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SUPLEMENTAÇÃO DE TANINO DE *Acacia mearnsii* EM RAÇÕES PARA FRANGOS DE CORTE

Santa Maria, RS
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Dissertação de mestrado apresentada ao Programa de Pós-Graduação em Zootecnia da Universidade Federal de Santa Maria (UFSM), como requisito parcial para a obtenção do título de **Mestre em Zootecnia.**

Orientadora: Profª Drª Catarina Stefanello

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Santa Maria, RS
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RESUMO

SUPLEMENTAÇÃO DE TANINO DE *Acacia mearnsii* EM RAÇÕES PARA FRANGOS DE CORTE

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Devido à pressão pela retirada do uso de antibióticos da produção avícola, cresce a busca por aditivos naturais que exerçam função similar. Nesta realidade, o tanino de *Acacia mearnsii* pode ser uma alternativa aos antibióticos, devido seu efeito antimicrobiano. Entretanto, os taninos não possuem histórico de utilização na nutrição de aves devido aos seus efeitos antinutricionais, que levam ao baixo desempenho. Porém, os recentes avanços tecnológicos demonstram que estes efeitos podem ser evitados, uma vez que se encontram intimamente relacionados a fonte e dosagem de tanino utilizada, a categoria animal e ao *status* sanitário. Diante disso, o presente estudo foi conduzido no Laboratório de Avicultura da UFSM, com o objetivo de avaliar os efeitos da suplementação de níveis crescentes de taninos de *Acacia mearnsii*, na dieta de frangos de corte submetidos a desafio com *Clostridium perfringens*. Um total de 1620 pintos de corte de um dia de idade foram divididos em 6 tratamentos com 10 repetições de 27 aves cada. Os tratamentos consistiram em: controle não desafiado; controle desafiado; controle desafiado recebendo ração suplementada com 300, 500, 700 ou 900 mg/kg de tanino de *Acacia mearnsii.* O desafio foi realizado com vacina comercial para coccidiose no dia 1 e inóculo de *C. perfringens* via oral nos dias 11, 12 e 13. O desempenho foi avaliado semanalmente até 43 dias de idade. Aos 21 dias foi realizado coleta de sangue para análise bioquímica e de permeabilidade intestinal, através do marcador isotiocianato de fluoresceína (FITC-d), além de coletas de conteúdo ileal para determinar a digestibilidade de nutrientes e amostras do jejuno para morfometria. Aos 28, 35 e 43 dias, foram realizadas análises de qualidade de cama e pododermatite. No dia 44 as aves foram abatidas para avaliação de carcaça e coleta da coxa para análise de oxidação lipídica, durante 6 meses de armazenamento a - 20ºC. Os dados foram submetidos à análise de variância usando o programa SAS, com comparação de médias pelo teste de Tukey ($P<0,05$) e modelos de regressão linear e polinomial quadrática. Foram observados menor desempenho, parâmetros sanguíneos e maior permeabilidade do intestino, nos frangos desafiados em relação aos não desafiados ($P<0,05$). A suplementação com tanino causou aumento quadrático no ganho de peso (GP), melhora na conversão alimentar (CA) e diminuição linear da oxidação lipídica da carne ($P<0,05$). A suplementação ótima foi de 310, 444, 466 e 528 mg/kg de tanino para GP, CA, permeabilidade intestinal e altura de vilosidades, respectivamente ($P<0,05$). A digestibilidade da proteína melhorou com 374 mg/kg de tanino ($P<0,05$). Os resultados indicaram que baixos níveis de suplementação de tanino na dieta de frangos de corte promovem melhorias no desempenho, digestibilidade e saúde intestinal em desafio, enquanto níveis crescentes de tanino retardam a oxidação lipídica da carne.

Palavras-chave: Avicultura. Aditivos naturais. Desempenho. Saúde intestinal. Polifenóis.

ABSTRACT

DIETARY SUPPLEMENTATION WITH *Acacia mearnsii* TANNIN FOR BROILER CHICKENS

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Due to the pressure to antibiotics removal from poultry production, the search for natural additives that perform a similar function is growing. In this reality, tannin from *Acacia mearnsii* is an alternative to antibiotics due to its antimicrobial effect. However, tannins do not have a history of use in poultry nutrition due to its anti-nutritional effects that induce to poor performance. However, recent technological advances demonstrate that these effects can be avoided, since they are closely related to the source and dosage of tannin used, animal category and health status. In view of this, the present study was conducted at the UFSM Poultry Laboratory with the objective of evaluating the effects of supplementation of increasing levels of tannins from *Acacia mearnsii* for broiler chickens submitted to an experimental intestinal challenge with *Clostridium perfringens*. A total of 1620 day-old broiler chicks were divided into 6 treatments with 10 replicates of 27 birds each. Treatments consisted of non-challenged control, challenged control, and the challenged control fed diets supplemented with 300, 500, 700, or 900 mg/kg of *Acacia mearnsii* tannin. The challenge was done with a cocci vaccine on day 1 and oral gavage with *C. perfringens* on days 11, 12, and 13. Performance was evaluated weekly for 43 days of age. At 21 days, blood was collected for biochemical analysis and intestinal permeability was analyzed with fluorescein isothiocyanate-dextran (FITC-d) as marker, in addition to digesta collection to determine nutrient digestibility, and jejunum sample for morphometrics. At 28, 35 and 43 days, litter quality and footpad dermatitis were analyzed. On day 44, broilers were slaughtered for carcass evaluation and thigh samples were collected for lipid oxidation during 6 months of storage at - 20ºC. Data were subjected to analysis of variance using SAS, means were compared using the Tukey's test ($P<0.05$), and linear and polynomial quadratic regression models. Decreased performance, blood parameters, and higher intestinal permeability were observed in challenged broilers compared to non-challenged ($P<0.05$). Tannin supplementation caused a quadratic increase in weight gain (WG), improvement in feed conversion (FCR) and a linear decrease in lipid oxidation of meat ($P<0.05$). Optimal supplementation was 310, 444, 466, 528 mg/kg of tannin for BWG, FCR, intestinal permeability, and villus height, respectively ($P<0.05$). Protein digestibility improved at 374 mg/kg tannin ($P<0.05$). The results indicated that low levels of tannin supplementation in the broiler diet promote improvements in performance, digestibility and intestinal health in challenge, while increasing levels of tannin retard meat lipid oxidation.

Keywords: Aviculture. Natural additives. Performance. Gut health. Polyphenols.

SUMÁRIO

1. INTRODUÇÃO

A elevação no consumo mundial da carne de frango nas últimas décadas tem atuado como propulsor para várias propostas inovadoras no mercado avícola, que sustentem o aumento produtivo à custo compatível e responsabilidade ambiental. Nessa passagem de tempo, os mercados importadores elevaram suas preocupações com a qualidade e segurança dos produtos, principalmente com relação ao uso de antibióticos na produção animal, o que afeta significativamente o modo de produção avícola brasileiro por ser o maior exportador de carne de frango do mundo, exportando para mais de 150 países (ABPA, 2022).

Os antibióticos, quando utilizados em doses subterapêuticas, atuam como promotores de crescimento, mantendo os animais saudáveis através do seu efeito antimicrobiano. Devido às preocupações com o aumento da resistência bacteriana, a União Europeia proibiu o uso de antibióticos como promotores de crescimento em 2006. Desde então, sua utilização tem diminuído em vários países e muitos aditivos naturais que tenham efeito antimicrobiano passaram a ser estudados e utilizados na indústria avícola, como óleos essenciais e extratos herbais. Nesta nova geração de bioativos, os taninos, por demonstrarem efeitos benéficos comprovados em ruminantes, tiveram um grande revés de interesse técnico-científico, sendo promovido de antinutricional para bioativo, nos estudos sobre nutrição de animais monogástricos (CARRASCO *et al.*, 2018; HIDAYAT *et al.*, 2021).

Neste novo cenário, o presente estudo foi conduzido com o objetivo de avaliar os efeitos da suplementação de níveis crescentes de tanino de *Acacia mearnsii* nas rações para frangos de corte submetidos a desafio intestinal, sobre respostas zootécnicas, metabólicas e sanitárias.

2. REVISÃO DE LITERATURA

2.1 *Clostridium perfringens*

Dentre as diversas bactérias que podem acometer as aves, o *Clostridium perfringens* tornou-se um patógeno recorrente na avicultura comercial. É uma bactéria gram-positiva em forma de bastonete com 2 a 6 µm de comprimento, sendo

encontrado de forma isolada ou em pares. Embora seja considerada anaeróbica, não necessita condições rigorosas de anaerobiose para que ocorra o seu desenvolvimento, possuindo resistência a temperaturas de até 47ºC (ITURRINO; ISHI; VITTORI, 2009). Comumente encontrada no meio ambiente, é capaz de formar esporos e alojar-se no intestino de animais e humanos, causando doenças gastrointestinais.

Ao contrário de outras bactérias patogênicas, o *C. perfringens* não invade as células saudáveis, mas sim, produz diversas toxinas que variam de acordo com a cepa, causando as lesões e sintomas (PETIT; GIBERT; POPOFF, 1999). Por esse motivo, quando inoculado em aves para gerar desafio intestinal, normalmente vem acompanhado de desafio prévio com protozoários de *Eimeria* spp. (Stefanello et al., 2020), o que contribui para que aconteça a disbiose. Nos lotes comerciais, a presença concomitante de patógenos é uma realidade constante.

Por estar presente na microbiota intestinal de aves sadias, o surgimento de efeitos patológicos depende do crescimento bacteriano e da produção elevada das toxinas. Uma das doenças causadas pelo *C. perfringens* que acomete frangos de corte é a enterite necrótica, causando redução do apetite, apatia e prostração, podendo levar a morte. Além dos fatores clínicos, podem ocorrer perdas econômicas devido aos fatores subclínicos da doença, que resultam na diminuição da absorção dos nutrientes associados a elevada conversão alimentar e baixo ganho de peso (GOMES *et al.*, 2008).

