UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
FACULDADE DE AGRONOMIA
PROGRAMA DE PÓS-GRADUAÇÃO EM ZOOTECNIA

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FITOGÊNICOS: UMA ALTERNATIVA PARA O CONTROLE DA COCCIDIOSE AVIÁRIA

Porto Alegre
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Tese apresentada como requisito para obtenção do Grau de Doutor em Zootecnia, na Faculdade de Agronomia, Universidade Federal do Rio Grande do Sul.

Orientadora: Prof.ª Dr.ª Andréa Machado Leal Ribeiro

Porto Alegre
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RESUMO

A coccidiose é uma enterite potencialmente grave causada por espécies de parasitas intracelulares obrigatórios do gênero *Eimeria*. Esses parasitas causam perdas econômicas significativas para a avicultura, principalmente devido ao comprometimento da eficiência da produção e do custo em controlá-los. O controle da coccidiose é, portanto, um esforço importante e estratégias alternativas ao uso isolado de anticoccidianos e/ou vacinas são abordagens interessantes para serem aplicadas à indústria avícola. Os aditivos fitogênicos, comumente definidos como aditivos vegetais ou botânicos, representam um grupo de substâncias naturais, derivados de plantas, ervas e seus extratos, que podem apresentar efeitos positivos sobre a produção e a saúde dos animais. O objetivo geral deste projeto foi avaliar os efeitos de dois diferentes compostos fitogênicos associados ou não a ionóforos na dieta de frangos de corte desafiados por oocistos esporulados de *Eimeria* spp. e o impacto no desempenho, na microbiota e no sistema imune. Nos dois experimentos que compõem este projeto, as aves foram expostas a um desafio intestinal com *E. tenella* (1×10^4 oocistos), *E. acervulina* (2×10^5 oocistos) e *E. maxima* (8×10^4 oocistos) por via oral aos 14 dias de idade. O consumo de ração e o ganho de peso para cada repetição foram medidos semanalmente. Aos 28 dias, o conteúdo intestinal dos frangos foi coletado para análise da microbiota, realizada pela plataforma Miseq Illumina. No experimento do artigo 1, uma porção da junção jejuno-íleo foi usada para análise de expressão gênica, realizada no StepOnePlus™ Real-Time PCR System. No artigo 1, o aditivo fitogênico (à base de *Acacia concinna* e *Saccharum officinarum*) e a monensina aumentaram a abundância de grupos bacterianos importantes para o estabelecimento de uma microbiota intestinal favorável. Já no artigo 2, observou-se que a salinomicina em combinação à mistura comercial de óleos funcionais (líquido da casca de castanha de caju e óleo de mamona) mostrou efeito sinérgico no desempenho e na modulação da microbiota intestinal de frangos de corte submetidos a desafio intestinal com *Eimeria*, minimizando o impacto da coccidiose.

Palavras-chave: Aditivos; *Eimeria*; Frangos de corte; Microbiota intestinal.

ABSTRACT

Coccidiosis is a potentially serious enteritis caused by species of obligate intracellular parasites of the genus *Eimeria.* These parasites cause significant economic losses for the poultry industry, mainly due to the compromise of production efficiency and cost of control. Coccidiosis control is therefore an important effort and alternative strategies to the isolated use of anticoccidials and/or vaccines are interesting approaches to be applied to the poultry industry. Phytogenic feed additives, commonly defined as plant-based feed additives or botanicals, represent a group of natural substances, derived from herbs, spices, other plants and their extracts, that can have positive effects on production and animal health. The general objective of this project was to evaluate the effects of two different phytogenic products associated or not with ionophores in the diet of broilers challenged by sporulated oocysts of *Eimeria* spp. and the impact on performance, microbiota and the immune system. In the two experiments that make up this project, birds were exposed to an intestinal challenge with *E. tenella (*1×10^4 oocysts), *E. acervulina* (2×10^5 oocysts) and *E. maxima* (8×10^4 oocysts) administered orally at 14 days old. Feed consumption and weight gain for each repetition were measured weekly. At 28 days, the intestinal contents of the chickens were collected for microbiota analysis, performed by the Miseq Illumina platform. In the experiment in article 1, a portion of the jejunum-ileum junction was used for gene expression analysis, performed on the StepOnePlus™ Real-Time PCR System. In article 1, the phytogenic additive (based on Acacia concinna and Saccharum officinarum) and monensin increased the abundance of bacterial groups important for the establishment of a favorable intestinal microbiota. In article 2, it was observed that salinomycin in combination with a commercial mixture of functional oils (cashew nutshell liquid and castor oil) showed a synergistic effect on the performance and modulation of the intestinal microbiota of broilers submitted to challenge. intestinal tract with Eimeria, minimizing the impact of coccidiosis.

Keywords: Additives; Brolilers; *Eimeria*; intestinal microbiota.

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LISTA DE ABREVIATURAS E SIGLAS

AGP – Antibiotic growth promoter
APC – Antibióticos promotores de crescimento
AST – Teste de sensibilidade a antimicrobianos
CD – Cluster of differentiation
CNSL – Cashew nutshell liquid
COX – Cyclooxygenase
DNA – Deoxyribonucleic acid
dNTP – Deoxyribonucleotide triphosphate
FO – Functional oil
IFN-γ – Interferon-gamma
IL - Interleukin
INOS – Inducible nitric oxide synthase
GAPDH – Glyceraldehyde-3-phosphate-dehydrogenase
GALT – Gut-associated lymphoid tissue
mRNA – Messenger ribonucleic acid
MUC – Mucin
NCBI – National biotechnology information center
NK – Células natural killer
OTU - Taxonomic operational units
PFA – Phytogenic feed additives
ppm – Partes por milhão
qPCR – Quantitative polymerase chain reaction
RNA – Ribonucleic acid
rRNA – Ribosomal ribonucleic acid
SCARB – Scavenger receptor class B member
SCFA – Short-chain fatty acid
SS66 – Sodium salinomycin 66 ppm
TFF – Trefoil factor family
TGI – Trato gastrointestinal
TLR – Receptor do tipo toll-like
TNFSF – Tumor necrosis factor superfamily

LISTA DE SÍMBOLOS

γ – Gamma
® – Registrado
μ – Micro
% – Porcentagem
°C – Grau Celsius

SUMÁRIO

CAPÍTULO I

1. INTRODUÇÃO

A manutenção de um ambiente intestinal saudável é um pré-requisito para o desempenho eficiente de frangos de corte (MURUGESAN et al., 2015). No entanto, saúde e bem-estar são ameaçados constantemente por diversos patógenos e protozoários parasitas, que representam um desafio contínuo à avicultura mundial (SOUTTER et al., 2020). A coccidiose, uma infecção causada por protozoários do gênero *Eimeria*, é uma das doenças parasitárias mais significativa na indústria de frangos e um grande problema em todo o mundo, acarretando enormes perdas anuais (MADLALA; OKPEKU; ADELEKE, 2021).

A infecção por *Eimeria* é caracterizada pela colonização da mucosa intestinal do hospedeiro seguida de destruição celular, o que resulta em elevada permeabilidade, prejudicando a digestão e a absorção de nutrientes (DALLOUL; LILLEHOJ, 2005; MADLALA; OKPEKU; ADELEKE, 2021). Esse dano intestinal não apenas afeta as células epiteliais, mas também ocasiona grande desequilíbrio das comunidades microbianas intestinais, promovendo a colonização e a proliferação de outros patógenos, como *Clostridium perfringens* (COLLIER et al., 2008) e *Campylobacter jejuni* (MACDONALD et al., 2019).

As infecções causadas por *Eimeria* podem ser facilmente transmitidas entre os hospedeiros pela via fecal-oral direta por meio da ingestão de oocistos esporulados presentes na cama ou na ração (DALLOUL; LILLEHOJ, 2005). Sete espécies reconhecidas de *Eimeria* causam coccidiose em vários graus de severidade (QUIROZ-CASTAÑEDA; DANTÁN-GONZÁLEZ, 2015), sendo a *E. acervulina*, a *E. maxima* e a *E. tenella* as principais espécies relacionadas a frangos de corte. Cada espécie tem seu próprio espectro de hospedeiro, sua própria predileção por locais específicos no intestino e sua própria capacidade de causar diferentes tipos de lesões no trato gastrointestinal (TGI) (TEWARI; MAHARANA, 2011).

Os principais métodos de controle da coccidiose, além da higiene e da biosseguridade, são a quimioprofilaxia e a vacinação das aves. Entretanto, ainda que diferentes estratégias sejam empregadas para reduzir a resistência anticoccidiana,

cepas resistentes aos principais compostos químicos utilizados na avicultura são frequentemente encontradas (FERDJI et al., 2022; KRAIESKI et al., 2021). Nesse sentido, estratégias alternativas seguras necessitam ser estudadas constantemente para controlar a coccidiose aviária (ABBAS; COLWELL; GILLEARD, 2012), entre as quais estão os aditivos fitogênicos.

O termo fitogênico refere-se às diferentes partes de plantas, ervas aromáticas e especiarias, bem como aos seus respectivos extratos vegetais na forma de óleos essenciais (WINDISCH et al., 2008). Muitas propriedades benéficas dos compostos fitogênicos derivam de suas moléculas bioativas, como alcaloides, flavonoides, saponinas, taninos fenólicos, terpenoides, carvacrol, timol, piperina, entre outros (WINDISCH et al., 2008). As atividades biológicas dessas fitomoléculas já foram documentadas, assim como suas funções antibacterianas, antioxidantes, antivirais, antiparasitárias e inseticidas.

Nesse contexto, o presente estudo tem como objetivo avaliar diferentes compostos fitogênicos associados ou não a ionóforos na dieta de frangos de corte desafiados por coccídios vivos e o impacto no desempenho, na microbiota e no sistema imune.

2. REVISÃO BIBLIOGRÁFICA

Neste capítulo encontra-se uma análise sobre os principais aspectos da coccidiose aviária, entre eles agente etiológico, ciclo da espécie, patogenia, controle, resistência e alternativas para o controle dessa enfermidade. Esses assuntos são relevantes para o entendimento do comportamento da doença e seu reflexo no desempenho e na modulação da microbiota intestinal de frangos de corte.

2.1 Coccidiose aviária

A coccidiose é uma doença entérica causada por protozoários intracelulares obrigatórios do gênero *Eimeria*, caracterizada como uma doença de alto impacto econômico na indústria avícola mundial (QUIROZ-CASTAÑEDA; DANTÁN-GONZÁLEZ,

2015). Atualmente, sete espécies de *Eimeria* são conhecidas por infectarem aves domésticas (*E. acervulina*, *E. brunetti*, *E. maxima*, *E. mitis*, *E. necatrix*, *E. praecox* e *E. tenella*), diferindo quanto à patogenicidade, ao local de invasão no intestino e ao tipo de lesão produzida (TEWARI; MAHARANA, 2011).

E. acervulina, *E. maxima* e *E. tenella*, são consideradas as mais importantes devido à patogenicidade, à prevalência global e ao impacto econômico causado (ATTREE et al., 2021).

A coccidiose varia significativamente em sua severidade e em impacto na saúde e na produtividade individual dos frangos. Dependendo da espécie do parasita, dose infectante, idade e estado imunológico do hospedeiro, as aves infectadas podem mostrar leves sinais clínicos ou podem sofrer efeitos que resultam em redução no ganho de peso esperado, conversão alimentar prejudicada, crescimento retardado devido à má absorção ou à diarreia, enterite grave e, em poucos casos, morte (ATTREE et al., 2021).

Uma consideração importante sobre a coccidiose é o reconhecimento, como uma doença economicamente significativa à produção animal. Em 1999, Williams publicou uma das estimativas mais abrangentes para o custo da coccidiose em aves, apresentando um modelo compartimentado para os custos de profilaxia, tratamento e perdas produtivas, o qual indicou um custo total superior a £ 38 milhões ao Reino Unido em 1995. Após 25 anos dessa análise, a aplicação do mesmo modelo a dados de Brasil, Egito, Guatemala, Índia, Nova Zelândia, Nigéria e Estados Unidos resultou em estimativas que, quando extrapoladas por região geográfica, indicam um custo global de aproximadamente £ 10,4 bilhões a preços de 2016, equivalente a £ 0,16 / frango produzido (BLAKE et al., 2020).

Assim, devido aos impactos significativos na economia mundial e no bem-estar dos animais, a necessidade de manejo e controle contínuos dos parasitas de *Eimeria* spp. permanece essencial. O controle adequado dessa enfermidade requer uma compreensão do complexo ciclo de vida e da transmissão de *Eimeria* spp. (KEETON; NAVARRE, 2018).

2.2 Ciclo de vida

O ciclo de vida da *Eimeria* inclui basicamente dois estágios: uma fase exógena (esporogonia) e uma fase endógena (esquizogonia e gametogonia), sendo esta composta por estágios assexuado e sexuado (Figura 1) (TEWARI; MAHARANA, 2011).

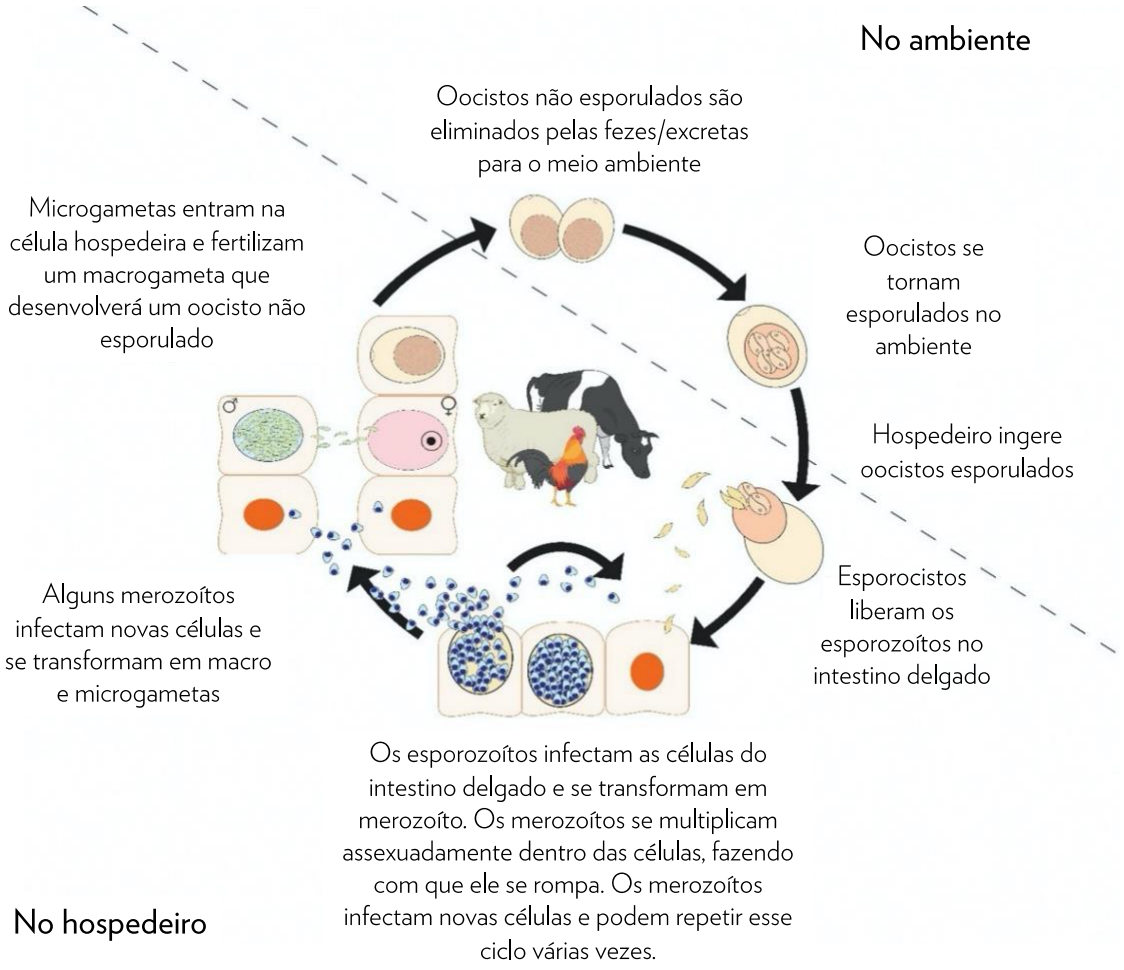

Figura 1. Ciclo de vida da *Eimeria* (Adaptado de KEETON; NAVARRE, 2018).

O ciclo de vida da *Eimeria* é iniciado quando os hospedeiros suscetíveis ingerem oocistos esporulados de ambientes contaminados (ATTREE et al., 2021). O oocisto, ao ser ingerido, é rompido na moela por trituração mecânica, liberando os esporocistos. No intestino, devido à ação de sais biliares e tripsina, os esporozoítos saem ativamente do esporocisto e invadem as células epiteliais intestinais, permanecendo dentro de vacúolos parasitóforos durante seu desenvolvimento em trofozoítos. Os trofozoítos iniciam a

replicação assexuada, ponto em que o parasita é referido como um esquizonte. Cada esquizonte é capaz de formar milhares de merozoítos de primeira geração. Após o término de um ciclo de esquizogonia, as células hospedeiras são destruídas e os merozoítos entram no lúmen intestinal, onde infectam novas células epiteliais. Após várias gerações de merogonia, o parasita entra na replicação sexuada, formando os estágios dimórficos de macrogametas e microgametas. Os microgametas entram na nova célula hospedeira e fertilizam os macrogametas para produzir zigotos (FERGUSON et al., 2003). Após o zigoto se tornar um oocisto, ele é então liberado no ambiente externo por meio das fezes (SHIRLEY; SMITH; TOMLEY, 2005). Os coccídios realizam uma série de atividades vitais no intestino do hospedeiro, incluindo colonização, crescimento e reprodução, perturbando assim o equilíbrio do ambiente intestinal.

No ambiente externo, ou seja, fora do hospedeiro, sob condições favoráveis de temperatura, oxigênio e umidade, o oocisto sofre um processo de esporulação, envolvendo meiose e mitose, que resulta na formação do oocisto esporulado, o qual contém quatro esporocistos com dois esporozoítos em cada (KEETON; NAVARRE, 2018). De maneira geral, o ciclo de vida da *Eimeria* leva em torno de 7 dias para ser concluído, dependendo da espécie e das condições ambientais (CHAPMAN et al., 2013).

2.3 Patogenicidade de *Eimeria* sp.

As sete espécies de *Eimeria* apresentam graus variados de patogenicidade aos seus hospedeiros, sendo que *E. mitis* e *E. praecox* são pouco ou não patogênicas, *E. acervulina* e *E. maxima* apresentam média patogenicidade e *E. brunetti*, *E. necatrix* e *E. tenella* são de alta patogenicidade, esta última capaz de causar mortalidade das aves quando em alto grau de infecção. Além disso, cada espécie possui características próprias quanto a sítio de infecção no intestino, lesão, imunogenicidade, tamanho de oocistos, entre outras diferenças (Quadro 1) (VERMEULEN; SCHAAP; SCHETTERS, 2001).

Quadro 1. Lesões e patogenicidade de *Eimeria* spp. em frangos de corte.

Eimeria	**Local – lesões**	**Patogenicidade***
E. acervulina	Duodeno, Jejuno. As lesões incluem várias manchas esbranquiçadas, ovais ou alongadas na metade superior do intestino delgado.	++
E. brunetti	Íleo, Reto. A mucosa pálida, podendo estar espessada. Em infecções graves, necrose coagulativa e descamação da mucosa ocorrem em quase todo o intestino delgado.	+++
E. máxima	Duodeno, Jejuno, Íleo. Causa dilatação e espessamento da parede, hemorragia petequial e um exsudato mucoso viscoso avermelhado, laranja ou rosa.	++
E. mitis	Duodeno, Jejuno. As lesões são indistintas, mas podem se assemelhar a infecções moderadas por *E. brunetti*.	+
E. necatrix	Jejuno, Ceco. Lesões na forma de pequenos pontos brancos, geralmente misturados com pontos arredondados, brilhantes ou vermelhos de vários tamanhos, podem ser vistos na superfície serosa.	+++
E. praecox	Duodeno, Jejuno. Não produzem lesões macroscópicas.	+
E. tenella	Ceco. Inflamação local com sangramento e espessamento da parede intestinal.	+++

*+, pouco patogênica; ++, moderadamente patogênica; ++, altamente patogênica.

As alterações patológicas variam desde desintegração local da barreira mucosa, associada à inflamação do tecido subjacente, até efeitos sistêmicos (VERMEULEN; SCHAAP; SCHETTERS, 2001). Durante um quadro de infecção por coccidiose, a alta mortalidade não é frequente, uma vez que, na maioria dos casos, ocorre a infecção subclínica, o que dificulta o diagnóstico da doença em um tempo hábil para começar um tratamento antes que ocorra a perda de desempenho. Durante a infecção subclínica, ocorre piora na digestão e na absorção de nutrientes em virtude das lesões no trato gastrointestinal (TGI) e, assim, queda no desempenho (CORNELISSEN et al., 2009; LEE et al., 2013).

Os aspectos zootécnicos mais comuns observados durante a infecção por coccidiose são a baixa taxa de crescimento e a redução no ganho de peso, entretanto há uma

complexidade de fatores que podem atuar modulando o desempenho produtivo em animais infectados. Um estudo metanalítico realizado por Kipper et al. (2013) avaliou as variações no desempenho produtivo em frangos desafiados com diferentes espécies de *Eimeria*. Os autores observaram que os animais desafiados apresentaram ganho de peso diário em média 10% menor que os não desafiados, com exceção do grupo desafiado por *E. maxima*, que apresentou redução de 23% em relação ao grupo controle, ou seja, o dano causado por *E. maxima* foi o que mais influenciou no ganho de peso. A idade (idade média de 19 dias) foi a variável de maior efeito na variação do consumo de ração em animais desafiados, mostrando que a faixa etária do desafio é importante para determinar o impacto da infecção por *Eimeria*.

Em geral, os animais jovens são mais suscetíveis à coccidiose e demonstram mais prontamente sinais da doença, enquanto os frangos mais velhos são relativamente resistentes à infecção (LILLEHOJ, 1998). Os animais jovens que se recuperam da coccidiose podem, posteriormente, compensar parcialmente a perda de crescimento corporal, mas o potencial de produtividade permanece gravemente comprometido. Além do dano celular primário causado pela coccidiose, a saúde e o bem-estar das aves podem ser ainda mais comprometidos pelo efeito destrutivo no revestimento celular do intestino, pela resposta inflamatória ao desafio parasitário e pela perturbação da microbiota intestinal normal.

2.4 Relação entre o sistema imune e a microbiota durante um desafio por *Eimeria* sp.

O microbioma intestinal é uma rede complexa de microrganismos simbióticos com diversas funções benéficas ao hospedeiro, incluindo absorção de nutrientes, síntese de compostos orgânicos essenciais, proteção contra patógenos e desenvolvimento do sistema imunológico intestinal (LU et al., 2021). A coexistência da microbiota comensal e de coccídios no intestino oferece amplas oportunidades de interação, tanto positivas quanto negativas (LEUNG; GRAHAM; KNOWLES, 2018). Coletivamente, os resultados presentes na literatura sugerem que os coccídios e a microbiota têm uma relação complexa e interagem através de uma barreira mecânica (células epiteliais, proteínas de

junção firme), uma barreira química (mucina, proteínas antimicrobianas) e por meio da ativação do sistema imunológico (Figura 2).

