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META-ANÁLISE DAS RESPOSTAS DE DESEMPENHO E INTEGRIDADE INTESTINAL DE FRANGOS DE CORTE DESAFIADOS POR *Eimeria spp.* E *Clostridium perfringens*

PORTO ALEGRE
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Dissertação apresentada como requisito para obtenção do Grau de Mestre em Zootecnia, na Faculdade de Agronomia, da Universidade Federal do Rio Grande do Sul.

ORIENTADOR: Prof. Dra. Ines Andretta

PORTO ALEGRE

2022

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META-ANÁLISE DAS RESPOSTAS IMUNOLÓGICAS E DE DESEMPENHO DE FRANGOS DE CORTE DESAFIADOS POR *Eimeria spp.* E *Clostridium perfringens*[1]

Autor: Alexandre Bonadiman Mariani
Orientadora: Ines Andretta

Este trabalho teve como objetivo realizar duas extensas revisões sistemáticas, seguidas de meta-análises, acerca do impacto da infecção de duas das principais doenças que acometem frangos de corte no mundo, coccidiose e enterite necrótica causadas pelos respectivos patógenos, *Eimeria* spp. e *Clostridium perfringens*. Os estudos foram selecionados nas seguintes plataformas de pesquisa indexadoras, PubMeb, Web of Science e Scopus. E após uma criteriosa avaliação dos trabalhos encontrados foram selecionados 132 e 93 artigos para compor as bases de *Eimeria* spp. e *Clostridium perfringens*, respectivamente. Os dados foram tabulados e posteriormente analisados seguindo as seguintes etapas: analise gráfica para observar coerência biológica, correlação e variância-covariância, comparando apenas animais infectados e não infectados aonde os tratamentos aplicados só foram incluídos apenas se apresentassem um controle positivo e negativo dentro dos trabalhos. Em ambos os trabalhos foram encontrados resultados significantes na redução de desempenho dos animais nas variáveis ganho de peso e eficiência alimentar ($P<0.01$). Os modelos gerados demonstraram uma relação linear entre a variação do consumo alimentar com a variação do ganho de peso dos animais infectados e através deste estudo foi possível quantificar qual os valores destas variações para as diferentes infecções. Outra variável que apresentou resultados significativos para as infecções por *Eimeria spp*. e enterite necrótica foi em relação a integridade intestinal, aonde os animais infectados apresentaram uma redução de tamanho de vilos e um aumento de criptas nas diferentes porções do intestino delgado. Estes resultados são de grande importância uma vez que através da sumarização destes trabalhos é possível realizar uma analise mais profunda nas variabilidades presentes nos trabalhos em diferentes países, além de que a mensuração do efeito da variação de consumo sobre o desempenho dos animais é difícil de quantificar em experimentos convencionais.

[1] Dissertação de mestrado em Zootecnia – Produção Animal, Faculdade de Agronomia, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brasil (49p) Agosto, 2022.

Palavras-chave: Enterite Necrótica; Meta-análise; Coccidiose; Revisão

META-ANALYSIS OF IMMUNOLOGICAL AND PERFORMANCE RESPONSES OF BROKER CHICKENS CHALLENGED BY *Eimeria* spp. AND *Clostridium perfringens*[2]

Author: Alexandre Bonadiman Mariani
Supervisor: Ines Andretta

This study aimed to carry out two extensive systematic reviews, followed by meta-analyses, about the impact of infection of two of the main diseases that affect broilers in the world, coccidiosis and necrotic enteritis caused by the respective pathogens, *Eimeria* spp. and *Clostridium perfringens*. Studies were selected from the following indexing search platforms, PubMeb, Web of Science and Scopus. After a careful evaluation of the works found, 132 and 93 articles were selected to compose the bases of *Eimeria* spp. and *Clostridium perfringens*, respectively. The data were transfer to online tables and further analyzed using the following steps: graphical analysis to observe biological coherence, correlation and variance-covariance, comparing only infected and non-infected animals where the treatments applied were only included if they presented a positive and negative control within the works. In both studies, significant results were found in the reduction of animal performance in the variables of weight gain and feed efficiency ($P<0.01$). The generated models showed a linear relationship between the variation of feed intake with the variation of weight gain of infected animals and through this study it was possible to quantify the values of these variations for the different infections. Another variable that showed significant results for infections by *Eimeria* spp. and necrotic enteritis was in relation to intestinal integrity, where infected animals showed a reduction in the villi height and an increase in crypts depth in different portions of the small intestine. These results are of great importance since through the summarization of these works it is possible to carry out a deeper analysis of the variability present in the works in different countries, in addition to the fact that the measurement of the effect of the variation in intake on the performance of the animals is difficult to quantify in conventional experiments.

Keywords: Necrotic Enteritis; Meta-analysis; Coccidiosis; Review

[2] Master of Science dissertation in Animal Science, Faculdade de Agronomia, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil. (49p) August, 2022.

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RELAÇÃO DE ABREVIATURAS E SÍMBOLOS

ADG – Average Daily Gain

ADFI – Average Daily Feed Intake

***C. perfringens* –** *Clostridium perfringens*

EN – Enterite Necrótica

FE – Feed efficiency

Δ - Variation

CAPÍTULO I

INTRODUÇÃO

O crescimento da avicultura de corte tem sido exponencial nas últimas décadas devido ao avanço genético, da nutrição e da sanidade, que fornecem animais de alta produtividade com menor custo de produção.

O sistema intensivo de produção de frangos de corte, mesmo com uso de alto padrão tecnológico, não assegura que o ambiente de criação das aves esteja livre de patógenos. Quando presentes, os patógenos prejudicam a eficiência do aproveitamento dos nutrientes das rações, em decorrência do possível surgimento de desordens entéricas (RAMOS et al, 2011).

Dentre as doenças entéricas, a coccidiose é uma das mais frequentes e segundo ABBAS et al (2012), constitui-se como um dos principais prejuízos econômicos para indústria avícola. Segundo PEEK & LANDMAN (2011), a importância econômica da coccidiose é atribuída à diminuição da produtividade animal (pior conversão alimentar, redução do ganho de peso e aumento da mortalidade) e aos custos envolvidos no tratamento e prevenção. Os custos anuais com coccidiose em aves comerciais são estimados em seis bilhões de dólares a nível global (ABDELAZIZ, 2011).

A enterite necrótica (EN) é uma enterotoxemia que afeta a produção de aves com uma perda estimada acima de 6 bilhões de dólares anualmente, principalmente devido à redução do crescimento e desempenho, assim como medicação das aves afetadas (BROOM, 2017; JONES et al., 2019). Os clostrídios fazem parte da microbiota normal das aves, mas sob condições propicias, quando o microambiente digestivo é alterado, ocorre à proliferação dessas bactérias, produção de toxinas e invasão. Fatores predisponentes podem ser dietas de fácil fermentação ou que reduzam o peristaltismo, estresse e drogas que alteram a microbiota intestinal e, como na maioria dos casos relatados na avicultura industrial, a coccidiose é apontada como um dos principais fatores predisponentes ao desenvolvimento da EN (JONES et al.,2019).

Com a massiva quantidade de estudos envolvendo ambas as doenças citadas, encontramos diversos resultados, mas como cita Sauvant et. al. 2005, a transformação dos resultados de uma pesquisa em algo utilizável não pode ser baseado apenas de um único experimento, pois os resultados refletem as condições experimentais especificas. Apesar das metodologias serem desenvolvidas para

possibilitar a extrapolação dos resultados à população, na maioria das vezes os pesquisadores confirmam os mesmos através de novos experimentos, com isso diversos resultados são obtidos.

Além disso, a meta-análise pode evidenciar o efeito de um tratamento que individualmente não possibilita a determinação de uma resposta, pela falta de potência analítica (baixo número amostral). Em casos como este, a meta análise amplifica o poder analítico do modelo, por trazer um maior número amostral e assim possibilitando evidenciar mais precisamente as diferenças entre os tratamentos, caso existam (LOVATTO, 2007).

Na literatura cientifica encontram-se diversos estudos abordando os impactos da coccidiose e da enterite necrótica no desempenho dos frangos de corte, porém vários dos estudos são contraditórios e imprecisos nos resultados apresentados. e então, faz-se necessário o desenvolvimento de uma abordagem meta analítica para uma determinação mais precisa e concisa dos resultados referentes a ambas as doenças, trazendo não apenas os valores ligados ao desempenho, mas também os dados que remontam as questões nutricionais dos animais que estão sob desafios sanitários.

REVISÃO BIBLIOGRÁFICA

1.1 Coccidiose aviária

O desenvolvimento tecnológico empregado na avicultura industrial melhorou sobremaneira os índices de conversão alimentar e precocidade das aves ao abate (LUCHESE, 2007). Por outro lado, as doenças entéricas tornaram-se um dos maiores desafios para a avicultura industrial mundial nos últimos anos devido às perdas de produtividade e ao aumento de mortalidade (SANTOS, 2005).

Os protozoários do gênero *Eimeria* são responsáveis por causar uma das principais doenças entéricas aviária, a coccidiose ou eimeriose. Por ser um parasito intracelular, causa destruição de células do epitélio intestinal em processo de replicação prejudicando a digestão e a absorção dos alimentos, o que resulta em diarreia aquosa ou hemorrágica (MARTINS et al., 2012). As perdas econômicas envolvendo a coccidiose são decorrentes ou da mortalidade dos animais infectados com alta carga parasitária ou devido o desempenho insatisfatório dos animais, incluindo a coccidiose entre as doenças responsáveis pelos maiores prejuízos (LIMA, 2004).

A coccidiose apresenta caráter endêmico nas granjas. São reconhecidas sete espécies do gênero *Eimeria* spp. que parasitam aves doméstica: *E. acervulina, E. brunetti, E. maxima, E. mitis, E. necatrix, E. praecox* e *E. tenella* (SCHNITZLER, 1999; CASTAÑÓN, 2006; LUCHESE, 2007).

1.2. Ciclo biológico

As aves se infectam com espécies de *Eimeria* quando ingerem água ou ração contaminada com oocistos esporulados, contendo a forma infectante, o esporozoíto, este desenvolve em ciclo completo em um único hospedeiro, com fase de multiplicação assexuada e sexuada ocorrendo dentro das células do hospedeiro (KAWAZOE, 2009).

O ciclo parasitário inicia-se quando os oocistos esporulados são ingeridos e recebem uma série de estímulos, iniciando-se pela ação mecânica exercida pela moela das aves por trituração mecânica da moela, liberando os esporocistos. Em seguida inicia-se uma fase altamente dependente de ativação enzimática, conhecida como excitação. No intestino do animal, devido à ação de sais biliares e enzimas proteolíticas como a tripsina, os esporozoítos são liberados ativamente do esporocisto. A mobilidade do parasito é estimulada por sais biliares e pela temperatura interna do animal (CASTAÑÓN, 2006; ALLEN, 2002; DUBREMETZ, 1993).

Segundo Levine (1998), a esquizogonia inicia-se a partir de esporozoítos liberados de oocistos ingeridos, que penetram na parede intestinal e invadem células epiteliais, formando os merontes ou esquizontes. Essa etapa corresponde a fase do ciclo de reprodução assexuada ou também chamada de esquizogonia (KAWAZOE, 2009). De 2 a 4 dias após a infecção surgem os esquizontes de segunda geração que são diferenciados em microgametas e macrogametas (ITO et al., 2004). A fase gametogonia ou sexuada inicia-se ao final da fase assexuada, na qual o esquizonte que é diferenciado em macrogametas (correspondente a gametas femininos) e microgametas (gametas masculinos). Após a maturação, os microgametas flagelados rompem as células hospedeiras e vão fecundar os macrogametas, dando origem aos zigotos, e posteriormente aos oocistos imaturos que são eliminados juntamente com as fezes do hospedeiro.

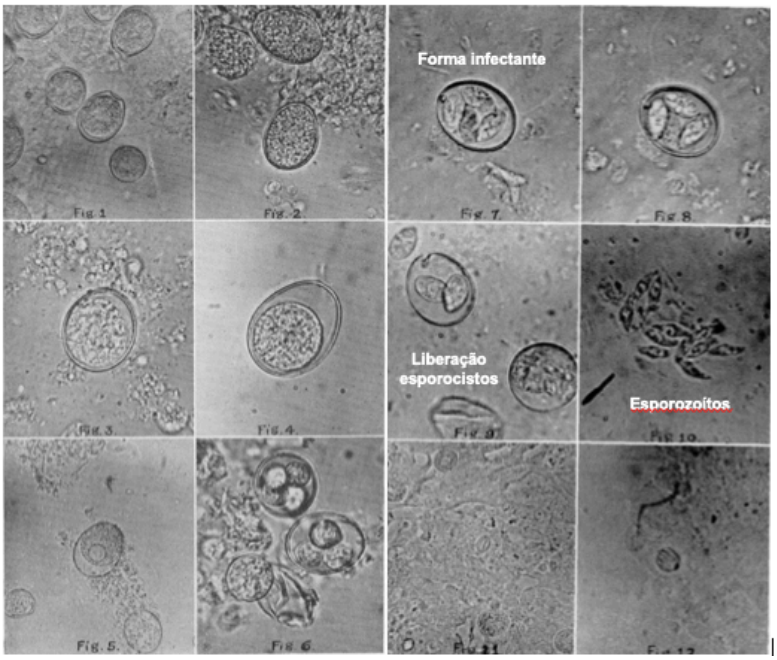

Figura 1. Imagens microscópicas de oocistos e esquizontes de Eimeria no intestino de frangos (Johnson, 1923)

Os oocistos podem ser liberados por vários dias, após período de pré-patente, que corresponde a um primeiro ciclo completo da *Eimeria*. O ciclo completo da *Eimeria* ocorre entre 96 a 150 horas (ITO et al., 2004). Após sair da célula epitelial e ser eliminado nas fezes, sob condições ambientais adequadas, passa por um processo de esporulação ou esporogonia (FERNANDO, 1990). Ao final do processo, cada oocisto esporulado contém quatro esporocistos cada qual com dois esporozoítos no seu interior. Oocistos esporulados podem ser imediatamente reingeridos para iniciar uma nova infecção.

1.3. Patogenicidade e imunidade

As espécies de *Eimeria* que possuem a galinha doméstica como o único hospedeiro natural e de maior importância econômica para a avicultura de corte brasileira são *E. acervulina*, *E. maxima* e *E. tenella*, cada qual se apresenta ocasionando lesões intestinais em locais distintos (PINHEIRO et al., 2014).

A coccidiose aviária pode ocorrer em diferentes graus, dependendo da sanidade do hospedeiro e da patogenicidade do parasito, sendo que as lesões que a

infecção provoca no intestino das aves são o diferencial entre as várias espécies de *Eimeria i*ntestinal (PINHEIRO et al. 2014). A coccidiose ainda pode ser dividida em subclínica e aguda. Na maioria das criações avícolas é possível verificar casos de coccidiose subclínica, observada pela redução na eficiência metabólica e imunológica da ave. Porém nos casos agudos, Allen (2002) relata que os sinais clínicos variam conforme as espécies de coccídios envolvidos na infecção. Algumas espécies apresentam maior grau de patogenicidade, causando diarreia que varia de mucóide a sanguinolenta, desidratação, penas arrepiadas, anemia, despigmentação da pele e prostração, dentre outros sinais clínicos.

Segundo Pinheiro (2014), infecções oriundas de agentes coccidianos causam uma modificação nas estruturas das vilosidades intestinais provocando o encurtamento na altura das mesmas, diminuindo a capacidade de absorção. Muitas vezes ocorre a destruição das células epiteliais do intestino, impedindo a renovação das vilosidades levando a perda de fluidos, hemorragia e susceptibilidade a outras doenças.

O dano tecidual causado, pela infecção de *E. tenella* ocorre no momento da divisão mitótica dos esquizontes. Já no caso de *E. acervulina* e *E. maxima*, a liberação dos esquizontes não provocam danos, mas durante a fase de divisão sexuada produz uma forte reação como a infiltração nas células e no tecido e espessamento tecidual (ITO et al., 2004). As fases críticas para a sanidade do hospedeiro estão relacionadas com a proliferação do parasito.

1.4. Espécies

Eimeria acervulina

Conforme descrito por KAWAZOE (2009), a *E. acervulina* é a espécie que invade as células epiteliais do duodeno e intestino delgado anterior. Os oocistos da E. acervulina possuem formato alongado, quando comparado aos oocistos das demais Eimerias. Em aves acometidas encontram-se, macroscopicamente, pontos esbranquiçados transversais no duodeno e, em casos mais graves, acomete o jejuno

ocorrendo formação de muco e perda das vilosidades intestinais (ITO et al., 2004). A patogenicidade e as lesões causadas por *E. acervulina* estão relacionadas a quantidade de oocistos que a ave é exposta, sendo que principal sintomatologia apresentada no lote de aves acometidas é a severa depressão no ganho de peso.

