UNIVERSIDADE ESTADUAL PAULISTA - UNESP
CÂMPUS DE JABOTICABAL

EFEITO DO USO DE PROBIÓTICO, β-MANANASE E ANTIBIÓTICO SOBRE O DESEMPENHO E SAÚDE INTESTINAL DE FRANGOS DE CORTE DESAFIADOS POR *Eimeria maxima* E *Clostridium perfringens*

Larissa Pereira Maria

Zootecnista

2023

UNIVERSIDADE ESTADUAL PAULISTA - UNESP
CÂMPUS DE JABOTICABAL

EFEITO DO USO DE PROBIÓTICO, B-MANANASE E ANTIBIÓTICO SOBRE O DESEMPENHO E SAÚDE INTESTINAL DE FRANGOS DE CORTE DESAFIADOS POR *Eimeria máxima* E *Clostridium perfringens*

Discente: Larissa Pereira Maria

Orientadora: Profa. Dra. Nilva Kazue Sakomura

Dissertação apresentada à Faculdade de Ciências Agrárias e Veterinárias – Unesp, Câmpus de Jaboticabal, como parte das exigências para a obtenção do título de Mestre em Ciência Animal

2023

M332e

Maria, Larissa Pereira

Efeito do uso de probiótico, B-mananase e antibiótico sobre o desempenho e saúde intestinal de frangos de corte desafiados por Eimeria maxima e Clostridium perfringens / Larissa Pereira Maria. -- Jaboticabal, 2023

74 p. : tabs.

Dissertação (mestrado) - Universidade Estadual Paulista (Unesp), Faculdade de Ciências Agrárias e Veterinárias, Jaboticabal

Orientadora: Nilva Kazue Sakomura

1. Aves domésticas alimentação e rações. 2. Aves criação. 3. Coccidiose. 4. Enterite. 5. Mucosa intestinal. I. Título.

Sistema de geração automática de fichas catalográficas da Unesp. Biblioteca da Faculdade de Ciências Agrárias e Veterinárias, Jaboticabal. Dados fornecidos pelo autor(a).

Essa ficha não pode ser modificada.

IMPACTO POTENCIAL DESTA PESQUISA

Uma das dificuldades da cadeia produtiva de frangos de corte, é controlar as doenças entéricas presentes na criação. A coccidiose e a enterite necrótica são consideradas enfermidades globais que atingem a produção de aves e geram perdas econômicas significativas para o setor.

Portanto o uso de cepas probióticas vem ganhando força com a proibição de vários países sobre o uso desenfreado de antimicrobianos melhoradores de desempenho em dietas para frangos, principalmente por um mercado consumidor cada vez mais exigente, que impõe padrões ao sistema produtivo a para obter características desejadas da carne e isentas de possibilidades de infortúnios à saúde humana. Por outro lado, o uso de enzimas como a β-mananase também surge para minimizar as perdas geradas pela coccidiose avícola, e o estudo da sua atuação deve ser analisada quanto para a saúde intestinal das aves e desempenho zootécnico.

Deste modo, compreender os benefícios da utilização desses compostos, gerará uma maior assertividade no enfretamento das adversidades do setor produtivo, estabelecendo maiores ganhos produtivos no setor.

POTENTIAL IMPACT OF THIS RESEARCH

One of the difficulties in the broiler production chain is controlling enteric diseases present in breeding. Coccidiosis and necrotic enteritis are considered global diseases that affect poultry production and generate significant economic losses for the sector.

Therefore, the use of probiotic strains has been gaining strength with the ban in several countries on the rampant use of performance-enhancing antimicrobials in chicken diets, mainly due to an increasingly demanding consumer market, which imposes standards on the production system to obtain desired characteristics. of the flesh and free from the possibility of misfortunes to human health. On the other hand, the use of enzymes such as β-mannanase also appears to minimize the losses generated by poultry coccidiosis, and the study of their action must be analyzed about the birds' intestinal health and zootechnical performance.

Therefore, understanding the benefits of using these compounds will generate greater assertiveness in facing adversities in the production sector, establishing greater productive gains in the sector.

UNIVERSIDADE ESTADUAL PAULISTA

Câmpus de Jaboticabal

CERTIFICADO DE APROVAÇÃO

TÍTULO DA DISSERTAÇÃO: EFEITO DO USO DE PROBIÓTICO, -MANANASE E ANTIBIÓTICO SOBRE O DESEMPENHO E SAÚDE INTESTINAL DE FRANGOS DE CORTE DESAFIADOS POR *Eimeria maxima* E *Clostridium perfringens*

AUTORA: LARISSA PEREIRA MARIA
ORIENTADORA: NILVA KAZUE SAKOMURA

Aprovada como parte das exigências para obtenção do Título de Mestra em Ciência Animal, área: Nutrição Animal pela Comissão Examinadora:

Profa. Dra. NILVA KAZUE SAKOMURA (Participaçao Presencial)
Departamento de Zootecnia / FCAV UNESP Jaboticabal

Pesquisador Dr. MARCOS KIPPER DA SILVA (Participaçao Virtual)
Departamento de Nutrição e Saúde de Aves / Elanco Animal Health Incorporated Company - São Paulo/SP

Pesquisadora Dra. LETÍCIA SOARES (Participaçao Virtual)
Departamento de Aves e Suinos / Natural Resources Institute Finland/LUKE - Jokioinen/Finlândia/UE

Jaboticabal, 13 de dezembro de 2023

gov.br Documento assinado digitalmente
MARCOS KIPPER DA SILVA
Data: 14/12/2023 17:08:26-0300
Verifique em https://validar.iti.gov.br

DADOS CURRICULARES DO AUTOR

Larissa Pereira Maria, nascida no dia 28 de agosto de 1998 em Bebedouro – SP, Brasil, filha de Ligia Maria Pereira e Leonardo Rodrigo Maria. Zootecnista pela Universidade Estadual Paulista “Júlio de Mesquita Filho”, Faculdade de ciências agrárias e veterinárias, campus Jaboticabal, São Paulo – título obtido em junho de 2022. Durante a graduação foi bolsista do Programa de Educação Tutorial durante 2 anos e meio, realizou monitoria em disciplina do curso de zootecnia – Avicultura (2021). Realizou treinamento técnico no Laboratório de Ciências Avícolas- Lavinesp, coordenado pela Profª. Drª. Nilva Kazue Sakomura (2020/2021), no qual defendeu seu trabalho de conclusão de curso -TCC. Realizou seu estágio final de graduação (2º/2021) na empresa Embrapa Suínos e Aves, em Concórdia, Santa Catarina. Iniciou o curso de mestrado pelo Programa de Pós-Graduação em Ciência Animal em janeiro de 2022 da Faculdade de Ciências Agrárias e Veterinárias – Unesp, Câmpus Jaboticabal sob a orientação da Profª. Drª. Nilva Kazue Sakomura, sendo bolsista da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

“A maior recompensa para o trabalho do homem não é o que ele ganha com isso, mas o que ele se torna com isso” - John Ruskin

A minha tão amada família,

A vocês, dedico

AGRADECIMENTOS

Primeiramente agradeço a Deus por estar sempre comigo e me conceder o maior de todos os bens: a vida!

A minha família, o bem mais tocável e precioso que possuímos em vida terrestre.

A minha mãe, Ligia, exemplo de mulher, forte e batalhadora. Que esteve ao meu lado nessa caminhada árdua, mas cheia de bons frutos.

A minha irmã, Louise, que foi minha inspiração e paz, ao lidar com as adversidades do caminho. Minha luz!

Ao meu namorado, Nicolas, o meu companheiro que esteve comigo incansavelmente em todos os meus passos, apoiando firmemente minha trajetória.

Ao meu pai, Leonardo, que me apoiou em todas as escolhas da minha vida, e sempre torceu para o meu crescimento pessoal e profissional.

Aos meus avós, que tenho o privilégio de tê-los em minha vida ainda, e que permaneceram comigo nesse processo, Maria Santa, Maria do Carmo, Cícero e Nivaldo.

A minha orientadora, Profª Drª Nilva Kazue Sakomura, pela oportunidade de vivenciar o mundo acadêmico e a pesquisa. Por todos os desafios e tarefas impostas a mim, onde estás me agregaram em inúmeros aspectos da minha carreira profissional e pessoal. Deixo aqui minha gratidão!

Aos meus amigos e companheiros do Lavinesp, pela caminhada e ajuda nesse período, por todo aprendizado adquirido juntos, e pela grande troca de experiências.

Em especial a minha amiga, companheira de casa, e de mestrado, Bárbara Marçal, por cada instante que esteve comigo, cada palavra amiga, cada gesto de amizade. Obrigada por sempre permanecer!

A Elanco Animal Health Incorporated, pelo apoio financeiro ao estudo, ao Marcos Kipper da Silva pelo apoio técnico durante a condução do estudo.

Aos membros da minha banca de defesa, Dr. Marcos Kipper e Dra. Letícia Soares muito obrigada pela contribuição, com certeza ajudaram a enriquecer minha dissertação.

A Coordenação de Aperfeiçoamento de Pessoal de Nível Superior-Brasil (CAPES) pelo apoio concessão da bolsa de estudos: 88887.662245/2022-00.

O presente trabalho foi realizado com apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Código de Financiamento 001.

A Faculdade de Ciências Agrárias e Veterinárias de Jaboticabal, pela estrutura e acolhimento, sendo minha segunda casa nesses dois anos.

E a todas as pessoas que pude conviver e conhecer, obrigada a cada um que de forma direta ou indiretamente contribuiu para esse trabalho.

SUMÁRIO

UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
Câmpus de Jaboticabal

CEUA – COMISSÃO DE ÉTICA NO USO DE ANIMAIS

CERTIFICADO

Certificamos que o projeto de pesquisa intitulado **"Efeito de aditivos nutricionais sobre o desempenho e saúde intestinal de frangos de corte desafiados"**, protocolo nº 1061/22, sob a responsabilidade da Profª Drª Nilva Kazue Sakomura, que envolve a produção, manutenção e/ou utilização de animais pertencentes ao Filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica (ou ensino) - encontra-se de acordo com os preceitos da lei nº 11.794, de 08 de outubro de 2008, no decreto 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA), e foi aprovado pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA), da FACULDADE DE CIÊNCIAS AGRÁRIAS E VETERINÁRIAS, UNESP - CÂMPUS DE JABOTICABAL-SP, em reunião ordinária de 15 de junho de 2022.

Vigência do Projeto	01/07/2022 a 05/01/2024
Espécie / Linhagem	*Gallus gallus domesticus* / Ross AP 95
Nº de animais	2400
Peso / Idade	40g/ 1 dia
Sexo	Machos
Origem	Incubatório Pluma Agroavícola – Descalvado- SP

Jaboticabal, 15 de junho de 2022.

Fabiana Pilarski

Profª Drª Fabiana Pilarski
Coordenadora – CEUA

Faculdade de Ciências Agrárias e Veterinárias
Via de Acesso Prof. Paulo Donato Castellane, s/n CEP 14884-900 - Jaboticabal - SP - Brasil
Tel. 16 3209-7100 - www.fcav.unesp.br

EFEITO DO USO DE PROBIÓTICO, B-MANANASE E ANTIBIÓTICO SOBRE O DESEMPENHO E SAÚDE INTESTINAL DE FRANGOS DE CORTE DESAFIADOS POR *Eimeria máxima* E *Clostridium perfringens*

RESUMO - O uso de antibióticos na cadeia avícola tem sido associado à resistência bacteriana em humanos, levando à proibição ou redução da sua inclusão como promotor de crescimento nas dietas animais. Portanto, este estudo teve como objetivo avaliar os efeitos de probióticos e β-mananase sobre o desempenho de crescimento e saúde intestinal de frangos de corte desafiados por um modelo de *Eimeria maxima* associada a *Clostridium perfringens.* Foram utilizados 2.100 pintos machos de um dia de idade (Ross 308) distribuídos aleatoriamente em seis tratamentos com 10 repetições de 35 aves cada, totalizando 60 unidades experimentais. Os tratamentos foram: T1 – Controle negativo (CN) (sem desafio); T2 - Controle positivo (CP) (desafiado com *E. máxima* e *C. perfringens*); T3 – CP+Antibiótico; T4 - CP+β-mananase; T5 - CP+probiótico T6 - CP+β-mananase+probiótico. O protocolo de desafio consistiu na inoculação de *Eimeria maxima* ($7x10^3$ oocistos/mL) aos 14 dias de idade e inoculação de *Clostridium perfringens* ($2,5x10^6$ UFC) aos 20 dias. O desempenho produtivo na fase de 15 a 28 dias, mostra que houve redução no consumo de ração (CR) e no ganho de peso (GP), exceto no tratamento não desafiado ($P<0,05$). Aos 29-42d dias as variáveis (PV) peso vivo e CR, as aves do grupo CN apresentaram valores maiores em comparação às aves dos grupos CP e CP+probiótico ($P<0,05$) e a (CA) conversão alimentar das aves no tratamento CP+antibiótico foi menor em comparação aos grupos CN, CP e CP+probiótico. Diferenças significativas ($P<0,05$) foram observadas de 1 a 42 dias nas variáveis PC, GP e CR, o tratamento NC apresentou valores maiores em comparação aos grupos CP e CP+probiótico. A relação vilo:cripta no duodeno aumentou no tratamento CP+β-man+prob, diferindo do tratamento CN, CP e CP+probiótico ($P<0,05$). A relação *Firmicutes/Bacteroidota* aos 22 dias foi maior nos tratamentos que receberam probiótico ou β-man+prob, quando comparado às aves desafiadas sem qualquer suplementação ($P<0,05$). Nas aves da família *Lactobacillaceae* houve aumento na sua proporção no microbioma das aves que foram suplementadas com β-mananase ($P<0,05$). O gênero *Prevotellamassilia* foi mais abundante em aves não desafiadas, desafiadas sem adição de aditivos ou desafiadas recebendo β-mananase dietética em comparação com animais desafiados recebendo antibiótico dietético aos 22 dias de idade ($P<0,05$). O uso de β-mananase ou probiótico pode ser uma alternativa eficaz na saúde intestinal das aves, pois que gera modulação benéfica na microbiota intestinal e atenua os efeitos do desafio relacionado à melhoria do desempenho produtivo.

Palavras-chave: Aves criação, aves domésticas alimentação e rações, coccidiose, enterite, mucosa intestinal

EFFECT OF THE USE OF PROBIOTICS, B-MANANASE AND ANTIBIOTICS ON THE PERFORMANCE AND INTESTINAL HEALTH OF BROIL CHICKENS CHALLENGED BY *Eimeria maxima* AND *Clostridium perfringens*

ABSTRACT- The use of antibiotics in the poultry chain has been associated with bacterial resistance in humans, leading to the prohibition or reduction of their inclusion as growth promoters in animal diets. Therefore, this study aimed to evaluate the effects of probiotics and β-mannanase on the growth performance and intestinal health of broiler chickens challenged by a model of Eimeria maxima associated with Clostridium perfringens. 2,100 one-day-old male chicks (Ross 308) were used, randomly distributed in six treatments with 10 replications of 35 birds each, totaling 60 experimental units. The treatments were: T1 – Negative control (NC) (no challenge); T2 - Positive control (PC) (challenged with *E. maxima* and *C. perfringens*); T3 – PC+Antibiotic; T4 - PC+β-mannanase; T5 - PC+probiotic T6 - PC+β-mannanase+probiotic. The challenge protocol consisted of inoculation of *Eimeria maxima* ($7x10^3$ oocysts/mL) at 14 days of age and inoculation of *Clostridium perfringen*s ($2.5x10^6$ CFU) at 20 days of age. Productive performance in the 15 to 28-day phase shows that there was a reduction in feed intake (FI) and body weight gain (BWG), except in the unchallenged treatment ($P<0.05$). At 29-42d days, the variables (BW) live weight and FI, the birds in the CN group showed higher values compared to the birds in the PC and PC+probiotic groups ($P<0.05$) and the (CA) feed conversion of the birds in the PC+antibiotic treatment it was lower compared to the NC, PC and PC+probiotic groups. Significant differences ($P<0.05$) were observed from 1 to 42 days in the variables BW, BWG and FI, the NC treatment presented higher values compared to the PC and PC+probiotic groups. The villus:crypt ratio in the duodenum increased in the CP+β-man+prob treatment, differing from the NC, PC and PC+probiotic treatments ($P<0.05$). The *Firmicutes/Bacteroidota* ratio at 22 days was higher in treatments that received probiotic or β-man+prob, when compared to birds challenged without any supplementation ($P<0.05$). In birds of the *Lactobacillaceae* family, there was an increase in its proportion in the microbiome of birds that were supplemented with β-mannanase ($P<0.05$). The genus *Prevotellamassilia* was more abundant in unchallenged birds, challenged without added additives or challenged receiving dietary β-mannanase compared to animals challenged receiving dietary antibiotics at 22 days of age ($P<0.05$). The use of β-mannanase or probiotics can be an effective alternative for the intestinal health of birds, as they generate beneficial modulation in the intestinal microbiota and mitigate the effects of the challenge related to improving production performance.

Keywords: Coccidiosis, enteritis, intestinal mucosa, poultry breeding, poultry feed and feed

CAPÍTULO 1 – CONSIDERAÇÕES GERAIS

1.1. ***Introdução***

Segundo dados da ABPA (2022), o Brasil atualmente lidera o ranking de maior exportador de carne de frango e é o terceiro maior produtor mundial. Isso ocorre de forma significativa pois a avicultura Brasileira mostra-se como uma grande potência no desenvolvimento de tecnologias, nas áreas de nutrição, sanidade, ambiência e bem-estar animal. Entretanto, a alta intensificação na produção avícola desencadeou o desenvolvimento de processos patológicos, que são ocasionados pela imediata taxa de crescimento e pela alta densidade de criação. Esses aspectos levam a falhas nas respostas fisiológicas ou sistêmicas, tornando os animais vulneráveis a diferentes patógenos, acentuados, principalmente pela baixa resposta do sistema imunológico (Jaenisch et al*.,* 2001; Rubio, 2018).

Conforme a taxa de crescimento e a conversão alimentar melhoram, as aves se tornam mais exigentes, os cuidados com a nutrição e saúde (Choct et al., 1999). Logo, esses aspectos mencionados estão ligados com a saúde intestinal, onde o sistema imunológico está relacionado, assim como o equilíbrio e a integridade intestinal a níveis macro e microestrutural. A saúde do trato gastrointestinal atinge diretamente a digestão, absorção e metabolismo dos nutrientes, resposta imune e resistência a patógenos (Kelly & Conway; 2001, Yegani & Korver, 2008). As alterações desses processos são propícias a resultar em doenças entéricas (Dekich, 1998).

Um dos exemplos mais comuns de doenças entéricas é a infecção por coccidiose, doença parasitária causada pelo protozoário de gênero *Eimeria*, sendo um dos fatores que desencadeia a enterite necrótica, devido a lesões ocasionadas na mucosa intestinal, que são locais propícios para o desenvolvimento inicial de *Clostridium perfringens* (Ribas, 2020). O contato com a matriz extracelular, que atua como substrato para a bactéria, transborda o soro para o lúmen, que se torna um meio de cultura para o crescimento bacteriano, provocando a formação de muco rico em proteínas que fortalece a multiplicação de *C. perfringens* (Williams, 2005; Moore, 2016).

É relatado que a enterite ocorre em frangos de corte com idade entre duas e seis semanas (Songer, 1996; Cooper et al., 2010), apresentando os sinais clínicos, desidratação, diarreia, penas eriçadas e menor ingestão de alimento (anorexia)

(Songer, 1996; Oliveira, 2019). São descritas duas abordagens da doença, a clínica e subclínica (Kaldhusdal et al., 1992; Songer, 1996; Keyburn et al., 2010). A primeira forma aparece com os sinais citados acimas com alta mortalidade dos animais, já a subclínica apresenta um baixo desempenho, que caracteriza em redução da eficiência alimentar e crescimento do animal, mas sem levar a morte. Logo, esse estado da patogenia pode ser diagnosticado pelo aumento da conversão alimentar, através de danos macroscópicos no intestino delgado e pela comunidade bacteriana (Kaldhusdal et al., 1992). Mediante este aspecto, perdas econômicas estão alinhadas a enterite necrótica, relacionado a doença de forma subclínica, inclusive pontua-se também o alto custo que se demanda para combate à doença com antibióticos.

A crescente preocupação em substituir o uso de antibióticos como melhoradores de desempenho por substâncias alternativas, se dá pelo resultado da legislação que se estabeleceu na Europa em 2006 (Castanon, 2007). Assim, tem ocorrido a busca por alternativas aos antibióticos, para manter o desempenho e o equilíbrio da microbiota em frangos de corte (Chowdhury et al., 2009).

Nesse sentido, os probióticos têm apresentado efeitos positivos na saúde animal (Voung et al., 2016), além dos aditivos enzimáticos. Os efeitos benéficos dos probióticos no geral compreendem: preservar a microbiota intestinal por exclusão competitiva e antagonismo; modificando o metabolismo, elevando a atividade de enzimas digestivas e reduzindo a ação de enzimas bacterianas e geração de amônia; aumentar o consumo de ração e melhorar a digestão do alimento; além de e neutralizar as enterotoxinas (Jin et al., 1998a). As várias classes de probióticos como *C. butyricum*, tem expressado potencial para troca aos antibióticos, uma vez que promove a resposta imune, melhora a função da barreira intestinal e a digestão, tanto em modelo experimental em desafio sanitário (Zhang et al, 2016; Hayashi et al., 2018) como utilização na suplementação (Mountzouris et al., 2007; Wu et al., 2018; Manafi et al., 2018), corroborando quase sempre com melhorias no desempenho animal, em especial na conversão alimentar.

Uma alternativa que está sendo implantada nas dietas de frangos desafiados imunologicamente, é a enzima β-mannanase, que pode atuar sobre a reação imunológica dos animais pela redução da geração de imunoglobulinas (Li et al., 2010) e leucócitos (Mehri et al., 2010). Essa reação é em relação aos mananos encontrados

nos ingredientes, que são amplos estimuladores do sistema imune. A atenuação da resposta imunológica e a diminuição da viscosidade pelo aumento desta enzima, possibilita resultados superiores em relação ao desempenho, como o ganho de peso (Daskiran et al., 2004; Kim et al., 2003; Kong et al., 2011) e melhoria da conversão alimentar (Lee et al., 2003; Zou et al., 2006).

Desse modo, no intuito de alcançar resultados mais significativos na produção avícola, a utilização destes produtos na forma associada pode trazer efeitos favoráveis, pois se espera uma potencialização dos efeitos de suas atividades, maior impacto da atuação sobre os microrganismos e, portanto, uma melhora na produtividade dos frangos de corte.

1.2. ***Desafio sanitário na cadeia avícola***

O Brasil é visto mundialmente pela sua alta produção e crescimento da avicultura de corte, ocupando o terceiro lugar entre os países produtores de frango e o maior exportador de carne de frangos do mundo (ABPA, 2022). O segmento de produção do frango de corte é uma das maiores fontes de captação de recursos para o agronegócio brasileiro, portanto, o aperfeiçoamento de técnicas de manejo, melhoria no controle sanitário, ambiência das instalações, melhoramento genético, nutrição e alimentação, são características cruciais que são levadas em consideração para um maior desempenho das aves.

Entretanto a cadeia avícola atual sofre com desafios sanitários, causadores de impactos negativos na produtividade das aves, além de diversos prejuízos no setor. A enfermidade conhecida como coccidiose é um desafio rotineiro em frangos de corte, causada pelo protozoário de gênero *Eimeria spp.*, considerada uma das principais doenças da avicultura, ela é responsável por reduzir o ganho de peso, piorar a conversão alimentar, e em casos severos, alta mortalidade (Yin et al., 2011).

A multiplicação do parasita na célula epitelial do intestino, lesiona a parede do trato gastrointestinal, reduzindo a absorção de nutrientes (McDougald, 2008). De forma adicional, a ruptura de células epiteliais provocada pela *Eimeria* spp., desorganizam as junções oclusivas, pelo aumento da permeabilidade da barreira intestinal, facilitando a invasão de patógenos na circulação sistêmica (Santos et al., 2020).