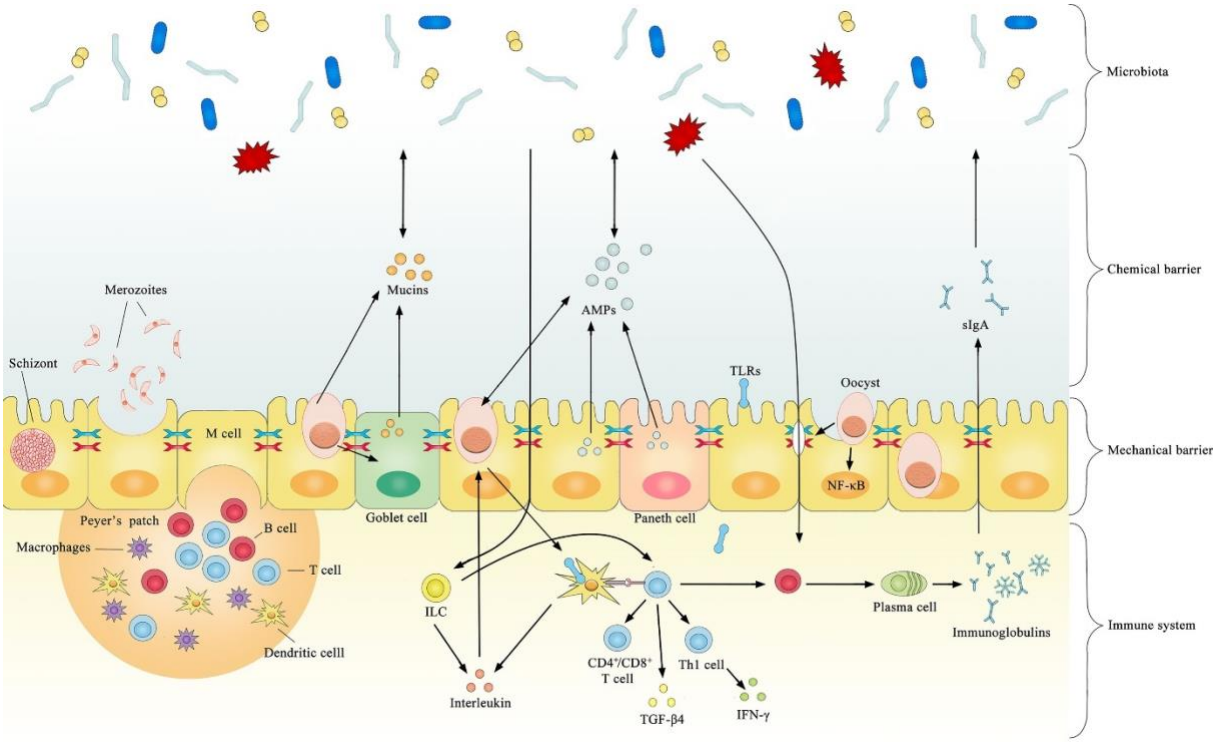

Figura 2. Resumo dos mecanismos documentados pelos quais a infecção por coccídios pode interagir indiretamente com a microbiota intestinal e o sistema imune (LU et al., 2021).

A barreira mecânica intestinal é regulada por proteínas de junções firmes (que consistem em várias proteínas únicas, incluindo a proteína transmembrana ocludina, os membros da família claudina e as proteínas ligantes, como a família de proteínas zônula de oclusão), que desempenham papel importante na absorção de nutrientes, bem como na manutenção da integridade da barreira intestinal e na proteção do intestino contra a translocação de patógenos entéricos (PETERSON; ARTIS, 2014). Recentemente, Lin e Olukosi (2021) demonstraram que uma infecção por *Eimeri*a (*E. máxima, E. acervulina* e *E. tenella*) foi capaz de alterar a expressão de proteínas de junções firmes e de genes transportadores de nutrientes, além de aumentar a permeabilidade do epitélio intestinal, revelando o comprometimento das barreiras epiteliais do intestino quando os animais estão sob um desafio por coccidiose.

A barreira química do intestino consiste em mucinas, peptídeos antimicrobianos, proteínas, lisozimas e outros fatores (OKUMURA; TAKEDA, 2017). A camada de muco intestinal, produzida pelas células caliciformes, é um elemento chave na manutenção da proteção funcional do epitélio (MELHEM; REGAN-KOMITO; NIESS, 2021). Os membros da família das mucinas são amplamente agrupados em mucinas secretoras (MUC2, MUC5 e MUC6) e mucinas associadas à membrana (MUC1, MUC3A/b, MUC12, MUC13 e MUC17), dependendo da sua estrutura e localização.

A expressão/secreção de mucina pode ser influenciada por mediadores como citocinas do tipo 1 (IL-2, IL-12 e IFN-γ) ou do tipo 2 (IL-4, IL-9, IL-10 e IL-13), fatores inerentes à dieta, além de bactérias comensais e patogênicas (MELHEM; REGAN-KOMITO; NIESS, 2021).

Durante a infecção por coccidiose, uma secreção maior de muco pode ser esperada como mecanismo de prevenção da adesão de patógenos nas superfícies epiteliais e proteção de novas infecções. No entanto, o aumento da mucogênese no intestino promove uma condição propícia para a proliferação de *Cl. perfringens,* uma vez que a camada de muco fornece um substrato nutricional capaz de favorecer o crescimento desta bactéria (COLLIER et al., 2008).

Devido ao complexo ciclo de vida que o parasita desenvolve dentro do hospedeiro, a resposta imune é também bastante complexa, envolvendo imunidade mediada por células, produção de anticorpos e de citocinas (GALHA; BONDAN; LALLO, 2008). O sistema imunológico das mucosas representa a primeira linha de defesa contra vários antígenos patogênicos. Como a *Eimeria* é um parasita intestinal, a resposta de defesa contra a coccidiose começa no tecido linfoide associado ao intestino ou GALT (do inglês, *Gut-associated lymphoid tissue*). O GALT possui três funções principais na defesa do hospedeiro contra a infecção por coccidiose: processamento de antígenos e apresentação de epítopos imunogênicos, liberação de anticorpos intestinais e ativação da imunidade mediada por células (SHIVARAMAIAH et al., 2014).

Nesse sentido, a imunidade adaptativa inclui respostas imunes humorais e mediadas por células, que são mediadas por anticorpos solúveis e linfócitos T, respectivamente. Ambas as respostas desempenham papéis importantes contra patógenos intracelulares,

no entanto a resposta mediada por células parece estar predominantemente envolvida na defesa do hospedeiro.

As células T desempenham papel importante na resposta à infecção primária ou ao desafio por coccídio (KIM; CHAUDHARI; LILLEHOJ, 2019). Diversas citocinas e quimiocinas envolvidas na imunorregulação do hospedeiro durante a infecção primária ou secundária foram caracterizadas, entre elas interleucinas IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, IL-15, 17 e IL-18, Interferon-gama (IFN-γ), Fator de necrose tumoral (TNF)-α, TNF-α superfamília 15 (TNFSF15).

As proteínas estruturalmente homólogas IL-1β e IL-18 são notáveis citocinas envolvidas na resposta inflamatória inicial, sendo elas responsáveis pela indução da produção de quimiocinas e pela secreção de IFN-γ, respectivamente (LEE; LU; LILLEHOJ, 2022). O IFN-γ é um imunomodulador representativo e apresenta efeito inibitório direto sobre o desenvolvimento intracelular de *Eimeria* (LEE; LU; LILLEHOJ, 2022).

A IL-6 é considerada uma citocina indicativa de início de uma resposta de fase aguda, sendo importante nos processos inflamatórios e na resposta imune (HONG et al., 2006). Sob um desafio por coccidiose, a *Eimeria* pode causar uma resposta inflamatória massiva na mucosa intestinal através da invasão e, subsequente, dano às células epiteliais. Assim, como a inflamação é um componente da resposta de fase aguda, a IL-6 é produzida durante as respostas imunes à inflamação causada pelo parasita (LYNAGH; BAILEY; KAISER, 2000). A expressão aumentada do gene IL-6 foi relatada nas células epiteliais de aves infectadas com *E. acervulina*, *E. maxima* ou *E. tenella* (HONG et al., 2006).

A infecção por *Eimeria* destrói a estrutura e o revestimento dos tecidos intestinais, causando um distúrbio conhecido como disbiose na comunidade microbiana do intestino (DUCATELLE et al., 2015). A disbiose é caracterizada por uma flutuação significativa de bactérias benéficas e pelo acúmulo de bactérias nocivas, a ponto de se tornarem uma ameaça potencial para o hospedeiro, causando um desequilíbrio na homeostase (CUI et al., 2017).

A disbiose associada à coccidiose aumenta a suscetibilidade do hospedeiro a outros patógenos. Alguns estudos indicam que a infecção por *Eimeria* sozinha tem pouco ou nenhum efeito na diversidade alfa do ceco (MACDONALD et al., 2017), no entanto, coinfecções entre *Eimeri*a e outros fatores predisponentes que afetam o TGI, como *C. perfringens*, podem remodelar ou alterar a microbiota intestinal (MADLALA; OKPEKU; ADELEKE, 2021). A infecção coccidiana afeta a composição da microbiota intestinal do hospedeiro direta ou indiretamente, e alterações na microbiota intestinal também podem influenciar a infectividade dos coccídios.

2.5 Anticoccidianos ionóforos

Os compostos anticoccidianos são substâncias farmacologicamente ativas destinadas a eliminar ou inibir os protozoários do gênero *Eimeria* que afetam o TGI de muitos animais de criação, como frangos, perus, bovinos, suínos, ovelhas e outros animais (ANNUNZIATA et al., 2017). Segundo Allen e Fetterer (2002) os anticoccidianos podem ser classificados em duas categorias distintas e subdividido dentro delas:

(1) Ionóforos ou poliéter, que são produzidos pela fermentação de *Streptomyces* spp. ou *Actinomadura* spp. e agem por meio de mecanismos gerais de alteração do transporte de íons e perturbação do equilíbrio osmótico no parasita:

(a) Ionóforos monovalentes (Monensina, Narasina, Salinomicina);
(b) Ionóforos glicosídicos monovalentes (Maduramicina, Semduramicina);
(c) Ionóforo divalente (Lasalocida).

(2) Compostos sintéticos, que são produzidos por síntese química e têm um modo de ação específico contra o metabolismo do parasita:

(a) Inibição da respiração mitocondrial do parasita (Decoquinato, Clopidol);

(b) Inibição da via do ácido fólico (Sulfonamidas);

(c) Inibição competitiva da captação de tiamina (Amprolium);

(d) Modo de ação não totalmente elucidado (Diclazuril, Halofuginona, Nicarbazina, Robenidina).

A descoberta e o desenvolvimento dos ionóforos na segunda metade da década de 1960 forneceram uma interessante estratégia para prevenção e controle da coccidiose aviária (CONWAY; MCKENZIE, 2007). Os ionóforos são definidos como agentes quelantes lipofílicos capazes de transportar cátions como sódio (Na^+), potássio (K^+), cálcio (Ca^{++}) e magnésio (Mg^{++}) através das membranas celulares do parasita, e esse mecanismo é apenas eficiente contra coccídios (ANNUNZIATA et al., 2017).

O mecanismo de ação dos ionóforos é baseado no transporte desses íons para o citoplasma celular, aumentando as concentrações intracelulares, o que altera, portanto, o equilíbrio osmótico do coccídio. Como resultado, funções vitais das mitocôndrias dos parasitas são inibidas e a entrada de água, atraída pela alteração da osmolaridade, pode levar à ruptura da membrana do parasita (SILVA, 2012).

Estudos com monensina, salinomicina e lasalocida demonstraram que esses três ionóforos causam um aumento nos níveis de Na + e uma diminuição nos níveis de K^+ dentro das células, alterando a bomba de Na^+/K^+ na membrana citoplasmática (SMITH; GALLOWAY, 1983). Assim, o influxo de Na^+ excede a capacidade da célula do parasita de removê-lo, levando-o à morte.

Ainda nesse sentido, outra pesquisa envolvendo lasalocida, monensina, narasina e salinomicina mostrou que o pré-tratamento de esporozoítos de *E. tenella* com esses compostos inibiu a capacidade de invasão da célula hospedeira pelos esporozoítos e, subsequentemente, a capacidade de desenvolvimento na célula hospedeira (SMITH; GALLOWAY; WHITE, 1981). Micrografias eletrônicas de um esporozoíto não tratado de *E. tenella* e um esporozoíto tratado com monensina mostrando o vacúolo grande e o inchaço são apresentados na Figura 3.

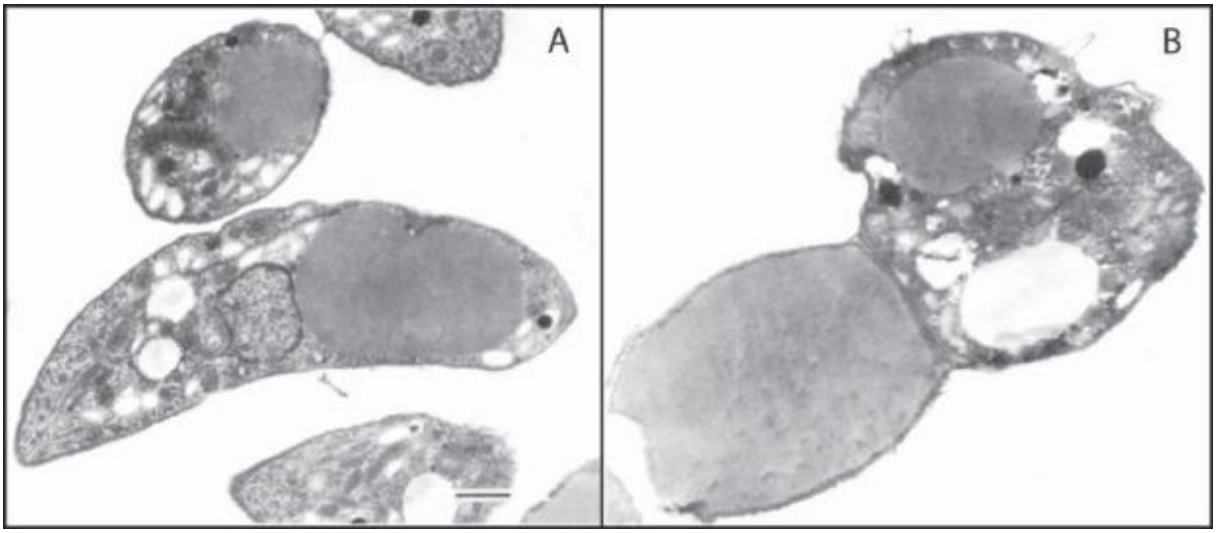

Figura 3. A) Esporozoíto íntegro de *E. tenella*. B) Esporozoíto de *E. tenella* tratado com 1,0 µg / mL de monensina. Observe a vacuolização e o abaulamento típico (SMITH; GALLOWAY; WHITE, 1981).

2.5.1 Monensina sódica

A monensina é um poliéter de ácido monocarboxílico produzida a partir da fermentação de uma cepa de *Streptomyces cinnamonensis* originalmente isolada de uma amostra de solo coletada no Arizona, Estados Unidos (CONWAY; MCKENZIE, 2007). A monensina foi o primeiro ionóforo usado no controle da coccidiose aviária, provocando uma revolução nos métodos de prevenção dessa doença em aves comerciais desde o início do seu emprego na indústria avícola, em 1971 (CHAPMAN; JEFFERS; WILLIAMS, 2010).

A monensina tem a habilidade de formar complexos com os íons monovalentes, apresentando preferência pelos íons Na^+ seguida por K^+, rubídio (Rb^+) e lítio (Li^+) (CHAPMAN; JEFFERS; WILLIAMS, 2010). O mecanismo de ação da monensina é baseado no influxo de íons Na + para dentro do esporozoíto e esse acúmulo de íons Na^+ possibilita que água entre por osmose e o parasita inche e, eventualmente, exploda. O local de ação dos ionóforos foi considerado a membrana externa (película) do esporozoíto. Além de um efeito contra os esporozoítos, a monensina é eficaz contra os merozoítos, presumivelmente pelo mesmo mecanismo. Merozoítos são as formas

móveis que são liberadas no lúmen intestinal após a esquizogonia, como demonstrado anteriormente na Fig. 1 (LONG; JEFFERS, 1982).

2.5.2 Salinomicina

A salinomicina sódica é um poliéter do ácido carboxílico, produzido por fermentação a partir de uma cepa de *Streptomyces albus* isolada de uma amostra de solo coletada na província de Shizuoka, Japão (CONWAY; MCKENZIE, 2007). A salinomicina é um ionóforo com seletividade estrita por cátions alcalinos monovalentes e tem forte preferência por K^+, interferindo no potencial transmembrana e promovendo o efluxo de íons K^+ da mitocôndria e do citoplasma (DINIZ, 2008).

A ação da salinomicina se manifesta logo nos primeiros estágios do ciclo de vida da *Eimeria*, destruindo esporozoítos, trofozoítos e esquizontes de primeira geração, atuando tanto na forma clínica como na subclínica da doença (DUTRA, 2002). A eficácia da salinomicina no controle da coccidiose, na diminuição da presença de oocistos, na recuperação das lesões da enfermidade, na melhoria no ganho de peso e na conversão alimentar é amplamente demonstrada na literatura mundial (CONWAY et al., 1993). Além do efeito sobre a coccidiose aviária, a salinomicina exerce efeito sobre *C. perfringens*, diminuindo a incidência de enterite necrótica em frangos e reduzindo a prevalência de *Salmonella* (ENGBERG et al., 2000).

2.6 Resistência aos ionóforos

A resistência é geralmente reconhecida como uma falha dos quimioterápicos em controlar o parasitismo, mas a definição formal de resistência é uma mudança na suscetibilidade a uma droga (ABBAS et al., 2011). Vários métodos diferentes são usados para analisar a resistência aos medicamentos, que, na maioria das vezes, é expressa em termos de sobrevivência de parasitas após a administração de um fármaco que seria esperado ser eficaz, ou que, em outras palavras, pode ser aceita como uma redução na sensibilidade de um parasita a um medicamento específico.

Os mecanismos que levam ao desenvolvimento de cepas resistentes aos anticoccidianos não são totalmente conhecidos. Chapman (1997) citou que, para o amprolium, acredita-se que mudanças a nível molecular podem alterar o receptor alvo, impedindo a ligação do anticoccidiano às cepas resistentes. Já para os ionóforos, acredita-se que os mecanismos envolvam mudanças bioquímicas na composição da membrana do parasita ou na expressão de genes que produzem uma proteína, chamada de Pglicoproteína, responsável por aumentar a atividade da bomba de Na^+/K^+.

Em um estudo recente, Ferdji et al. (2022) determinaram a resistência de coccídios aos principais ionóforos usados em granjas de frangos de corte na Argélia. Os autores observaram a presença de resistência total à monensina (120 ppm) e à robenidina (33 ppm) e resistência parcial à salinomicina (60 ppm) e à combinação narasina-nicarbazina (80 ppm). No Brasil, Kraieski et al. (2021) avaliaram a sensibilidade de *E. acervulina* e *E. maxima* em frangos de corte de três diferentes regiões do país (São Paulo, Paraná e Minas Gerais) utilizando testes de sensibilidade anticoccidiana (ASTs). Diferentes níveis de sensibilidade foram identificados nos isolados, porém, de maneira geral, os menores níveis de sensibilidade desses isolados foram observados nos grupos maduramicina (6 ppm) e monensina (110 ppm).

2.7 Aditivos fitogênicos

Anticoccidianos e vacinas vivas têm sido aplicados na indústria avícola comercial como métodos convencionais para prevenir a coccidiose; entretanto, com a ocorrência de resistência e a proibição ao uso de determinados compostos químicos na produção animal, muitos produtos naturais estão sendo investigados como alternativas potenciais para manter adequadas as funções dos sistemas digestório e imunológico das aves frente a um desafio sanitário (MUTHAMILSELVAN et al., 2016). Entre as alternativas mais populares estão os aditivos fitogênicos (BAJAGAI et al., 2020).

O termo fitogênico refere-se às diferentes partes de plantas, ervas aromáticas e especiarias, bem como aos seus respectivos extratos vegetais na forma de óleos essenciais (WINDISCH et al., 2008). Muitas propriedades benéficas dos compostos fitogênicos derivam de suas moléculas bioativas, como alcaloides, flavonoides,

saponinas, taninos fenólicos, terpenoides, carvacrol, timol, piperina, entre outros tantos (WINDISCH et al., 2008). As atividades biológicas dessas fitomoléculas já foram documentadas, assim como suas funções antibacterianas, antioxidantes, antivirais, antiparasitárias e inseticidas.

Desde a antiguidade, plantas e ervas aromáticas têm desempenhado um papel crucial na atenção primária à saúde humana como agentes terapêuticos para o tratamento de muitas doenças. Os efeitos positivos de plantas aromáticas, ervas e seus óleos essenciais em várias doenças foram evidenciados ao longo da história (GIACOMETTI et al., 2018; OLIVEIRA et al., 2018). Plantas aromáticas e ervas foram os primeiros compostos farmacológicos usados na antiguidade para tratar doenças ou outras condições anormais e mesmo agora ainda são usados na medicina (CHRISTAKI et al., 2012). Quase todas as civilizações antigas demonstraram alguma evidência de consciência do uso medicinal das plantas.

Nas últimas décadas, a popularidade de alternativas naturais em relação aos aditivos sintéticos para rações tem crescido rapidamente com o futuro da indústria animal e, principalmente, orientada pelas demandas do mercado importador. Anteriormente, metabólitos secundários de plantas foram considerados fonte de fatores antinutricionais, mas mudanças na legislação mundial têm estimulado o interesse nesses extratos bioativos, amplamente consistentes com o atual pensamento sobre o futuro da agricultura e da alimentação humana.

Vários compostos bioativos derivados de plantas e produtos naturais parecem ter atividades anticoccidianas contra as espécies de *Eimeria* comumente encontradas em frangos de corte (NAIDOO et al., 2008), como saponinas, flavonoides, papaína, taninos, entre outros, os quais podem atuar em diferentes fases do ciclo de vida do protozoário. Muitos desses compostos se ligam à superfície celular da *Eimeria*, alterando a estabilidade estrutural do parasita (ABBAS; COLWELL; GILLEARD, 2012).

Embora os modos precisos de ação dos fitogênicos ainda não estejam totalmente elucidados, estudos têm demonstrado os efeitos no crescimento, características de carcaça e qualidade da carne (ATTREE et al., 2021). Entretanto, tais benefícios e a eficácia dos aditivos fitogênicos são influenciados por uma série de fatores, como

composição química, efeito sinérgico entre os diferentes compostos, parte da planta usada (sementes, folha, raiz ou casca), época de colheita e origem geográfica, variação genética da planta, idade da planta, dosagem usada, método de extração, tempo de colheita e compatibilidade com outros ingredientes (YITBAREK, 2015). Além disso, o efeito benéfico dos fitogênicos pode ser influenciado pelo estado nutricional dos animais, pela infecção, pela composição da dieta e pelas condições ambientais (GIANNENAS et al., 2003)

Conforme Hashemi e Davoodi (2011), os aditivos fitogênicos são classificados de acordo com a parte utilizada (planta inteira, raiz, caule, casca, folha, flor, fruto e semente), o hábito (gramíneas, juncos, ervas, arbustos, trepadeiras e árvores), o habitat (tropical, subtropical e temperado), o valor terapêutico (antibacteriano, antifúngico, anti-inflamatório, anticoccidiano, antioxidante, antiviral, anticâncer.) e as vias de administração (tintura, decocção, maceração, xarope, inalação e tisanas), além da classificação botânica usual.

2.7.1 Acacia concinna e Saccharum officinarum

Acacia é o segundo maior gênero da família Leguminosae (SULAIMAN et al., 2013), compreendendo mais de 1350 espécies em todo o mundo (NASRI et al., 2012). Essa planta contém uma variedade de componentes bioativos, como compostos fenólicos, alcaloides, terpenos, taninos, saponinas e flavonoides, os quais são responsáveis pelas propriedades biológicas e farmacológicas (TODKAR; CHAVAN; KULKARNI, 2010; SULAIMAN et al., 2013; LEE RANGEL, 2022).

O extrato aquoso de *A. concinna* demonstrou apresentar potente atividade antimicrobiana contra diversas bactérias, como *E. coli*, *Klebsiella pneumoniae*, *Bacillus subtilis*, *Pseudomonas aeruginosa* e *Staphylococcus aureus* (TODKAR et al., 2010; LEE-RANGEL et al., 2022), além de atividade antifúngica contra *Aspergillus niger*, *Penicillium* sp. e *Candida albicans* (TODKAR et al., 2010).

A cana-de-açúcar (*Saccharum officinarum* L.) é uma cultura bem conhecida da família *Poaceae* e seus extratos são mundialmente caracterizados por conterem uma

variedade de fitocompostos com diferentes atividades biológicas e imunológicas (SINGH et al., 2015). Os compostos fenólicos mais comuns encontrados em *S. officinarum* pertencem à classe das flavonas, como naringenina, apigenina, tricina e derivados de luteolina, sendo eles, parcialmente responsáveis pelos potentes efeitos antioxidantes e anti-inflamatórios (DUARTE-ALMEIDA et al., 2007).