Eimeria maxima

Essa espécie é denominada de máxima devido ao tamanho grande dos seus oocistos, de formato ovóide de parede lisa e coloração amarelada (ITO et al., 2004). Este tipo de *Eimeria* apresenta como lesões característica na região mediana do intestino delgado e ocasionais lesões no duodeno e íleo. A enterite hemorrágica oriunda da infecção por *E. maxima* é associada ao espessamento da parede intestinal (KAWAZOE, 2009). Como sintomatologia, o animal apresenta redução de peso, aumento da conversão alimentar, petéquias na camada serosa do jejuno e íleo, inapetência e despigmentação cutânea. As lesões são observadas após 5 a 8 dias após a infecção, na fase sexuada do ciclo evolutivo da *Eimeria*. Além disso, a presença de conteúdo alaranjado devido a descamação e lesões da mucosa pode ser observado em necropsia da ave e avaliação das fezes (ITO et al., 2004)

Eimeria tenella

Essa espécie causa lesões de mucosa mais profundas no ceco, sendo considerado o patógeno que provoca maiores danos às aves (PINHEIRO et al., 2014). A clínica é caracterizada por fezes sanguinolentas, mortalidade elevada, alta morbidade, perda de peso expressivo e perda de pigmentação da pele (ITO et al., 2004). A mortalidade causada pela *E. tenella*, de forma fulminante, pode ultrapassar 20% das aves em um período de 2 a 3 dias. O intestino das aves afetadas apresenta encurtamento na altura das vilosidades epiteliais, o que resulta no impedimento da renovação da vilosidade epitelial e desencadeia a perda contínua de fluídos e maior vulnerabilidade a invasão bacteriana (ITO et al., 2004). Uma infecção por *E. tenella* provoca modificações no mecanismo de coagulação sanguínea da ave, o que afeta a demanda por vitamina K, resultado da hemorragia intestinal (KAWAZOE, 2009).

1.5. Identificação

Abaixo é apresentado um quadro desenvolvido por JOHNSON & REID (1970) utilizado como o padrão de avaliação para coccidiose durante as necropsias. Estes escores foram desenvolvidos de acordo com cada uma das três espécies principais de Eimerias e com as formas de lesões intestinais que as mesmas causam nos frangos.

QUADRO 1. Descrição dos escorres de lesão intestinal causados pelas Eimeria acervulina, Eimeria maxima e Eimeria tenella segundo JOHNSON & REID, (1970).

Escores de Lesão	*Eimeria acervulina*	*Eimeria maxima*	*Eimeria tenella*
Escore 0	Ausência de lesões.	Ausência de lesões.	Ausência de lesões.
Escore 1	Pontos ou estrias brancas, vistas da serosa ou mucosa, esparsas (até cinco porcentímetro quadrado) econfinadas aoduodeno.	Pequenas petéquias vistas da serosa no intestino médio. Pode haver pequena quantidade de muco alaranjado. Ausência de embalonamento e engrossamento do intestino.	Poucas petéquias dispersas na parede cecal. Ausência de engrossamento da parede cecal. Conteúdo cecalnormal.
Escore 2	Pontos ou estrias brancas mais numerosas, mas não coalescentes, que se estendem, entre duodeno e divertículo. conteúdo intestinal normal.	Superfície serosa com numerosas petéquias. O intestino pode estar cheio de muco alaranjado. Algum embalonamento e engrossamento do intestino.	Número maior de petéquias epresença de sangue aquoso no conteúdo cecal. Parede cecal com um leve engrossamento. Trabéculas cecais aparentemente normais.
Escore 3	Pontos ou estrias brancas já coalescendo com redução de tamanho, que se estendem até o divertículo. Parede intestinal engrossada e conteúdo intestinal aquoso.	Parede intestinal com embalonamento e engrossamento. Superfície mucosa áspera. Conteúdo dointestino com pequenos coágulos.	Grande quantidade de sangue ou tampão cecal presente. Parede cecal bastante engrossada. Pouco ou nenhum conteúdo fecal no ceco. Trabéculas cecaisdisformes.

Escore 4	Pontos ou estrias brancas completamente coalescentes, dandoà mucosa do intestino uma coloração acinzentada. Presença de lesões típicas somente no intestino médio. Parede intestinal engrossada e conteúdo cremoso.	Parede intestinal engrossada e embalonada em quase toda sua extensão. Presença de coágulos no conteúdo intestinal.	Parede cecal distendida com sangue ou tampão caseoso, contendo áreas de necrose. Material fecalausente ou incluído no tampão caseoso. Perca das trabéculas cecais.

2. Enterite Necrótica

Uma das doenças mais comuns na avicultura moderna e que gera grandes prejuízos econômicos devido a sua alta patogenicidade é a Enterite Necrótica (EN), causada pelo microrganismo *Clostridium perfringes* tipo A e C (Van Immerseel et al., 2004).

O *C. perfringens* é uma bactéria gram-positiva, em forma de bastonete, anaeróbia encapsulada, que causa um amplo espectro de doenças humanas e veterinárias. Clostridium perfringens difere de muitos outros clostrídios por não ter motilidade, reduzir o nitrato e realizar uma alta fermentação da glicose, frutose, galactose, inositol, maltose, manose, amido e sacarose. Os produtos da fermentação incluem ácidos acético e butírico com ou sem butanol (referencia). Clostridium perfringens cresce em uma ampla faixa de pH, variando de 5,5 a 8,5, enquanto o crescimento ideal dessa bactéria ocorre em pH 6 a 7. (WRIGLEY, 2001)

A ocorrência de *C. perfringens* é natural no intestino de animais de sangue quente, mas sua presença não é o único fator determinante para o desenvolvimento da doença. Vários fatores predisponentes desempenham papel vital na proliferação global desta bactéria, como diferentes níveis de nutrientes e ingredientes da dieta, presença de coccidioses, o status imune e estresse (além do estresse nutricional, qualquer fator que cause estresse em frangos de corte os predispõem a enterite necrótica; SONGER, 1996).

Além disso, o dano ao epitélio causado por *Eimeria* leva à liberação de soro e outros nutrientes das células hospedeiras ou causa mucogênese, o que ajuda a auxiliar na proliferação de Clostridium sp. e leva a mudanças no perfil microbiano do trato gastrointestinal (WU, 2014).

2.1 Patogenia

A virulência de *C. perfringens* resulta amplamente de sua alta capacidade de produzir toxinas. O sistema de classificação para *C. perfringens* é de acordo com a capacidade de liberar 1 ou todas as 4 toxinas de "tipagem" (α, β, ε e ı) (WRIGLEY, 2001).

A EN é causada pela ação de toxinas produzidas quando, em condições favoráveis, há rápida multiplicação de *C. perfringens* no intestino delgado (THOMPSON et al., 2006). As lesões características da EN são produzidas pela toxina α (WILLIAMS, 2005), a qual vem sendo associada com a doença (TITBALL et al., 1999), sendo considerada o principal fator de patogenicidade da bactéria (DAHIYA et al., 2006; KEYBURN et al., 2006; THOMPSON et al., 2006). A toxina, que destrói a membrana celular dos enterócitos devido a sua propriedade de fosfolipase C (STERNE & BATTY, 1975), é uma metalofosfolipase que possui dois domínios, o C-terminal, que penetra na membrana celular sendo responsável pela fixação da proteína na célula, e o N-terminal, que desempenha a função enzimática propriamente dita e hidrolisa os fosfolipídios das membranas celulares separando as porções polar e apolar, formando di-acil-glicerol e ácido fosfatídico, provocando a lise da membrana celular (SAKURAI et al., 2004).

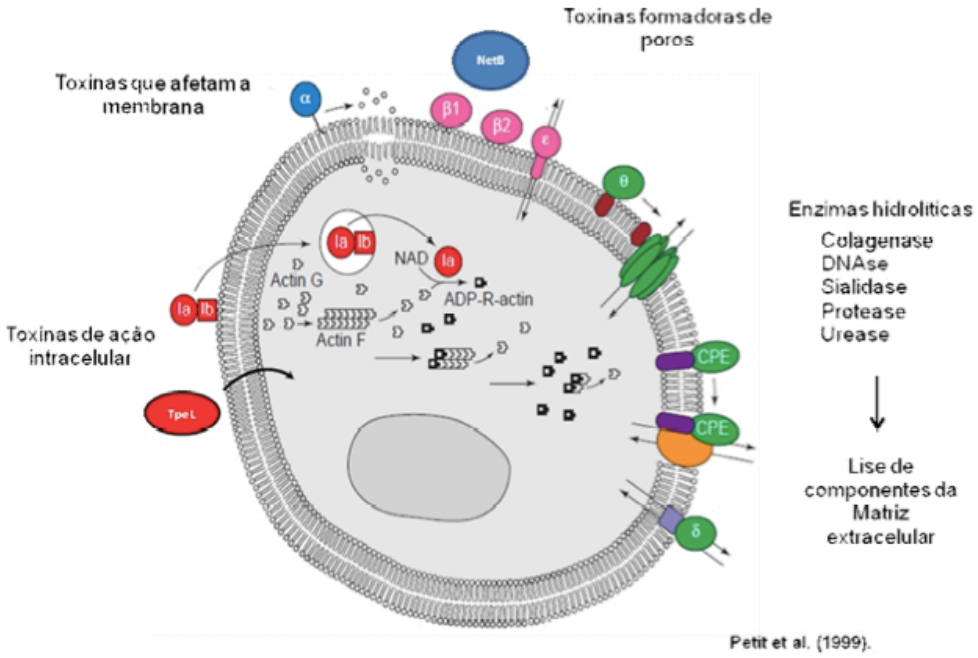

Figura 2. Mecanismo de ação das principais toxinas de C. perfringens.(Petit et al., 1999)

Posteriormente, Keyburn et al. (2008), analisando a sequência do cromossomo de *C. perfringens* relataram a presença do gene netB, e realizando experimentos genéticos de mutação/complementação, demonstraram que a citotoxicidade deste gene estaria relacionada à produção da toxina NetB, sugerindo que a mesma, também seria essencial para o desenvolvimento da EN, descartando o papel atribuído exclusivamente à toxina α.

2.2. Sinais clínicos e diagnóstico

O desenvolvimento da EN é rápido e grave e os sinais clínicos observados são inespecíficos (FUKATA et al., 1988). Os principais sinais relatados em aves incluem diminuição da movimentação, do consumo de ração, apatia, diarreia e eriçamento das penas. Com exceção da diarreia, os demais sinais são reflexos inespecíficos do comportamento doentio (JOHNSON, 2002).

Os principais sinais clínicos desta doença são as lesões necróticas de diferentes graus no intestino delgado, principalmente no jejuno e íleo, e algumas vezes

no duodeno. A redução da digestão e absorção dos alimentos, com maior conversão alimentar, levam ao dano da mucosa intestinal causado pela forma subclínica da doença (CALY et al., 2015).

O exame necroscópico de aves com EN mostrou distensão das alças intestinais por gás com aparecimento de lesões características e mais restritas ao intestino delgado; no entanto, foram descritas também lesões com menor frequência no ceco, no fígado e em outros órgãos (PARISH, 1961). A mucosa intestinal apresenta-se com coloração enegrecida a arroxeada. Segundo Helmboldt e Bryant (1971), a distribuição e a intensidade das lesões variam com a gravidade da doença.

A friabilidade do tecido intestinal e o dano às vilosidades culminam com a formação de uma pseudomembrana de coloração verde amarelada denominada de “toalha turca” ou “toalha felpuda” (GOLDER et al., 2011). Em casos mais graves ocorre fibronecrose intestinal difusa (SHANE et al., 1985). O destacamento da serosa, a alteração na espessura da parede intestinal, a presença de conteúdo descamativo e a irregularidade no contorno da mucosa são outros achados macroscópicos encontrados na EN.

Nos casos subclínicos, os achados são mais focais, com formação de regiões de coloração acinzentada a enegrecida ou, às vezes, discretamente avermelhadas (LOVLAND; KALDHUSDAL, 2001). Como o próprio nome da doença sugere, a principal característica da NE é o desenvolvimento de necrose multifocal coalescente da mucosa; próximo à região de necrose ocorre a formação de um tecido fibrinoso em associação com o debriamento resultante do processo infeccioso (TSAI; TUNG, 1981). O processo de necrose de coagulação ocorre preferencialmente no topo dos vilos, estendendo-se da superfície do epitélio à lâmina própria. Junto das lesões é possível encontrar uma quantidade abundante de bacilos pleomórficos Gram positivos aderidos à superfície das regiões lesionadas, devido à capacidade de adesão que eles têm às moléculas da matriz extracelular (ROOD, 1998).

O processo inflamatório é caracterizado pela migração massiva de heterófilos para as regiões em que ocorre a necrose, e intenso infiltrado linfoplasmocitário na lâmina basal, fatos acompanhados por edema, hiperemia, congestão e hemorragia da lâmina própria, submucosa e serosa. O processo de regeneração tecidual produz, de

modo geral, fusão e atrofia das vilosidades com diminuição das células de Globet e perda acentuada dos microvilos (LONG; PETTIT; BARNUM, 1974).

O diagnóstico é realizado através das lesões macroscópicas, microscópicas, pelo isolamento do agente etiológico, através de ELISA ou PCR. O isolamento do agente é realizado utilizando-se conteúdo intestinal, raspado da mucosa intestinal ou dos nódulos linfóides hemorrágicos (JÚNIOR, 2000).

Até alguns anos atrás, o tratamento mais utilizado para combater a enterite necrótica era o uso de antibióticos, mas com a crescente pressão para a redução do uso de antibióticos na produção animal, novos meios têm sido estudados para realizar esse controle, tais como, probióticos, prebióticos, ácidos orgânicos, e mais recentemente vem sendo desenvolvidos estudos avaliando a possibilidade do uso de diferentes níveis de alguns nutrientes específicos para o aumento da eficiência das respostas imunológicas dos animais desafiados por esta doença.

3. Revisão sistemática e meta-análise

A revisão sistemática é reprodutível e tende a ser imparcial, a mesma busca reduzir o viés através do uso de métodos específicos para realizar uma pesquisa bibliográfica abrangente, avaliando criticamente os estudos. A revisão sistemática busca responder uma questão de investigação bem definida e é caracterizada por ser metodologicamente abrangente, transparente e replicável. Ou seja, a revisão sistemática é uma busca cientifica menos dispendiosa, é um artigo de busca metódica e sistemática pré-definidos para possibilitar a identificação de todos os documentos relevantes publicados e não publicados para responder a questão de investigação, avaliando a qualidade desses artigos, extraindo os dados e sintetizando os resultados dos mesmos (FIGUEIREDO, 2014).

Uma descrição detalhada do problema de pesquisa que está se investigando é de suma importância para todos os dias de pesquisa, principalmente quando se trata de uma meta-análise. A questão de pesquisa envolve a definição das variáveis e do padrão de associação entre elas (COOPER, 2010).

PICo representa um acrônimo para População, Interesse e Contexto. Estes elementos são fundamentais para a construção e busca bibliográfica da pergunta da pesquisa, esta, quando bem construída possibilita a definição correta de que informações são necessárias para a sua resolução, maximizando a recuperação de evidências nas bases de dados, foca o escopo da pesquisa e evita a realização de buscas desnecessárias (SANTOS, 2007).

Revisões sistemáticas e meta-análises devem ser construídas através de um protocolo que descreva a razão, hipótese e métodos planejados na revisão. Um protocolo bem detalhado e descrito pode facilitar efetivamente o entendimento dos métodos de revisão, bem como auxiliar na identificação de modificações dentro dos métodos e no compilado de materiais acessados.

Para facilitar o desenvolvimento e realização das revisões sistemáticas foi desenvolvido uma diretriz de protocolo, conhecida como Preferred Reporting Items for Systematic reviews and Meta-Analyses for Protocols 2015 (PRISMA-P 2015). O PRISMA consiste em um checklist de itens, o qual facilita a preparação e descrição de um protocolo mais robusto e completo para a revisão sistemática (MOHER, 2015).

Nele podemos identificar as seguintes informações: número de trabalhos retirados por base de busca; número de trabalhos removidos por serem repetidos; triagem dos trabalhos de maneira superficial e quantos foram excluídos nessa triagem; artigos que foram lidos por completo e quantos foram retirados por motivos estabelecidos pelos autores; estudos incluídos na síntese qualitativa; e estudos incluídos na síntese quantitativa (meta-análise).

A meta-análise é uma ferramenta que sintetiza ou combina informações de múltiplos estudos para obter informações que possam ser aplicadas à grandes populações (BORENSTEIN, 2019). Uma das grandes vantagens da meta-análise na produção animal, ou em qualquer outra grande área, é o aumento no tamanho da amostra (pela combinação de vários estudos e delineamentos), o que diminui o grau de incertezas que existem nos resultados e os apresentando de maneira mais concisa (LOVATTO et al., 2007).

Levando em consideração a quantidade de informações disponíveis na literatura cientifica moderna sobre os temas coccidiose e enterite necrótica, faz-se de

grande ajuda a utilização das ferramentas de revisão sistemática e meta-análise na sumarização e melhor entendimento dos resultados encontrados. Pois estas ferramentas possibilitam a conclusão de algumas informações que experimentos tradicionais não possuem a capacidade de encontrar.

1. OBJETIVO

Estes estudos foram desenvolvidos para fornecer um resumo completo da literatura disponível sobre coccidiose e enterite necrótica em frangos de corte, avaliando seus impactos em relação a desempenho, histologia e respostas imunológicas.