A capacidade de causar doenças pode ir além da produção de toxinas no trato gastrointestinal. O baixo tempo de duplicação do *C. perfringens* resulta em uma elevada taxa de multiplicação para atingir uma maior carga patogênica, resultando na ocorrência de contaminação dos alimentos (MCCLANE; ROBERTSON; LI, 2013), o que se agrava por se tratar de uma bactéria comumente encontrada no meio ambiente. Logo, é possível verificar que a incidência de contaminação está relacionada com as medidas de sanidade dos estabelecimentos comerciais e biosseguridade das granjas. De acordo com Martel *et al.* (2004), diversas cepas de *C. perfringens* obtidas de frangos de corte possuem algum grau de resistência a determinados antibióticos e coccidiostáticos comumente utilizados na indústria avícola, demonstrando a importância dos estudos voltados a avaliar os efeitos de aditivos naturais e alternativos aos medicamentos usuais.

2.2 TANINOS

Aditivos são comumente adicionados nas rações visando suprir alguma necessidade específica do animal, melhorar o processamento de ração nas fábricas ou atribuir um efeito que pode ou não ser nutricional. Nos últimos anos, dentre as diversas opções de aditivos, destacam-se as pesquisas com aditivos fitogênicos, que são substâncias extraídas de plantas e podem possuir diversos efeitos, como influenciar a digestibilidade, a secreção de enzimas, bem como atuar como antimicrobiano e antioxidante. Consequentemente, seu uso pode melhorar os índices zootécnicos, como ganho de peso e conversão alimentar, e melhorar a saúde intestinal dos animais (PERIC; ŽIKIC; LUKIC, 2009). Dentre os diversos aditivos fitogênicos disponíveis, os taninos são utilizados a longo tempo na nutrição de animais ruminantes, atuando principalmente no metabolismo do nitrogênio reduzindo suas perdas na urina, além de melhorar o suprimento de aminoácidos da dieta (ORLANDI, *et al.*, 2015), porém ainda pouco explorados para monogástricos.

Taninos são compostos polifenólicos de alto peso molecular produzidos naturalmente pelas plantas como mecanismo de defesa gerando sabor adstringente, além de retardar a decomposição teciduais e danos devido seus efeitos antioxidativos e antimicrobianos (MONTEIRO *et al.*, 2005). Comercialmente, os taninos são geralmente obtidos através da casca das plantas devido sua qualidade e concentração elevada, onde podem perfazer até 40% da massa seca das cascas, dependendo da espécie utilizada (PAES *et al.*, 2006).

2.2.1 Características e funções

Os taninos são caracterizados como metabólitos secundários encontrados nos vacúolos das plantas, atuando como mecanismo de defesa na proteção contra possíveis ataques de predadores, patógenos e condições adversas (FRAGA-CORRAL *et al.*, 2020). Metabólitos secundários são compostos que não participam diretamente das vias metabólicas principais responsáveis pelo desenvolvimento das plantas, agindo após alguma lesão, o que resulta em uma ampla variação na sua composição e quantidade (MAGALHÃES; DURÃES, 2003).

Por estarem presentes em vários segmentos das plantas, os tecidos que contém taninos, como folhas, frutos e casca, apresentam uma das principais

características dos taninos: a adstringência. O sabor adstringente das folhas e frutos visa evitar seu consumo por animais através da interação entre os polifenóis e as proteínas salivares, causando redução na lubrificação e evitando que plantas em crescimento sofram danos (HASLAM, 1977). Heldt (1997 apud MONTEIRO *et al.*, 2005) relatou que antílopes da savana africana se alimentam de folhas de acácia, que ao serem predadas pelos animais, aumentam a síntese de taninos, demonstrando que a sua produção pode aumentar de acordo com desafios ambientais que acometam a planta, reforçando o seu importante papel como mecanismo de defesa.

Já na casca e madeira, os taninos se destacam pela ação antimicrobiana, atuando na resistência dos tecidos ao ataque de microrganismos patogênicos como bactérias e fungos, inativando enzimas ou formando uma barreira protetora que impede o ataque microbiano em resposta à infecção onde ocorre dano à planta (HASLAM, 1977). De acordo com o estudo conduzido por Rawchal *et al.* (2001) sobre a produtividade de *Acacia mearnsii* em diferentes tipos de solos, foi observado que a casca das árvores que se desenvolveram em solos com características físicas mais desfavoráveis ao desenvolvimento vegetal apresentaram maior produção de taninos, confirmando que condições de solo adversas induzem a planta a aumentar a concentração de taninos na casca.

2.2.2 Classificação e estruturas

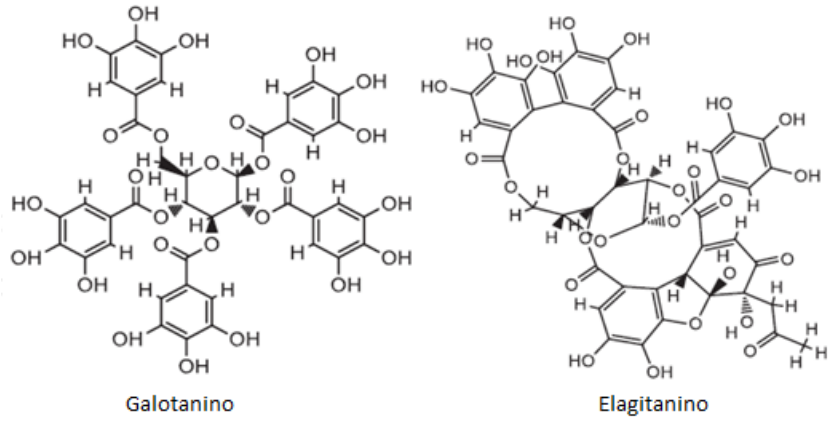
A estrutura e a composição química dos taninos variam de acordo com a fonte vegetal que o produto foi extraído, sendo possível classificá-los através da divisão em dois grupos distintos: hidrolisáveis ou condensados.

Taninos hidrolisáveis são polímeros compostos por ácidos fenólicos, caracterizados principalmente pela presença de ácido gálico e açúcares simples (geralmente glicose), com massa molecular menor do que os taninos condensados, variando de 600 a 3000 Dalton (TAIZ; ZEIGER, 2009). A estrutura básica dos taninos hidrolisáveis é composta de uma unidade poli alcoólica e grupos de hidroxila, unidos através de ligações éster-carboxila, onde é possível realizar o processo de hidrólise em ambientes ácidos ou básicos. A partir da hidrólise, que pode ser química ou enzimática, os taninos hidrolisáveis podem ser classificados em dois grupos: galotaninos (liberam ácido gálico) e elagitaninos (liberam ácido elágico) (GONÇALVES; LELIS; VIEIRA, 2017). Existem outras variações sobre a formação

química após a hidrólise, visto que os taninos possuem a capacidade de reagir com uma ampla variedade de moléculas, formando macromoléculas mais complexas (SERRANO *et al.*, 2009).

A Figura 1 apresenta a estrutura química dos principais ácidos oriundos dos taninos hidrolisáveis.

Figura 1 – Estrutura química dos galotaninos e elagitaninos.



Fonte: Adaptado de Bule *et al.* (2020).

Os taninos hidrolisáveis são encontrados geralmente nas folhas, frutos, vagens e sementes das plantas, de onde é feito o processo de extração. O ácido tânico é um exemplo de tanino hidrolisável que corresponde ao composto de vários taninos, geralmente gálicos, comumente utilizado por diversos segmentos da indústria de alimentos, farmacêutica e até para produção de enzimas (COUTO *et al.*, 2021).

A Tabela 1 apresenta alguns dos principais exemplos de plantas que apresentam taninos hidrolisáveis.

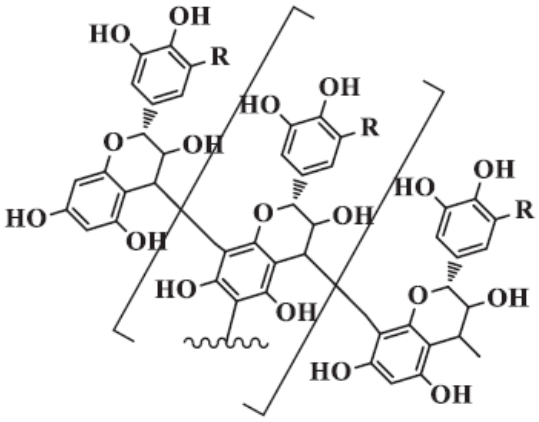
Tabela 1 – Plantas que apresentam taninos hidrolisáveis.

Família	Espécies	Tipo de tanino
Anacardiaceae	*Rhus sp.*	Galotaninos e Elagitaninos
Leguminosae	*Caesalpinea sp.*	Galotaninos e Elagitaninos
Fagaceae	*Quercus sp.*	Galotaninos e Elagitaninos
	Castanea sp.	Elagitaninos
Combretaceae	*Terminalia sp.*	Elagitaninos
Myrtaceae	*Eucalyptus sp.*	Elagitaninos

Fonte: Adaptado de Mueller-Harvey, (2001).

Os taninos condensados são constituídos pela polimerização de diversos flavonoides, principalmente flavan-3-ol ou flavan-3,4-diol (catequina e leucoantocianinas, respectivamente) (PIZZI, 2019). Também conhecidos como proantocianidinas, os taninos condensados possuem estruturas químicas complexas, composto de até cinquenta unidades flavonoides. Apesar de resistentes à hidrólise, podem ser solúveis em solventes orgânicos, dependendo dos componentes de sua estrutura (BATTESTIN; MATSUDA; MACEDO, 2004). A Figura 2 apresenta a estrutura de um tanino condensado.

Figura 2 – Estrutura química de um tanino condensado.



Fonte: Adaptado de Bule *et al.* (2020).