A utilização de extratos da cana-de-açúcar (etanólico e aquoso) contra a coccidiose em frangos de corte mostrou eficácia imunoterapêutica por meio da melhora do ganho de peso e da redução na excreção de oocistos e no escore de lesão intestinal (AWAIS et al., 2011). Além disso, em condições in vitro, o extrato de *S. officinarum* mostrou atividade anticoccidiana contra quatro espécies de *Eimeria* (*E. tenella*, *E. necatrix*, *E.* mitis e *E. brunetti*) e foi capaz de inibir a esporulação dos oocistos e prejudicar a morfologia e a forma normal dos oocistos (ABBAS et al., 2015).

Devido às propriedades químicas presentes na *A. concinna* e na S*. officinarum*, recentemente, estudo com o uso combinado dessas plantas foi realizado para avaliar a eficácia sinérgica em controlar a coccidiose em frangos de corte (SÁNCHEZ-HERNÁNDEZ et al., 2019). A suplementação da mistura de plantas na dieta de frangos desafiados com *Eimeria* demonstrou efeito positivo na redução da excreção de oocistos e na manutenção do desempenho produtivo após o desafio, indicando ser uma opção para ser usada em quadros de coccidiose.

2.7.2 Líquido da casca da castanha de caju (*Anacardium occidentale* L.) e óleo de mamona (*Ricinus communis L.*)

Anacardium occidentale L. (Anacardiaceae), o cajueiro, é uma espécie tropical nativa do Nordeste do Brasil, onde seu cultivo é uma das atividades agrícolas mais importantes (MAZZETTO; LOMONACO; MELE, 2009). O principal produto dessa planta é o fruto, conhecido como castanha de caju, que é comercializado e consumido em todo o mundo. O líquido da casca da castanha de caju (CNSL) é um líquido viscoso marrom escuro contendo uma mistura de lipídios fenólicos, obtido do mesocarpo esponjoso da casca do caju (MAZZETTO; LOMONACO; MELE, 2009).

A composição do líquido pode variar conforme o modo de extração. O CNSL tipicamente extraído com solvente é composto principalmente por ácido anacárdico (60-70%), cardol (15-20%), cardanol (10%) e traços de 2-metilcardol. No processo industrial, quando as cascas são aquecidas com a castanha durante a torra, o ácido anacárdico é convertido em cardanol por meio de descarboxilação, alterando o conteúdo do CNSL para traços de ácido anacárdico, cardanol (60-70%), cardol (15-20%), material polimérico (10%) e vestígios de 2-metilcardol (VOIRIN et al., 2014).

Essas propriedades químicas possibilitam que o CNSL desempenhe importantes atividades antibacterianas, antiprotozoáricas e antifúngicas (STASIUK; KOZUBEK, 2010). O cardanol e o cardol, que apresentam propriedades antioxidantes, atuam como ionóforos naturais, desnaturando as camadas lipídicas da parede celular, com ação principalmente em bactérias gram-positivas (PARAMASHIVAPPA et al., 2001). Esses compostos são classificados como componentes do grupo dos fenóis com alta atividade antibacteriana devido à alta solubilidade em membranas biológicas, tornando-os notórios bactericidas (NAZZARO et al., 2013).

A mamona (*Ricinus communis* L.) é uma oleaginosa pertencente à família Euforbiaceae. O óleo de mamona, conhecido também como óleo de rícino, é um dos óleos essenciais extraído por uma combinação de prensagem mecânica e extração com solventes. A molécula farmacologicamente ativa presente em maior concentração no óleo é o ácido ricinoleico, que compreende aproximadamente 90% dos ácidos graxos presentes.

O ácido ricinoleico é caracterizado por apresentar propriedades analgésicas, antiinflamatórias e antimicrobianas (PABIŚ; KULA, 2016). A ação antimicrobiana dessa molécula acontece, principalmente, por meio da sua capacidade de interagir com a membrana microbiana, atuando inibição de enzimas envolvidas na biossíntese da parede celular bacteriana (KUPPALA et al., 2016).

Em virtude desses benefícios, uma mistura de óleos funcionais à base do CNSL e do óleo de mamona foi desenvolvida a fim de potencializar os efeitos antimicrobianos e antioxidantes dos dois subprodutos. Interessantes resultados no desempenho e na microbiota foram observados com o uso da mistura do CNSL e do óleo de mamona em

dietas para frangos de corte desafiados com coccidiose. Comparado a um ionóforo amplamente utilizado, a monensina sódica (100 ppm), o óleo funcional (1.500 ppm) teve efeito semelhante ao ionóforo no período de criação (até 42 dias de idade) e foi capaz de compensar o efeito negativo causado pela coccidiose (MORAES et al., 2019). Além disso, a mistura de CNSL e óleo de mamona mostrou ser uma boa opção no desafio da coccidiose, atuando como moduladora da microbiota intestinal, com ação antimicrobiana contra bactérias gram-positivas, principalmente *C. perfringens* e *S. aureus*.

2.8 HIPÓTESES E OBJETIVOS

2.8.1 Hipóteses

As hipóteses específicas desta pesquisa são:

a) O uso de um determinado blend de compostos fitogênicos melhora o desempenho de frangos desafiados por coccidiose, modulando o sistema imune e a microbiota intestinal.
b) O uso de uma determinada mistura de óleos funcionais potencializa o efeito da salinomicina frente ao desafio por coccidiose, modulando a microbiota intestinal.

2.8.2 Objetivo Geral

O objetivo geral desta pesquisa é avaliar os efeitos de dois diferentes compostos fitogênicos associados ou não a ionóforos na dieta de frangos de corte desafiados por oocistos esporulados de *Eimeria* spp. e o impacto no desempenho, na microbiota e no sistema imune.

2.8.3 Objetivos Específicos

Os objetivos específicos desta pesquisa são:

a) Comparar um blend comercial à base de Acacia concinna e Saccharum officinarum com o ionóforo monensina sódica no controle da coccidiose e avaliar os reflexos no

desempenho, na microbiota intestinal e na resposta imune de frangos de corte desafiados ou não por coccidiose.

b) Avaliar a capacidade sinérgica de um produto composto pelo líquido da casca de castanha de caju e pelo óleo de mamona somado à salinomicina sódica no desempenho e na microbiota de frangos de corte desafiados ou não por coccidiose.

CAPÍTULO II

Artigo científico nas normas da revista *Frontiers*

Molecular Characterization Of The Intestinal Microbiota And Mucosal Gene Expression In Broilers Fed Phytogenic Additive Or Sodium Monensin In Response To An Intestinal *Eimeria* Challenge

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Abstract
The present study evaluated the effect of Sodium Monensin or Phytogenic additive (based on *Acacia concinna* and *Saccharum officinarum*) on the gut microbiota and immune response of broiler chickens infected with *E. tenella*, *E. acervulina*, and *E. maxima*. A total of 120 male one-day-old male broiler chickens were divided in 3 groups: Control group (without infection and no additives); Ionophore group (sodium monensin, 100 ppm + *Eimeria* challenged) and Phytogenic group (poly-herbal product at 750 ppm + *Eimeria* challenged). At 28 days, the intestinal content of four birds per treatment was collected for microbiota analysis by the Miseq Illumina platform. Samples of jejunum-ileum were collected for mRNA gene transcription reactions using the StepOnePlus™ Real-Time PCR System. The intestinal microbiota was dominated by members of the phylum Firmicutes, regardless of treatment. However, the Phytogenic group had a higher relative proportion of Firmicutes in the ileum-jejunum, and a higher proportion of Bacteroidetes in the cecum, while the Ionophore group exhibited a higher relative proportion of Proteobacteria and Cyanobacteria. At the family level, the Phytogenic group enhanced the relative abundances of Lactobacillaceae, Bacteroidaceae, and Peptostreptococcaceae, while monensin enhanced Lactobacillaceae, Rikenellaceae, Bacteroidaceae, and Clostridiaceae. Under a coccidiosis challenge, the ionophore and phytogenic additives modulated the immune system by significantly increasing *IL-6* and *MUC13* gene transcription compared to the Control group. Different profiles were detected in infected birds supplemented with different additives (Ionophore and Phytogenic groups) when compared to unchallenged birds. The phytogenic product, a mixture composed by *A. concinna* and *S. officinarum*, and sodium monensin enhanced

the abundance of important bacterial groups for the establishment of a favorable intestinal microbiota in the face of an intestinal challenge.

1 Introduction

The gut microbiota is a complex community of microorganisms that establish a close symbiotic relationship with the host's health (Nicholson et al., 2012). The crucial role played by the gut microbiota has been well-defined with respect to the regulation of homeostasis and host performance. Therefore, it is known that the gut bacterial populations may modulates the progression of protozoan infection and host response upon infection.

Coccidiosis, an intestinal disease in poultry, is caused by several apicomplexan parasites of the genus *Eimeria* (family *Eimeriidae*) that infect the gut and are transmitted between birds via ingestion of infective oocysts (Dalloul and Lillehoj, 2006). The invasion of *Eimeria* sp. damages the intestinal epithelial cells and disrupts gut homeostasis, resulting in malabsorption, enteritis, bloody diarrhea, and hampered weight gain (Gilbert et al., 2011).

Currently, ionophores drugs and vaccines have been applied in the commercial poultry industry as conventional methods to prevent coccidiosis, however, viable alternatives are being sought improve performance, protect animal health, and maintain profit margins in the face of a sanitary challenge (Muthamilselvan et al., 2016). Among the available alternatives, phytogenic feed additives (PFA) are emerging as a strategy to combat coccidiosis. Plants mixture, including *Acacia concinna* and *Saccharum officinarum*, are composed of bioactive substances that provide health benefits and protection against *Eimeria* infections in broiler chickens (Singh et al., 2015; Abbas et al., 2017; Shaikh et al., 2022).

S. officinarum extracts contains polysaccharides and other compounds with immunostimulant effects (Awais et al., 2018). The use of *S. officinarum* extracts against coccidiosis in broilers showed immunotherapeutic efficacy by improving weight gain and reducing oocyst excretion and intestinal lesion score (Awais et al., 2011). Furthermore, under *in vitro* conditions, this extract showed anticoccidial activity against four *Eimeria* species (*E. tenella*, *E. necatrix*, *E. mitis* and *E. brunetti*) and was able to inhibit oocyst sporulation and harm the morphology and normal shape of the oocysts (Abbas et al., 2015).

A. concinna contains saponins and polyphenolic components with immunomodulatory properties to interrupt the lipid structure of the cell membrane of parasites affecting the enzymatic and metabolic activity and resulting in cell death (Kukhetpitakwong et al., 2006; Hassan et al., 2010). The extract of *A. concinna* showed potent antimicrobial activity against several bacteria such as E. coli, Klebsiella pneumoniae, Bacillus subtilis, Pseudomonas aeruginosa and Staphylococcus aureus (Todkar et al., 2010; Lee-Rangel et al., 2022).

Due to the chemical properties present in *A. concinna* and *S. officinarum*, recently, study with the combined use of these plants were carried out to evaluate the synergistic effectiveness in controlling coccidiosis in broilers (Sánchez-Hernández et al., 2019). The supplementation of the plant mixture in the diet of chickens challenged with *Eimeria* showed a positive effect in reducing the excretion of oocysts and in maintaining productive performance after the challenge, indicating that it is an option to be used in coccidiosis cases.Therefore, this study aimed to investigate the effects of phytogenic feed additive (based on *Acacia concinna* and *Saccharum officinarum)* or sodium monensin on broiler's intestinal microbiota and gene expression of signaling molecules using an intestinal challenge with *Eimeria* spp.

2 Material and methods

2.1 Experimental procedures

A total of 120, one-day-old male broiler chicks (Cobb 500) were obtained from a commercial hatchery and housed in a controlled-temperature room, composed of 12 pens with an initial density of 10 birds per pen. Each group was housed in a 1 m^2 pen equipped with two nipple drinkers and one tubular feeder. The experimental design was completely randomized, composed of three treatments groups: Control group (basal diet without additives inclusion in no coccidiosis-challenged birds); Ionophore group (sodium monensin supplementation at 100 ppm - 0.025% - in coccidiosis challenged birds) and Phytogenic group (commercial poly-herbal product at 750 ppm - 0.075% - in coccidiosis-challenged birds).

The commercial poly-herbal product was composed of a mixture of *Acacia concinna* and *Saccharum officinarum* (Peptasan – Nutriquest Technofeed Nutrição Animal LTDA., São Paulo, Brazil). Both feed additives, the oil blend and sodium monensin (Elancoban – Elanco Animal Health, Greenfield, IN, USA) were included by replacing an inert ingredient (kaolin) in the basal diet for all phases.

The nutritional program consisted of four phases: pre-starter (1 to 7 d), starter (8 to 21 d), grower (22 to 35 d) and finisher (36 to 42 d), formulated to provide the nutritional requirements recommended by the Brazilian Tables of Poultry and Swine (Rostagno et al., 2017) (Table S1. Supplementary material).

2.2 Challenge and sample collection

At 14 days of age, all chickens of the Ionophore and Phytogenic groups were inoculated by oral gavage with 1 mL of a saline solution containing sporulated oocysts of *E. tenella* (1×10^4 oocysts), *E. acervulina* (20×10^4 oocysts), and *E. m*axima (8×10^4 oocysts). The oocysts were acquired from the Laboratório Biovet (Laboratório BIO-VET LTDA, São Paulo, Brazil). To similarly stress all treatments, unchallenged broilers (Control group) received 1 mL of saline. The *Eimeria* inoculum dosage for the current study were chosen based on previous studies (Moraes, 2019; Moraes, 2019; Vieira, 2020), and this dosage was able to cause a drop in performance without causing high mortality.

At 28 days of age, four chickens per treatment group (four replicates) within the average weight of each replicate were euthanized, totaling 12 animals. A 10 cm portion of each segment: jejunum (descending duodenal loop to the Meckel's diverticulum), ileum (diverticulum to ileocecal insertion), and caecum were removed with the intestinal contents inside and immediately stored at -20°C until analysis of microbiota sequencing.

For gene transcription, a 5 cm portion of the jejunum-ileum junction were carefully excised aseptically, placed in cryogenic vials, snap frozen in liquid nitrogen, delivered to the laboratory, and stored at -80°C until RNA extraction.

2.3 DNA Extraction, PCR amplification and sequencing

Total microbial genomic DNA samples (jejunum-ileum junction of 4 animals/treatment = totaling 12 samples) were extracted using the E.Z.N.A. Stool DNA Kit (Omega Bio-Tek, Norcross, Georgia, USA) according to the manufacturer's instructions. The genomic DNA was

quantified using a Qubit® 3.0 Fluorometer (Life Technologies, Carlsbad, CA), and stored at -20°C.
The V4 region of bacterial 16S rRNA gene was amplified using 515F and 806R primers to characterize the intestinal bacterial composition, both modified to contain an Illumina adapter region as described by Caporaso et al. (2010). Each PCR amplification was performed in a 25 µl mixture, consisting of ~1 ng of genomic DNA, 1.0 mM MgCl2, 0.5 µM of each primer, 0.2 mM of each dNTP, 2U Platinum Taq DNA Polymerase (Life Technologies), and 1x reaction buffer. Amplification was carried out in a BioRad My Cycler Thermocycler (BioRad, USA) according to the following program: initial denaturation at 94°C for 2 min, followed by 25 cycles of 45 sec at 94ºC, 45 sec at 55ºC 1 min at 72ºC and a final cycle at 72°C for 6 min. Five microliters of each PCR product was used to verify amplification by gel electrophoresis on a 1 % agarose gel. Amplicons were purified using Agencourt AMPure XP beads following manufacturer instructions. Purified products were again quantified and checked in Qubit® Fluorometric Quantitation. Indexes were added to DNA libraries following the manufacturer instructions (Illumina Inc., San Diego, California, USA). Sequencing was conducted on platform Illumina MiSeq with a v2 500 kit.

2.4 RNA Extraction and RT-qPCR in jejunum-Ileum tissue

Total RNA in jejunum-ileum tissue was first homogenized in 1 mL of TRIzol (Invitrogen; Thermo Fisher Scientific, Inc.) using an electric homogenizer. RNA was purified from the lysate using a Universal RNA Purification Kit (EURx, Gdańsk, Poland), according to manufacturer's instruction, and concentration of the RNA was measured using a Qubit® 3.0 Fluorometer (Life Technologies, Carlsbad, CA, USA). RNA samples were treated with DNase I (QIAGEN, Valencia, CA, USA) during isolation procedure to prevent DNA contamination. The GoScript Reverse Transcription Mix (Promega Corporation, Madison, WI, USA) was used to reverse transcribe RNA to cDna.
Each reaction used 1 µl of cDNA, 0.2 µl of each primer, (forward and reverse primers), 5 µL of PowerUp™ SYBR® Green Master Mix (Applied Biosystems, Austin, TX, USA) and Ultrapure™, RNase and DNase free water (Invitrogen, Carlsbad, CA, USA) to reach a total reaction volume of 10 µl. Oligonucleotide primers are presented in Table 2.
RT-qPCR reactions were performed in the StepOnePlus™ Real-Time PCR System (Applied Biosystems, Foster City, USA) and consisted of reverse transcription at 50ºC for 2 min and initial denaturation at 95ºC for 2 min, 40 cycles of amplification (denaturation at 95ºC for 15 s and annealing at 60ºC for 1 min) and melting curve. The annealing temperature was 60ºC for all target genes. Fluorescence was measured at the end of each elongation step.
The relative expression was calculated by the comparative Ct ($2^{-\Delta\Delta Ct}$) method using the glyceraldehyde-3-phosphate-dehydrogenase (*GAPDH*) gene as an endogenous reference gene. Gene transcription levels were quantified relatively to the expression of the GAPDH according to the formula as followed (Livak and Schnittgen, 2001). The values obtained for each gene were normalized and gene transcription was calculated relative to the Control group (unchallenged birds fed no additives).
The genes evaluated were MUC2, MUC5AC, MUC13, IL-1β, IL-6, TNFSF15 and TFF2. Quantitative RT-PCR oligonucleotide primers for chicken cytokines, chemokines, and GAPDH control are listed in Table 1.

2.5 Bioinformatics analysis and statistical analyses

The bioinformatics analysis used the QIIME2 2020.11 framework (Bolyen et al., 2019). The data for the analysis was 16S rRNA amplicons. Q2-dada2 plugin was used with DADA2 pipeline to filter, denoise and chimera filter the raw sequence data (Callahan et al., 2016).
The reads were truncated using a score less than or equal to 12 on the read length filtering, the 5' nucleotide bases were trimmed (forward and reverse read sequences) due to low quality and reads that had expected errors higher than 2 were discarded. In addition, forward and reverse sequences had their resulting reads that were shorter than 250bp length discarded.
Chimera removal used the "consensus" method. In this way, each chimera is individually analyzed and detected in samples. For the OTU's alignment, were used mafft, via the q2-alignment plugin that allows its uses (Katoh and Daron, 2013). Building the phylogeny for the samples, we use the q2-phylogeny plugin (choosing fasttree2 to perform the construction) (Pearson et al. 2019). The classifier used to sign the taxonomy for the OTUs was Naive Bayes via q2-feature-classifier (Rish, 2001) and was trained with Greengenes 13_8 99% OTU's reference sequences.
On diversity analysis, after the samples were rarefied, the metrics used were alpha diversity metrics (Shannon diversity index and Faith's Phylogenetic Diversity), beta diversity metric (bray-curtis dissimilarity (Clarke et al., 2006)) and Principal Coordinate Analysis was performed (PCoA). For this, the q2-diversity plugin was used to perform the analyses, and the significance for alpha diversity was estimated by Kruskal-Wallis test and for beta diversity by PERMANOVA non-parametric multivariate analysis (Anderson et al., 2013).
The graphic visualization was made using the resulting archives (feature table, rooted tree and taxonomy classification) that were imported from QIIME2 to R v4.0.5 environment. The R packages that have functions for biological analysis used were qiime2R, phyloseq, microbiome (R Core Team, 2021; McMurdie and Holmes, 2013).
Gene expression data were analyzed by ANOVA and contrasts between Control group and each treatment were assessed using Tukey test in R (v. 3.6.3) (R Core Team, 2018). For all analyzes, the level of significance considered was $P < 0.05$ and $P < 0.10$.

3 Results

3.1 Operational taxonomic

A total of 2,069,993 quality sequences were produced with an average number of sequences per sample of 59,142. Sample T27 (Phytogenic - jejunum) showed low sequencing depths and was disregarded from subsequent analyses.

3.2 Alpha diversity analysis

The Faith's phylogenetic index was used to estimate species richness; Shannon's index was used to indicate species diversity. There was no statistical difference between groups regarding the estimated richness (P=0.23) or diversity (P=0.64) considering the pool of intestinal segments (Figure 1). Pairwise comparison between treatments and intestinal segments were done, and also no differences were observed on Faith's and Shannon's indices.

3.3 Beta diversity analysis

PCoA based on Bray-Curtis distances was performed to determine the formation of clusters according to the treatments. In diversity metric, the treatment groups were well separated with 27.6% and 17.8% variation by the principal components PCoA1 and PCoA2, respectively (Figure 2).

The PERMANOVA test based on treatment groups resulted in a p-value 0.027 and a test statistic of 1.905 for Bray-Curtis. These metrics indicated that clustering based on treatment is weak (ie, differences can be explained by randomness). However, despite the lack of homogeneity in the dispersion, the PCoA graphics showed that the microbial populations of jejunum and ileum were more similar than that of the cecum.

3.4 Relative abundance

To elucidate the effect of feed additives associated with *Eimeria* infection on the composition of the cecal, ileal and jejunal microbiota, we analyzed the bacteria at the phylum, class and family levels to characterize the dynamics of microbial taxonomic distribution.

3.4.1 Jejunum and Ileum

Firmicutes was the dominant phylum in the jejunal and ileal microbial community in all groups (Figure 3). Broilers fed diets supplemented with Ionophore showed a higher relative proportion of Proteobacteria in the jejunum (3% vs. 0.8% and 0.1% for the Control, and Phytogenic groups, respectively) and ileum (1% vs. 0.2% and 0% for the Control, and Phytogenic groups, respectively), in addition to greater Cyanobacteria in jejunum (21.2% vs. 1.5% and 0.7% for the Control and Phytogenic groups, respectively) and ileum (15% vs. 2% and 0.1% for the Control and Phytogenic groups, respectively). The highest relative abundance of Actinobacteria was observed in the microbiota of the Control group.

At the class level (Figure 4), Bacilli was the most abundant class in all groups. Broilers from the Control and Ionophore groups exhibited the largest proportion of Actinobacteria in jejunum (Control: 21%, Ionophore: 14%, and Phytogenic: 0.2%) and Clostridia in ileum, compared Phytogenic group (Control: 22%, Ionophore: 25%, and Phytogenic: 0.8%).

Lactobacillaceae was the most abundant family in the microbiota of all groups, however the highest proportion was observed in birds supplemented with phytogenic (Figure 5). The Phytogenic group had lower Corynebacteriaceae proportion (0.2% vs. 19% and 10% for the Control and Ionophore groups, respectively). The highest relative abundance of Corynebacteriaceae was observed in the jejunal and ileal microbiota of the Control group. In the ileum, the birds from the Ionophore group showed a higher percentage of Turicibacteraceae when compared to the other groups.

