Parabacteroides spp. from a healthy fecal donor.**Nutrients**, v. 12, n. 4, p. 935, 2020.

Hong *et al*. Compound Sophorae Decoction: treating ulcerative colitisby affecting multiple metabolic pathways. **Chinese Journal of Natural Medicines**, v.19, n.4, 267–83, 2021.

HUANGFU, L. X. *et al.* Irisin attenuates inflammation in a mouse model of ulcerative colitis by altering the intestinal microbiota. **Experimentaland Therapeutic Medicine**, v. 22, n. 6, p. 1-15, 2021.

JU, T.; KONG, J. Y,; STOTHARD, P.; WILLING, B. Defining the role of Parasutterella, a previously uncharacterized member of the core gut microbiota. **The ISME journal**, v. 13, n. 6, p. 1520-1534, 2019.

KAAKOUSH, N. O. Insights into the role of Erysipelotrichaceae in the human host. **Frontiers in cellular and infection microbiology**, v. 5, p. 84, 2015.

KOBAYASHI, T. *et al.* Ulcerative colitis (Primer). **Nature Reviews: Disease Primers**, v. 6, n. 1, 2020.

KOSTIC, A. D.; XAVIER, RAMNIK J.; GEVERS, D. The microbiome in inflammatory bowel disease: current status and the future ahead. **Gastroenterology**, v. 146, n. 6, p. 1489-1499, 2014.

KUSHKEVYCH, I.; DORDEVIĆ, D.; KOLLÁR, P. Analysis of physiological parameters of Desulfovibrio strains from individuals with colitis. **Open life sciences**, v. 13, n. 1, p. 481-488, 2018.

KUSHKEVYCH, I.; DORDEVIĆ, D.; VÍTĚZOVÁ, M. Possible synergy effect of hydrogen sulfide and acetate produced by sulfate-reducing bacteria on inflammatory bowel disease development. **Journal of Advanced Research**, v. 27, p. 71-78, 2021.

LAGRANGE, V.; CLARK, D. C. Nutritive and Therapeutic Aspects ofWhey Proteins. *In*: DEETH, H. C.; BANSAL, N. **Whey Proteins: From Milk to Medicine**. 1 ed. Elsevier Inc., 2019. p 549-577.

LAN, A. *et al.* Dual effects of a high-protein diet on DSS-treated miceduring colitis resolution phase. **American Journal of Physiology-Gastrointestinal and Liver Physiology**, v. 311, n. 4, p. G624-G633, 2016.

LIU, K. Y. *et al.* Vitamin E alpha-and gamma-tocopherol mitigate colitis, protect intestinal barrier function and modulate the gut microbiota inmice.

Free Radical Biology and Medicine, v. 163, p. 180-189, 2021.

LIU, X. *et al*. Blautia—a new functional genus with potential probiotic properties?. **Gut microbes**, v. 13, n. 1, p. 1875796, 2021.

LIU, Y.; WANG, X.; HU, C. A. A. Therapeutic potential of amino acidsin inflammatory bowel disease. **Nutrients**, v. 9, n. 9, p. 1–18, 2017.

ORDÓÑEZ, J. A. *et al.* Características gerais do leite e componentes fundamentais. In: Tecnologia de Alimentos: Volume 2 - Alimentos de Origem Animal. Porto Alegre. p. 279. 2007.

MARTIN-GALLAUSIAUX, C. *et al*. SCFA: mechanisms and functional importance in the gut. **Proceedings of the Nutrition Society**, v. 80, n. 1, p. 37-49, 2021.

MUKHOPADHYA, I.; HANSEN, R.; EL-OMAR, E. M.; HOLD, G. L. IBD-what role do Proteobacteria play? **Nature reviews. Gastroenterology & hepatology**, England, v. 9, n. 4, p. 219–230, 2012.

NG, S. C. *et al.* Worldwide incidence and prevalence of inflammatorybowel disease in the 21st century: a systematic review of population-based studies. **The Lancet**, v. 390, n. 10114, p. 2769-2778, 2017.

PARK, H.; YEO, S.; KANG, S.; HUH, C. S. Longitudinal microbiomeanalysis in a dextran sulfate sodium-induced colitis mouse model. **Microorganisms**, v. 9, n. 2, p. 370, 2021.