Sete espécies de *Eimeria* spp. acometem a cadeia produtiva de frangos de corte, nas quais podem causar diferentes graus de severidade da doença (coccidiose). Do ponto de vista econômico as três principais são: *Eimeria tenella, Eimeria acervulina* e *Eimeria maxima* (Pirali-kheirabadi et al., 2008).

A contaminação por *Eimeria* spp. pode agir como fonte de novas infecções, principalmente pelo surgimento de uma microbiota com microrganismos não desejáveis, como bactérias e vírus que possuem características de atuação e lesões. Exemplificando, o aumento da produção de muco, é a primeira resposta do sistema imune inato a infecção por *Eimeria* spp., pela indução local das respostas de células T inflamatórias. Bactérias mucolíticas como *Clostridium perfringens*, utilizam do muco como substrato. Assim alta concentração desse muco, estimula a proliferação do *Clostridium perfringens,* agente causador de enterite necrótica (Collier et al., 2008).

1.3. ***Enterite necrótica***

A crescente preocupação pela substituição de antibióticos é atribuída pela legislação e pelos consumidores, que exigem pela criação de animais sem o uso de antibióticos melhoradores de desempenho. Diante do exposto percebe-se o aumento de doenças entéricas que impactam negativamente o desempenho de frangos de corte, entre elas um destaque para a enterite necrótica (EN) causada por cepas de *Clostridium perfringens* tipo G, cujo as perdas econômicas mundialmente chegam até US$ 6 bilhões por ano (Wade & Keyburn, 2015).

A preocupação se dirige principalmente à forma subclínica, pela falta de sintomas visíveis e, assim, falha no tratamento (Shojadoost et al., 2012). As Toxina α CPA e do tipo B (NetB) são produzidas pelas cepas do tipo G, causadoras da EN (Keyburn et al., 2008; Smyth & Martin., 2010). Está é uma doença complexa denominada como multifatorial, abrange não somente a toxina NetB, mas também fatores associados à virulência (Lepp et al., 2021).

A EN não é gerada somente pela existência da cepa patogênica do *Clostridium perfringens* (Flores-díaz et al*.* 2016; Moore, 2016). Eventualmente, a sua ocorrência está associada a infecções por coccídeos, com relevância para *Eimeria brunetti e Eimeria maxima*, agravando o quadro pela ocorrência de coccidiose (Cooper & Songer, 2009; Moore, 2016). *Eimeria* spp*.* em geral tem a capacidade de danificar a

mucosa intestinal, conduzindo para dentro do intestino o extravasamento do plasma sanguíneo (Moore, 2016). O trato gastrointestinal em resposta a inflamação pela coccidiose aumenta a produção de muco através das células caliciformes que estão ali distribuídas na mucosa (Ohland & Macnaughton, 2010). Esse muco que está em conjunto com o plasma extravasado, contribui como substrato para o *Clostridium perfringens*, potencializando sua proliferação (Silva et al., 2015).

Outros motivos podem propiciar de forma individual ou associada a incidência da EN, seja para desencadear quadros subclínicos, quanto clínicos (M'sadeq et al., 2015; Moore, 2016). Dentro da nutrição animal, fatores como a utilização de cereais com níveis altos de polissacarídeos solúveis não amiláceos (Annett et al., 2002; Timbermont et al., 2011; Barekatain et al., 2013; Latorre et al., 2015) e inclusão de farinha de peixe, implicando no uso de proteína animal, predispõem sua ocorrência (Drew et al., 2004; Fernandes da Costa et al., 2013; Stanley et al., 2014). Inclui-se também fatores como: condições fisiológicas de pH gástrico e intestinal das aves; aviários contaminados; estresse animal, ligado a troca de dieta e ocorrência de doenças (Allaart, et al. 2013).

Esses motivos juntamente com a coinfecção entérica por agentes como *Eimeria spp.* ampliam as possibilidades de surgimento da enterite necrótica (Wu et al., 2014; Zhou et al., 2017), promovendo condições propicias a multiplicação de oocistos de *Eimeria* e *Clostridium perfringens*, logo após a sua fixação na mucosa do intestino, levando a necrose deste tecido (Rehman et al. 2006; Prescott et al. 2016; Józefiak et al. 2016).

1.4. ***Microbiologia do trato gastrointestinal***

Oliveira et al. (2019) relataram que um bom desempenho zootécnico tem relação direta com a qualidade da saúde intestinal das aves. É visto que, a dinâmica de barreira da mucosa do intestino delgado é usada como linha inicial de defesa do corpo e essencialmente, por sua vez, pode manter uma barreira à invasão microbiana, conferindo proteção as células do tecido epitelial do intestino (Elphick et al., 2005).

Logo, o equilíbrio dinâmico entre o conteúdo luminal e a mucosa intestinal, expressa o que define por saúde intestinal, nas quais estas características funcionais e estruturais da mucosa devem ser mantidas ou preservadas (Ito et al., 2007).

Conforme Oviedo-Rondón (2019), a saúde intestinal deve existir para manter a eficiência da fisiologia do trato gastrointestinal, isto é, funções digestivas, metabólicas, absortivas, imunológicas e endócrinas do organismo animal.

A microbiologia do trato gastroentérico, é um sistema que por sua vez possui uma microbiota bacteriana normal e agentes normais, na qual a sua composição pode ser alterada pela dieta, melhoradores de crescimento, anticoccidianos ou vacinas coccidiostáticas (Prada, 2011). A microbiota do intestino possui papel fundamental na regulação da eficiência de absorção, resposta do sistema imune, tempo de retenção da digesta, maturação intestinal e uso de nutrientes não digeridos totalmente pelas enzimas endógenas das aves (Amit-Romach et al., 2004). De acordo com Macari et al. (2014), no intestino as bactérias que habitam esse meio dependem dos componentes da dieta, principalmente aqueles nutrientes resistentes ao processo digestivo ou mal absorvidos, para obtenção de energia para o crescimento das mesmas.

No intestino como diversas populações bacterianas coexistem e possuem preferencias por substrato, as mudanças na dieta irão modificar a diversidade, distribuição e composição dos microorganismos no intestino das aves (Apajalahti et al., 2004). Visto que esses microorganismos e antígenos alimentares estão em constate contato com a mucosa intestinal, uma população de bactérias estável pode impedir a proliferação de patógenos e fortalecer as defesas imunológicas (Song et al., 2012).

A taxonômia da microbiota é influenciada por diversos fatores, como os órgãos, a idade do animal, a dieta e o uso de antimicrobianos na ração. Tipos distintos de aditivos regulam a comunidade microbiana na alimentação, incluem-se probióticos e prebióticos (Clavijo et al., 2018)

*1.5. **Aditivos alternativos***

O uso de aditivos nas dietas tem sido uma das aplicações mais utilizadas para preservar as funções fisiológicas e a digestibilidade dos nutrientes das aves (Souza et al., 2020). Determinados nutrientes da dieta são capazes de modificar, de maneira benéfica, com renovação da mucosa intestinal, levando a melhoria da absorção e digestão dos nutrientes pelos animais (Imperatori, 2018).

A microbiota benéfica, atua inibindo o crescimento de bactérias indesejáveis, estimulando a formação de ácidos graxos voláteis, como o ácido lático principalmente, produzido em grande escala por lactobactérias como o *Lactobacillus acidophillus* e *Lactobacillus latis* (Prada, 2011).

Esses elementos são conhecidos como agentes tróficos, nos quais estimulam o processo mitótico, e ampliam a quantidade de células e o tamanho dos vilos (Maiorka et al., 2008). Algumas fontes que possuem essa ação sobre a mucosa intestinal, são elas: ácidos graxos de cadeia curta, prebióticos, probióticos, aminas biogênicas, aditivos fitogênicos e o aminoácido glutamina (Furlan et al., 2004; Viola et al., 2008; Zavarize et al., 2010; Lemos et al., 2016). O modo de atuação da maioria dos agentes é através de mecanismos que induzem a transcrição gênica, onde ocorre ativação de enzimas relevantes no movimento mitótico na região cripta-vilo. Outros possuem atividade não direta, pois contribuem com os meios de manutenção da integridade epitelial por conceder maior saúde para mucosa, por meio de métodos (exclusão competitiva) que proporcionam o equilíbrio da microbiota intestinal (Furlan et al., 2004).

Dessa maneira a aplicação de produtos alternativos como moduladores de desempenho nas rações é um meio nutricional para fortalecer a produtividade e suavizar os custos da produção de aves para corte, visto que a nutrição se caracteriza como um dos principais fatores que impactam diretamente os custos finais de produção, totalizando em aproximadamente 70% dos gastos, o que onera a produção (Gewehr et al., 2014).

1.5.1. Antibióticos

O emprego de antibióticos como aditivos melhoradores de desempenho na avicultura tem sido bastante contestado atualmente (Machado et al., 2007). Todavia o antibiótico Enramicina é permitido pela legislação nacional para uso como antimicrobianos melhoradores de desempenho em suínos e aves no mercado interno desde que o produto esteja registrado corretamente no Ministério da agricultura, pecuária e abastecimento, e seja respeitado seu modo de administração, dosagem e período de carência (MAPA, 2018). A Enramicina é um polipeptídico produzido por *Streptomyces fungicidus*, a qual é amplamente utilizada na alimentação de aves

e suínos. Ele age no impedimento de organismos causadores de infecções subclínicas (*Clostridium perfringerns*) e diminui inflamações no epitélio intestinal (Brock et al*.,* 1994). Este quando utilizado em doses subterapêuticas na alimentação animal, promove melhora na conversão alimentar, ganho de peso e redução da mortalidade do lote (Iafigliola et al., 2000).

Porém antibióticos trazem desvantagens na administração contra bactérias patogênicas, apresentando poder antibacteriano não seletivo que se ampliam além do patógeno de interesse. Sendo assim, são capazes de desequilibrar a microbiota intestinal, contribuindo para a seleção de microrganismos resistentes. Dessa maneira, ocorre uma proliferação de microrganismos resistentes a esses medicamentos, classificados como não desejáveis, podendo ocorrer o aumento desses invasores na microbiota intestinal (Allaart et al. 2013).

Por conta da restrição do uso de antibióticos na nutrição animal e no impacto que eles trazem sob o desempenho produtivo das aves, torna-se indispensável o conhecimento e utilização de produtos alternativos, que mantenham o desempenho ocasionado pelo tratamento com antibióticos (Lourençon et al., 2007).

1.5.2. Probióticos

A definição de probiótico vem sendo discutida a algum tempo, devido ao seu amplo uso na cadeia animal. Atualmente a definição utilizada mais correta, seria “microrganismos vivos que, quando administrados em quantidades adequadas, conferem um benefício à saúde do hospedeiro” (FAO, 2002).

Nos últimos anos a discussão sobre o não uso de antibióticos vem sendo abordada, entretanto estudos provam os resultados positivos, entre eles o uso de probióticos melhoradores de desempenho. Em dose subterapêutica quimioterápicos são utilizados para melhora do desempenho, colaborando com a prevenção de doenças das aves principalmente as entéricas. Seu uso se mostra eficaz, em razão de suas vantagens para os animais, tanto fisiológicas, nutricionais e metabólicas (Anderson et al., 1999; Gaskins et al., 2002; Applegate et al., 2010), além de ter influência sobre as atividades corporais, visando melhora da saúde animal, ampliando seus resultados a níveis fisiológicos (Al-khalaifah et al., 2018). Dessa maneira, a utilização de aditivos alimentares se faz uma alternativa para redução desses efeitos negativos.

Estudos com o uso de probiótico na alimentação animal trazem efeitos benéficos na saúde dos animais e melhora do ganho de peso das aves (Silva & Pinheiro, 2008; Abdel-hafeez et al., 2017). Esse se mostra eficaz em não deixar resíduos nos produtos de origem animal e não favorecem a resistência a antimicrobianos (Kołożyn-krajewska & Dolatowski, 2012; Roberfroid, 2000; Vohra et al., 2016).

Os meios de ação dos probióticos não são bem elucidados ainda, pelo fato da sua vasta atividade com o lúmen intestinal (Machado, et al., 2019). Contudo, são relatados na literatura possíveis mecanismos, os quais incluem a competição por sítios de ligações, onde os probióticos aderem-se a parede do epitélio intestinal, desfavorecendo a aderência de microrganismos patogênicos (Roth & Kirchgessner, 1998).

Os probióticos tem ação direta sob bactérias patogênicas como *Clostridium perfringens* e *Salmonella*, inibindo as mesmas de habitarem o intestino por meio do método de exclusão competitiva (Teo & Tan, 2006; Abudabos et al., 2013). Em um estudo usando diferentes doses de compostos probióticos apresentaram mudanças nas respostas de parâmetros de desempenho e carcaça, demonstrando que possivelmente a concentração ideal de um probiótico na dieta de frangos de corte muda conforme o microrganismo usado na constituição do produto (Pourakbari et al., 2016).

A via de aplicação não é o ponto determinante, o que se leva em consideração principalmente para uma boa aplicabilidade do produto é o tempo de aplicação, que deve ser feito o mais rápido possível, a fim de que as bactérias benéficas habitem o trato gastrintestinal do hospedeiro, previamente a atuação dos patógenos (Silva & Pinheiro, 2008). Pesquisas têm demonstrado vantagens em relação a utilização de probióticos na dieta animal, incluindo na criação de frangos de corte, onde observa-se melhora na eficácia alimentar e ganho de peso nas aves de corte (Barbosa et al., 2011; Foley et al., 2011).

1.5.3. β-mananase

Os β-mananos, são conhecidos também como β-galactomananos e estão presentes nos ingredientes das dietas de frangos de corte (Hsiao et al., 2006). A

principal fonte proteica é o farelo de soja, usado pela maioria dos países como ingrediente principal das rações, e que por sua vez possui quantidade significativa de β-mananos (Stein et al., 2008). Estes estão ligados a efeitos antinutricionais devido suas propriedades, aumentando a viscosidade da digesta, ocasionando piora na conversão alimentar das aves (Reid, 1985).

A enzima Endo-β-1,4-mananase tem se mostrado primordial para a despolimerização de mananos, galactomananos e galacto-glicomananos. Ela age catalisando, através da hidrolise aleatória de ligações β-1,4-manano, na cadeia principal de polímeros de mananos (Stalbrand et al, 1993; De vries & Visser, 2001), no qual quebra os B-mananos e assim reduz a viscosidade da digesta no lúmen intestinal, obtendo melhora da digestibilidade de nutrientes e maior aporte para o desempenho animal. A utilização da β-mananase degenera os mananos, causando redução da carga sobre o sistema imunológico, consequentemente levado há uma maior reserva da energia metabolizável (Jackson et al., 2003). Daskiran et al. (2004) confirmou que β-mananase beneficia a conversão alimentar e reduz o consumo de ingestão de água por ração ingerida em frangos de corte.

O incremento da enzima nas rações para frangos de corte favorece a redução da quantidade de células caliciformes em toda parte do intestino delgado, ocasionando a produção de uma menor quantia de muco (MherI et al., 2010). Logo, essa estratégia nutricional contribui com a economia na produção, reduzindo a inclusão de alimentos energéticos, permitido pela utilização da enzima para agregar custo-benefício na formulação de dietas, obtendo níveis adequados de energia, sem comprometer o desempenho do animal (Li et al., 2010).

Desse modo, o intuito principal de avaliar os aditivos disponíveis para substituição de antibióticos melhoradores de desempenho é pensando nos benefícios que eles trazem à produção de frango de corte, contribuindo para melhoria do desempenho animal, permitindo melhores valores de conversão alimentar, frente às patogenias, como a enterite necrótica, encontradas na avicultura atual.

1.6. Referências bibliográficas

Abdel-hafeez, H. M.; Hassan, M.; Elham, S. E. S.; Samar, S. T.; Ibrahim, M. I. Y.; Asmaa, S. A. Effects of probiotic, prebiotic, and symbiotic with and without feed restriction on performance, hematological indices, and carcass characteristics of broiler chickens. **Asian-Australasian Journal of Animal Sciences**, v. 30, n. 5, p. 672-682, 2017.

Abudabos, Alaeldein. Alyemni. Abdullah H, Marshad B.A. AL Marshad. *Bacillus subtilis* PB6 based probiotic (CloSTATTM) improves intestinal morphological and microbiological status of broiler chickens under *Clostridium Perfringens* challenge**. International Journal of Agriculture and Biology**. 15(6):978-982, 2013.

Allaart, Janneke G.; Van Asten, Alphons Jam; Gröne, Andrea. Predisposing factors and prevention of *Clostridium perfringens*-associated enteritis. **Comparative immunology, microbiology and infectious diseases**, v. 36, n. 5, p. 449-464, 2013.

Al-khalaifah H. S. Benefits of probiotics and/or prebiotics for antibiotic-reduced poultry. **Poultry science**, 97(11): 3807-3815, 2018.

Amit-Romach, E., Sklan, D., & Uni, Z. (2004). Microflora ecology of the chicken intestine using 16S ribosomal DNA primers. ***Poultry science***, *83*(7), 1093–1098. https://doi.org/10.1093/ps/83.7.1093

Anderson, D. B, McCracken VJ, Aminov RI, Simpson JM, Mackie RI, Vestegen MWA, Gaskins HR. Gut microbiology and growth-promoting antibiotics in swine. **Pig News Information**, 20:115N–122N, 1999.

Annett, C. B, Viste, JR, Chirino-Trejo, M., Classen, HL, Middleton, DM, & Simko, E. Enterite necrótica: efeito de dietas de cevada, trigo e milho na proliferação de *Clostridium perfringens* tipo A. **Avian Pathology**, *31* (6), 598-601, 2002.

ANUAL, ABPA Relatório. Associação Brasileira de Proteína Animal, 2022.

Applegate, T. J, Klose V, Steiner T, Ganner A, Schatzmayr G. Probiotics and phytogenics for poultry: Myth or reality? **The Journal of Applied Poultry Research**, 19(2):194–210, 2010.

Arabkhazaeli F, Nabian S, Modirsaneii M, MansoorI B, Rahbari S. Biopathologic Characterization of Three Mixed Poultry *Eimeria* spp. Isolates. **Iranian Journal of Parasitology** 6(4) 23- 32, 2011.

Barbosa, F. H. F., Barbosa, L. P. J. L., Bambirra, L. H. S. & Aburjaile, F. F. Probióticos microrganismos a favor da vida. **Revista de Biologia e Ciências da Terra**, 11(1):11–21, 2011.

Barekatain, M. R, Antipatis, C., Choct, M., & IJI, PA. Interação entre protease e xilanase em dietas de frangos de corte contendo grãos secos de destilaria de sorgo com solúveis. **Animal Feed Science and Technology**, *182* (1-4), 71-81, 2013.

Barker, K. J., C.D. Coufal, J. L. Purswell, J. D. Davis, H. M. Parker, M. T. Kidd, C. D. McDaniel, A. S. Kiess. In-house windrowing of a commercial broiler farm during early

spring and its effect on litter composition. **Journal of Applied Poultry Research**, Volume 22, Issue 3, Pages 551-558, 2013.

Brock, T. D.; Madigan, M. T.; Martinko, J. M.; Parker, J. **Biology of microoganisms**. 7a ed, Prendice-Hall, New Jersey, 1994. 909p, 1994.

Castanon J. I. R. History of the use of antibiotic as growth promoters in European poultry feeds. **Poultry science**, 86(11):2466-2471, 2007.

Choct, Mingan; Hughes, Robert J.; Bedford, MR. Efeitos de uma xilanase na variação individual de aves, digestão de amido ao longo do intestino e produção de ácidos graxos voláteis ileal e cecal em galinhas alimentadas com trigo. **British Poultry Science**, v. 40, n. 3, pág. 419-422, 1999.

Chowdhury R, Islam K, Khan M, Karim M, Haque M, Khatun M. Efeito do ácido cítrico, avilamicina e sua combinação no desempenho, cinzas da tíbia e estado imunológico de frangos de corte. **Poultry Science**. 88(8):1616–22, 2009.

Clavijo, Viviana., Flórez, Martha. The gastrointestinal microbiome and its association with the control of pathogens in broiler chicken production: A review, **Poultry Science**, Volume 97, Issue 3, 2018, Pages 1006-1021, ISSN 0032-5791, https://doi.org/10.3382/ps/pex359.

Collier, C. T., Hofacre, C. L., Payne, A. M., Anderson, D. B., Kaiser, P., Mackie, R. I., & Gaskins, H. R. (2008). Coccidia-induced mucogenesis promotes the onset of necrotic enteritis by supporting *Clostridium perfringens* growth. **Veterinary immunology and immunopathology**, 122(1-2), 104-115. https://doi.org/10.1016/j.vetimm.2007.10.014.

Cooper, K. K.; Songer, J. G. Necrotic enteritis in chickens: a paradigm of enteric infection by *Clostridium perfringens* type A. **Anaerobe**, v. 15, n. 1-2, p. 55-60, 2009.

Cooper K. K, Songer. J. G: Virulence of Clostridium perfringens in an experimental model of poultry necrotic enteritis. **Vet Microbiol**, 142:323–328, 2010.

Daskiran, M.; Teeter, R. G.; Fodge, D.; Hsiao, H. Y. An evaluation of endo-β-mannanase (Hemicell) effects on broiler performance and energy use in diets carrying in β-mannan content. **Poultry Science**, v.83, p.662-668, 2004.

Dekich, M. A. Broiler industry strategies for control of respiratory and enteric diseases. **Poultry science**, v. 77, n. 8, p. 1176-1180, 1998.

De Vries, R. P.; Visser, Jaap. Enzimas de *Aspergillus* envolvidas na degradação de polissacarídeos da parede celular vegetal. **Revisões de Microbiologia e Biologia Molecular**, v. 65, n. 4, pág. 497-522, 2001.

Drew, M. D., Syed, N. A., Goldade, B. G., Laarveld, B. And Van Kessel, A. G. Effects of dietary protein source and level on intestinal populations of *Clostridium perfringens* in broiler chickens. **Poultry Science**, 83:414-420, 2004.

Elanco, Animal Heath. Guia da referência de doenças de frangos de corte, 2010.

Elphick D, Mahida Y. Paneth cells: their role in innate immunity and inflammatory disease. **Gut**. 54(12):1802–9, 2005.

Fernandes Da Costa, S. P., Mot, D., Bokori-brown, M., Savva, C.G., Basak, A.K., Van immerseel, F. And Titball, R.W. Protection against avian necrotic enteritis after immunisation with NetB genetic or formaldehyde toxoids. **Vaccine**, 31:4003-4008, 2013.

Flores-díaz, M., Monturiol-gross, L., Naylor, C., Alape-girón, A., & Flieger, A. Bacterial sphingomyelinases, and phospholipases as virulence factors. **Microbiology and Molecular Biology Reviews**, *80*(3), 597-628, 2016.

Foley, S. L., Nayak, R., Hanning, I. B., Johnson, T. J., Han, J. & Ricke, S. C. Population dynamics of *Salmonella enterica* serotypes in commercial egg and poultry production. **Applied and Environmental Microbiology**, 77(13):4273-4279, 2011.

Furlan, R. L., Macari, M., & LuquettI, B. C. Como avaliar os efeitos do uso de prebióticos, probióticos e flora de exclusão competitiva. In: 5° Simpósio Técnico de Incubação, Matrizes de Corte e Nutrição, 04, 05 a 06 de outubro de 2004. **Anais**... Balneário Camboriú-SC, 06-28, 2004.

Gaskins H.R, Collier CT, Anderson DB. Antibiotics as growth promotants: mode of action, **Animal Biotechnology**, 13(1):29-42, 2002.

Gewehr, C. E.; Rosniecek, M.; Follmann, D. D.; Cezaro, A. M.; Gerber, M. S.; Schneider, A. F. Complexo multienzimático e probióticos na dieta de frangos de corte. **Revista Brasileira de Saúde e Produção Animal**, Salvador, v.15, n.4, p.907-916, 2014.

Gordon H.M.L. E Whitlock H.N. A new technique for counting nematode egg in sheep faeces. **Journal of Scientific Industry Research**. Austrália n.12(1), p.50-52, 1939.

Hayashi, R. M, Lourenço, M. C, Kraieski, A. L, Araujo, R. B, Gonzalez-Esquerra, R., Leonardecz, E. Da Cunha. AF, Carazzolle M. F, Monzani PS and Santin E. Effect of Feeding *Bacillus subtilis* Spores to Broilers Challenged with *Salmonella* enterica serovar Heidelberg Brazilian Strain UFPR1 on Performance, Immune Response, and Gut Health. **Frontiers in Veterinary Science**, 5:1-13, 2018.