3.4. Cecum

In cecum, following Firmicutes, the most abundant phylum group is Bacteroidetes, followed by a small proportion of Cyanobacteria, Actinobacteria, Proteobacteria, and Tenericutes (Figure 3). Ionophore group had lower Firmicutes (Control: 80%, Ionophore: 51%, and Phytogenic: 57%) proportions, while Proteobacteria (Control: 0.9%, Ionophore: 6%, and Phytogenic: 2%) and Cyanobacteria (Control: 0%, Ionophore: 34%, and Phytogenic: 0.3%) proportions were higher, compared to the Phytogenic and Control group. The Phytogenic and Ionophore groups had

higher Bacteroidetes (Control: 13%, Ionophore: 38%, and Phytogenic: 40%) compared to Control group.
At the class level (Figure 4), Clostridia was the most abundant class in all groups (Control: 66%, Ionophore: 49%, and Phytogenic: 49%). The Ionophore groups had higher Gammaproteobacteria proportion, compared to other groups (2.5% vs. 0.8% and 0.1% for the Control, and Phytogenic groups, respectively), whereas the Phytogenic group had higher Erysipelotrichi (4.0% vs. 0.46% and 0.8% for the Control, and Ionophore groups, respectively).
At Family level (Figure 5), Peptostreptococcaceae showed the higher percentage in Control and Phytogenic groups (Control: 37%, Ionophore: 15%, and Phytogenic: 32%), whereas Bacteroidaceae was the most abundant in Ionophore and Phytogenic groups (Control: 7%, Ionophore: 29%, and Phytogenic: 39%). The highest relative abundance of Clostridiaceae was observed in the jejunal and ileal microbiota of the Ionophore group. As in the jejunum and ileum segments, the Control group showed a higher percentage of Corynebacteriaceae in cecum when compared to the other groups.

3.5 Relative gene expression of intestinal barrier function

The level of inflammatory gene transcript for *IL-6* (Pro-inflammatory) were increased ($P < 0.05$) in the Ionophore and Phytogenic groups compared to Control group (Figure 6). Furthermore, broilers of the Ionophore group showed an increase in the level of *MUC13* gene transcription, but statistically similar to the Phytogenic group. No difference between treatments was observed for gene transcription of *MUC2*, *MUC5AC*, *IL-1β*, *TNFSF15*, and *TFF2* ($P > 0.05$).

4 Discussion

Phytogenic feed additive effects on broiler performance, gut microbiota composition, and metabolic activities as well as on gut signaling molecules and host immune responses are currently in worldwide scientific focus. In the current study, microbiotas were characterized in intestinal segments associated with two different commercial additives in response to an intestinal challenge with *Eimeria* spp., and the relation between the broiler inflammatory responses. Microbiota was also characterized in non-challenged chickens fed without additives.

4.1 Microbiome

The Faith's and Shannon's diversity indices in the gut microbiota decreased in the Phytogenic group. This was consistent with the literature results, which showed that supplementation with essential oils (Yin et al., 2017), and phytogenic extract (carvacrol, thymol) (Zhu et al., 2019) decreased Shannon's and Simpson's indices. Evaluation of the microbiota population has revealed that phytogenic additives alike reduced total bacterial load in the gut (Murugesan et al., 2015). This inhibitory effect of phytogenic on bacterial load may alleviate pressure on the immune system, thus allowing the reallocation of energy toward improving performance. Firmicutes was the major phyla for the three gut sections analyzed. Firmicutes species are associated with the decomposition of polysaccharides and the production of butyrate. Some recent in vivo experiments have suggested that phytogenic extracts modify the composition of the intestinal microbiota, increasing the relative abundance of Firmicutes in the gut (Salaheen et al., 2017; Li et al., 2018).

The cecal microbiota of the Phytogenic and Ionophore groups showed a greater relative abundance of the phylum Bacteroidetes and lower abundance of Firmicutes, compared to the Control group (non-challenged). These results confirm that the infection of *Eimeria* induces differential changes in microbiota, as well as noted by Huang et al., (2018) and Vieira et al. (2020), in which the *Eimeria* infection reduced the frequency of Firmicutes and increased the abundance of Bacteroidetes. In situations of dysbiosis, such as those caused by the *Eimeria* challenge, some genera of Bacteroidetes can proliferate and become pathogenic, and consequently reduce the feeding efficiency of birds (Betancourt et al., 2019). In our study, a higher ratio of Firmicutes: Bacteroidetes in the challenged groups (Phytogenic and Ionophore groups) was observed when compared to the Control group, a proportion that have been associated with a poor performance (Vieira et al., 2021).

An interesting result observed was the highest relative abundance of Proteobacteria in the jejunal and cecal microbiota of the Ionophore group. As noted in the present study, Lu et al. (2006) also showed that monensin supplementation produced a greater relative abundance of gram-negative bacteria, such as the Proteobacteria phylum and the predominance of the Clostridia class. The phylum Proteobacteria is formed by gram-negative bacteria and includes a wide variety of pathogenic species such as *Shigella* spp., *Escherichia* spp., *Campylobacter* spp., *Salmonella* spp. and *Pseudomonas* spp. In humans, the increased prevalence of the bacterial phylum Proteobacteria it is associated a compromised ability to maintain a balanced intestinal microbial community, causing this phylum to be considered a marker for an unstable microbial community (dysbiosis) and a potential diagnostic criterion for disease (Shin et al., 2015). Meanwhile, in broilers, lower percentage of Proteobacteria have been associated with a healthy intestinal environment and better performance (Orso et al., 2021).

The class Bacilli and family Lactobacillaceae was observed in the highest relative abundance in jejunal and ileal microbiota of all groups, mainly i in the Phytogenic group. These results were consistent with those of Yin et al. (2017) and Li et al. (2018), who reported that phytogenic treatment increased the relative abundance of Lactobacillales after intestinal challenged. A relative increase in the abundance of the Lactobacillaceae family was showed in the microbiota (at 28 d) of coccidiosis-challenged broilers supplement with functional-oil blend or anticoccidial antibiotics (enramycin and tylosin), however, at 42 d, all treatments showed a reduction in the relative abundance of this family (Pires et al., 2022). Vieira et al. (2020) also observed a higher proportion of Lactobacillaceae in broilers challenged with *Eimeria*. Members of this family, such as *Lactobacillus* spp., play a positive role in improving gut health, immunological characteristics, and production performance (Joat et al., 2021) and can selectively exclude the pathogens from adhering due to their fast colonization, proliferation, and acidifying properties in the gastrointestinal tract (McReynolds et al., 2009).

The Peptostreptococcaceae family, a member of the phylum Firmicutes, is an important group of butyric acid-producing bacteria. Short-chain fatty acids, such as butyric acids, are end products of non-starch polysaccharides by the gut bacterial fermentation. Functionally, butyric acid is a main energy source for the colonic mucosa and can increase the expression of tight junction proteins to support the integrity of the gut barrier (2021). Peptostreptococcaceae is usually considered as normal commensal bacteria, their higher proportion in the gut microbiota has been linked to a healthy animal (Fan et al., 2017), indicating that Peptostreptococcaceae helps maintain intestinal homeostasis.

4.2 Immune responses

To evaluate the protective effect of additives supplementation against coccidiosis, we examined the gene expressions of pro-inflammatory cytokines and repair proteins of gut tissue injury in jejunum-ileum. We demonstrated that the ionophore and phytogenic additives modulated the immune system by significantly increasing *IL-6* and *MUC13* gene transcription compared to the Control group.

IL-6 is generally considered a cytokine indicative of the onset of an acute phase response, being important in inflammatory processes and in the immune response (Hong et al. 2006). Under a challenge by coccidiosis, *Eimeria* can causes a massive inflammatory response in the intestinal mucosa through invasion and subsequent damage to epithelial cells. Thus, as inflammation is a component of the acute phase response, *IL-6* is produced during immune responses to parasitic infection (Lynagh et al., 2000). Enhanced expression of the *IL-6* gene has been reported in the epithelial cells of chickens infected with *E. acervulina*, *E. maxima* or *E. tenella* (Hong et al., 2006).

Mucins are a major component of mucus, and in this study, the transcription of *MUC13*, a transmembrane mucin, was increased in both groups submitted to coccidiosis. Macdonald et al. (2019) also observed an increased transcription of *MUC13* in the presence of nonattenuated *E. tenella*. This observed increase in *MUC13* expression may be related to intestinal inflammation caused by *Eimeria*, since results of studies position *MUC13* as an important participant in the epithelial response to injury and inflammation. These results position Muc13 as an important participant in the epithelial response to injury and inflammation. In humans, *MUC13* mRNA expression was significantly increased in inflamed intestinal mucosa, and it can be assumed that this increase in expression during inflammation occurs to protect epithelial cells from apoptosis (Sheng et al., 2011).

The *TFF2* is believed to have the ability to interact with mucin to maintain barrier function (Suzuki et al., 2006) and plays an important role in the repair and restoration of damaged intestinal mucosa, with its expression increased after mucosal damage (Kurt-Jones et al., 2007). Although *TFF2* mRNA transcripts did not differ between treatments, an increase in their expression in both groups challenged by coccidiosis (Ionophore and Phytogenic groups) could be observed, showing that these birds would possibly be trying to restore damage caused by the infection.

Also, the levels of inflammatory intestinal gene transcript for *IL-1β* (Pro-inflammatory) were increased in both groups challenged by coccidiosis (Ionophore and Phytogenic). The *IL-1β* cytokines are involved in the initial inflammatory response and were evidenced to participate in the avian *Eimeria* infection (Hong et al., 2006; Jiao et al, 2018). *IL-1β* induces chemokine production, which promotes the recruitment of inflammatory cells at the inflammation site (Yamada et al., 2001). In chickens infected with *E. maxima* and *E. tenella*, highly upregulated expression of *IL-1β* was found in the duodenum, jejunum, and cecum post primary infection (Laurent et al., 2001; Hong et al., 2006).

The commercial product used in this project is composed of a mixture of plants, such as *A. concinna* and *S. officinarum*, which provide a range of active compounds (saponins, tannins and polyphenols). These active compounds may be one of the responsible for the variation of the immune responses studied. Saponins are natural steroids or triterpene glycosides with

immunomodulatory properties (Sander et al., 2019), which can act in synergistic or antagonistic manner in the interactions between the other compounds present in the commercial product.

5 Conclusion

The results of the present study showed that dietary supplementation with phytogenic feed additive or sodium monensin and coccidiosis challenge altered the composition of the intestinal microbiota. The phytogenic product, a mixture composed by *A. concinna* and *S. officinarum*, enhanced the relative abundances of Lactobacillaceae in duodenal and ileal microbiotas, and the relative abundances of Bacteroidaceae and Peptostreptococcaceae in cecum, while monensin, enhanced the relative abundances of Lactobacillaceae in ileal microbiota and Rikenellaceae, Bacteroidaceae, and Clostridiaceae in cecum. Both additives increased the abundance of important bacterial groups for the establishment of a favorable intestinal microbiota in the face of an intestinal challenge.

6 Data Availability Statement

The datasets presented in this study can be found in online repositories. NCBI BioProject accession number: PRJNA623634.

7 Ethics Statement

The animal study was approved by the Ethics Committee on Animal Use of the Universidade Federal do Rio Grande do Sul, Brazil (36347).

8 Conflict of Interest

The authors declare that the research was conducted in the absence of conflict of interest.

9 Author Contributions

TBS, CO, and AMLR designed the research. TBS, CO, CHF, JPS, and YY worked on the animal handling and collected and processed the samples. TBS, CO, MBM, APMV, and JF processed the samples by 16S rRNA sequencing and qPCR. JS conducted the bioinformatics analysis. TBS and AMLR final review and approval for manuscript publication. All authors contributed to the article and approved the submitted version.

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Table 1. Oligonucleotide primers used for the study of gene expression of selected targets by quantitative real time PCR.

Gene target[1]	**Function in intestinal mucosa**	**Primer sequences (5'-3')[2]**	**GenBank accession[3]**
GAPDH	Cytosolic enzyme that produces NADPH in reductive biosynthetic reactions	GGTGGTGCTAAGCGTGTTAT ACCTCTGTCATCTCTCCAC	KO1458
MUC2	Thought to provide a protective, lubricating barrier against particles and infectious agents at mucosal surfaces	ATGCGATGTTAACACAGGACTC GTGGAGCACAGCAGACTTTG	IX284122.1
MUC13	Epithelial and hemopoietic transmembrane mucin that may play a role in cell signaling	GCATTCCTCAAGCAGAGGTG CTCAGGCTGCCGTGATATTT	XM_003641585
MUC5AC	Protect the mucosa from infection and chemical damage by binding to inhaled microorganisms that are subsequently removed by the mucociliary system	TGTGGTTGCTATGAGAATGGA TTGCCATGGTTTGTGCAT	XM_003641322
IL-1β	Potent proinflammatory cytokine. Induces neutrophil influx and activation, T-cell activation and cytokine production, B-cell activation and antibody production	TGGGCATCAAGGGCTACA TCGGGTTGGTTGGTGATG	AF111631
IL-6	Plays an essential role in the final	CAAGGTGACGGAGGAGGAC TGGCGAGGAGGGATTTCT	AJ309540

	differentiation of B-cells into Ig-secreting cells		
IL-17	It is important for proliferation during certain stages of B-cell maturation	CTCCGATCCCTTATTCTCCTC AAGCGGTTGTGGTCCTCAT	AJ493595
TNFSF15	Mediates activation of NF-kappa-B	CCTGAGTATTCCAGCAACGCA ATCCACCAGCTTGATGTCACTAAC	AB194710
CD36	Involved in inflammatory response, fatty acid metabolism, taste and dietary fat processing in the intestine	GAATTGCTGTGGAAGTGCTG TGGTCCCAACAGACTCACTG	NM_001030731
TFF2	Could function as a structural component of gastric mucus, possibly by stabilizing glycoproteins in the mucus gel through interactions with carbohydrate side chains	GCTGTAGCCCTCATCAGCTC CTGGCAGCTATTTTGCACTG	XM_416743

[1] gene function derived from Gene Cards (http://www.genecards.org)
[2] F–Forward primer, R–Reverse primer
[3] ID GenBank access number

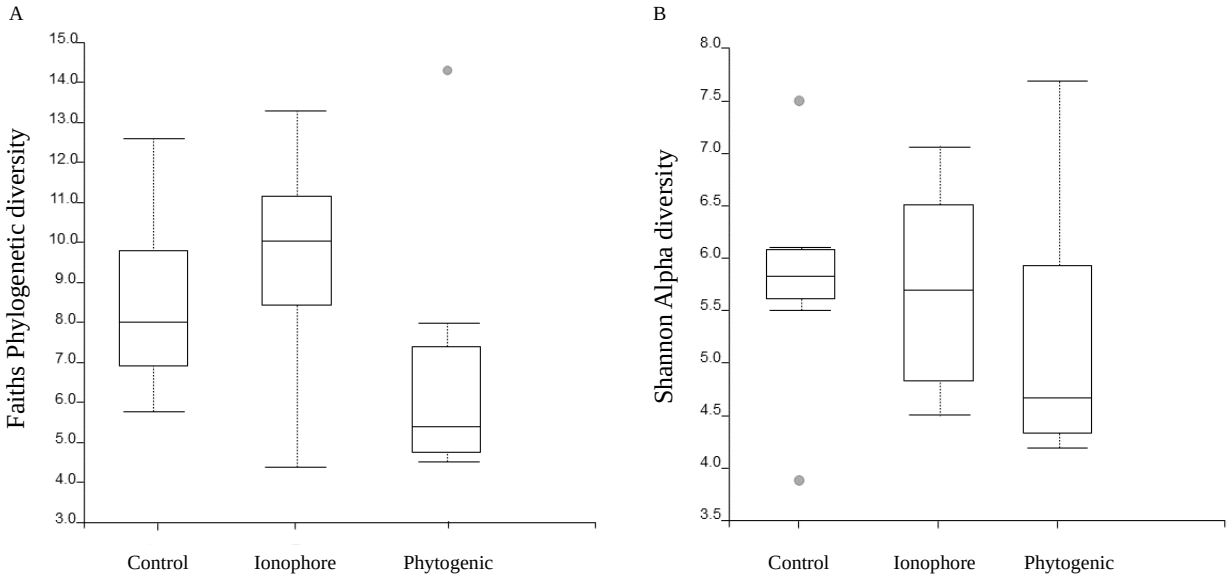

Figure 1. Alpha diversity in the intestinal digesta of broiler chickens at 28 days of age. Boxplots showing distribution of Shannon and Faith's phylogenetic alpha diversity measures in broilers samples. Control group (without infection and no additives), Ionophore group (Sodium Monensin + *Eimeria* challenged) and Phytogenic group (Phytogenic additive + *Eimeria* challenged).

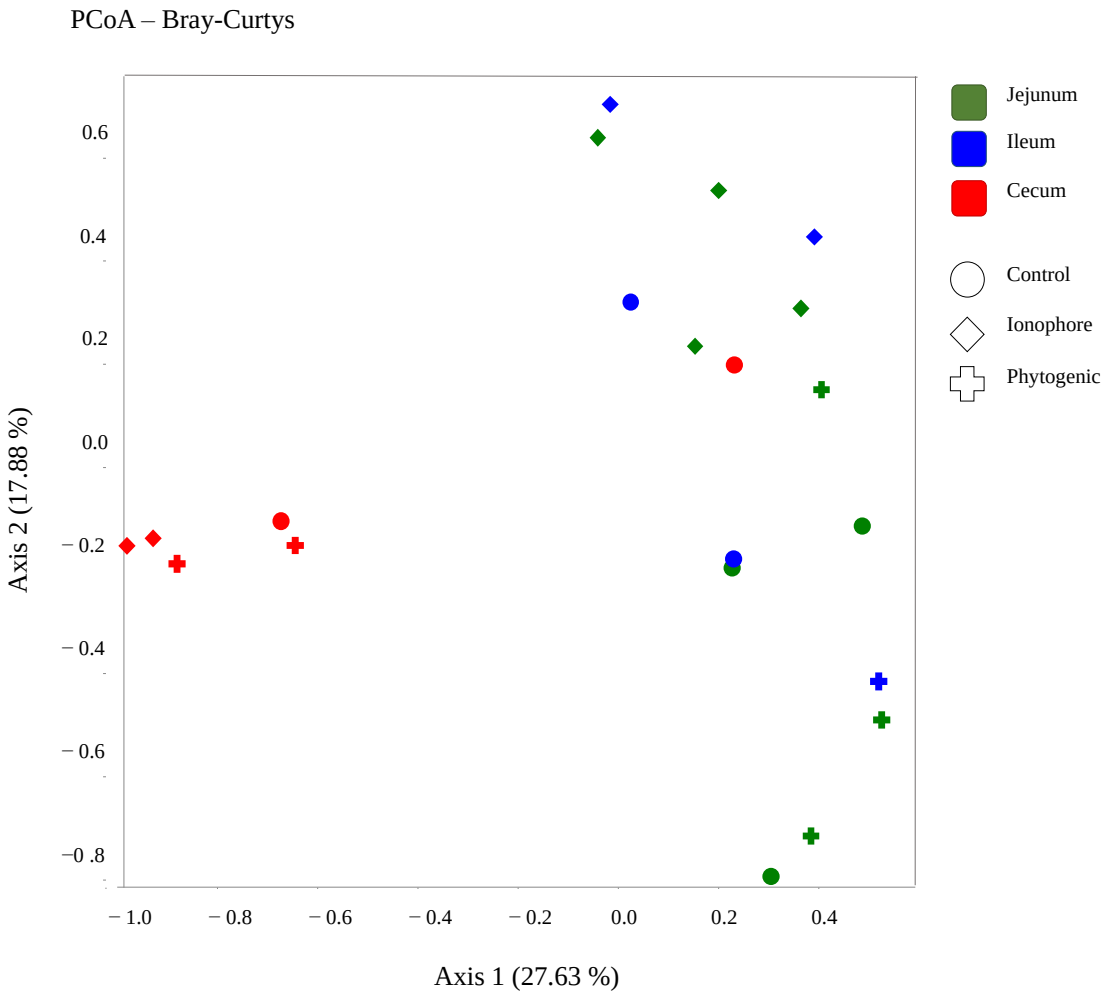

Figure 2. Network inference of microbiome using Bray-Curtis distance distance constructed in QIIME and visualized in EMPeror. Control group (without infection and additives), Ionophore group (Sodium Monensin + *Eimeria* challenged) and Phytogenic group (Phytogenic additive + *Eimeria* challenged).

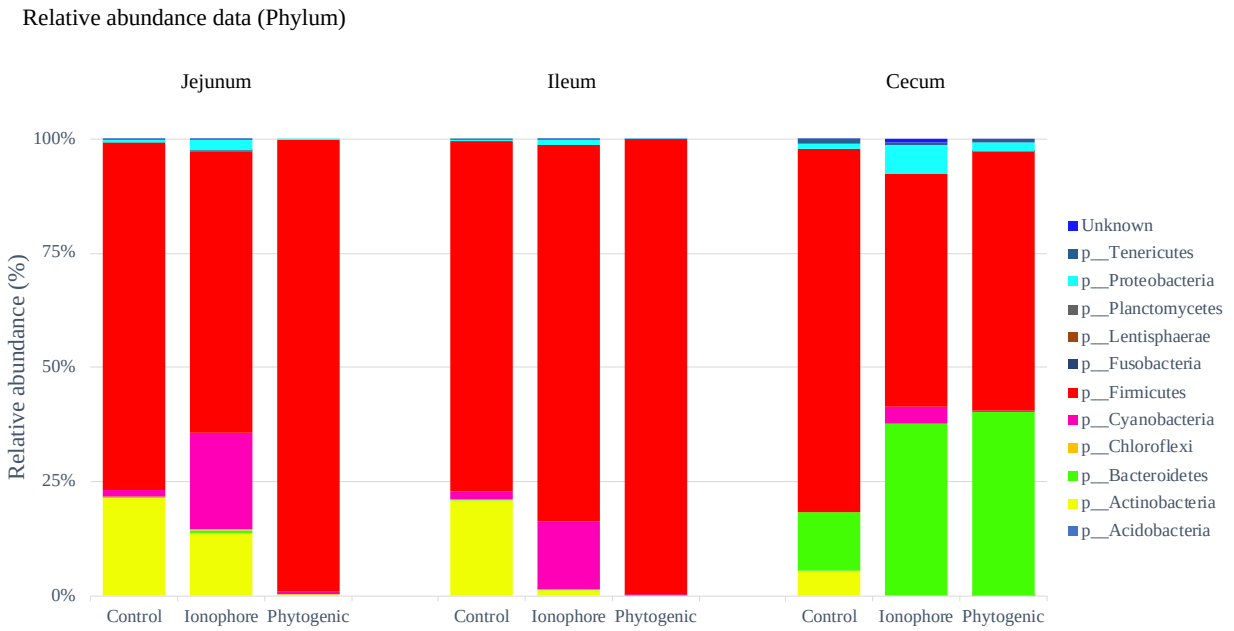

Figure 3. Bar graph of the relative abundances of the gut microbiota at the phylum level. The relative abundances of the gut bacteria presented here were calculated by averaging the data obtained from the replicates within each group. Minor bacterial genera and unassigned values were included as "others". Control group (without infection and no additives), Ionophore group (Sodium Monensin + *Eimeria* challenged) and Phytogenic group (Phytogenic additive + *Eimeria* challenged).

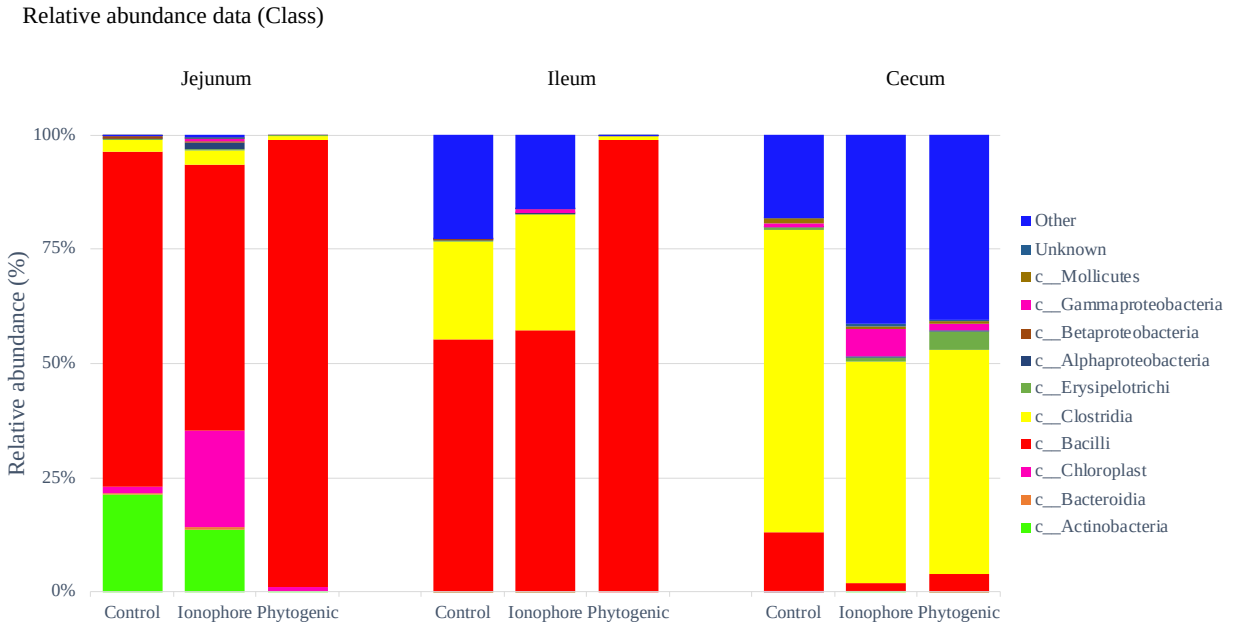

Figure 4. Bar graph of the relative abundances of the gut microbiota at the class level. The relative abundances of the gut bacteria presented here were calculated by averaging the data obtained from the replicates within each group. Minor bacterial genera and unassigned values were included as "others". Control group (without infection and no additives), Ionophore group (Sodium Monensin + *Eimeria* challenged) and Phytogenic group (Phytogenic additive + *Eimeria* challenged).