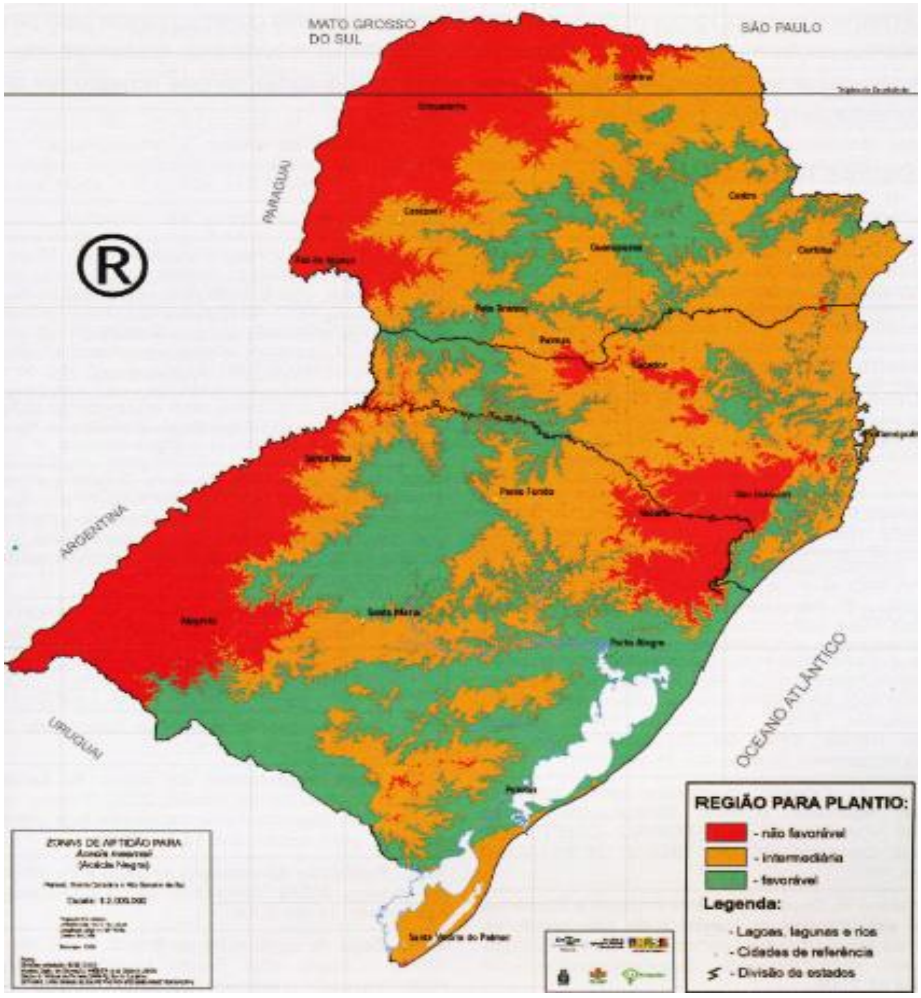
Os taninos condensados não sofrem hidrólise e possuem uma estrutura molecular mais complexa e de maior peso molecular quando comparados aos hidrolisáveis, podendo apresentar uma baixa biodisponibilidade, o que lhe caracteriza como um potencial antimicrobiano para frangos de corte (CHOI; KIM, 2020). Dentre as principais fontes vegetais de taninos condensados, os mais utilizados comercialmente nos diversos segmentos da indústria são quebracho (até 25% de tanino), carvalho (até 15%) e acácia negra (até 40%) (GONÇALVES; LELIS; VIEIRA, 2017).

2.2.3 *Acacia mearnsii*

A *Acacia mearnsii*, também conhecida como acácia negra, é uma espécie de árvore originária da Austrália, que se destaca pelo rápido crescimento, com sua taxa máxima entre o terceiro e quinto ano de vida, atingindo aproximadamente 6 a 10 metros de altura (HIGA *et al.*, 2009). É uma espécie utilizada em diversos segmentos da indústria, principalmente visando a produção de taninos e a exportação de madeira. A acácia negra se destaca na produção de taninos devido ao extrato de sua casca apresentar qualidade superior aos demais, tornando-se a principal fonte de extração do mundo, seguida pelo quebracho e castanheira, cultivados principalmente na Argentina e Europa, respectivamente (CHAN *et al.*, 2015).

Originária da Austrália, a acácia negra é muito cultivada em vários países do mundo, especialmente na África e Brasil devido seu rápido crescimento e madeira de alta qualidade com potencial para produção de lenha e carvão. No Brasil, o cultivo da acácia se concentra na região Sul do País, devido a adaptação da cultura às condições ambientais da região (ROVERSI *et al.*, 2002), conforme representado na Figura 3.

Figura 3 – Regiões de cultivo de acácia negra no Brasil.



Fonte: HIGA *et al.* (2009).

Embora esteja presente em diversos tecidos das plantas, a casca e a madeira de espécies lenhosas costumam apresentar maior concentração de tanino em relação aos demais tecidos. Calegari *et al. (*2016), ao avaliarem a casca da acácia negra, encontraram concentrações superiores a 40% de taninos, demonstrando seu elevado potencial tanífero. Estes compostos possuem inúmeras funções na indústria, sendo utilizados nos mais diversos setores. A primeira utilização dos taninos foi no curtimento do couro dos animais devido a característica de complexação com determinadas proteínas, garantindo maior resistência e impermeabilização das peles. Atualmente, também são utilizados para perfuração de poços de petróleo, tratamento de águas, fabricação de adesivos de resina, antioxidantes e pela indústria farmacêutica principalmente como antidiarreico, cicatrizante e anti-inflamatório (GONÇALVES; LELIS; VIEIRA, 2017).

Os taninos podem apresentar diversas propriedades que geram efeitos benéficos quando consumidos por humanos. Em 2001, foi comprovada sua ação antioxidante, atuando na prevenção da oxidação e esgotamento das vitaminas, além de auxiliar na prevenção de determinadas doenças (OGAWA; YAZAKI, 2018). A ação antimicrobiana, antibacteriana e antifúngica, são outras características muito evidenciadas aumentando ainda mais a atenção para seu potencial uso na nutrição animal.

A Figura 4 apresenta o tanino obtido da extração da casca da acácia negra.

Figura 4 – Tanino de acácia negra.

Fonte: Arquivo pessoal do autor.

2.2.4 Uso de taninos na alimentação animal

A utilização de taninos na nutrição animal, tanto na forma de aditivos como no uso de ingredientes que os contém em sua composição, foi considerado por muito tempo como fator antinutricional para monogástricos, porém é uma prática comum na nutrição de ruminantes por apresentar efeitos benéficos no rúmen, gerando melhor desempenho e aproveitamento do nitrogênio, além de reduzir a emissão de gás metano entérico (GOMES; SILVA; ARCANJO, 2023).

O principal motivo para sua utilização na nutrição animal, baseia-se na sua capacidade em complexar com determinadas proteínas e moléculas, fator este que pode ser benéfico ou prejudicial, dependendo principalmente da espécie animal, tipo de tanino e da quantidade ingerida. Os ruminantes possuem a capacidade de tolerar concentrações mais elevadas de taninos, pois os microrganismos presentes no rúmen, de forma geral, conseguem transformar alguns fatores antinutricionais em

substâncias mais simples, que não são nocivas ao animal (CORDÃO *et al.*, 2010). A complexação dos taninos com as proteínas ocorre através de pontes de hidrogênio, que são formadas no rúmen para, em seguida, serem dissociadas no abomaso e duodeno devido às diferenças de pH entre as estruturas do trato gastrointestinal. Com isso, pode ocorrer melhor aproveitamento dos nutrientes devido a redução da degradação da proteína dietética no rúmen, que acabará ocorrendo no intestino (FONSECA *et al.*, 2023).

Ainda em ruminantes, os aditivos de taninos apresentaram ação antiparasitária, resultando em reduções na contagem de ovos fecais, viabilidade dos ovos e número de larvas de *Trichostrongylus colubriformis* em ovinos suplementados com tanino de acácia negra (Minho *et al.*, 2010). Assim, com a comprovação desses efeitos, pesquisas foram realizadas para verificar se resultados similares podem ocorrer em animais monogástricos, visando avaliar os efeitos desses taninos na produção de aves. Poletti *et al.* (2021) ao avaliarem a eficácia de 150, 300 e 450 mg/kg de extrato de acácia negra no controle de endoparasitas em aves de postura no sistema de produção orgânico, obtiveram redução na infestação por *Ascaris spp.* e *Heterakis spp.*, porém não apresentou eficácia contra *Cappilaria spp.*

Em frangos de corte, ao substituir parcialmente milho por sorgo, fonte comum de taninos em grãos, Kumar; Elangovan; Mandal (2005) relataram queda no desempenho das aves em relação às aves alimentadas com dieta milho-farelo de soja, principalmente nas fases iniciais. Os autores relataram que a adstringência é um dos fatores antinutricionais mais comuns dos taninos, proporcionando menor palatabilidade dos alimentos. No entanto, devido ao baixo número de papilas gustativas das aves em relação aos outros animais, este pode não ser um fator limitante. Conforme relatado por Moyle *et al.* (2012), ao incluir 5, 10 ou 20% de folhas secas e trituradas de *Sericea lespedeza* (taninos condensados) na dieta de frangos de corte, foi observado redução no ganho de peso dos grupos 10 e 20%, enquanto o grupo que recebeu 5% não apresentou diferença em relação ao controle sem taninos. Entretanto, não houve diferença no consumo de ração entre todos os grupos estudados, demonstrando que a adstringência do tanino não interferiu neste parâmetro zootécnico. Com isso, é mais provável que a queda de desempenho ocorra por fatores de digestibilidade do que pela adstringência.

Com o avanço na tecnologia proporcionando melhor entendimento dos mecanismos de ação e processos de extração dos taninos, novas pesquisas são feitas

afim de obter os efeitos benéficos sem prejudicar o desempenho das aves, principalmente por ser um potencial substituto aos antibióticos. Através de testes *in vitro*, estudos demonstraram que o tanino da acácia negra possui ação contra diversas bactérias que podem acometer tanto a saúde pública quando dos animais, como *Escherichia coli* e *Salmonella* (OLAJUYIGBE; AFOLAYAN, 2012). O mecanismo de ação do tanino ocorre através da inibição de enzimas e substratos essenciais para o desenvolvimento dos microrganismos patógenos, dificultando sua multiplicação e favorecendo o rompimento da membrana microbiana (YAO *et al.*, 2006).

Em pesquisas mais recentes, Redondo *et al.* (2022) avaliaram a eficácia de um composto de taninos de quebracho e castanha (1Kg/ton), em comparação a um promotor de crescimento convencional, em granjas comerciais de frangos de corte. Os autores relataram que não houve diferença no desempenho das aves. Quanto a saúde intestinal, houve redução na frequência e gravidade das lesões intestinais nas aves alimentadas com taninos e melhor desenvolvimento da morfometria intestinal, principalmente na fase final. Em outro estudo similar induzindo enterite necrótica através do desafio com *C. perfringens*, o grupo com taninos apresentou menor frequência e gravidade de lesões macroscópicas no intestino em relação às aves que não receberam tanino e foram desafiadas.