Hsiao, H.-Y.; Anderson, D. M.; Dale, N.M. Levels of β-mannan in soybean meal. **Poultry Science**, v. 85, p. 1430-1432, 2006.

Iafigliola, M. C. Menten, J.F.M., A.M.C RacaniccI, J.B Gaiotto. Cobre e Antibiótico como Promotores de Crescimento em Rações para Frangos de Corte**. Brazilian Journal of Poultry Science**. 2000, v. 2, n. 3, pp. 201-208, 2000.

Imperatori, F. **Efeito da adição de butirato de sódio microencapsulado sobre o desempenho produtivo e a regeneração da mucosa intestinal de frangos de corte submetidos a um desafio experimental**. (83p.) Marechal Cândido Rondon-PR: Universidade Estadual do Oeste do Paraná, 2018.

Ito, N. M. K., Miyaji, C. I., & Okabayashi, S. M. Saúde intestinal em frangos de corte. **Circular Técnica** Aviagen Brasil, 2007.

Jackson, M. E., Anderson, D. M., Hsiao, H. Y., Mathis, G. F., & Fodge, D. W. Beneficial effect of β-mannanase feed enzyme on performance of chicks challenged with *Eimeria* sp. and Clostridium perfringens. **Avian Diseases**, Kenett Square, v. 47, n. 1, p. 759-763, 2003.

Jaenisch, F. R. F, ÁVILA V. S, MAZZUCO H. Síndrome da Hipertensão Pulmonar: a Ascite em Frangos de Corte. Concórdia: **Embrapa Suínos e Aves**, 16 p, 2001.

Jin, L. Z., Ho, Y. W., Abdullah, N., Ali, M. A. & S. Jalaludin. Effects of adherent *Lactobacillus* cultures on growth, weight of organs and intestinal microflora and volatile fatty acids in broilers. **Animal Feed Science & Technology**, 70, 197–209, 1998a.

Józefiak D, Świątkiewicz S, Kierończyk B, Rawski M, Długosz J, Engberg RM And Højberg O. *Clostridium perfringens* challenge and dietary fat type modifies performance, microbiota composition and histomorphology of the broiler chicken gastrointestinal tract. **Archiv Fuer Gefluegelkunde**, 80:1-14, 2016.

Kaldhusdal M, Hofshagen M: Barley inclusion and avoparcin supplementation in broiler diets. 2. Clinical, pathological, and bacteriological findings in a mild form of necrotic enteritis. **Poultry Science**, 71:1145–1153, 1992.

Kasab-bachia H., Arruda, A. G., Roberts, T. E., & Wilson, J. B. The use of large databases to inform the development of an intestinal scoring system for the poultry industry. **Preventive Veterinary Medicine**, 146, pp.130–135, 2017.

Kelly, D.; Conway, S. Genomics at work: the global gene response to enteric bacteria. **Gut**, v. 49, n. 5, p. 612-613, 2001.

Keyburn A. L, Bannam T. L, Moore R. J, Rood J. I: NetB, a pore forming toxin from necrotic enteritis strains of *Clostridium perfringens*. **Toxins**, 2:1913–1927, 2010.

Keyburn, Anthony L, Boyce, J. D, Vaz, P., Bannam, T. L, Ford, M. E, Parker, D., ... & MOORE, R. J. NetB, uma nova toxina que está associada à enterite necrótica aviária causada por Clostridium perfringens. **Patógenos PLoS**, v. 4, n. 2, pág. e26, 2008.

Kim, S. W.; Knabe, D. A.; Hong, K. J.; Easter, R. A. Use of carbohydrases in cornsoybean meal-based nursey diets. **Journal Animal Science**, v.81, p.2496-2504, 2003.

Kołożyn-krajewska, D.; Dolatowski, Z. J. Probiotic meat products and human nutrition. **Process Biochemistry**, 47(12):1761-1772, 2012.

Kong, C.; Lee, J. H.; Adeola, O. Supplementation of B-mannanase to starter and grower diets for broilers. **Canadian Journal of Animal Science**, v.91, p.389-397, 2011.

Latorre, J. D., Hernandez-Velasco, X., Bielke, L. R., Vicente, J. L., Wolfenden, R., Menconi, A., ... & Tellez, G. Evaluation of a *Bacillus* direct-fed microbial candidate on digesta viscosity, bacterial translocation, microbiota composition and bone

mineralisation in broiler chickens fed on a rye-based diet. **British Poultry Science**, v. 56, n. 6, p. 723-732, 2015.

Lee, J. T; Bailey, C. A.; Cartwright, A. L. β-mannanase ameliorates viscosity associated depression of growth in broiler chickens fed guar germ and hull fractions. **Poultry Science**, v.82, p.1925-1931, 2003.

Lemos, M. J., Calixto, L. F. L., Torres-cordido, K. A. A., & Reis, T. L. Uso de aditivo alimentar equilibrador da flora intestinal em aves de corte e de postura. **Arquivos do Instituto Biológico**, 83, 1-7, 2016.

Lepp, D., Zhou, Y., Ojha, S., Mehdizadeh Gohari, I., Carere, J., Yang, C., Prescott, J. F., & Gong, J. *Clostridium perfringens* Produces an Adhesive Pilus Required for the Pathogenesis of Necrotic Enteritis in Poultry. **Journal of bacteriology**, *203*(7), e00578-20, 2021.

Li, Y.; Chen, X.; Chen, Y, Li, Z.; Cao, Y. Effects β-mannanase expressed by Pichia pastoris in corn-soybean meal diets on broiler performance, nutrient digestibility, energy utilization and immunoglobulin levels. **Animal Feed Science and Technology**, v.159, p.59-67, 2010.

Lourençon, L., Vianna Nunes, Ricardo; Pozza, Paulo Cesar; Dos Santos Pozza, Magali Soares; Djalma Appelt, Mathias; Mozer da Silva, Wagner Thiago. Utilização de promotores de crescimento para frangos de corte em rações fareladas e peletizadas. **Acta Science Animal**. Maringá, v. 29, n. 2, p. 151- 158, 2007.

Machado, A. M. B., Dias E. S., Santos É. C. S. & Freitas R. T. F. Composto exaurido do cogumelo *Agaricus blazei* na dieta de frangos de corte. **Revista Brasileira de Zootecnia**. 36:1113-1118, 2007.

Machado, Noédson de Jesus Beltrão; Lima, Cristina Amorim Ribeiro de; Brasil, Ronner Joaquim Mendonça; Ramos, Juliano Carneiro; Santarém, Luis Eduardo Sá; Macêdo, Valéria Carneiro; REIS, Anna Cassia da Silva. New perspectives about the use of probiotics in diets to broilers. **Rev. Cient. Avic. Suin.,** v. 5, n. 2, p. 063-076, 2019.

Maiorka, A., Boleli, I. C., & Macari, M. Desenvolvimento e reparo da mucosa intestinal. In: Macari, M., Furlan, R. L., & Gonzales, E. **Fisiologia aviária aplicada a frangos de corte**. (pp. 113-123). Jaboticabal-SP: FUNEP/UNESP, 2008.

Manafi M, Hedayati M AND MIRZAIE S. Probiotic Bacillus species and *Saccharomyces boulardii* improve performance, gut histology, and immunity in broiler chickens. South African Journal of Animal Science, 48(2):379-389. Marshall BM and Levy SB (2011). Food animals and antimicrobials: impacts on human health. **Clinical microbiology reviews**, 24(4):718-733, 2018.

MAPA, Ministério da Agricultura, Pecuária e Abastecimento. Portaria nº 20, de 26 de julho de 2018, 2018.

McDougald, L. R.; FITZ-COY, S. H. Coccidiosis. Diseases Of Poultry. **Blackwell Publishing**, p. 1068 – 1085, 2008.

Mehri, M.; Adibmoradi, M.; Samie, A.; SHI. β- mannanase on broiler performace, gut morphology and immune system. **African Journal of Biotechnology**, v.9, p.6221-6228, 2010.

Moore, Robert J. Fatores predisponentes da enterite necrótica em frangos de corte. **Patologia Aviária**, v. 45, n. 3, pág. 275-281, 2016.

Mountzouris KC, Tsirtsikos P, Kalamara E, Nitsch S, Schatzmayr G And Fegeros K. Evaluation of the efficacy of a probiotic containing *Lactobacillus, Bifidobacterium, Enterococcus,* and *Pediococcus* strains in promoting broiler performance and modulating cecal microflora composition and metabolic activities. **Poultry science**, 86(2):309-317, 2007.

M'Sadeq, S. A., Wu, S., Swick, R. A., & Choct, M. Towards the control of necrotic enteritis in broiler chickens with in-feed antibiotics phasing-out worldwide. **Animal Nutrition**, v. 1, n. 1, pág. 1-11, 2015.

Ohland, Christina L.; Macnaughton, Wallace K. Probiotic bacteria and intestinal epithelial barrier function. **American journal of physiology-gastrointestinal and liver physiology**, v. 298, n. 6, p. G807-G819, 2010.

Oliveira, Marllon José Karpeggiane. **Avaliação de probiótico comercial como alternativa aos antibióticos promotores de crescimento, utilizando um modelo de desafio sanitário em frangos de corte**. (Dissertação). Repositório Unesp, 2019.

Oviedo-rondón, E. O. Holistic view of intestinal health in poultry. **Animal Feed Science and Technology**, 250, 1-8, 2019.

Pirali-kheirabadi, K., Zamani-Moghadam, A., Abdi, F., & Bahonar, AR. O efeito da administração de drogas anticoccidianas na eliminação de oocistos e desempenho na coccidiose experimental em frangos de corte. **Int J Vet Res**, v. 2, p. 67-73, 2008.

Pourakbari, Mohammadreza, & Seidavi, Alireza & Asadpour, Leila & Martínez MARÍN, Andrés. Probiotic level effects on growth performance, carcass traits, blood parameters, cecal microbiota, and immune response of broilers. **Anais da Academia Brasileira de Ciências**. 88. 10.1590/0001-3765201620150071, 2016.

Prada luengas, Jorge armando. **Suplementação de probióticos em dietas de frangos de corte: desempenho e digestibilidade da proteína**. Dissertação - Viçosa, MG- 2011.

Prescott JF, Parreira VR, Mehdizadeh Gohari I, Lepp D And Gong J. The pathogenesis of necrotic enteritis in chickens: what we know and what we need to know: a review. **Avian Pathology**, 45(3):288-294, 2016.

Rehman H, Awad WA, Lindner I, Hess M, Zentek J. *Clostridium perfringens* alpha toxin affects electrophysiological properties of isolated jejunal mucosa of laying hens. **Poultry Science**, 85:1298–302, 2006.

Reid, J. S. G. “Galactomannans”, em “Biochemistry of storage carbohydrate in green plants” (Dey, P.M. & R.A. Dixon, eds.) **Academic Press**, London, Cap.7, págs. 265-88, 1985.

Ribas, C. B. **Estudo das alterações histopatológicas e ultraestruturais do intestino delgado de frangos infectados experimentalmente com *Eimeria spp. e Clostridium perfringens* para desenvolvimento de enterite necrótica**. 2020. Dissertação, (Universidad de São Paulo, São Paulo, 2020.

Roberfroid, M. B. Prebiotics and probiotics: are they functional foods? **The American Journal of Clinical Nutrition**, 71(6):1682s-1687s, 2000.

Rostagno, H. S.; Albino, L. F. H.; Hannas, M. I.; Donzele, J. L.; Sakomura, N. K.; Perazzo, F. G.; Saravia, A.; De Abreu, M. L. T.; Rodrigues, P. B.; De Oliveira, R. F.; Barreto, S. L. T. E Brito, C. D. Tabelas Brasileiras para aves e suínos: Composição de Alimentos e exigências nutricionais. Editora UFV, Viçosa, MG, 2017.

Roth, F.X.; Kirchgessner, M. Organic acids as feed additives for young pigs: nutritional and gastrintestinal effects**. Journal of Animal and Feed Science**, v. 7, n. 8, p. 25-33, 1998.

Rubio, L. A. Possibilities of early life programming in broiler chickens via intestinal microbiota modulation. **Poultry science**, p. 416, 2018.

Sakomura, N. K., Rostagno, H. S. Métodos de pesquisa em nutrição de monogástricos. Jaboticabal: Funep, p.262, 2016.

Santos, T. S. D., Teng, P. Y., Yadav, S., Castro, F. L. D. S., Gould, R. L., Craig, S. W., ... & Kim, W. K. (2020). Effects of Inorganic Zn and Cu Supplementation on Gut Health in Broiler Chickens Challenged With Eimeria spp. **Frontiers in Veterinary Science**, 7, 230. https://doi.org/10.3389/fvets.2020.00230.

Shawkat A. M'Sadeq, Shubiao Wu, Robert A. Swick, Mingan Choct, Towards the control of necrotic enteritis in broiler chickens with in-feed antibiotics phasing-out worldwide, **Animal Nutrition**, Volume 1, Issue 1, Pages 1-11, 2015.

Shojadoost, B., Vince, A.R. & Prescott, J.F. The successful experimental induction of necrotic enteritis in chickens by *Clostridium perfringens*: a critical review. **Veterinary Research,** 43**,** 74, 2012.

Silva, C. R.; Pinheiro, A. L. B. C. Utilização de probióticos como melhoradores de desempenho em aves. **Revista Eletrônica Nutritime**, 5(6):690-706, 2008.

Silva, Rodrigo Otávio Silveira; Oliveira Junior, Carlos Augusto; Guedes, Roberto Maurício Carvalho e Lobato, Francisco Carlos Faria. *Clostridium perfringens*: uma revisão da doença em suínos, cavalos e frangos de corte. **Ciencia Rural**, Santa Maria, v. 45, n. 6, pág. 1027-1034, 2015.

Smyth, Joan A.; Martin, Thomas G. Capacidade de produzir doença de isolados netB positivos de C. perfringens recuperados de galinhas normais e de uma vaca, e de isolados netB positivos e negativos de galinhas com enterite necrótica. **Microbiologia veterinária**, v. 146, n. 1-2, pág. 76-84, 2010.

Songer J. G: Clostridial enteric diseases of domestic animals. **Clinical Microbiology Reviews**, 9:216–234, 1996.

Souza, C. S., Vieites, F. M., Justino, L. R., De Lima, M. F., Chaves, A. S., Da Silva Cardoso, V., & De Resende Sousa, F. D. Importance of intestinal health in broilers Importancia de la salud intestinal en pollos de engorde. **Research, Society and Development**, v. 9, n. 3, p. e86932475, 2020.

Stålbrand, Henrik., Siika-aho, M., Tenkanen, M., & Viikari, L. Purificação e caracterização de duas β-mananases de *Trichoderma reesei*. **Revista de biotecnologia**, v. 29, n. 3, pág. 229-242, 1993.

Stanley D, Wu, S.B, Rodgers N, Swick R. A, & Moore R. J. (2014b). Differential responses of cecal microbiota to fishmeal, Eimeria and Clostridium perfringens in a necrotic enteritis challenge model in chickens. **PloS ONE**, 9: e104739, 2014.

Stein, H. H.; Berger, L. L.; Drackley, J. K.; Fahey J. R, G. F.; Hernot, D. C.; PARSONS, C.M. Nutricional properties and feeding values of soybeans and their coproducts. IN: JOHNSON, L.A.; WHITE, P.J.; GALLOWAY, R. Soybeans chemistry, production, processing, and utilization. Urbana: AOCS Press, p. 613-660, 2008.

Teo, A. Y. L, Tan, HM. Effect of *Bacillus subtilis* PB6 (CloSTAT) on broilers infected with a pathogenic strain of *Escherichia coli*. **Journal of Applied Poultry Research**; 15: 229-235, 2006.

Timbermont, L., Haesebrouck, F., Ducatelle, R., & Van Immerseel, F. Necrotic enteritis in broilers: an updated review on the pathogenesis. **Avian Pathology**, *40*(4), 341-347, 2011.

Vicuña, E. A., Kuttappan, V. A., Galarza-seeber, R., Latorre, J. D., Faulkner, O. B., Hargis, B. M., ... & Bielke, L. R. Effect of dexamethasone in feed on intestinal permeability, differential white blood cell counts, and immune organs in broiler chicks. **Poultry science**, v. 94, n. 9, p. 2075-2080, 2015.

Viola, E. S., Vieira, S. L., Torres, C. A., Freitas, D. M. F., & Berres, J. Desempenho de frangos de corte sob suplementação com ácidos lático, fórmico, acético e fosfórico no alimento ou na água. **Revista Brasileira de Zootecnia**, 37(2), 296-302, 2008.

Vohra, Ashima; Syal, Poonam; Madan, Anshu. ET AL. Probiotic yeasts in livestock sector. **Animal Feed Science and Technology**, v. 219, p. 31-47, 2016.

Vuong, C. N., Chou, W. -., Hargis, B. M., Berghman, L. R., & Bielke, L. R. Role of probiotics on immune function and their relationship to antibiotic growth promoters in poultry, a brief review**. International Journal of Probiotics and Prebiotics***, 11*(1), 1-6, 2016.

Wade, B.; Keyburn, A. The cost of necrotic enteritis is huge. **World Poultry News**, v. 31, n. 5, 2015.

Williams, R. B. Intercurrent coccidiosis and necrotic enteritis of chickens: rational, integrated disease management by maintenance of gut integrity. **Avian pathology**, v. 34, n. 3, p. 159-180, 2005.

Wu S. B, Stanley D, Rodgers N, Swick R. A And Moore R. J. Two necrotic enteritis predisposing factors, dietary fishmeal and *Eimeria* infection, induce large changes in the caecal microbiota of broiler chickens. **Veterinary microbiology**, 169(3-4):188-197, 2014.

Wu Y, Shao Y, Song B, Zhen W, Wang Z, Guo Y, Shahid Ms And Nie W. Effects of Bacillus coagulans supplementation on the growth performance and gut health of broiler chickens with Clostridium perfringens-induced necrotic enteritis. **Journal of animal science and biotechnology**, 9:9, 2018.

Yegani, M.; Korver, D. R. Factors affecting intestinal health in poultry. **Poultry science**, v. 87, n. 10, p. 2052-2063, 2008.

Yin G, Liu X, Zou J, Huang X, Suo X. Co- expression of reporter genes in the widespread pathogen *Eimeria tenella* using a double-cassette expression vector strategy. **International Journal for Parasitology, Elmsford**, v. 41, n. 8, p. 813-816, 2011.

Zhang L, Zhang L, Zeng X, Zhou L, Cao G AND Yang C. Effects of dietary supplementation of probiotic, *Clostridium butyricum*, on growth performance, immune response, intestinal barrier function, and digestive enzyme activity in broiler chickens challenged with Escherichia coli K88. **Journal of animal science and biotechnology**, 7:3, 2016.

Zavarize, K. C., Menten, J. F. M., Traldi, A. B. T., Santarosa, J., & Silva, C. L. S. Utilização de glutamina na nutrição de monogástricos. **Revista Ciência Portuguesa de Ciências Veterinárias**, 105(573-576), 5-10, 2010.

Zhou, H., Lepp, D., Pei, Y., Liu, M., YIN, X., Ma, R., Prescott, J.F. And Gong, J. Influence of pCP1NetB ancillary genes on the virulence of *Clostridium perfringens* poultry necrotic enteritis strain CP1. **Gut pathogens**, 9:6, 2017.

Zou, X.T.; Qiao, X. J.; Xu, Z.R. Effect of β-mannanase (Hemicell) on growth performance and immunity of broilers. **Poultry Science**, v.85, p.2175-2179, 2006.

Food and Agricultural Organization of the United Nations and World Health Organization. Joint FAO/WHO working group report on drafting guidelines for the evaluation of probiotics in food. ***Food and Agricultural Organization of the United Nations*** [online], ftp://ftp.fao.org/es/esn/food/wgreport2.pdf (2002).

CAPÍTULO 2- EFFECT OF PROBIOTIC AND B-MANNANASE SUPPLEMENTATION ON THE PRODUCTIVE PERFORMANCE AND INTESTINAL HEALTH OF BROILER CHICKENS CHALLENGED BY *EIMERIA MAXIMA* AND *CLOSTRIDIUM PERFRINGENS*

Este capítulo corresponde ao artigo científico submetido à revista “Poultry - MDPI”

Effect of probiotic and β-mannanase supplementation on the productive performance and intestinal health of broiler chickens challenged by *Eimeria maxima* and *Clostridium perfringens*

Larissa Pereira Maria[1], Rony Riveros Lizana[1], Rosiane de Souza Camargos[1], Bruno Balbino Leme[1], Bárbara Vitória Marçal[1], Nilva Kazue Sakomura[1] and Marcos Kipper da Silva[2]*

[1]Universidade Estadual Paulista (Unesp), Faculdade de Ciências Agrárias e Veterinárias, Jaboticabal, São Paulo, Brazil;

[2]Elanco Animal Health Incorporated Company, São Paulo, Brazil.

*Correspondence: marcos.kipper@elancoah.com; Tel.: +55 51 9881-5658.

Abstract: The use of antibiotics in poultry farming has been associated with bacterial resistance in humans, leading to a ban on their inclusion in chicken diets. Therefore, the objective was to evaluate the effects of probiotics and β-mannanase on the growth performance and intestinal health of broiler chickens challenged by *Eimeria maxima* and *Clostridium perfringens*. 2,100 one-day-old male Ross 308 chicks were used. The treatments were: T1- Negative control (NC) unchallenged birds; T2- Positive control (PC) challenged with *E. maxima+C. perfringens*; T3- PC+Antibiotic (Enramycin 8%- 125g/ton); T4-PC+β-mannanase (Hemicell^{HT}; 300g/ton); T5- PC+probiotic (Protexin^{TM}; 150g/ton); T6- PC+β-mannanase+probiotic. Significant differences (P<0.05) were observed from 1 to 42 days in the variables BW, BWG and FI, the NC treatment presented higher values compared to the PC and PC+probiotic groups. The villus:crypt ratio in the duodenum increased in the PC+β-man+prob treatment, differing from the NC, PC and PC+probiotic (P<0.05). The use of β-mannanase, probiotic or both together is effective to mitigate the effects of production challenges, through the maintenance of the intestine by modulating action on the cecum microbiome and intestinal morphometry.

Keywords: coccidiosis; intestinal permeability; necrotic enteritis; natural alternative

1. Introduction

Coccidiosis is a parasitic disease caused by an apicomplexan parasite of the genus Eimeria, which infects different parts of the intestinal tract depending on the species of *Eimeria* [1]. The *Eimeria Tenella* species invades intestinal cells and causes physical damage to the intestinal epithelium, producing hemorrhagic diarrhea and disrupting the normal functioning of the intestinal tract [2]. This leads to impaired nutrient absorption and com-promised gut health [3]. On the other hand, necrotic enteritis (NE) is a significant and widespread bacterial disease in poultry that causes economic losses in commercial pro-duction [4]. This disease is caused by *Clostridium perfringens*, a gram-positive bacterium, in which the disease causes necrotic lesions of enterocytes [5].

Both diseases can occur in interaction and establish a symbiotic relationship, exacerbating the negative effects on gut integrity and leading to dysbiosis [6]. The damage caused by coccidia weakens the gut barrier and makes the birds more susceptible to *C. perfringens* infection [7]. Coccidial parasitism creates an environment favorable to the proliferation of C. perfringens and the production of its toxins, leading to the development of NE, resulting in increased mucogenesis, severe intestinal lesions, decreased intestinal permeability and, in more serious cases, death of birds [8-9-10].

Years ago, antibiotics and anticoccidial medications have been used to prevent and control many infectious diseases in birds. Such as, the ban on antibiotics as growth promoters has led to the development and evaluation of alternative additives [11-12]. One of these alternatives is the use of probiotics in the diet to prevent and control coccidiosis and NE [13]. Some probiotics create a physical barrier by occupying binding sites on the intestinal mucosa, thus excluding pathogenic bacteria through competitive exclusion [14]. Additionally, probiotics exhibit antimicrobial activity by producing substances such as bacteriocins, carbon dioxide, hydrogen peroxide, and organic acids (acetic and lactic acids). Probiotics compete with pathogenic bacteria for nutrients, effectively reducing their colonization and growth in the intestinal mucosa [13-15]. The mechanism of microbial balance improved by probiotics has a beneficial effect on gut health and, consequently, on nutrient absorption [16].