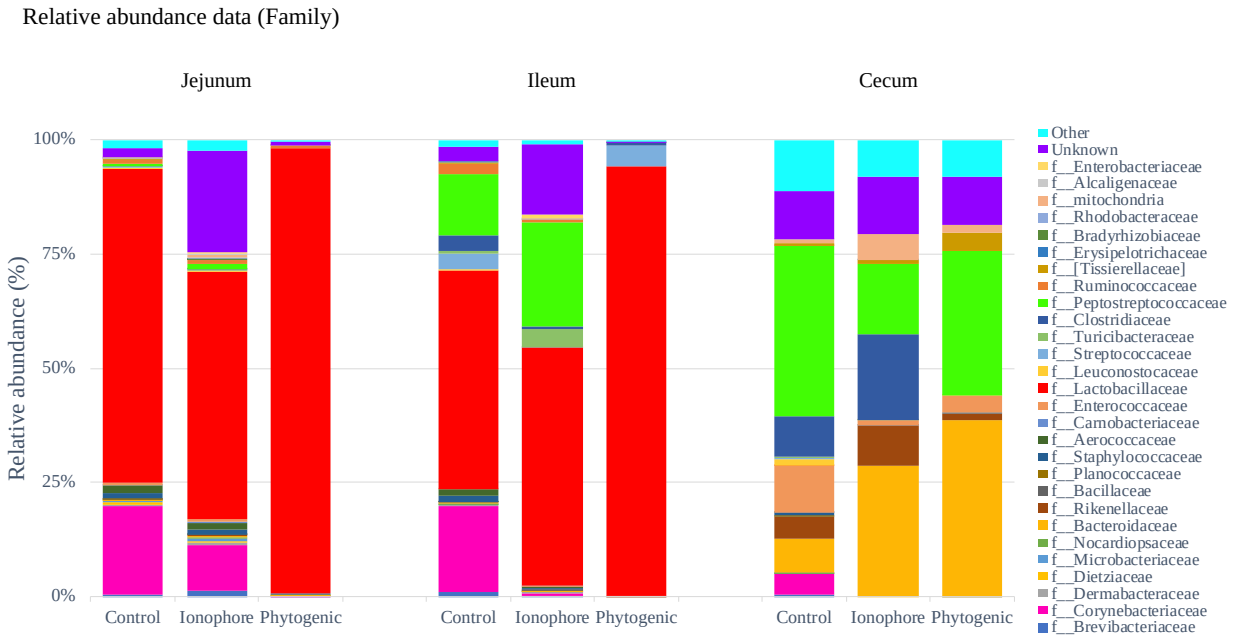

Figure 5. Bar graph of the relative abundances of the gut microbiota at the family level. The relative abundances of the gut bacteria presented here were calculated by averaging the data obtained from the replicates within each group. Minor bacterial genera and unassigned values were included as "others". Control group (without infection and no additives), Ionophore group (Sodium Monensin + *Eimeria* challenged) and Phytogenic group (Phytogenic additive + *Eimeria* challenged).

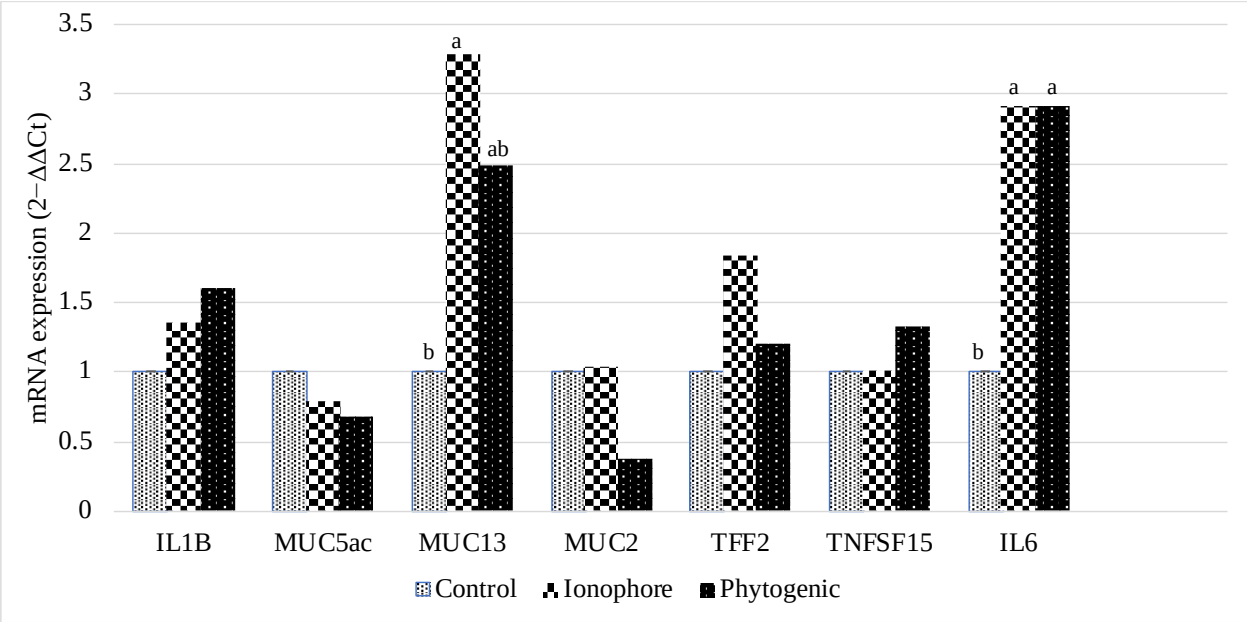

Figure 6. Relative mRNA expression of intestinal immune-related genes of broilers challenged with coccidiosis. Relative gene expression ($2^{-\Delta\Delta Ct}$) was calculated using the ΔΔCt method with GAPDH as the endogenous control and the average ΔCt value for the negative control birds on each day as the calibrator. Control group (without infection and no additives), Ionophore group (Sodium Monensin + *Eimeria* challenged) and Phytogenic group (Phytogenic additive + *Eimeria* challenged).

11 Supplementary Material

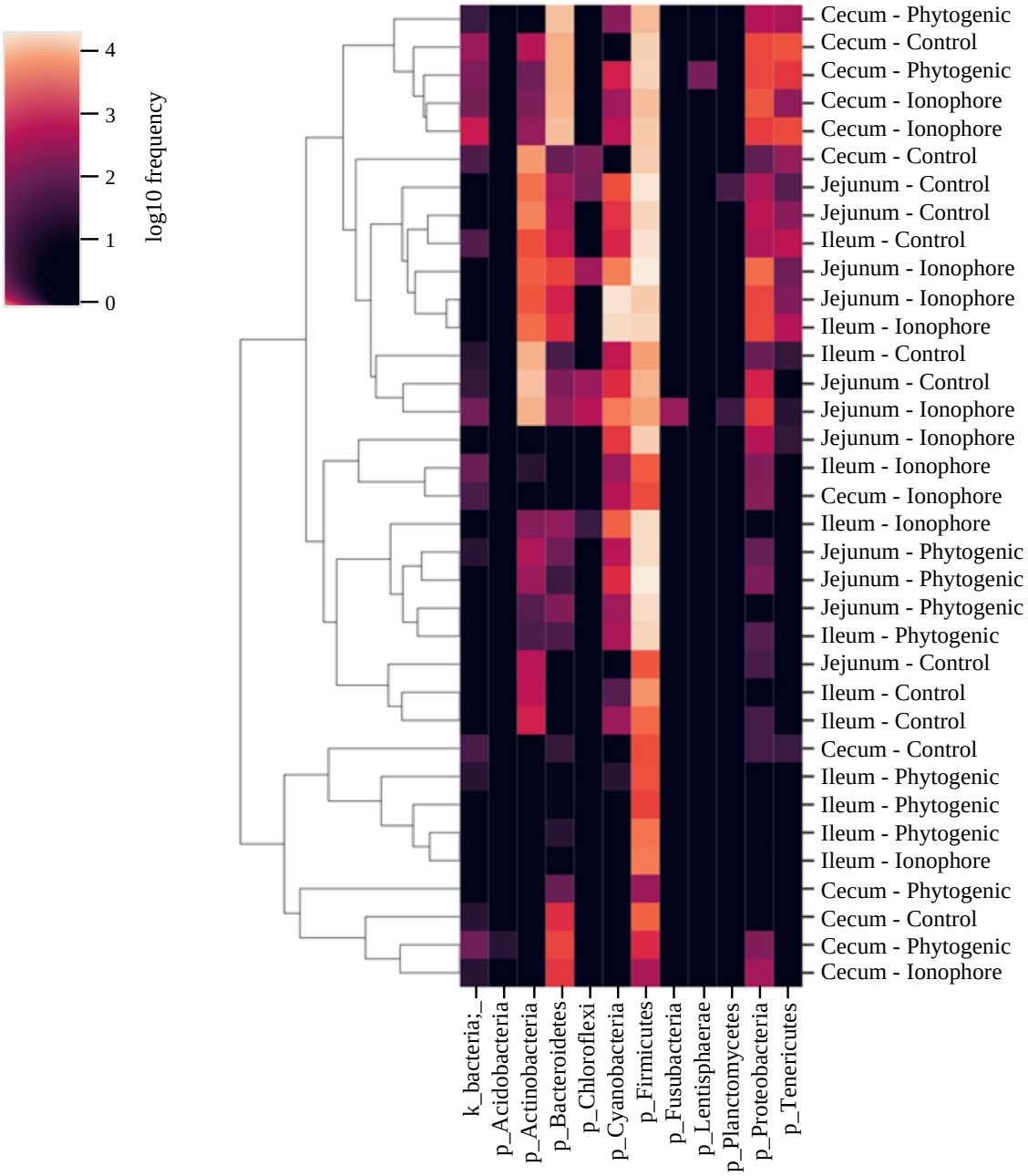

Figure S1. Heatmap ordinated using NMDS with Bray-Curtis microbial abundance of bacterial phylum in all intestinal samples (n = 35). Normalization of the frequency of the genera in log10. The color scale represents the stepped abundance of each variable, indicated by the score, with light pink indicating high abundance and dark blue indicating low abundance.

Table S1. Dietary compositions and nutrient levels of broilers (as-fed basis).

Ingredient (%)	Pre-starter (1-7 days)	Starter (8-21 days)	Grower (22-35 days)	Finisher (36-42 days)
Maize, %	46.759	48.607	51.183	61.082
Soybean meal, %	45.481	42.924	39.700	31.494
Soybean oil, %	3.692	4.784	5.768	4.658
Limestone, %	1.151	1.061	1.016	0.889
Phosphate, %	1.535	1.274	1.025	0.636
NaCl, %	0.525	0.511	0.486	0.460
DL-Methionine, %	0.366	0.347	0.323	0.262
L-Lysine, %	0.135	0.140	0.149	0.180
L-Treonine, %	0.062	0.059	0.056	0.047
Biocholine, %	0.050	0.050	0.050	0.050
Vit Premix, %	0.034	0.034	0.034	0.034
Min Premix, %	0.100	0.100	0.100	0.100
Phytase, %	0.010	0.010	0.010	0.010
Inert/Ionophore/Phytogenic[3]	0.10	0.10	0.10	0.10
Calculated nutrition levels, %				
CP	24.63	23.61	22.34	19.40
Ca	1.011	0.907	0.822	0.661
P disp	0.482	0.432	0.384	0.309
Na	0.227	0.221	0.211	0.201
EM, kcal	3000	3100	3200	3250
Dig Lys	1.364	1.306	1.235	1.067
Dig Met	0.680	0.649	0.612	0.522
Dig Met+Cis	1.009	0.966	0.914	0.790

Dig Thr	0.900	0.862	0.815	0.704
Dig Tr	0.287	0.273	0.256	0.213

Table S2. Relative abundance of phylum, class, family and genus present in the intestinal microbiota of broilers.

Intestinal segment	Jejunum			Ileum			Cecum		
Treatment group	Control	Ionophore	Phytogenic	Control	Ionophore	Phytogenic	Control	Ionophore	Phytogenic
Phylum									
p__Acidobacteria	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.1%
p__Actinobacteria	21.4%	13.7%	0.2%	20.8%	1.2%	0.0%	5.5%	0.1%	0.0%
p__Bacteroidetes	0.2%	0.6%	0.1%	0.2%	0.3%	0.0%	12.7%	37.5%	40.0%
p__Chloroflexi	0.1%	0.3%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
p__Cyanobacteria	1.5%	21.2%	0.7%	1.7%	14.7%	0.1%	0.0%	3.7%	0.2%
p__Firmicutes	75.9%	61.6%	98.9%	76.9%	82.6%	99.6%	79.7%	51.1%	56.9%
p__Fusobacteria	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
p__Lentisphaerae	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
p__Planctomycetes	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
p__Proteobacteria	0.8%	2.5%	0.1%	0.2%	1.2%	0.0%	1.0%	6.2%	1.8%
p__Tenericutes	0.1%	0.0%	0.0%	0.1%	0.1%	0.0%	1.1%	0.8%	0.6%
Unknown	0.0%	0.0%	0.0%	0.0%	0.0%	0.2%	0.0%	0.6%	0.2%
Class									
c__Holophagae	0.0%	0.0%	0.0%	20.8%	1.2%	0.0%	5.5%	0.0%	0.0%
c__Actinobacteria	21.4%	13.6%	0.2%	0.0%	0.0%	0.0%	0.0%	0.1%	0.0%
c__Coriobacteriia	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
c__Thermoleophilia	0.0%	0.0%	0.0%	0.2%	0.3%	0.0%	12.7%	37.5%	40.0%
c__Bacteroidia	0.2%	0.6%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
c__Cytophagia	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
c__Flavobacteriia	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
c__Sphingobacteriia	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
c__Thermomicrobia	0.1%	0.3%	0.0%	0.1%	0.0%	0.0%	0.0%	0.3%	0.2%
c__4C0d-2	0.0%	0.2%	0.0%	1.6%	14.6%	0.1%	0.0%	3.4%	0.0%
c__Chloroplast	1.5%	21.0%	0.7%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
c__Bacilli	73.1%	58.2%	97.9%	55.2%	57.3%	98.8%	13.0%	1.8%	4.0%
c__Clostridia	2.7%	3.3%	0.9%	21.6%	25.2%	0.8%	66.1%	48.6%	49.0%
c__Erysipelotrichi	0.1%	0.1%	0.1%	0.2%	0.1%	0.0%	0.5%	0.8%	4.0%
c__Fusobacteriia	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
c__[Lentisphaeria]	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
c__Planctomycetia	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
c__Alphaproteobacteria	0.2%	1.5%	0.0%	0.1%	0.3%	0.0%	0.0%	0.4%	0.3%
c__Betaproteobacteria	0.4%	0.2%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%

c__Deltaproteobacteria	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
c__Epsilonproteobacteria	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
c__Gammaproteobacteria	0.2%	0.8%	0.0%	0.1%	0.9%	0.0%	1.0%	5.8%	1.5%
c__Mollicutes	0.1%	0.0%	0.0%	0.1%	0.1%	0.0%	1.1%	0.8%	0.6%
Unknown	0.0%	0.0%	0.0%	0.0%	0.0%	0.2%	0.0%	0.6%	0.3%
Family									
f__Brevibacteriaceae	0.5%	1.3%	0.0%	0.9%	0.2%	0.0%	0.4%	0.0%	0.0%
f__Corynebacteriaceae	19.3%	10.0%	0.2%	19.0%	0.6%	0.0%	4.5%	0.0%	0.0%
f__Dermabacteraceae	0.4%	0.4%	0.0%	0.1%	0.1%	0.0%	0.1%	0.0%	0.0%
f__Dietziaceae	0.6%	0.4%	0.0%	0.1%	0.1%	0.0%	0.1%	0.0%	0.0%
f__Microbacteriaceae	0.1%	0.7%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%
f__Nocardiopsaceae	0.2%	0.2%	0.0%	0.2%	0.0%	0.0%	0.1%	0.0%	0.0%
f__Bacteroidaceae	0.1%	0.3%	0.1%	0.2%	0.2%	0.0%	7.4%	28.8%	38.7%
f__Rikenellaceae	0.0%	0.2%	0.0%	0.0%	0.1%	0.0%	4.8%	8.7%	1.4%
f__Bacillaceae	0.0%	0.0%	0.0%	0.1%	0.1%	0.0%	0.0%	0.0%	0.3%
f__Planococcaceae	0.3%	0.1%	0.0%	0.0%	0.0%	0.0%	0.4%	0.0%	0.0%
f__Staphylococcaceae	1.2%	1.2%	0.4%	1.5%	0.3%	0.0%	0.4%	0.1%	0.0%
f__Aerococcaceae	1.7%	1.2%	0.0%	1.2%	0.4%	0.0%	0.1%	0.0%	0.0%
f__Carnobacteriaceae	0.1%	0.3%	0.0%	0.0%	0.0%	0.0%	0.1%	0.0%	0.0%
f__Enterococcaceae	0.3%	0.5%	0.0%	0.1%	0.1%	0.0%	10.1%	1.2%	3.6%
f__Lactobacillaceae	68.7%	54.3%	97.5%	47.9%	52.2%	94.2%	0.1%	0.0%	0.0%
f__Leuconostocaceae	0.3%	0.1%	0.0%	0.5%	0.0%	0.0%	1.3%	0.0%	0.0%
f__Streptococcaceae	0.4%	0.2%	0.0%	3.2%	0.0%	4.6%	0.3%	0.0%	0.0%
f__Turicibacteraceae	0.0%	0.1%	0.0%	0.6%	3.8%	0.0%	0.3%	0.0%	0.0%
f__Clostridiaceae	0.1%	0.2%	0.0%	3.5%	0.8%	0.5%	8.9%	18.8%	0.0%
f__Peptostreptococcaceae	0.5%	1.0%	0.0%	13.5%	22.7%	0.0%	37.3%	15.3%	31.9%
f__Ruminococcaceae	0.8%	0.9%	0.6%	2.2%	0.6%	0.2%	0.1%	0.0%	0.0%
f__[Tissierellaceae]	0.3%	0.0%	0.0%	0.3%	0.0%	0.0%	0.5%	0.8%	4.0%
f__Erysipelotrichaceae	0.1%	0.1%	0.1%	0.2%	0.1%	0.0%	0.0%	0.1%	0.0%
f__Bradyrhizobiaceae	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
f__Rhodobacteraceae	0.1%	0.4%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
f__mitochondria	0.1%	0.6%	0.0%	0.0%	0.2%	0.0%	1.0%	5.8%	1.5%
f__Alcaligenaceae	0.2%	0.2%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
f__Enterobacteriaceae	0.1%	0.4%	0.0%	0.0%	0.8%	0.0%	0.0%	0.0%	0.0%
Unknown	2.0%	22.1%	0.8%	3.0%	15.3%	0.3%	10.5%	12.5%	10.6%
Other	1.6%	2.4%	0.2%	1.5%	1.0%	0.2%	11.1%	8.0%	8.1%

Genus									
g__Brevibacterium	0.5%	1.3%	0.0%	0.9%	0.2%	0.0%	0.4%	0.0%	0.0%
g__Corynebacterium	19.2%	10.0%	0.2%	19.0%	0.6%	0.0%	4.5%	0.0%	0.0%
g__Brachybacterium	0.4%	0.4%	0.0%	0.1%	0.0%	0.0%	0.1%	0.0%	0.0%
g__Dietzia	0.6%	0.4%	0.0%	0.1%	0.1%	0.0%	0.1%	0.0%	0.0%
g__Glycomyces	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
g__Gordonia	0.1%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
g__Leucobacter	0.0%	0.4%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
g__Pseudoclavibacter	0.1%	0.1%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%
g__Arthrobacter	0.1%	0.1%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%
g__Mycobacterium	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
g__Nocardioides	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
g__Nocardiopsis	0.2%	0.2%	0.0%	0.2%	0.0%	0.0%	0.1%	0.0%	0.0%
g__Streptomyces	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
g__Yaniella	0.1%	0.1%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%
g__Bacteroides	0.1%	0.3%	0.1%	0.2%	0.2%	0.0%	7.4%	28.8%	38.7%
g__Parabacteroides	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.4%	0.0%	0.0%
g__Alistipes	0.0%	0.2%	0.0%	0.0%	0.1%	0.0%	4.8%	8.7%	1.4%
g__Dyadobacter	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
g__Bacillus	0.1%	0.0%	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%
g__Kurthia	0.2%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
g__Jeotgalicoccus	0.3%	0.4%	0.0%	0.9%	0.1%	0.0%	0.2%	0.0%	0.0%
g__Macrococcus	0.0%	0.0%	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%
g__Staphylococcus	0.8%	0.8%	0.4%	0.6%	0.2%	0.0%	0.2%	0.0%	0.0%
g__Aerococcus	0.2%	0.4%	0.0%	0.1%	0.0%	0.0%	0.2%	0.0%	0.0%
g__Atopostipes	0.2%	0.0%	0.0%	0.2%	0.0%	0.0%	0.0%	0.0%	0.0%
g__Facklamia	1.3%	0.9%	0.0%	0.8%	0.4%	0.0%	0.2%	0.0%	0.0%
g__Enterococcus	0.3%	0.4%	0.0%	0.1%	0.1%	0.0%	0.1%	0.0%	0.0%
g__Lactobacillus	68.7%	54.1%	97.5%	47.8%	52.2%	94.2%	10.1%	1.2%	3.6%
g__Weissella	0.3%	0.1%	0.0%	0.5%	0.0%	0.0%	0.1%	0.0%	0.0%
g__Streptococcus	0.4%	0.2%	0.0%	3.2%	0.0%	4.6%	1.3%	0.0%	0.0%
g__Turicibacter	0.0%	0.1%	0.0%	0.6%	3.8%	0.0%	0.3%	0.0%	0.0%
g__Caloramator	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
g__Candidatus Arthromitus	0.0%	0.1%	0.0%	3.0%	0.7%	0.5%	0.0%	0.0%	0.0%
g__Clostridium	0.1%	0.0%	0.0%	0.5%	0.0%	0.0%	0.3%	0.0%	0.0%
g__Blautia	0.1%	0.0%	0.1%	0.2%	0.0%	0.0%	3.8%	0.3%	0.3%

g__Clostridium	0.1%	0.0%	0.0%	0.1%	0.1%	0.0%	0.3%	0.7%	0.4%
g__Coprococcus	0.0%	0.0%	0.0%	0.1%	0.0%	0.0%	0.3%	0.2%	0.6%
g__Defluviitalea	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.1%	0.2%	0.5%
g__Ruminococcus	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.1%	0.0%	0.0%
g__[Ruminococcus]	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.2%	0.1%	0.2%
g__Clostridium	0.1%	0.2%	0.0%	2.9%	6.1%	0.0%	1.9%	3.4%	0.0%
g__Peptostreptococcus	0.1%	0.1%	0.0%	0.1%	0.0%	0.0%	0.0%	0.1%	0.0%
g__Anaerotruncus	0.1%	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%	0.4%	0.2%
g__Butyricicoccus	0.1%	0.0%	0.0%	0.2%	0.1%	0.0%	0.3%	1.0%	1.0%
g__Clostridium	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.2%	0.3%	0.2%
g__Faecalibacterium	0.2%	0.3%	0.3%	0.4%	0.2%	0.2%	16.9%	3.1%	17.8%
g__Oscillospira	0.1%	0.2%	0.0%	0.3%	0.1%	0.0%	6.3%	3.6%	4.1%
g__Ruminococcus	0.1%	0.0%	0.0%	0.1%	0.0%	0.0%	0.7%	2.0%	1.5%
g__Subdoligranulum	0.1%	0.0%	0.1%	0.1%	0.0%	0.0%	0.4%	0.2%	0.7%
g__Phascolarctobacterium	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.8%
g__Gallicola	0.3%	0.0%	0.0%	0.3%	0.0%	0.0%	0.1%	0.0%	0.0%
g__Clostridium	0.1%	0.0%	0.1%	0.1%	0.0%	0.0%	0.1%	0.5%	1.0%
g__Coprobacillus	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.1%	0.1%	0.5%
g__Ochrobactrum	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
g__Paenochrobactrum	0.1%	0.3%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
g__Aquamicrobium	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
g__Oceaniovalibus	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
g__Pseudorhodobacter	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.1%	0.0%
g__Alcaligenes	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
g__Oligella	0.2%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
g__Oceanimonas	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
g__Morganella	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
g__Providencia	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
g__Shigella	0.0%	0.2%	0.0%	0.0%	0.8%	0.0%	0.7%	5.5%	1.4%
g__Marinomonas	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
g__Acinetobacter	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
g__Psychrobacter	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Other	0.1%	0.3%	0.0%	0.3%	0.2%	0.1%	0.3%	0.3%	0.2%
Unknow	3.9%	25.8%	1.1%	15.6%	33.1%	0.4%	36.1%	39.3%	25.1%