A partir dos dados descritos, pode-se observar que taninos oriundos de diferentes fontes respondem de forma distinta no organismo das aves de acordo com a dosagem fornecida. Isso demonstra a necessidade de estudos mais aprofundados sobre a disponibilidade das fontes de taninos, seus mecanismos de ação no metabolismo dos animais e principalmente a quantidade ótima a ser suplementada que poderá trazer melhorias na saúde intestinal das aves e no desempenho produtivo.

3. ARTIGO

Effects of *Acacia mearnsii* tannins on growth performance, footpad dermatitis, nutrient digestibility, intestinal permeability, and meat quality of broiler chickens[1]

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Effects of *Acacia mearnsii* tannins on growth performance, footpad dermatitis, nutrient digestibility, intestinal permeability, and meat quality of broiler chickens

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Abbreviations: BW, body weight; d, days; DM, dry matter; FCR, feed conversion ratio; FI, feed intake; FITC-d, systemic fluorescein isothiocyanate-dextran; GE, gross energy; IDE, ileal digestible energy; L, linear regression; MDA, malondialdehyde; QP, quadratic polynomial regression; PBS, phosphate buffered saline; TBA, thiobarbituric acid; TBARS, thiobarbituric acid reactive substances.

ABSTRACT

An experiment was conducted to evaluate the effects of increasing dietary supplementation of tannins from *Acacia mearnsii* on broiler growth performance, footpad dermatitis, litter moisture, and ileal digestibility as well as on intestinal permeability, jejunal morphometric, and lipid oxidation measurements. A total of 1,620 Cobb 500 one-day-old male chicks were fed 6 experimental diets with 10 replicates of 27 birds each in a 4-phase feeding program. Dietary treatments were as follow: non-challenged control, challenged control, and challenged supplemented with 300, 500, 700, or 900 mg/kg tannin from *Acacia mearnsii*. All birds on challenged groups were challenged with *Eimeria* spp. on d 1 and *Clostridium perfringens* on d 11, 12, and 13. Performance was evaluated weekly until d 43. On d 21, blood samples were collected for biochemistry analyses and the intestinal permeability was evaluated using FITC-d as marker. Ileal digestibility and jejunal morphometric were also determined on d 21. Footpad dermatitis, litter moisture, and litter pH were evaluated on d 28, 35, and 43. On d 44, carcass and commercial cuts weights and yields were evaluated and thigh samples were collected for lipid oxidation (TBARS) through a 6 months storage period. Statistical analysis was conducted using linear and quadratic polynomial regression models and mean comparisons were done using the Tukey test ($P < 0.05$). Broilers under intestinal challenge had lower growth performance, higher intestinal permeability, and lower serum albumin, cholesterol, glucose, and triglycerides compared to non-challenged birds ($P < 0.05$). From d 0 to 43, increasing tannin supplementation for broilers under intestinal challenge led to quadratic increases ($P < 0.05$) in BW gain, with optimal supplementation at 310 mg/kg as well as optimized estimations for FCR at 444 mg/kg tannin. Dietary tannin supplementation that reduced intestinal permeability and improved villus height at 21 d were 466 and 528 mg/kg, respectively ($P < 0.05$). Tannin supplementation that improved protein and energy digestibility were at 374 and 294 mg/kg, respectively ($P < 0.05$). No effects of tannin were observed on blood biochemistry, crypt depth,

litter pH, carcass yield, and lipid oxidation until the storage d 60. However, increasing levels of tannin supplementation led to a linear ($P < 0.05$) decrease on lipid oxidation of broiler meat after 90 d of storage. In conclusion, low levels of supplemental tannins from *Acacia mearnsii* improved growth performance, nutrient digestibility, and intestinal permeability in broilers under an intestinal challenge.

Keywords: Broiler; Lipid oxidation; Litter moisture; Performance; Tannin.

1. Introduction

The global ban of antibiotics as growth promoters in broiler production has been a driving effort to increase the utilization of natural substances as feed additives (Brown et al., 2017). Several additives have been evaluated as alternatives to improve gut heath, immune functions, and growth performance; however, variable results are observed depending on the type and inclusion rates of natural additives, environmental challenges, and diet types as well as the differences of birds' age and production systems (Choi and Kim, 2020).

The aromatic and medicinal properties of plants and herbal extracts have been observed since antiquity, and this knowledge increased the utilization of plant extracts as additives in animal production, especially due to their antimicrobial properties. Tannins are the fourth most abundant constituent of plants as components of wood leaves, fruit skins, seeds, and bark, being characterized as water-soluble phenolic compounds with high molecular weight. Tannins deposition in plants have been reported to increase as a defensive response to predators, pathogens, and radiation (Fraga-Corral et al., 2020).

Acacia mearnsii is a medium-sized tree, native to southeastern Australia which has rapid adaptation to different environmental conditions and high productivity. This species was introduced into different regions of the world and, nowadays, it is mainly planted in Brazil and

South Africa for woodchip exports and tannin production on a significant international scale (Chan et al., 2015). The majority of tannins in *Acacia mearnsii* are condensed tannins, which are defined as polymeric flavonoids consisting of flavan-3-ols (fisetinidol, robinetinidol, catechin, and gallocatechin) or flavan-3,4-diols (leucoanthocyanins) units. Condensed tannins may contain from 2 to 50 flavonoid units, in a complex structure resistant to hydrolysis depending on its structure (Botha et al., 1979).

Low amounts of tannins in poultry diets have shown positive effects on intestinal health, gut microbiota, and litter quality as well as on meat quality and growth performance due to its antimicrobial, antioxidant, anti-inflammatory, and immunostimulatory functions (Huang et al., 2018; Choi and Kim, 2020). On the other hand, high amounts of tannins have been reported to reduce feed intake and performance due to its astringent properties, which typifies them as antinutrients (Hidayat et al., 2021). The majority of publications exploring tannin supplementation has been done in ruminants and recent studies with monogastric animals have revealed that if tannins are used with caution, they can be of benefit to performance and intestinal health (Huang et al., 2018). However, unlike in ruminants, the mode of action of tannins for broiler chickens is unclear and the results are still inconsistent.

The difference between the amount of tannin that can be beneficial and the excess that can be harmful is narrow. For this reason, this study was conducted to evaluate the effects of increasing dietary supplementation of tannin from *Acacia mearnsii* on broiler growth performance, footpad dermatitis, litter moisture, and ileal digestibility as well as on blood biochemistry, intestinal permeability, jejunal morphometric, and lipid oxidation measurements.

2. Material and methods

2.1 Broiler husbandry, experimental feeds, and intestinal challenge

All procedures used in this present experiment were approved by the Animal Care, Ethics, Use, and Research Committee of the Federal University of Santa Maria, Santa Maria, RS, Brazil. A total of 1,620 slow-feathering (Cobb 500 × Cobb 500) one-day-old male chicks (Agrogen, Montenegro, RS, Brazil), vaccinated for Marek's disease at the hatchery, were placed into 60 floor pens (9.0 birds/m^2) in a climate-controlled poultry house. Chicks (45 ± 1 g) were weighed and separated into groups of 27 birds per pen with a variation between the live weights not exceeding 3% in each experimental unit (floor pens).

Broilers were fed 6 experimental diets, with 10 replicates of 27 broilers each, distributed in a completely randomized design. Each pen was equipped with one tube feeder and 5 nipple drinkers. New wood shavings were used as litter. Broilers had *ad libitum* access to water and corn-soy all vegetable mash feeds. Average temperature was 32ºC at placement, being reduced 1ºC every 2 d to provide bird comfort using thermostatically controlled heaters, evaporative pads, and exhaust fans. A continuous lighting schedule was used until d 14 and a 18L:6D cycle light program was used from d 15 to 43.

The dietary treatments consisted of a non-challenged control; a challenged control; and 4 levels of tannins (300, 500, 700, or 900 mg/kg) from *Acacia mearnsii* (73.5 g/kg minimum guarantee of condensed tannins; NutreSet PNT, SETA S.A., Estancia Velha, RS, Brazil) supplemented on the challenged control. Except those on non-challenged control treatment, all birds were challenged on d 1, via individual oral gavage (10x regular dose) with a commercially approved coccidial vaccine containing *Eimeria acervulina*, *E. maxima*, *E. tenella*, *E. mitis*, *E. necatrix*, *E. praecox*, and *E. brunetti*. On d 11, 12, and 13, birds were individually orally gavaged with 1 mL/bird of *Clostridium perfringens* (MercoLab, Cascavel, PR, Brazil) with analyzed concentration at 1.0×10^8 cfu/mL. This intestinal challenge model was previously used by the research group (Stefanello et al., 2020).

A four-phase feeding program with pre-starter (d 0 to 7), starter (d 8 to 21), grower (d 22 to 35), and finisher (d 36 to 43) feeds was used. Celite at 1% was added to the started feeds and it was used as an indigestible marker to calculate the ileal digestibility on d 21. Feeds were formulated as usual in the Brazilian broiler industry (Table 1).

2.2 Growth performance

Birds weight and feed intake (FI) averaged by pen were recorded per week (7, 14, 21, 28, 35, and 43 d). Based on this data, the body weight gain (BW gain) of birds was calculated. The feed conversion ratio (FCR) was calculated considering the FI per replicate group, the weight gain of birds, and the weight of dead birds.

2.3 Intestinal permeability and blood histochemistry

On d 21, one bird per pen, with the average body weight (BW) of the experimental unit (n = 60), was orally gavaged with 2.2 mg/kg of BW of systemic fluorescein isothiocyanate-dextran (FITC-d, 3–5 kDa; 4,000 mol weight; Sigma-Aldrich, Saint Louis, MO, US) dissolved in 1 mL Milli-Q water. Blood samples were collected from the wing vein 1.5 h after gavage and centrifuged (500 × g for 15 min) to separate the serum. Fluorescence levels of diluted serum [1:1 in phosphate buffered saline (PBS)] were measured at an excitation wavelength of 485 nm and emission wavelength of 528 nm. The FITC-d concentration (µg/mL) was calculated based on a standard curve (Gilani et al., 2018; Stefanello et al., 2020; Stefanello et al., 2022).