On the other hand, the utilization of enzymes in feed has demonstrated an improvement in nutrient absorption by reducing antinutritional components and having a beneficial effect on gut integrity [17]. Supplementation of exogenous enzymes, such as β-mannanase, in broiler chicken diets offers an alternative to mitigate these effects [17]. β-Mannanase is an endohydrolase enzyme that breaks down β-mannans, making previously indigestible nutrients available, β-mannans, which are non-starch polysaccharides (NSPs) [18]. β-mannanases have been used to act on specific polysaccharide targets and break down β-mannans found in plant cell walls of the many ingredients that are part of the feed formula.

The negative effects of β-mannans impact nutrient absorption and nutrition, increasing the particularity of intestinal mucus, leading to reduced absorption and negative effects on intestinal integrity. They also interfere with intestinal microorganisms, damaging the intestinal microbiota [19-20]. As reported by [21] and [22], a β-mannanase found microbial modulating activity in broiler chickens fed corn and soy diets and challenged with *C. perfringens* and *Eimeria*. β-mannanase decreased the magnitude of Eimeria and Clostridium infection, proving a significant increase in body weight gain and a reduction in some intestinal lesions compared to the enzyme-free diet. Supplementation of β-mannanase at the level of 200 and 400 ppm in the poultry diet beneficially improved the homeostasis of anabolic hormones, blood glucose, digestible energy, digestible amino acids, and feed conversion [23]. The authors [24] found that β-mannanase supplementation at the level of (200 or 400/ton) improved ileal digestible energy, provided intestinal vitamin, and improved growth performance of broiler chickens.

The additive interaction between β-mannanase and probiotics has not been explored for broiler chickens challenged by an NE model. In this sense, we hypothesize that supplementing broiler diets with β-mannanase may result in reduced viscosity. β-mannanase used in conjunction with a probiotic blend, can serve as a robust alternative for improving gut health by contributing to the development of beneficial microbial communities, im-proving nutrient absorption, and ultimately leading to better performance. growth. There-fore, this study aims to evaluate the effects of the concomitant application of probiotics and β-mannanase on intestinal integrity,

microbiota establishment and productive performance responses in broiler chickens challenged by *E. maxima* in association with *C. perfringens.*

2. Materials and Methods

2.1. Ethics approval

The experiment was carried out at the Laboratory of Poultry Science, São Paulo State University, Jaboticabal. This study received approval (Protocol no: 1061/22) and was conducted in strict adherence to the guidelines established by the Animal Ethics Committee of the Faculty of Animal Science and Veterinary Medicine, São Paulo State University, Brazil.

2.2. Management and facilities

The experiment was conducted within a controlled-environment poultry house, with environmental conditions adjusted in accordance with the recommendation of the genetic management guidelines [25]. The birds were allocated to collective pens with a stocking density of 39 kg/m^2 throughout the test period, using new wood shavings as bedding material. The birds were provided free access to fresh water via nipple-type drinkers and *ad libitum* feeding. A lighting regime was implemented as recommended by the lineage manual [25]. Throughout the experimental period, daily monitoring and recording of environmental temperature and relative humidity were conducted, resulting in an average temperature of 24.6 °C and an average relative humidity of 52.1%.

2.3. Birds, experimental design, and dietary treatments

A total of 2,100 one-day-old sexed male Ross 308 broiler chicks were obtained from a local commercial hatchery. These birds were subject to the vaccination program, including immunization against Marek on their first day and subsequent vaccinations against Newcastle and Gumboro diseases at seven days of age. The birds were randomly allocated to 60 pens, each measuring (3.0 m x 1.5 m) consisting of experimental units that guarantee the same body weight (42 g ± 0.5). Each unit comprises 35 birds per pen. Subsequently, each experimental unit was assigned one of the six experimental treatments, with ten replicates for each treatment. The feeding regimen encompassed three phases: the initial (days 1-14), grower (days 15-28), and finisher (days 29-42). Across all experimental treatments, the feed consisted of corn-

soybean meal basal diets, formulated to meet the nutritional requirements as recommended elsewhere [26] (Table 1). A representative feed sample was collected from each batch for further analysis.

Table 1. Composition of diets provided throughout the trial (%).

Ingredients	Start (1-14 d)	Grower (15-28 d)	Finisher (29-42 d)
Corn (7.88% CP)	54.88	56.29	61.30
Soybean meal (45% CP)	35.00	33.30	29.00
Meat and bone meal (48% CP)	6.55	5.76	4.67
Soybean oil	2.00	3.34	3.71
Limestone	0.15	0.11	0.20
Salt	0.42	0.41	0.40
DL-Methionine (99%)	0.35	0.30	0.25
L-Lysine HCl (78%)	0.29	0.16	0.16
L-Threonine (98%)	0.07	0.04	0.03
[1]Vitamin Premix	0.10	0.10	0.10
[2] Mineral Premix	0.10	0.10	0.10
Choline Chloride (60%)	0.05	0.05	0.05
[3]Innert or tested products	0.05	0.04	0.04
Nutritional composition			
Metabolizable Energy, (Kcal/kg)	3,000	3,100	3,175
*Crude Protein, (%)	23.79 (24.14)	22.62 (23.35)	20.50 (20.90)
Lysine (%)	1.282	1.190	1.069
Met+Cys (%)	0.948	0.881	0.791
Methionine (%)	0.653	0.596	0.526
Threonine (%)	0.846	0.786	0.706
Valine (%)	0.957	0.916	0.830
Tryptophan (%)	0.245	0.235	0.706
Isoleucine (%)	0.864	0.827	0.746
Arginine (%)	1.467	1.396	1.247
Leucine (%)	1.730	1.673	1.560
Histidine (%)	0.531	0.511	0.468
Phenyl+tyrosine (%)	1.755	1.685	1.535
Gly+serine (%)	2.150	2.032	1.811
Calcium (%)	0.925	0.818	0.714
Available Phosphorus (%)	0.441	0.397	0.335
Sodium (%)	0.222	0.213	0.203
Electrolytic balance	214	197	177

[1]Content/kg of premix= Vitamin A (min) 11.000.000 IU; Vitamin D3 (min) 4.000.000 IU; Vitamin E (min) 55.000 IU; Vitamin K3 (min) 3.000 mg. Vitamin B1 (min) 2.300 mg. Vitamin B2 (min) 7.000 mg, Pantothenic Acid (min) 12g. Vitamin B6 (min) 4.000 mg. Vitamin B12 (min) 25.000 mcg. Nicotinic Acid (min) 60g. Folic Acid (min) 2.000 mg. Biotin (min) 250 mg. Selenium (min) 300 mg; [2]Content/kg of premix= iron (min) 100g. Cuprum (min) 20g. Manganese (min) 130g. Zinc (min) 130g. Iodine (min) 2.000mg; [3]Inert: washed sand, Antibiotic: Enramycin, β-mannanase and probiotic: The probiotic was composed by *Lactobacillus acidophilus* (2.06 x 10^8 CFU/g), *Lactobacillus bulgaricus* (2.06 x 10^8 CFU/g), *Lactobacillus rhamnosus* (2.06 x 10^8 CFU/g), *Lactobacillus plantarum* (1.26 x 10^8 CFU/g), *Bifidobacterium bifidum* (2.0 x 10^8 CFU/g), *Enterococcus faecium* (6.46 x 10^8 CFU/g), *Streptococcus thermophilus* (4.10 x 10^8 CFU/g);*Values calculated and analyzed respectively.

The six experimental treatments were as follows: T1 – Negative control (NC) (Non-challenged birds without antibiotics or additives); T2 - Positive control (PC) (birds challenged with *Eimeria maxima* + *Clostridium perfringens* without antibiotic or additive); T3 – PC + antibiotic; T4 - PC + β-mannanase; T5 - PC + probiotic; and T6 - PC + β-mannanase + probiotic. The antibiotic used was enramycin, administered at a rate of 125 g/ton. β-mannanase was supplemented at a rate of 300 g/ton, while the probiotic was included at 150 g/ton during the starter phase, 100 g/ton during the grower phase, and 50 g/ton during the finisher phase, following the brand recommendations (Elanco ®, SP, Brazil). β-mannanase is the active ingredient, and its source is through a patented enzyme produced from the fermentation of the bacteria *Paenibacillus lentus* (Elanco ®, SP, Brazil). All additives were administered from the birds' first day of life until the end of the test. The probiotic used was composed of *Lactobacillus acidophilus* (2.06 x 10^8 CFU/g), *Lactobacillus bulgaricus* (2.06 x 10^8 CFU/g), *Lactobacillus rhamnosus* (2.06 x 10^8 CFU/g), *Lactobacillus plantarum* (1.26 x 10^8 CFU/g), *Bifidobacterium bifidum* (2.0 x 10^8 CFU/g), *Enterococcus faecium* (6.46 x 10^8 CFU/g), *Streptococcus thermophilus* (4.10 x 10^8 CFU/g) (Elanco ®, SP, Brazil).

2.4. Eimeria maxima and Clostridium perfringens challenge protocol

The health challenge protocol entailed individual inoculation of the challenged treatments at 14 days of age with 1 mL of inoculum containing *E. maxima* at a concentration of 7 x 10^3 sporulated oocysts/mL, which was diluted in a phosphate-buffered saline solution. Subsequently, at 18, 19, and 20 days of age, the chickens received individual daily inoculations of 1 mL each, containing *C. perfringens* at a concentration of 2.5 x 10^6 CFU/ mL. All inoculations were administered orally, using a syringe attached to a probe inserted into the birds' beaks.

2.5. Productive performance collection

The productive performance variables, covering body weight (BW) and feed intake (FI), were recorded at 14, 21, 28 and 42 days of age, in which all birds in the trial were weighed to obtain body weight (n =2,100). Subsequently, the body weight gain (BWG) for this designated period was calculated and employed in the determination of the feed conversion rate (FCR). At the same time, daily mortality checks were carried out and integrated into the FCR correction, following the

methodology elucidated by [27] in which the procedure generally applied consists of recording the day of death, the number of dead birds and their weights respectively.

2.6. Oocyst count analysis

Oocyte counting was conducted at six days post-inoculation (dpi) with *E. maxima*. To do this, fresh excreta were sampled from various areas within the pen and stored in labeled plastic bags at -4°C for subsequent analysis. Oocyst counting was carried out at the Laboratory of Parasites (LabEPar, São Paulo State University, Brazil), using 2 grams of homogenized sub-samples to determine oocysts per gram of feces (OPG). This process followed the protocol described by [28], which involved sub-sample dilution in 28 mL of NaCl solution, homogenization, and allowing it to settle for 15 minutes. Subsequently, an aliquot of the solution was taken and placed in the McMaster chamber, and oocysts were counted in each area using an electronic microscope with a 10 X objective (Olympus CX31, Evident Corporation, Tokyo, Japan). The final count was multiplied by 50 to determine the number OPG.

2.7. Intestinal morphometry

The assessment of intestinal morphology was conducted at both 21 and 42 days of age. To perform this assessment, one bird per pen was euthanized via cervical dislocation. Following the opening of the celomic cavity, fragments from various segments of the gastrointestinal tract were carefully collected to prevent tissue autolysis.

Sampling from each gut section (duodenum, jejunum, and ileum) was executed with precision to ensure uniformity, with each section measuring approximately 1.5 cm to 2.0 cm. These sections were selected within the middle two-thirds of each respective segment. The gathered samples were meticulously cleansed with a 10% formaldehyde solution to eliminate intestinal contents. They were then placed in labeled containers filled with formalin solution, ensuring complete immersion to maintain sample integrity. Subsequently, the samples were transported to the laboratory (Mercolab®, Cascavél - Paraná) for further analysis.

For analysis purposes, the collected samples underwent three distinct sectioning techniques: hemicylinder, transverse, and longitudinal, each with a thickness of approximately 1 mm. Additionally, the longitudinal sections were 15 mm

in length, as recommended by [29]. The samples were subsequently subjected to a dehydration process using ascending alcohol concentrations, followed by clarification in xylene, impregnation, and embedding in paraffin. Microtomy was employed to produce semi-serial sections with a thickness of 4 µm, and these sections were subsequently stained with hematoxylin and eosin, following [30] method.

Microscopic observations were carried out using an optical microscope (Olympus CX31, Evident Corporation, Tokyo, Japan), with the examination of fields at a 10 X objective. To measure villus height and crypt depth, the Toup View Software was utilized, with 10 readings being taken on complete villi for each fragment evaluated.

2.8. Gut microbiota of the cecum

The determination and evaluation of the cecal microbiota in birds, at 22 and 43 days of age, involved the collection of samples of cecal content. One bird per box was used at the respective ages. The cecal content was sampled and subsequently stored at -80°C for further analyses.

For microbial community identification, DNA extraction from the samples was executed using the "ZR Quick DNA Fecal/Soil Microbe MiniPrep™" commercial kit provided by (Zymo Research Corporation D6010, USA), following the manufacturer's prescribed protocol. The quantification of extracted DNA was performed using spectrophotometry at 260 nm. To assess DNA integrity, all samples underwent 1% agarose gel electrophoresis.

The amplification of the V3V4 hypervariable region of the ribosomal 16S rRNA gene was achieved using universal primers as detailed in the methodology of PCR [31]. The amplified samples were employed to construct the metagenomic library utilizing the "Nextera DNA Library Preparation Kit" commercial kit by Illumina®. The pooled samples were subsequently subjected to sequencing on the Illumina® "MiSeq" sequencer [32].

The dataset obtained from the sequencer underwent analysis using the QIIME2 platform (Quantitative Insights into Microbial Ecology) [33]. The analysis workflow included the removal of low-quality sequences, filtration, elimination of chimeras, and taxonomic classification. Sequences were categorized into bacterial genera via

Amplicon Sequence Variants (ASVs) by comparing them against a dataset. Specifically, the SILVA database of ribosomal sequences [34], particularly the 2019 update (SILVA 138), was utilized for sequence comparison. To generate bacterial community classifications based on ASV identification, a normalization of 20,536 reads per sample was applied to ensure data comparability among samples with varying read numbers.

2.9. Health Tracking System analysis

The Health Tracking System analysis (HTSi) was conducted on two birds per enclosure at both 21 and 42 days of age, following the methodology established by Elanco Animal Health [35]. This analysis employs a quantitative metric referred to as "Intestinal Integrity" (I^2), which serves as an index comprising various intestinal lesions impacting avian health. These lesions encompass gizzard erosion, oral cavity lesions, proventriculitis, intestinal tone, excessive intestinal fluid, conditions of the small intestine and large intestine, excessive bile, excessive mucus, cell desquamation, food passage, necrotic enteritis, hyperemia, and intestinal hemorrhage. Additionally, the presence of *Eimeria* genus (*E. acervulina, E. maxima* and *E. tenella*) was evaluated based on the typical lesions produced by these three genera.

Each condition is allocated a weight, with scores ranging from 0-1 (indicating presence/absence) for most of the aforementioned items, 0-2 for proventriculitis and necrotic enteritis, 0-3 for gizzard erosion and intestinal hemorrhage, and 0-4 for the severity of lesions across all species of *Eimeria*. The initial score of the evaluated individuals is 100, meaning a healthy digestive system. The presence of each lesion listed corresponds to a decrease in the score, and therefore the lower the score, the less healthy the bird is [35].

2.10. Intestinal permeability

On day 42, the chickens underwent oral administration of a marker known as FITC-dextran to evaluate intestinal permeability. Serum levels of FITC-dextran were quantified using the methodology proposed by [36]. This trial was performed using one bird per experimental unit selected according to a body weight close to the average body weight of the pen (±10%).

Two hours and thirty minutes before blood sampling, each bird received 500 µL (0.5 mL) dose of FITC-dextran (PM 3000–5000; Sigma Aldrich Co., St. Louis, MO) through oral gavage and this content was allocated on the crop with a fine and flexible oral tube. On sequence, the blood sample collection was taking from the jugular artery following the administration of the FITC-dextran marker. To prevent clotting, the collected blood (2 to 3 mL) was gently homogenized by 5 to 8 times.

To detect the presence of FITC-d in the serum, the blood was left at room temperature for 3 hours to facilitate clot formation, after which it was centrifuged at 500xg for 15 min to separate the serum. Fluorescence levels of diluted serum (1:1 in PBS) were measured using an excitation wavelength of 485 nm and an emission wavelength of 528 nm (Synergy HT, multimode microplate reader, BioTek Instruments, Inc., VT). The concentration of FITC-d per mL of serum was determined based on a standard curve [36]. Consequently, a higher concentration of Dextran-FITC in the plasma/serum indicates a greater degree of intestinal permeability and intestinal damage.

2.11. Statistical analysis

Statistical analyses were carried out utilizing SAS version 9.4 statistical software. The data was subject to analysis of variance (ANOVA) via the PROC MIXED procedure. The normality of errors and homoscedasticity was checked before ANOVA was performed. Mean comparisons between treatments were performed using the Tukey test, with a significance level set at $P < 0.05$. Non-parametric methods were employed to analyze oocyst count. The Kruskal-Wallis test was initially applied, followed by the Dwass-Steel-Critchlow-Fligner (DSCF) multiple comparison test. Significance was attributed to $P < 0.05$.

In the case of gut microbiome analysis, alpha diversities were calculated using the "phyloseq" package [37] and the "vegan" library [38] and were compared using the Kruskal-Wallis non-parametric test [39], followed by the post-hoc by Dunn [40]. Results with $P < 0.05$ were considered statistically significant. Beta diversity was assessed using permutational multivariate analysis of variance (perMANOVA) through the QiimeII pipeline, involving 10,000 permutations. All additional numbers and statistical analyzes were performed in the R software environment.

3. Results

3.1. Growth performance

The performance responses are presented in Table 2 and are detailed for each evaluation period.

Table 2. Performance responses of broiler chickens subject to the experimental treatment.

	1 to 14d				15 to 28d				29 to 42d			
Treatments	**BW[1] 14d (kg)**	**BWG[2] (kg)**	**FI[3] (kg)**	**FCR[4] (kg/kg)**	**BW 28d (kg)**	**BWG (kg)**	**FI (kg)**	**FCR (kg/kg)**	**BW 42d (kg)**	**BWG (kg)**	**FI (kg)**	**FCR (kg)**
NC	0.489^{abc}	0.446^{abc}	0.490^{ab}	1.101	1.769^{a}	1.250^{a}	1.743^{a}	1.393^{ab}	3.365^{a}	1.596	2.889^{a}	1.810^{a}
PC	0.481^{c}	0.438^{c}	0.481^{b}	1.096	1.701^{b}	1.203^{b}	1.644^{b}	1.368^{b}	3.208^{b}	1.509	2.731^{b}	1.814^{a}
PC+Ant	0.507^{a}	0.465^{a}	0.510^{a}	1.098	1.730^{ab}	1.200^{b}	1.655^{b}	1.376^{ab}	3.313^{ab}	1.590	2.774^{ab}	1.745^{b}
PC+β-man	0.492^{abc}	0.449^{abc}	0.501^{ab}	1.116	1.707^{ab}	1.188^{b}	1.661^{b}	1.400^{a}	3.257^{ab}	1.546	2.767^{ab}	1.793^{ab}
PC+prob	0.486^{bc}	0.443^{bc}	0.494^{ab}	1.114	1.698^{b}	1.178^{b}	1.647^{b}	1.398^{a}	3.223^{b}	1.517	2.741^{b}	1.809^{a}
PC+β-man+prob	0.502^{ab}	0.460^{ab}	0.504^{a}	1.095	1.711^{ab}	1.190^{b}	1.656^{b}	1.390^{ab}	3.260^{ab}	1.554	2.757^{ab}	1.775^{ab}
SEM	0.002	0.002	0.002	0.005	0.007	0.005	0.008	0.003	0.015	0.010	0.016	0.006
P - value	0.003	0.003	0.002	0.705	0.023	<0.001	<0.001	0.002	0.013	0.064	0.028	0.008

[1]Body weight; [2]Body weight gain; [3]Feed intake; [4]Feed conversion rate. ^{abc} Different letters in the same column represent statistical difference by the Tukey test (*P*-value <0.05 was considered statistically different). Means that do not follow a letter are significantly different. Negative control (NC) (birds without challenge); Positive control (PC) (birds challenged with *Eimeria maxima* + *Clostridium perfringens*); PC + (antibiotic); PC + β-mannanase; PC + probiotic; PC+ β-mannanase + probiotic; SEM: Standard error of the mean.

During the period from 1 to 14 days of age, significant differences ($P < 0.05$) were observed in BW, FI and BWG between experimental treatments. The PC+Antibiotic treatment showed higher BW, BWG and FI values compared to the PC group. Meanwhile, the combination of β-mannanase and probiotics resulted in higher FI, like the PC+Antibiotic treatment.

From 15 to 28 years old, corresponding to the most critical period of the challenge, significant differences ($P < 0.05$) were observed in productive performance responses. For the BW variable, the NC treatment presented a higher value compared to the PC and PC+probiotic groups. Furthermore, non-challenged birds (NC) showed higher BWG and FI than all other treatments. For the FCR variable, PC birds achieved improved conversion compared to PC+β-mannanase and PC+probiotic treatments.

In the final period, from 29 to 42 days of age, only the BWG variable did not differ between treatments ($P > 0.05$). For the variables BW and FI, birds in the NC group presented higher values compared to birds in the PC and PC+probiotic groups ($P < 0.05$). The feed conversion of birds in the PC+antibiotic treatment was lower compared to the NC, PC and PC+probiotic groups.

Significant differences ($P < 0.05$) in all productive variables were observed throughout the entire trial (1 to 42 days). For the variables BW, BWG and FI, birds in the NC treatment exhibited higher values compared to the PC and PC+probiotic groups. However, the FCR of birds that received PC+antibiotic was lower compared to birds from NC. (Table 3).

Table 3. Performance of broilers throughout the trial (1 to 42 days old).

Treatments	**BW[1] (kg)**	**BWG[2] (kg)**	**FI[3](kg)**	**FCR[4] (kg/kg)**
NC	3.365^{a}	3.322^{a}	5.134^{a}	1.545^{a}
PC	3.208^{b}	3.165^{b}	4.856^{b}	1.534^{ab}
PC+Ant	3.331^{ab}	3.269^{ab}	4.928^{ab}	1.508^{b}
PC+β-man	3.258^{ab}	3.215^{ab}	4.941^{ab}	1.537^{ab}
PC+prob	3.222^{b}	3.180^{b}	4.883^{b}	1.536^{ab}
PC+β-man+prob	3.261^{ab}	3.218^{ab}	4.916^{ab}	1.527^{ab}
SEM	0.015	0.015	0.023	0.003
P - value	0.015	0.015	0.004	0.021

[1]Body weight; [2]Body weight gain; [3]Feed intake; [4]Feed conversion rate. ^{abc} Different letters in the same column represent statistical difference by the Tukey test (P-value <0.05 was considered statistically different). Means that do not follow a letter are significantly different. Negative control (NC) (birds without challenge); Positive control (PC) (birds challenged with *Eimeria maxima* + *Clostridium perfringens*); PC + (antibiotic); PC + β-mannanase; PC + probiotic; PC+ β-mannanase + probiotic; SEM: Standard error of the mean

3.2. Oocyst count

Figure 1 presents the results of the oocyst count analysis, revealing statistical significance (*P* <0.05). The NC treatment demonstrated the absence of oocysts, indicating the effectiveness of the physical barriers and the biosafety procedure adopted to avoid potential cross-contamination. However, *E. maxima* oocysts were detected in the excreta of the challenged treatments, with greater abundance in the PC+Antibiotic group and lower numbers of oocysts in the PC, PC+probiotic and PC+β-man+prob groups.

