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ADIÇÃO DE PRODUTOS NATURAIS NA FORMA ISOLADA (SILIMARINA) E COMBINADA (BLEND FITOGÊNICO) NA DIETA DE FRANGOS DE CORTE CONTAMINADA POR MICOTOXINAS: IMPACTOS SOBRE DESEMPENHO, SAÚDE DAS AVES E QUALIDADE DE CARNE

EDUARDO HENRIQUE ARMANINI

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Orientador (a): Dr. Aleksandro Schafer da Silva

Corientador(s): Dr. Marcel Manente Boiago

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Elaborada por

Eduardo Henrique Armanini

como requisito parcial para obtenção do grau de

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Comissão Examinadora:

Aleksandro Schafer da Silva, Presidente (UDESC)

Dr. Régis Adriel Zanette (UFRGS)

Dr. Tiago Goulart Petrolli (UNOESC)

Chapecó, 16 de março de 2021

RESUMO

Dissertação de Mestrado
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Autor: Eduardo Henrique Armanini
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As micotoxinas são responsáveis por contaminar diversas culturas a campo e no seu armazenamento, apresentando risco a saúde do consumidor, seja ele animal ou humano, elas também são responsáveis por ocasionar percas econômicas incalculáveis em nível mundial. As aflatoxinas e fumonisinas são micotoxinas que normalmente acometem problemas na produção dos animais domésticos. Dentre os problemas, destacam-se as lesões hepáticas e intestinais, imunodepressão e redução nos índices zootécnicos. Portanto, para manter a alta produção avícola do Brasil conhecer aditivos com a capacidade de mitigar os efeitos negativos das micotoxinas é de grande vantagem ao setor. Os compostos fitogênicos são compostos a base de extratos herbais, que possuem uma vasta gama de propriedades medicinais, como ação antioxidante, antimicrobiana e hepatoprotetora, os quais podem ser utilizados de forma isolada ou combinada em forma de blend. Os ácidos orgânicos, taninos, curcumina, óleos essenciais e a silimarina são compostos fitogênicos que se destacam por suas propriedades benéficas quando adicionados na dieta de frangos de corte. O objetivo deste estudo foi avaliar se a adição de produtos naturais de forma isolada (silimarina) ou combinada (ácidos orgânicos, taninos, curcumina, óleos essenciais microencapsulados) na dieta de frangos de corte podem reduzir os impactos negativos ocasionados pelas micotoxinas. Para o estudo foram realizados dois experimentos distintos. Experimento I: Foram utilizados 160 pintainhos de um dia, machos, divididos em 4 tratamentos e 4 repetições, com 10 aves/repetição, sendo eles o tratamento controle, T250, T500 e T100 representando doses de teste de 250, 500 e 1000 mg/kg de ração do blend fitogênico a base de ácidos orgânicos, taninos, curcumina, óleos essenciais microencapsulados, o experimento teve duração de 34 dias. No 22 dia de experimento, as aves foram desafiadas com 500 ppb de aflatoxinas na dieta. Na fase inicial (até os 21 dias), não houve diferença entre os grupos em termos de ganho de peso, consumo de ração ou conversão alimentar. Após a adição da micotoxina na ração, foi observado que doses de 250 a 500 mg/kg minimizaram os efeitos negativos no consumo de ração e na conversão alimentar ocasionados pelas aflatoxinas. Nas aves que consumiram o blend fitogênico foram observados redução dos leucócitos totais devido à menor contagem de heterofilos, linfócitos e monócitos; redução de oxidantes e aumento dos antioxidantes séricos e da carne de peito; redução da contagem de bacteriana na cama; maiores concentrações de ácidos graxos saturados e menores concentrações de ácidos graxos insaturados na carne; maiores relações vilosidades/criptas intestinais. Experimento II: Para o estudo foram utilizados 240 pintinhos de 1 dia, machos, divididos em 4 tratamentos e 4 repetições, com 15 pintos por repetição, os tratamentos foram: ração sem aditivos (NoMyc-NoSil); ração suplementada com 100 mg/kg de silimarina (NoMyc-Sil); ração contaminada com aflatoxinas e fumonisinas (Myc-NoSil); ração contaminada com micotoxina e suplementada com silimarina (Myc-Sil). A contaminação de aflatoxinas foi de 0,05 ppm e de fumonisinas de 20 ppm. O consumo de ração contaminada com micotoxinas atrasou o ganho de peso e aumentou a conversão alimentar; no entanto, a adição de silimarina evitou esses efeitos adversos nos sistemas de produção dos frangos de

corte. Os níveis de ALT foram maiores em frangos de corte Myc-NoSil do que em outros grupos. A ingestão de silimarina em aves saudáveis aumentou os níveis de globulina sérica e reduziu os níveis de albumina, ALT e AST. Os frangos do grupo NoMyc-Sil obtiveram maiores alturas de vilosidades e profundidades de cripta. A luminosidade e a perda de água pelo cozimento foram afetadas pela ingestão de micotoxinas, não ocorreram mudanças na carne das aves que foram suplementadas com silimarina. A soma dos ácidos graxos saturados e monoinsaturados na carne não se alterou entre os tratamentos, ao contrário da soma dos ácidos graxos poliinsaturados mais elevada na carne das aves que consumiram silimarina. Em conclusão a esta dissertação, a utilização dos compostos fitogênicos tanto em forma isolada ou combinada, são potenciais aditivos para utilização na cadeia avícola do Brasil como redutores dos impactos negativos ocasionados pelas micotoxinas, gerando efeitos positivos no desempenho zootécnico e na saúde das aves. A utilização da silimarina na forma isolada, impede o crescimento retardado e melhora a qualidade da carne das aves. A associação dos compostos fitogênicos (ácidos orgânicos + taninos + curcumina + óleos essenciais microencapsulados) potencializa as propriedades biológicas de cada composto, a associação tem alto potencial para melhorar o desempenho dos frangos de corte nos estágios iniciais e de crescimento.

Palavras-chave: Aflatoxina, Estresse oxidativo, Fitobióticos, Fumonisina, Micotoxicoses, Saúde avícola.

ABSTRACT
Master's Dissertation
Programa de Pós-Graduação em Zootecnia
Universidade do Estado de Santa Catarina

ADDITION OF NATURAL PRODUCTS IN ISOLATED (SILYMARIN) AND COMBINED (PHYTOGENIC BLEND) IN THE DIET OF BROILERS CONTAMINATED BY MYCOTOXINS: IMPACTS ON PERFORMANCE, POULTRY HEALTH AND MEAT QUALITY

AUTHOR: Eduardo Henrique Armanini
ADVISER: Aleksandro Schafer da Silva
Chapecó 16 de março de 2021

Mycotoxins are responsible for contaminating various crops in the field and not their storage, which risks the health of the consumer, whether animal or human, they are also responsible for causing incalculable economic losses worldwide. Aflatoxins and fumonisins are mycotoxins that normally affect problems in the production of domestic animals. Among the problems, liver and intestinal injuries, immunosuppression and reduction in zootechnical indexes stand out. Therefore, to maintain high poultry production in Brazil, knowing additives with the ability to mitigate the negative effects of mycotoxins is of great advantage to the sector. Phytogenic compounds are compounds based on herbal extracts, which have a wide range of medicinal properties, such as antioxidant, antimicrobial and hepatoprotective action, which can be used alone or combined as a mixture. Organic acids, tannins, curcumin, essentials and silymarin are phytogenic compounds that stand out for their beneficial properties when made in the diet of broilers. The objective of the study was to evaluate whether the addition of natural products in isolation (silymarin) or combined (organic acids, tannins, curcumin, microencapsulated essential oils) in the diet of broilers can reduce the impacts caused by mycotoxins. Two experiments were carried out for the study. Experiment I: 160 male day-old chicks were used, divided into 4 treatments and 4 repetitions, with 10 birds/repetition, being the control treatment, T250, T500 and T100 representing test doses of 250, 500 and 1000 mg/kg of feed of the phytogenic blend based on acids, tannins, curcumin, microencapsulated essentials, the experiment lasted 34 days. On the 22nd day of the experiment, the birds were challenged with 500 ppb of aflatoxin in the diet. In the initial phase (up to 21 days), there was no difference between the groups in terms of weight gain, feed intake or feed conversion. After adding mycotoxin to the feed, it was observed that doses of 250 to 500 mg/kg minimized the negative effects on feed intake and feed conversion caused by aflatoxin. In birds that consumed the phytogenic blend, a reduction in total leukocytes was observed due to the lower count of lymphocyte and monocyte heterophils; reduced oxidants and increased serum antioxidants and breast meat; reduction of bacterial count in bed; higher certificates of saturated fatty acids and lower Preferences of unsaturated fatty acids in meat; greater villus/intestinal crypt relationships. Experiment II: For the study 240 male 1-day old chicks were used, divided into 4 treatments and 4 repetitions, with 15 chicks per repetition, the treatments were: feed without additives (NoMyc-NoSil); diet supplemented with 100 mg of silymarin per kg (NoMyc-Sil); ration contaminated with aflatoxin and fumonisin (Myc-NoSil); ration contaminated with mycotoxin and supplemented with silymarin (Myc-Sil). The aflatoxin contamination was 0.05 ppm and fumonisin was 20 ppm. The consumption of feed contaminated with mycotoxins delayed weight gain and increased feed conversion; however, the addition of silymarin prevented these adverse effects on chicken production systems. ALT levels were higher in Myc-NoSil broilers than in other groups. The intake of silymarin in birds increased the levels of serum globulin and reduced the levels of albumin, ALT and AST. The chickens of the NoMyc-Sil group had higher villus heights and crypt depths. The luminosity and the loss of