PARKER, B. J.; WEARSCH, P. A.; VELOO, A. C. M.; RODRIGUEZ-PALACIOS, A. The genus Alistipes: gut bacteria with emerging implications to inflammation, cancer, and mental health. **Frontiers in immunology**, v. 11, p. 906, 2020.

PEYRIN-BIROULET, Laurent *et al.* Defining disease severity in inflammatory bowel diseases: current and future directions. **Clinical Gastroenterology and Hepatology**, v. 14, n. 3, p. 348-354. e17, 2016.

PORTUNE, K. J. *et al.* Gut microbiota role in dietary protein metabolism and health-related outcomes: the two sides of the coin. **Trendsin Food Science & Technology**, v. 57, p. 213-232, 2016.

QIN, H. *et al.* Enterochromaffin cell hyperplasia in the gut: Factors, mechanism and therapeutic clues. **Life sciences**, v. 239, p. 116886, 2019.

RAHAMAN, T.; VASILJEVIC, T.; RAMCHANDRAN, L. Effect of processing on conformational changes of food proteins related to allergenicity. **Trends in Food Science & Technology**, v. 49, p. 24–34, 2016.

RAJPUT, M. *et al.* Determining the association between gut microbiota and its metabolites with higher intestinal Immunoglobulin A response. **Veterinary and Animal Science**, v. 19, p. 100279, 2023.

RANKIN, S. A. *et al*. The application of alkaline phosphatase assaysfor the validation of milk product pasteurization. **Journal of dairy science**,United States, v. 93, n. 12, p. 5538–5551, 2010.

RINNINELLA, E. *et al.* What is the healthy gut microbiota composition? A changing ecosystem across age, environment, diet, anddiseases. **Microorganisms**, v. 7, n. 1, p. 14, 2019.

RIZZATTI, G. *et al.* Proteobacteria: a common factor in human diseases. **BioMed research international**, v. 2017, 2017.

RUDLOFF, S.; LONNERDAL, B. Solubility and digestibility of milk proteins in infant formulas exposed to different heat treatments. **Journal of pediatric gastroenterology and nutrition**, United States, v. 15, n. 1, p. 25–33, 1992.

SAH, Baidya Nath Prasad; MCAINCH, A. J.; VASILJEVIC, Todor. Modulation of bovine whey protein digestion in gastrointestinal tract: A comprehensive review. **International dairy journal**, v. 62, p. 10-18, 2016.

SHANG, J. *et al.* Exploring the mechanism of action of Sanzi formulain intervening colorectal adenoma by targeting intestinal flora and intestinal metabolism. **Frontiers in Microbiology**, v. 13, p. 01-13, 2022.

SHANG, L. *et al.* Core altered microorganisms in colitis mouse model:A comprehensive time-point and fecal microbiota transplantation analysis. **Antibiotics**, v. 10, n. 6, p. 643, 2021.

SHIN, N.; WHON, T. W.; BAE, J. Proteobacteria: microbial signature of dysbiosis in gut microbiota. **Trends in biotechnology**, v. 33, n. 9, p. 496-503, 2015.

SMITHERS, G. W. Whey and whey proteins—From 'gutter-to-gold'. **International dairy journal**, v. 18, n. 7, p. 695-704, 2008.

SPRONG, R. C.; SCHONEWILLE, A. J.; VAN DER MEER, R. Dietary cheese whey protein protects rats against mild dextran sulfate sodium–induced colitis: Role of mucin and microbiota. **Journal of dairy science**, v. 93, n. 4, p. 1364-1371, 2010.

SUN, J*. et al.* Anti-inflammatory properties and gut microbiota modulation of an alkali-soluble polysaccharide from purple sweet potato inDSS-induced colitis mice. **International journal of biological macromolecules**, v. 153, p. 708-722, 2020.

TEAM, R. Core. R: The R Project for Statistical Computing [Internet]. 2021 [cited 2022 Aug 22]. **Available from: Available from: https://www. r-project. org**.

TIERNEY, B. T. et al. The landscape of genetic content in the gut andoral human microbiome. **Cell host & microbe**, v. 26, n. 2, p. 283-295. e8, 2019.

TRIANTAFILLIDIS, J. K.; TZOUVALA, M.; TRIANTAFYLLIDI, E. Enteral nutrition supplemented with transforming growth factor-β, colostrum, probiotics, and other nutritional compounds in the treatment of patients with inflammatory bowel disease. **Nutrients**, v. 12, n. 4, p. 1048, 2020.