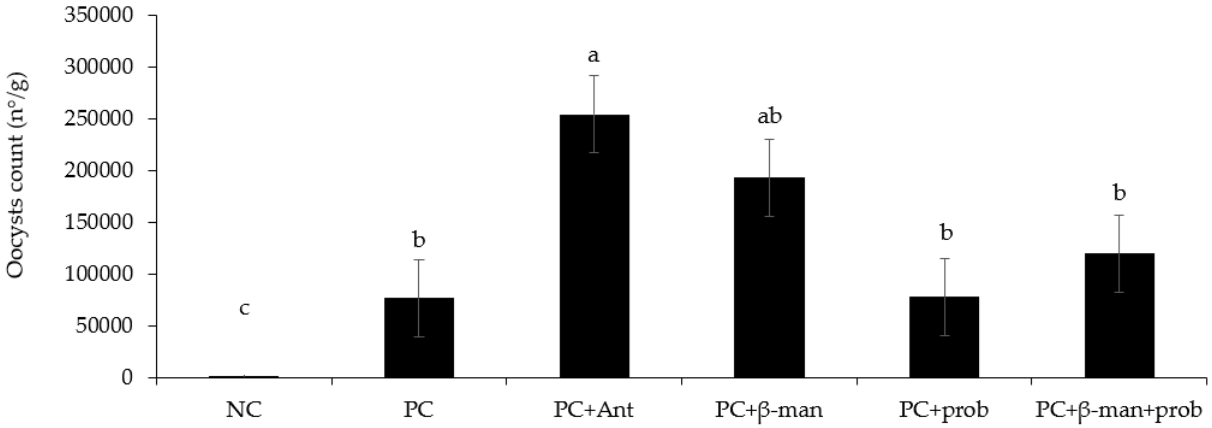

Figure 1. Boxplot of oocyst count (oocysts/g of feces) of 20-day-old broilers fed diets supplemented or not with β-mannanase, Probiotic, Antibiotic and challenged or not with oocysts of *Eimeria maxima* and *Clostridium perfringens*. [a,b] Different letters represent statistical difference by a non-parametric Kruskal-Wallis test. Negative control (NC) (birds without challenge); Positive control (PC) (birds challenged with *Eimeria maxima* + *Clostridium perfringens*); PC + (antibiotic); PC + β-mannanase; PC + probiotic; PC+ β-mannanase + probiotic.

3.3. Intestinal morphometry

The morphometric variables of the intestine sections (duodenum, jejunum, and ileum) at both collection ages (21 and 42 days of age) were statistically significant (*P* <0.05) (Table 4). At 21 days of age, the variable villous height (VH) in the duodenum was higher in the NC and PC+probiotic groups, in relation to the PC, PC+β-man and PC+β-man+prob treatments, also in the jejunum the NC was higher among all treatments. However, in the ileum, VH is higher in birds that received PC+β-man+prob. The variable crypt depth (CD) at 21 days indicates that in the duodenum birds from NC had the lowest among treatments, but in contrast in the jejunum they obtained the highest value, and the PC and PC+β-mannanase treatments had the lowest values. Also, CD in the ileum was lower in PC compared to the PC+antibiotic and PC+β-

man+prob groups. In addition, the results of the villus:crypt ratio at this age showed that in the three segments of the intestine, NC birds obtained higher values between treatments.

At 42 days of the test, there was an increase in the VH variable in the duodenum in the NC, PC and PC+β-man+prob treatments, differing from the birds that received PC+β-mannanase. In the jejunum and ileum segments, VH was higher in NC and PC treatments. The CD at 42 days in the duodenum, jejunum and ileum was lower in the PC+B-mannanase treatment compared to the other treatments. The villus:crypt ratio in the duodenum increased in the PC+β-man+prob treatment, differing from the NC, PC and PC+prob groups. In the jejunum, this relationship was greater in the NC and PC+β-man+prob groups of birds, and in the NC ileum it was greater, differing from PC+β-mannanase and PC+probiotic.

Table 4. Morphometric measurements of the mucosa of the duodenum, jejunum, and ileum of broilers at 21 days and 42 days of age fed diets supplemented or not with Antibiotic, β-mannanase, probiotic and challenged or not with oocysts of *Eimeria maxima* and *Clostridium perfringens.*

	21 days									**42 days**								
Intestinal segment	Duodenum			Jejunum			Ileum			Duodenum			Jejunum			Ileum		
Treatments	VH[1] (µm)	CD[2] (µm)	V:C[3] (µm)	VH (µm)	CD (µm)	V:C (µm)	VH (µm)	CD (µm)	V:C (µm)	VH (µm)	CD (µm)	V:C (µm)	VH (µm)	CD (µm)	V:C (µm)	VH (µm)	CD (µm)	V:C (µm)
NC	1.914^{b}	0.159^{c}	12.363^{a}	1.115^{a}	1.119^{a}	8.139^{a}	0.824^{bcd}	0.812^{bc}	7.042^{a}	2.524^{a}	2.507^{a}	14.720^{b}	1.386^{a}	1.389^{a}	9.071^{a}	1.043^{a}	1.043^{a}	6.788^{a}
PC	2.067^{a}	0.194^{a}	10.872^{b}	0.916^{d}	0.928^{c}	5.968^{bc}	0.793^{d}	0.794^{c}	5.140^{c}	2.577^{a}	2.577^{a}	15.082^{b}	1.240^{b}	1.259^{b}	7.463^{bc}	0.956^{b}	0.938^{bc}	6.033^{abc}
PC+Ant	1.998^{ab}	0.199^{a}	10.435^{b}	1.032^{bc}	1.004^{b}	6.475^{b}	0.859^{ab}	0.890^{a}	6.274^{b}	2.515^{ab}	2.512^{a}	15.984^{ab}	1.443^{a}	1.440^{a}	8.117^{ab}	1.012^{a}	1.012^{a}	6.604^{ab}
PC+β-man	1.923^{b}	0.179^{b}	11.110^{b}	0.980^{c}	0.948^{c}	6.114^{bc}	0.814^{cd}	0.814^{bc}	5.420^{c}	2.396^{b}	2.347^{b}	15.872^{ab}	1.121^{c}	1.125^{c}	6.956^{c}	0.894^{c}	0.897^{c}	5.732^{c}
PC+prob	2.075^{a}	0.194^{ab}	10.991^{b}	1.022^{bc}	1.027^{b}	6.027^{bc}	0.842^{abc}	0.821^{bc}	5.277^{c}	2.515^{ab}	2.497^{a}	14.971^{b}	1.262^{b}	1.270^{b}	6.959^{c}	0.988^{ab}	0.990^{ab}	5.840^{bc}
PC+β-man+prob	1.930^{b}	0.192^{ab}	10.354^{b}	1.039^{b}	1.050^{b}	5.666^{c}	0.868^{a}	0.847^{b}	6.073^{b}	2.563^{a}	2.560^{a}	17.394^{a}	1.256^{b}	1.216^{b}	8.481^{a}	1.040^{b}	1.040^{a}	6.399^{abc}
SEM	0.009	0.002	0.097	0.006	0.005	0.072	0.004	0.004	0.066	0.012	0.013	0.189	0.008	0.008	0.101	0.006	0.006	0.081
P-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

[1]Villus height; [2]Crypt depth; [3]Villus Crypt Ratio; Negative control (NC) (birds without challenge); Positive control (PC) (birds challenged with *Eimeria maxima* + *Clostridium perfringens*); PC + (antibiotic); PC + β-mannanase; PC + probiotic; PC+ β-mannanase + probiotic. ^{abcd} Different letters in the same column represent statistical difference by the Tukey test (P-value <0.05 was considered statistically different). Means that do not follow a letter are significantly different.

3.4. Cecal microbiota diversity

The cecal contents microbiome was assessed for alpha and beta diversity, taxonomic composition, and differential taxon abundance.

3.4.1. Alpha diversity

Alpha diversity analysis, encompassing Chao1, Observed OTUs, Fisher, Simpson, Shannon, and Evenness Pielou metrics, unveiled statistically significant distinctions ($P < 0.05$) among treatments on their respective sampling dates (Figure 2A to 2F).

At 22 days, discernible differences manifested between treatments in birds subjected to challenges. Specifically, birds from the PC and PC+probiotic exhibited higher diversity compared to the PC+Antibiotic treatment, as evidenced by the Simpson ($P = 0.04$) and Pielou ($P = 0.02$) metrics (Figure 2D and 2F). Moreover, distinctions in alpha diversity were evident between the NC and PC+Antibiotic, particularly in the Pielou metric ($P = 0.04$) at this age.

Upon reaching 43 days, variations across all metrics were observed between NC and PC+Antibiotic treatment, with the latter displaying greater alpha diversity. The NC treatment exhibited lower alpha diversity than the PC+β-mannanase treatment in all metrics except for Chao1 (Figure 2B to 2F). Furthermore, the group of challenged birds receiving diets supplemented with the association of PC+β-man+prob showed reduced diversity ($P = 0.002$) compared to the PC+Antibiotic treatment. However, the PC+β-mannanase treatment group demonstrated heightened diversity ($P = 0.03$) regarding the Simpson, Shannon, and Pielou metrics (Figures 2D, 2E, and 2F). Significant distinctions were also apparent between PC treatment ($P = 0.008$) and the PC+Antibiotic in Simpson's metric (Figure 2D). Additionally, differences surfaced between the NC treatment, exhibiting lower alpha diversity compared to the PC+Antibiotic ($P = 0.02$) or PC+β-mannanase ($P = 0.04$) in the Fisher metric (Figure 2C).

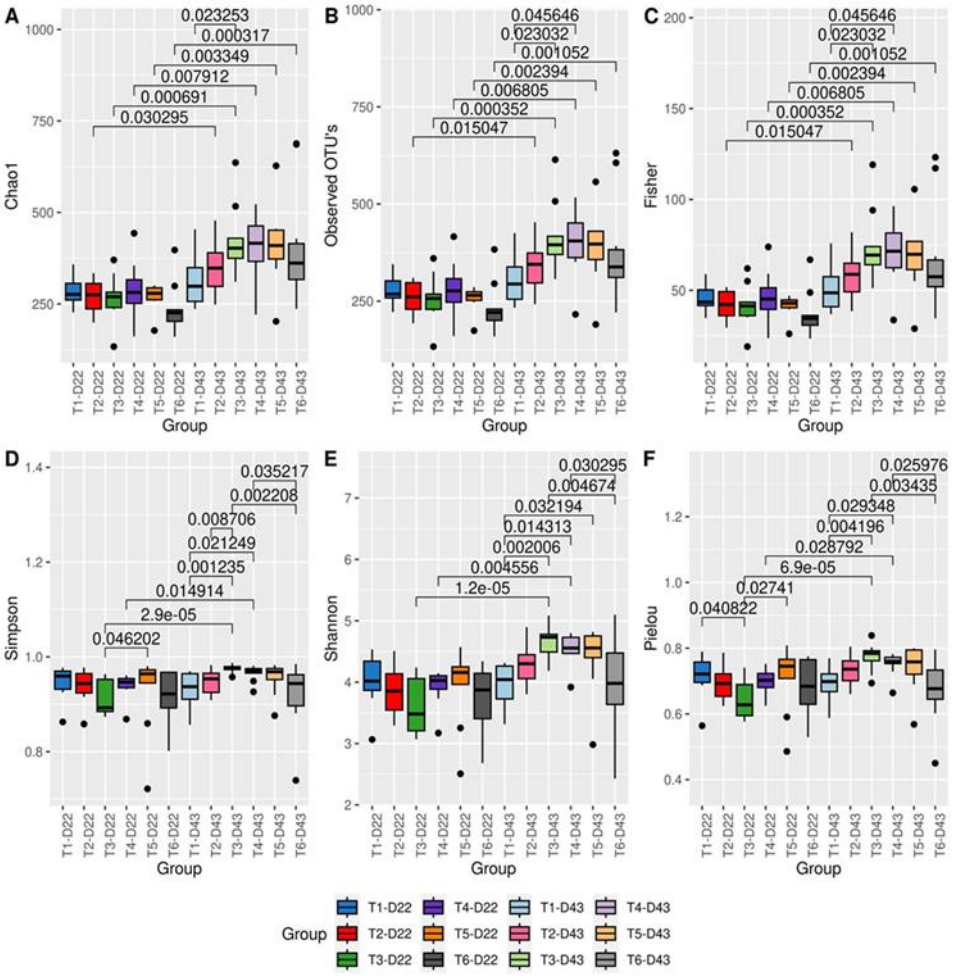

Figure 2. Alpha-diversity estimated by Chao1 parameters (**A**), Observed OTUs (**B**), Fischer (**C**), Simpson index (**D**), Shannon Entropy (**E**) and Evenness Pielou (**F**). Statistical comparison between groups was performed using the non-parametric Kruskal-Wallis and Post-Hoc Dunn tests. Statistical results below 0.05 were accepted as statistically significant. The treatments were: T1 – Negative control (NC) (birds without challenge); T2 Positive control (PC) (birds challenged with *Eimeria maxima* + *Clostridium perfringens*); T3 – PC + (antibiotic); T4 - PC + β-mannanase; T5 - PC + probiotic; T6 - PC+ β-mannanase + probiotic.

3.4.2. Beta Diversity

Beta diversity analysis, as illustrated in Figure 3, was conducted using parameters with statistical significance, including Bray-Curtis (P = 0.0001), Jaccard (P = 0.0001), UniFrac (P = 0.0001), and Weighted UniFrac (P = 0.0001). Principal component analyses (PCoA) based on Bray-Curtis distances were carried out to elucidate cluster formation according to the treatments.

At 22 days, the PC+β-mannanase dietary treatment exhibited dissimilarities compared to the NC treatment, as well as to the PC treatment without supplementation

and PC+probiotic, as indicated by both the Bray-Curtis and Jaccard metrics (Figure 3A and 3B). Furthermore, the group of birds fed the combination diet of PC+β-man+probiotic showed distinctions compared to the NC and PC treatments, as reflected in the Jaccard and UniFrac metrics (Figure 3B and 3C). Just the Jaccard metric revealed differences between the NC treatment and the PC, PC+Antibiotic, and PC+probiotic treatments. Additional disparities were observed in the PC+β-mannanase treatment compared to the PC+Antibiotic and PC+β-mannanase+probiotic treatments, as well as between PC and PC+probiotic treatments (Figure 3B).

At 43 days of age, the NC treatment group exhibited differences compared to the PC+Antibiotic and PC+probiotic treatments across all metrics (Figure 3A to 3D). Specifically, the NC treatment group displayed dissimilarities compared to the PC, PC+β-mannanase, and PC+β-man+prob groups. Additionally, antibiotic supplementation in challenged birds differed from the PC+probiotic treatment in terms of the Bray-Curtis, Jaccard, and UniFrac metrics (Figure 3A to 3C). When considering the Jaccard, UniFrac, and Weighted UniFrac metrics, the PC+Antibiotic group exhibited distinctions when compared to the PC+β-man+prob treatment (Figure 3B to 3D). Furthermore, the PC group differed from the PC+probiotic treatment in terms of the Jaccard and UniFrac metrics (Figure 3B and 3C). Unique differences were observed in the PC+β-mannanase treatment compared to the PC+probiotic and PC+β-man+prob groups. In a complementary way, significant differences were found between the PC+Antibiotic and PC+β-mannanase dietary treatments based on the Jaccard metric (Figure 3B). All comparisons considered the abundance and phylogenetic relationships among taxa.

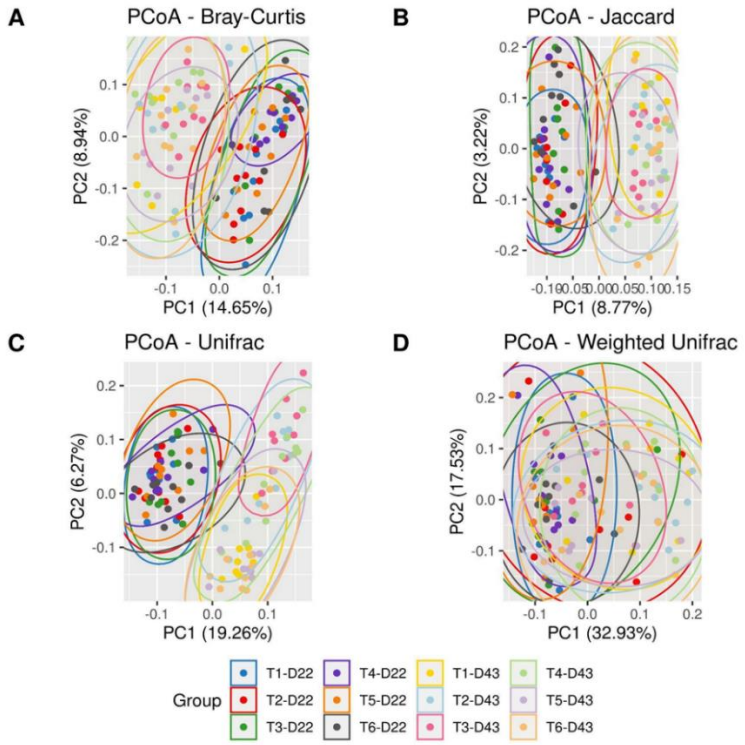

Figure 3. Beta diversity estimated by Bray-Curtis parameters (A). Jaccard (B). UniFrac (C) and weighted Unifrac (D). Colored ellipses were automatically added via the ggforce library in R. The treatments were: T1 – Negative control (NC) (birds without challenge); T2 Positive control (PC) (birds challenged with *Eimeria maxima* + *Clostridium perfringens*); T3 – PC + (antibiotic); T4 - PC + β-mannanase; T5 - PC + probiotic; T6 - PC+ β-mannanase + probiotic.

3.*4.3. Composition of the bacterial community*

The *Firmicutes/Bacteroidota* ratio was computed for each analyzed sample (Figure 4). Significant distinctions were observed among all treatments at their respective sampling ages. Furthermore, at 22 days, the group of birds of the treatment PC exhibited a statistically significant difference compared to the treatments PC+probiotic and PC+β-man+prob.

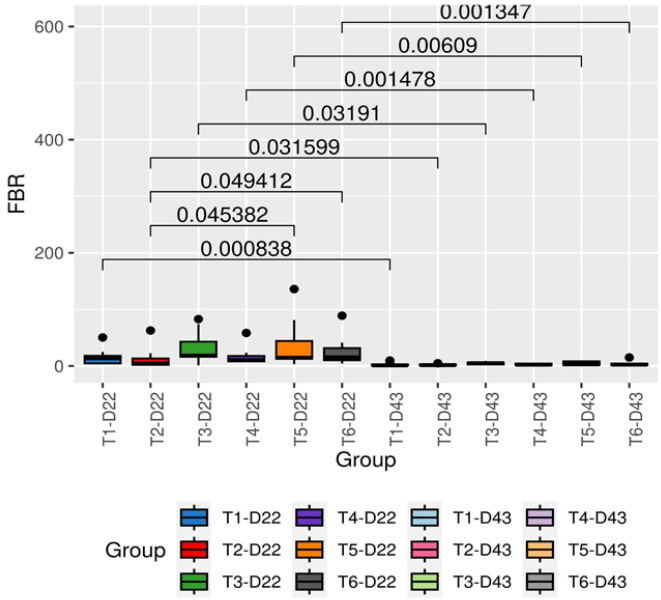

Figura 4. The bar graph shows the ratio between *Firmicutes* and *Bacteroidota* taxa in the tested groups. Differences with p value below 0.05 were considered statistically significant. The treatments were: T1 – Negative control (NC) (birds without challenge); T2 Positive control (PC) (birds challenged with *Eimeria maxima* + *Clostridium perfringens*); T3 – PC + (antibiotic); T4 - PC + β-mannanase; T5 - PC + probiotic; T6 - PC+ β-mannanase + probiotic.

3.4.4. Differences in the abundance of taxa

Only taxa with statistically significant differences in relative abundance Kruskal -Wallis *P* <0.05) were evaluated.

Family *Acutalibacteraceae*, statistically significant differences were observed between the treatments for NC and PC+β-mannanase at the corresponding sample ages. At 43 days there were differences between the NC treatment and the PC+Antibiotic treatment, with a greater quantity of this family being found in the PC+Antibiotic treatment (Figure 5A). In the *Bacteroidaceae* family, except for NC, there were significant differences between treatments at both bird ages (22 and 43 days old). At 22 days, the PC+Antibiotic treatment differed from the NC and PC treatments (Figure 5B). From the *Lactobacillaceae* family, the treatments PC+Antibiotic, PC+probiotic and PC+β-man+prob differ at the two ages of the birds (22 and 43 days old). At age 43, there were differences between the PC+β-mannanase and PC+probiotic treatments and the PC+β-man+prob treatment (Figure 5C).

Oscillospiraceae family, the undisputed treatment differed between the two ages of the birds. At 22 days of age, the NC and PC+β-mannanase treatments differed, and there was a statistical difference between the PC+probiotic and PC+β-man+prob treatments. At 43 days, there were statistical differences between PC+Antibiotic and NC compared to the PC and PC+β-man+prob treatments (Figure 5D). In the *Butyricicoccaceae* family, the PC treatment showed differences between the two corresponding sampling ages (22 and 43 days). At 22 days of age, there were differences between PC+β-mannanase treatments and PC+probiotic treatment. At 43 days, there were differences in relation to the PC+probiotic treatment (Figure 5E). In the *Lachnospiraceae* family, the PC+β-mannanase and PC+β-man+prob treatments differed at the respective sampling ages. At 22 days, the PC treatment differed from the PC+β-mannanase, PC+probiotic and PC+β-man+prob treatments. Furthermore, at the same age, NC showed differences in relation to PC+β-mannanase (Figure 5F). In the *Rikenellaceae* family, differences occurred in all groups, except in the PC+Antibiotic treatment, which showed differences only between the respective sampling ages of the birds (Figure 5G). At 43 days, the *Ruminococcaceae* family showed a significant difference between the PC treatment and the PC+probiotic treatment (Figure 5H).

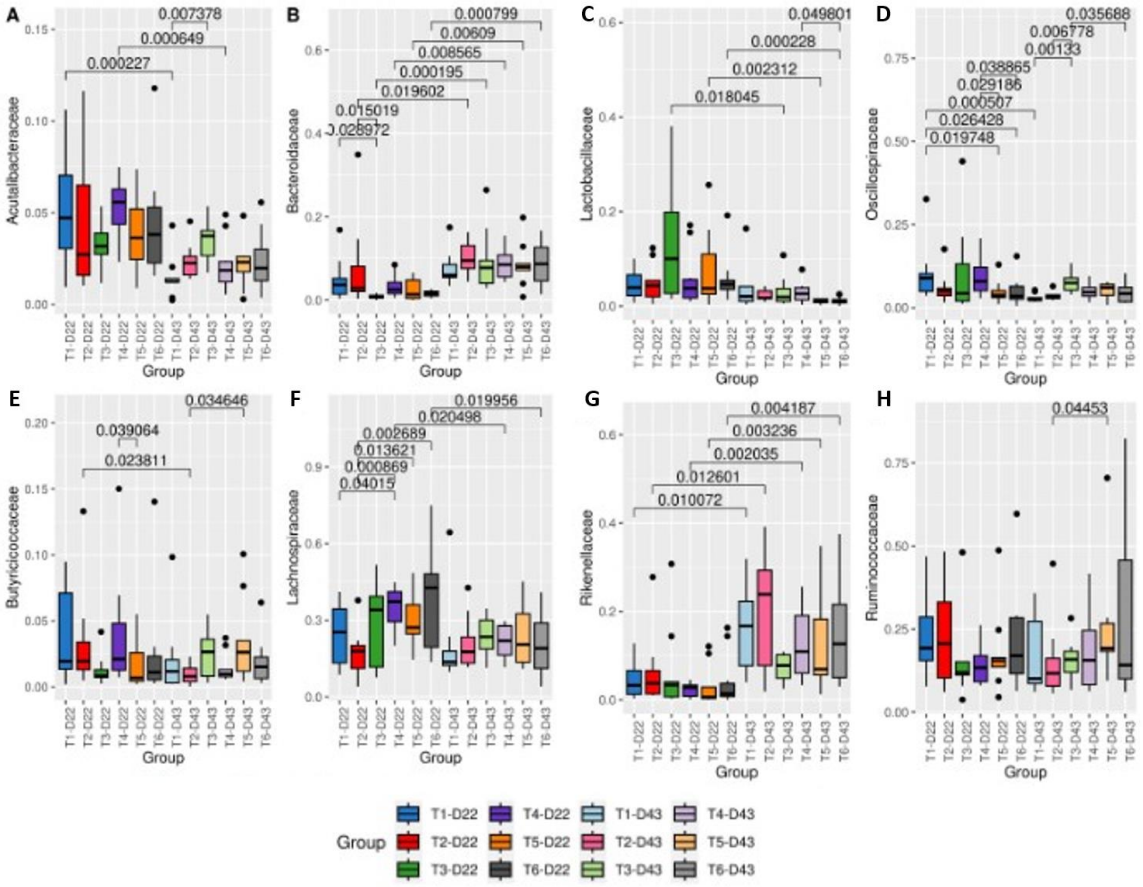

Figure 5. Differential abundance of the *Acutalibacteraceae* family (**A**), *Bacteroidaceae* (**B**), *Lactobacillaceae* (**C**), *Oscillospiraceae* (**D**), *Butyricicoccaceae* (**E**), *Lachnospiraceae* (**F**), *Rikenellaceae* (**G**) and *Ruminococcaceae* (**H**). Statistical comparison between groups was performed using the non-parametric Kruskal-Wallis and Post-Hoc Dunn tests. Statistical results below 0.05 were accepted as statistically significant. The treatments were: T1 – Negative control (NC) (birds without challenge); T2 Positive control (PC) (birds challenged with *Eimeria maxima* + *Clostridium perfringens*); T3 – PC + (antibiotic); T4 - PC + β-mannanase; T5 - PC + probiotic; T6 - PC+ β-mannanase + probiotic.