water by cooking were affected by the intake of mycotoxins, there were no changes in the meat of the birds that were supplemented with silymarin. The sum of saturated and monounsaturated fatty acids in the meat did not change between treatments, unlike the sum of the higher polyunsaturated fatty acids in the meat of birds that consumed silymarin. In conclusion to this dissertation, the use of phytogenic compounds, either in isolation or in combination, are additives for use in the poultry chain in Brazil as reducers of the impacts caused by mycotoxins, generating positive effects on zootechnical performance and bird health. The use of silymarin in isolation, prevents delayed growth and improves the quality of poultry meat. The association of phytogenic compounds (organic acids + tannins + curcumin + microencapsulated essential oils) enhances the biological properties of each compound, an association has a high potential to improve the performance of broilers in the early and growing stages.

Keywords: Aflatoxin, Fumonisin, Oxidative stress, Phytobiotics, Poultry health, Mycotoxicosis.

SUMÁRIO

CAPÍTULO I

REVISÃO DE LITERATURA

1.1 AVICULTURA DE CORTE

O Brasil é um dos maiores produtores de proteína animal em âmbito global, no qual foi responsável por produzir 13.845 mil ton de carne de frango no ano de 2020, deste volume 69% foi destinado ao abastecimento do mercado interno e 31% para exportação, conferindo ao Brasil o titulo de maior exportador mundial da proteína (ABPA, 2021). A grande produção avícola brasileira é resultado em grande parte da melhora na gética, sanidade, manejo e nutrição, fatores os quais são cruciais para a produção avícola de corte (Gonzalez et al., 2012).

Com a grande produtividade do setor avícola, a demanda por alimentos seguros e de alta qualidade nutricional é alta, porem os grãos como o milho e a soja, os quais apresentam os maiores níveis de inclusão perante os grãos nas rações, podem apresentar contaminações advindos do campo e da sua armazenagem pela sua alta suceptibilidade aos microrganismos, como contaminações de carater fúngico (Reges et al., 2016). Dos grãos utilizados para a obtenção das rações destinadas a produção de frangos de corte, estimasse que a safra 2020/21 tenha um total de 106,4 milhões de toneladas de milho e 135,4 milhões de tonadas de soja produzidos (CONAB, 2021). A perspectiva de produção de ração para avicultura de corte para 2021 é de 34,6 milhões de toneladas, 0,2 milhões de toneladas a mais que o produzido em 2020 (SINDIRAÇÕES, 2021). Estima-se que cerca de 25% das culturas alimentícias do mundo tenham sua produtividade e qualidade diminuídas por contaminações fúngicas (FAO, 2001).

O Brasil por apresentar clima predominante quente e úmido, propicia um ambiente ideal para que os grãos produzidos tenham suceptibilidade para contaminações fúngicas (Cruz, 2010). O milho, por apresentar características como a umidade e a alta disponibilidade de nutrientes, é umas das culturas mais afetadas por contaminações fúngicas e de micotoxinas (Reges et al., 2016). Alimentos com umidade acima de 14% propiciam a colonização de fungos produtores de micotoxinas, como o teor de umidade em que o milho é colhido situa-se entre 18 a 24%, erros acometidos no processo de secagem dos grãos podem impactar diretamente no desenvovimento fúngico durante o armazenamento do alimento (Oliveira, 2016; Back, 2010; Hirsh e Zee, 2009).

1.2 MICOTOXINAS

As micotoxinas são oriundas do metabolismo secundário de fungos filamentosos, são responsáveis por contaminar diversas culturas de alimentos no campo e também em seu armazenamento, visto a sua alta toxidez e capacidade de acumulo em variados tecidos, apresentam riscos a produtividade animal e também a saúde humana (Bánáti et al., 2017; Kongkapan et al., 2015; Tardieu et al., 2019). Dentre as micotoxinas as mais frequentemente encontradas nos alimentos e produtos agrícolas destacam-se as aflatoxinas, fumonisinas, ocratoxinas e tricotecenos (deoxinivalenol, T-2, HT-2, diacetoxyscirpenol e nivalenol, fusarenon-X) (Thanushree et al., 2019).

A alta resistência das micotoxinas, resulta na sua permanência na cadeia alimentar contaminando uma ampla quantidade de produtos agrícolas na sua colheita e no armazenamento, também sendo responsável por contaminar rações e diversos alimentos no seu armazenamento (Prado, 2014). As micotoxinas são geradas em condições limitadas de substrato, temperatura e umidade, durante períodos de estiagem, acompanhados de temperaturas elevadas e baixa umidade os grãos de milho ficam mais sujeitos a proliferação fúngica, pois se tem um maior transporte dos esporos do fungo até a espiga, por meio do vento e dos insetos que danificam a estrutura dos grãos possibilitando a sua colonização e posterior contaminação pelas micotoxinas (Back, 2010).

As micotoxicoses são capazes de prejudicar a imunidade, causar anormalidades endócrinas e carcinogênese, dependendo de sua concentração e ocorrência (Yu et al., 2018). As toxicidades geradas pela ingestão incluem estresse oxidativo, apoptose, inflamação e lesão hepática aguda ou crônica e redução dos índices zootécnicos (Li et al., 2021; Dazuk et al., 2020; Sousa et al., 2020). Alem de percas em produção e os voltados a sanidade dos animais, também foi observado alteração nos perfis de ácidos graxos em filés, aumentando os níveis de ácidos graxos saturados e diminuindo os ácidos graxos poliinsaturados, está alteração afeta de modo negativo o seu consumo, impactando diretamente na qualidade do produto gerado e a aceitabilidade do mercado consumidor (Baldissera et al., 2019).

As micotoxinas não impactam somente na saúde animal e humana, elas também são responsáveis por ocasionar percas econômicas incalculáveis em nível mundial. Estimasse que a contaminação pelas aflatoxinas no Estado Unidos da América na indústria do milho pode chegar à casa de 52,1 milhões a 1,68 bilhões de dólares ao ano (Mitchell et al., 2016). Visto a sua distribuição, atuar na prevenção da formação das micotoxinas é quase

impossível em âmbito mundial, porem agir na redução do seu impacto após a absorção é possível (Oliveira et al., 2018).

1.2.1 Aflatoxina

As aflatoxinas, produzida principalmente pelo *Aspergillu*s spp. em especial pelas espécies *A. flavus*, *A. parasiticus*, *A. nomius*, *A bombycis*, *A. pseudotamarii* e *A. ochraceoroseus*, é uma micotoxina que possui uma gama de atividades biológicas, como teratogenicidade, mutagenicidade e carcinogenicidade (Mclean e Dutton, 1995; Prestes et al., 2019). Conforme Trabulsi e Alterthum (2005), os principais compostos da aflatoxina são B1, B2, B3, G1, G2, M1 e M2. Os tipos das aflatoxinas são diferenciados com base na fluorescência sob luz ultravioleta, diververgindo de azul a verde, no qual o tipo B referense a cor azul (blue) e o do tipo G a coloração verde (Green) (Prestes et al., 2019).

Segundo a Agência Internacional de Pesquisa do Câncer (International Agency for Research on Câncer – IARC, pelo potencial carcinogênico que as micotoxinas apresentam, elas são classificadas conforme seu potencial carcinogênico, no qual as aflatoxinas pertecem ao grupo 1 o qual apresenta maior potencial, seguido pelas fumonisinas e ocarotoxinas pertencentes ao gurpo 2B, os tricotecenos classificados como grupo 3 (IARC, 2010). aflatoxicoses quando aguda apresenta grande risco a saúde humana, podendo levar ao óbito, após 6 horas da ingestão da micotoxina inicia-se o quadro clínico, gerando sintomas como severa depressão, fezes sanguinolentas, hipertermina e tremores musculares, quando apresentada cronicamente acarreta potologias com aparecimento tardio, como câncer e imunossupressão (Pereira e Dos Santos, 2011)

As aflatoxinas e a fumonisinas possuem a característica de lesionar principalmente os tecidos hepáticos, visto que a sua biotransformação ocorre no fígado. As aflatoxinas, em especial a B1, ao serem ingeridas em conjunto com os alimentos são absorvidas pelo trato gastrointestinal, no fígado vai ocorrer a sua briotransformação, no qual é ativada pelo citocromo p450 presente na fração do citosol e microssômica da célula hepática, formando um reativo intermediario denominado de AFB1-8,9-epóxido (Baertschi et al., 1988; Pereira e Dos Santos, 2011). O reativo pode reagir com o DNA formando dutos, estes dutos intitulados de AFB1-DNA, o qual é considerado a forma de iniciação tumorigênese (Choy, 1993). A formação dos dutos provocam alterações no códon 249, o que gera a substituição da base nitrogenada da guanina por timina, esse códon integra o gene supressor p53, com a alteração a proliferação celular é aumentada iniciando a foração de tumores, sendo característico em especial do carcinoma hepático (Ferreira et al., 2007). A aflatoxina B1

necessita da sua ativação para manifestar seu efeito carcinogênico, sendo classificada como um pró-neoplasto (Pereira e Dos Santos, 2011)

As micotoxinas tem característica de liberarem radicais livres, esses responsáveis por iniciar a peroxidação lipídica, visto que todas as células possuem em sua membrana ácidos graxos, no momento em que acontece a peroxidação lipídica a integridade da membrana celular diminui, ocorrendo a apoptose celular (Shen et al., 1994; Rastogi et al., 2001; Fraschini et al., 2002). Antioxidantes como a vitamina E, podem ser opções para ajudar a reduzir a peroxidação lipídica e consequentemente seus impactos, protegendo órgãos vitais para o corpo, como o fígado (Shen et al., 1994).