CAPÍTULO III

Artigo científico nas normas da revista *Poultry Science*

MICROBIOTA, FUNCTIONAL OIL, COCCIDIOSIS

Dietary supplementation of different levels of functional oils associated with salinomycin in broiler undergoing an intestinal challenge with *Eimeria*: the dynamics on growth performance and intestinal microbiota

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ABSTRACT The present study evaluated the single effect of salinomycin sodium and the impact of its combination with functional oils (cashew nutshell liquid (CNSL) and castor oil) on the performance and microbiota of broiler chickens infected with mixed *Eimeria* species. In total, 512 Cobb500 male broilers were randomly allocated to 4 treatments (8 replicates, 16 birds/pen) including Control group (basal diet without additives); Ionophore group [salinomycin supplementation at 66 ppm (SS66)]; Ionophore + 0.075% Functional oil (FO) group (SS66 + FO supplementation at 750 ppm); Ionophore + 0.10% FO group (SS66 + FO supplementation at 1,000 ppm). All experimental groups were under a coccidiosis challenge. At 14 days of age, all birds were orally gavaged with 1 mL of a saline solution containing sporulated oocysts of *E. tenella*, *E. acervulina* and *E. maxima*. Feed intake and weight gain for each replicate were measured weekly. At 28 days, the intestinal content of three birds per treatment was collected for microbiota analysis conducted by the Miseq Illumina platform. In the weeks following the intestinal challenge (15 to 28 days of age), broilers of the Control group presented the worst performance for BW, BW gain and feed intake. However, broilers of Ionophore + FO (0.075% and 0.10%) groups exhibited a higher BW at 28 days of age. Over days 29-42, no differences between treatments were observed ($P>0.05$). However, at 42 d, broilers fed with Ionophore + FO (0.075% and 0.10%) had significantly higher final BW. In the accumulated period (1-42 d) the BW gain was higher for the same groups. The intestinal microbiota was dominated by the phylum Firmicutes, regardless of treatment. The Ionophore + 0.075% FO group had a higher relative proportion of Firmicutes and a lower proportion of Actinobacteria in the ileum-jejunum. *Lactobacillus* dominated the jejunal and ileal microbial community in all groups, while for cecum, *Bacteroides* and *Faecalibacterium* were found more frequently. In jejunum and ileum, the Ionophore + 0.075% FO group increased the number of *Lactobacillus* and decreased *Corynebacterium*. Therefore, salinomycin in combination with functional oil showed synergistic effect on performance and modulation of intestinal microbiota of broilers undergoing an intestinal challenge with *Eimeria*, minimizing the impact of coccidiosis.

Key words: 16S rRNA sequencing, broiler, coccidiosis, *Eimeria*.

INTRODUCTION

Maintaining a healthy intestinal environment is a prerequisite for the efficient performance of broilers (Murugesan et al., 2015). However, health and well-being are constantly threatened by a series of pathogens and parasitic protozoa (Soutter et al., 2020), which represent an ongoing challenge to the world's poultry industry. Coccidiosis, an infection caused by protozoa of the genus *Eimeria*, is one of the most significant parasitic diseases in the chicken industry and a major problem worldwide, resulting in huge losses (Madlala et al., 2021).

For many years, the use of prophylactic doses of anticoccidials was the main choice for the treatment and prevention of this intestinal disease (Chapman et al., 2010). However, due to increasing changes in legislation and industry pressure to reduce the use of antibiotics growth promoters (AGP) and anticoccidials, an increasing demand was created in the poultry industry for new alternative strategies to improve performance and disease resistance including ways to establish a favorable gut microbiota (Yadav and Jha, 2019).

Among the alternative nutritional strategies, the phytogenic feed additives comprise one of the scientific areas of great interest, since these products have beneficial bioactive compounds that can protect animals against bacteria and parasite infections (Sidiropoulou et al., 2020). Functional oils, including cashew nutshell liquid (CNSL) and castor oil, are chemical substances extracted that provide health benefits extend beyond their nutritional value. The commercial CNSL and Castor oil mixture is composed of bioactive substances such as cardanol, cardol, ricinoleic acid, terpenes and phenols that, synergistically, exhibit potential antioxidant and antimicrobial activity and interact with the cell membrane of microorganisms limiting their growth (Andrade et al., 2011; Osmari et al., 2017).

Under a coccidiosis challenge, positive outcomes on performance and microbiota modulation were observed with the use of CNSL and Castor oil mixture. The blend was able to compensate the negative effect caused by coccidiosis, showing a similar effect to the ionophore monensin on the performance in the rearing period (up to 42 d) (Moraes et al., 2019b). In addition, better energy utilization, higher survival rate of animals and lower intestinal damage caused by *Eimeria* were also observed with the same functional oil compared to the control group without any additive (Murakami et al., 2014). Despite the positive results obtained with the use of CNSL and Castor oil mixture or other phytogenic feed additives, the process of removing AGP and anticoccidials from the poultry production chain is a gradual process and, often, the resistance from producers and the high price of phytogenic additives products makes their single use unfeasible. Additionally, in many situations, avian coccidiosis cannot be treated or controlled using just one compound, but requires a combination of products and protocols to achieve the necessary efficiency (Quiroz-Castañeda, 2018).

Therefore, the present study evaluated the single effect of salinomycin sodium and the impact of its combination with functional oils (CNSL - Castor oil blend) on the performance and microbiota of broiler chickens infected with mixed *Eimeria* species.

MATERIAL AND METHODS

Ethics Statement

The work described here was conducted under protocol number 36475 approved by the Ethics Committee on Animal Use from the Universidade Federal do Rio Grande do Sul, Brazil, following the legislation for the protection of animals used for scientific purposes.

Treatments, Bird Husbandry And Experimental Design

A total of 512, one-day-old male broiler chicks (Cobb 500) were obtained from a commercial hatchery and housed in a controlled-temperature room, composed of 32 pens with 16 birds per pen. Each group was housed in a 1 m^2 pen equipped with two nipple drinkers and one tubular feeder. The nutritional program consisted of four phases (Table 1): pre-starter (1 to 7 d), starter (8 to 21 d), grower (22 to 35 d) and finisher (36 to 42 d), formulated to provide the nutritional requirements recommended by the Brazilian Tables of Poultry and Swine (Rostagno et al., 2017).

The experimental design was completely randomized, composed of four treatments groups: Control group (basal diet without additives); Ionophore group (sodium salinomycin supplementation at 66 ppm - SS66); Ionophore + 0.075% FO group (SS66 + FO supplementation at 750 ppm); Ionophore + 0.10% FO group (SS66 + FO supplementation at 1,000 ppm in coccidiosis-challenged birds). All treatments groups were challenged with coccidiosis.

The commercial product of functional oils was composed of a mixture of cashew nutshell liquid (*Anacardium occidentale*) and castor oil (*Ricinus communis* L.), which provide active components, such as 4% of cardol, 20% of cardanol and 9% of ricinoleic acid (Essential, Oligo Basics Agroind. Ltda, Cascavel, Brazil). Both feed additives, the oil blend and sodium salinomycin (Coxistac 12% – Phibro Animal Health Corporation, Brazil), were included by replacing an inert ingredient (kaolin) in the basal diet for all phases varying the dosage of each treatment group.

Intestinal Challenge

At 14 days of age, all chickens were inoculated by oral gavage with 1 mL of a saline solution containing sporulated oocysts of *E. tenella* (1×10^4 oocysts), *E. acervulina* (20×10^4 oocysts), and *E. maxima* (8×10^4 oocysts). The oocyst inoculum was acquired from the Laboratório Biovet (Laboratório BIO-VET LTDA, São Paulo, SP, Brazil) and was consisted of field strains, but multiplied in the laboratory, produced exactly to simulate a coccidiosis similar to what occurs in commercial breeding. The *Eimeria* inoculum dosage for the current study were chosen based on previous studies (Moraes et al., 2019a; Moraes et al., 2019b; Vieira et al. 2020), and this dosage was able to cause a drop in performance without causing high mortality.

Performance

FI and BW gain for each replicate were measured weekly.

Oocyst Counts In Litter

At 28 days of age, representative litter samples (a mixed sample of five handfuls taken in five sections per pen) samples were collected, forming a pool with these samples. The mean number of oocysts per gram (OPG) of litter was determined using the technique of Long and

Rowell (1975). Each sample was thoroughly mixed with a paddle stirrer in 2000 mL of water for 10 minutes and two 1 mL aliquots were removed with a pipette and placed in 10 mL glass test tubes. They were diluted with 9 mL of saturated salt solution and mixed by repeated inversion. Oocyst counting was performed in a McMaster chamber using the 10x objective of a compound microscope.

Sample Collection, DNA Extraction, PCR Amplification, And Sequencing

At 28 days of age, three broiler chickens per treatment within the average weight of each replicate were euthanized, totaling 12 animals. A 10 cm portion of each segment: jejunum (descending duodenal loop to the Meckel's diverticulum), ileum (diverticulum to ileocecal insertion), and caecum were removed with the intestinal contents inside and immediately stored at -20°C until further analysis.

Total microbial genomic DNA samples were extracted using the E.Z.N.A. Stool DNA Kit (Omega Bio-Tek, Norcross, Georgia, USA) according to the manufacturer's instructions. The genomic DNA was quantified using a Qubit® 3.0 Fluorometer (Life Technologies, Carlsbad, CA), and stored at -20°C. After DNA extraction, the samples were sent to Imunova Análises Biológicas (Curitiba-PR, Brazil) for PCR amplification.

The V4 region of bacterial 16S rRNA gene was amplified using the universal primers 515F and 806R (Caporaso et al., 2010). Amplification was carried out according to the following program: initial denaturation at 94°C for 3 min, followed by 18 cycles of 45 s at 94ºC, 30 s at 50ºC and 60 s at 68ºC and a final cycle at 72°C for 10 min. Sequencing was performed by Illumina MiSeq (Illumina, San Diego, CA, USA). which generates paired end reads of 460 bp.

Raw sequencing reads of this study was deposited in the National Biotechnology Information Center (NCBI) under the accession number PRJNA854667.

Bioinformatic and Statistical Analysis

Sequencing reads were analyzed using the QIIME (Quantitative Insights Into Microbial Ecology) platform. Sequences were classified into bacterial genera through the recognition of operational taxonomic units (OTU) based on the homology of the sequences when compared to the SILVA 128 ribosomal sequence database (Yilmaz et al., 2014).

For diversity analysis, after the samples were rarefied, alpha diversity metrics (Shannon entropy, Simpson index, ACE, Chao1, Fisher and total number of observed OTUs), beta diversity metrics (weighted and unweighted UniFrac (Lozupone et al., 2011) and Bray-curtis dissimilarity (Clarke et al., 2006) were used. The statistical comparison between the groups in the alpha diversity analysis was performed using the nonparametric Wilcoxon test, accepting as statistically significant results, values lower than 0.05 ($P < 0.05$). Statistical analyzes for beta diversity were performed using perMANOVA of the adonis function, present in the vegan library, using several 10,000 permutations (Anderson & Walsh, 2013). For beta diversity comparison between two groups, the values obtained by the perMANOVA analysis were corrected by Bonferroni. All figures and statistical analyzes were performed in R version 3.6 (https://www.R-project.org/). The calculation of phylogenetic diversity was performed by the spicy library, while the alpha and beta

diversity were calculated using the phyloseq, vegan and microbiome libraries. Rarefaction curves, phylogenetic tree and correlation analyzes are performed using the Microbiome Analyst tool (Dhariwal et al., 2017).

All performance data were subjected to one-way analysis of variance (ANOVA) using XLSTAT statistical software (Addinsoft, Paris, France). When significant differences were noted, Tukey test was performed to separate means and significance accepted at $P \leq 0.05$. The oocyst count data were performed by the nonparametric Kruskal-Wallis test.

RESULTS

Performance

The performance data are presented in Table 2. In the first weeks (d 1 to 7; d 8 to 14), period before the intestinal challenge, there was no statistical difference in performance among treatments. However, in the week following the challenge (d 15 to 21) there was a statistical difference between the additives tested for all analyzed variables, except for FCR. Broilers of the Control group (challenge with coccidiosis non-supplemented diet) presented the worst performance for BW, BW gain and FI, even though these variables did not differ from the Ionophore group. The challenged broilers fed diets supplemented with Ionophore + 0.075% FO exhibited a higher BW, BW gain and FI ($P<0.05$) than other groups.

In the second week post-challenge (d 21 to 28), broilers fed with Ionophore + 0.075% FO and Ionophore + 0.10% FO additives had a significant higher BW, BW gain and FI, even though this last variable did not differ from the Ionophore group. Similarly, to the previous week, the broilers in the Control group presented the worst performance compared to other groups, still as a reflection of the intestinal challenge with coccidiosis.

From d 29 to 35 and d 35-42, broilers fed diets supplemented with Ionophore + 0.075% FO and Ionophore + 0.10% FO additives exhibited a higher BW ($P<0.05$) than broilers from the other treatments. Broilers in the Control group did not have enough time to compensate the loss of performance caused by coccidiosis, thus reflecting in the lower final BW. In a similar way, broilers in the Ionophore group also presented lower final BW compared to the groups fed with Ionophore + FO.

In the accumulated period from d 1 to 42, a statistical difference was observed in performance among treatments for BW gain. Broilers fed Ionophore + 0.075% FO and Ionophore + 0.10% FO resulted in a higher BW gain when compared to the Control and Ionophore groups. The ionophore used alone exhibited a 6% and 5% reduction in BW gain compared to Ionophore + 0.075% FO and Ionophore + 0.10% FO, respectively.

Oocyst Litter Count

The oocyst counts in the litter of broilers fed diets supplemented with Ionophore alone, Ionophore + 0.075% FO and Ionophore + 0.10% FO decreased compared to broilers in the Control group ($P<0.1$) (Figure 1).

Operational Taxonomic

A total of 412,688 frequency were obtained from 35 samples with an average of 11,791 per sample of. Sample 15609 (Control - jejunum) showed low sequencing depths and was disregarded from subsequent analyses.

Alpha Diversity Analysis

The alpha diversity of a sample is determined by its richness and uniformity. Statistically significant microbiota modulations ($P < 0.05$) were observed between the Control and Ionophore groups using the Simpson Index (Figure 2). Furthermore, a trend ($P = 0.093$) was observed among the Ionophore and Ionophore + 0.075% FO groups. We found no statistical difference between groups regarding the other applied alpha diversity tests considering the pool of intestinal segments. Conversely, independently of the statistical significance, Shannon's, ACE, Chao1 and Fisher's graphics showed a larger distribution and diversity in Ionophore + 0.075% FO group, while showed a lower, but more uniform α-diversity species for the microbiota of the Ionophore + 0.10% FO group.

In addition, the graphic of the total number of observed OTUs showed a higher distribution of the number of different species in the Ionophore + 0.075% FO group that can be translated into a greater richness of an ecosystem.

Beta Diversity Analysis

Analysis of similarity (PERMANOVA) were performed on the weighted and unweighted Unifrac distances and Bray-Curtis metric obtained from the beta diversity workflow in QIIME2 (Figure 3, 4). The PERMANOVA test based on treatment groups resulted in a p-value of 0.791 and a test statistic of 0.621 for weighted, a p-value of 0.561 and test statistic of 0.966 for unweighted Unifrac analysis and of p-value 0.194 and a test statistic of 1.248 for Bray-Curtis, so no difference was found regarding the dissimilarity based on treatment groups. These metrics showed that the treatments had a similar microbial composition and indicated that grouping based on treatment is weak (i.e., the differences can be explained by randomness). Despite the lack of homogeneity in the dispersion, the PcoA graphics showed that the microbial populations of the broilers challenged with coccidiosis that received the additives were presented closer microbial compositions.

In the weighted Unifrac PcoA analysis, the treatment groups were well separated with 38.9% and 26.1% variation by the principal components PcoA1 and PcoA2, respectively, and for the unweighted Unifrac were well separated with 13.8% and 5.9% variation by the principal components PcoA1 and PcoA2, respectively. In PcoA based on Bray–Curtis diversity metric, the treatment groups were well separated with 32.3% and 15.1% variation by the principal components PcoA1 and PcoA2, respectively.

Relative Abundance

To elucidate the effect of feed additives associated with *Eimeria* infection on the composition of the intestinal microbiota (jejunum, ileum and cecum), we analyzed the bacteria at the phylum and genus levels to characterize the dynamics of microbial taxonomic distribution.

All sequences were classified into nine phyla, although four phyla were more common (> 1%): *Firmicutes*, *Actinobacteria*, *Bacteroidetes*, and *Proteobacteria* (Figure 5).

Jejunum And Ileum. Broilers fed diets supplemented with Ionophore + 0.075% FO showed a higher proportion of *Firmicutes* and lower abundance of *Actinobacteria*, compared to the other groups. *Actinobacteria* was more abundant in the jejunum and ileum of the Control group.

Lactobacillaceae was the dominant family in the jejunal, and ileal microbiotas of the broilers fed diets supplemented with Ionophore, Ionophore + 0.075% FO and Ionophore + 0.10% FO (Figure 6). Control group birds showed a lower proportion of Lactobacillaceae and a higher abundance of Corynebacteriaceae.

Lactobacillus genus was more abundant in the microbiota of the broilers supplemented with Ionophore + 0.075% FO, compared to the other treatments (69% vs. 20%, 58% and 43% for the Control, Ionophore, and Ionophore + 0.10% FO groups, respectively), while *Corynebacterium* was less abundant for the same group (11% vs. 33%, 23% and 22% for Control, Ionophore, and Ionophore + 0.10% FO, respectively) (Figure 7). In the ileum, we found the same behavior for the Ionophore + 0.075% FO group, showing a higher proportion of *Lactobacillus* and lower *Corynebacterium.* The highest relative abundance of *Corynebacterium* was observed in the jejunal and ileal microbiota of the Control group. Furthermore, in these two segments (jejunum and ileum), the birds from the Control group showed a reduced percentage of *Lactobacillus* when compared to the other groups.

Cecum. Broilers fed diets supplemented with Ionophore showed a higher relative abundance of Firmicutes, while the Bacteroidetes were more abundant for the Ionophore + 0.075% FO group. In addition, broilers challenged and supplemented with additives showed a cecal microbiota with a higher percentage of *Proteobacteria* when compared to the Control group.

Ruminococcaceae was the dominant family in the cecal microbiotas of all groups, followed by Lachnospiraceae. Control group birds exhibited a greater abundance of Lactobacillaceae compared to the other groups (14% vs. 2%, 6% and 14% for Ionophore, Ionophore + 0.075% FO, and Ionophore + 0.10% FO groups, respectively).

At genus level, the Ionophore + 0.075% FO group showed a higher proportion of *Bacteroides*, compared to the other groups (13% vs. 7%, 1% and 4% for Control, Ionophore, and Ionophore + 0.10% FO groups, respectively). The Ionophore group showed a lower abundance of *Bacteroides* and *Oscillospira*.

DISCUSSION

Studies with the addition of CNSL and Castor oil to poultry diet improved growth performance, metabolizable energy and gut morphometry (Bess et al., 2012; Murakami et al., 2011). Under an intestinal challenge with coccidiosis, the functional oils improve energy utilization and survival rate and decrease the intestinal lesions caused by *Eimeria* in supplemented broilers (Murakami et al., 2014).

Encouraging positive outcomes on the performance and microbiota were observed in our laboratory using CNSL and Castor oil blend in diets for broilers challenged with coccidiosis. Compared with one of the widely used ionophore anticoccidials, sodium monensin (100 ppm), the functional oil (1,500 ppm) had a similar effect to the ionophore in the rearing period (up to 42 day of age) and was able to compensate the negative effect caused by coccidiosis (Moraes et al., 2019b). In addition, CNSL and Castor oil blend showed to be a good option in a coccidiosis challenge, acting as a modulator of the intestinal microbiota, with antimicrobial action against gram-positive bacteria, mainly *C. perfringens* and *S. aureus*.

The search for alternatives to synthetic anticoccidial drugs is an important field of study for researchers, producers and the poultry industry (Qaid et al., 2021), especially due to increasing changes in legislation and pressure to reduce the use of AGP and anticoccidials. However, we understand that the process of removing these compounds from the poultry production chain is a gradual process and, often, the easiest way to encourage these changes is through a transition stage in which AGP and anticoccidials are combined with an alternative additive.

Additionally, we also focused on evaluating an herbal solution with anticoccidial properties that could potentially be used in poultry diets, such as the blend of CNSL and Castor oil. Cashew oil contains cardanol and cardol, which present antioxidant properties, act as natural ionophores, denaturing the lipid layers of the cell wall, with action mainly in gram-positive bacteria (Sosa et al., 2020; Paramashivappa et al., 2001). These oils are classified as components of phenols group with high antibacterial activity as the result of high solubility in biological membranes, making them notorious bactericides (Nazarro, et al., 2013). Conversely, castor oil, which is rich in ricinoleic acid, can interact with the microbial membrane and inhibit microbial growth by dissolving chitin, which is a component of cell membranes.

Performance

In the present study, at the end of d 42, the broilers of the Control group did not have enough time to compensate the loss of performance caused by coccidiosis, reflected in the low final BW. We can also observe that the group of broilers supplemented only with ionophore also presented a low final BW, statistically similar to the group without additive. However, when ionophore was combined with functional oils in both dosages (0.075% and 0.10%), it was possible to observe a higher final weight at the end of 42 days of age. Under the conditions of this study, this result evidence a synergistic action between the ionophore sodium salinomycin and the functional oils on the growth performance.

The combination of natural additives and anticoccidial drugs to verify the synergistic effects was also the objective of other studies in broilers. The combination of berberine synergistically increased the effectiveness of amprolium (a synthetic anticoccidial) against coccidian oocysts, confirmed by reduction in the number of coccidian oocysts shed in the feces and better weight gain and feed conversion, possibly explained by their different mechanisms of action (Malik et al., 2016). Conversely, Bozkurt et al. (2016) did not observe a synergistic effect associating essential oil derived from the herb *Origanum minutiflorum* and monensin sodium in the diet of broilers challenged with coccidiosis.

In this study, it is possible to notice that before the challenge, the inclusion of the ionophore alone or in combination with the functional oil did not affect performance. However, faced with

the intestinal challenge, we believe that the inclusion of these additives from the first day of age prepared the broilers for the intestinal stress that came in the sequence.