CAPÍTULO II[1]

[1]Artigo escrito nas normas da revista *Veterinary Parasitology*

Comprehensive study of the impact of coccidiosis in broiler production – systematic review and meta-analysis

Abstract

A systematic review followed by a meta-analysis was carried out to study the impacts of coccidiosis in poultry production, seeking to see the relationship of the variation in feed intake and weight gain in different species of *Eimeria*, including, *Eimeria acervulina*, *Eimeria maxima*, *Eimeria tenella,* and a pool of *Eimeria* species. After a narrow search, a database was developed composing articles using coccidia infection in broilers and presenting animal performance and histological results. The database was composed by 141 articles, totalizing 46,354 birds. Meta-analysis followed three sequential analyses: graphical for biological coherence, correlation, and variance-covariance. The results showed that the weight gain reduced for all the groups infected by coccidia (P<0,05). Control groups presented higher feed efficiency when compared with infected groups (P<0,05), but for feed intake the group infected with *E. maxima* did not show significant differences when compared with control group (P>0,05). This infection caused the highest losses in weight gain in a simulated scenario in which the challenge did not impair feed intake (in other works, if the variation in feed intake was set to zero in the obtained equations), despite of that all the species presented reduction in weight gain even without reduction in feed intake. In the matter of intestinal integrity, all the infections presented significant responses in the variable lesion score, but *E. tenella* presented the highest values among all, mostly in the cecum. Even though there is a reduction in feed intake due to the infection, is correct to say that the impacts caused by coccidiosis come from different causes beyond that, including damage in the intestinal epithelium, which can lead to other problems.

Key-words: *Eimeria spp*., health challenge, intestinal lesion, nutrient requirements, performance, poultry.

Introduction

The most important intestinal disease in broilers production is caused by a protozoon of the genus Eimeria. This infection is associated not only with animal performance reduction, but also with epithelial cell damage, oxidative stress, malabsorption of nutrients, and in severe cases mortality (Yegani and Korver, 2008; McDougald et al., 2013). The main species that affect broilers are *Eimeria acervulina*, *Eimeria maxima,* and *Eimeria tenella* due to high prevalence and reduced susceptibility to anticoccidial drugs (Györke et al., 2013). Subclinical coccidiosis is identified more often than clinical and visible signs. So, it is often difficult to diagnose the disease at an appropriate moment to begin a treatment before the animal start to show major losses on performance.

This health challenge may interfere on the nutritional requirements of the animals. Even tough, these implications are not fully understood. Birds challenged by Eimeria frequently shown reduced feed intake (Kipper, 2013). In addition, these protozoa can reduce the nutrients availability to the animals though lesions in the gastrointestinal tract and can also alter the animal metabolism by deviation of nutrients that would be used for growth, and thus compromising animal performance (Cornelissen et al., 2009; Kipper, 2013). The growth impairment is another way to modify the nutritional requirements, as few nutrients are used for tissue deposition. For this reason, the feed formulated to sick animals when calculated based in the requirements of healthy animals will probably be disbalanced, with a lack of nutrients to cope the immune system and keep the productivity for these animals (Perez-Carbajal et al., 2010).

Over the last two decades, a considerable amount of research has been conducted investigating the effects of coccidiosis in epithelial intestinal damage, microbiota composition, immunological responses, and performance losses. The systematic assessment of all these information can be very useful to further understand the implications of coccidiosis infection

of the nutritional requirements of the broilers. Seeing this accumulative of information developed in the last decades the objective of this work was to evaluate through a meta-analysis approaches the real impacts of *Eimeria spp*. infection in performance, histological damages, and the relationship between the variation in feed intake and weight gain of broilers challenged.

Material and methods

The present work is a sistematic review and meta-analysis study about the impacts of coccidiosis in poultry production, evaluating the losses on performance of the animals in relation to feed efficiency and histological parameters, from studies published from 2000 to 2020.

Search strategy, screening, and inclusion/exclusion criteria

In 2020, two authors searched in three international databases (PubMed, Scopus, and Web of Science) using the PICo method (reference to population, intervention, and context). The key search used for this research is presented in the **Table 1**. Furthermore, the references of the included articles were studied in order to find the relevant studies to include in this research. The gathered information was entered into the EndNote version X9 from which the repeated articles were excluded. An initial search was performed through the studies by two authors. The screening of the studies, the extraction of results, and also the assessment of quality control of the articles was accomplished individually by these researchers. If there was no consistency in the inclusion of an article, the authors together determined the final decision for that article.

All the resources used for this paper were found by a narrow analytical view, where the papers which fitted with the criteria created by the authors was reviewed by full text reading and further discussion. The criteria used for this project was that the original study should have

been published from 2000; have animals in any growth phase 1-50 days old; infection with *Eimeria* spp., *E. acervulina*, *E*. maxima, *E. tenella,* or a pool of them; the infection couldn't be from overdose vaccine; and the article should report at least one of the target variables (body weight gain, feed intake, feed efficiency, intestinal morphology, or immunological responses).

For the variable lesion score of coccidiosis is used the method created by Johnson and Reid, 1970. Where the animals are euthanized and after this different portion of the small intestine is taken for observation, then according to the severity of the lesions they are classified in 0 when there is no sign of infection and 4 with the worse lesions in the intestine (duodenum, jejunum and ileum).

Data extraction, calculations, and statistical analysis

The relevant data from each selected study were inserted into pre-designed tables. Information about the publication, design, performance, country of the experiment, were some of the variables collected. Then, data were categorized using a sequential code for classification, adjustments in the variance-covariance analysis and undergone statistical analysis. This sequential code was used as moderating variables in the analysis with the purpose of considering the variability of compiled studies, articles, inter and intra effects as well.

At first, we used a method for relativization the data, intending to reduce the variability among studies. For these processes the responses of the animal used as control in the paper was set to 'zero', and by using a simple rule of three, comparing the variation of the responses from the infected animals. The procedure allowed calculating the 'effect of heath challenge' (%) in each response, which will be further indicated by the symbol Δ.

Statistical analyses were performed using Minitab (Minitab for Windows, v. 19). The relationships between predetermined variables (e.g., experimental characteristics: animal age; and animal performance: feed intake, body weight gain) were accessed using scatter charts.

This procedure was performed to evaluate database quality and observe the biological coherence of data. Unusual information or patterns were revised in the database. Outliers were not removed, as they may represent pathological responses and higher variability may be expected in the performance of challenged animals. Pearson correlation were accessed between variables, and significant results ($P < 0.05$) were used to identify related factors. Then, the variance analyses were performed using the 'General Linear Model' procedure to compare the treatments and to obtain the prediction equations. The statistical model for variance analyses included the fixed effect of treatment and the random effect of experiment and the code of experiment was considered as random in the model.

Control and challenged groups were compared only if the difference between them was limited to the *Eimeria* spp. inoculation. Treatments that received any kind of extra treatment (i.e., drugs or vaccines) were excluded from the main analysis if there were not a correspondent control group (i.e., a control group containing the same feed additive tested in the challenged treatment). Equations were used to study the relationship between the variations (Δ%) in feed intake and weight gain. The partition between these two effects was calculated considering the corrected Δ% in weight gain (obtained using the equations) and the average Δ% in feed intake observed in the database.

Results

Literature research results

The PRISMA diagram describing the studies found during each step of literature search is presented in **Figure 1**. A total of the 2,905 papers was found in the digital databases PubMed, ScienceDirect, and Web of Science. From these, 1,439 papers were removed due to duplicity in the database. After, 1,166 papers that were not related to the database objectives were also removed during the evaluation of titles and abstracts. During the full-text evaluation, other 81

papers were removed, from which: 2 were abstracts, 3 were meta-analyzes, 12 were literature reviews, 62 studies did not have a control (unchallenged) group, 55 studies used high dose of coccidiosis vaccine as a challenge, and 23 papers did not include the target-responses of the project.

After applying the selection criteria, 143 articles were included in the database, from which 131 papers evaluated performance responses. The final database occupied 1,970 lines on the electronic spreadsheet. Each line of the database referred to a treatment in the original publication. However, some treatments occupied more than one line when the measures were taken repeatedly over time.

Most of the studies were conducted in the United States and China. The selected studies included 46,354 birds, of these 29% were mixed groups (female and male), 44% were males, 3% were females, and 24% were not described **(Figure 2)**. A great diversity of genetic lines was described in the papers. From them, the main cited were Ross, Cobb, Arbor Acres, and Hubbard. A summary of the studies is presented in **Table 2**.

Database limitations

Some effects were not further evaluated in this study due to limited information availability. In this particular, it is important to highlight the effect of infection doses. Only 13 studies evaluated different challenging doses of *Eimeria*. From these trials, three studies evaluated infection with different doses of *E. acervulina,* two studies evaluated *E. maxima*, and only one study evaluated infection by different doses of *E. tenella.* In addition, doses varied greatly among studies and the pathogenicity of each stream could not be quantified/classified, neither considered in the analytical models.

Exploratory analysis

The effect of *Eimeria* spp. infection on the performance responses was evaluated in terms of variation (Δ%) of challenged groups compared to their respective control group. A preliminary description of the relative effect of *Eimeria* spp. infection (Δ%) on performance responses of broilers is shown in **Table 5**.

The effect of *Eimeria* challenge on broiler performance varied widely across studies, probably due to the heterogeneity of experimental conditions applied in the trials that composed the database. When accessing the variation (Δ%) between challenged groups and their respective control group, it was possible to observe numerical decrease in feed intake in 78% of the original comparisons, while 87% of the contrasts showed worsening in weight gain and 85% in feed efficiency. **(Figure 3)**

Performance of broilers challenged or not by *Eimeria* spp. are presented in **Table 3**. Repeated assessments taken within treatment over time were not considered in this analysis. So, just means from the complete experimental period were used in order to avoid a large variation in terms of 'treatments per study' included in the analysis (e.g., studies with repeated measures would contribute with much more data/lines in the analysis, in contrast to the papers that reported only means for the overall period).

Broilers challenged by *Eimeria* spp. reduced ($P<0.001$) the feed intake by 6.27%, the weight gain by -19.73%, and the feed efficiency by -11.11% compared to control group. All the studied effects were highly significant ($P<0.008$), despite the challenges with *E. maxima* that did not influence ($P>0.05$) the feed intake.

Residuals of the models previously presented were correlated ($P<0.05$) with age. Considering the overall database, the correlations with broiler age were 0.646 for feed intake, 0.439 for weight gain, and -0.442 for feed efficiency. The effect of age was tested in the same models previously presented and found significant ($P<0.05$) for all responses. So, equations

were fitted using the measures repeated in time to evaluate the impact of bird age on *Eimeria* effect. However, obtained equations showed very low coefficient of determination and no linear effect was found ($P>0.05$).

Relation between weight gain and in feed intake in challenged animals

The correlation between variation (Δ%) in weight gain and in feed intake caused by *Eimeria* challenge was 0.705 ($P<0.05$) for the overall database, 0.795 ($P<0.05$) in challenges with *E. acervulina,* 0.309 ($P<0.05$) in challenges with *E. maxima*, 0.973 ($P<0.05$) in challenges with *E. tenella*, and 0.887 ($P<0.05$) in challenges with pool of the *Eimeria* spp.

The variation in weight gain (Δ%G) showed a linear relationship with variation in feed intake (Δ%FI) in both challenges. The intercepts of the equations were different from zero and negative in all analyses (**Figure 4**).

The approach used in this study evaluated the relationship between Δ%FI and Δ%G. When Δ%FI was set to zero in the equations (i.e., simulating a scenario without changes in feed intake), the Δ%G was estimated to be 6.27% for general *Eimeria* challenges, 4.41% in challenges caused by *E. acervulina,* 16.8% for *E. maxima*, 4.30% for *E. tenella*, and 5.23% for pool of *Eimeria* spp. These reductions represented 32% of total ADG impairment for the overall database, 23% for *E. acervuline*, 65% for *E. maxima*, 18% for *E. tenella*, and 26% for pool of *Eimeria* spp.

The partition of the effects on Δ%G corrected (adjusted using the equations previously presented to the average Δ% in feed intake observed in the database) is presented in **Figure 5**. Considering the overall database, 68% of Δ%G was related with reduction of feed intake. Otherwise, the fraction related to feed efficiency was estimated to be 77% in challenges caused by *E. acervulina,* 31% for *E. maxima*, 81% for *E. tenella*, and 74% for pool of *Eimeria* spp.

Intestinal Integrity

At the **Figure 6** presents the results in relation to the lesion scores used to quantify the intensity of the infection, using the Johnson and Reid (1970) scores. As expected, the infected animals showed higher values than the control animals in all intestinal regions analyzed. It is worth noting that the highest degrees of injury were found in the cecum, because it is the site with the highest infection of *Eimeria tenella*, which causes greater damage to the intestinal epithelium of the animals and, consequently, presents more visible lesions, that are evaluated on this type of scale.

The analyzes of the intestinal morphometry portion are presented in **Table 4**, where we can observe in the duodenum that there was a significant difference in the variable's crypt depth and villus:crypt ratio, where the animals infected by *Eimeria* spp. showed a greater crypt depth of 54,82um than uninfected animals and, consequently, a greater villus:crypt ratio, demonstrating an epithelium damaged by parasitic infection. Another important point to note is that although there was no significant difference in the villus variable, there was a reduction in the villus height of 8,15% on infected animals, further reinforcing the impacts caused by an infection.

In the jejunum portion, in which significant results can be observed in relation to villus size and villus:crypt ratio. There was a reduction of approximately 90um (>0.05%) in the height of the villi of animals infected by the protozoan and a reduction in the villus:crypt ratio of the same animals. And although not statistically significant, there was an increase in the depth of the crypts of infected animals in 34,46um.

For the ileum portion of the intestine there was no significant difference between the variables, however, they showed the same behavior as the other intestinal portions. Where the infected animals showed a reduction in the height of the villi and an increase in the depth of the

crypts in 18,65um when compared to non-infected animals, and with that a reduction in the relationship between them.

Discussion

The impacts of coccidiosis excel mortality, in addition to the money spent for control or prevention of this infection, the financial losses are mainly related to subclinical losses of asymptomatic animals, which keep environmental contamination through oocysts elimination in their feces. Even though the animals don't present clinical signs they loss performance due to the malabsorption syndrome and other impairments caused by the challenge (Maiorka, 2005).

The specie *Eimeria acervulina* develops in the epithelial cells in the proximal region of the small intestine, mainly in the duodenum. (Kant V., 2013) Is also know from previous works that the *Eimeria acervulina* may cause loss of fluids and a decrease in nutrient absorption, mostly because of the intestine site that this specie targets in the animals (Chapman 2014; Joyner 1974). The results found in this research showed a great reduction of body weight gain, even when the variation in feed intake was set to zero, agreeing with Kipper (2013) research, this is probably because the duodenum is a site of high nutrient absorption.

Poultry infected by *Eimeria maxima* targets the intermediate region of the intestine and is easily recognizable due to the size of its oocysts (the largest), they can cause swelling of the intestinal wall with petechiae and loosening of the epithelium (Conway, 2007; Joyner, 1974). *E. maxima* affect a large portion of the intestine, from a small portion of the duodenum to a large region of the ileum, due to this long involvement, there is a great reduction in food efficiency (Idris et al., 1997), which is the Eimeria species that presented the greatest reduction in efficiency among all species in our research. Another important result found in this work was that unlike the other species, *E. maxima* didn't show any significant result in the variable feed intake, even though presented a great reduction in the body weight gain.

Eimeria tenella is known for its infection in the cecum and causes bloody diarrhea, this is due to the complete destruction of villi (Chapman, 2014; Conway, 2007). These conclusions go in the same line as from our work where we found that the higher lesion scores were found in the cecum from infected animals. Another important highlight is that the reduction in body weight gain for this specie infection in highly corelated with feed efficiency, in other words the main impact in performance caused by *E. tenella* is due to the reduction in feed intake.

The infection from more than one species of Eimeria is the most common found out of experimental barns and in this research showed some similarities results as the infection by only one specie. It was presented that there is a greater reduction in feed intake when compared to others, but the feed efficiency showed results similar to *E. tenella* and *E. acervuline*. It is also important to highlight that this multiple infection can be harder to deal in the field, due to multiple targets for the antimicrobial or other forms of treatment that can be effective for one specie but not for others.

The equations generated by this research setting the feed intake to zero allowed us to see that the losses in performance are not only related to the reduction in the feed efficiency, these results are very important since the relationship between ADFI and ADG impairment are very difficult to quantify in conventional experimental designs. But also raises some questions about what increase these losses. Some hypothesis that are already been studied are the alteration in the microbiota due to the infection, also this alteration can be caused many times by the disruptive impact on the intestinal epithelium as showed in this work. And another important point to be taken in account for future studies is the variation in nutrients requirements for animals during sanitary challenges, by the fact that occurs a mobilization of nutrients for the development in the immunological responses (Orso, et al., 2021; Huang, et al., 2018; Hume, et al.,2006; Oviedo-Rondón, et al., 2006).

Conclusion

The variation in feed intake is responsible for most of the reduction in weight gain during *Eimeria spp.* infection. Although when this variation is set to zero the *E. maxima* infection cause the highest losses in animal performance. This reinforces the importance of study the variation of nutritional requirements during any kind of infection, aiming to reduce the impacts in the animal production.

Declaration of interest

The authors declare no conflict of interests.

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Attachments

Table 1 – Keyword developed for narrow search using PICo method.