A contaminação de 2 ppb de aflatoxinas em rações destinas a frangos de corte, são suficientes para diminuir índices zootécnicos das aves, como o aumento da taxa de mortalidade, diminuição do peso corporal e aumento da conversão alimentar (Solis-Cruz et al., 2019). Wang et al. (2018), observaram autofagia hepatocelular em frangos de corte, alimentados com ração contaminada por aflatoxina B1. Segundo estudo de Vaccari et al. (2017), observaram correlação de lesões de lingua grau 1 com a quantidade de micotoxina presente na ração de frangos de corte.

1.2.2 Fumonisina

As fumonisinas produzidas em especial por fungos do gênero *Fusarium* ssp., em sua maioria pelo *F. verticillioides*, *F. proliferatum* e *F. moniliforme*, seus compostos são A1, A2, A3, B1, B2, B3, B4, AK1, C1, C3, C4, P1, P2, P3, PH1a, PH1b (Bánáti et al., 2017; Revolledo e Ferreira, 2009; Pozzi et al, 2002), sendo que B1 é o membro mais toxico das fumonisinas, visto sua capacidade de interferir no metabolismo dos esfingolipios(Liu et al., 2019).

As fumonisinas ao contrário das aflatoxinas, tem seu potencial carcinogênico ligado a intervenção na biossíntese dos esfingolipios e não a interação da micotoxina com o DNA. A fumonisina B1 atua principalmente na interrupção do metabolismo dos esfingolipideos e na indução do estresse oxidativo, o que ocasiona danos no tecido hepático e no renal (Liu et al., 2019). A intervenção da síntese dos esfingolipideos pelas fumonisinas atua no bloqueio da síntese de ceramida, os quais são moléculas de esfingosina ou esfinganina ligadas a um acido graxo, a semelhança estrutural das fumonisinas com a esfingosina e a esfinganina faz com que haja uma competição pelo sitio de ligação da enzima Cers, o que bloqueia a síntese de ceramina pela esfinganina e a reversão da esfingosina em ceramina (Wanget al., 1991). Assim a um aumento nas concentrações de esfinganina e diminuição dos níveis de esfingolipios, a esfinganina vai ser bioconvertida em esfinganina-1 fosfato

(S1P), o aumento de S1P vai elevar a trandução de sinal de crescimento e divisão celular (Spiegel e Merril, 1996; Taha et al., 2006; Turner et al., 1999). Uma vez que os esfingolipideos são essenciais para a composição da membrana e a comunição celular, a inibição acaba ocasionando danos à atividade celular (Merrill et al., 1993).

As fumonisinas são responsáveis por induzir efeitos nefrotóxicos, genotóxicos, neurotóxicos, carcinogênicos, prejudicando também o sistema imunitário e o trato intestinal dos animais (Masching et al., 2016). A presença das fumonisinas pode aumentar a susceptibilidade de infecções parasitárias em frangos de corte, como a coccidiose (Grenier et al., 2016). Lesões de língua grau 3 (altamente severos) estão correlacionados com a presença de contaminações em rações de frangos de corte com fumonisinas, vomitoxina e T-2 (Vaccari et al., 2017). A B1 quando combinada com outras toxinas do funsarium, tem seus efeitos tóxicos agravados, como a zearalenona, desoxinivalenol, nivalenol, T-2 e micotoxinas de outros fungos, como as aflatoxinas (Corrêa et al., 2018; Liu et al., 2019). A B1 também é um agente teratogênico, o qual pode perturbar o desenvolvimento do embrião ou feto, a toxidade é transmitida da mãe ao embrião ou feto, o que causa defeitos no tubo neural levando a morte do mesmo (Lumsangkul et al., 2019). As fumonisinas também são capazes de levar a lesões na córnea (Sharma et al., 2019).

O tratamento adequado dos efeitos tóxicos das fumonisinas, com compostos capazes de adsorver ou reduzir os danos aos tecidos alvos são de grande interesse da cadeia de produção de proteína animal, visto sua capacidade de causar diversos danos nos mais variados tecidos, impactando tanto na saúde quanto na produtividade dos animais.

1.3 ADITIVOS

Os aditivos utilizados na produção animal, possuem os mais variados objetivos, o seu efeito farmacológico vai depender muito do tipo de aditivo utilizado e das moléculas presentes. Um dos aditivos que tem sido utilizados na produção avícola são os fitogênicos, os quais são derivados de uma grande variedade de ervas e especiarias, apresentando os mais variados ganhos, como aumento da palatabilidade, ação antioxidante, antibiótica, antifúngica e melhoradores de desempenho zootécnico (Windisch et al,. 2007).

A utilização de produtos naturais tem aumentado, visto a necessidade de substituição dos antibióticos como promotores de crescimento na produção animal, em razão a políticas de restrição de uso nacionais e restrições impostas pelos mercados consumidores (Stella et al., 2020; Cardinal et al., 2020). Os efeitos dos aditivos fitogênicos na produção animal ainda não são totalmente compreendidos, visto a gigantesca quantidade de moléculas existentes. Segundo Orlowski et al. (2018), a utilização de aditivos fitogênicos na produção de frangos de corte

melhora a qualidade da carne em nível sensorial, via modulações relacionadas ao estresse e aos antioxidantes.

Conforme apresentado no experimento de Pirgozliev et al. (2019), frangos de corte suplementados com um composto fitogênico a base de 5% de carvacrol, 3% de cinamaldeído e 2% de oleorresina de capsicum, melhorou as variáveis gerais dos índices zootécnicos, retenção de energia e nutrientes, assim como melhorou a expressão de citocinas intestinais. Hazrati et al. (2020), observaram que a suplementação de óleo essencial de ajwain (*Trachyspermum ammi*), probióticos e mananoligossacarídeos tem efeito positivo no crescimento de codornas japonesas, e a suplementação com óleos essenciais de ajwain e endro (*Anethum graveolens*) reduziram a peroxidação lipídica da carne e aumentaram os índices morfométricos intestinais. Segundo experimento efetuado por Reis et al. (2018), frangos de corte suplementados com uma combinação comercial de óleos essenciais, como carvacrol, timol e aldeído cinâmico, pode ser uma alternativa para substituição dos antibióticos como promotores de crescimento, sem perder em nível de produtividade zootécnica das aves e atuando na redução da carga bacteriana ambiental gerada.

1.3.1 Ácidos orgânicos

Os ácidos orgânicos estão sendo cada vez mais utilizados como fontes alternativas para os antibióticos utilizados como promotores de crescimento para frangos de corte, visto sua gama de atividades antimicrobianas, dentre os ácidos orgânicos temos destaque para o ácido cítrico, fumárico, sórbico e málico (Yang et al., 2018; Schnurer & Magnusson, 2005).

Os ácidos orgânicos de cadeia curta, como ácidos fórmico, acético, propiônico e isobutírico possuem a capacidade de inibir o crescimento fúngico nos grãos durante o armazenamento e consequentemente a produção de micotoxinas (Vieira, 2003; Sherwood & Peberdy, 1974; Schnurer & Magnusson, 2005). As bactérias ácido láticas possuem a capacidade de produção de ácidos orgânicos, como o ácido lático, acético, fórmico, propiônico, butírico e outros, o que as conferem a capacidade de ação antifúngica e na redução da produção de aflatoxinas (Guimarães et al., 2018). Segundo apresentado por Yang et al., (2017), a adição de ácido sórbico, ácido fumárico e timol na dieta de frangos de corte podem substituir o uso de enramicina como promotor de crescimento, pois apresentam a capacidade de aumentar a eficiência e a morfologia intestinal, assim como a atividade das enzimas digestivas.