TULIPANO, G. Role of bioactive peptide sequences in the potential impact of dairy protein intake on metabolic health. **International Journal of Molecular Sciences**, v. 21, n. 22, p. 8881, 2020.

VALDES, A. M.; WALTER, J.; SEGAL, E.; SPECTOR, T. D. Role of the gut microbiota in nutrition and health. **BMJ**, v. 361, 2018.

VERHOECKX, K. C. *et al.* Food processing and allergenicity. **Foodand Chemical Toxicology**, v. 80, p. 223-240, 2015.

VESTER-ANDERSEN, M. K. *et al*. Increased abundance of proteobacteria in aggressive Crohn's disease seven years after diagnosis. **Scientific reports**, v. 9, n. 1, p. 1-10, 2019.

WONG, J. *et al*. Expansion of urease-and uricase-containing, indole- and p-cresol-forming and contraction of short-chain fatty acid-producing intestinal microbiota in ESRD. **American journal of nephrology**, v. 39, n. 3,p. 230-237, 2014.

WU, Y. *et al.* Probiotics (Lactobacillus plantarum HNU082) supplementation relieves ulcerative colitis by affecting intestinal barrier functions, immunity-related gene expression, gut microbiota, and metabolic pathways in mice. **Microbiology Spectrum**, v. 10, n. 6, p. e01651-22, 2022.

XU, R. *et al.* Metagenomic approach reveals the fate of antibiotic resistance genes in a temperature-raising anaerobic digester treating municipal sewage sludge. **Journal of Cleaner Production**, v. 277, p. 123504, 2020.

YOUNG, V. B.; SCHMIDT, T. M. Overview of the gastrointestinal microbiota. **GI microbiota and regulation of the immune system**, p. 29-40,2008.

ZHAO, S. *et al.* Sodium Alginate Prevents Non-Alcoholic Fatty Liver Disease by Modulating the Gut–Liver Axis in High-Fat Diet-Fed Rats**.** **Nutrients**. v. 14, n. 22, p. 4846. 2022

ZHAO, Y.; NAKATSU, C.; JONES-HALL, Y.; JIANG, Q. Supplementation of polyphenol-rich grapes attenuates colitis, colitis-associated colon cancer, and disease-associated dysbiosis in mice, but fails to mitigate colitis in antibiotic-treated mice. **The Journal of Nutritional Biochemistry**, v. 109, p. 109124, 2022.

ZITOMERSKY, N. L. *et al.* Characterization of adherent bacteroidales from intestinal biopsies of children and young adults with inflammatory bowel disease. **PLOS one**, v. 8, n. 6, p. e63686, 2013.

SUPPLEMENTARY FILES

Supplementary Table 1 - The weight/length of the ileocecal junction at the anal margin of the animals.

Groups	Weight/length ratio (mg/mm)
H	3.46 ± 0.96 ^{B}
C	4.77 ± 1.52 ^{A}
W	4.11 ± 0.83 ^{A}
WU	4.52 ± 0.84 ^{A}

Note: Values are expressed as median ± standard deviation (ANOVA one way, Tukey post test, α = 0.05). Group H: healthy animals that did not receive the products under study;Group C: animals with chemically induced colitis that did not receive the products under study; Group W: animals with chemically induced colitis that received the whey protein without UV-C treatment; Group WU: animals with chemically induced colitis that received the whey protein without UV-C treatment. Source: own authorship.

Supplementary Table 2 - Alpha-Diversity measures of fecal microbiota between groups and during the timeline of experiment.