In the genus *Agathobaculum*, the PC showed differences in their corresponding sampling ages. At 43 days, the treatment PC differed from the PC+probiotic (Figure 6A). In *Alistipes*, the treatments PC+β-mannanase, PC+probiotic, and PC+β-man+prob showed differences between sampling ages. At 22 days, the NC differed from the PC+β-mannanase treatment (Figure 6B). In the genus *Lactobacillus*, the PC+Antibiotic and PC+β-man+prob showed differences between the corresponding sampling ages. At 43 days, the PC+β-mannanase differed from the PC+probiotic and PC+β-man+prob treatments (Figure 6C). In *Mediterraneibacter*, differences were observed that the NC, PC, and PC+β-mannanase differed between bird ages. At 22 days, the treatment of PC+β-mannanase differed from the PC+β-man+prob (Figure 6D). In *Faecalibacterium*, the NC and PC+β-man+prob treatments showed differences

between the two ages. At 22 days, the NC treatment differed from PC+Antibiotic supplemented treatment (Figure 6E). In *gemmiger*, there were differences between treatments of PC+Antibiotics and PC+β-mannanase at both corresponding sampling ages (Figure 6F). In *Prevotellamassilia*, the PC+Antibiotic differed between bird ages. At 22 days of age, there were differences between treatments for NC, PC, and PC+β-mannanase (Figure 6G). In *Tidjanibacter*, the NC, PC+β-mannanase, and PC treatments differed in their respective ages. At 22 days, there were differences between NC and PC treatments (Figure 6H).

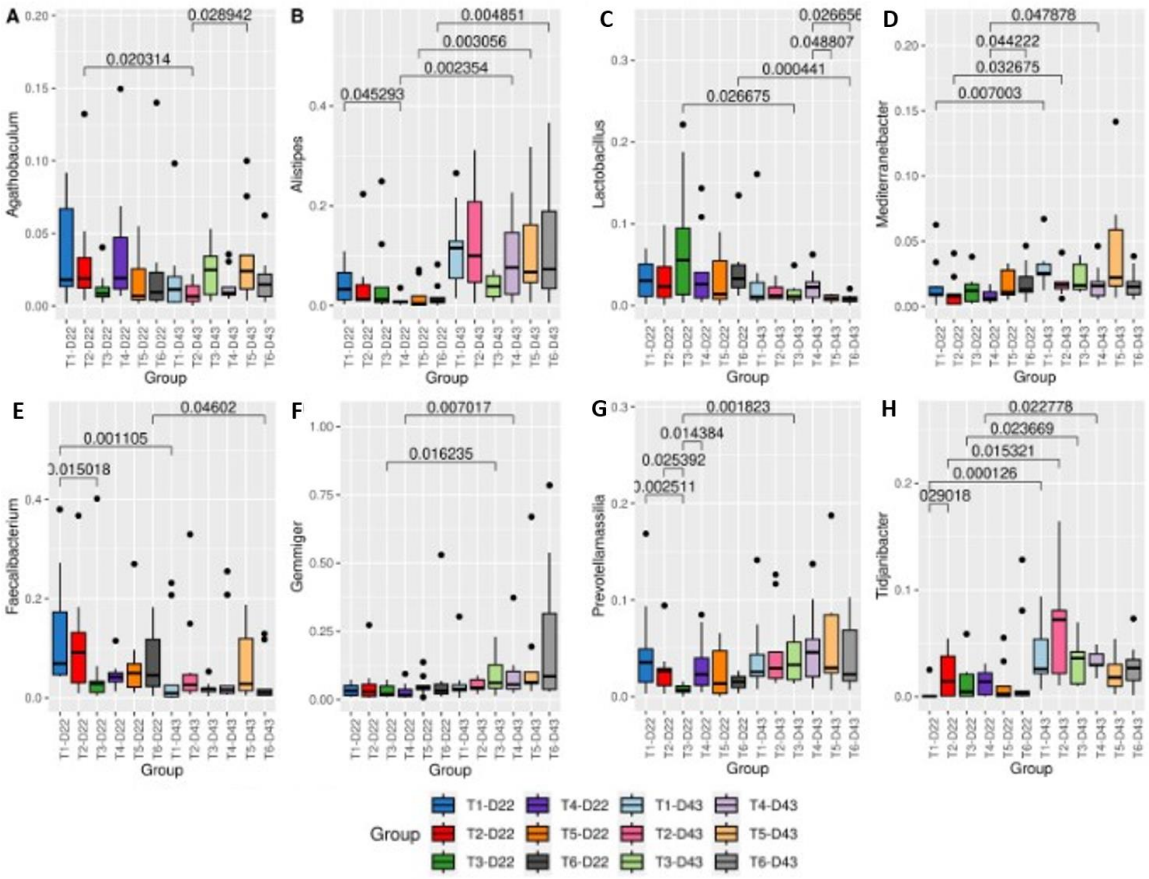

Figure 6. Differential abundance of *Agathobaculum* generum (**A**), *Alistipes* (**B**), *Lactobacillus* (**C**), *Mediterraneibacter* (**D**), *Faecalibacterium* (**E**), *Gemmiger* (**F**), *Prevotellamassilia* (**G**) and *Tidjanibacter* (**H**). Statistical comparison between groups was performed using the non-parametric Kruskal-Wallis and Post-Hoc Dunn tests. Statistical results below 0.05 were accepted as statistically significant. The treatments were: T1 – Negative control (NC) (birds without challenge); T2 Positive control (PC) (birds challenged with *Eimeria maxima* + *Clostridium perfringens*); T3 – PC + (antibiotic); T4 - PC + β-mannanase; T5 - PC + probiotic; T6 - PC+ β-mannanase + probiotic.

3.5. HTSi (Health Tracking System)

There was a significant influence of treatments ($P < 0.05$) on the HTSi analysis at 22 days of age (Figure 7). The birds in the PC+probiotic group demonstrated a value closer to 100%, that is, smaller intestinal lesions. The NC treatment showed a lower percentage of HTSi, indicating greater intestinal lesions in the birds in this group.

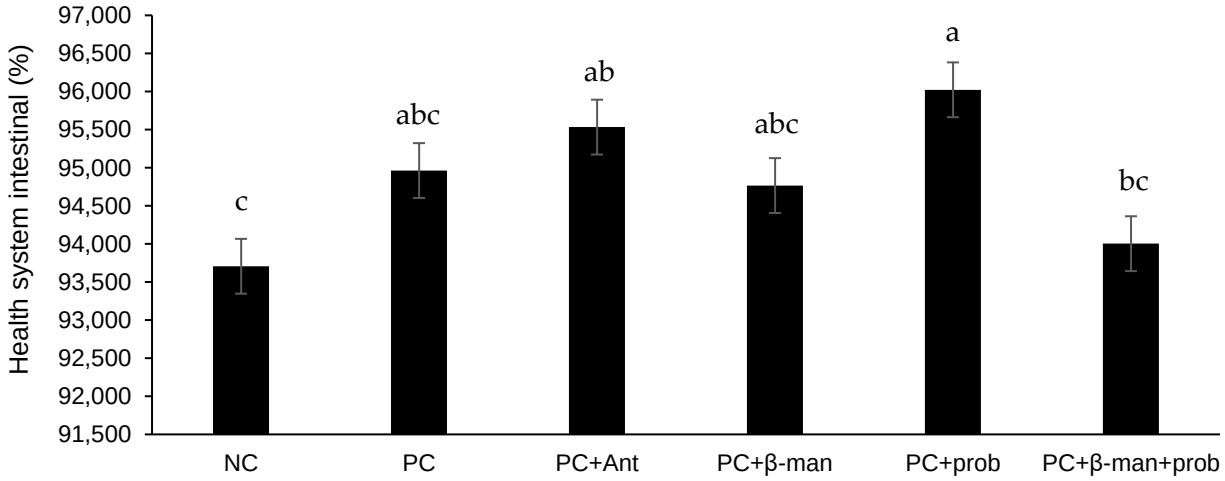

Figure 7. The graph shows through the analysis of intestinal integrity (HTSi) that there was a significant difference between treatments, in broilers challenged or not by *Eimeria maxima* and *Clostridium perfringens*. Negative control (NC) (birds without challenge); Positive control (PC) (birds challenged with *Eimeria maxima* + *Clostridium perfringens*); PC + (antibiotic); PC + β-mannanase; PC + probiotic; PC+ β-mannanase + probiotic.

3.6. Intestinal permeability (FITC-dextran)

The results for intestinal permeability were not significant ($P > 0.05$) as demonstrated (Table 5).

Table 5. Hematological parameters of intestinal permeability of 42-day-old broiler chickens

Treatments	**FITC-d (µg/mL)**
NC	0.476
PC	0.404
PC+Ant	0.418
PC+β-man	0.438
PC+prob	0.402
PC+β-man+prob	0.400
SEM	0.010
P-value	0.190

Negative control (NC) (birds without challenge); Positive control (PC) (birds challenged with *Eimeria maxima* + *Clostridium perfringens*); PC + (antibiotic); PC + β-mannanase; PC + probiotic; PC+ β-mannanase + probiotic. SEM: Standard error of the mean. Means that do not follow a letter are significantly different.

4. Discussion

4.1. Performance Response

Coccidiosis, caused by protozoa of the genus *Eimeria* spp, damages the barrier of the intestinal epithelium, leading to severe inflammatory responses and the appearance of lesions in the villi, interfering with the performance responses and health of birds [41]. To evaluate the effectiveness of the inoculation, *E. maxima* oocyst counts were performed at 20 days. The inoculation protocol was demonstrated to be effective since all challenged treatments showed the presence of oocysts six days post-inoculation, corresponding to the incubation cycle of E. *maxima*, and that the performance responses are due to the challenge depending on the treatments. We believe that the greater abundance of oocysts in the excreta of birds in the PC+Antibiotic treatment is due to the antibiotic's non-selective mechanism of action, which reduced or eliminated an entire healthy microbial population [42] promoting an increase in *E.maxima* in the gastrointestinal tract of these birds.

For the productive performance responses before the challenge, in the period from 1 to 14 days of age, the results demonstrate that the birds that received PC+antibiotic obtained higher BW, BWG and FI values than the PC treatment, indicating that the previous supplementation of antibiotics from the beginning of the birds' life, benefits productive performance responses.

On the other hand, in relation to productive performance in the age group of 15 to 28 years that corresponds to the challenged period, the challenge negatively affected the reduction of the FI and consequently the BWG. A decrease of about 10% in FI was previously observed by [43], who used a similar challenge model. Furthermore, the reduced FI directly reflects the lower BWG, due to the damage caused sub clinically by *E. maxima* and *C. perfringens*, in the small intestine of challenged birds that influenced nutrient utilization [43-44]. Reduction in FI and BWG due to the association of *E. maxima* and *C. perfringens* challenge models has been demonstrated laterally in broiler chickens [44-45]. Previous studies by [44-46-43] have shown that the most drastic reduction in FI occurs about three to seven days after inoculation. A study developed by [21] reports that broiler chickens immunologically challenged with *E. acervulina, E. maxima* and *C. perfringens*, from 8 to 21 days of age, broiler chickens that received the diet containing β-mannanase showed a reduction in FI compared to birds that received the same diet without enzyme supplementation. Our study corroborates this finding, since birds that received β-mannanase also had reduced FI, due to the proposed challenge.

In the period from 29 to 42 days, the challenge is less intense, and the results demonstrated that an increase in FI and BW were achieved in challenged birds fed with PC+Antibiotic, PC+β-mannanase and β-man+prob. The authors [47-48] demonstrated that broiler chickens aged four to six weeks, supplemented with β-mannanase in diets based on soybean meal and corn, increased the daily weight gain of the animals, evidencing a partial recovery in the performances after three weeks of the challenge. Authors [48], evaluating probiotic supplementation in broiler chicken diets, reported that probiotics significantly increased average daily weight gain during the first three-old-weeks but not during the next few weeks of growth (4-6). In a study conducted by [49] it was demonstrated that broiler chickens fed diets supplemented with 10^{8} CFU of *Bacillus subtilis* /kg had greater live weight gain, while other studies did not report the same positive effect [50-51].

The response (from 1 to 42 years) demonstrates that unchallenged birds had a greater productive performance in relation to the PC and PC+probiotic treatments, except in the FCR variable. This is related to the damage that coccidiosis, through *Eimeria*, causes to the epithelial tissue [52], causing desquamation of the epithelium,

resulting in damage to the intestinal villi, which impairs the absorption of nutrients and an energy expenditure for the repair of intestinal cells injured. Regarding the use of probiotics in broiler chickens, although several studies demonstrate their efficiency and viability, there is some conflicting literature on productive performance responses [53-54]. Several factors can alter the effectiveness of the probiotic, such as the route of administration, strains used, handling conditions when preparing the product, animal category and physiological state, in addition to the environment, which will jointly influence the response to the use of probiotics [55-56-57].

4.2. Gut health

The Advances in broiler genetics have made it possible to maximize weight gain and protein deposition. However, for broilers to reach their full genetic potential, we must pay attention to intestinal health. Intestinal health is of fundamental importance for the good performance of animals in the production chain, and there appears to be a direct relationship between a healthy intestine and productive performance. Although, there is still no clear definition that encompasses all physiological functions of the intestinal tract, such as digestion and absorption, host metabolism and energy production.

Variations in diet composition, as well as physical characteristics, can affect intestinal integrity. For example, the internal epithelium of the intestine is constantly changing (turn over) and the characteristic of the diet such as the higher fiber content can increase the turnover rate, reducing the physical barrier and becoming susceptible to the actions of pathogenic microorganisms [58]. Furthermore, depending on the remaining substrate in the feed, it can be a fuel for the proliferation of microorganisms, altering the composition of the microbiota [59]. Moreover, intestinal integrity is strongly correlated with microbiome diversity because intestinal functions are positively regulated by the microbiome, which has adequate mucosal wall development, functional intestinal barrier, and mucosal immune response [59]. The intestine regulates physiological homeostasis, allowing the animal to resist harmful and nonmalignant stressors [60]. Therefore, a healthy intestine has intact villi and crypts, a healthy microbiome and decreased intestinal permeability.

4.2.1. Intestinal morphometry

Villus height and crypt depth constitute the morphology of intestinal tissue and are indicators for measuring intestinal health, injury, and recovery [61]. Crypts are also measuring of cell multiplication, and shallower crypts indicate better intestinal health [62]. The size and density of villi are related to cell loss and renewal by the intestinal mucosa [63]. When there is a balance between these processes, a constant turnover occurs, that is, the maintenance of the size of the villi, also generating maintenance in the digestive and absorptive capacity of the intestine. In this sense, when we talk about the morphometric variables of villus height and crypt depth in general, our results demonstrated that supplementation with β-mannanase, probiotic or both together positively affect the morpho functional integrity of the digestive system.

At 21 days, supplementation with probiotics stimulated greater villi height in the duodenum due to the action of probiotics on microbiological development, in which the intestinal epithelium inhibits the colonization of pathogens, causing changes in the barrier against microorganisms, triggering benefits to the intestinal mucosa, thus favoring the structure of the villi [64-65]. It is hypothesized that the probiotic reduced the inflammatory process, resulting in greater height of the duodenal villi. Furthermore, CD in the jejunum was lower in PC+β-mannanase birds, a beneficial effect also demonstrated by [66-67].

At 42 days, in the three segments of the intestine the CD variable of the birds that received β-mannanase was lower compared to the other treatments. The villus-crypt ratio (V:C) in the duodenum and jejunum demonstrated that challenged birds receiving β-man+prob had a higher V:C value, that is, recovery in intestinal integrity. The beneficial effect of enzymatic and probiotic supplementation was due to the interaction of both additives, where the probiotic positively influenced the establishment of beneficial microbiota, protecting the integrity of the intestine, and the enzyme acts in the improvisation of nutrients from the feed [68-69-70]. The beneficial action of probiotic supplementation, such as *Lactobacillus acidophilus* and *C. butyricum*, was evidenced by the increase in the renewal of intestinal morphology, observed through the increase in the height of the villi and the reduction in the depth of the crypts [49-71-72].

In general, the joint use of additives provided broiler chickens with greater resistance to damage to the intestinal epithelium caused by *E. maxima* and *C. perfringens*. This is due to the modulation of intestinal health to maximize the defense of the immune system in terms of protection of epithelial tissues, demonstrating an immunomodulatory action that modifies the morphological structure of the intestine to reduce the harmful effects of immunological challenges.

4.2.2. Cecum microbiome

It was previously demonstrated that *Eimeria* spp. can cause major changes in microbiota composition in birds affected by necrotic enteritis [73-74-75]. Studies claim that the composition of the diet use of antibiotics, and other performance enhancers affect the amounts and the set of bacterial populations in the intestinal tract [76]. Incidentally, a stable and healthy intestinal ecosystem weakens the colonization of harmful microbial populations, enhancing intestinal barrier function and increasing growth performance [60].

Alpha diversity depicts a synopsis of diversity in a unique population. It has also been reported that alpha diversity can be related to baseline inferences about the mechanisms and functionalities of the microbiome [77]. Diversity richness refers to the amount of rare operational taxonomic units (OTUs) present in the samples. Therefore, the higher the index, the greater the number of rare OTUs. The diversity index is calculated, considering the abundance and number of OTUs present in a fragment. Additionally, the increase in the Shannon index concerns the increase in species richness and uniformity and, thus, diversity [78]. Authors [79] identified that greater richness is linked to good health, but species richness and diversity reduction may be directly related to pathologies.

In our study, the analysis of alpha diversity (Figure 2) reveals that at 43 days of age, the animals that received antibiotics, β-mannanase, or probiotics in the diet showed greater richness and microbial diversity in the cecum. It is believed that this greater richness is related to the action of the antibiotic, enzyme, and probiotic in modulating the microbiome of these birds, even in a situation of immunological challenge, to minimize the effects of harmful bacteria on the host. This result corroborates the findings of [21-22-10], who also report in their studies that β-mannanase had a modulating action on the microbiota of broilers fed corn-soy diets

and challenged with *C. perfringens* and *E. maxima.* Studies have shown that probiotics are effective in optimizing intestinal health and performance in birds, establishing a healthy microbiome, as they regulate the microbial community through competitive exclusion, producing antibodies, allowing intestinal development of beneficial microorganisms, as well as intestinal immunity [80-81-82]. Author [83] observed in their study that at 42 days the balance between the bacteria that make up the microbiome, regardless of the enzyme supplementation or the challenge occurred four weeks ago. And it indicates that the proper balance of microorganisms is probably not seen in high health challenges.

β-diversity is used when comparing the microbiome to determine the number of (OTU) or the shared rate, thus understanding how the actions of microbial species vary in numerous microbiomes. A dissimilarity index is provided as a response, for example, Bray -Curtis, which considers additional information such as the extent to which functions change in diverse microbiomes and shared memberships between microbiome profiles of numerous communities [84]. Furthermore, derivation interpretations of βeta diversity can be quantitative (weighted UniFrac) or qualitative (unweighted UniFrac) [77]. In our study, it was observed that the βeta diversity is very expressive in all treatments, showing that it changes with the advancing age of the animal and possibly with the presence of undesirable microorganisms as the challenge proposed in this study. In a healthy intestine, bacteria act synergistically, where each bacterium is an integral link in the generation of metabolites that the host uses. However, during an imbalance of gut microbiome, the result of inflammation by the host targets commensal microorganisms, causing a decrease in bacterial diversity and essential functions, thus a decrease in metabolic activity, inhibiting the host from numerous end products that limit intestinal health and bird performance [85-86]. In other words, at 22 days in the present study, a homogeneous population of bacteria according to Bray-Curtis and Jaccard parameters was observed in the cecum microbiome of birds that received β-mannanase in the diet compared to unchallenged birds or challenged without supplementation. This reflects less dissimilarity in the birds that received β-mannanase, that is, that the microbial communities have similar functions, in which they modulate the microorganisms in the cecum to bring benefits to the host.

It has been determined that a higher *Firmicutes/Bacteroidetes* (F/B) ratio favors chicken growth [87-88-89]. Consequently, the relationship between the amount of *Firmicutes* and *Bacteroidetes* has been used as evidence of bird's efficiency in synthesizing dietary nutrients. When comparing in our study the *Firmicutes/Bacteroidota* ratio between the treatments and the respective ages of the birds, in all treatments, this ratio was higher at 22 days of age when compared to 43 days. It is also observed that at 22 days this relationship was higher in treatments that received probiotic or β-man+prob, when compared to birds challenged without any supplementation. Therefore, we observe that even during a challenge by *E. maxima* and *C. perfringens* at 22 days, the birds tried to modulate their microbiota to minimize the losses related to the immunological challenge, and the animals that received the probiotic or β-mannanase associated with the probiotic, managed to achieve this beneficial modulation more effectively (Figure 4).

Studies report a significant continuous change in the taxonomic composition, which is more abundant and taxonomically varied as the bird ages [90]. Taken together, part of the modulations that occur in birds come from families, genera and species that play a crucial role in the homeostasis of the gastrointestinal tract. In our study, the main families that showed a significant difference between the treatments that were challenged without supplementation and the treatments supplemented with antibiotic, probiotic or β-mannanase were the families described in (Figure 5). This corroborates the findings of authors who describe that the families *Bacteroidaceae, Lactobacillaceae, Clostridiaceae, Ruminococcaceae, Lachnospiraceae, Enterobacteriaceae* and *Prevotellaceae* are common members of the cecal microbiota of chickens [91-92-93].

Bacteroidaceae family belonging to the Phylum *Bacteroidota was* present in greater quantity in the microbiome of birds challenged without supplementation at 22 days of age. Some bacteria of this family, in situations of dysbiosis, as caused by *Eimeria*, can multiply, and become pathogenic, consequently reducing the feed efficiency of the birds [94]. In our study, at 22 days of age, there was a significant decrease in the *Lachnospiraceae* family*,* belonging to the phylum *Firmicutes* in negative control birds, or challenged without supplementation, and an increase in those that received β-mannanase, probiotic and β-man+prob. This is one of the main

families whose function is to ferment non-digestible polysaccharides in the cecum, producing short-chain fatty acids that lead to the growth of epithelial cells [95], being the main generators of butyrate [96], considered an anti-inflammatory metabolite. Studies in pigs reported that the *Lachnospiraceae* family was abundant in the microbiota of dietary treatments containing B-mannanase [97]. Another study using the enzyme with broiler chickens reported that at 21 days of age, the cecal microbiome was compromised mainly by members of the *Ruminococcaceae* and *Lachnospiraceae* families [10].