O acido tânico extraído da *Acacia mearnsii*, é uma alternativa para suprimir o desenvolvimento de patógenos na avicultura de corte, agindo como possíveis substitutivos a utilização de antibióticos, porem podem apresentar fatores antinutricionais (Mannelli et

at., 2019; Harvey, 2006). Segundo apresentado no trabalho efetuado por Mannelli et at. (2019), a suplementação de tanino de castanha e monoglicerídeos SN1 é eficiente na redução da proliferação de *C. perfringens*, *Salmonella typhymurium*, *Escherichia coli*, *Campylobacter jejunie* em frangos de corte. Os taninos apresentam valiosos potencializadores de respostas imunes humorais em galinhas e podem ser usados como protetores contra a coccidiose (Kaleem, 2014). O ácido tânico é utilizado na avicultura de corte como modulador da microbiota intestinal, melhorando o desempenho zootécnico, o qual é uma alterativa para a substituição dos antimicrobianos como promotores de crescimento (Petrolli et al., 2019).

1.3.2 Óleos essenciais

Os extratos das plantas, como os óleos essenciais, possuem uma vasta gama de efeitos farmacológicos, sendo utilizados como medicamentos para várias enfermidades, porem seus efeitos dependem da composição química e da sua concentração (Marchese et al., 2017). A suplementação de óleos essenciais na dieta de frangos de corte, ajuda a melhorar os índices zootécnicos, como no ganho de peso diário e conversão alimentar (Kim et al., 2016). Segundo Restuccia et al., (2019), os óleos essenciais de limão, laranja e bergamota possuem capacidade de inibir o crescimento fúngico, como o *A. flavus* e podendo inibir a formação da aflatoxina b1. O eugenol em conjunto de outros óleos essenciais, como o carvacrol e mentol, possuem a capacidade de interferir nas funções da membrana e na supressão de toxinas, enzimas, fatores de virulência e no biofilme bacteriano e fúngico (Marchese et al., 2017).

O carvacrol é um isopenil fenol constituinte natural de plantas aromáticas e seus óleos essenciais, é encontrado principalmente no orégano (*Origanum vulgare*), a sua utilização é aprovada pelo FDA (Food and Drug Administration) (De Vincenzi et al., 2004). O carvacrol possui capacidade de inibiação do crescimento de patógenos de origem alimentar (Kim et al., 1995). O carvacrol possui a característica de ser hidrofóbico, o que permite se acumular na membrana celular, além disto possui sua capacidade de ligação de hidrogênio e de libreação de prótons induzem a modificação na membrana em nível de permeabilidade, o que ocasiona a morte e inibição do crescimento nos microorganismos (Arfa et al., 2006). Segundo estudo efetuado por Fukayama et al. (2005), a inclusão de óleo essencial de orégano como aditivo substituto dos promotores de crescimento convencionais, não ocasiona diferenças estatiticas em nível de desempenho, qualidade da carne e saúde intestinal. A inclusão de carvacrol e cinamaldeico microencapsulados em dietas de frangos de corte pode substituir a utilização de virginiamicina como promotor de

crescimento, sem ter perdas no desempenho zootécnico, rendimento de carcaça e dos seus cortes, além de apresentar melhora na saúde intestinal das aves e qualidade da carne das aves (Bosseti, 2020).

O timol é um terpeno, presentes nos óleos essenciais de tomilho (*Thymus vulgaris*) com diversas ações farmacológicas, como antimicrobiana, antifúngica, antioxidante, antiinflamatória, antiviral e cicatrizante (Najafloo et al, 2020; Souza et al, 2020; Santana et al, 2018). A suplementação de óleos essenciais de timol (Thymus vulgaris), eugenol (Cinnamomum spp.) e piperina (Piper spp.) na dieta de frangos de corte aumenta o desempenho, aumentando a população ileal de lactobacillus e redução da população de E. coli, além de reduzir a excreção de amônia (Park & Kim, 2018).

A canela (*Cinnamomum zeylanicum*) é uma planta da família das Laureaceae, o qual de sua casca é estraido o óleo essencial cinamaldeído (Souza et al, 2020). A canela é utilizada na culinária e na medicina tracional, possui propriedade antialérgicas, antimicrobianas, antifúngicas, antioxidante, antiinflamatória e anticarcinogenica, empregada como alternativa aos pediculicidas sintéticos comumente usados por não apresentar toxidade na sua utilização (Wong et al, 2014). Segundo estudo efetuado por Souza et al. (2020), a suplementação do óleo essencial de canela em 78 ppm em frangos de linhagem caipira no período de criação de 70 dias pode substituir a utilização dos antibióticos na produção, não ocasionando perdas nos índices zootécnicos das aves. Segundo Salah et al. (2019), os óleos essenciais do alho e da canela possuem a capacidade de ação anti-carcinoma hepatocelular, sua utilização é útil para controlar a proliferação cancerosas no fígado.

1.3.3 Curcumina

A curcumina é um dos constituintes do açafrão (*Curcuma longa*), geralmente utilizado como corante e antioxidante, podendo ser utilizada como promotor natural de crescimento quando suplementada em dietas de frangos de corte (Khan et al., 2012). A curcumina tem capacidade de ação antioxidade relacionada a capacidade de doação de hidrogênio, o qual reage com espécias reativas de oxigênio (ERO) prevenindo danos a célula pela oxidação (Santiago et al., 2015). A suplementação de curcumina e ácido acetilsalicílico, melhoram o desempenho, índices bioquímicos séricos e o efeito antioxidante de frangos de corte quando desafiados a estresse térmico positivo (Salah et al., 2019).

A curcumina induz a reução significatica do colesterol total, colesterol-LDL, colesterol VLDL e triacilglicerol, possivelmente pelo aumento da atividade da enzima 7α-hidroxilase, aumentando a conversão do colesterol em sais bileares consequentemente reduzindo os seus níveis sanguíneos (Da Silva et al., 2001). Segundo o experimento de Xei et al., (2019), a curcumina tem papel importante no decréscimo da deposição de gordura abdominal, no perfil lipídico e plasmático em frangos de corte. Em um estudo efetuado por De Souza et al. (2017), a suplementação de açafrão em pó na dieta de frangos de corte possui efeito análogo ao antimicrobianos utilizados como promotores de crescimento, em desempenho zootécnico, rendimento de carcaça e cortes, assim como saúde intestinal.

A utilização de subtancias com ação antioxidante como a curmina pode ser um meio de reduzir os impactos ocasionados pelas micotoxinas. A curcumina alivia os efeitos negativos da aflatoxina b1, inibindo a biotransformação das aflatoxinas em metabólicos epóxidos e reverte os danos hepáticos causados (Muhammad et al., 2017). Alem da ação nos efeitos das micotoxinas, a curcumina tem capacidade de inibir o desenvolvimento fungico e na produção das micotoxinas, como a redução no desenvolvimento do *A. parasiticus* e na sua produção de aflatoxinas (Soni et al., 1992).

1.3.4 Silimarina

A silimarina, um flavolignano presente em sementes e frutos de *Silybum marianum* ou Cardo de leite da Família das Asteraceas, a qual é uma planta anual, presente na região mediterrânea, Europa Central, América do Sul e Austrália, utilizado há mais de 2000 anos como rémédio a base de plantas para uma vasta quantidade de doenças (Ramellini e Meldolesi, 1976; Cunha et al., 2003; Kazazis et al., 2014). Dentre as características da silimarina, destaca-se a sua capacidade de antioxidação, regulação e estabilização da permeabilidade dos hepatócitos, promotor da síntese de RNA ribossômico e inibidor da transformação de hepatócitos estrelados em miofibroblastos (Fraschini et al., 2002).

A silimarina é capaz de reduzir o acúmulo de gordura intra-hepática, inflamação lobular, balonamento e gordura sérica, assim como melhorar a homeostase, visto sua capacidade de modulação dos genes envolvidos no metabolismo lipídico, assim como no controle do estresse oxidativo (Tsai et al., 2017). A silimarina atua como inibidora da enzima aldose redutase o que reduz o acumulo de água intracelular e dano aos tecidos auxiliando na prevenção catarata e da neuropatia diabética, além disto a silimarina tem a capacidade de evitar a diferenciação de pré-adipócitos em adipócitos, com subsequente redução na concentração de triglicerídeos, o qual atua como marcador de acúmulo de lipídeos e hipertrofia dos adipócitos (Kazazis et al., 2014).

Conforme apresentado por Din et al. (2012), frangos alimentados com 10g de sementes de *Silybum marianum* por kg de ração, foram capazes de reduzis os efeitos negativos nos índices zootécnicos ocasionados pela contaminação de 600 ppb de aflatoxina B1. Segundo Rastogi et al. (2001), ratos contaminados com 2000 ppb de aflatoxina B1 suplementados com 20000 ppb de silimarina, apresentaram parâmetros de peroxidação lipídica idêntica a ratos do grupo controle, indicando que a silimarina é um forte redutor dos impactos ocasionados pelas aflatoxinas em nível hepático. O experimento de Jahanian et al. (2016), mostrou que a silimarina quando suplementada em 500 ppm a pintainhos que receberam ração contaminada com 0,5 e 2 ppb de aflatoxinas, reduziu os efeitos ao tecido intestinal, aumentando a área de superfície absorvente do epitélio jejunal, quando comparado a pintainhos que não receberam a suplementação. Segundo o trabalho efetuado por Fakhar-ud-Din et al. (2012), os danos hepáticos ocasionados pela peroxidação lipídica proveniente da contaminação de ocratoxina em rações de aves White Legorn é diminuído quando suplementado na ração silimarina e vitamina E.