PICo	
Population	(broiler OR broilers OR chicks OR chicken OR chickens)
Interest	(" Eimeria" OR " coccidiosis" OR " E. acervulina" OR "E. tenella" OR "E. maxima")
Context	(performance OR "body weight" OR "average daily gain" OR "weight gain" OR "average daily feed intake" OR "feed intake" OR “feed consumption" OR "feed conversion" OR "feed to gain" OR "feed : gain" OR "feed efficiency" OR "gain to feed" OR "gain : feed" OR ADFI OR ADG OR BW OR FCR OR cytokines OR interleukin OR antibody OR immunoglobulin OR “intestinal health”)

Figure 1. Flow-chart describing the inclusion and exclusion of studies

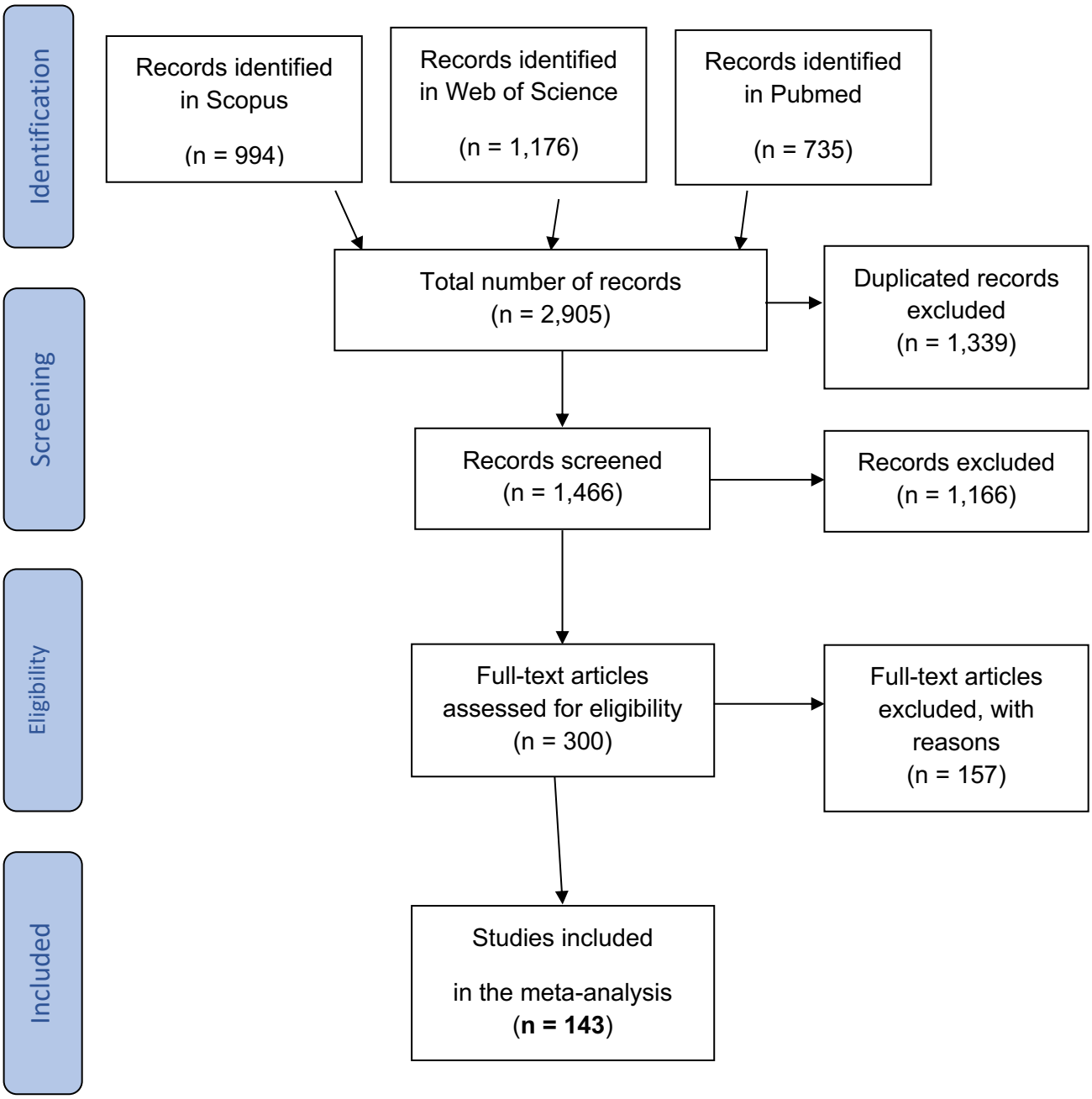


Figure 2. Origen of the studies that were used to build the database

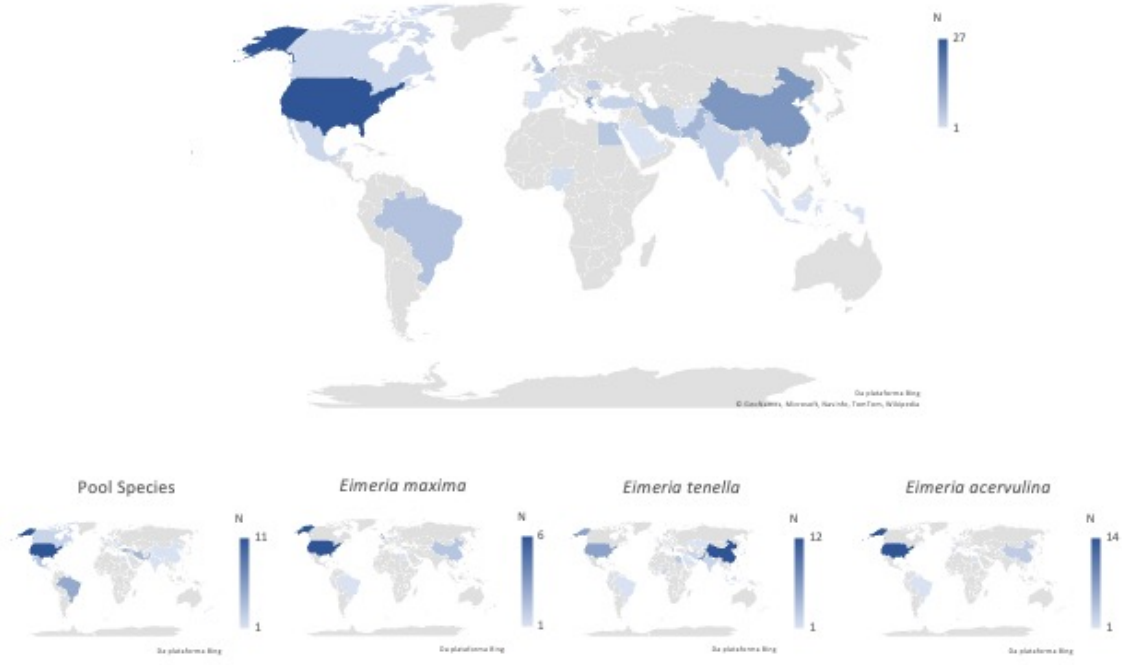


Table 2. Description of the papers used in the database

Code [1]	Citation	N of Broilers	Challenge		
			Species	Dose	Age of inoculation/ Post hatch
2	Hu et al. 2000	1260	*E. acervulina* + *E. maxima*	-	14, 13, 14, 25
3	Matthews et al. 2000	450	*E. acervulina*	-	1
4	Zhu et al. 2000	100	*E. maxima*	1,000; 5,000; 10,000; 20,000	-
6	Yadav et al. 2001	140	*E. tenella*	10,000	10
7	Klasing et al. 2002	336	*E. acervulina*	46,000	14
8	Allen et al., 2002	225	*E. maxima*	17,500; 40,000	14
9	Banfield et al. 2002	1152	*E. acervulina*	15,000	22
11	Kidd et al. 2003	384	*E. acervulina*	10,000	7, 8
12	Mathis et al., 2005	2000	*E. acervulina* + *E. maxima* + *E. tenella*	60,000 + 10,000 + 60,000	1
13	Guo et al., 2005	180	*E. tenella*	-	18
14	Koynarski et al. 2005	100	*E. acervulina*	300	12
15	Li et al. 2005	660	*E. acervulina* + *E. maxima* + *E. tenella*	650,000	24
17	Watsonet al. 2005	240	*E. acervulina* + *E. maxima* + *E. tenella*	400,000	1
19	Oviedo et al. 2006	288	*E. acervulina* + *E. maxima* + *E. tenella*	200,000 + 100,000 + 50,000	19
20	Persia et al. 2006	192	*E. acervulina*	15,000; 50,000	9, 11

22	Alfaro et al. 2007	600	*E. acervulina + E. maxima + E. tenella*	2,300	1
23	Elmusharaf et al. 2007	256	*Pool*	1,640	15
24	Estrada et al. 2007	80	*Pool*	105,000	21
25	Guo et al. 2007	360	*E. tenella*	50,000	14
26	Koynarski et al. 2007	44	*E. acervulina*	40,000	1
27	Lee et al. 2007	90	*E. acervulina, E. tenella*	5,000; 5,000	10
28	Lee et al. 2007	190	*E. acervulina*	5,000; 10,000	10, 12
29	Nollet et al. 2007	720	*E. acervulina + E. maxima + E. tenella*	100,000 + 10,000 + 15,000	15
30	Parker et al. 2007	500	*E. acervulina + E. maxima + E. tenella*	200,000 + 100,000 + 100,000	17
31	Abbas et al. 2008	286	*E. tenella*	75,000	14
32	Bafundo et al. 2008	2496	*E. acervulina + E. maxima + E. tenella*	100,000	14
33	Zulpo et al.2007	39	*E. acervulina; E. maxima*	20,000	-
35	Wanh et al. 2008	342	*E. tenella*	50,000; 100,000; 50,000; 10,000	8, 14
36	Cornelissen et al. 2009	400	*E. acervulina; E. maxima; E. tenella*	50,000; 20,000; 75;000	7
37	Gao et al. 2009	360	*E. tenella*	60,000	21
39	Lee et al. 2009	150	*E. maxima; E. tenella*	10,000; 8,000	7
40	Nweeze et al. 2009	25	*E. tenella*	8,000	-
41	Peek et al. 2009	80	*E. acervulina; E. maxima; E. tenella*	10,000; 6,310; 1,995	1
42	Song et al. 2009	1200	*E. acervulina; E. maxima; E. tenella*	50,000	28
43	Bera et al. 2009	49	*E. tenella*	1,000; 5,000; 25,000	16, 24
44	Berezin et al. 2010	96	*E. tenella*	50,000	14
45	Cox et al. 2010	1440	*E. acervulina + E. maxima + E. tenella*	50,000 + 10,000 + 2,500	8
47	Jang et al. 2010	18	*E. maxima*	20,000	21
48	Jang et al. 2010	140	*E. acervulina*	10,000	21
49	Shah et al. 2010	192	*E. acervulina*	100,000	28
50	Abbas et al. 2011	198	*E. tenella*	75,000	12
51	Bun et al. 2011	384	*E. tenella*	15,000	21
52	Ellakany et al. 2011	180	*E. tenella*	5,000	14
53	Kuçukyilmaz, et al. 2011	624	*Pool*	190,000	19
54	Lillehoj et al. 2011	80	*E. acervulina*	2,000	14
55	Shaw et al. 2011	1008	*E. acervulina + E. tenella*	100,000+5,000	10
56	Yin et al. 2011	135	*E. maxima*	10,000	5
57	Bozkurt et al. 2012	624	*Pool*	35,000	14
58	Faber et al. 2012	160	*E. acervulina*	100,000	9
59	Giannenas, et al. 2012	300	*E. tenella*	20,000	-
60	Orengo et al. 2012	72	*E. acervulina*	100,000	25
61	Willis et al. 2012	180	*E. acervulina + E. maxima + E. tenella*	49,000	14
62	Arczewska et al. 2013	400	*Pool*	19,000	12
63	Gao et al. 2009	300	*E. tenella*	60,000	21
64	Wils-Plotz et al. 2013	432	*E. maxima*	1,500	10
65	Abdelrahman et al. 2014	360	*E. acervulina + E. maxima + E. tenella*	75,000 + 25,000 + 75,000	15

66	Ali et al. 2014	180	*E. maxima*	10,000	14
67	Bozkurt et al. 2014	2400	*Pool*	50,000	14
68	Ritzi et al. 2014	1008	*E. acervulina + E. maxima + E. tenella*	65,000	15
69	Abu-Akkada et al. 2015	108	*E. tenella*	40,000	21
70	Amerah et al. 2015	256	*E. acervulina + E. maxima + E. tenella*	180,000 + 6,000 + 18,000	14
71	Aziza et al. 2016	180	*E. tenella*	40,000	21
72	Chand et al. 2016	300	*E tenella*	30,000	14
73	Dersjant-Li et al. 2016	192	*E. acervulina + E. maxima + E. tenella*	-	5
74	Habibi et al. 2016	180	*E. tenella*	3,000	22
75	Pender et al. 2016	400	*E. acervulina*	100,000	15
76	Rochell et al. 2016	400	*E. acervulina*	25,000; 50,000; 10,000,000	15
77	Wang et al. 2016	270	*E. tenella*	100,000	15
78	Kim et al. 2017	216	*E. acervulina + E. tenella*	25,000+5,000	5
79	Wang et al. 2017	270	*E. tenella*	100,000	15
80	Aziza et al. 2018	120	*E. tenella*	40,000	21
81	Ott et al. 2018	1280	*E. acervulina + E. maxima + E. tenella*	50,000 + 10,000 + 5,000	15
82	Sakkas et al. 2018	288	*E. maxima*	2,500; 7,000	-
83	Tonda et al. 2018	3300	*E. acervulina + E. maxima + E. tenella*	760,000	14
84	Awais et al. 2019	280	*E. acervulina + E. maxima + E. necatrix*	67,500	20
85	Behnamifar et al. 2019	480	*E. tenella + E. maxima + E. necatrix*	340,000	28
86	Leung et al. 2019	672	*E. acervulina + E. maxima*	125,000 + 65,000	10
87	Moraes et al. 2019	864	*E. tenella + E. acervulina + E. maxima*	10,000 + 200,000 + 80,000	14
88	Sakkas et al. 2019	336	*E. tenella + E. acervulina + E. maxima*	7,000	11
97	Manafi et al. 2011	200	*E. tenella*	50,000	21
98	Swaggerty et al. 2011	160	*E. tenella*	15,000	14
102	Laika and Jahanian, 2016	288	*E. acervulina + E. maxima + E. Necatrix*	65,000 + 65,000 + 65,000	16
105	Fortuoso et al. 2019	30	*Pool*	35,000	27
106	Khatlab et al. 2019	384	*Pool*	17,000	14
107	Oelschlager et al. 2019	576	*E. acervulina + E. maxima + E. tenella*	100,000 + 40,000 + 30,000	14
108	Oikeh et al. 2019	72	*E. maxima*	7,000	12
109	Pop et al.2019	150	*E. acervulina + E. maxima + E. tenella*	5,000	14
110	Giannenas et al. 2003	120	*E. tenella*	50,000	14
111	Giannenas et al. 2004	210	*E. tenella*	50,000	14
112	Tipu et al. 2002	240	*Pool*	30,000	22
113	Du and Hu. 2004	50	*E. tenella*	100,000	14
114	Florou-Paneri et al. 2004	150	*E. tenella*	60,000	14
115	Christaki et al. 2004	450	*E. tenella*	60,00	14
116	Elmusharaf et al.2006	120	*E. tenella*	3,500	12, 8
117	Ogbe et al.2009	120	*E. tenella*	36,250	42

118	Papazahariadou et al. 2010	105	*E. tenella*	31,000	11
119	Abbas et al. 2010	90	*E. tenella*	100,000	20
121	Ellakany et al. 2011	180	*E. tenella*	500,000	14
122	Tsinas et al. 2011	375	*E. acervulina* + *E. maxima*	100,000	14
123	Haq et al. 2011	240	*E. tenella*	30,000	22
124	Bun et al. 2011	384	*E. tenella*	1,500	21
125	Arczewska et al.2012	280	*Pool*	17,000	12
129	Ashley et al.,2012	1008	*E. acervulina* + *E. tenella*	10,000+ 50,000	12
132	Faber et al., 2012	200	*E. acervulina*	1,000,000	9
133	Giannenas et al. 2012	300	*E. tenella*	20,000	14
134	Scheurer et al.2013	1080	*E. acervulina* + *E. maxima* + *E. tenella*	250,000 + 25,000 + 25,000	15
135	Kim et al. 2013	80	*E. acervulina*	50,000	10
137	Kheirabadi et al. 2014	120	*Pool*	200,000	21
138	Pourali et al. 2014	480	*Pool*	50,000	14
160	Ali et al.2019	240	*Pool*	30,000	8
162	Park et al. 2019	196	*E. maxima*	10,000	21
163	Srinivasu et al. 2020	420	*Pool*	50,000	21
164	Conway et al. 2002	2500	*E. acervulina* + *E. maxima* + *E. tenella*	100,000	-
166	Elmusharaf et al. 2007	288	*E. acervulina* + *E. maxima* + *E. tenella*	37,600	15
168	Awais et al. 2020	280	*Pool*	67,500	20
140	Almeida et al. 2014	1140	*E. acervulina* + *E. maxima*	250,000	8
141	Bortoluzzi et al. 2015	1440	*E. maxima* + *E. acervulina*	25,000	14
142	Pop et al. 2015	504	*E. acervulina* + *E. maxima* + *E. tenella*	20,000 + 10,000 + 2,000	14
143	Singh et al. 2015	288	*E. acervulina* + *E. maxima* + *E. tenella*	87,500 + 3,500 + 35,000	21
145	Homg et al. 2016	30	*E. tenella*	10,000	10
146	Liu et al. 2016	144	*E. acervulina*	10,000	21
148	Wiedosari & Wardhana. 2016	35	*E. tenella*	2,000	6
147	Guven et al. 2016	160	*Pool*	300,000	16
149	Rochell et al. 2017	149	*E. acervulina*	630,000	15
150	Kaingu et al. 2017	60	*E. tenella*	75,000	24
151	Abbas et al. 2017	315	*E. tenella*	60,000	14
152	Rochell et al. 2016	384	*E. acervulina*	350,000	15
120	Major et al. 2010	120	*E. acervulina*	25,000	12
170	Willis et al. 2010	144	*E. acervulina* + *E. maxima* + *E. tenella*	50,000	28
169	Conway et al. 2002	210	*E. tenella*	100,000	-

[1] Used in the database to identify each study and across this document to refer to each publication. The order presented here is the same applied in the database, even if a crescent numerical order was not always used.