However, when we analyze the *Butyricicoccaceae* family at 43 days of age, the treatment that received probiotics had a higher concentration of this family in the microbiome, compared to birds challenged without supplementation. The genus *Butyricoccus* has been described as beneficial for the intestinal microbiome [98]. Therefore, its greater predominance shows that the use of the probiotic was effective in the intestinal modulation of the cecum even after the immunological challenge. *Lactobacillaceae* family, there was an increase in their proportion in the microbiome of birds that were supplemented with β-mannanase. In a study where birds received β-mannanase, they also recorded an increase in the microbiota of the *Lactobacillaceae* and *Ruminococcaceae* family*,* and a reduction in bacteria associated with low feed efficiency [10]. It has already been seen that exogenous enzyme, including β-mannanase, have been supplemented in broiler diets to improve the digestibility of feed ingredients and modulate the intestinal microbiota of birds [99]. The *Lactobacillus* family at 22 days it was abundant in animals that received antibiotics. At 43 days of age, there was a reduction in this family in all treatments, compared to the age of 22 days of the birds. Members of this family, such as *Lactobacillus spp.,* play a beneficial role in intestinal health, immunological parameters and zootechnical performance [100] as well as selectively exclude pathogens from adhering to the intestine, due to their rapid proliferation and acidifying characteristics in the GIT [101]. One study shows an increase in the abundance of the *Lactobacillales* family in the microbiota at 28 days of broilers challenged with coccidiosis, receiving antibiotics (Enramycin and Tylosin), however, at 42 days, all treatments showed a reduction in the relative abundance of this family [102], corroborating our findings. The author [103] also observed a higher proportion of *Lactobacillaceae* in broiler chickens challenged with *Eimeria.* The

Ruminococcaceae family was in greater abundance at 43 days of age in the microbiome of birds that received probiotics in the diet compared to birds challenged without inclusion of any performance enhancer. It has been suggested that the increase in this family is related to broilers in those with lower feed conversion ratio [104]. That is, the results of the present study show that the animals challenged with the probiotic treatment, even though their feed conversion was higher, and a lower weight gain compared to other treatments, the probiotic had a positive action in the modulation of the microbiome, as *Ruminococcaceae* is known to produce butyrate and therefore helped in the regulation of inflammation [105]. In a study administering postbiotics in the feed, it was observed that the dominance of *Ruminococcaceae* at 28 days of age in the birds decreased by 24.2% in the birds of this treatment, and they obtained lower weight and higher feed conversion rate [106].

Certain pathological states induce the loss of diversity, with the increase in the concentration of certain bacterial genera to the detriment of others [78]. The genera that predominate in the cecum are *Clostridium, Lactobacillus, Bacteroides* [107], *Ruminococcus* [108] and *Prevotella* [109]. The genus *Agathobaculum* at 43 days of age it showed greater abundance in challenged birds that received probiotic in the diet, compared to challenged birds without supplementation. This genus is linked to butyrate -producing anaerobic bacteria [110]. This genus belongs to the *Firmicutes phylum,* so it is believed that its higher concentration in the treatment that received probiotics in this study is positively linked to the modulation of the microbiome of the cecum, since in general bacteria of this phylum can inhibit the growth of opportunistic pathogens and some are known to be involved in the breakdown of complex carbohydrates [111]. This may also be correlated with the benefits of probiotics, as they reduce and prevent colonization by enteric pathogens, through competitive exclusion and formation of bacteriostatic and bactericidal substances [108-109].

In our study, the genus *Faecalibacterum* at 22 days it was higher in the treatment of negative control birds compared to birds that received PC+Antibiotic. This genus has been described as having anti-inflammatory properties [112-113]. Decrease in the richness of *Faecalibacterium,* like butyrate producing bacteria, may impede the development of the immune response and depress the synthesis of butyrate used as an energy source [71]. A study using bulk sequencing techniques established the

composition of the microbiota, and on days 21 and 42, the genus *Faecalibacterium* apparently predominated [114]. The genus *Alistipes* at 22 days of age was more abundant in negative control birds when compared to challenged birds receiving β-mannanase in the diet. This genus, like its species, is known to have anti-inflammatory properties [115]. However, these bacteria produce butyrate and short chain fatty acids through two main pathways, the butyryl pathway (CoA: acetate CoA transferase) and the butyrate kinase pathway, in which these substances collaborate with physiological processes and energy homeostasis of the host [116]. A study by [10] found an inverse response when birds challenged at 21 days received β-mannanase obtained in the microbiota of the cecal content, the enzyme or increase of *Alistipes.* In general, with the aim of replacing antibiotics, the use of β-mannanase or probiotics can be an effective alternative in beneficially modulating the intestinal microbiota and mitigating the effects of the challenge.

4.2.3. HTSi (Health Tracking System)

The presence of visual intestinal lesions at necropsy can be used as a good measure to evaluate intestinal health disorders and can be related to the results of morphometric and microbiological analyzes, enabling the classification of intestinal health status. In the study, the incidence of abnormal characteristics in NC treatment birds was notably high compared to the group that received PC+probiotic in the feed at 22 days of age (Figure 7). In other words, through the HTSI score, birds supplemented with probiotics in their feed had a lower incidence of intestinal lesions at the time of the challenge. Therefore, the probiotic promoted an increase in intestinal protection and barrier functions, demonstrating improved intestinal health.

4.2.4. Intestinal permeability (FITC-Dextran)

According to [59], the action of the intestinal barrier is necessary to maintain the functionality of the intestine cells. Consisting of a single layer of epithelial cells trapped in columns, they perform the first line of protection of the organism against pathogens and harmful products present in the lumen [117]. Lymphoid tissue provides immune cells to help against pathogenic microorganisms [118]. The outer layer, which has a beneficial microbiota, and the inner layer, which defends the intestine, consisting of mucus rich in mucin and IgA, help preserve and control health, thus inhibiting the entry of pathogens. Furthermore, they protect the epithelium from anti-nutritional factors and

the effects of toxins [119]. Multiple conditions regulate intestinal permeability and determine which molecules can cross this barrier and enter the bloodstream [120]. Damage to the epithelial barrier has the potential to cause increased intestinal permeability that directly transports intraluminal macromolecules and pathogens into the blood [121]. Coccidiosis afflicts intestinal barrier function, which is critical to host health and defense, as demonstrated. Thus, an ideal food additive can promote effective gut barrier action [122]. The permeability test can influence the experimental conditions, which can be considered an index of the gut integrity and the absorption capacity of the enterocytes. Also, the induced lesions can be a rapid response on terms of permeability test and depending on what moment post-infection it occurs and the degree of challenge [123]. Thus, cell breakdown allows greater intestinal permeability, which was observed in a study by [43], and was corroborated by [123] who showed greater passage of FITC-dextran in the blood of birds challenged by *E.maxima.*

Another study observed by [124], reported that diets containing probiotics with 1 x 10^{6} CFU of *Bacillus amyloliquefaciens CECT 5940* per g of feed, through FITC-dextran analysis did not have a significant effect, so there was no significant influence on intestinal permeability. Similarly, [125] obtained the same effect in the birds using a *B. amyloliquefaciens* strain at 42 days of age. This result may be related to the fact that after the period of infection by *E. maxima* and *C. perfringens* birds regain intestinal health at the level of more serious injuries that affect the intestine. In an assay with a dose of 50,000 sporulated oocysts per bird, [123] they reported that if the birds in the experiment survived severe infection between five and seven dpi, gastrointestinal permeability would return to normal levels within nine dpi, regardless of the challenge dose administered. In our study, the challenge dose is not to cause mortality in the birds, so we can observe that they recover after the challenge. Through this, it is believed that the broilers in the present study recovered their intestinal health, and the non-significant concentration of Fic-dextran shows that intestinal permeability was not affected at this age (42 days).

5. Conclusions

The challenge with *E. maxima* and *C. perfringens* reduces feed consumption and weight gain of birds, negatively affecting production performance responses.

However, based on our findings, supplementation with β-mannanase, probiotic or β-mannanase+probiotic can prevent damage to intestinal integrity, modulating intestinal morphometry and the microbiome of the cecum, beneficially impacting the health of the gastrointestinal tract of these birds.

Author Contributions: All authors have read and agreed to the published version of the manuscript. “Conceptualization, L.P.M., N.K.S. and M.K; methodology, L.P.M., N.K.S., M.K. and B.B.L; validation, L.P.M., R.S.C., B.B.L., R.R.L. and B.V.M; formal analysis, L.P.M., B.B.L., R.S.C; resources, L.P.M., R.S.C., B.B.L. and B.V.M; data curation, L.P.M., R.R.L; writing—original draft preparation, L.P.M. and R.R.L; writing—review and editing, L.P.M., R.S.C., B.B.L., R.R.L. and B.V.M; visualization, L.P.M., R.S.C., B.B.L., R.R.L. and B.V.M; supervision, N.K.S. and M.K; project administration, L.P.M., N.K.S. and M.K. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Elanco Animal Health ®.

Institutional Review Board Statement: The animal study protocol was approved by the Animal Ethics Committee of the Faculty of Animal Science and Veterinary Medicine of the Universidade Estadual Paulista, Brazil (Protocol nº: 1061/22 - June 15, 2022).

Informed Consent Statement: Not applied.

Data Availability Statement: Data available upon request.

Acknowledgments: O presente trabalho foi realizado com apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Código de Financiamento: 88887.662245/2022-00.

Conflicts of Interest: The authors declare that there are no conflicts of interest regarding the publication of this article.

6. References

1. Fatoba, A. J., & Adeleke, M. A. Diagnosis and control of chicken coccidiosis: a recent update. *J. Parasit. Dis.: official organ of the Indian Society for Parasitology*. **2018** *42*, 483–493. https://doi.org/10.1007/s12639-018-1048-1.

2. Pham, H. H. S.; Matsubayashi, M., Tsuji, N.; & Hatabu, T. Relationship between Eimeria tenella associated-early clinical signs and molecular changes in the intestinal barrier function. *Veterinary immunology and immunopathology*. **2021**, *240*, 110321. https://doi.org/10.1016/j.vetimm.2021.110321.

3. Sharman, P.A.; Smith, N.C.; Wallach, M.G.; Katrib, M. Chasing the golden egg: vaccination against poultry coccidiosis. *Parasite Immunol.* **2010,** *32*, 590–598. https://doi.org/10.1111/j.1365-3024.2010.01209.x.

4. Wade, B.; Keyburn, A.L. The True Cost of Necrotic Enteritis. *World Poult.* **2015,** *31*, 6-7.

5. Allen, P.C.; Fetterer, R.H. Recent advances in biology of *Eimeria* species and diagnosis and control of infection with these coccidian parasites of poultry. *Clin. Microbiol. Rev.* **2002**, *15*, 58–65. https://doi.org/10.1128/cmr.15.1.58-65.2002.

6. DeGruttola, A.K.; Low, D.; Mizoguchi A.; Mizoguchi E. Current Understanding of Dysbiosis in Disease in Human and Animal Models. *Inflamm. Bowel. Dis*. **2016**; 22 :1137-1150. https://doi:10.1097/MIB.0000000000000750.

7. Williams, R.B. Intercurrent coccidiosis and necrotic enteritis of chickens: rational, integrated disease management by maintenance of gut integrity. *Avian pathol.* **2005**, *34*, 159–180. https://doi.org/10.1080/03079450500112195.

8. Collier, C.T.; Hofacre, C.L.; Payne, A.M.; Anderson, D.B.; Kaiser, P.; Mackie, R.I.; Gaskins, H.R. Coccidia-induced mucogenesis promotes the onset of necrotic enteritis by supporting *Clostridium perfringens* growth. *Vet. Immunol. Immunopathol*. **2008**, *122*, 104–115. https://doi.org/10.1016/j.vetimm.2007.10.014.

9. Zhou, Z.; Nie, K.; Huang, Q.; Li, K.; Sun, Y.; Zhou, R.; Wang, Z.; Hu, S. Changes of cecal microflora in chickens following *Eimeria tenella* challenge and regulating effect of coated sodium butyrate. *Exp. Parasitol.* **2017**, *177*, 73–81. https://doi.org/10.1016/j.exppara.2017.04.007.

10. Bortoluzzi, C.; Scapini, L.B; Ribeiro, M.V.; Pivetta, M.R.; Buzim, R.; Fernandes, J.I.M. Effects of β-mannanase supplementation on the intestinal microbiota composition of broiler chickens challenged with a coccidiosis vaccine. *Livest. Sci*. **2019**, *228*, 187–194. https://doi.org/10.1016/j.livsci.2019.09.001.

11. Companyó, R.; Granados, M.; Guiteras, J.; Prat, M.D. Antibiotics in food: legislation and validation of analytical methods. *Bioanal. Chem.* **2009**, *395*, 877-891. https://doi.org/10.1007/s00216-009-2969-4.

12. Menten, J.F.M. Probióticos, prebióticos e aditivos fitogênicos na nutrição de aves. In Simpósio sobre ingredientes na alimentação animal, Uberlândia, Brasil.

13. Adhikari, P., Kiess A., Adhikari R., Jha R., An approach to alternative strategies to control avian coccidiosis and necrotic enteritis*, J. Appl. Poult. Res.* **2020**, *29*, 515-534. https://doi.org/10.1016/j.japr.2019.11.005.

14. Furlan, R.L.; Macari, M.; Luquetti, B.C. Como avaliar os efeitos do uso de prebióticos, probióticos e flora de exclusão competitiva. In Simpósio técnico de incubação, matrizes de corte e nutrição. Balneário Camboriú, Brasil (**2004**).

15. Pelicia, K.; Mendes, A.A.M.; Saldanha, E.S.P.B.; Pizzolante, C, C; Takahashi, S.E.; Garcia, R.G.; Paz, I.C.L.A.; Quintero, R.R. Utilização de promotores biológicos para frangos de corte tipo colonial. *Ver. Bras. Ciên. Avíc.* **2004**, *6*, 21.

16. Hemarajata, P.; Versalovic, J. Effects of probiotics on gut microbiota: mechanisms of intestinal immunomodulation and neuromodulation. *Therap. Adv. Gastroenterol*. **2013**;*6* :39-51. https://doi:10.1177/1756283X12459294.

17. Yaqoob, M.U.; Yousaf, M.; Khan, M.I.; Wang, M. Effect of β-Mannanase Supplementation on Growth Performance, Ileal Digestibility, Carcass Traits, Intestinal Morphology, and Meat Quality in Broilers Fed Low-ME Diets. *Animals (Basel)*. **2022**; *12*, 1126. https://doi:10.3390/ani12091126.

18. Choct, M. Enzymes for the feed industry: past, present, and future. *Worlds J. Poult. Sci.* **2006**, *62*, 5–16. https://doi.org/10.1079/WPS200480.

19. Slominsk, B.A. Recent advances in research on enzymes for poultry diets. *Poult. Sci.* **2011**, *90*, 2013-2023. https://doi.org/10.3382/ps.2011-01372.

20. Ferreira Júnior, H.C. Avaliação da β-mannanase em dietas para frangos de corte. Master, Degree-Granting Universidade Federal de Viçosa, feb, 2014.

21. Jackson, M.E.; Anderson, D.M.; Hsiao, H.Y.; Mathis, G.F.; Fodge, D.W. Beneficial Effect of β-Mannanase Feed Enzyme on Performance of Chicks Challenged with *Eimeria* sp. and *Clostridium perfringens*. *Avian Dis.* **2003**, 47, 759-763. https://doi.org/10.1637/7024.

22. Latorre, Juan & Hernandez-Velasco, Xochitl & Bielke, Lisa & Vicente-Salvador, Jose & Wolfenden, R & Menconi, Anita & Hargis, B & Tellez, Guillermo. Evaluation of a *Bacillus* direct-fed microbial candidate on digesta viscosity, bacterial translocation, microbiota composition and bone mineralisation in broiler chickens fed on a rye-based diet. *Br. Poult. Sci.*, **2015**, *56.* https://doi.org/10.1080/00071668.2015.1101053.

23. Caldas, J.V.; Vignale, K.; Boonsinchai, N.; Wang, J.; Putsakum, M.; England, J.A. et al. The effect of β-mannanase on nutrient utilization and blood parameters in chicks fed diets containing soybean meal and guar gum. *Poult Sci.,* **2018**; 97 :2807-2817. https://doi.org/10.3382/ps/pey099.

24. Latham, R.E.; Williams MP, Walters HG, Carter B, Lee JT. Efficacy of β-mannanase on broiler growth performance and energy utilization in the presence of increasing dietary galactomannan. *Poult. Sci.,* **2017**; *97*: 549-556. https://doi.org/10.3382/ps/pex309.

25. Aviagen*. Ross Broiler Pocket Guide: The Pocket Guid*e. Aviagen, Huntsville, Alabama, USA, 2018.

26. Rostagno, H.S.; Albino, L.F.H.; Hannas, M.I.; Donzele, J.L.; Sakomura, N.K.; Perazzo, F.G.; Saravia, A.; De abreu, M.L.T.; Rodrigues, P.B.; De Oliveira, R.F.; Barreto, S.L.T.; Brito, C. D. *Tabelas Brasileiras para aves e suínos: Composição de Alimentos e exigências nutricionais,* 3nd ed.; Editor UFV, Viçosa, 2017.

27. Sakomura, N.K.; Rostagno, H.S. *Métodos de pesquisa em nutrição de monogástricos,* 2nd ed.; Editor FUNEP, 2016; pp262.

28. Gordon H.M.L. E Whitlock H.N. A new technique for counting nematode egg in sheep faeces. *J. Sci. Ind. Res*, **1939**, *12*, 50-52.

29. Gava, M.S. Metodologia de morfometria intestinal em frangos de corte. Master, Degree-Granting Universidade Federal do Rio Grande do Sul, apr, 2012.

30. Luna, L.G. *Manual of histologic staining methods of the Armed Forces Institute of Pathology*, 3nd ed.; Editor McGraw-Hill, New York. 1968.

31. INNIS, Michael A. Gelfand, D.H., Sninsky, J.J.; White, T.J. (Ed.). PCR protocols: a guide to methods and applications. *Academic press,* 2012.

32. Degnan, P.H.; Ochman, H. Illumina-based analysis of microbial community diversity. ISME J. **2012**, *6,* 183–194. https://doi.org/10.1038/ismej.2011.74.

33. Caporaso, J.G.; Kuczynski, J.; Stombaugh, J.; Bittinger, K.; Bushman, F.D.; Costello, E.K.; Fierer, N.; Peña, A.G.; Goodrich, J.K.; Gordon, J.I. QIIME allows analysis of high-throughput community sequencing data*. Nat. Methods,* **2010**, 7, 335–336. https://doi.org/10.1038/nmeth.f.303.

34. Yilmaz, P.; Parfrey, L.W.; Yarza, P.; Gerken, J.; Pruesse, E.; Quast, C.; Schweer, T.; Peplies, J.; Ludwig, W.; Glöckner, F.O. The SILVA and "All-species Living Tree Project (LTP)" taxonomic frameworks. *Nucleic Acids Res*. **2014,** 42, 643-648. https://doi.org/10.1093/nar/gkt1209.

35. Kasab-bachia H.; Arruda, A.G.; Roberts, T.E.; Wilson, J.B. The use of large databases to inform the development of an intestinal scoring system for the poultry industry. *Prev. Vet. Med.* **2017**, *146*, 130–135. https://doi.org/10.1016/j.prevetmed.2017.07.012.

36. Vicuña, E.A.; Kuttappan, V.A.; Galarza-seeber, R.; Latorre, J. D.; Faulkner, O. B.; Hargis, B. M.; ... Bielke, L. R. Effect of dexamethasone in feed on intestinal permeability, differential white blood cell counts, and immune organs in broiler chicks. *Poult. Sci*. **2015**, *94*, 2075-2080. https://doi.org/10.3382/ps/pev211.

37. McMurdie, P.J., Holmes, S. phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS One.,* **2013**, 8, 612-617. https://doi.org/10.1371/journal.pone.0061217.

38. Oksanen, J.; Kindt, R.; Legendre, P.; O'hara, B.; Stevens, M.H.H.; Oksanen, M.J.; Suggests, M. The vegan package*. Commun. Ecol. Packag*. **2007**, *10*, 719.

39. KruskaL, W.H.; Wallis, W.A. Use of Ranks in One-Criterion Variance Analysis. *J. Am. Stat. Assoc*. **1952**, *47*, 583–621. https://doi.org/10.1080/01621459.1952.10483441.

40. Dunn, O.J. Multiple Comparisons Using Rank Sums. *Technometrics,* **1964**, *6*, 241–252. https://doi.org/10.1080/00401706.1964.10490181.

41. Yuan, J.; Johnson, T.A.; Ajuwon, K.M.; Adeola, O. *Eimeria* infection-related intestinal dynamics and microbiome, growth performance, and nutrient utilization in broiler chickens fed diets supplemented with multienzyme. *Can. J. Anim. Sci.* **2022**, 103, 81-91. https://doi.org/10.1139/cjas-2022-0046.

42. Graña, A.L. Use of probiotics in broiler chicken feed. Magister, Concessão de Grau Universidade Federal de Viçosa" Viçosa, February, 2006.

43. De Freitas, L.F.V.B. Impact of coccidiosis challenge and balanced protein levels on the responses of broiler chickens. PhD, Degree-Granting Universidade Estadual Paulista "Júlio de Mesquita Filho", Jaboticabal, feb, 2023.

44. Kipper, M.; Andretta, I.; Lehnen, C.R.; Lovatto, P.A.; Monteiro, S.G. Meta-analysis of the performance variation in broilers experimentally challenged by *Eimeria* spp. *Vet. Parasitol*. **2013**, *196*, 77–84. https://doi.org/10.1016/j.vetpar.2013.01.013.

45. Bortoluzzi, C. Desempenho produtivo e microbiota intestinal de frangos de corte suplementados com B-ácidos do lúpulo (*Humulus lupulus*) após desafio com *Eimeria acervulina* e *E. tenella*. Master, Degree-Granting Escola Superior de Agricultura "Luiz de Queiroz", Piracicaba, feb, 2013.

46. Teng, P.Y.; Yadav, S.; de Souza Castro, F. L.; Tompkins, Y. H.; Fuller, A. L.; Kim, W. K. Graded Eimeria challenge linearly regulated growth performance, dynamic change of gastrointestinal permeability, apparent ileal digestibility, intestinal morphology, and tight junctions of broiler chickens. *Poult. Sci.* **2020**, *99*, 4203-4216. https://doi.org/10.1016/j.psj.2020.04.031

47. Zou, X.T.; Qiao, X.J.; XU, Z.R. Effects of β-mannanase (Hemicell) on growth performance and immunity of broilers. *Poult sci*. **2006**, *85*, 2175-2179. https://doi.org/10.1093/ps/85.12.2176.

48. Yeo, J.; Kim, K.I. Effect of feeding diets containing an antibiotic, a probiotic or cassava extract on growth and intestinal urease activity in broiler chicks. *Poult. Sci.* **1997**, *76*, 2, 381-385. https://doi.org/10.1093/ps/76.2.381.

49. Zhang, Z.F.; Zhou, T.X.; Ao, X.; Kim, I.H. Effects of β-glucan and *Bacillus subtilis* on growth performance, blood profiles, relative organ weight and meat quality in broiler chickens fed diets based on corn and soybean meal. *Livest. Sci.* **2012**, *150*, 419-424. https://doi.org/10.1016/j.livsci.2012.10.003.

50. Willis, W. L.; Reid, L. Investigating the effects of probiotic diets on broiler production and the presence of *Campylobacter* jejune. *Poult. Sci.* **2008**, *87*, 606-611. https://doi.org/10.3382/ps.2006-00458.

51. Lee, K.W.; Lee, S.H.; Lillehoj, H.S.; Li, G.X.; Jang, S.I.; Badu, U.S.; Park, M.S.; Kim, D.K.; Lillehoj, E.P.; Neurnann, A.P.; Rehberger, T.G.; Siragusa, G.R. Effects of direct-fed microbials on growth performance, gut morphometry, and immune characteristics in broiler chickens. *Poult. Sci.* **2010**, *89*, 203-216. https://doi.org/10.3382/ps.2009-00418.

52. Berto, Rafaela. Probióticos a base de *Bacillus* em rações de frangos de corte desafiados com Clostridium perfringens e Eimeria vacinal. Master, Degree-Granting Universidade Estadual do Oeste do Paraná, Marechal Cândido Rondon, Paraná, mar, 2023.

53. Majidi-Mosleh, A.; Sadeghi, A.A; Mousavi, S.N.; Chamani, M.; Zarei, A. Ileal MUC2 gene expression and microbial population, but not growth performance and immune 23 response, are influenced by in ovo injection of probiotics in broiler chickens. *British Poult. Sci.* **2017**, *58*, 40-45. https://doi.org/10.1080/00071668.2016.1237766.

54. Wu, Y.; Shao, Y.; Song, B.; Zhen, W.; Wang, Z.; Guo, Y.; Shahid, M.S.; Nie, W. Effects of *Bacillus coagulans* supplementation on the growth performance and gut health of broiler chickens with *Clostridium perfringens*-induced necrotic enteritis. *J. Anim. Sci. Biotechnol. 2***018**, 9, 1-9. https://doi.org/10.1186/s40104-017-0220-2.