1.4 OBJETIVOS

1.4.1 Geral:

Averiguar se a adição de produtos naturais na forma isolada (silimarina) e combinada (blend fitogênico) na dieta de frangos de corte contaminada por micotoxinas tem impacto sobre o desempenho, saúde e qualidade da carne das aves.

1.4.2 Específicos:

- Verificar se a suplementação de silimarina de forma isolada na dieta de frangos de corte inibe os efeitos negativos causados pelas micotoxinas no desempenho zootecnico e saúde das aves;
- Averiguar se a suplementação de ácidos orgânicos, taninos, curcumina e óleos essenciais (blend fitogênico) reduzem os efeitos das micotoxinas no desempenho zootecnico e saúde em frangos de corte;
- Avaliar se a qualidade de carne dos frangos de corte é alterada com a suplementação de silimarina e com o blend fitogênico.

CAPÍTULO II

ARTIGO E MANUSCRITO

Os resultados desta dissertação são apresentados na forma de um artigo e um manuscrito com suas formatações de acordo com as orientações das revistas as quais foram publicados e submetidos:

Artigo I – Inclusion of a phytogenic bend in broiler diet as a performance enhancer and anti-aflatoxin agent: impacts on health, performance, and meat quality

Publicado: Research in Veterinary Science

Manuscrito I - Protective effects of silymarin in broiler feed contaminated by mycotoxins: growth performance, meat antioxidant status, and fatty acid profiles

Submetido: Tropical Animal Health and Production

2.1 ARTIGO I

Inclusion of a phytogenic bend in broiler diet as a performance enhancer and anti-aflatoxin agent: impacts on health, performance, and meat quality

Eduardo H. Armanini[a], Marcel M. Boiago[a, b], Paulo Vinícius de Oliveira[a], Eduardo Roscamp[a], João V. Strapazzon[a], Andressa G. de Lima[a], Priscila M. Copetti[c], Vera M. Morsch[c], Fernanda C. de Oliveira[d], Roger Wagner[d], Janio M. Santurio[e], Gilnéia da Rosa[a], Aleksandro S. Da Silva[a, b*]

[a] Graduate Program in Animal Science, Universidade do Estado de Santa Catarina (UDESC), Chapecó, SC, Brazil.

[b] Department of Animal Science, UDESC, Chapecó, SC, Brazil.

[c] Graduate Program in Toxicological Biochemistry, Universidade Federal de Santa Maria (UFSM), Santa Maria, Brazil.

[d] Department of Food Science, UFSM, Santa Maria, Brazil.

[e] Department of Microbiology and Parasitology, UFSM, Santa Maria, Brazil.

*Corresponding author: aleksandro.silva@udesc.br

Abstract

The objective of this study was to determine whether a phytogenic blend (PB), formulated based on organic acids, tannins, curcumin, and essential oils, could replace the antimicrobials commonly used as growth promoters in the poultry industry without compromising zootechnical performance, health, or meat quality. In addition, our goal was to report the anti-aflatoxin effect of this phytogenic blend. Four treatments were used: TC, or control; T250, T500, and T1000, representing test doses of 250, 500, 1000 mg PB/kg of feed, respectively, or a 34-day experiment (initial and growth phases). On day 22 of the study and age of the birds, 500 ppb of aflatoxin was included in the diet to represent an intestinal challenge and to evaluate the growth-promoting effects of PB. In the initial phase (up to 21 days), there were no differences between groups in weight gain, feed intake, or feed conversion. After adding an aflatoxin-contaminated feed, doses of 250 and 500 mg/kg minimized the adverse effects on

feed consumption and feed conversion caused by aflatoxin; but 1000 mg/kg did not differ between groups. In birds that consumed PB (T250, T500, and T1000) compared to the control, there were the following changes: 1) lower counts of heterophiles, lymphocytes, and monocytes; 2) lower lipid peroxidation and high non-protein thiols levels in breast meat; 3) lower bacteria counts in broiler litter; and 4) lower ALT levels. Greater intestinal villus/crypt ratios were observed at T250 and T500. The dose of 250 mg/kg reduced saturated fatty acids and increased unsaturated fatty acids. The chemical-physical composition of the meat did not differ between treatments. The findings suggest that the addition of a PB has a high potential to improve performance for chickens in the growing stage and minimize the adverse effects of aflatoxicosis.

Keywords: aflatoxicosis, broilers, additives, phytobiotics, meat quality.

1. INTRODUCTION

Antibiotics are frequently used as growth promoters in poultry production. These additives are associated with productivity gains and suppression of damage caused by microbes, thereby increasing the effectiveness of the feeding and the associated zootechnical indexes (Kostadinović et al., 2001; Lorençon et al., 2007; Bezerra et al., 2017). Nevertheless, this use of antibiotics production is partially or totally banned in several countries, including Brazil, where there is already a plan for a total reduction in antibiotic use in animal production as a growth promoter. The antibiotic ban is mainly aimed at decreasing bacterial multidrug resistance to antibiotics in humans (Shrestha et al., 2017), and many antimicrobial residues have been found in food (Diarra and Malouin, 2014). For these reasons, alternative and natural compounds have gained market share as replacements for antibiotics. In particular are phytogenic additives that are formulated from compounds derived from plants and their parts, including extracts and essential oils; these have potent antimicrobial action, in addition to antioxidant effects that have overall benefits for animal health (Oladeji et al., 2019; Salah et al., 2019; Mannelli et al. 2019).

For many years, researchers have tested natural products in animal feed in isolation; however, in recent years, commercial products have gained prominence, mainly in the form of phytogenic blends, that is, products formulated using ingredients that have synergism and enhance the broad-spectrum antimicrobial effect, and that often have functional and nutritional effects. Although our primary objective of this experiment was to determine if the (PB) could replace conventional antimicrobials, at this moment, based on our results, we would like to

draw attention to the anti-mycotoxin effects of PB. There are many studies with phytogenics; however, there are few studies of blends as an alternative to minimize the effect of mycotoxin.

Essential oils have a wide range of pharmacological effects that allow them to be used as treatments for various diseases (Marchese et al., 2017); in particular, there are applications in the poultry chain: a) improvement zootechnical parameters (Kim et al., 2016), regulation of the intestinal flora of broilers that helps decrease ammonia excretion (Park and Kim, 2018), and inhibiting fungal proliferation, thereby controlling aflatoxin in animal feed (El-Aziz et al., 2015). Organic acids also have antimicrobial activity and the ability to protect intestinal integrity against pathogenic lesions (Yang et al., 2018; Schnurer and Magnusson, 2005; Calaça et al., 2019), which makes this additive extremely attractive as a performance enhancer in the poultry diet. Turmeric extract has gained space in the animal feed market due to positive results when used as a natural growth promoter for broilers (Khan et al., 2012), reducing inflammatory processes (Almeida et al., 2018), exerting intense antioxidant action (Liu et al., 2017), and protecting the liver (Uzunhisarcikli and Aslanturk, 2019).

We hypothesized that combining these and other components could generate a multifunctional commercial product that functions as a substitute for antibiotics in the feed. The results showed that it also has anti-aflatoxin action; the latter is a silent problem that damages poultry farms. Therefore, in the present study, we determined whether the inclusion of a PB formulated with organic acids, tannins, curcumin, and essential oils can replace antimicrobials commonly used as growth promoters in the poultry industry, without compromising zootechnical performance, health, or meat quality. In addition, based on the results, it became our goal to report the anti-aflatoxin effect of this PB added to the chicken diet.

2. MATERIALS AND METHODS

This project was approved by the Committee for the Use of Animals in Research (CEUA) of the State University of Santa Catarina (UDESC) under protocol number 7562021219. It complies with the rules of the National Council for the Control of Animal Experimentation (CONCEA).

2.1. Products and mycotoxin

2.1.1. Commercial product

We used a blend with carvacrol, thymol, and cinnamaldehyde (pure components - Sigma-Aldrich), Curcuma longa extract, as well as tannic acid (extracted from Acacia mearnsi),

citric acid, fumaric acid, sorbic acid, and malic acid (pure components). This commercial product (Acidosan®, Konkreta, Brazil) was defined as the PB.

2.1.2. Aflatoxin

Aflatoxins were produced by fermentation in rice, converted under constant agitation and at controlled temperature. The NRLL 2999 strain of *Aspergillus parasiticus* was used, according to the method described by West et al. (1973). After autoclaving with the valve open, the material was dried in a forced ventilation oven and ground in a laboratory mill equipped with a 1-mm sieve. The concentration of aflatoxins was subsequently determined by HPLC (Thorpe et al., 1982).