On d 21, blood samples were collected from another one bird per pen (n = 60) for biochemistry analysis. Blood was centrifuged (3,400 × for 20 min) and the obtained serum was stored at −80ºC. Albumin, total cholesterol, glucose, and triglycerides serum concentration were determined using a BS-120 automatic biochemical analyzer (Mindray Headquarters,

Nanshan, Shenzhen, China). Commercial kits (Bioclin, Quibasa, Belo Horizonte, MG, Brazil) were used following methodology proposed by the manufacturer.

2.4 Ileal digestibility and jejunal morphometric measurements

On d 21, four birds per pen, with the average BW of the experimental unit (n = 240) were euthanized for ileal digesta collection. Ileal contents, from the 2/3 distal ileum, were flushed with distilled water into plastic containers, pooled by pen, immediately frozen and stored at −20°C. Digesta samples were dried in a forced air oven at 55ºC and ground prior analyses. Dry matter (DM) of feeds and ileal digesta was performed after oven drying the samples at 105ºC for 16 h (method 934.01; AOAC International, 2000). Gross energy (GE) of feeds and ileal digesta was determined using an adiabatic bomb calorimeter (IKA Werke C200, Staufen, Germany). Nitrogen of feeds and ileal digesta was determined using the combustion method (Thermo-Finnigan Flash EA 1112, Waltham, MA, US). Acid insoluble ash was analyzed to determine the indigestible marker in feeds and ileal digesta. Coefficients of apparent ileal digestibility of DM, energy, and protein as well as ileal digestible energy (IDE, in MJ/kg) were calculated as previously described by Kong and Adeola (2014) and Stefanello et al. (2019, 2020).

On d 21 and 43, a medial section of jejunum was collected from one bird per pen for morphometry measurements. On d 21, the jejunum samples were collected from the same birds sampled for intestinal permeability. The jejunal tissues were washed with PBS and fixed in a solution with 10% formaldehyde and phosphate buffer 0.1 M with pH 7.3. Then, samples were histologically processed, stained with hematoxylin and eosin, and analyzed under a light microscope. Digital images were captured with ImageJ 1.52v (NIH-developed image processing software) and, in each sample (n = 120), 20 well-oriented villi and 20 crypts were measured (Stefanello et al., 2022).

173 *2.5 Footpad dermatitis, litter moisture, and litter pH*

174 On d 28, 35, and 42, representative samples of litter were collected in 5 different points
175 of each pen and preserved individually at -20ºC. To determine litter moisture, two 100 g
176 samples were weighed and dried in an oven at 105ºC for 16 h (method 934.01; AOAC
177 International, 2000). To obtain litter pH, 10 g of fresh samples were homogenized with 100 mL
178 distilled water for 30 min as described by Brauer-Vigoderis et al. (2014). Then, the pH was
179 measured (twice per sample) with a pH meter equipped with an electrode (InLab 413 SG;
180 Mettler-Toledo GmbH).

181 On d 28, 35, and 42, 10 birds from each pen were randomly selected, wing banded,
182 examined for the presence of footpad dermatitis, and given a 4-lesion score according to the
183 Welfare Quality Assessment Protocol for Poultry (Welfare Quality, 2009). The same birds were
184 examined in each age, data was taken from both feet. Footpad dermatitis was classified as 0 (no
185 evidence), 1 and 2 (minimal evidence), and 3 and 4 (evidence of footpad dermatitis).

186

187 *2.6 Carcass yield and lipid oxidation*

188 On d 44 d, five birds were selected from each pen (with averaged BW) and processed for
189 carcass and commercial cuts evaluation at a commercial poultry slaughterhouse. Broilers were
190 fasted for 8 h, individually weighed, and wing banded prior to processing. Carcasses were
191 chilled in pre-chiller and chiller tanks. Eviscerated carcasses (without feet and neck) were then
192 hung to remove excess water prior weighing and the abdominal fat was weighed within the
193 carcass. Commercial cuts were performed by a crew of industry-trained personnel into deboned
194 breast as well as bone-in drumsticks, thighs, and wings. Carcass yield was expressed relative to
195 the fasted live weight while commercial cuts were expressed as percentage of the eviscerated
196 carcass.

After carcass processing and weighing, thighs were collected from 2 carcasses per experimental unit (n = 600) to determine lipid oxidation using thiobarbituric acid reactive substances (TBARS). In the presence of thiobarbituric acid (TBA), malonaldehyde and other aldehyde products of lipid oxidation (TBA reactive substances; TBARS) form pink chromogens with maximum absorbance at 532 to 535 nm. Lipid oxidation was expressed as mg of malondialdehyde (MDA) per kg of sample following the method described by Sinnhuber et al. (1958) and Buege and Aust (1978). Lipid oxidation was determined on fresh meat (d 0), thighs were separated in different samples and stored at -20ºC. Samples were analyzed every 30 days until 6 months of storage.

2.7 Statistical analysis

The data was tested for homoscedasticity and normality of the variance prior to statistical analyses and square root transformed for analyses when necessary. This data submitted for analysis of variance using the MIXED procedure of SAS (SAS, 2015) and mean separation was done using Tukey multiple-range test using a 95% confidence interval. Orthogonal contrast analysis was also conducted to compare the non-challenged control against the challenged control.

Estimations of optimal responses of supplemented tannin (0, 300, 500, 700, or 900 mg/kg) were done using linear (L) and quadratic polynomial (QP) regression models. The L model ($Y = \beta1 + \beta2 \times X$) had Y as the dependent variable, X as the dietary supplementation of tannin, β1 as the intercept, and β2 as the linear coefficient. The QP model ($Y = \beta1 + \beta2 \times X + \beta3 \times (X)^2$) had Y as the dependent variable as a function of dietary supplementation of tannin; β1 as the intercept; β2 as the linear coefficient and β3 as the quadratic coefficient. The optimal response for tannin supplementation was defined as $X = -\beta2 \div (2 \times \beta3)$.

3. Results

Effects of the increasing supplementation of tannin from *Acacia mearnsii* on cumulative growth performance of broiler chickens are shown in Table 2. Mean comparisons between treatments for BW gain, FI, and FCR showed no effects of dietary tannin from d 0 to 7 and d 22 to 43 ($P > 0.05$). Mean comparisons did not demonstrate effects of dietary treatments on FI from d 0 to 43 ($P > 0.05$); however, the FCR was improved when tannin was supplemented at 300 or 500 mg/kg compared to the non-supplemented challenged group ($P = 0.0042$). From d 0 to 21, broilers on the non-challenged control had greater BW gain and improved FCR then broilers on the challenged control ($P < 0.001$). Mortality (Grand mean = 2.6%) was not affected by the dietary treatments throughout the study ($P > 0.05$).

Coefficients of ileal digestibility, blood biochemistry, and serum FITC-d of 21-d-old broilers are presented in Table 3. Dietary treatments did not affect albumin and cholesterol concentrations ($P > 0.05$). Mean comparisons demonstrated that IDE and energy digestibility of broilers fed diets supplemented with 300, 500, or 700 mg/kg tannin were similar to broilers on the non-challenged or challenged controls. However, broilers fed the Challenged + 900 mg/kg tannin had the lowest IDE and ileal digestibility of DM, protein, and energy ($P < 0.0001$). Ileal digestibility of DM and protein were greater when broilers were fed 500 mg/kg tannin than the non-challenged and challenged controls ($P < 0.0001$). The intestinal permeability, expressed as the serum FITC-d concentration, was higher in the challenged control and Challenged + 900 mg/kg tannin compared to broilers fed diets supplemented with 300, 500, or 700 mg/kg tannin ($P < 0.05$).

The litter pH evaluated on d 28, 35, and 43 was not affected by the intestinal challenge and tannin supplementation ($P > 0.05$) as shown in Table 4. Litter moisture and footpad dermatitis also were not affected by the treatments on d 43; however, differences were observed

246 on d 28 where broilers fed diets supplemented with 300, 500, or 700 mg/kg tannin had lower
247 footpad dermatitis and higher litter moisture than the non-challenged control ($P < 0.05$).

248 Jejunal morphometric measurements from broilers on d 21 and 43 are presented in Table
249 5 and no differences were observed on crypt depth. On d 21, the villus:crypt ratio decreased in
250 the challenged control compared to the non-challenged control ($P = 0.0145$). On d 43, the lowest
251 villus height was observed in the challenged control compared to non-challenged broilers and
252 all supplementation levels of tannins ($P = 0.0444$).

253 Carcass and commercial cut weights and yields at 44 d showed no differences by the
254 mean comparison tests (Table 6). The lipid oxidation was similar among treatments in
255 refrigerated thighs on d 0 and in frozen thigh samples stored for 30 and 60 d (Table 7). The
256 Challenged + 900 mg/kg tannin resulted in lower lipid oxidation with 90 and 120 d of storage
257 compared to both challenged and non-challenged groups ($P < 0.05$). With 150 and 180 d
258 storage, the Challenged + 700 mg/kg tannin and Challenged + 900 mg/kg tannin had lower lipid
259 oxidation than the controls ($P < 0.05$).

260 The Table 8 shows contrasts between the non-challenged against the challenged control
261 and demonstrates that BW gain from d 0 to 21 and d 0 to 43 was greater in the non-challenged
262 group ($P < 0.05$). The FCR was improved in the non-challenged control compared to the
263 challenged control from d 0 to 21 and d 0 to 43 ($P < 0.01$). Serum albumin, cholesterol, glucose,
264 and triglycerides as well as FITC-d were lower in the challenged control than in the non-
265 challenged ($P < 0.01$). Footpad dermatitis on d 28 was higher in challenged broilers ($P =$
266 0.0053) and lipid oxidation of refrigerated thighs also was higher in the challenged control (P
267 $= 0.0087$).