Microbioma

Firmicutes, Actinobacteria, Bacteroidetes and *Proteobacteria* were the most abundant phyla in all treatments. Some recent in vivo experiments have suggested that phytogenic products, such as functional oils, modify the composition of intestinal microbiota, increasing the relative abundance of *Firmicutes* in the gut (Salaheen et al., 2017; Li et al., 2018). Broilers of the Ionophore + 0.075% FO group showed a higher proportion of *Firmicutes* and less abundance of *Actinobacteria*, compared with other treatments. Firmicutes constitutes a heterogeneous phylum containing bacterial groups with different metabolic activities and are associated with the decomposition of polysaccharides and the production of butyrate (Ducatelle et al. 2018). Also this same group, regarding the genus level, increased the relative abundance of *Lactobacillus* and decreased *Corynebacterium*. Conversely, challenged birds that did not receive additives showed a reduced percentage of *Lactobacillus* in the jejunum and ileum when compared to the other groups. Members of the *Lactobacillus* genus produce lactic acid which, in addition to providing an inhibitory environment to the growth of many bacteria, also potentiates the antimicrobial activity of host lysozyme by disrupting the bacterial outer membrane (Alakomi et al., 2000). In a study developed by Macdonald et al. (2017), the authors examined the effect of *Eimeria* infection on intestinal microbiome diversity and observed that coccidial infection did not affect microbial alpha diversity but reduced the microbial population of *Bacillales* and *Lactobacillales*. Microbial shift may result in depletion of crucial microbiota, such as *Lactobacillus*, and disrupt metabolic processes of providing energy, while favoring overgrowth of pathogenic bacterial strains (Chen et al., 2020).

Furthermore, ionophore alone or in combination with functional oils increased the cecal population of genus *Bacteroide*s and *Oscillospira*, compared to the Control. Broilers that receive salinomycin normally have a higher abundance of phyla and genera related to short-chain fatty acids (SCFAs) production, resulting in the improvement of intestinal health besides controlling pathogenic bacteria growth (Orso et al., 2021). The genus *Bacteroides* is related to the ability to degrade indigestible fiber in the cecum (Lee et al., 2017). Meanwhile, *Oscillospira* is likely to be a genus capable of producing all kinds of SCFAs dominated by butyrate, which has potential for development as prebiotics (Yang et al., 2021). The fermentation of indigestible fibers may increase the production of SCFAs, helping the host-beneficial cecal microbiota (Orso et al., 2021).

The results of previous studies showed that the blend composed of CNSL and Castor oil affects gram-positive bacteria and modulate the intestinal microbiota (Moraes et al., 2019a; Moraes et al., 2019b; Vieira et al. 2020). In our study, the synergistic effect observed between the blend of functional oils and the ionophore can be explained by the mode of action of these additives. In particular, the antimicrobial effect of salinomycin and functional oils is connected to their capacity to diffuse through the bacterial cell. According to Abbas et al. (2012), the liquid cashew nutshell components, cardol and anacardic acid, have a similar action to a monovalent ionophore (such as salinomycin), causing damage to the bacterial cell membrane. In addition, the ricinoleic acid, present in castor oil, has an antimicrobial effect that denatures and coagulates proteins of the bacterial cell wall.

The cell membrane of gram-positive bacteria is 90–95% composed of peptidoglycan, allowing hydrophobic molecules, such as functional oils and salinomycin, to easily penetrate the cells, causing a disruption of the structure and function of bacteria cell membranes (Nazzaro et al., 2013). This disruption disturbs the ions concentrations in the cytoplasm.

Based on previous and current studies, positive outcomes on the performance and microbiota were observed with the use of CNSL and Castor oil blend in diets for broilers challenged with coccidiosis, which supports the importance of considering this blend's inclusion in poultry diets. Thus, due to the cost and the results obtained, the inclusion of the CNSL and Castor oil blend at the level 0.075% seems to be the most indicated.

This study demonstrated the effectiveness of the use of ionophores and functional oils for broilers under a coccidiosis challenge. The combination of salinomycin sodium and CNSL – Castor oil blend showed a synergistic effect on growth performance and microbiota modulation in broilers challenged with sporulated oocysts of *Eimeria*. The positive effect of this combination may be associated with the complementary mechanism of action against intestinal challenges, resulting in the improvement of intestinal health as well as controlling pathogenic bacteria growth. Thus, due to the cost of the product and the results obtained, we can suggest the inclusion of the CNSL and Castor oil blend at the level 0.075% in broiler diets.

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Table 1. Dietary compositions and nutrient levels of broilers (as-fed basis).

Ingredient (%)	Pre-starter (1-7 days)	Starter (8-21 days)	Grower (22-35 days)	Finisher (36-42 days)
Maize	44.53	46.37	48.94	58.80
Soybean meal	45.85	43.29	40.07	31.87
Soybean oil	5.022	6.122	7.117	6.045
L-Lysine	0.127	0.133	0.141	0.172
DL-Methionine	0.368	0.349	0.325	0.264
L-Threonine	0.072	0.067	0.062	0.047
NaCl	0.526	0.511	0.486	0.460
Limestone	0.915	0.825	0.780	0.653
Phosphate	2.188	1.927	1.678	1.290
Bicholine	0.050	0.050	0.050	0.050
Vit Premix[1]	0.034	0.034	0.034	0.034
Min Premix[2]	0.100	0.100	0.100	0.100
Inert/Ionophore/Functional oil	0.205	0.205	0.205	0.205
Calculated nutrition levels, %				
EM, kcal/kg	2975	3050	3150	3200
CP	24.71	23.68	22.41	19.44
Ca	1.011	0.90	0.82	0.66
Available P	0.48	0.43	0.38	0.30
Dig Lys	1.364	1.30	1.23	1.06
Dig Met	0.680	0.65	0.61	0.52
Dig Met+Cis	1.009	0.96	0.91	0.79
Dig Thr	0.900	0.86	0.81	0.70
Dig Trp	0.287	0.27	0.25	0.21
(Na+K)-Cl, mEq/kg[3]	228.39	216.91	202.51	166.96

[1]Vitamin premix containing the following per kilogram of diet: vitamin A, 10,000 IU; vitamin D3 (cholecalciferol), 3,500 IU; vitamin E (DL-α-tocopheryl acetate), 60 mg; vitamin K (menadione), 3 mg; thiamine, 3 mg; riboflavin, 6 mg; pyridoxine, 5 mg; vitamin B12 (cyanocobalamin), 0.01 mg; niacin, 45 mg; pantothenic acid (D-calcium pantothenate), 11 mg; folic acid, 1 mg; biotin, 0.15 mg; choline chloride, 500 mg; ethoxyquin (antioxidant), 150 mg.
[2]Mineral premix containing the following per kilogram of diet: Fe, 60 mg; Mn, 100 mg; Zn, 60 mg; Cu, 10 mg; I, 1 mg; Co,0.2 mg; Se, 0.15 mg.
[3]Dietary electrolyte balance.

Table 2. Growth performance of broilers chickens.

	Control	Ionophore	Ionophore + 0.075% FO	Ionophore + 0.10% FO	SEM	P-value
1-7d						
BW1d	43	43	43	43	0.09	0.901
BW	132	134	139	137	1.96	0.651
FI	100	96	105	100	1.55	0.301
BWG	89	91	96	94	1.95	0.641
FCR	1.132	1.069	1.097	1.068	0.02	0.438
7-14d						
BW	354	362	373	353	5.31	0.551
FI	289	285	295	284	3.98	0.790
BWG	221	228	234	217	3.84	0.434
FCR	1.312	1.252	1.264	1.314	0.01	0.258
14-21d						
BW	620^{b}	650^{b}	695^{a}	647^{b}	9.76	**0.047**
FI	469^{b}	473^{b}	531^{a}	497^{b}	7.58	**0.007**
BWG	267c	287^{bc}	322^{a}	293^{b}	6.27	**0.011**
FCR	1.776	1.661	1.653	1.705	0.03	0.474
22-28d						
BW	1095^{c}	1196^{b}	1273^{a}	1270^{a}	17.41	**<0.0001**
FI	754b	835a	861a	880a	17.36	**0.045**
BWG	475c	546b	578ab	624a	13.94	**0.000**
FCR	1.610	1.530	1.509	1.429	0.04	0.505
29-35d						
BW	1895^{c}	1993^{b}	2092^{a}	2106^{a}	22.27	**0.000**
FI	1222	1124	1186	1238	30.12	0.569
BWG	791	787	840	836	15.23	0.475
FCR	1.608	1.429	1.414	1.490	0.05	0.599
35-42d						
BW	2794^{b}	2825^{b}	2978^{a}	2984^{a}	28.27	**0.017**
FI	1447	1373	1446	1447	14.95	0.204
BWG	899	846	886	878	14.88	0.654
FCR	1.614	1.632	1.641	1.659	0.02	0.905
1-42d						
FI	4282	4186	4424	4446	49.642	0.206
BWG	2742^{b}	2786^{b}	2955^{a}	2941^{a}	29.255	**0.010**
FCR	1.546	1.489	1.478	1.498	0.013	0.261

Each value represents the mean of four replicates. Control group (no additives in coccidiosis-challenged birds); Ionophore group (sodium salinomycin -SS66- in coccidiosis-challenged birds); Ionophore + 0.075% FO group (SS66 + FO at 750 ppm in coccidiosis-challenged birds); Ionophore + 0.10% FO group (SS66 + FO at 1,000 ppm in coccidiosis-challenged birds).

[a,b] Different letters in same row indicate significant differences between the respective means ($P<0.05$; Tukey test)

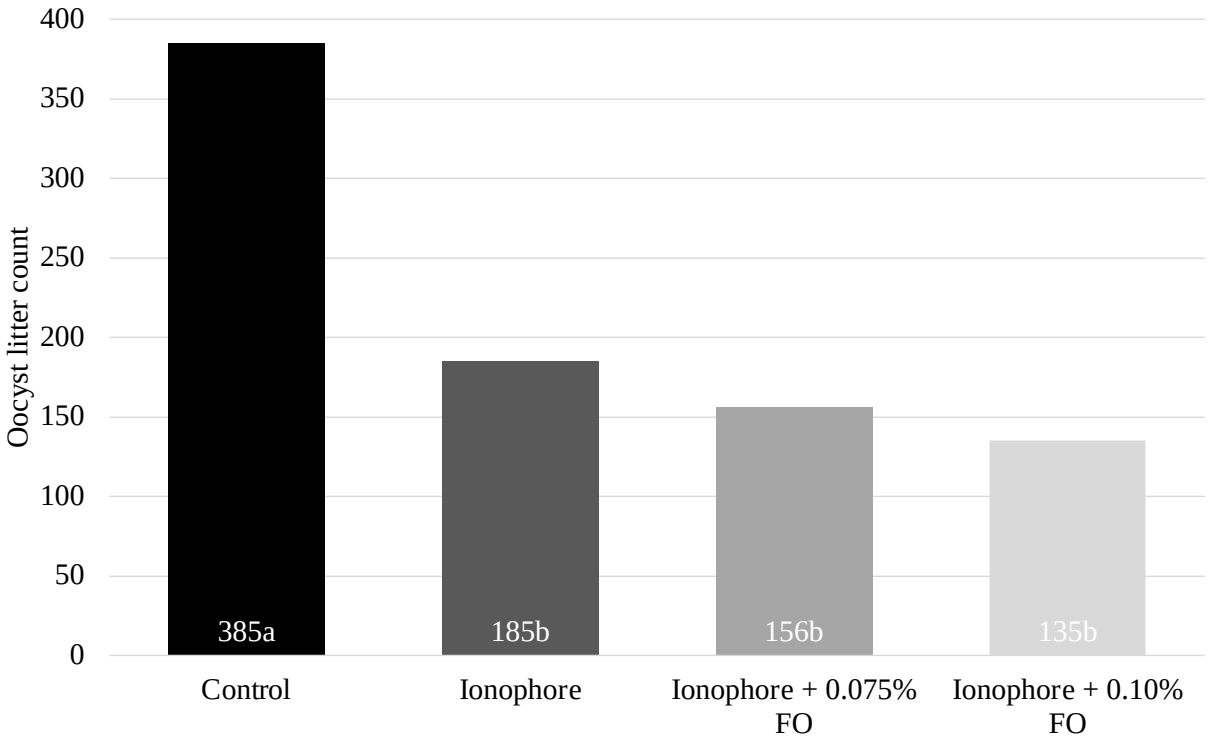

Figure 1. Number of *Eimeria* spp. OPG in litter at d 28. Each value represents the mean of eight replicates. Control group (no additives in coccidiosis-challenged birds); Ionophore group (sodium salinomycin -SS66- in coccidiosis-challenged birds); Ionophore + 0.075% FO group (SS66 + FO at 750 ppm in coccidiosis-challenged birds); Ionophore + 0.10% FO group (SS66 + FO at 1,000 ppm in coccidiosis-challenged birds). [a,b] Different letters indicate significant differences between the respective means ($P<0.1$; Kruskal-Wallis test)

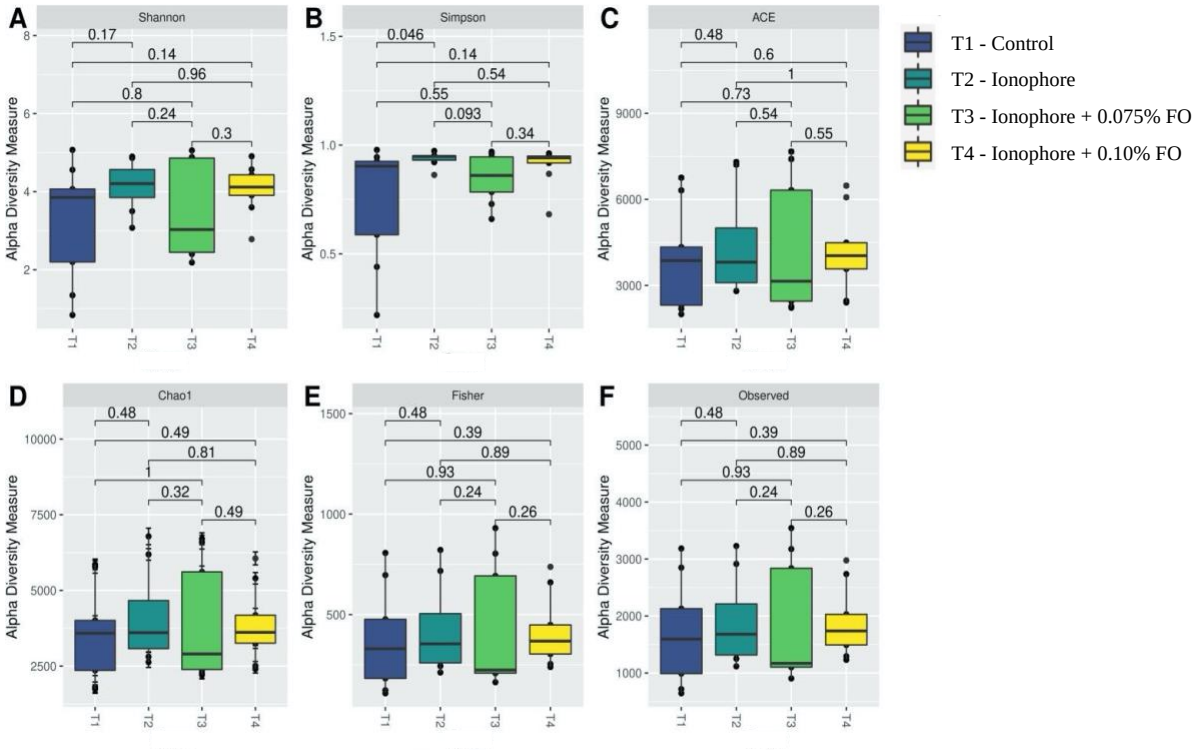

Figure 2. Alpha diversity, estimated by the parameters Shannon entropy (A), Simpson index (B), ACE (C), Chao1 (D), Fisher (E) and total number of observed OTUs (F). Statistical tests were performed using the Wilcoxon test. Differences that presented a p value lower than 0.05 were considered statistically significant. Control group (no additives in coccidiosis-challenged birds); Ionophore group (sodium salinomycin -SS66- in coccidiosis-challenged birds); Ionophore + 0.075% FO group (SS66 + FO at 750 ppm in coccidiosis-challenged birds); Ionophore + 0.10% FO group (SS66 + FO at 1,000 ppm in coccidiosis-challenged birds).

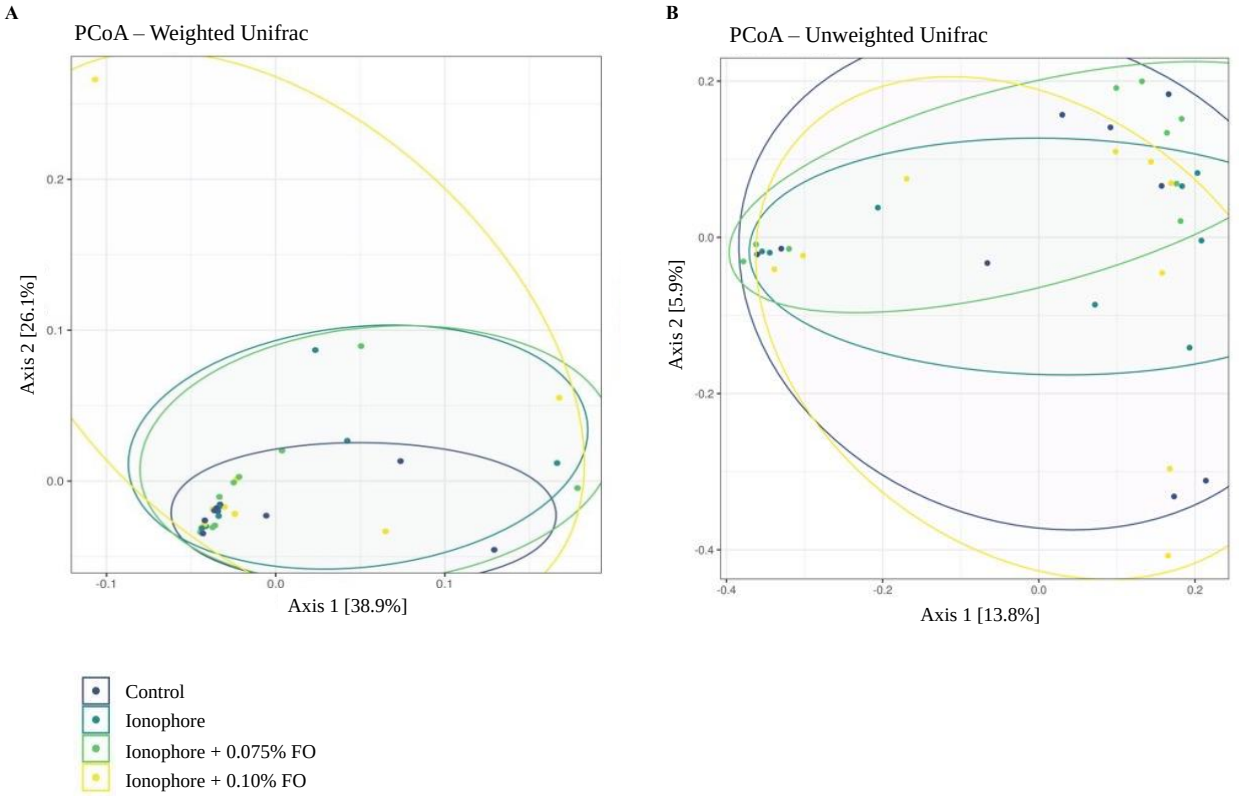

Figure 3. Beta diversity, estimated by Weighted Unifrac dissimilarity (A) (p-value = 0.791) and the Unweighted Unifrac dissimilarity (B) (p-value = 0.561). Colored ellipses were automatically added using the Rggforce library. Control group (no additives in coccidiosis-challenged birds); Ionophore group (sodium salinomycin -SS66- in coccidiosis-challenged birds); Ionophore + 0.075% FO group (SS66 + FO at 750 ppm in coccidiosis-challenged birds); Ionophore + 0.10% FO group (SS66 + FO at 1,000 ppm in coccidiosis-challenged birds).

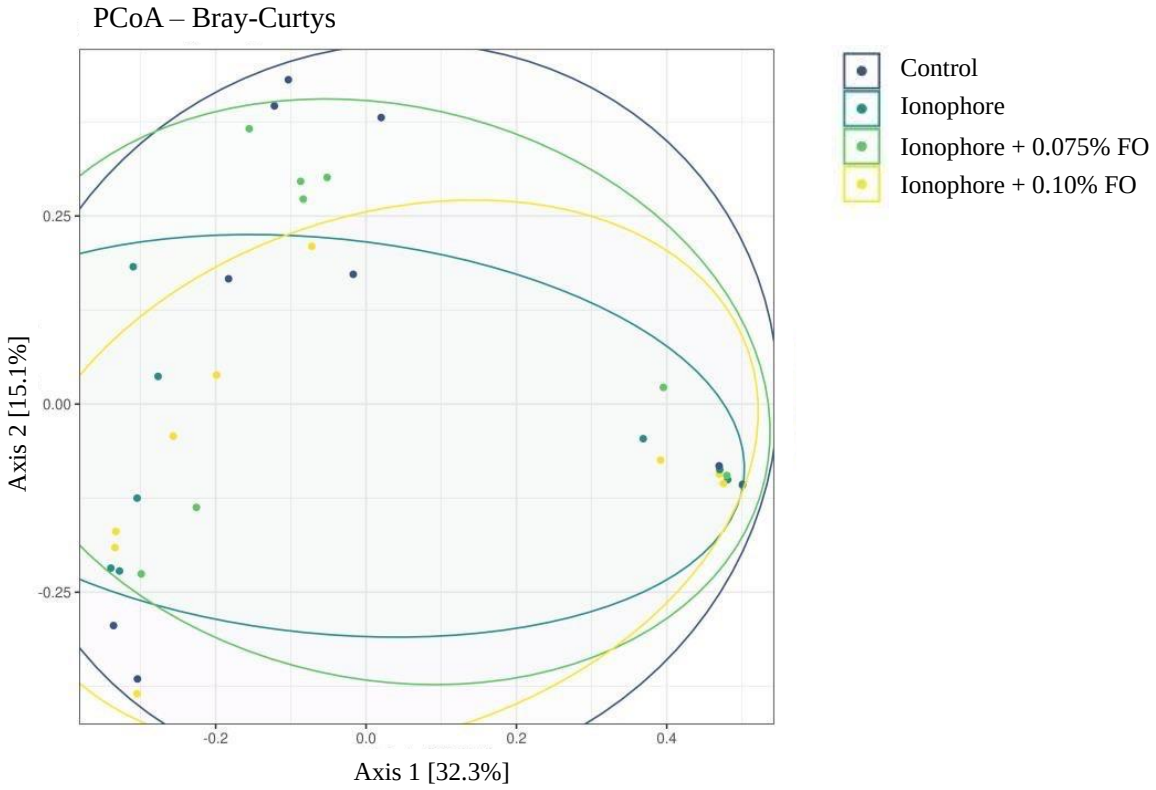

Figure 4. Beta diversity, estimated by Bray-Curtis dissimilarity (p-value = 0.194). Colored ellipses were automatically added using the Rggforce library. Control group (no additives in coccidiosis-challenged birds); Ionophore group (sodium salinomycin -SS66- in coccidiosis-challenged birds); Ionophore + 0.075% FO group (SS66 + FO at 750 ppm in coccidiosis-challenged birds); Ionophore + 0.10% FO group (SS66 + FO at 1,000 ppm in coccidiosis-challenged birds).