2.2. Animals, accommodation and food

We used 160 male day-old chicks of the Cobb-500 lineage, acquired from a commercial hatchery located in Chapecó, SC, Brazil. Upon reaching the experimental shed, the birds were immediately weighed and distributed in a completely randomized design, including four treatments, four replicates per treatment, and ten birds per repetition. The birds were kept in pens measuring 1.88 m2, heated by a gas bell for the first 14 days of life. We provided hanging feeders appropriate for the age of the chicks and water consumption with nipple-type drinkers for feeding. The light program used during the experiment was based on the lineage manual.

The basal feed used in the present study was formulated based on crushed corn and soybean meal (Table 1), according to the nutritional requirements for broilers described in the Brazilian poultry tables (Rostagno et al., 2017). All groups received the same basal diet. The treatments formed were as follows: TC, control diet containing conventional performance enhancers (80 mg/kg of salinomycin + 2 mg/kg flavomycin); T250, T500, and T1000 corresponded to test treatments with phytogenic product at 250 mg/kg, 500 mg/kg, and 1000 mg/kg, respectively. The experimental period was 34 days when the experimental food was supplied according to the production phase (initial and growth). Water was provided ad libitum.

To create a challenge for birds, at 22 days of age, beginning of the growth phase, the diet of all treatments was contaminated with aflatoxin at 250 µg/kg. Aflatoxin was chosen for the challenge because it is frequently found in poultry feed, causing intestinal damage. There is a hypothesis that the PB can directly or indirectly inhibit the action of mycotoxin.

2.3. Zootechnical data

The animals were weighed on days 1, 10, 21, and 34 of life. Feed intake (FI) (g/animal/day) was calculated as the difference between the weight of food provided at the

beginning and the weight of leftovers obtained at the end of the period. Based on these zootechnical data, weight gain (WG) and feed conversion (FC) were calculated; that is, the WG was determined using the formula: (final weight – initial weight)/number of birds per repetition. FC was calculated using the formula: the amount of food consumed/live weight of the animals.

2.4. Sample collection

Eight blood samples were collected per treatment on day 34 of the experiment. Blood was collected via puncture of the branchial vein, allocated into two tubes, one containing EDTA as an anticoagulant for analysis of blood count and antioxidant enzymes and another tube without anticoagulant to obtain serum. Blood and serum were separated after centrifugation at 3500 rpm for 10 minutes, stored at –20 °C until biochemical analysis.

On day 34, eight birds were also slaughtered per treatment (two per repetition). The slaughtered animals' feed was removed before slaughter (10 hours), carried out in a room near the aviary for this purpose. The ethics committee approved the slaughter methodology on the use of animals at the institution, characterized by electronarcosis followed by cervical dislocation. Then, the skin was removed along with the feathers, leaving the carcass exposed. Liver and intestine fragments were collected for histopathological analysis, and the pectoralis major muscle was analyzed for fatty acid profile, meat quality, and composition. The left breast was used for the physicochemical composition, while the oxidative status and fatty acids were performed in the right breast. The muscle samples used were free of skin and joints, and cuts of muscle fragments were standardized for all treatments.

2.5. Histopathology

Liver and intestinal samples (jejunum) from the eight birds were preserved in vials containing 10% formaldehyde. It is important to note that the location (fragment) of the intestine collected was standardized (25 cm from the end of the duodenum), to obtain similar and representative samples from the same segment. Slides with histological sections were made and stained with hematoxylin and eosin. Villus height and crypt depth were determined according to Caruso and Demonte (2005) and described in detail by Galli et al. (2020).

2.6. Blood analysis

2.6.1. Leukogram

Leukocyte counts were performed according to the methodology described by Thrall et al. (2015), using a Neubauer chamber. For the leukocyte differential, blood smears were made,

stained with commercial dye (Rapid Panotic), and the differential was determined using an optical microscope (1000x).

2.6.2. Serum biochemistry

Serum levels of total protein and albumin were measured using commercial analysis kits (Analisa®), using a semi-automatic biochemical analyzer BioPlus (Bio-2000®). The globulin concentration was calculated using the following formula: globulins = total protein – albumin.

2.6.3. Serum oxidant/antioxidant status

The liver was used for oxidative and antioxidant analyses. The tissue was placed in 10 mM Tris-HCl solution, pH 7.4 for analysis of reactive oxygen species (ROS), reactive to thiobarbituric acid (TBARS), protein thiols (PSH), non-protein thiols (NPSH) and glutathione S-transferase (GST). The samples were homogenized using a Potter glass homogenizer with specific buffer solution, and then centrifuged at 10,000 rpm at 4 °C for 10 minutes to produce S1, which was used for analysis. The methodology to determine ROS levels was as described by Halliwell & Gutteridge (2007). To measure GTS activity, the methodology detailed by Habig et al. (1974) was used. TBARS determination was performed according to the method reported by Jentzsch et al. (1996) and Ohkawa et al. (1978). The methodologies applied to obtain PSH and NPSH was as described by Ellman (1969).

2.7. Meat analysis

2.7.1. Chemistry-physic composition

For meat analysis, the boned breast muscle was used. The pH of the meat was evaluated using an electrode pH meter. To determine the color (L*, a*, b*), a Konica Minolta CR-400 colorimeter was used, measuring the sample bands at three different points. We calculated the average obtained from three points. The color parameter a* varied from red (+a) to green (–a*), parameter b* varied from yellow (+b*) to blue (–b*), and parameter L* represents the intensity of luminosity. Higher values of L* indicate lighter meat and lower values indicate darker meat. Water retention was measured as the release of water by the muscle after application of pressure, using 2 grams of the chest muscle placed between two filter papers, then exerting a pressure of 10 kg through an acrylic plate for 5 minutes. The weight of the exudate in relation to the initial weight of the muscle was considered, expressed as a percentage (2 g). The evaluation of water loss due to thawing was analyzed as described by Fortuoso et al. (2019).

2.7.2. Oxidant and antioxidant status

In the meat, the concentrations of ROS (Halliwell and Gutteridge 2007), TBARS (Ohkawa et al. 1978), GST (Habig et al. 1974), PSH, and NPSH (Ellman 1969) were measured, as detailed in section 2.6.3.

2.7.3. Fatty acids

The fatty acid profiles of samples were determined according to Bligh and Dyer (1959) with modifications. Four grams of sample (feed or meat) were weighed in 50-mL polypropylene tubes and we added 6 mL of distilled water and 16 mL of methanol. This mixture was triturated in a crushing disperser (Tecnal TE-102, Brasil), and we added 8 mL of chloroform. The samples were shaken for 1 h on an orbital table shaker. Then, 8 mL of chloroform and 8 mL of 1.5% sodium sulfate in aqueous solution were added. The samples were subjected to a homogenization-type vortex and centrifuged. The chloroform phase was collected, and set aside for lipid determination by drying at 105 °C and a aliquot containing 30 mg of lipid was dried under N_2 flow at 40 °C for fatty acid analysis.

The lipids were derivatized to methyl esters by adding 1 mL of KOH (0.4 M) in methanol and heating to 100 °C for 10 min. Then, 3 mL of H_2SO_4 (1 M) in methanol was added and the mixture was immersed in a water bath under the same conditions. FAME were partitioned suing 2 mL of hexane and after stirring and centrifuged. Samples were analyzed in a gas chromatograph equipped with a flame ionization detector (GC-FID) (Varian, Star, 3600, USA) and an automatic sampler (Varian, 8200, USA). We injected 1 μL of extract in split mode (20:1). The FAME separations occurred in a HP-88 capillary column (100 m × 0.25 mm; 0.20 μm of thickness film; Agilent Technologies, USA). The initial column temperature was 50 °C maintained for 1 min, then increasing to 180 °C at 15 °C/min, following by an increase of 0.5 °C/min to 195 °C, and finally to 230 °C at 15 °C/min, remaining for 5 min. The detector and injector temperature were both maintained at 250 °C. Results were expressed as percentage of total area of the chromatograms, considering the correction factors of the flame ionization detector and conversion of ester to acid according to Visentainer (2012).

2.8. Microbiology in poultry litter

On days 21 and 34, fecal samples were collected in litter for total bacterial count, total coliform count, and total count of *Escherichia coli*, using Petrifilm 3M EC 6404 TM®. The results obtained were expressed as colony-forming units (CFU) per gram of feces.

2.9. Statistical analysis

We use a completely randomized design. All variables were subjected to normality testing (Shapiro–Wilk); variables that did not have a normal distribution were transformed into logarithms. Subsequently, the data were subjected to analysis of variance and Tukey's test, in which differences between treatments were considered when $P < 0.05$.

Structured linear and quadratic contrasts test were planned for zootechnical performance. These tests considered four doses of the phytogenic; however, the absence of differences between treatments in the tests for feed intake, feed conversion, weight, and weight gain contradicted the test results.

3. RESULTS

3.1. Performance data

The treatments did not generate significant differences in performance variables when we from days 1 to 10, days 1 to 21, and days 1 to 35 of the experiment (Table 2). However, after the challenge with aflatoxin in the diet (between days 22 and 35), there was less feed consumption and less feed conversion in treatments T250 and T500 when compared to TC (Table 2).