268 Estimations of optimized responses of performance, carcass yield, ileal digestibility, and
269 litter and meat quality by regression analysis are presented in Table 9. Increases in supplemental
270 tannin allowed linear or quadratic adjustments ($P < 0.05$) for most responses. Dietary tannin

supplementation that maximized BW gain were 349 mg/kg (R^2 = 0.21) and 310 mg/kg (R^2 = 0.36) from d 0 to 21 and d 0 to 43, respectively. The FCR was improved from d 0 to 21, d 22 to 43, and d 0 to 43 with optimal responses at 436, 446, and 444 mg/kg, respectively. Estimations of dietary tannin supplementation that maximized breast yield and weight were 428 and 409 mg/kg, respectively.

Significant regressions ($P < 0.05$) allowed for the estimation of tannin supplementation that maximized nutrient and energy utilization on d 21 as follow: IDE and energy digestibility at 274 mg/kg (R^2 = 0.43) and 294 mg/kg (R^2 = 0.42), respectively; DM digestibility at 316 mg/kg (R^2 = 0.44) and protein digestibility at 374 mg/kg (R^2 = 0.50). Estimations of dietary tannin that increased villus height on d 21 and d 43 were 528 (R^2 = 0.22) and 628 mg/kg (R^2 = 0.20), respectively. The intestinal permeability decreased at 466 mg/kg tannin (R^2 = 0.22). Tannin supplementation led to linear decreases on litter moisture on d 28, 35, and 43 as well as linear decreases on lipid oxidation in thigh meats stored for 90, 120, 150, and 180 d ($P < 0.05$).

4. Discussion

Tannins are plant secondary metabolites, characterized as water-soluble phenolic compounds with high molecular weight that serve as a part of plant chemical defense system. Chemical structures and concentrations of tannins vary among plant species, growth stages, and harvesting conditions (Berard et al., 2011; Li et al., 2014). Additionally, there are variable types of tannins, which have different bioavailability, and different tannin concentrations and types were previously attributed to beneficially modulate growth performance, gut health, and gut microbiota of poultry (Choi and Kim, 2020). Most of the studies supplementing tannins in broiler diets have been conducted with chestnut and tannic acid as hydrolyzed tannin sources, or grape pomace and grape seed extract as condensed tannins. There is a lack of data showing the effects of tannins from *Acacia mearnsii* as an additive for poultry and more data is needed

296 to better understand their effects. Most of the discussion of the present study was explored
297 comparing condensed tannin concentrations in broiler diets.

298 Results in the present experiment indicated that the supplementation of tannins from
299 *Acacia mearnsii* for broilers led to improvements in BW gain and FCR from d 0 to 21 and 0 to
300 43. In the overall period, BW gain and FCR were optimized with 310 and 444 mg/kg tannin,
301 respectively, whereas the higher supplementation levels at 900 mg/kg resulted in the lowest
302 BW gain. Previous researches indicated that the BW gain of 42-day-old broiler chickens was
303 impaired when fed a diet containing 560 mg/kg tannin (Woyengo and Nyachoti, 2012). Hidayat
304 et al. (2021), in a meta-analysis, also indicated that high levels of dietary tannins negatively
305 affected BW gain and FI of broilers.

306 In the present study, the FI of broilers was not affected by tannin supplementation from d
307 0 to 7, d 22 to 43, and d 0 to 43. However, from d 0 to 21, broilers fed diets supplemented with
308 900 mg/kg tannin had lower FI compared to birds fed the non-supplemented feeds. There are
309 some reports indicating that low concentrations of tannins decreased FI, given the astringent
310 nature of tannins; however, for broilers it seems not justified that feed palatability can influence
311 FI (Huang et al., 2018). On the other hand, the effects of tannins on broiler FI can be more
312 related to the system of production, physiological status, type of diets, type of tannins, and their
313 concentrations in the diets.

314 Tannins were considered as anti-nutritional factors in the past, but several reports
315 indicated that tannins at appropriate dosages have potentials to improve growth performance
316 and health status of broilers (Schiavone et al., 2008; Starčević et al., 2015). The mechanisms of
317 growth promoting effects of tannins in poultry are much less understood compared with those
318 in ruminants since tannins have traditionally been considered as antinutrients in monogastric
319 nutrition.

Antinutrients are commonly known as compounds that interfere with the absorption of nutrients. Condensed tannins can inhibit endogenous amylases, lipases, and proteases (Bhat et al., 2013) and, for this reason, they can negatively influence the digestibility of fats, starch, and amino acids (Garcia et al., 2004). Woyengo and Nyachoti (2012) reported a decrease of aminopeptidase secretion in broilers fed high tannin contents. In a meta-analysis evaluating different types of tannins for broilers, Hidayat et al. (2021) indicated that high levels tannins had a negative effect on amino acid digestibility due to the ability of tannins to form tannin-protein complexes in the intestine; however, no effects were observed with concentrations up to 120 mg/kg. Optimal tannin supplementations observed in the current study were 316, 294, and 374 mg/kg for digestibility of dry matter, energy, and protein, respectively, in 21-d-old broilers under an intestinal challenge.

The model of intestinal challenge proposed in this study was previously applied in other researches, and it was created to generate a dysbiosis (Belote et al., 2018; Stefanello et al., 2020). *Clostridium perfringens* is an important poultry pathogen that results in expressive economic impacts on broiler production, as a causative agent of necrotic enteritis and sub-clinical disease (Ficken, 1997). In the present study, the intestinal challenge with *Eimeria* vaccine and *Clostridium perfringens* negatively affected BW gain and FCR from d 0 to 21 and d 0 to 43. Coccidiosis is closely associated with necrotic enteritis, which is predominately induced by *Clostridium perfringens* with the presence of *Eimeria* spp. (Prescott et al., 2016). The negative effects of coccidiosis on gut health of chickens can be related to oxidative stress, as *Eimeria* infections cause lipid peroxidation and excessive production of reactive oxygen species.

Tannins are known to have anticoccidial effects, forming complexes with parasitic enzymes and metal ions that are essential for *Eimeria* spp. (Scalbert, 1991; Chung et al., 1998). Condensed tannins present flavanols with a trihydroxy B ring (gallocatechin), which have an

345 inhibitory effect on *Streptococcus*, *Clostridium*, *Proteus*, and *Staphylococcus* species
346 (Sakanaka et al., 1989). According to Choi and Kim (2020), although many studies reported
347 benefits of supplemental tannins in broilers, more comprehensive studies are required to better
348 understand the mechanisms under anticoccidial effects of tannins; to investigate the effects of
349 tannins in a necrotic enteritis challenge model with *Eimeria* spp. and *Clostridium perfringens*,
350 and to elucidate mechanisms of the beneficial effects of tannins on the gut health.

351 The antimicrobial activities of tannins have also been recognized with activity against
352 Gram-negative bacteria. Authors reported that *Escherichia coli*, *Salmonella*, *Shigella*,
353 *Staphylococcus*, *Pseudomonas*, and *Helicobacter pylori* were sensitive to tannins (Funatogawa
354 et al., 2004; Liu et al., 2013). The antimicrobial activity of tannins was associated to the
355 inhibition of extracellular microbial enzymes, deprivation of the substrates required for
356 microbial growth, direct action on microbial metabolism, and increasing membrane
357 permeability in bacteria cell walls (Scalbert, 1991; Liu et al., 2013). In the present study,
358 challenged broilers had higher intestinal permeability on day 21 compared with non-challenged
359 birds. This negative effect was ameliorated when tannins were supplemented in the control diets
360 from d 0 to 21, with reduced intestinal permeability at 466 mg/kg tannin and improved villus
361 height at 528 mg/kg.

362 Linear increases of tannin supplementation from 0 to 900 mg/kg led to decreases in litter
363 moisture. This is an interesting evaluation because high litter moisture and increased excreta
364 viscosity have been strongly related to the incidence of footpad dermatitis in broiler chickens
365 (Shepherd and Fairchild, 2010; Cengiz et al., 2012). In this context, tannins have been
366 supplemented in broiler diets to relieve the incidence and severity of footpad dermatitis by
367 enhancing excreta dry matter contents and litter quality (Redondo et al., 2014). Rezar and
368 Salobir (2014) indicated that 700 mg/kg and 2,000 mg/kg of tannin-rich sweet chestnut wood
369 extract increased excreta dry matter in broilers, whereas Cengiz et al. (2012) reported that 2,000

mg/kg tannic acid supplementation reduced the incidence and severity of footpad dermatitis without affecting litter quality and intestinal viscosity of broilers.

Positive effects of low levels of tannins have also been reported to stimulate the antioxidant status, to improve of intestinal morphology, and to positively modulate intestinal microbiota (Ebrahim et al., 2015). Tannins can result in enhanced antioxidant enzyme activities, increasing the antioxidant capacity in liver and plasma, and improving the antioxidant status of muscle (Huang et al., 2018). In the current study, linear increases of tannin supplementation led to decreases in broiler meat lipid oxidation after 90 d-storage. Metabolic antioxidant activities provided by natural additives can reduce muscle lipid oxidation by preventing free radical production. Dominguez et al. (2019) reported that evaluating lipid oxidation is important since it can reduce the nutritional value of meats due to the production of potentially toxic compounds that reduce its shelf life and compromise meat quality.

5. Conclusions

The evaluated levels of *Acacia mearnsii* tannins (from 0 to 900 mg/kg) supplemented in corn-soy diets allowed us to determine that low levels of tannins improved growth performance and nutrient digestibility in broilers under an intestinal challenge. The average tannin levels that optimized growth performance was 377 mg/kg, whereas 374 mg/kg was needed for better protein digestibility; however, higher tannin levels were needed to improve villus height and intestinal permeability. There was a linear decrease of litter moisture and lipid oxidation of broiler meat stored from 90 to 180 days when broilers were fed diets supplemented with increasing levels of tannins.

CRediT authorship contribution statement

Guilherme Godoy: Investigation, Data Curation, Formal analysis. **Beatriz Rodrigues**: Investigation, Data Curation. **Jessica Agilar**: Investigation, Data Curation. **Valeria Biselo**: Methodology, Investigation. **Kelly Castro**: Investigation**. Danielle Bruti:** Writing - Review & Editing. **Greicy Maysonnave**: Conceptualization, Visualization, Writing - Review & Editing. **Catarina Stefanello**: Supervision, Project administration, Writing - Original Draft, Writing - Review & Editing.

Declaration of Competing Interest

All authors declare that they have no competing interests.