55. Fuller, R. Nature of the determinant responsible for the adhesion of *lactobacilli* to chicken crop epithelial cells. *J. Gen. Microbiol.* **1975**, *87*, 245-250. https://doi.org/10.1099/00221287-87-2-245.

56. Otutumi, L.K.; Góis, M.B.; Garcia, E.R.M.; Loddi, M.M.. Variations on the efficacy of probiotics in poultry. *Prob. Anim.* **2012**, 203-230. https://doi.org/10.5772/50058.

57. Clavijo, V.; Flórez, M.J.V. The gastrointestinal microbiome and its association with the control of pathogens in broiler chicken production: A review. *Poult. Sci.* **2018***, 97*, 1006-1021. https://doi.org/10.3382/ps/pex359.

58. Singh, A.K., Kim, W.K. Effects of Dietary Fiber on Nutrients Utilization and Gut Health of Poultry: A Review of Challenges and Opportunities. *Animals (Basel)*. **2021**, 11 :181. https://doi:10.3390/ani11010181.

59. Song, J.; Xiao, K.; Ke, Y.; Jiao, L.F.; Hu, C.H.; Diao, Q.Y.; Shi, B.; Zhou, X. T. Effect of a probiotic mixture on intestinal microflora, morphology, and barrier integrity of broilers subjected to heat stress. *Poult. Sci.* **2014**, *93,* 581–588. https://doi.org/10.3382/ps.2013-03455.

60. Kogut, M.H. The effect of microbiome modulation on the intestinal health of poultry. *Anim. Feed Sci. Technol.* **2019**, 250, 32-40. https://doi.org/10.1016/j.anifeedsci.2018.10.008.

61. Golder, H.M.; Geier, M.S.; Forder, R.E.A.; Hynd, P.I.; Hughes, R.J. Effects of necrotic enteritis challenge on gut microarchitecture and mucin profile*. Br. Poult. Sci.* **2011**, *52*, 500-506. https://doi.org/10.1080/00071668.2011.587183.

62. Viola, E.S.; Vieira, S.L. Suplementação de acidificantes orgânicos e inorgânicos em dietas para frangos de corte: desempenho zootécnico e morfologia intestinal. *Ver. Bras. Zoot.* **2007**, 36, 1097-1104.

63. Maiorka, A.; Boleli, I.C.; Macari M. *Fisiologia Aviária Aplicada a Frangos de Corte*. 1RD ED.; Publisher: Funep/Unesp, Jaboticabal, 2002, pp.113-123.

64. Pelicano, E.R.L.; Souza, P.A.; Souza, H.B.A.; Figueiredo, D.F.; Boiago, M.M.; Carvalho, S.R.; Bordon, V.F. Intestinal mucosa development in broiler chickens fed natural growth promoters. *Braz. J. Poult. Sci.* **2005**, *7*, 221-229. https://doi.org/10.1590/S1516-635X2005000400005.

65. Khan, S.H. Probiotic microorganisms-identification, metabolic and physiological impact on poultry. *Worlds. Poult. Sci. J*, **2013**, *69,* 601–612. https://doi.org/10.1017/S0043933913000603.

66. Jeurissen, S.H.M.; Lewis, F.; Klis, J.D.V.; Mroz, Z.; Rebel, J.M.J.; Huurne, A.A.H.M. Parameters and Techniques to Determine Intestinal Health of Poultry as Constituted by Immunity, Integrity, and Functionality. *Current Issues of Intestinal Microbiology*, **2002**, *3*, 1-14.

67. Soares, E.S.R. Efeito continuado de simbiótico em dietas para poedeiras da fase de cria à produção. PhD, Degree-Granting Universidade Federal Rural de Pernambuco, Recife, 2023, pp.134.

68. Chaveerach, P., Keuzenkamp, DA Lipman, LJA e Van Knapen, F. Effect of organic acids in drinking water for young broilers on *Campylobacter* infection, volatile fatty acid production, intestinal microflora, and histological cellular changes. *Poult. Sci*, **2004**, *83*, 330-334. https://doi.org/10.1093/ps/83.3.330.

69. Van immerseel, Filip et al. Microencapsulated short-chain fatty acids in feed modify colonization and invasion shortly after *Salmonella enteritidis* infection in young chickens. *Poult. Sci*, **2004**, *83*, 69-74. https://doi.org/10.1093/ps/83.1.69.

70. Izat, A. L. et al. Effects of a buffered propionic acid in diets on the performance of broiler chickens and on microflora of the intestine and carcass. *Poult. Sci*, **1990**, *69*, 818-826. https://doi.org/10.3382/ps.0690818.

71. Chen, H. L., X. Y. Zhao, G. X. Zhao, H. B. Huang, H. R. Li, C. W. Shi, W. T. Yang, Y. L. Jiang, J. Z. Wang, L. P. Ye, Q. Zhao, C. F. Wang, and G. L. Yang. Dissection of the cecal microbial community in chickens after *Eimeria tenella* infection. *Parasit. Vectors.* **2020**, 13:56. https://doi.org/10.1093/ps/85.12.2106.

72. Lin, Y., S. Xu, D. Zeng, X. Ni, M. Zhou, Y. Zeng, H. Wang, Y. Zhou, H. Zhu, K. Pan, and G. Li. Disruption in the cecal microbiota of chickens challenged with *Clostridium perfringens* and other factors was alleviated by *Bacillus licheniformis* supplementation. *PLoS One*, **2018**, *12*:1–18. https://doi.org/10.1371/journal.pone.0182426.

73. Hume, EU; Clemente-Hernández, Sergio; Oviedo-rondón, EO. Effects of feed additives and *Eimeria* mixed species infection on the intestinal microbial ecology of broiler chickens. *Poult. Sci*, **2006**, *85*, 2106-2111. https://doi.org/10.1093/ps/85.12.2106.

74. Oviedo-Rondón, E.O. M.E. Hume, M.E. C. Hernández, C. S. Clemente-Hernández, Intestinal Microbial Ecology of Broilers Vaccinated and Challenged With Mixed *Eimeria* Species, and Supplemented with Essential Oil Blends, *Poult. Sci*, *85*, **2006**, 854-860, ISSN 0032-5791, https://doi.org/10.1093/ps/85.5.854.

75. Stanley, D., Hughes, R.J., Moore, R.J. Microbiota of the chicken gastrointestinal tract: influence on health, productivity, and disease. *Appl. Microbiol. Biotechnol*, **2014**, *98*, 4301–4310. https://doi.org/10.1007/s00253-014-5646-2.

76. Feye, K.M.; Baxter, M.F.A.; Tellez-isaias, G.; Kogut, M.H.; Ricke, S.C. Influential factors on the composition of the conventionally raised broiler gastrointestinal microbiomes. *Poult. Sci*, **2020**, *99,* 653-659. https://doi.org/10.1016/j.psj.2019.12.013.

77. Lozupone CA, Knight R. Global patterns in bacterial diversity. *Proc. Natl. Acad. Sci.* USA. **2007**, *104*: 11436–40. https://doi.org/10.1073/pnas.0611525104.

78. Derami, Mariana Silveira. Comparação entre o microbioma do trato respiratório de aves saudáveis e aves diagnosticadas com colibacilose/ Mestre- Mariana Silveira Derami- Universidade Estadual de Campinas- Campinas, SP: [s.n], **2019**.

79. Blaut, M., & Clavel, T. Metabolic diversity of the intestinal microbiota: implications for health and disease. J. Nutr, **2007**, *137*, 751S–5S. https://doi.org/10.1093/jn/137.3.751S.

80. Zhang ZF., Kim IH. Effects of multistrain probiotics on growth performance, apparent ileal nutrient digestibility, blood traits, cecal microbial excretion and excreted odor content in broiler chickens. *Poult. Sci*, **2014**, 93, 364 – 370. https://doi.org/10.3382/ps.2013-03314.

81. Pedroso, A. A., Batal, A. B., & Lee, M. D. Effect of in ovo administration of an adult-derived microbiota on establishment of the intestinal microbiome in chickens. *Am. J. Vet. Res.*, **2016**, *77*, 514–526. https://doi.org/10.2460/ajvr.77.5.514.

82. Pender, CM., Kim S., Potter TD, Ritszi MM, Young M, Dalloul, RA. Efeitos da suplementação in ovo de probióticos sobre o desempenho e imuno competência de frangos de corte ao desafio de *Eimeria*. *Benef. Microbes*, **2016**a *7*, 699 – 705. https://doi.org/10.3920/BM2016.0080.

83. Scapini, Lidiane Boareto. Suplementação de β-mannanase em dietas para frangos de corte criados em condições experimentais e comerciais, **2015.** Universidade Estadual do Paraná, Palotina. Dissertação de mestrado. Disponível em: < http://hdl.handle.net/1884/41318 >, 2023.

84. Martiny JBH, Eisen JA, Penn K, Allison SD, Horner-Devine MC. Os drivers da beta-diversidade bacteriana dependem da escala espacial. *Proc. Natl. Acad. Sci.* US A, **2011**, *108*:7850–4. https://doi.org/10.1073/pnas.1016308108.

85. Cisek, A. A.; Binek, Marian. Chicken intestinal microbiota function with a special emphasis on the role of probiotic bacteria. *Pol. J. Vet. Sci.*, **2014**, *17*, 2. https://doi.org/10.2478/pjvs-2014-0057.

86. Ducatelle, R., Eeckhaut, V., Haesebrouck F., Van Immerseel., F. A review on prebiotics and probiotics for the control of dysbiosis: present status and future perspectives. *Animal*, **2015**, *9*, 43-48, ISSN 1751-7311, https://doi.org/10.1017/S1751731114002584.

87. Singh, P.; Karimi, A.; Devendra, K.; Waldroup, P.W.; Cho, K.K.; Kwon, Y.M. Influence of penicillin on microbial diversity of the cecal microbiota in broiler chickens. *Poult. Sci.* **2013**, *92*, 272-276. https://doi.org/10.3382/ps.2012-02603.

88. Salaheen, S.; Kim, S.W.; Haley, B.J.; Van kessel, J.A.S.; Biswas, D. Alternative Growth Promoters Modulate Broiler Gut Microbiome and Enhance Body Weight Gain. *Front. Microbiol,* 26, **2017**. https://doi.org/10.3389/fmicb.2017.02088.

89. Hong, Y.; Cheng, Y.; Li, Y.; Li, X.; Zhou, Z.; Shi, D.; Li, Z.; Xiao, Y. Preliminary Study on the Effect of *Bacillus amyloliquefaciens* TL on Cecal Bacterial Community Structure of Broiler Chickens. *Biomed Res. Int.* **2019**, 1-11. DOI: 10.1155/2019/5431354.

90. Wielen, P. W. J. J., Keuzenkamp, D. A., Lipman, L. V., Knapen, F., & Biesterveld, S. Spatial and temporal variation of the intestinal bacterial community in commercially raised broiler chickens during growth. *Microbial ecology*, **2002**, *44*, 286-293. https://doi.org/10.1007/s00248-002-2015-y.

91. Callaway, T.R.; Dowd, S.E.; Wolcott, R.D.; Sun, Y.; McReynolds, J.L.; Edrington, T.S.; BYRD, J.A.; Anderson, R.C.; Krueger, N.; Nisbet, D.J. Evaluation of the bacterial diversity in cecal contents of laying hens fed various molting diets by using bacterial tag-encoded FLX amplicon pyrosequencing. *Poult. Sci.* **2009**, *88*, 298-302. https://doi.org/10.3382/ps.2008-00222.

92. Borda-molina, D.; Seifert, J.; Camarinha-silva, A. Current Perspectives of the Chicken Gastrointestinal Tract, and Its Microbiome. *Comput. Struct. Biotechnol*, **2018**, *16*, 131-139. https://doi.org/10.1016/j.csbj.2018.03.002.

93. Giacobbo, F.C.N.; Eyng, C.; Nunes, R.V.; De-souza, C.; Teixeira, L.V.; Pilla, R.; Suchodolski, J.S.; Bortoluzzi, C. Different enzymatic associations in diets of 69 broiler chickens formulated with corn dried at various temperatures. *Poult. Sci*. *100,* **2021**. https://doi.org/10.1016/j.psj.2021.01.035.

94. Betancourt, Liliana., Hume, Michael., Fernando Rodríguez, David Nisbet, Muhammad Umar Sohail, Germán Afanador-Tellez. Effects of Colombian oregano essential oil (Lippia origanoides Kunth) and *Eimeria* species on broiler production and cecal microbiota. *Poult. Sci*. **2019**, 98, 4777-4786, ISSN 0032-5791, https://doi.org/10.3382/ps/pez193.

95. Richards, P.; Fothergill, J.; Bernardeau, M.; Wigley, P. Development of the Caecal Microbiota in Three Broiler Breeds. *Front. vet. sci.* **2019,** *6*. https://doi.org/10.3389/fvets.2019.00201.

96. Antonissen, G Eeckhaut, V.; Van Driessche, K.; Onrust, L.; Haesebrouck, F.; Ducatelle, R.; Moore, R.J.; Van-immerseel, F. Microbial shifts associated with necrotic enteritis. *Avian Path*, **2016**, *45*, 308-312. https://doi.org/10.1080/03079457.2016.1152625.

97. Genova JL, Rupolo PE, Azevedo LBd, Henz D, Carvalho ST, Kipper M, Gonçalves GdAC, Vilela HLO, Pasquetti TJ, Oliveira NTEd, Dietrich ARM and Carvalho PLdO. β-mannanase supplementation in diets reduced in 85 kcal metabolizable energy/kg

containing xylanase-phytase improves gain to feed ratio, nutrient usage, and backfat thickness in finisher pigs. *Front. Vet. Sci*, **2023**, 10:1144692. https://doi.org/10.3389/fvets.2023.1144692.

98. Alvarenga, Bauer Oliveira. Relação entre o aspecto morfológico de fezes de frangos de corte e sua composição bacteriana. Mestrado. Universidade de São Paulo. Faculdade de Medicina Veterinária e zootecnia. Departamento de Patologia, São Paulo, 72 f. **2020**.

99. Adeola O., Cowieson AJ. Board invited review: Opportunities and challenges in the use of exogenous enzymes to improve non-ruminant animal production. *J. Anim. Science*, **2011**, *89*, 3189–3218. https://doi.org/10.2527/jas.2010-3715.

100. Joat, N., Van, T. T. H., Stanley, D., Moore, R. J., & Chousalkar, K. Temporal dynamics of gut microbiota in caged laying hens: a field observation from hatching to end of lay. *Appl. Microbiol. Biotechnol.*, **2021**, 105, 4719-4730. https://doi.org/10.1007/s00253-021-11333-8.

101. McReynolds, J., Waneck, C., Byrd, J., Genovese, K., Duke, S., & Nisbet, D. Efficacy of multistrain direct-fed microbial and phytogenetic products in reducing necrotic enteritis in commercial broilers. *Poult. Sci.* **2009**, *88*, 2075-2080. https://doi.org/10.3382/ps.2009-00106.

102. Pires, P. G. D. S., Torres, P., Teixeira Soratto, T. A., Filho, V. B., Hauptli, L., Wagner, G., ... & Moraes, P. D. O. Comparison of functional-oil blend and anticoccidial antibiotics effects on performance and microbiota of broiler chickens challenged by coccidiosis. *Plos one*, **2022**, *17*, e0270350. https://doi.org/10.1371/journal.pone.0270350.

103. Vieira, Alexandre. Mistura de óleos funcionais como promotor de crescimento e seu efeito na microbiota intestinal de aves e suínos. **2020**. Graduação. Universidade Estadual de Santa Catarina, Florianólis- SC. 122 p.

104. Singh, K.M.; Shah, T.; Deshpande, S.; Jakhesara, S.J.; Koringa, P.G.; Rank, D.N.; Joshi, C.G. High through put 16S rRNA gene-based pyrosequencing analysis of the fecal microbiota of high FCR and low FCR broiler growers. *Mol. Biol. Rep.*, **2012**, *39*, https://doi.org/10.1007/s11033-012-1947-7.

105. Morgan, X. C., Tickle, T. L., Sokol, H., Gevers, D., Devaney, K. L., Ward, D. V., Reyes, J. A., Shah, S. A., LeLeiko, N., Snapper, S. B., Bousvaros, A., Korzenik, J., Sands, B. E., Xavier, R. J., & Huttenhower, C. Dysfunction of the intestinal microbiome in inflammatory bowel disease and treatment. *Genome Biol*. **2012**, *13*, R79. https://doi.org/10.1186/gb-2012-13-9-r79.

106. Kaufmann, Cristine. Níveis de energia metabolizável de dietas para frangos de corte suplementadas com enzimas exógenas e pósbiótico- Mestre. Universidade Estadual do Oeste do Paraná. Marechal Cândido Rondon, **2023**. 84 p.

107. Stanley, D.; Geier, M.S.; Chen, H.; Hughes, R.J.; Moore, R.J. Comparison of fecal and cecal microbiotas reveals qualitative similarities but quantitative

differences. *BMC Microbiology*, **2015**, *15*, 51. https://doi.org/10.1186/s12866-015-0388-6.

108. Wei, S.; Morrison, M.; Yu, Z. Bacterial census of poultry intestinal microbiome. *Poult. Sci*. **2013**, *92*, 671-683. https://doi.org/10.3382/ps.2012-02822.

109. Pan D, Yu Z. Gut microbiome of birds and its interaction with host and diet. *Micróbios Intestinais***. 2014**; *5*:108–19. DOI: 10.4161/gmic.26945.

110. Ahn, S., Jin, T. E., Chang, D. H., Rhee, M. S., Kim, H. J., Lee, S. J., Park, D. S., & Kim, B. C. *Agathobaculum butyriciproducens* gen. nov. sp. nov., a strict anaerobic, butyrate-producing gut *bacterium* isolated from human faeces and reclassification of *Eubacterium* desmolans as *Agathobaculum* desmolans comb. nov. *Int. J. Syst. Evol. Microbiol.* , **2016**, *66*, 3656–3661. https://doi.org/10.1099/ijsem.0.001195.

111. Kirsty B, Daniella D, Erin M, Gibson DL. Diet-induced dysbiosis of the intestinal microbiota and the effects on immunity and disease. *Nutrients*. **2012**; *4*: 1095–119. doi: 10.3390/nu4081095.

112. Sokol, H.; Pigneur, B.; Watterlot, L. et al. *Faecalibacterium prausnitzii* is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of Crohn disease patients. *PNAS*, **2008**, *105*, 16731-16736. doi: 10.1073/pnas.0804812105.

113. Fiagá, Dany Alberto Mesa. Microbiota intestinal em frangos de corte. Doutorado- Universidade Federal do Paraná. Curitiba-PR, 107 f. **2018**.

114. Oakley, B.B.; Buhr R.J.; Ritz, C.W.; Kiepper., B.H.; Berrang M.E.; BS, Seal, N.A. Cox. Successive changes in chicken cecal

microbiome during 42 days of growth are independent of organic acid additives. *Veterinary BMC. Res.,* **2014**, *10*, 282. https://doi.org/10.1186/s12917-014-0282-8.

115. Jiang, H., Ling, Z., Zhang, Y., Mao, H., Ma, Z., Yin, Y., Wang, W., Tang, W., Tan, Z., Shi, J., Li, L., & Ruan, B. Altered fecal microbiota composition in patients with major depressive disorder. *Brain Behav. Immun*., **2015**, *48*, 186–194. https://doi.org/10.1016/j.bbi.2015.03.016.

116. Du, X., Xiang, Y., Lou, F., Tu, P., Zhang, X., Hu, X., Lyu, W., & Xiao, Y. Microbial Community and Short-Chain Fatty Acid Mapping in the Intestinal Tract of Quail. *Animals (Basel)*, **2020**, *10*, 1006. https://doi.org/10.3390/ani10061006.

117. Moeser, A. J., C. V. Klok, K. A. Ryan, J. G. Wooten, D. Little, V. L. Cook, And A. T. Blikslager. “Stress signaling pathways activated by weaning mediate intestinal dysfunction in the pig.” *Am. J. Physiol. Cell Physiol.* Gastrointestal and Liver Physiology, **2007**, *292*: G173–G181. https://doi.org/10.1152/ajpgi.00197.2006.

118. Brisbin, J. T., Gong, J. And Sharif, S. “Interactions between commensal bacteria and the gut-associated immune system of the chicken.” *Anim. Health Res. Rev.,* **2008**, *9*: 101-110. https://doi.org/10.1017/s146625230800145x.

119. Celi, P., Verlhac, V., Pérez C. E., Schmeisser, J. And Kluenter, A. M. "Biomarcadores da funcionalidade gastrointestinal na nutrição e saúde animal." *Rev. Acad. Ciên. Anim.,* **2019**, *250*: 9-31. doi: https://doi.org/10.1016/j.anifeedsci.2018.07.012.

120. Camilleri M. Leaky gut: mechanisms, measurement, and clinical implications in humans. *Gut*, **2019**, *68*, 1516–1526. https://doi.org/10.1136/gutjnl-2019-318427.

121. Hornbuckle, W. E., Simpson, K. W., & Tennant, B. C. Gastrointestinal Function. *Clin. Biochem of Dom. Anim.*, **2008**, 413–457. https://doi.org/10.1016/B978-0-12-370491-7.00014-3.

122. Madlala, T., Okpeku, M., & Adeleke, M. A. Understanding the interactions between *Eimeria* infection and gut microbiota, towards the control of chicken coccidiosis: a review. *Parasite* (Paris, France), **2021**, *28*, 48. https://doi.org/10.1051/parasite/2021047.

123. Teng, P. Y., Choi, J., Tompkins, Y., Lillehoj, H., & Kim, W. Impacts of increasing challenge with Eimeria maxima on the growth performance and gene expression of biomarkers associated with intestinal integrity and nutrient transporters. *Vet. Res.*, **2021**, *52*, 1-12. https://doi.org/10.1186/s13567-021-00949-3.

124. Gharib-Naseri, K., de Paula Dorigam, J. C., Doranalli, K., Kheravii, S., Swick, R. A., Choct, M., & Wu, S. B. Modulations of genes related to gut integrity, apoptosis, and immunity underlie the beneficial effects of *Bacillus amyloliquefaciens CECT 5940* in broilers fed diets with different protein levels in a necrotic enteritis challenge model. *J. Anim. Sci. Biotechnology*. **2020**, *11*, 104. https://doi.org/10.1186/s40104-020-00508-4.

125. Maria, Larissa Pereira. Avaliação do efeito da suplementação de um probiótico associado a diferentes níveis dietéticos de proteína balanceada em frangos de corte desafiados por *Eimeria maxima*. Graduation, **2021**, Universidade Estadual Paulista "Júlio de Mesquita Filho" Unesp, Jaboticabal, Brazil.

CAPÍTULO 3 – IMPLICAÇÕES

O cenário atual da cadeia avícola, demonstra uma crescente nos desafios sanitários que acometem as aves de corte, impactando de forma negativa sua saúde intestinal e o desempenho produtivo. Para minimizar as perdas referentes aos danos causados pela coccidiose aviária, o uso de antibióticos é empregado. Entretanto, o uso desenfreado de promotores de crescimento trouxe à tona questões relacionadas a resistência animal e humana aos antibióticos.

Sendo assim, surgisse alternativas aos antibióticos, a fim de evitar as perdas do desempenho e a saúde das aves em desafio entérico. A β-mannanase age estimulando a quebra dos β-mananos que estão presentes nos ingredientes das rações das aves e são considerados fatores antinutricionais por aumentarem a viscosidade da digesta dificultando assim a digestibilidade e absorção dos nutrientes. De maneira distinta o probiótico é composto por microrganismos vivos que são capazes de beneficiar o animal hospedeiro pelo equilíbrio da microbiota intestinal, através de um de seus mecanismos em que os probióticos aderem-se a parede do epitélio intestinal, dificultam a aderência de microrganismos patogênicos.

Apesar do modo de ação dos aditivos serem distintos, ambos aditivos nutricionais demonstraram serem eficientes na saúde intestinal das aves. Salienta-se que através do presente estudo, foi possível comprovar a eficácia da suplementação de β-mannanase e probiótico nas dietas de frango de corte desafiados imunologicamente por *E. maxima* e *C. perfringens*.