Groups	Alpha-diversity	Days				Total
		T1	T2	T3	T4	
Healthy group	Shannon	5.21 ± 0.42 ^{Aa}	5.31 ± 0.41 ^{Aa}	5.44 ± 0.87 ^{Aa}	5.33 ± 0.77 ^{Aa}	5.32 ± 0.62 ^{A}
	Chao1	83.00 ± 25.02 ^{Aa}	80.00 ± 29.80 ^{Aa}	84.50 ± 41.00 ^{Aa}	107.00 ± 39.52 ^{Aa}	83.50 ± 33.54 ^{A}
	Number of observation	83.00 ± 25.02 ^{Aa}	80.00 ± 29.80 ^{Aa}	84.50 ± 41.00 ^{Aa}	107.00 ± 39.52 ^{Aa}	83.50 ± 33.54 ^{A}
Colit group	Shannon	5.15 ± 0.54 ^{Ab}	5.37 ± 0.77 ^{Ab}	4.37 ± 0.55 ^{Aa}	5.00 ± 0.78 ^{Aab}	4.99 ± 0.73 ^{A}
	Chao1	89.50 ± 29.99 ^{ABab}	83.00 ± 34.79 ^{Ab}	58.5 0 ± 12.94 ^{Aa}	68.00 ± 34.08 ^{Aab}	64.50 ± 30.92 ^{A}
	Number of observation	89.50 ± 29.99 ^{ABab}	83.00 ± 34.79 ^{Ab}	58.5 0 ± 12.94 ^{Aa}	68.00 ± 34.08 ^{Aab}	64.50 ± 30.92 ^{A}
W group	Shannon	5.53 ± 0.30 ^{Ab}	5.37 ± 1.21 ^{Aab}	5.03 ± 0.71 ^{Aa}	5.18 ± 0.76 ^{Aab}	5.31 ± 0.80 ^{A}
	Chao1	87.00 ± 18.35 ^{Ba}	78.50 ± 35.58 ^{Aa}	65.50 ± 33.47 ^{Aa}	84.00 ± 29.11 ^{Aa}	84.00 ± 28.58 ^{A}

	Number of observation	87.00 ± 18.35 ^{Aa}	78.50 ± 35.58 ^{Aa}	65.50 ± 33.47 ^{Aa}	84.00 ± 29.11 ^{Aa}	84.00 ± 28.58 ^{A}
WU group	Shannon	5.40 ± 0.32 ^{Ab}	5.08 ± 0.50 ^{Aab}	4.60 ± 0.52 ^{Aa}	5.06 ± 0.64 ^{Ab}	5.06 ± 0.57 ^{A}
	Chao1	78.00 ± 26.27 ^{ABa}	64.00 ± 29.78 ^{Aa}	45.00 ± 37.81 ^{Aa}	56.00 ± 34.44 ^{Aa}	61.50 ± 31.36 ^{A}
	Number of observation	78.00 ± 26.27 ^{Aa}	64.00 ± 29.78 ^{Aa}	45.00 ± 37.81 ^{Aa}	56.00 ± 34.44 ^{Aa}	61.50 ± 31.36 ^{A}
Total	Shannon	5.33 ± 0.41 ^{b}	5.28 ± 0.76 ^{b}	4.74 ± 0.70 ^{a}	5.11 ± 0.70 ^{ab}	
	Chao1	84.00 ± 24.53 ^{b}	76.50 ± 31.45 ^{ab}	59.00 ± 32.60 ^{a}	78.50 ± 34.23 ^{ab}	
	Number of observation	84.00 ± 24.53 ^{b}	76.50 ± 31.45 ^{ab}	59.00 ± 32.60 ^{a}	78.50 ± 34.23 ^{ab}	

Note: Values are expressed as the median ± standard deviations. p values were adjusted using Bonferroni's method. Different uppercase letters within a column indicate significant differences among different groups between the same day and same parameter ($p < 0.05$). Different lowercase letters within a line indicate significant differences among different days between the same group ($p < 0.05$). Group H: healthy animals that did not receive the products under study; Group C: animals with chemically induced colitis that did not receive the products under study;Group W: animals with chemically induced colitis that received the whey protein without UV-C treatment; Group WU: animals with chemically induced colitis that received the whey protein without UV-C treatment. T1: first of protocol. T2: 7 days of protocol. T3: 11 days of protocol. T4: last day of protocol.

Supplementary Table 3 - Permutational multivariate analysis of variance (PERMANOVA) tests of the bacterial gut microbiota on the Bray-Curtis dissimilarity according to comparison between groups and timeline of experiment.