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526 **Table 1**

527 Ingredient and nutrient composition of the experimental control diet, as-fed.

Item	Pre-starter (d 0 to 7)	Starter (d 8 to 21)	Grower (d 22 to 35)	Finisher (d 36 to 42)
Ingredients, g/kg				
Corn	527.5	590.8	643.5	661.3
Soybean meal, 45%	409.4	347.8	300.0	277.5
Soybean oil	28.7	28.9	26.9	31.7
Dicalcium phosphate	8.8	7.8	7.1	7.3
Limestone	13.0	12.5	10.8	10.9
Salt	4.9	4.7	4.5	4.2
DL-Methionine, 99%	3.1	2.7	2.3	2.2
L-lysine sulphate, 60%	1.8	2.1	2.3	2.3
L-Threonine, 98.5%	0.5	0.4	0.3	0.2
Choline chloride, 60%	0.4	0.7	0.7	0.8
Vitamin and mineral premix[a]	1.9	1.6	1.6	1.6
Nutrient and energy composition, g/kg or as shown				
ME, MJ/kg	12.44	12.76	12.97	13.18
Crude protein	231.2	208.3	190.6	181.7
Ca	9.0	8.4	7.6	7.6
Av. P	4.3	4.0	3.8	3.8
Na	2.1	2.0	1.9	1.8
Choline, mg/kg	1,600	1,600	1,500	1,500
Dig. Lys[b]	12.5	11.2	10.2	9.7
Dig. Met + Cys	9.3	8.3	7.5	7.2
Dig. Thr	8.3	7.4	6.7	6.4
Dig. Trp	2.7	2.3	2.1	2.0
Dig. Arg	14.5	12.8	11.5	10.9
Dig. Val	9.6	8.6	7.9	7.5
Dig. Ile	9.0	8.0	7.2	6.8
Dig. Leu	17.6	16.3	15.3	14.7

528 [a] Composition per kilogram of feed: vitamin A, 9,000 IU; vitamin D3, 2,500 IU; vitamin E, 20 IU; vitamin K3, 2,5
529 mg; thiamine, 2 mg; riboflavin, 6 mg; pyridoxine, 3.8 mg; cyanocobalamin, 0.015 mg, pantothenic acid, 12 mg;
530 niacin, 35 mg; folic acid, 1,5 mg; biotin, 0.1 mg; iron, 40 mg; zinc, 80 mg; manganese, 80 mg; copper, 10 mg;
531 iodine, 0.7 mg; selenium, 0.25 mg. Phytase with 10,000 fungal phytase units/g, using available P and total Ca
532 (g/kg) matrix values of 1,500 and 1,700, respectively.
533 [b] Ratios of digestible amino acids to digestible Lys were maintained according to Cobb-Vantress (2018).

Table 2

Cumulative growth performance of broilers fed diets supplemented with increasing levels of tannins from *Acacia mearnsii*.

Item	BW gain/bird, g				Feed intake/bird, g				Feed conversion ratio			
	d 0 to 7	d 0 to 21	d 22 to 43	d 0 to 43	d 0 to 7	d 0 to 21	d 22 to 43	d 0 to 43	d 0 to 7	d 0 to 21	d 22 to 43	d 0 to 43
Non-challenged	158	1,239[a]	2,417	3,656[a]	174	1,620[a]	3,812	5,431	1.106	1.307[b]	1.577	1.485[ab]
Challenged control[a]	154	1,164[bc]	2,440	3,604[a]	176	1,609[a]	3,903	5,511	1.145	1.383[a]	1.599	1.529[a]
Challenged + 300 mg/kg tannin[b]	154	1,182[ab]	2,461	3,643[a]	172	1,581[ab]	3,812	5,394	1.119	1.339[ab]	1.550	1.480[b]
Challenged + 500 mg/kg tannin	152	1,186[ab]	2,432	3,618[a]	174	1,576[ab]	3,785	5,361	1.146	1.329[ab]	1.557	1.482[b]
Challenged + 700 mg/kg tannin	151	1,168[bc]	2,423	3,591[a]	170	1,575[ab]	3,819	5,393	1.127	1.349[ab]	1.576	1.501[ab]
Challenged + 900 mg/kg tannin	150	1,114[c]	2,393	3,507[b]	170	1,547[b]	3,810	5,358	1.128	1.392[a]	1.593	1.527[ab]
SEM	0.79	7.59	8.10	9.94	0.72	5.91	13.50	17.45	0.007	0.007	0.007	0.005
P-value	0.0836	0.0001	0.2503	0.0001	0.0914	0.0033	0.1825	0.1038	0.6228	0.0015	0.3037	0.0042

[a,b,c] Means with different superscript letters differ ($P \leq 0.05$) based on Tukey's test.

[a] Challenged = coccidiosis vaccine at 10× the manufacturer recommendation dose on d 1 and *Clostridium perfringens* inoculation at 11, 12, and 13 d of age).

[b] Tannin from *Acacia mearnsii* (with 73.5 g/kg minimum guarantee of condensed tannins) supplemented on the challenged control.

Table 3

Coefficients of ileal digestibility, blood biochemistry parameters, and serum FITC-d of 21-d-old broilers fed diets supplemented with increasing levels of tannins from *Acacia mearnsii*.

Item	Ileal digestibility[a]				Blood biochemistry				Serum FITC-d[d], µg/mL
	IDE[b], MJ/kg	DM[c], %	Protein, %	Energy, %	Albumin, g/dL	Cholesterol, mg/dL	Glucose, mg/dL	Triglycerides, mg/dL	
Non-challenged	14.35^{ab}	0.70^{b}	0.77^{b}	0.72^{a}	1.46	127	328^{a}	44^{a}	0.276^{ab}
Challenged control[e]	14.68^{ab}	0.70^{b}	0.76^{b}	0.74^{a}	1.26	111	235^{b}	33^{ab}	0.317^{a}
Challenged + 300 mg/kg tannin[f]	15.12^{a}	0.73^{ab}	0.77^{b}	0.76^{a}	1.39	115	232^{b}	31^{b}	0.261^{c}
Challenged + 500 mg/kg tannin	14.72^{ab}	0.75^{a}	0.82^{a}	0.76^{a}	1.28	111	231^{b}	31^{b}	0.265^{c}
Challenged + 700 mg/kg tannin	14.20^{b}	0.70^{b}	0.78^{ab}	0.72^{a}	1.33	113	229^{b}	32^{ab}	0.270^{bc}
Challenged + 900 mg/kg tannin	13.00^{c}	0.63^{c}	0.66^{c}	0.66^{b}	1.32	115	212^{b}	27^{b}	0.312^{a}
SEM	0.14	0.007	0.007	0.008	0.02	2.12	7.09	1.35	0.007
P-value	0.0001	0.0001	0.0001	0.0001	0.2189	0.2550	0.0001	0.0046	0.0479

[a,b,c] Means in the same column with different superscript letter are significantly different by Tukey test ($P \leq 0.05$).

[a] Means (on dry matter basis) were obtained from 10 replicate pens and 4 birds each.

[b] IDE = ileal digestible energy.

[c] DM = dry matter.

[d] FITC-d = systemic fluorescein isothiocyanate-dextran.

[e] Challenged = coccidiosis vaccine at 10× the manufacturer recommendation dose on d 1 and *Clostridium perfringens* inoculation at 11, 12, and 13 d of age.

[f] Tannin from *Acacia mearnsii* (with 73.5 g/kg minimum guarantee of condensed tannins) supplemented on the challenged control.

Table 4

Scores of footpad dermatitis and litter moisture and pH of broilers fed diets supplemented with increasing levels of tannins from *Acacia mearnsii*.

Item	Footpad dermatitis scores[a]			Litter moisture, %			Litter pH		
	d 28	d 35	d 43	d 28	d 35	d 43	d 28	d 35	d 43
Non-challenged	0.70[a]	1.37	1.67	37.2[a]	41.3[a]	35.2	8.2	8.7	9.0
Challenged control[b]	0.34[ab]	1.19	1.61	37.2[a]	40.2[ab]	35.0	7.9	8.7	9.0
Challenged + 300 mg/kg tannin[c]	0.28[b]	1.05	1.53	36.6[ab]	39.4[ab]	32.5	8.0	8.6	9.0
Challenged + 500 mg/kg tannin	0.27[b]	1.18	1.61	36.2[b]	37.8[ab]	31.6	7.9	8.6	8.9
Challenged + 700 mg/kg tannin	0.30[b]	1.19	1.66	35.3[b]	37.7[ab]	31.6	8.0	8.6	9.0
Challenged + 900 mg/kg tannin	0.26[b]	1.17	1.48	35.1[b]	37.2[b]	31.0	8.0	8.6	8.9
SEM	0.03	0.06	0.06	0.48	0.42	0.38	0.05	0.03	0.02
P-value	0.0055	0.7233	0.9471	0.0282	0.0267	0.4640	0.7310	0.8914	0.9909

[a,b] Means with different superscript letters differ ($P \leq 0.05$) based on Tukey's test.

[a] Average of scores of footpad dermatitis evaluated in 10 broilers per experimental unit (n = 600). The same broilers were evaluated across ages.

[b] Challenged = coccidiosis vaccine at 10× the manufacturer recommendation dose on d 1 and *Clostridium perfringens* inoculation at 11, 12, and 13 d of age.

[c] Tannin from *Acacia mearnsii* (with 73.5 g/kg minimum guarantee of condensed tannins) supplemented on the challenged control

Table 5

Jejunal morphometric measurements of broilers fed diets supplemented with increasing levels of tannins from *Acacia mearnsii*[a].

Item	d 21			d 43		
	Villus height, µm	Crypt depth, µm	V:C	Villus height, µm	Crypt depth, µm	V:C
Non-challenged	1,214	138	9.0[a]	1,192[a]	144	8.5
Challenged control[b]	1,162	178	6.6[b]	1,070[b]	136	7.9
Challenged + 300 mg/kg tannin[c]	1,207	159	7.7[ab]	1,179[a]	138	8.6
Challenged + 500 mg/kg tannin	1,225	173	7.2[ab]	1,197[a]	144	8.5
Challenged + 700 mg/kg tannin	1,271	165	8.1[ab]	1,201[a]	140	8.7
Challenged + 900 mg/kg tannin	1,181	176	7.1[ab]	1,185[a]	141	8.4
SEM	12.39	4.50	0.21	14.31	2.31	0.15
P-value	0.1671	0.0851	0.0145	0.0444	0.9386	0.7367

[a,b] Means with different superscript letters differ ($P \leq 0.05$) based on Tukey's test.