Figure 3. Relative effect of *Eimeria* spp. infection (Δ%) on performance of challenged broilers compared to their respective control group – data presented according to the year of publication

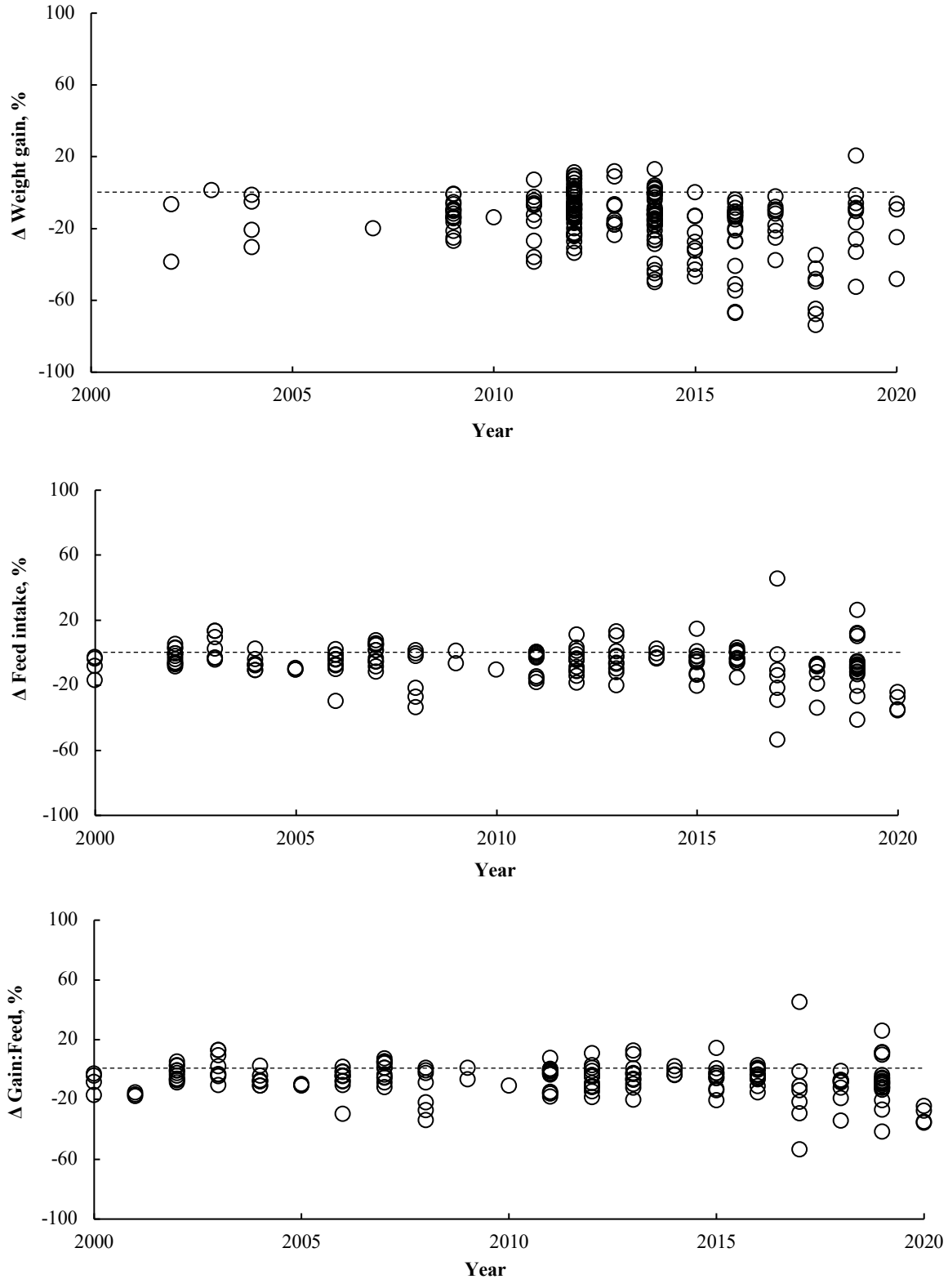
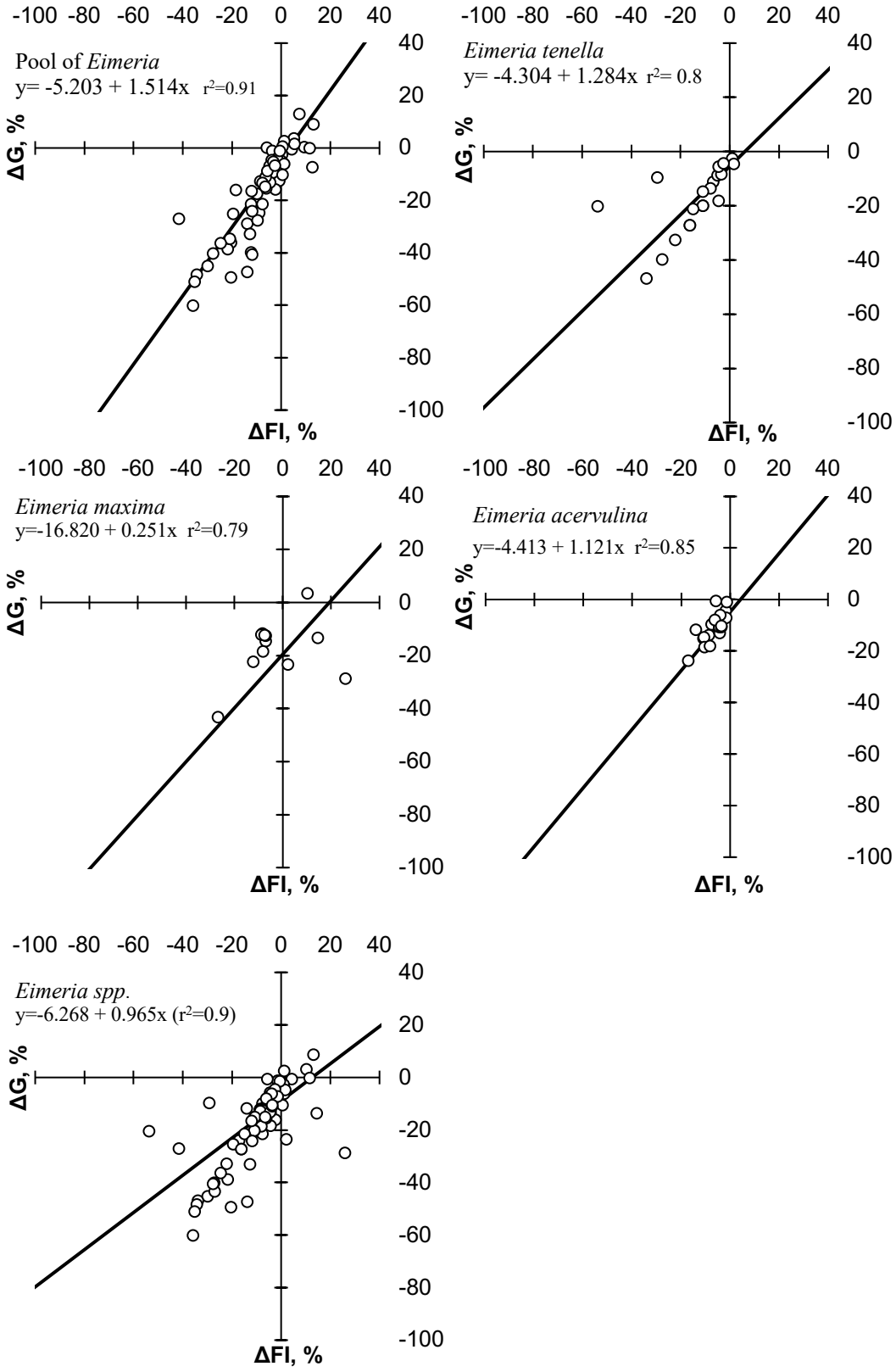


Table 3. Performance of broilers challenged by *Eimeria* spp.

Variables	Total	*E. acervulina*	*E. maxima*	*E. tenella*	Pool
Average feed intake, g/d					
Control	82.42	56.92	101.01	81.36	88.81
Challenged	77.25	53.47	95.76	75.15	80.63
Δ%[1], %	-6.27	-6.06	-5.20	-7.63	-9.21
P-value [2]	<0.001	<0.001	0.400	0.002	0.008
RSE [3]	8.48	1.93	14.05	6.36	9.57
R^{2} [(4)]	0.96	0.99	0.82	0.98	0.94
N means [5]	213	37	22	58	79
N studies [6]	86	11	8	29	35
Average daily gain, g/d					
Control	51.71	46.89	55.29	48.21	55.28
Challenged	41.51	37.87	40.89	36.96	44.32
Δ%, %	-19.73	-19.24	-36.04	-23.34	-19.83
P-value	<0.001	0.001	0.002	<0.001	<0.001
RSE	9.33	9.21	16.06	9.97	8.79
R^{2}	0.89	0.87	0.76	0.90	0.89
N means	323	63	36	99	99
N studies	120	22	15	42	45
Feed efficiency, kg/kg					
Control	0.63	0.67	0.62	0.57	0.63
Challenged	0.56	0.62	0.53	0.52	0.56
Δ%, %	-11.11	-7.46	-14.52	-8.77	-11.11
P-value	<0.001	0.006	0.003	<0.001	<0.001
RSE	0.05	0.05	0.06	0.03	0.05
R^{2}	0.93	0.86	0.90	0.98	0.92
N means	205	37	22	54	77
N studies	83	11	8	28	34

[1] Variation between challenged and unchallenged performance.
[2] Probabilities of challenge effect. Models also included the effect of study ($P<0.05$).
[3] Residual standard errors.
[4] Adjusted coefficients of determination.
[5] Number of means considered in each analysis.
[6] Number of studies that originated the means used in the analysis.

Figure 4 - Relationship between variation in weight gain (Δ%G) and variation in feed intake (Δ%FI) of broilers challenged by different infections of *Eimeria spp.*



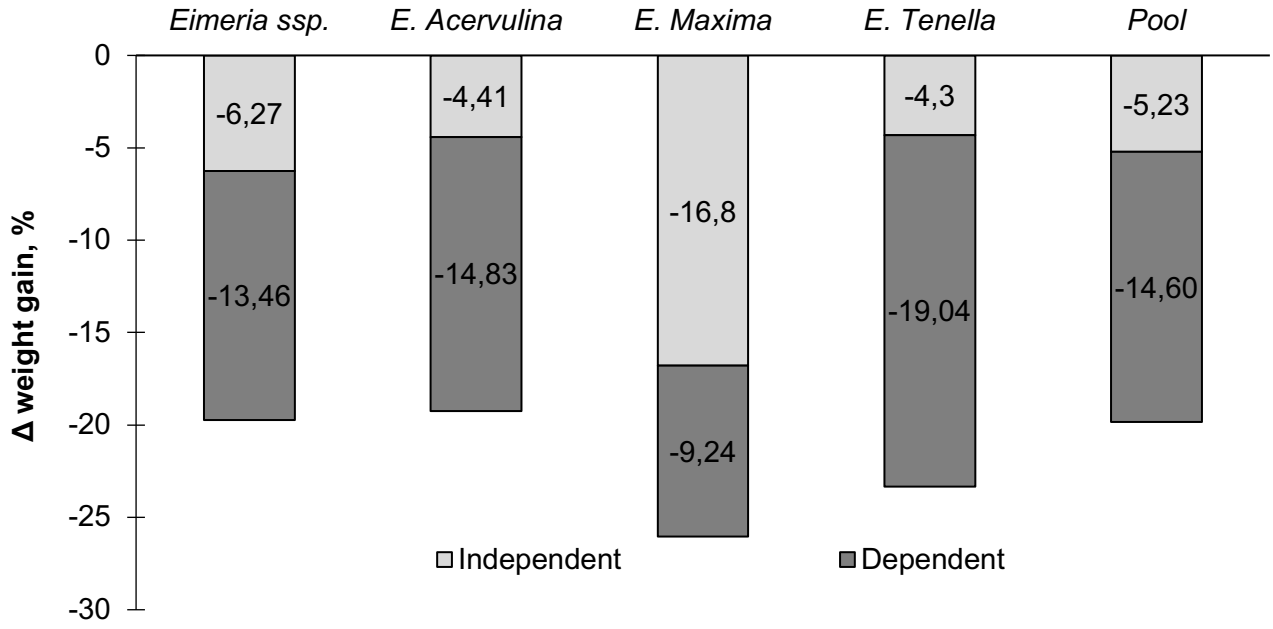


Figure 5. Partitioning of the reduction in average weight gain of broilers chicks challenge by *Eimeria* spp., between the fraction due to the change in maintenance (not associated with feed intake) or change in feed intake (associated with feed intake)

Figure 6. Intestinal lesion scores in broilers challenged with *Eimeria* spp

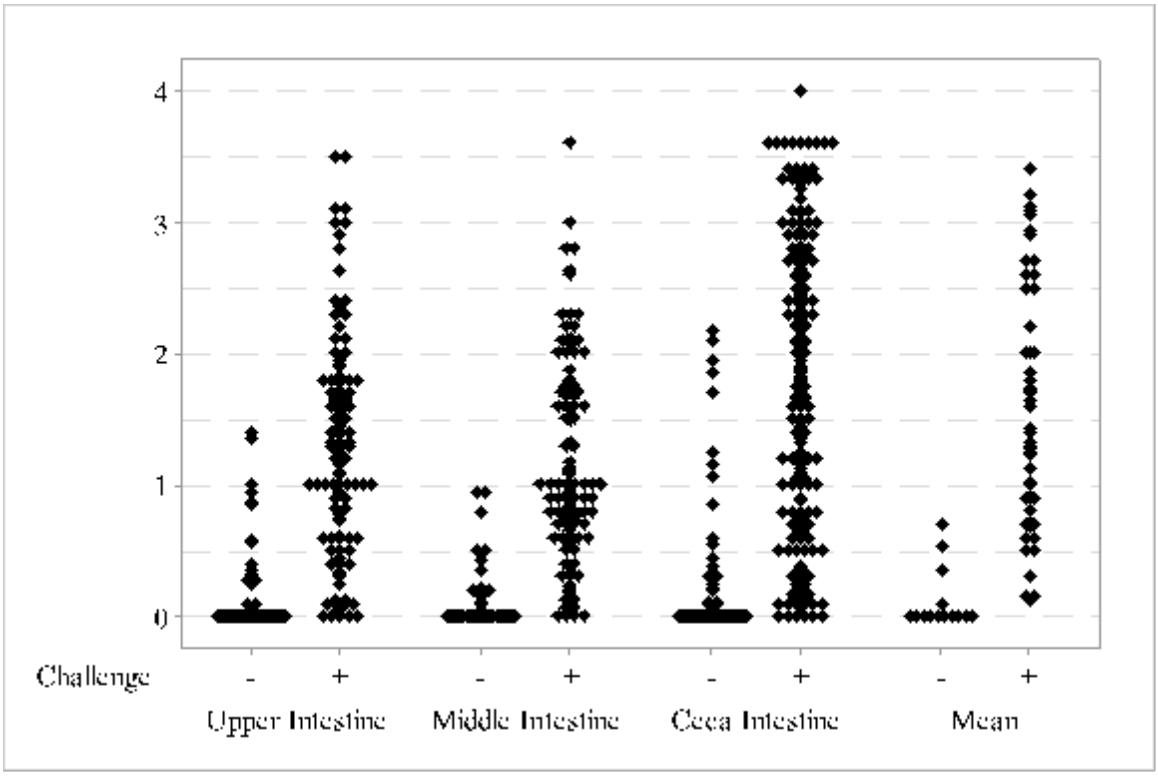


Table 4. Intestinal morphometry in broilers challenged with *Eimeria spp.*

Variables	Vilus, um	Crypt, um	Vilus:Crypt
	Duodenum		
Control	1401.13	133.70	9.93
Challenged	1286.89	188.52	6.93
P-value	0.085	0.024	0.042
SEM	126	22.9	0.64
	Jejunum		
Control	922.15	176.23	5.97
Challenged	812.63	210.69	4.70
P-value	0.042	0.080	0.050
SEM	76.2	24.5	0.44
	Ileum		
Control	437.37	94.01	4.87
Challenged	448.54	112.65	4.50
P-value	0.697	0.060	0.374
SEM	59.2	8.26	0.41

CAPÍTULO III

[1]Artigo escrito nas normas da revista *Poultry Science*

Reassessing the impact of necrotic enteritis on broiler performance and immunological responses using a meta-analytic approach

Abstract

The objective of the study was to evaluate the effects of necrotic enteritis in animal performance of poultry production, and the relation between variation of feed intake and weight gain during this type of infection, through of a systematic review and meta-analysis. The PubMed, Web of Science, and Scopus databases were searched in August of 2020 by using a keyword based on PICo method. After a critical selection, the database was composed by 93 articles, totalizing 55,275 birds. Meta-analysis followed three sequential analyses: graphical for biological coherence, correlation and variance-covariance of clear comparisons where all kinds of treatments was excluded unless there was a control group receiving the same treatment. Great variation in the models of pre-infection was found in the systematic review. The results showed a decrease ($P<0.01$) in weight gain in animals infected, but didn't present significant reduction in feed intake. The variation in feed intake presented a linear influence over the variation of weight gain. It was also observed a reduction in the intestinal integrity of challenged animals, regarding histological responses and lesion scores. This summarization of results is really important to understand more how this important disease impacts the poultry production and also to highlight some important topics that need further studies and understanding.