Pairwise comparison	p-value
C *vs* H	0.001*
C *vs* W	0.686
C *vs* WU	0.138
H *vs* W	0.028*
H *vs* WU	0.001*
W *vs* WU	0.158
T1 *vs* T2	0.227
T1 *vs* T3	0.003*
T1 *vs* T4	0.003*
T2 *vs* T3	0.003*
T2 *vs* T3	0.003*
T3 *vs* T4	0.0096*

Note: * show significative statistical values (p< 0.05) , Bonferroni corrected p values. Group H: healthy animals that did not receive the products under study; Group C: animals with chemically induced colitis that did not receive the products under study;Group W: animals with chemically induced colitis that received the whey protein without UV-C treatment; Group WU: animals with chemically induced colitis that received the whey protein without UV-C treatment.T1: first of protocol. T2: 7 days of protocol. T3: 11 days of protocol. T4: last day of protocol.

Supplementary Table 4 - Permutational multivariate analysis of variance (PERMANOVA) tests of the bacterial gut microbiota on the Bray-Curtis dissimilarity according to comparison between groups on different days of experiment and between days on specific groups.

Pairwise Comparisons		
Days	Groups	p-value
T1	C *vs* H	0.100
	C *vs* W	0.642
	C *vs* WU	0.644
	H *vs* W	0.771
	H *vs* WU	0.467
	W *vs* WU	0.897
T2	C *vs* H	0.850
	C *vs* W	0.771
	C *vs* WU	0.494
	H *vs* W	0.803
	H *vs* WU	0.132
	W *vs* WU	0.21
T3	C *vs* H	0.131
	C *vs* W	0.705
	C *vs* WU	0.592
	H *vs* W	0.076
	H *vs* WU	0.053
	W *vs* WU	0.792
T4	C *vs* H	0.048*

	C *vs* W	0.649
	C *vs* WU	0.261
	H *vs* W	0.147
	H *vs* WU	0.095
	W *vs* WU	0.666
H group	T1 *vs* T2	0.522
	T1 *vs* T3	0.580
	T1 *vs* T4	0.684
	T2 *vs* T3	0.862
	T2 *vs* T3	0.939
	T3 *vs* T4	0.862
C group	T1 *vs* T2	0.771
	T1 *vs* T3	0.103
	T1 *vs* T4	0.048*
	T2 *vs* T3	0.372
	T2 *vs* T3	0.248
	T3 *vs* T4	0.243
W group	T1 *vs* T2	0.115
	T1 *vs* T3	0.079
	T1 *vs* T4	0.051
	T2 *vs* T3	0.051
	T2 *vs* T3	0.079
	T3 *vs* T4	0.103
WU group	T1 *vs* T2	0.372
	T1 *vs* T3	0.132
	T1 *vs* T4	0.174
	T2 *vs* T3	0.048*

	T2 *vs* T3	0.064
	T3 *vs* T4	0.430

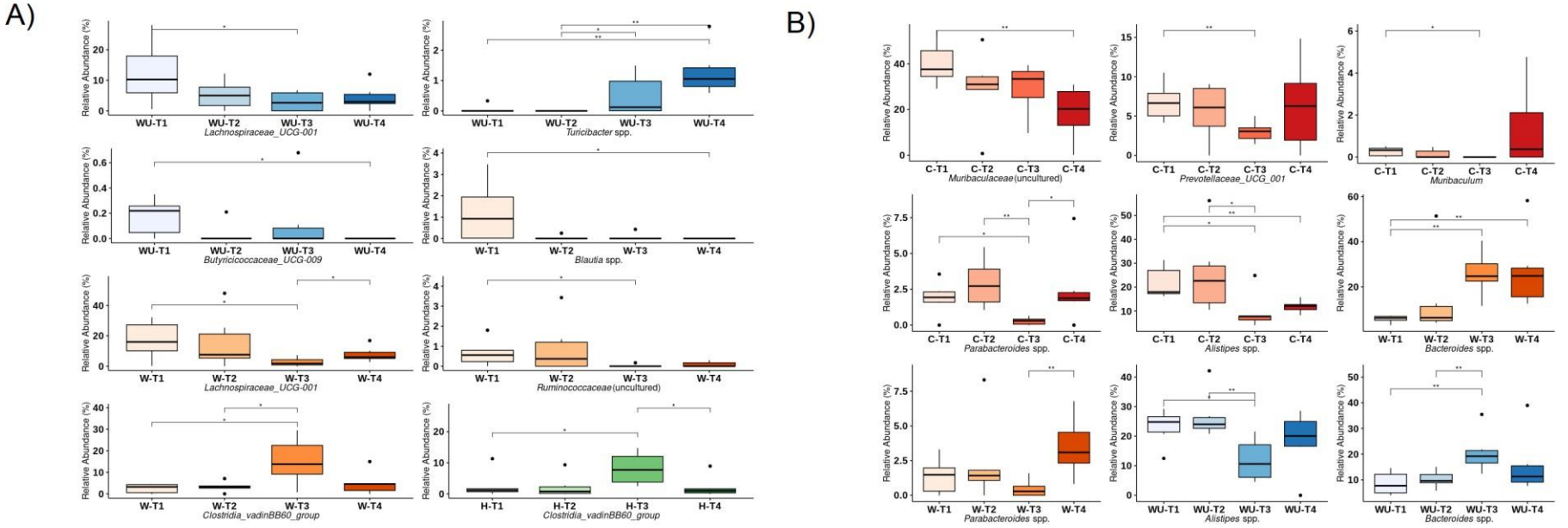
Note: * show significative statistical values (p< 0.05) , Bonferroni corrected p values. Group H: healthy animals that did not receive the products under study; Group C: animals with chemically induced colitis that did not receive the products under study;Group W: animals with chemically induced colitis that received the whey protein without UV-C treatment; Group WU: animals with chemically induced colitis that received the whey protein without UV-C treatment.T1: first of protocol. T2: 7 days of protocol. T3: 11 days of protocol. T4: last day of protocol.