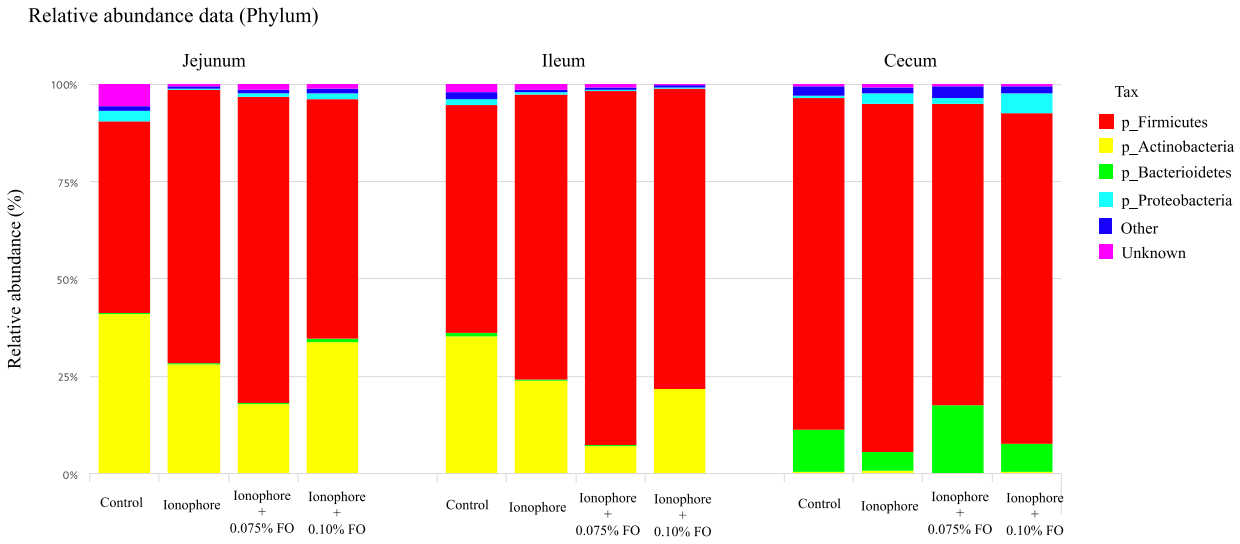

Figure 5. Bar graph of the relative abundances of the gut microbiota at the phylum level. The relative abundances of the gut bacteria presented here were calculated by averaging the data obtained from the replicates within each group. Minor bacterial genera and unassigned values were included as “others”. Control group (no additives in coccidiosis-challenged birds); Ionophore group (sodium salinomycin -SS66- in coccidiosis-challenged birds); Ionophore + 0.075% FO group (SS66 + FO at 750 ppm in coccidiosis-challenged birds); Ionophore + 0.10% FO group (SS66 + FO at 1,000 ppm in coccidiosis-challenged birds).

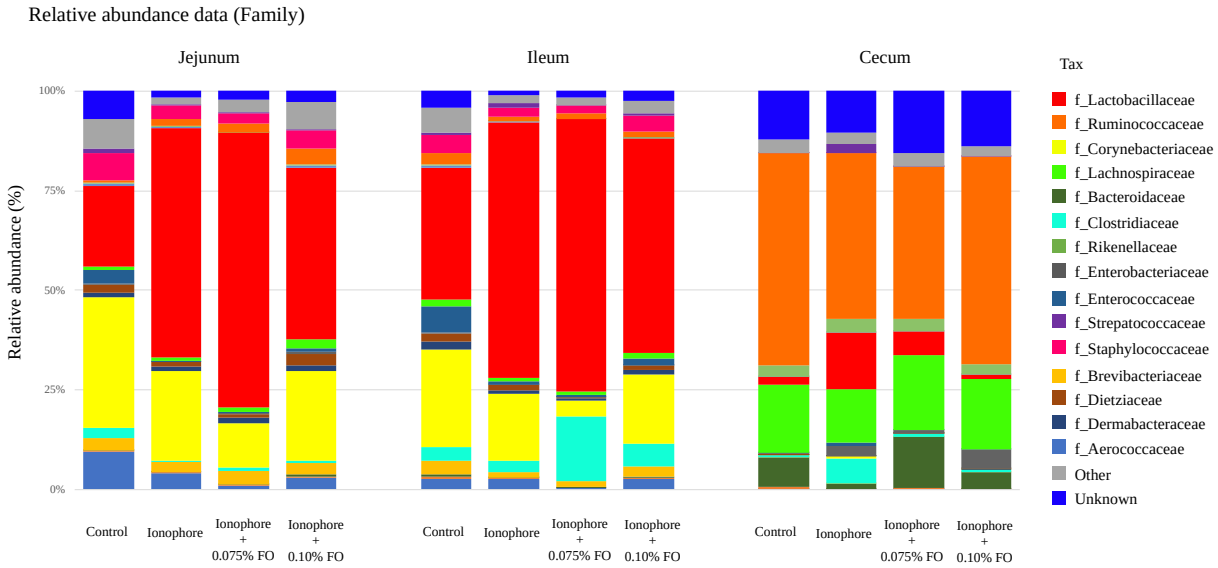

Figure 6. Bar graph of the relative abundances of the gut microbiota at the family level. The relative abundances of the gut bacteria presented here were calculated by averaging the data obtained from the replicates within each group. Minor bacterial genera and unassigned values were included as “others”. Control group (no additives in coccidiosis-challenged birds); Ionophore group (sodium salinomycin -SS66- in coccidiosis-challenged birds); Ionophore + 0.075% FO group (SS66 + FO at 750 ppm in coccidiosis-challenged birds); Ionophore + 0.10% FO group (SS66 + FO at 1,000 ppm in coccidiosis-challenged birds).

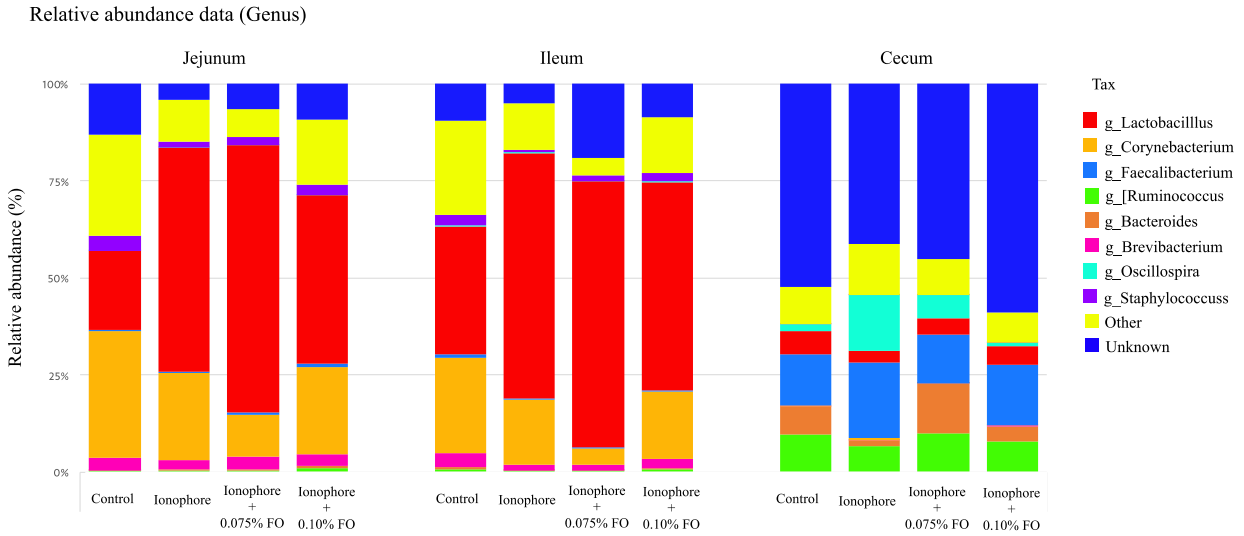

Figure 7. Bar graph of the relative abundances of the gut microbiota at the genus level. The relative abundances of the gut bacteria presented here were calculated by averaging the data obtained from the replicates within each group. Minor bacterial genera and unassigned values were included as “others”. Control group (no additives in coccidiosis-challenged birds); Ionophore group (sodium salinomycin -SS66- in coccidiosis-challenged birds); Ionophore + 0.075% FO group (SS66 + FO at 750 ppm in coccidiosis-challenged birds); Ionophore + 0.10% FO group (SS66 + FO at 1,000 ppm in coccidiosis-challenged birds).

SUPPLEMENTARY MATERIAL

Table S1. Relative abundance of phylum, class, family and genus present in the intestinal microbiota of broilers

Intestinal segment	Jejunum				Ileum				Cecum			
Treatment group	Control	Ionophore	Ionophore + 0.075 % FO	Ionophore + 0.10% FO	Control	Ionophore	Ionophore + 0.075 % FO	Ionophore + 0.10% FO	Control	Ionophore	Ionophore + 0.075 % FO	Ionophore + 0.10% FO
Phylum												
p__Actinobacteria	41 %	28%	18%	34%	24 %	22%	7%	35%	0%	1%	0%	1%
p__Bacteroidetes	0%	0%	0%	1%	0%	0%	0%	1%	11 %	5%	17%	7%
p__Chloroflexi	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
p__Cyanobacteria	1%	0%	0%	0%	0%	0%	0%	1%	1%	0%	0%	0%
p__Firmicutes	49 %	70%	78%	61%	73 %	77%	91%	59%	85 %	89%	78%	85%
p__Proteobacteria	3%	0%	1%	1%	1%	0%	0%	2%	1%	3%	1%	5%
p__Tenericutes	0%	0%	0%	0%	0%	0%	0%	0%	1%	1%	2%	1%
Other	0%	0%	1%	1%	0%	0%	0%	1%	0%	0%	1%	0%
Unknown	6%	1%	2%	1%	1%	0%	1%	2%	1%	1%	1%	1%
Class												
c__4C0d-2	0%	0%	0%	0%	0%	0%	0%	0%	1%	0%	0%	0%
c__Actinobacteria	41 %	28%	18%	34%	35 %	22%	7%	24%	0%	1%	0%	0%
c__Alphaproteobacteria	0%	0%	0%	1%	1%	0%	0%	0%	0%	0%	0%	0%
c__Bacilli	43 %	67%	74%	53%	49 %	72%	72%	64%	3%	18%	7%	1%
c__Bacteroidia	0%	0%	0%	1%	1%	0%	0%	0%	11 %	5%	17%	7%
c__Betaproteobacteria	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
c__Chloroplast	1%	0%	0%	0%	1%	0%	0%	0%	0%	0%	0%	0%
c__Clostridia	6%	3%	5%	8%	9%	5%	19%	9%	80 %	69%	70%	82%
c__Erysipelotrichi	0%	0%	0%	0%	0%	0%	0%	0%	2%	2%	1%	2%
c__Gammaproteobacteria	2%	0%	0%	0%	0%	0%	0%	0%	0%	3%	1%	5%
c__Mollicutes	0%	0%	0%	0%	0%	0%	0%	0%	1%	1%	2%	1%
Other	1%	0%	1%	1%	1%	0%	0%	1%	1%	0%	1%	1%
Unknown	6%	1%	2%	1%	2%	0%	1%	1%	1%	1%	1%	1%

Family												
f__Aerococcaceae	9%	4%	1%	3%	3%	3%	0%	3%	0%	0%	0%	0%
f__Bacillaceae	0%	0%	0%	0%	1%	0%	0%	0%	1%	0%	0%	0%
f__Bacteroidaceae	0%	0%	0%	1%	0%	0%	0%	0%	7%	1%	13%	4%
f__Brevibacteriaceae	3%	2%	3%	3%	4%	1%	1%	2%	0%	0%	0%	0%
f__Burkholderiaceae	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
f__Clostridiaceae	3%	0%	1%	0%	3%	3%	16%	6%	1%	6%	1%	1%
f__Corynebacteriaceae	33%	23%	11%	22%	25%	17%	4%	17%	0%	1%	0%	0%
f__Dermabacteraceae	1%	1%	2%	2%	2%	1%	0%	1%	0%	0%	0%	0%
f__Dietziaceae	2%	1%	1%	3%	2%	2%	0%	1%	0%	0%	0%	0%
f__Enterobacteriaceae	0%	0%	0%	0%	0%	0%	0%	0%	0%	3%	1%	5%
f__Enterococcaceae	3%	0%	0%	1%	7%	1%	0%	2%	0%	1%	0%	0%
f__Erysipelotrichaceae	0%	0%	0%	0%	0%	0%	0%	0%	2%	2%	1%	2%
f__Glycomycetaceae	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
f__Lachnospiraceae	1%	1%	1%	2%	2%	1%	1%	1%	17%	13%	19%	18%
f__Lactobacillaceae	20%	58%	69%	43%	33%	64%	69%	54%	2%	14%	6%	1%
f__Leuconostocaceae	1%	0%	0%	0%	1%	0%	0%	0%	0%	0%	0%	0%
f__Microbacteriaceae	0%	0%	0%	1%	1%	0%	0%	0%	0%	0%	0%	0%
f__Nocardiopsaceae	0%	0%	0%	0%	1%	0%	0%	0%	0%	0%	0%	0%
f__Peptostreptococcaceae	1%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
f__Planococcaceae	1%	0%	0%	1%	1%	0%	0%	0%	0%	0%	0%	0%
f__Porphyromonadaceae	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	1%	0%
f__Rikenellaceae	0%	0%	0%	0%	0%	0%	0%	0%	3%	3%	3%	3%
f__Ruminococcaceae	1%	2%	2%	4%	3%	1%	1%	2%	53%	41%	38%	52%
f__Staphylococcaceae	7%	3%	3%	5%	4%	2%	2%	4%	0%	0%	0%	0%
f__Streptococcaceae	1%	0%	0%	0%	1%	1%	0%	0%	0%	2%	0%	0%
f__Yaniellaceae	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
Other	5%	1%	2%	4%	3%	1%	1%	2%	1%	1%	1%	1%
Unknown_Family	7%	2%	2%	3%	4%	1%	2%	2%	12%	10%	15%	14%
Genus												

g__[Ruminococcus]	0%	0%	0%	1%	1%	0%	0%	1%	9%	7%	10%	8%
g__Aerococcus	4%	2%	0%	1%	1%	1%	0%	1%	0%	0%	0%	0%
g__Bacillus	0%	0%	0%	0%	0%	0%	0%	0%	1%	0%	0%	0%
g__Bacteroides	0%	0%	0%	1%	0%	0%	0%	0%	7%	1%	13%	4%
g__Blautia	0%	0%	0%	0%	0%	0%	0%	0%	3%	2%	1%	2%
g__Brachybacterium	1%	1%	2%	2%	2%	1%	0%	1%	0%	0%	0%	0%
g__Brevibacterium	3%	2%	3%	3%	4%	1%	1%	2%	0%	0%	0%	0%
g__Burkholderia	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
g__Candidatus Arthromitus	0%	0%	0%	0%	3%	1%	0%	3%	0%	0%	0%	0%
g__cc	0%	0%	0%	0%	0%	0%	0%	0%	1%	2%	0%	0%
g__Clostridium	0%	0%	0%	0%	0%	0%	0%	0%	0%	1%	1%	0%
g__Coprococcus	0%	0%	0%	0%	0%	0%	0%	0%	1%	0%	1%	1%
g__Corynebacterium	33%	23%	11%	22%	25%	17%	4%	17%	0%	1%	0%	0%
g__Dietzia	2%	1%	1%	3%	2%	2%	0%	1%	0%	0%	0%	0%
g__Dorea	0%	0%	0%	0%	0%	0%	0%	0%	1%	1%	1%	1%
g__Enterococcus	3%	0%	0%	1%	6%	1%	0%	2%	0%	1%	0%	0%
g__Facklamia	5%	2%	0%	1%	1%	1%	0%	2%	0%	0%	0%	0%
g__Faecalibacterium	0%	0%	1%	1%	1%	0%	1%	0%	13%	20%	13%	16%
g__Glycomyces	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
g__Jeotgalicoccus	3%	2%	1%	2%	2%	1%	1%	2%	0%	0%	0%	0%
g__Lactobacillus	20%	58%	69%	43%	33%	64%	69%	54%	2%	14%	6%	1%
g__Nocardiopsis	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
g__Oscillospira	0%	0%	0%	0%	0%	0%	0%	0%	6%	3%	4%	5%
g__Parabacteroides	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	1%	0%
g__Ruminococcus	0%	0%	0%	0%	0%	0%	0%	0%	2%	2%	2%	1%
g__Salinicoccus	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
g__Staphylococcus	4%	1%	2%	3%	3%	1%	1%	2%	0%	0%	0%	0%
g__Streptococcus	1%	0%	0%	0%	1%	1%	0%	0%	0%	2%	0%	0%
g__Weissella	1%	0%	0%	0%	1%	0%	0%	0%	0%	0%	0%	0%
g__Yaniella	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
Other	5%	1%	1%	4%	4%	2%	1%	2%	2%	1%	2%	1%
Unknown	13%	4%	7%	9%	9%	5%	19%	9%	52%	41%	45%	59%

CONSIDERAÇÕES FINAIS

Nas últimas décadas, a popularidade e a busca por alternativas naturais aos aditivos sintéticos para rações têm crescido rapidamente, principalmente devido às mudanças na legislação e à pressão para reduzir o uso de antibióticos promotores de crescimento e anticoccidianos. No entanto, sabe-se que quaisquer alterações no âmbito da produção animal envolvem a necessidade de adaptação, o que se aplica também ao processo de retirada desses compostos e inclusão de alternativas naturais na cadeia produtiva avícola.

Uma grande variedade de ervas e especiarias, além de óleos essenciais tem sido utilizada em aves, individualmente ou na forma de blends. No entanto, na literatura atual, há uma grande discrepância nos resultados obtidos com o uso de fitogênicos, o que pode ser atribuído a vários fatores, entre eles a variabilidade inerente à composição botânica, bem como as condições ambientais, de manejo e sanitárias. A ciência envolvida na elaboração de um produto baseado em fitogênicos reside na combinação ideal dos ingredientes e princípios ativos derivados dos vegetais, o que demanda um profundo entendimento dos efeitos biológicos e dos mecanismos de ação dos compostos nele presentes.

Esta pesquisa investigou a eficácia de estratégias nutricionais (diferentes compostos fitogênicos) associadas ou não a ionóforos na dieta de frangos de corte expostos a um desafio intestinal com oocistos esporulados de *Eimeria* spp. e o impacto no desempenho, na microbiota e no sistema imune. Os resultados obtidos evidenciaram um efeito positivo dos produtos fitogênicos utilizados neste projeto na modulação da microbiota intestinal de frangos de corte desafiados por coccidiose. Quanto ao desempenho dos animais, o produto comercial à base de plantas (*Acacia concinna* e *Saccharum officinarum* – Artigo 1) atuou mais lentamente na recuperação dos animais após um desafio intestinal, por isso sugere-se seu uso em animais com ciclo produtivo mais longo, como galinhas poedeiras ou matrizes de frangos de corte. Já a mistura comercial de óleos funcionais (líquido da casca de castanha de caju e óleo de mamona – Artigo 2) demonstrou efeito

sinérgico quando associada à salinomicina na recuperação do desempenho dos animais após o desafio.

A manutenção de um ambiente intestinal saudável é um pré-requisito para o desempenho eficiente de frangos de corte. Assim, é importante compreender a complexidade que há em relação ao alimento, à microbiota e ao animal, e como é possível utilizar esse conhecimento para produzir frangos de corte mais saudáveis e mais rentáveis. Os dados obtidos com esses estudos permitiram entender que não há uma única solução ou um produto mágico para substituir completamente o uso de APC e de anticoccidianos na produção animal, mas que é necessária uma metodologia de manejo integrado, em que seja possível conciliar técnicas como biosseguridade, bem-estar, matérias-primas de qualidade e ferramentas de nutrição intestinal de precisão.

Os estudos presentes nesta tese, assim como os outros tantos experimentos realizados no Laboratório de Ensino Zootécnico durante o doutorado, proporcionaram uma experiência engrandecedora para compreender a importância da ciência como parte da indústria animal. À medida que demandas da indústria e dos consumidores são levadas ao ambiente acadêmico, temos a chance de compartilhar e aplicar os resultados obtidos. Este projeto é resultado de uma parceria entre a Universidade pública e empresas privadas de nutrição animal, permitindo que pesquisas acadêmicas de relevância científica possam ter visibilidade e aplicabilidade técnica na indústria.

Os quatro anos de doutorado permitiram um desenvolvimento pessoal para relacionamento interpessoal, trabalho em grupo, organização de experimentos, aquisição de matérias-primas e contatos com laboratórios e empresas. Além disso, a oportunidade de realizar grande parte das análises laboratoriais deste projeto, incluindo as análises de microbiota intestinal e expressão gênica, possibilitaram um aprendizado enriquecedor.

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ANEXOS

Parecer da Comissão de Ética na Utilização de Animais (CEUA) – UFRGS

U F R G S
UNIVERSIDADE FEDERAL
DO RIO GRANDE DO SUL

PRÓ-REITORIA DE PESQUISA

Comissão De Ética No Uso De Animais

CEUA UFRGS

CARTA DE APROVAÇÃO

Comissão De Ética No Uso De Animais analisou o projeto:

Número: 36347
Título: EFICACIA DO PEPTASAN, UM ANTICOCCIDIANO A BASE DE ERVAS, NA DIETA DE FRANGOS DE CORTE DESAFIADOS COM OOCISTOS DE EIMERIA SPP.

Vigência: 01/06/2019 à 01/07/2020

Pesquisadores:

Equipe UFRGS:

ANDREA MACHADO LEAL RIBEIRO - coordenador desde 01/06/2019
INES ANDRETTA - pesquisador desde 01/06/2019
Kátia Maria Cardinal - Aluno de Doutorado desde 01/06/2019
Catiane Orso - Aluno de Doutorado desde 01/06/2019
Thais Bastos Stefanello - Aluno de Doutorado desde 01/06/2019

Comissão De Ética No Uso De Animais aprovou o mesmo , em reunião realizada em 13/05/2019 - Sala 223 do Prédio do Instituto de ciências Básicas da Saúde - ICBS - Campus Centro UFRGS- Bairro Farroupilha - Porto Alegre, em seus aspectos éticos e metodológicos, para a utilização de 512 pintos de um dia de idade, da linhagem Cobb 500, adquiridos comercialmente no incubatório da Cooperativa Languiru Ltda (CNPJ: 89.774.160/0001-00; Teutônia/RS); de acordo com os preceitos das Diretrizes e Normas Nacionais e Internacionais, especialmente a Lei 11.794 de 08 de novembro de 2008, o Decreto 6899 de 15 de julho de 2009, e as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), que disciplinam a produção, manutenção e/ou utilização de animais do filo Chordata, subfilo Vertebrata (exceto o homem) em atividade de ensino ou pesquisa.

Porto Alegre, Sexta-Feira, 24 de Maio de 2019

ALEXANDRE TAVARES DUARTE DE OLIVEIRA
Coordenador da comissão de ética

U F R G S
UNIVERSIDADE FEDERAL
DO RIO GRANDE DO SUL

PRÓ-REITORIA DE PESQUISA

Comissão De Ética No Uso De Animais

CEUA UFRGS

CARTA DE APROVAÇÃO

Comissão De Ética No Uso De Animais analisou o projeto:

Número: 36475
Título: Utilização do Essential ® em adição à monensina sódica como um modulador da microbiota intestinal de frangos de corte desafiados por coccidiose

Vigência: 01/05/2019 à 30/09/2020

Pesquisadores:

Equipe UFRGS:

ANDREA MACHADO LEAL RIBEIRO - coordenador desde 01/05/2019
INES ANDRETTA - pesquisador desde 01/05/2019
Catiane Orso - Aluno de Doutorado desde 01/05/2019
Thais Bastos Stefanello - Aluno de Doutorado desde 01/05/2019

Equipe Externa:

Priscila de Oliveira Moraes - pesquisador desde 01/05/2019

Comissão De Ética No Uso De Animais aprovou o mesmo , em reunião realizada em 27/05/2019 - Auditório Plenarinho- Saguão do Prédio da Reitoria- Campus Centro da UFRGS- Bairro Farroupilha - Porto Alegre , em seus aspectos éticos e metodológicos, para a utilização de 768 pintos de um dia de idade, da linhagem Cobb 500, oriundos do incubatório da Cooperativa Languiru Ltda (CNPJ:89774160000100; Teutônia, Rio Grande do Sul); de acordo com os preceitos das Diretrizes e Normas Nacionais e Internacionais, especialmente a Lei 11.794 de 08 de novembro de 2008, o Decreto 6899 de 15 de julho de 2009, e as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), que disciplinam a produção, manutenção e/ou utilização de animais do filo Chordata, subfilo Vertebrata (exceto o homem) em atividade de ensino ou pesquisa.

Porto Alegre, Sexta-Feira, 7 de Junho de 2019

ALEXANDRE TAVARES DUARTE DE OLIVEIRA
Coordenador da comissão de ética