[a] Results are the means of 20 spots corresponding to 10 birds each treatment group each age (n = 120).

[b] Challenged = coccidiosis vaccine at 10× the manufacturer recommendation dose on d 1 and *Clostridium perfringens* inoculation at 11, 12, and 13 d of age.

[c] Tannin from *Acacia mearnsii* (with 73.5 g/kg minimum guarantee of condensed tannins) supplemented on the challenged control.

Table 6

Carcass and commercial cuts weights (g) and yields (%) of 44-d-old broilers fed diets supplemented with increasing levels of tannins from *Acacia mearnsii*.

Item	Carcass[a]		Breast[b]		Drumsticks		Thighs		Wings	
	G	%	g	%	g	%	g	%	g	%
Non-challenged	2,762	75.0	918	33.2	355	12.8	483	17.5	348	12.6
Challenged control[c]	2,733	75.3	897	32.8	358	13.1	485	17.7	330	12.1
Challenged + 300 mg/kg tannin[d]	2,778	76.0	933	33.6	355	12.8	489	17.6	339	12.2
Challenged + 500 mg/kg tannin	2,738	75.6	917	33.5	353	12.9	476	17.4	337	12.3
Challenged + 700 mg/kg tannin	2,743	75.4	907	33.1	371	13.5	483	17.6	331	12.1
Challenged + 900 mg/kg tannin	2,721	76.2	894	33.8	358	13.2	472	17.3	329	12.1
SEM	10.89	0.14	5.18	0.11	2.64	0.08	2.36	0.07	2.38	0.06
P-value	0.7195	0.1453	0.2532	0.3043	0.4492	0.1177	0.2992	0.5129	0.1520	0.0941

[a] Eviscerated carcass as a percentage of body weight on d 44. There were 5 slaughtered birds per replicate pen and 10 replicates per treatment with a total of 300 evaluated carcasses.

[b] Skinless boneless *Pectoralis major* + *Pectoralis minor* as proportion of carcass on d 44.

[c] Challenged = coccidiosis vaccine at 10× the manufacturer recommendation dose on d 1 and *Clostridium perfringens* inoculation at 11, 12, and 13 d of age.

[d] Tannin from *Acacia mearnsii* (with 73.5 g/kg minimum guarantee of condensed tannins) supplemented on the challenged control.

Table 7

Lipid oxidation (TBARS; mg MDA/kg sample) of chicken thighs from broilers fed diets supplemented with increasing levels of tannins from *Acacia mearnsii*[a].

Item	Freezing storage at -18ºC, days						
	0	30	60	90	120	150	180
Non-challenged	0.072	0.124	0.141	0.161[a]	0.184[a]	0.189[a]	0.192[a]
Challenged control[b]	0.101	0.132	0.137	0.148[ab]	0.177[ab]	0.179[ab]	0.182[ab]
Challenged + 300 mg/kg tannin[c]	0.081	0.113	0.124	0.135[bc]	0.149[bc]	0.152[bc]	0.162[bc]
Challenged + 500 mg/kg tannin	0.070	0.110	0.115	0.129[bc]	0.137[bc]	0.145[c]	0.152[bc]
Challenged + 700 mg/kg tannin	0.067	0.112	0.117	0.123[bc]	0.147[bc]	0.139[c]	0.144[c]
Challenged + 900 mg/kg tannin	0.078	0.103	0.108	0.112[c]	0.128[c]	0.131[c]	0.138[c]
SEM	0.003	0.004	0.005	0.004	0.005	0.006	0.005
P-value	0.0637	0.3615	0.3535	0.0046	0.0015	0.0088	0.0030

[a,b,c] Means with different superscript letters differ ($P \leq 0.05$) based on Tukey's test.

[a] Thiobarbituric acid reactive substances performed in homogenate samples.

[b] Challenged = coccidiosis vaccine at 10× the manufacturer recommendation dose on d 1 and *Clostridium perfringens* inoculation at 11, 12, and 13 d of age.

[c] Tannin from *Acacia mearnsii* (with 73.5 g/kg minimum guarantee of condensed tannins) supplemented on the challenged control.

Table 8

Contrasts between the non-challenged control *vs*. the challenged control group of broilers[a].

Item	Contrasts (*P*-value)
d 0 to 21	
BW gain, g	0.0008
FCR	0.0008
d 21	
Footpad dermatitis	0.0053
FITC-d, µg/mL	0.0413
Albumin	0.0249
Cholesterol	0.0328
Glucose	0.0001
Triglycerides	0.0129
d 28	
Footpad dermatitis	0.0053
d 0 to 43	
BW gain, g	0.0443
FCR	0.0083
Lipid oxidation 0 d	0.0087

[a] The evaluated responses that are not listed in the table above were not significant ($P \leq 0.05$).

Table 9

Regression equations of the evaluated parameters from broilers fed diets supplemented with increasing levels of tannins from *Acacia mearnsii*.

Item	Model[a]	Regression equation[b]	R^2	*P*-value	Optimal response
d 0 to 21					
BW gain, g	Q	$Y = 1{,}161 + 0.1655x - 0.0002368x^2$	0.21	0.0060	349
FI, g	L	$Y = 1{,}606 - 0.0590x$	0.19	0.0036	-
FCR	Q	$Y = 1.38 - 0.000248x + 2.84102E^{-7}x^2$	0.20	0.0013	436
d 22 to 43					
FCR	Q	$Y = 1.60 - 0.000188x + 2.10974E^{-7}x^2$	0.21	0.0203	446
d 0 to 43					
BW gain, g	Q	$Y = 3{,}604 + 0.2317x - 0.0003738x^2$	0.36	0.0007	310
FCR	Q	$Y = 1.53 - 0.000211x + 2.38047E^{-7}x^2$	0.27	0.0001	444
Breast yield, %	Q	$Y = 33 + 0.0029x - 0.00000339x^2$	0.20	0.0268	428
Breast weight, g	Q	$Y = 901 + 0.1160x - 0.0001418x^2$	0.20	0.0357	409
d 21					
FITC-d, µg/mL	Q	$Y = 0.316 - 0.000252x + 2.7355E^{-7}x^2$	0.22	0.0017	466
Villus height, µm	Q	$Y = 1{,}154 + 0.3134x - 0.000297x^2$	0.19	0.0500	528
Litter moisture, %	L	$Y = 34.36 + 0.00423x$	0.24	0.0089	-
Ileal digestibility					
IDE, kcal/kg	Q	$Y = 3{,}510 + 0.6855x - 0.00125x^2$	0.43	0.0005	274
Dry matter	Q	$Y = 0.7152 + 0.000208x - 3.2876E^{-7}x^2$	0.44	0.0001	316
Energy	Q	$Y = 0.7396 + 0.000165x - 2.8083E^{-7}x^2$	0.42	0.0003	294
Protein	Q	$Y = 0.7437 + 0.000346x - 4.6159E^{-7}x^2$	0.50	0.0001	374
d 35					
Litter moisture, %	L	$Y = 40.13 + 0.00350x$	0.21	0.0177	-
d 43					
Villus height, µm	Q	$Y = 1{,}073 + 0.4274x - 0.000340x^2$	0.20	0.0430	628
Litter moisture, %	L	$Y = 37.28 + 0.00249x$	0.17	0.0548	-
Lipid oxidation[c]					
90 d	L	$Y = 0.1476 - 0.00003867x$	0.27	0.0026	-
120 d	L	$Y = 0.1701 - 0.00004655x$	0.29	0.0014	-
150 d	L	$Y = 0.1738 - 0.00005129x$	0.26	0.0038	-
180 d	L	$Y = 0.1788 - 0.00004899x$	0.29	0.0018	-

[a] Linear regression: $Y = \beta1 + \beta2 \times X$; where Y is the dependent variable, X is the dietary level of tannin product, $\beta1$ is the intercept, $\beta2$ and *is* the linear coefficients, respectively; Quadratic polynomial: $Y = \beta1 + \beta2 \times X + \beta3 \times X^2$; where Y is the dependent variable, X is the dietary level of tannin product, $\beta1$ is the intercept, $\beta2$ and $\beta3$ are the linear and quadratic coefficients, respectively; optimal responses were obtained by calculating: $-\beta2 \div (2 \times \beta3)$. Linear (L) or quadratic (Q) effect ($P \leq 0.05$).

[b] Regression equations for tannin supplementation levels (0, 300, 500, 700, 900 mg/kg). The coefficient of determination (r^2) was obtained using all data.

[c] Lipid oxidation was measured in mg MDA/kg sample in chicken thighs stored at -18ºC for 6 months.

4. CONSIDERAÇÕES FINAIS

Ao contrário dos resultados negativos obtidos em estudos passados, atualmente os taninos podem ser utilizados como aditivos em dietas para frangos de corte, apresentando diversos efeitos benéficos para as aves. Os principais benefícios dos taninos foram observados na melhoria do desempenho e da digestibilidade de nutrientes em frangos de corte submetidos a desafio intestinal com *C. perfringens*. Além disso, o tanino de acácia negra atuou melhorando a altura de vilos e a integridade intestinal, com diminuição da oxidação lipídica da carne de frangos. Assim, o tanino apresenta um grande potencial a ser explorado, principalmente como um aditivo substituto ao uso de antibióticos e antimicrobianos convencionais, além de possuir alta disponibilidade para aquisição do Brasil.

O grande desafio para a utilização de taninos na nutrição de monogástricos está na quantidade que pode ser fornecida aos frangos de corte, por isso há necessidade de pesquisas visando encontrar os níveis ideais de tanino que possibilitem explorar ao máximo os efeitos benéficos evitando a ocorrência dos efeitos antinutricionais. A partir dessa informação será possível aprofundar os conhecimentos sobre os mecanismos de ação, ampliar as análises realizadas e avaliar os resultados esperados em diferentes produções avícolas visando a máxima eficiência econômica e zootécnica.

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