Supplementary Table 5 - Phyla relative abundance (%) of fecal microbiota between groups during timeline of experiment.

Phylum	Groups	0 day	7 days	12 days	14 days	Total
Cyanobacteria	C	0.00 ± 0.69 ^{Aab}	0.00 ± 0.16 ^{Aab}	0.00 ± 0.00^{Aa}	0.64 ± 1.15 ^{Bb}	0.00 ± 0.73 ^{A}
	H	0.00 ± 0.40 ^{Aa}	0.00 ± 0.00 ^{Aa}	0.00 ± 0.00 ^{Aa}	0.00 ± 0.06 ^{ABa}	0.00 ± 0.21 ^{A}
	W	0.00 ± 0.18 ^{Aa}	0.00 ± 0.00 ^{Aa}	0.00 ± 0.33 ^{Aa}	0.00 ± 0.00 ^{Aa}	0.00 ± 0.19 ^{A}
	WU	0.00 ± 0.36 ^{Aa}	0.00 ± 0.09 ^{Aa}	0.00 ± 0.07 ^{Aa}	0.05 ± 0.92 ^{ABa}	0.00 ± 0.51 ^{A}
	Total	0.00 ± 0.43 ^{ab}	0.00 ± 0.09 ^{a}	0.00 ± 0.17 ^{a}	0.00 ± 0.79 ^{b}	
Desulfobacterota	C	0.31 ± 0.19 ^{Aa}	0.28 ± 0.88 ^{Aa}	0.06 ± 0.36 ^{Aa}	0.31 ± 0.29 ^{Aa}	0.24 ± 0.51 ^{A}
	H	0.91 ± 1.47 ^{Aa}	0.53 ± 0.47 ^{Aa}	0.80 ± 0.70 ^{Aa}	0.48 ± 0.78 ^{Aa}	0.71 ± 0.94 ^{B}
	W	0.56 ± 0.54 ^{Ab}	0.11 ± 0.64 ^{Aab}	0.15 ± 0.13 ^{Aab}	0.08 ± 0.11 ^{Aa}	0.17 ± 0.46 ^{A}
	WU	0.45 ± 0.55 ^{Ab}	0.63 ± 0.61 ^{Ab}	0.15 ± 0.47 ^{Aab}	0.00 ± 0.21 ^{Aa}	0.27 ± 0.52 ^{AB}
	Total	0.48 ± 0.89 c	0.43 ± 0.63 ^{bc}	0.17 ± 0.51 ^{ab}	0.16 ± 0.47 ^{a}	
Proteobacteria	C	0.41 ± 0.67 ^{Aab}	0.58 ± 0.35 ^{Aab}	0.26 ± 0.32 ^{Aa}	1.03 ± 3.18 ^{Ab}	0.52 ± 1.71 ^{A}
	H	0.08 ± 0.24 ^{Aa}	0.56 ± 0.53 ^{Ab}	0.37 ± 0.99 ^{Aab}	0.79 ± 1.20 ^{Aab}	0.34 ± 0.85 ^{A}
	W	0.48 ± 0.37 ^{Aa}	0.35 ± 0.67 ^{Aab}	0.85 ± 0.74 ^{Aab}	0.98 ± 1.13 ^{Ab}	0.62 ± 0.80 ^{A}
	WU	0.47 ± 0.54 ^{Aab}	0.61 ± 0.50 ^{Aab}	0.12 ± 0.78 ^{Aa}	1.82 ± 5.75 ^{Ab}	0.60 ± 3.22 ^{A}
	Total	0.25 ± 0.48 ^{a}	0.54 ± 0.49 ^{a}	0.30 ± 0.74 ^{a}	1.05 ± 3.42 ^{b}	
Firmicutes	C	16.41 ± 11.68 ^{Aa}	18.36 ± 10.28 ^{Aa}	19.92 ± 18.39 ^{Aa}	25.16 ± 8.77 ^{Ba}	20.73 ± 12.37 ^{AB}
	H	28.38 ± 15.80 ^{Aa}	20.01 ± 11.77 ^{Aa}	31.61 ± 16.14 ^{Aa}	22.00 ± 19.98 ^{ABa}	25.09 ± 15.70 ^{B}
	W	32.34 ± 22.57 ^{Aa}	23.50 ± 18.65 ^{Aa}	21.38 ± 10.22 ^{Aa}	20.61 ± 9.11 ^{ABa}	22.95 ± 15.86 ^{B}
	WU	26.90 ± 14.30 ^{Aa}	13.13 ± 4.85 ^{Aa}	15.17 ± 10.81 ^{Aa}	11.71 ± 5.43 ^{Aa}	13.73 ± 10.16 ^{A}
	Total	24.85 ± 16.47 ^{a}	18.36 ± 12.37 ^{a}	22.91 ± 14.62 ^{a}	20.51 ±12.54 ^{a}	