Key-words: *Clostridium perfringens, gut health, performance, poultry, systematic-review.*

Introduction

The growth of poultry production has been exponential in recent decades due to advances in genetics, nutrition, and health, which provide high-productivity animals with lower production costs. Even so, pathogens still impair the efficiency of using nutrients from feed due to the possible emergence of enteric disorders (Ramos et al., 2011).

One of the most common diseases in modern poultry farming is Necrotic Enteritis, caused by the microorganism *Clostridium perfringes* type A and C (Van Immerseel et al., 2004). In 2008, Keyburn et al., found another key virulence factor by using a *Clostridium perfringens* mutant unable to produce α-toxin, yet still causing necrotic enteritis. Thus, another toxin was identified occurring only in birds suffering from necrotic enteritis: *C. perfringens* necrotic enteritis B-like toxin (NetB), which is a pore-forming toxin. This disease causes considerable economic losses, an estimated approximately $6 billion per year in the poultry production sector due mainly to the reduced performance caused by this disease (Broom, 2017; Jones et al., 2019).

Furthermore, there are many predisposing factors for the proliferation of this bacterium and, consequently, for NE. Some factors include the different levels of nutrients and ingredients in the diet and readily fermentable diets. Moreover, coccidiosis is pointed out as one of the main predisposing factors to the development of NE (Jones et al.,2019). Besides this, the immune status and stress, or even factors that alter the natural microbiota in broilers, are predisposing factors to necrotic enteritis; (Songer, 1996).

Although discovered many years ago, and with several studies addressing the impacts of coccidiosis and necrotic enteritis on broiler performance, this disease is still not fully understood. Hence, developing a meta-analytical approach is necessary for a more precise and concise determination of the results regarding both diseases, bringing not only the values linked to performance but also the data that goes back to the nutritional issues of the animals are under sanitary challenges.

Material and Methods

Systematic review

The digital databases PubMed, ScienceDirect, and Web of Science were searched to identify studies reporting experimental challenges of broilers with Clostridium spp. during any growth phase. The review question was proposed using the 'PICo' framework. Thus, a set of keywords (Table 1) were combined in order to have elements designating population (e.g., broiler), interest (e.g., health challenge), and context (e.g., performance responses).

Table 1 – Keyword developed for narrow search using PICo method.

PICo	
Population	(broiler OR broilers OR chicks OR chicken OR chickens)
Interest	(" Clostridium" OR " Clostridium perfringens" OR " C. perfringens" OR "Eimeria and Clostridium perfringens" OR "E. tenella and C. perfringens")
Context	(performance OR "body weight" OR "average daily gain" OR "weight gain" OR "average daily feed intake" OR "feed intake" OR "feed consumption" OR "feed conversion" OR "feed to gain" OR "feed : gain" OR "feed efficiency" OR "gain to feed" OR "gain : feed" OR ADFI OR ADG OR BW OR FCR OR cytokines OR interleukin OR antibody OR immunoglobulin OR "intestinal health")

All references obtained in each database were exported to EndNote X9, where the title and abstract of each result were revised independently by two researchers in order to select the papers that were fully evaluated. The criteria used for this selection it was that the papers should have been published in scientific journals from 2000, containing broilers from 1 to 50 days old, with experimental challenges by *Clostridium* spp. alone or combined with Eimeria spp., assessment of at least one of the following variables: performance, gut morphology, and/or immunology responses and finally the paper should report a negative control, without any challenge for further comparisons.

The full version of the selected papers was critically evaluated as to their quality and relevance considering the systematic review objectives. Any additional removal was discussed with the team and, if accepted, registered in the PRISMA flow diagram. Negative or positive effects were not used as selection criteria.

To complete the database, all citations in the reference lists of selected publications were revised in order to search for additional studies that could meet the criteria to be included in the databases. If found, these papers were evaluated following the same criteria used in the previous step and their inclusion were also registered in the PRISMA flow diagram.

For the variable lesion score of necrotic enteritis is used visual methods of evaluation. Where the animals are euthanized and after that different portion of the small intestine is taken for observation, then according to the severity of the lesions they are classified in 0 when there is no sign of infection and 4 - 6 with the worse lesions in the intestine (duodenum, jejunum and ileum). For the statistical analysis, since there was more than one type of score used among the works, it was defined 4 as the highest score and 0 with absence of infection.

Database construction

The complete set of selected papers were printed and registered in a new EndNote database. The information related to the proposed theoretical model and other additional variables were copied from both ‘Material and Methods’ and ‘Results’ sections of the original publications and transferred to an electronic spreadsheet. Each row of the spreadsheet represented a treatment and each column represented a variable. Information relative to the objective of the study (e.g., animal performance [feed intake, weight gain, feed conversion ratio], gut morphology variables [scores of lesions], immune responses [interleukins, plasma proteins, immunoglobulins]) and other variables (e.g., bibliographic information, genetic strain, sex, dietary nutritional composition, and other relevant experimental characteristics) were included in the database in order to be considered in the meta-design and/or to provide a descriptive overview of the studies available in the research area.

Many papers described the bird responses previous do the health challenge. Although these data are very important for other objectives, only data collected after the challenge were considered and included in the database to avoid misinterpretation or bias in the analysis.

Codes were used with qualitative grouping criteria in the analytical models. In this item, the main codes were applied for challenge characterization (e.g., control or challenged groups). Other codes were used to consider the variability among all compiled experiments (e.g., the effect of study or trial).

Other codes were also used to indicate measures of the same treatment repeated in time. When this information was available in the papers, repeated measures were included in the database and assigned if a code in order to have an identification of means taken in the same group of animals. The repeated assessments taken within treatment over time were not considered in most of the analysis presented in the report (i.e., variance analysis) in order to avoid a large variation in terms of 'treatments per study' included in the analysis (e.g., studies with repeated measures would contribute with much more data/lines in the analysis, in contrast to the papers that reported only means for the overall period). If a given study presented performance responses by period, but did not report the means from the complete experimental period, these overall responses were calculated to allow the study to be part of the analysis.

Calculations

Performance responses were calculated whenever possible (i.e., feed conversion ratio was not presented, but weight gain and feed intake were available) to complete the database. Performance results were evaluated as raw data (as presented in the original papers) or as relativized information. In this case, the responses of challenged treatments were relativized to the respective control treatment, in order to be expressed as a percentage of variation between the treatments. The relativized responses were referred to as 'variation' (Δ%) and it were interpreted as the 'challenge effect on each performance response'. When calculating the Δ%, challenged treatments were always compared to the most similar control treatment (e.g., in a trial with a factorial design with challenge +/- and supplementation +/-, the

group + to challenge and + to supplementation was compared to the group – to challenge and + to supplementation) in an effort to reduce factors other than the challenge to affect the difference. This procedure was adopted because it considerably reduces the effect of heterogeneity (among experiments) in the database. However, it is important to highlight that despite the uppercase delta symbol (Δ) be frequently used to define "change" or "the change in" in math, here it is used always to address a relative variation, expressed in percentage.

Meta-analysis

Statistical analyses were performed using Minitab (Minitab for Windows, v. 19). All analyses were independently performed for each database. The relationships between predetermined variables (e.g., experimental characteristics: animal age; and animal performance: feed intake, body weight gain) were accessed using scatter charts. This procedure was performed to evaluate database quality and observe the biological coherence of data. Unusual information or patterns were revised in the database. Outliers were not removed, as they may represent pathological responses and higher variability may be expected in the performance of challenged animals.

Pearson correlation were accessed between variables, and significant results ($P < 0.05$) were used to identify related factors. Then, the variance analyses were performed using the 'General Linear Model' procedure to compare the treatments and to obtain the prediction equations. The statistical model for variance analyses included the fixed effect of treatment and the random effect of experiment. The correlations between the residuals of previously described models and some factors were tested (i.e., broiler age).

Control and challenged groups were compared only if the difference between them was limited to the *Clostridium* inoculation or *Clostridium* and *Eimeria* spp. inoculation. Treatments that received any kind of extra treatment (i.e., drugs or vaccines) were excluded from the main analysis if there were not a correspondent control group (i.e., a control group containing the same feed additive tested in the challenged treatment).

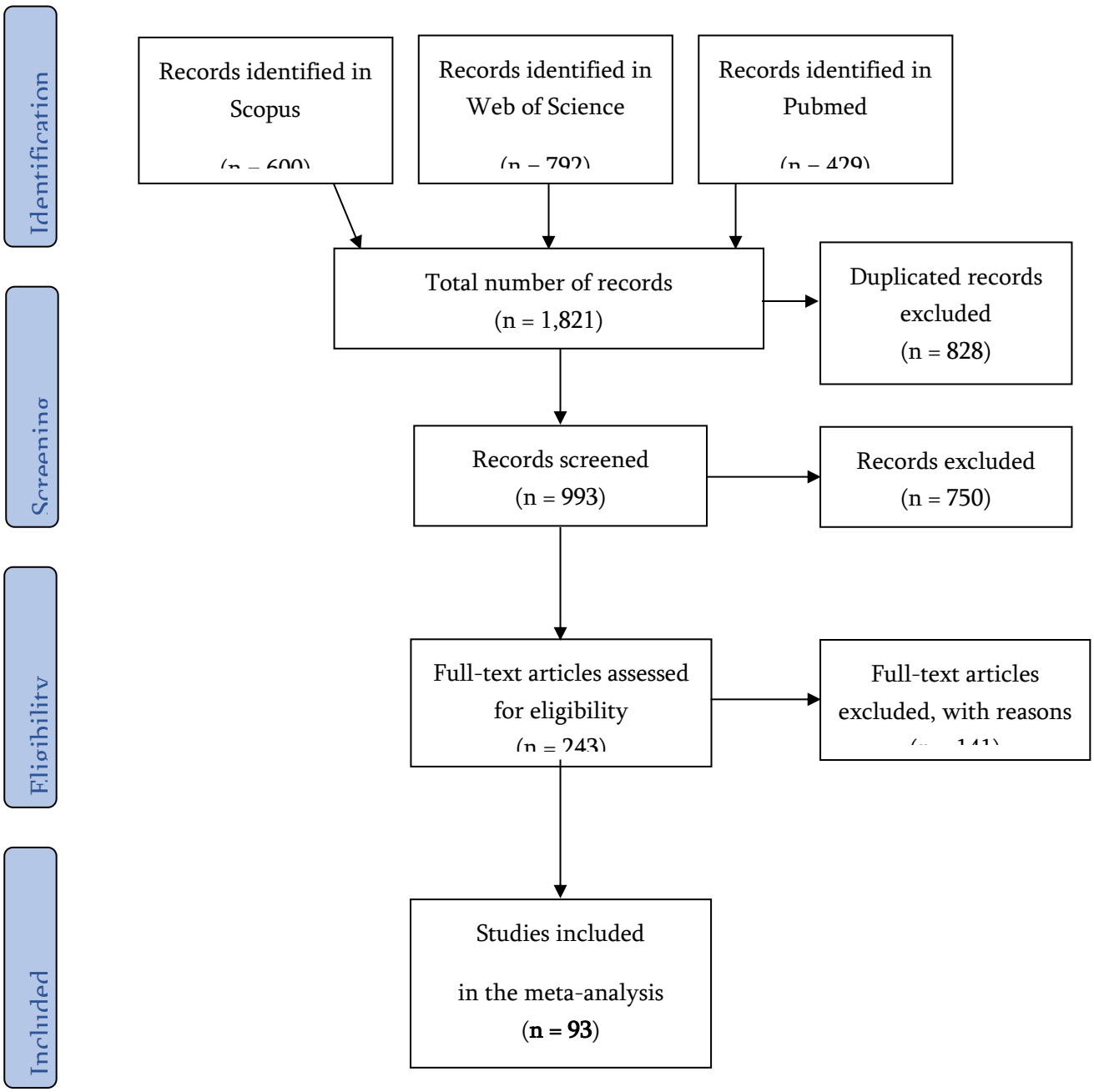
Equations were used to study the relationship between the variations (Δ%) in feed intake and weight gain. In this analysis, the intercepts of the equations were empirically associated with changes in maintenance requirements, while the slopes were interpreted as an indication of changes in feed efficiency. The partition between these two effects was calculated considering the corrected Δ% in weight gain (obtained using the equations) and the average Δ% in feed intake observed in the database.

Results and Discussion

The PRISMA diagram describing the studies found during each step of literature search is presented in **Figure 1**. A total of the 1,821 papers was found in the digital databases PubMed, ScienceDirect, and Web of Science. From these, 828 papers were removed due to duplicity in the database. After, 750 papers that were not related to the database objectives were also removed during the evaluation of titles and abstracts. During the full-text evaluation, other 190 papers were removed, from which: 5 were abstracts, 2 were literature reviews, 81 studies did not have a control (unchallenged) group, 7 studies were developing a challenge model and 55 papers did not include the responses focused in the project. After applying the selection criteria, 93 articles were included in the database, from which 82 papers evaluated performance responses. A summary of the studies is presented in **Table 2** on the attachment section.

The final database occupied 1,395 lines on the electronic spreadsheet. Each line of the database referred to a treatment in the original publication. However, some treatments occupied more than one line when the measures were taken repeatedly over time.

Figure 1. Flow-chart describing the inclusion and exclusion of studies



The selected studies included 55,275 birds, of these 10.95% were mixed groups (female and male), 73.16% were males, 1.79% were females, and 14.1% were not described. It is important to observe that there are few works using females, leaving the question if there is some influence of the sex on resistance or sensitivity for the disease.

A great diversity of genetic lines was described in the papers. From them, the main cited were Ross, Cobb and Arbor Acres. Most of the studies were conducted in the United States, Australia and China, as presented in the **Figure 2**.

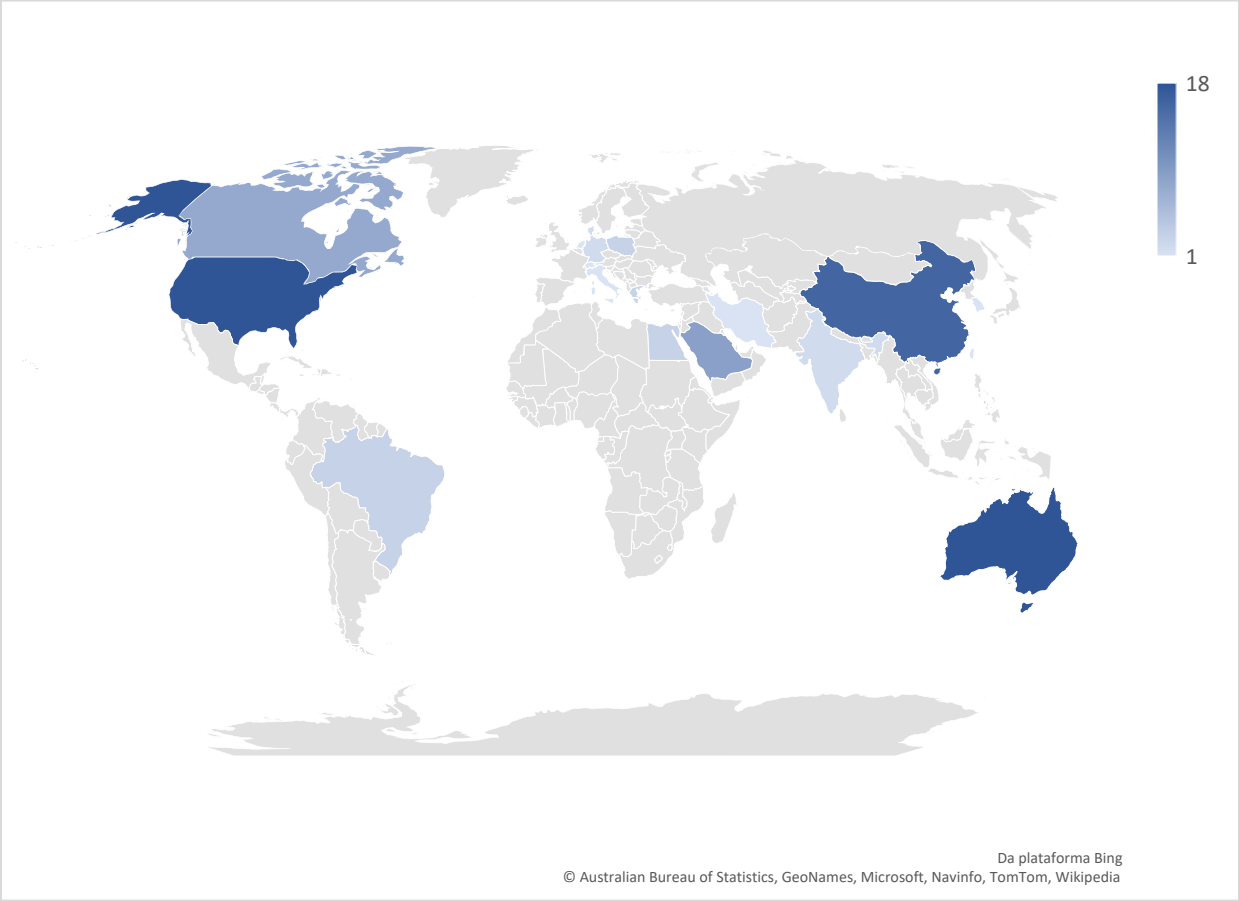


Figure 2. Origen of the studies that were used to build the database

Database limitations

Some effects were not further evaluated in this study due to limited information availability. In this particular, it is important to highlight the effect of pre-infection. The pre-infection varies among the

studies, some of the articles did not present any kind of pre-infection and others change the species used for pre-infecting the chickens, so this limited for a better evaluation of the results.