3.2. Histopathology

No changes/lesions were observed in the liver and intestines of broilers for all treatments. The intestinal villus height was greater in birds in groups T250 and T500 than in TC; the chickens from these two treatments also had lower crypt depths. Thus, the villus/crypt ratio was higher in the T250 and T500 groups than in the TC control. The highest dose tested (T1000) did not differ between treatments with respect to these variables (Table 3).

3.3. Hematology and serum biochemistry

Lower counts of leukocytes, heterophils, lymphocytes, and monocytes were observed in the blood of chickens in groups T250, T500, and T100 compared to TC. The eosinophil counts did not differ significantly between groups.

There was no significant difference between treatments in terms of levels of total protein and albumin. The chickens in the T1000 group had lower globulin levels than the T1000 group compared to the other groups. Birds that consumed the PB had lower serum ALT levels. Serum ROS levels were lower in the T250 than in TC and T1000. Birds from the T500 and T1000 groups showed a higher concentration of NPSH than in the TC. There were no differences between groups in terms of PSH or GST (Table 4).

3.5. Meat quality

The physicochemical composition is presented in Table 5. Water retention capacity, pH, luminosity (L), and red (a) and yellow (b) colors did not differ significantly between groups (P> 0.05). We observed a trend (P = 0.051) for cooking losses, with lower values in the meat of birds from the T500 and T1000 treatments.

3.5.1. Composition and status oxidant and antioxidant

Birds from groups T500 and T1000 showed significantly lower levels of ROS than in groups TC and T250. The chickens that consumed blend (T250, T500 to T1000) had significantly lower TBARS levels when compared to TC. The level of NPSH was significantly higher in the meat of birds that consumed the test product at all doses (T1000, T500, and T250) than in TC. The PSH and GTS variables did not show significant differences between treatments (Table 5).

3.5.2. Fatty acids

The amounts of fatty acids in meat were significantly higher in T250 birds than others (Table 6). The amount of saturated fatty acids (SFAs) was significantly lower in the T250 and T500 treatments than in the TC and T1000 groups. Among the SFAs that differed between treatments, we highlight tridecanoic acid, stearic acid, arachidic acid (C20:0), heneicosanoic acid, lignoceric acid (Table 6).

Levels of monosaturated fatty acids (MUFAs) were significantly higher in T250 and T500 and lower in TC; however, there was no significant difference between the meat of birds in the T1000 group treatments (Table 6). In the meat of these birds, the MUFAs that differed between treatments were palmitoleic acid, heptadecenoic acid, oleic acid, erucic acid, and nervonic acid (Table 6).

In the meat of birds of the T250 group, higher polyunsaturated fatty acids (PUFAs) were observed than in the other groups. The birds of the T500 and T1000 treatments had lower levels when compared to the TC. Among the PUFAs that differed between treatments, we highlight linoleic acid isomer, α-linolenic acid, cis-11,14-eicosadienoic acid, cis-8,11,14-eicosatrienoic acid, cis-11,14,17-eicosatrienoic acid, and arachidonic acid.

3.6. Microbiology in poultry litter

The treatments did not show significant differences among them in total bacterial counts on days 21 and 34 (Figure 1a). The total coliform count was significantly higher on day

21 in the TC group than in the other groups, which did not significantly differ. On day 34, TC samples showed higher total coliform counts than the other groups, with T500 being significantly higher than the T250 and T1000 groups (Figure 1b).

The Escherichia coli count on day 21 was significantly higher in TC than in the other groups, but it did not differ significantly between groups, regardless of the dose. On day 34, the TC birds obtained the highest E. coli count compared to the other groups. The T500 treatment obtained a significantly higher count than T1000, and the T250 group did not differ significantly from the T500 and T1000 groups (Figure 1c).

4. DISCUSSION

The zootechnical performance of chickens fed with a PB did not differ between treatments until they were challenged with a diet containing aflatoxin on day 22. Diets containing aflatoxin, even in small amounts such as 0.5 mg/kg, when compared to non-contaminated diets, showed less consumption of feed and consequently less WG in chickens (Nazaridadeh et al., 2019), similar to what we observed in our study. Although the FI was higher here in the TC treatment compared to T250 and T500, WG did not show any significant difference between the groups (trend only; $p = 0.071$), suggesting a decrease in the nutritional use of the diet; however, FC was lower in the birds that consumed the blend, a positive economic result for the productive sector, because food occupies the highest share of production costs. Based on the micrometric results of the intestine, it is possible to suggest that the explanation for the FI in birds in the T250 and T500 groups is related to the greater villus height and lower crypt depths when compared to the TC; that is, T250 and T500 resulted in greater contact surface for nutrient absorption, which explains the lower FI associated with the tendency of birds to have greater body WG. These results strongly suggest that the additive can replace conventional growth enhancers and can minimize the adverse effects on performance caused by aflatoxicosis, which is excellent. A product that has antimicrobial and anti-aflatoxin action is an excellent alternative to poultry farming.

Intestinal health has been associated with improved performance in chickens; however, many pathogenic microorganisms can negatively affect the intestinal lumen, interfering with the production of enterocytes (Petrolli et al., 2012). The use of additives has been an alternative for intestinal health. Nevertheless, the dose must be defined, as it is known that high doses may not be beneficial. As we saw in our T1000 treatment, they may not enhance the expected performance if we consider the villi/crypt relationship. As the higher the value of this relationship, it was determined that better conditions of intestinal absorption are reflected in WG. According to other studies, the combination of curcumin, thymol, cinnamaldehyde, and

carvacrol had antimicrobial effects. This combination protected the intestinal mucosa of broilers challenged with coccids from Eimeria spp. and E. coli (Galli et al., 2019); however, that study did not show a definitive explanation for what happened. Petrolli et al. (2012) added plant extracts and found that the quality of the intestinal mucosa improved, increasing the absorption areas and consequently the absorption of nutrients in broilers. These benefits to intestinal health can be related only to the antimicrobial action of the components present in the blend or be a direct and indirect joint action of the tested product. Many explanations are still needed to elucidate how the photogenic acts at the mucosa level. For example, the organic acids have antimicrobial activity related to lowering the pH of the medium. The pK of the acid determines its dissociation capacity. They are liposoluble, which gives them the ability to enter cells of microorganisms, reducing the intracellular pH and enzymatic action (Petrolli et al., 2019). In agreement with these results, Calaça et al. (2019) observed an increase in intestinal health of chickens supplemented with organic acids and subsequently challenged with Salmonella enterica serovar enteritidis and Eimeria tenella. In another study, researchers added tannins and organic acids to chickens' diets (Petrolli et al. 2019) and provided benefits to intestinal health. These data help explain our results regarding the reduction in the total coliform count and the E. coli count in broiler litter, given the capacity of the components of the phytogenic compound to act in an antimicrobial manner, consequently reducing environmental excretion. A similar result was described by Bourassa et al. (2018), who observed a reduction in the occurrence of Salmonella in the broiler litter following the addition of organic acids in the feed and the water.

Lower total leukocyte counts were due to lower neutrophil, lymphocyte, and monocyte counts in the blood of birds that consumed the PB. This phenomenon can be explained by the curcumin-related immunomodulatory factor that has been described in chickens (Galli et al., 2020). Curcumin reduces leukocyte counts and exerts inhibitory effects on chemotactic inflammatory mediators or molecules involved in the leukocyte migration process (De Almeida et al., 2018). This hypothesis is based on the fact that researchers have shown a reduction in inflammatory mediators induced by lipopolysaccharides in mice treated orally with 20 mg/kg curcumin, given the ability of curcumin to inhibit mIR-155 via PI3K/AKT. The anti-inflammatory effect is beneficial in farm animals under normal and healthy conditions, as the energy expenditure reduces to mount an inflammatory response. Consequently, more energy is left to deposit muscle mass in growing animals, as was the case in this study with animals that consumed PB.

Curcumin is known as an herbal component with hepatoprotective action, which may explain the lower activity of ALT in the T500 and T1000 treatments when compared to TC. As

is already well known, mycotoxins cause liver damage in chickens, especially aflatoxin, which can cause hepatocyte apoptosis and liver inflammation (Wang et al., 2018). In the current study, we confirmed our hypothesis: the addition of the PB reduced the impact on the liver caused by aflatoxin, in addition to reducing the inflammatory response and increasing non-enzymatic antioxidant levels.