Note: Values are expressed as the median ± standard deviations ($p < 0.05$). p values were adjusted using Bonferroni's method. Different uppercase letters within a column in the same Phylum indicate significant differences among different groups between the same day. Different lowercase letters within a line in the same Phylum indicate significant differences among different days between the same group. Group H: healthy animals that did not receive the products under study; Group C: animals with chemically induced colitis that did not receive the products under study;Group W: animals with chemically induced colitis that received the whey protein without UV-C treatment; Group WU: animals with chemically induced colitis that received the whey protein without UV-C treatment. Only Phyla with statistical differences were showed.

Supplementary Figure 1 - Genera relative abundance (%) in the groups by Fimicutes and Bacteroidota phylum over timeline of experiment.



Notes: Only genera that showed statistical differences between the times of the experiment within the same groups were presented. A) *Fimicutes* phylum, B) *Bacteroidota* phylum. Values are expressed as median ± IQR. (*) $p < 0.05$;(**) $p < 0.01$; (***) $p < 0.001$ and (****) $p < 0.0001$ by Bonferroni method. Group H: healthy animals that did not receive the products under study; Group C: animals with chemically induced colitis that did not receive the products under study; Group W: animals with chemically induced colitis that received the whey protein without UV-C treatment; Group WU: animals with chemically induced colitis that received the whey protein without UV-C treatment. Only genera with statistical differences were showed. T1: first of protocol. T2: 7 days of protocol. T3: 11 days of protocol. T4: last day of protocol. Source: own authorship.

Supplementary Table 6 - *Parasutterella* spp., *Gastranaerophilales* spp. and *Desulfovibrio* spp. genera abundance (%) between groups during timeline of experiment.