The results found during the systematic review described several pre-infection models for the development of necrotic enteritis, but the most used (38,7% of the studies) and the one that presented the best results in the development of the disease was the previous infection by coccidiosis, varying mainly between the species *Eimeria maxima*, *E. tenella,* and *E. brunetti*, with varying doses of oocysts (Shojadoost, 2012; Coller, 2008). Another pre-infection factor used alone or concomitantly with *Eimeria* infection was the provision of diets containing fish meal, which according to Rodgers et al. (2015) and Wu et al. (2014) generates a predisposition to the development of *Clostridium perfringens* bacteria in the intestine. The use of high doses of anticoccidial vaccines or Infectious Bursal Disease vaccine (IBD) has also been reported, where 23,7% of studies used as pre-infection model (Timbermont, 2009). An important point to address was the use of high doses of *Clostridium perfringens* as a means of pre-infection, but as the study by Tsouris (2016) reported, an increase in the bacteria alone is not always enough to trigger necrotic enteritis.

There are many predisposing factors which have been experimentally proved to increase the incidence and/or severity of necrotic enteritis. They have been categorized into infectious, nutritional, and management factors (Tsiouris, 2016). The pre-infection period ranged from days 1 to 28 days, with a higher concentration occurring at days 14 and 18 of age, representing 39,2 and 21,75% of works respectively. After the period of this pre-infection, inoculations of the *Clostridium perfringens* bacteria were carried out, which occurred on average 2 to 4 days after the initial infection, lasting a period of 2-3 days of inoculation of the bacteria. The *Clostridium* dosages used in the infection models varied a lot, with values ranging from $3x10^{12}$ to $1x10^{5}$ CFU. it is worth noting that, under natural conditions, the animal organism already has the presence of this bacterium, but in concentrations from 10^{2} to 10^{4} CFU/ g of digesta, and located mainly in the region of the large intestine (Kondo, 1988). The work developed by He (2022), showed that only pre-infection, using *Eimeria*, or immunosuppression by vaccines or the use of fish meal without infection with subsequent doses of *Clostridium perfringens* was enough to develop cases of necrotic enteritis, thus bringing a new possible experimental model for this disease.

Among all the studies, there were still 31 articles that did not described the pre-infection model used or did not perform it. However, this again brings up an important point of discussion, which is the fact that the bacteria alone will not necessarily generate necrotic enteritis, raising the question of a correct development of the disease model or the predisposing factors.

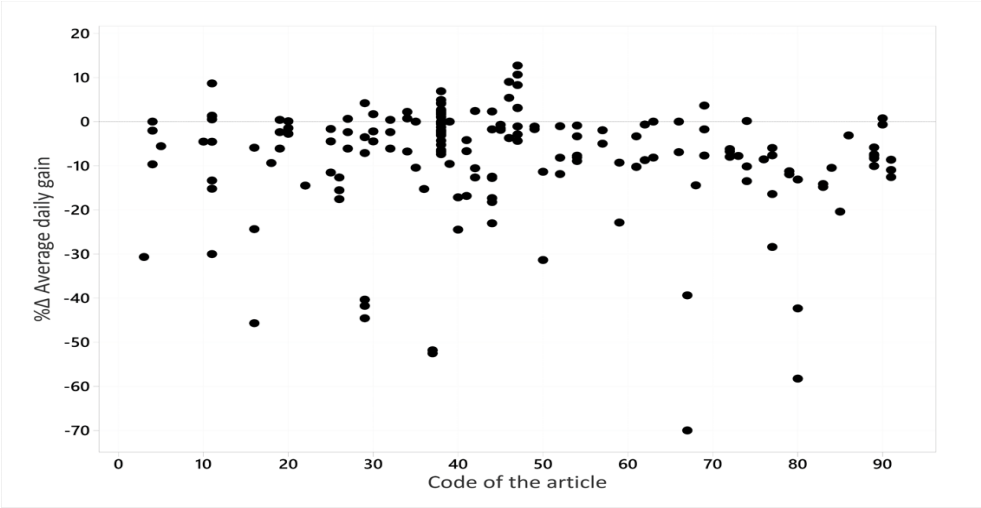
The type of bacteria reported in the literature until 2006 that caused necrotic enteritis is *C. perfringens* type A and C, but in studies developed by Keyburn (2006; 2008), it brought results which showed that bacteria A and C, producers of alpha type toxins and beta, respectively, are not essential for the triggering of necrotic enteritis, but the type G bacteria that produce the NetB toxin, now considered an essential toxin for the development of necrotic enteritis. A number of 51 articles presented the alpha toxin and only 14 used the NetB toxin, however, as stated by Rood, et al. (2016) horizontal gene transfer plays a role in the spread to NetB-negative strains, this means even though the *Clostridum perfringens* presented does not produce NetB toxin, this factor can be transfer through gene transfer of conjugative plasmids that produce NetB can to these strains of *Clostridium perfringens.*

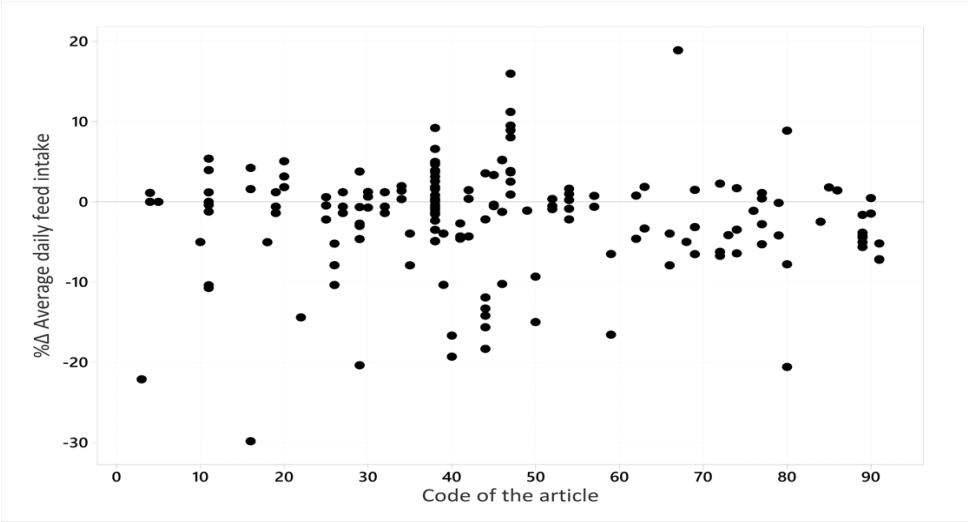
Another important limitation was found for the evaluation of 'challenge duration' and 'age at challenge'. Once both factors are correlated, it was not possible to further evaluate their interaction in the statistical models.

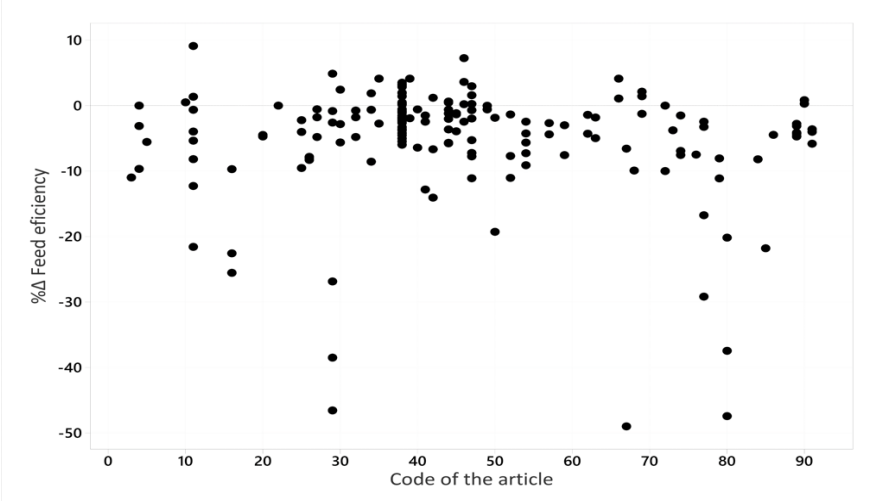
Performance effects

The effect of *Clostridium perfringens* infection on the performance responses was evaluated in terms of variation (Δ%) of challenged groups compared to their respective control group. The effect of *Clostridium perfringens* challenge on broiler performance varied widely across studies, probably due to the heterogeneity of experimental conditions applied in the trials that composed the database. When accessing the variation (Δ%) between challenged groups and their respective control group, it was possible to observe numerical decrease in feed intake in 57.95% of the original comparisons, while 76.64% of the contrasts showed worsening in weight gain and 81.98% in feed efficiency.

Figure 3. Relative effect of Necrotic Enteritis infection (Δ%) on performance of challenged broilers compared to their respective control group – data presented according to the year of publication







Performance of broilers challenged or not by *Clostridium* spp. are presented in **Table 3**. Repeated assessments taken within treatment over time were not considered in this analysis. So, just means from the complete experimental period were used in order to avoid a large variation in terms of 'treatments per study' included in the analysis (e.g., studies with repeated measures would contribute with much more data/lines in the analysis, in contrast to the papers that reported only means for the overall period).

Table 3. Performance of broilers challenged by *Clostridium perfringens*

	ADFI, g/d	***ADG, g/d***	***FE, kg/kg***
Control	82.84	49.55	0.646
Challenged	81.01	45.40	0.612
Δ%[1], %	-2.20	-8.37	-5.26
P-value [2]	0.407	<0.001	<0.001
RSE [3]	13.42	5.80	0.04
R^{2} [(4)]	0.84	0.93	0.87
N means [5]	74	79	72
N studies [6]	54	57	50

[1] Variation between challenged and unchallenged performance.
[2] Probabilities of challenge effect. Models also included the effect of study (P<0.05).
[3] Residual standard errors.
[4] Adjusted coefficients of determination.
[5] Number of means considered in each analysis.
[6] Number of studies that originated the means used in the analysis.
[7] ADFI – Average Daily Feed Intake. ADG – Average Daily Gain. FE – Feed Efficiency

Broilers challenged by *Clostridium perfringens* reduced (P<0.001) the weight gain by 8.37% and the feed efficiency by -5.26% compared to control group. However, no effect of challenge(P>0.05) was found for feed intake.

The cases of subclinical necrotic enteritis are the biggest cause of economic loss in the poultry production, this is due to the fact that the subclinical signs are late detected or often not even detected, so the animals do not receive any form of treatment (Timbermont et al., 2011; Kaldhusdal & Hofshagen, 1992; Dahiya et al., 2006).

In more severe cases of necrotic enteritis, the clinical signs that appear most frequently are dark diarrhea, which causes a wetter bedding with extreme odors and an increase in mortality as soon as clinical signs become visible (Ducatelle and Van Immerseel, 2010). In extreme cases, squad mortality rates can exceed 50% (Van der Sluis, 2013). The average mortality for infected animals found in the

database was 10.3%, but it varied greatly between experiments, with some papers presenting values higher than 60% of mortality and some with no mortalities, due to the presence of different treatments and infection intensities.

Unlike when clinical signs are visible, in subclinical cases NE does not show visible signs or peak mortality flock. Generally, the most subclinical cases are related to a reduction in weight gain and an increase in feed conversion, as demonstrated by the results found in this work.

Relation between the effects of challenge on weight gain and feed intake

The correlation between variation (Δ%) in weight gain and in feed intake caused by *Clostridium* challenge was 0.526 ($P<0.05$) for the overall database. The variation in weight gain (Δ%G) showed a linear relationship with variation in feed intake (Δ%FI) during challenges (**Figure 4**). This equation was fitted considering the effect of study and this fact, together with the data overlapping, may explain a visual impression of distance from equations to the data points. The intercepts of the equations were different from zero and negative in the analyses.

The result of the current study presents that the even though growth suppression in challenged animals is resulted largely from the capacity of health challenge to inhibit feed intake, this is not the main reason for this suppression for *Clostridium perfringens* challenge. This is important since the effects are difficult to quantify in conventional experimental designs. The approach used in this study evaluated the relationship between Δ%FI and Δ%G. When Δ%FI was set to zero in the equations (i.e., simulating a scenario without changes in feed intake), the Δ%G was estimated to be 4.74% for *Clostridium perfringens* challenge.

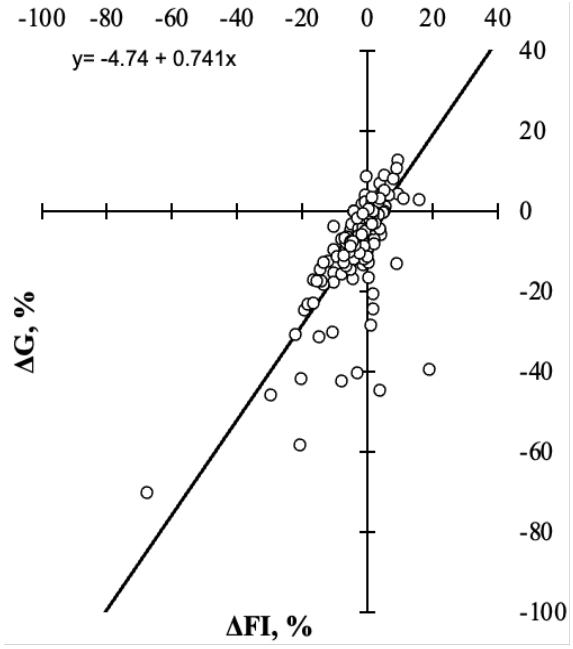


Figure 4. Relationship between variation in weight gain (Δ%G) and variation in feed intake (Δ%FI) of broilers challenged with pool of the *Clostridium perfringens*

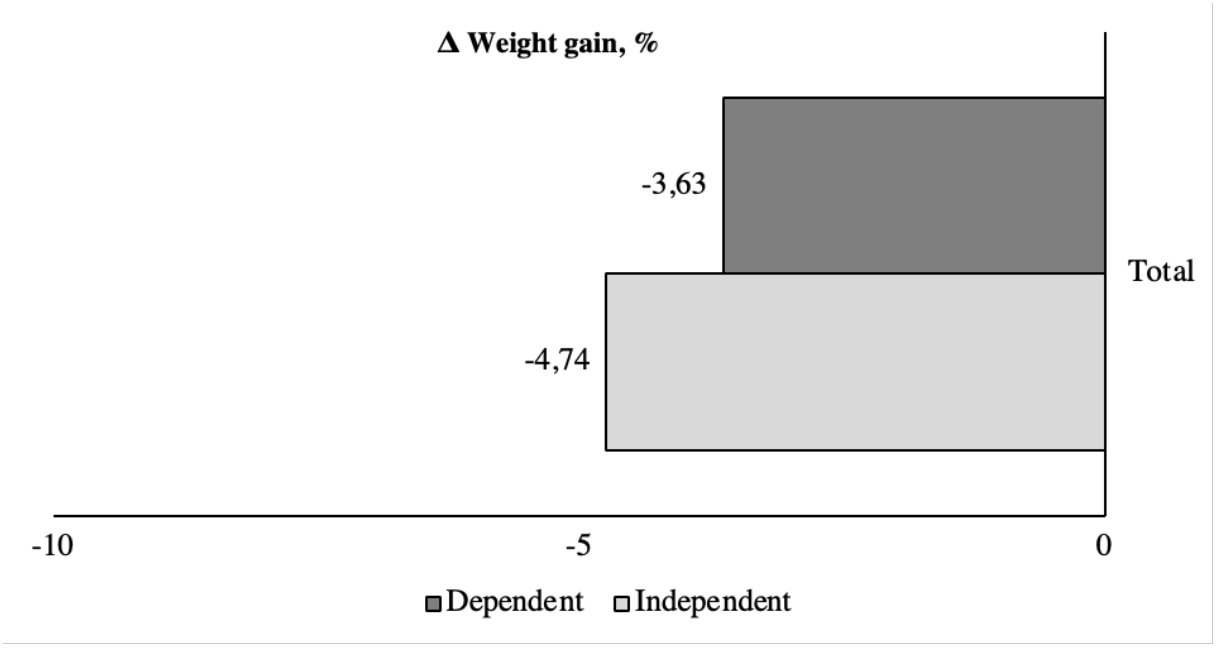


Figure 5. Partitioning of the reduction in average weight gain of broilers chicks challenge by *Clostridium perfringens* between the fraction due to the change in maintenance (not associated with feed intake) or change in feed intake (associated with feed intake)

The partition of the effects on Δ%G corrected (adjusted using the equation previously presented to the average Δ% in feed intake observed in the database) is presented in **Figure 5**. Considering the overall database, 43% of Δ%G was related with changes in feed intake, leaving 57% of the variation in body weight gain related with other factors, as such as, variation in nutrient absorption, mobilization of nutrients for immunological responses, reduction of some organs capacity.