Parasutterella spp. relative abundance (%)					
Groups	T1	T2	T3	T4	Total
C	0.41 ± 0.30 ^{Aa}	0.51 ± 0.35 ^{Aa}	0.17 ± 0.20 ^{ABa}	0.67 ± 0.49 ^{Aa}	0.45 ± 0.37 ^{A}
H	0.00 ± 0.11 ^{Aa}	0.47 ± 0.30 ^{Ab}	0.37 ± 0.72 ^{Bb}	0.65 ± 1.04 ^{Aab}	0.32 ± 0.68 ^{A}
W	0.47 ± 0.36 ^{Aa}	0.28 ± 0.67 ^{Aab}	0.02 ± 0.20 ^{Aab}	0.91 ± 0.23 ^{Ab}	0.43 ± 0.47 ^{A}
WU	0.39 ± 0.50 ^{Aab}	0.61 ± 0.43 ^{Ab}	0.06 ± 0.07 ^{Aa}	0.42 ± 0.44 ^{Aab}	0.22 ± 0.42 ^{A}
Total	0.20 ± 0.36 ^{a}	0.51 ± 0.43 ^{b}	0.12 ± 0.44 ^{a}	0.73 ± 0.61 ^{b}	
Gastranaerophilales spp. relative abundance (%)					
Groups	T1	T2	T3	T4	Total
C	0.00 ± 0.69 ^{Aab}	0.00 ± 0.16 ^{Aab}	0.00 ± 0.00 ^{Aa}	0.64 ± 1.15 ^{Bb}	0.00 ± 0.73 ^{A}
H	0.00 ± 0.40 ^{Aa}	0.00 ± 0.00 ^{Aa}	0.00 ± 0.00 ^{Aa}	0.00 ± 0.06 ^{Aba}	0.00 ± 0.21 ^{A}
W	0.00 ± 0.18 ^{Aa}	0.00 ± 0.00 ^{Aa}	0.00 ± 0.33 ^{Aa}	0.00 ± 0.00 ^{Aa}	0.00 ± 0.19 ^{A}
WU	0.00 ± 0.36 ^{Aa}	0.00 ± 0.09 ^{Aa}	0.00 ± 0.07 ^{Aa}	0.05 ± 0.92 ^{ABa}	0.00 ± 0.51 ^{A}
Total	0.00 ± 0.43 ^{ab}	0.00 ± 0.09 ^{a}	0.00 ± 0.17 ^{a}	0.00 ± 0.79 ^{b}	
Desulfovibrio spp. relative abundance (%)					
Groups	T1	T2	T3	T4	Total
C	0.31 ± 0.19 ^{Aa}	0.28 ± 0.88 ^{Aa}	0.06 ± 0.36 ^{Aa}	0.31 ± 0.29 ^{Aa}	0.23 ± 0.51 ^{A}

H	0.91 ± 1.47 ^{Aa}	0.53 ± 0.47 ^{Aa}	0.80 ± 0.70 ^{Aa}	0.48 ± 0.78 ^{Aa}	0.71 ± 0.94 ^{B}
W	0.56 ± 0.54 ^{Ab}	0.11 ± 0.64 ^{Aab}	0.15 ± 0.13 ^{Aab}	0.08 ± 0.11 ^{Aa}	0.17 ± 0.46 ^{AB}
WU	0.45 ± 0.55 ^{Ab}	0.63 ± 0.61 ^{Ab}	0.15 ± 0.47 ^{Aab}	0.00 ± 0.21 ^{Aa}	0.27 ± 0.52 ^{A}
Total	0.47 ± 0.89 ^{c}	0.43 ± 0.63 ^{Abc}	0.17 ± 0.51 ^{ab}	0.16 ± 0.47 ^{a}	

Only genera that showed statistical differences between the times of the experiment within the same groups were presented. Note: Values are expressed as the median ± standard deviations ($p < 0.05$). p values were adjusted using Bonferroni's method. Different uppercase letters within a column in the same Genus indicate significant differences among different groups between the same day. Different lowercase letters within a line in the same Genus indicate significant differences among different days between the same group. Group H: healthy animals that did not receive the products under study; Group C: animals with chemically induced colitis that did not receive the products under study;Group W: animals with chemically induced colitis that received the whey protein without UV-C treatment; Group WU: animals with chemically induced colitis that received the whey protein without UV-C treatment.T1: first of protocol. T2: 7 days of protocol. T3: 11 days of protocol. T4: last day of protocol. Source: own authorship.

5 CONSIDERAÇÕES FINAIS

Apoiados nos resultados obtidos nesta pesquisa, pode-se concluir que ambos suplementos auxiliaram na manutenção da microbiota intestinal durante a progressão da doença, porém com diferentes respostas ao longo do processo de inflamação nas vias metabolômicas e na microbiota intestinal. Recomenda-se maiores estudos na análise de diferentes concentrações de soro de leite tratado por UV-C para verificar eventuais sobrecargas nas funções renais, mas também avaliando as particularidades nas mudanças da microbiota intestinal.