The reduction in weight gain is directly related to the reduction in feed intake, however, as demonstrated in this study, often the reduction in feed intake represents only a part of reduction in performance. Several studies demonstrating the negative impacts on digestion and animal performance when the immune system is stimulated have been developed (Rochell et al., 2016). In addition to this immunological factor, studies such as the one by Iseri and Klasing (2013) demonstrated a significant increase in the damage caused by inflammation in the acute phase and later in the adaptive phase of the animal, resulting in significant impacts on feed consumption, weight gain, and also mostly of the times a consequent variation in the nutritional requirements of the animals (Klasing, 2007; Rochell et al., 2016; Remus et al., 2014).

The factors that generated the reduction in the performance of infected animals are diverse. As previously mentioned, the reduction in feed consumption is one of them, but as presented in this work, a great part of the performance reduction is not explained by this impact on feed consumption. Several factors have already been presented as contributors to performance reduction, such as increased requirements for the immune system; reduced intestinal integrity, which lead to reduced availability of essential amino acids for animals; among others.

A factor that has been heavily studied is the alteration of the intestinal microbiota of animals infected by the most varied diseases, such as necrotic enteritis. This factor is of great importance to study because it is directly related to the other impacts, an altered microbiota can alter the nutrients absorption, decreasing growth and metabolism, unprotecting against harmful bacteria and activate the immune system (Xu, S. et al., 2018).

Studies such as Stanley, et al. (2014); Latorre, et al. (2018); Antonissen, et al. (2016), demonstrate that NE causes a major change in the microbiota of infected animals, in different portions of the intestine and in different bacterial families. This leads to consequences for the animal's health and its performance and feed efficiency.

Intestinal Integrity

As can be seen in Table 5, the data referring to the duodenum and jejunum showed no significant differences in variables, villus, or crypt height. It was only possible to observe a tendency of reduction ($P<0.10$) in the variable duodenal villus crypt ratio of infected animals in relation to the control.

For the intestinal portion of the ileum, there was a significant difference ($P<0.05$) in the variable ileal crypt, where the contaminated animals presented a value of approximately 19 μm higher than the animals of the control group. This is mainly due to the disruptions caused in the crypts, consequently generating larger areas of it at the time of visual histological analysis.

Table 4. Intestinal morphometry in broilers challenged with *Clostridium perfringens*

	Vilus height, μm	**Crypt depth, μm**	**Vilus:Crypt**
	Duodenum		
Control	1298.27	205.19	6.40
Challenged	1348.45	217.19	7.57
P-value	0.572	0.113	0.07
SEM	207.1	29.20	1.10
	Jejunum		
Control	1001.78	174.92	14.14
Challenged	949.64	188.15	13.82
P-value	0.134	0.085	0.952
SEM	96.8	18.3	8.21
	Ileum		
Control	641.83	150.52	4.97
Challenged	662.45	169.15	4.93
P-value	0.345	0.004	0.902
SEM	60.9	20.7	0.8

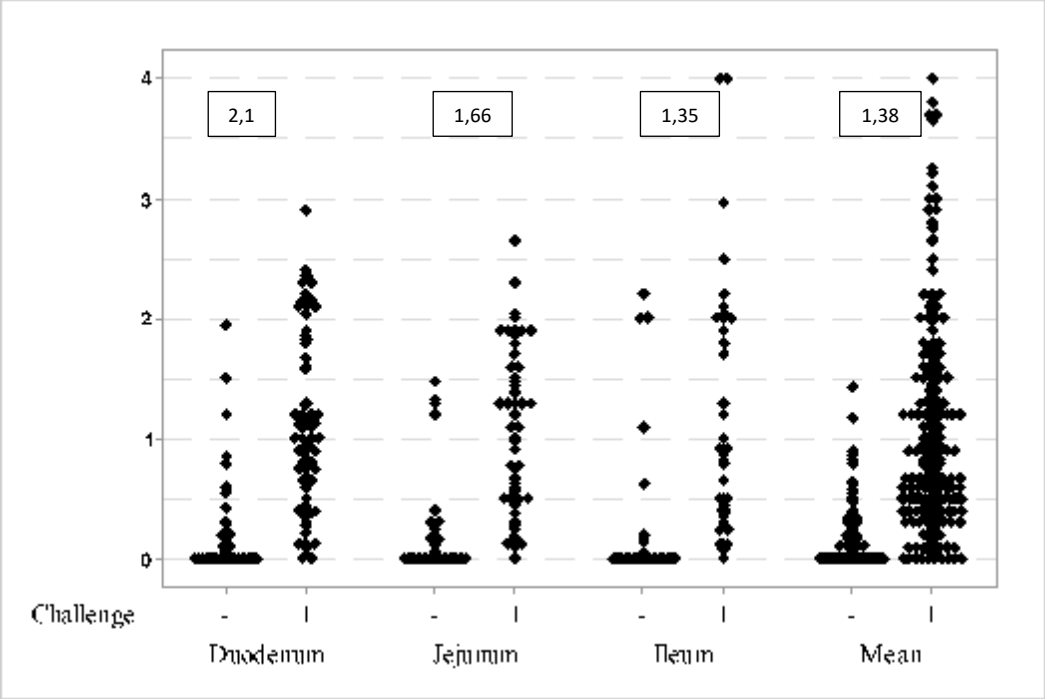
During the development of NE through microscopic examinations it is possible to observe large inflammatory signs in the intestine. The lamina propria presents hyperemia and infiltration with several inflammatory cells, mainly in the enterocytes (Olkowski et al., 2006).

As presented in this work and collaborating with some results found in literature, as such as, Broussard (1986), Olkowski (2006), and Golder (2011), is possible to see a reduction in the height of villus due to its necrosis, increase in villus width and increase crypt depth of challenged animals. As explain the work done by Pelícia, V. C. (2011), the increase of depth crypt is due to the higher cells proliferation in attempt to repair the damages caused by the infection, impacting on the intestinal integrity and consequently in the nutrient absorption and microbiota establishment.

Only 22 studies reported which type of score they were using for the assessment, 6 of which were using Johnson and Reid (1970), 9 using Prescott (2016) and the other studies were using other scores presented by other authors. For the analysis, a standardization was made between the scores, following the intensities of injuries of each one. Where 0 did not show any type of injury and 4 extreme levels of injury, it is important to note that most of the articles when the animals reached the last injury score, they were at infection levels that led to their mortality. In the studies found in the literature, the presence of 1 degree of lesion score in the intestine is already considered a case of subclinical necrotic enteritis.

In **Figure 6** is presented the analysis of the lesion scores in different parts of the intestine and its medians. For all the lesion responses it was found significance differences (<0.005) between unchallenged and challenged birds.

Figure 6. Intestinal lesion scores in broilers challenged with Necrotic Enteritis



As reported by Timbermont et al. (2011), the concentration spot of gross lesions during an necrotic enteritis infection occurs in the small intestine, mostly in the duodenum and jejunum, corroborating with this results found in our meta-analysis. The highest values found in this research was in the duodenum where we found a mean of 2.1 in the lesion score, where it presented lesions ranging from an intestinal wall that is thin and remains flat when opened to the standard "Turkish towel" appearance, which presents an extensive area of necrosis and ulceration. The intestine may also fill with a brownish liquid and become friable (Cooper et al., 2013; Hofacre et al., 2003).

During coccidiosis infection the animals present a great loss of performance. Despite there is a great relationship between the variation in feed intake with this performance reduction and also the impact in the intestinal epithelium, is really important to advance in studies about the nutritional requirements for animals during infection and the mobilization of nutrients for immunological responses, beyond that, to study the impact of disruptive intestinal microbiota

during enteric problems. Because with this further understanding, is possible to create new ways of treatments, prevention or reduction of this disease.

Conclusion

The reduction of performance and intestinal integrity during a necrotic enteritis infection is known, but through this work we are able to see that the variation in feed intake is not responsible for most of the reduction in weight gain during this infection. It was possible to confirm that the intestinal region most visible affected with necrotic enteritis is the duodenum.

Declaration of interest

The authors declare no conflict of interests.

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Attachment

Table 2. Description of the papers used in the database

Code [1]	Citation	N of Broilers	Pre-challenge (+/-)	Challenge Dose Clostridium	Age of inoculation/ Post hatch
1	Brennan et al. 2001	2000	-	1x10ˆ8	14, 15, 16
2	Hofacre et al. 2003	960	+	1x10ˆ8	18, 19, 20
3	Jackson et al. 2003	960	+	1,5x10ˆ8	11, 12, 13
4	Brennan et al. 2003	2000	-	1x10ˆ8	14, 15, 16
5	Gadbois et al. 2008	1600	-	1x10ˆ8	16
6	McDougald et al., 2008	90	+	2x10ˆ7	19, 20, 21
7	Collier et al. 2008	210	+	1x10ˆ8	18, 19, 20

8	Soon et al. 2008	75	+	1x10ˆ9	26
9	McReynolds et al., 2009	350	+	1x10ˆ5	17, 18, 19
10	Grilli et al., 2009	216	-	1x10ˆ8	11, 12, 13
11	Jia et al. 2009	2640	-	2x10ˆ9	14, 15
12	Jia et al. 2009	1216	-	8,9x10ˆ8	13
13	Miller al. 2009	481	+	1x10ˆ8	19, 20, 21
14	Geier et al. 2010	1200	+	3,5x10ˆ10	15
15	Lensing et al. 2010	600	+	-	14
16	Knap et al. 2010	384	+	1x10ˆ8	19, 20
17	Abd El-Ghany et al. 2007	200	-	1x10ˆ9	14
18	Liu et al. 2010	168	-	2x10ˆ8	17, 18, 19
19	Abudabos et al. 2012	150	+	4x10ˆ8	18, 19, 20
20	Józefiak et al. 2012	480	-	1x10ˆ8	18, 19, 20
21	Cao et al. 2012	240	-	1x10ˆ8	14, 15, 16, 17, 18, 19, 20, 21
22	Yitbarek et al. 2012	300	-	3x10^10	14
23	Liu et al. 2012	336	-	7x10^7	14, 15, 16, 17, 18, 19
24	Engberg et al. 2012	160	+	1x10ˆ6	17, 18, 19, 20
25	Barekatain et al. 2013	576	+	3,5x10ˆ8	14, 15, 16
26	Vidanarachchi et al. 2013	1050	+	3,5x10ˆ8	14, 15, 16
27	Abudabos et al.2013	100	+	4x10ˆ8	18, 19, 20
28	Jayaraman et al. 2013	216	+	1x10ˆ8	19, 20, 21
29	Shojadoost et al. 2013	240	+	2,6x10ˆ8	17, 18, 19
30	Abudabos et al.2013	100	+	4x10ˆ8	18, 19, 20
31	Alnassan et al. 2013	96	+	1x10ˆ9	22
32	Abudabos et al. 2013	100	+	4x10ˆ8	16
33	Lee et al. 2013	45	+	1x10ˆ9	18
34	Abudabos et al. 2013	120	+	4x10ˆ8	18
35	Tsiouris et al. 2014	240	+	4x10ˆ8	17, 18, 19
36	Lee et al. 2014	80	+	1x10ˆ9	18
37	Bangoura et al. 2014	60	+	1x10ˆ9	32
38	Józefiak et al. 2014	480	-	-	-
39	Tsiouris et al. 2015	240	+	4x10ˆ8	17, 18, 19, 20
40	Shawkat et al. 2015	480	+	4x10ˆ8	14
41	Rodgers et al. 2015	1344	+	1x10ˆ8	14, 15
42	Wang et al. 2015	252	-	2x10ˆ8	15, 16, 17, 18, 19
43	Sun et al. 2015	648	-	1,6x10ˆ8	14, 15, 16, 17, 18, 19, 20
44	Shawkat et al. 2015	720	+	3,8x10ˆ8	14
45	Tian et al. 2016	240	+	1x10ˆ8	16, 17, 18
46	Du et al. 2016	448	-	1x10ˆ8	14, 15, 16, 17, 18, 19
47	Józefiak et al. 2016	480	-	1x10ˆ8	18, 19, 20
48	Al-Baadani et al. 2016	240	-	4x10ˆ8	15, 16
49	Alizadeh et al. 2016	2640	-	8,9x10ˆ8	14
50	Paradis et al. 2016	-	-	1x10ˆ7	14
51	Eshak et al. 2016	800	-	4x10ˆ8	14, 15, 16, 17
52	Zhou et al. 2016	240	+	2,2x10ˆ8	18, 19, 20
53	Keerqin et al. 2017	180	+	1x10ˆ8	14
54	Song et al. 2017	180	+	1x10ˆ9	17, 18, 19
55	Richardson et al. 2017	192	+	1x10ˆ8	19, 20, 21

56	Keerqin et al. 2017	576	+	1x10^8	14, 15
57	Keerqin et al. 2017	396	+	1x10^8	14
58	Charal et al. 2017	360	-	2x10^9	10
59	Xue et al. 2017	476	+	1x10^9	14
60	Abudabos et al. 2017	480	-	4x10^8	1
61	Krueger et al. 2017	1250	-	1x10^12	11, 12, 13
62	Al Sagan et al. 2017	240	+	4x10^8	14
63	Xue et al. 2017	720	+	1x10^8	14, 15
64	Koli et al. 2017	400	-	1x10^7	17, 18, 19
65	Lee et al. 2018	300	+	1x10^9	18
66	Tsiouris et al. 2018	240	+	4x10^8	17, 18, 19, 20
67	Oh et al. 2018	168	+	1x10^9	18
68	Liu et al. 2018	360	-	1x10^8	8, 9, 10, 11
69	Kheravii et al. 2018	240	+	10x10^7	14, 15
70	Wu et al. 2018	240	+	1x10^9	18, 19, 20
71	Kang et al. 2018	350	-	4x10^8	14, 15, 16
72	Cheng et al. 2018	60	-	1x10^8	18, 19, 20
73	Li et al. 2018	308	-	2x10^8	14, 15, 16, 17, 18, 19, 20
74	Oliveira et al. 2019	1530	+	2,5x10^6	18, 19, 20
75	Bortoluzzi et al. 2019	384	+	1x10^8	19, 20, 21
76	Zhao et al. 2019	180	+	2,8x10^8	18, 19, 20, 21, 22
77	Sokale et al. 2019	320	+	1x10^8	18, 19, 20
78	Musa et al. 2019	480	+	1x10^8	14, 15, 16, 17, 18, 19, 20, 21
79	Bortoluzzi et al. 2019	1200	+	1x10^8	18, 19, 20
80	Liu et al. 2019	336	+	1x10^8	19, 20, 21
81	Ahiwe et al. 2019	576	+	4,5x10^7	14, 15
82	Naseri et al. 2019	468	+	1x10^8	14, 15
83	Oh et al. 2019	140	+	1x10^9	20
84	Cao et al. 2019	288	+	1x10^8	14, 15, 16, 17, 18, 19, 20, 21
85	Hussein et al. 2020	378	-	4x10^8	15
86	Shini et al. 2020	240	+	1,76x10^8	14, 15
87	Pham et al.2020	288	+	2,2x10^8	18, 19, 20
88	Zanu et al. 2020	768	+	1x10^8	14, 15
89	Stefanello et al. 2020	1080	+	3x10^9	11, 12, 13
90	Guaragni et al. 2020	400	-	4x10^9	21
91	Mohamed et al. 2020	270	+	4x10^8	14, 15, 16
92	Hilliar et al. 2020	972	+	1x10^8	14, 15
93	Zanu et al. 2020	768	+	1x10^8	14, 15

[1] Used in the database to identify each study and across this document to refer to each publication. The order presented here is the same applied in the database, even if a crescent numerical order was not always used.

CAPÍTULO IV

CONSIDERAÇÕES FINAIS

Revisões sistemáticas e meta-análises são ferramentas úteis na pesquisa animal. Um dos grandes auxiliadores desta metodologia é o fato de não haver a utilização de recursos, como animais e instalações para realizar o estudo, então torna-se economicamente viável e aceito do ponto de vista do bem-estar animal, principalmente nos casos aonde está sendo investigados fatores sanitários e infecciosos que apresentam uma grande barreira social para o desenvolvimento das experimentações cientificas.

Após estas duas extensas revisões sistemáticas, foi possível observar a grande variabilidade de resultados apresentados na literatura. A queda no desempenho dos animais é evidente perante as infecções, principalmente em questões como ganho de peso e eficiência alimentar. Porém outras variáveis importantes devem ser levadas em consideração, como a integridade intestinal, esta é responsável pela absorção dos nutrientes, regulação e fixação da microbiota e regulações imunológicas.

Por fim, entender como uma infecção afeta os animais, não apenas conhecer o impacto, mas sim conseguir quantificar fatores específicos que causam a perda de desempenho dos animais, são de difícil quantificação em experimentos convencionais, por isso a grande importância do desenvolvimento de estudos meta-analíticos.

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