One of the mechanisms by which aflatoxin damages liver tissue is the exacerbation of lipid peroxidation (Shen et al., 1994). Elevation in non-protein uncles (NPSH) concentration is a positive effect, as these non-enzymatic antioxidants act to maintain cell integrity, which protects against oxidative stress (Liu et al., 2016). Reduced lipid peroxidation and liver damage in rats supplemented with antioxidants have been shown (Shen et al., 1994). Curcumin has a great capacity to transfer electrons or protons from phenolic sites, thanks to its structure and functional groups such as β-diketone, allowing them to enter the polar environment of cells and protect them against oxidation, with excellent ability to eliminate ROS (Barzegar and Moosavi-Movahedi, 2012). This effect was also described in chickens that consumed a product phytogenic based on curcumin and essential oil components (Galli et al., 2020). As expected, the antioxidant capacity of phytogenic compound was verified here and may explain the lower levels of free radicals and lipid peroxidation combined with higher concentrations of NPSH in meat; similar findings were described in the meat of chickens fed with phytogenics (Galli et al. 2019; Reis et al. 2018). That phytogenic additive was based on essential oils, carvacrol, thymol, and cinnamic aldehyde. Its effects were comparable to those of antimicrobials commonly used as growth promoters in the poultry industry. Lipid peroxidation was also higher in groups supplemented with the phytogenic agent.

The profile of organic acids in chicken breast meat was evaluated in the present study. We found interesting results in terms of quality of the meat, "healthier" meat. Chicken breast meat has a relatively low-fat concentration compared to other types of meat; however, a greater amount of fat in meat in T250 birds was observed here, the mechanism of which is not known by the authors. In reality, we expected the opposite effect, as previously described by Xie et al. (2019). These researchers fed chickens with curcumin and found a reduction in the deposition of abdominal fat, reducing hepatic and plasma lipid content. Curcumin affects the expression of the ACC, FAS, SREBP-1c, ACLY genes, PPARα and CPT-EU linked to lipogenesis and lipolysis (Xie et al., 2019). Wang et al. (2015) also reported a reduction in pectoral fat with turmeric rhizome extract in Wenchang broilers. According to the literature, supplementation of 500 to 1000 mg/kg of curcumin inhibited the development of fatty liver in mice. However, the animals consumed high-fat diets, which allowed researchers to suggest that this herbal component may attenuate dyslipidemia, hyperglycemia, and hyperinsulinemia.

The consumption of SFAs is less desired by nutritionists. Therefore, it is less recommended for consumers compared to foods rich in unsaturated fatty acids, given their ability to increase total cholesterol and LDL (Menski et al., 2003). In the current study, the inclusion of the PB in the broiler diet was beneficial, evidenced by lower levels of SFAs and higher levels of PUFAs in T250 and MUFAs in meat from T250 and T500. Two studies (Salah et al., 2019; Galli et al., 2019) found similar results regarding reducing SFAs in the chest muscles using supplementation with curcumin or the combination of curcumin and components of encapsulated essential oils. The concentration of MUFAs and SFAs can be altered as stearic acid is converted to oleic acid (Bruce and Salter, 1996). This finding could explain the higher levels of oleic acid in poultry meat combined with a lower stearic acid concentration. In general, supplementation with 250 g of the PB per kg improves the fatty acid profile of chicken meat. However, because it is a "blend" of ingredients, it is difficult to establish precisely which component was responsible for the observed changes; therefore, we believe that there are synergistic effects of the commercial product Acidosan®, formulated based on curcumin, essential oils, and organic acids.

It is important to note that, in the present study, contamination with aflatoxin in the diet was a methodology used to impose a challenge on birds in order to compare the PB with an antimicrobial performance enhancer capable. However, the results of adding this PB were positive; i.e., the presence of the commercial product in the diet between the 22nd and 34th day of life was able to prevent or minimize the adverse effects caused by aflatoxicosis in the blood and poultry meat. The blend may be considered a candidate to replace antibiotics in chicken feed in addition to the desired antimicrobial activity. We believe that this commercial blend can exert functional, nutraceutical, growth-promoting, and anti-aflatoxin functions. Further studies will be conducted to explore the anti-toxin action and to technically understand the mechanisms involved in supplementing Acidosan for chickens and other animal species and whether it is also able to minimize the effect of other mycotoxins.

5. CONCLUSION

The substitution of conventional growth promoters with a phytogenic compound can occur without compromising zootechnical performance, intestinal health, or the quality of chicken meat. The inclusion of the phytogenic compound has antioxidant and antimicrobial effects, and it reduces the negative impacts caused by oxidation in broilers. The addition of 250 g/kg of the phytogenic compound increases fat in breast meat, reduces SFA, and increases unsaturated fatty acids, all of which are desirable and interesting for consumer health. The

challenge with aflatoxin in the diet of birds fed with a PB showed that this product protects against aflatoxicosis, minimizing adverse effects on the health and performance of the birds.

Ethics committee

The project was approved by the ethics committee for the use of animals in research at the State University of Santa Catarina, under protocol No. 7562021219.

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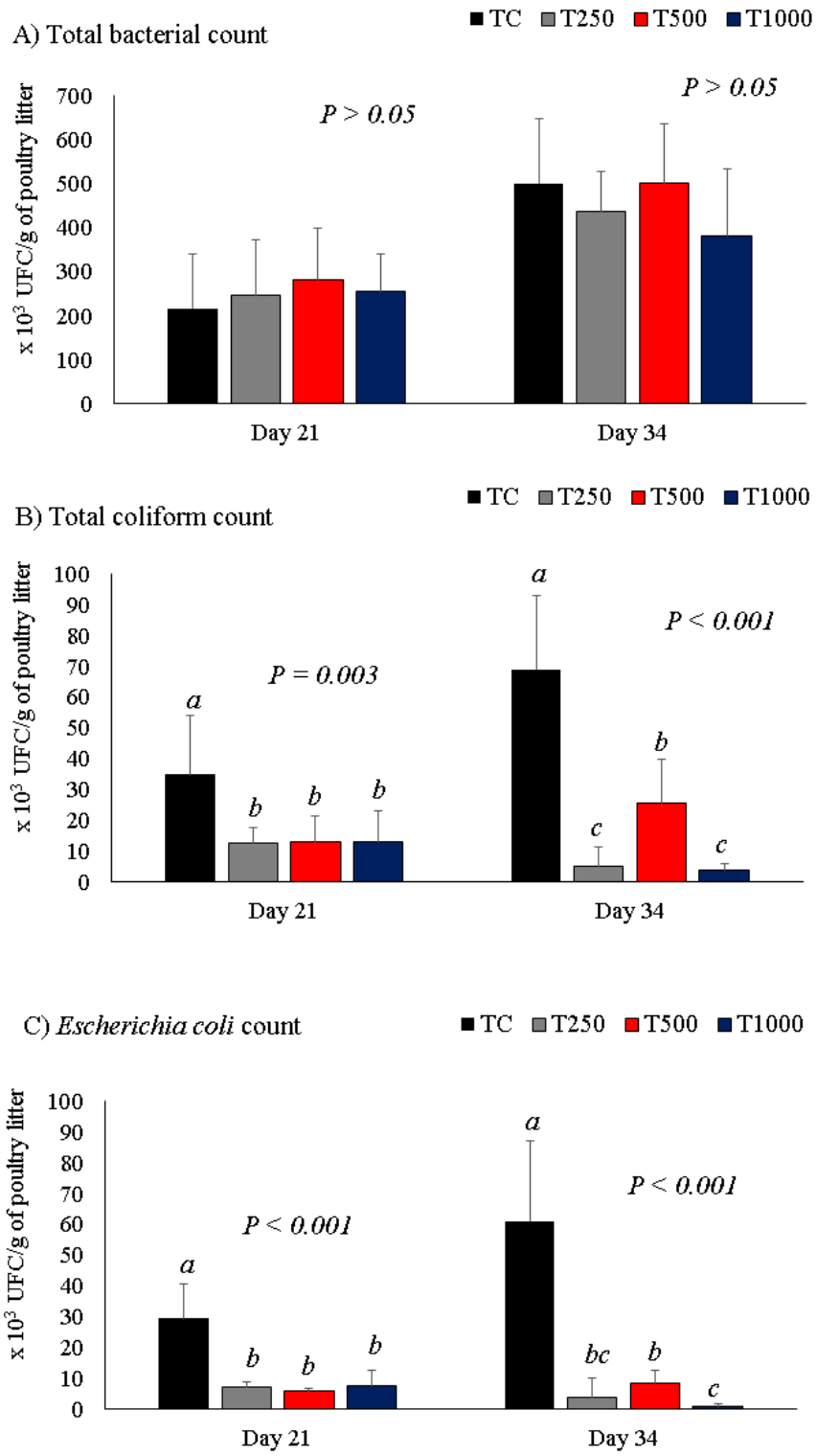

Figure 1: Total bacterial count, total coliforms, and E. coli in broiler litter at 21 and 34 days of age. The treatments formed were: TC, used as a control for the presence of conventional performance enhancers (80 mg/kg salinomycin + flavomycin 2 mg/kg); T250, T500, and T1000 correspond to the test treatments with phytogenic bend in the doses of 250 mg/kg, 500 mg/kg and 1000 mg/kg, respectively.

Table 1: Ingredients and basal feed used for all experimental treatments.

Ingredients (%)	Age (days)	
	1–21	22–34
Corn	55.15	58.00
Soybean meal	37.32	33.7
Soy oil	3.90	4.93
Bicalcium phosphate	1.27	1.30
Calcitic limestone	1.14	0.92
Iodized salt	0.48	0.43
DL-Methionine – 99%	0.29	0.28
L-lysine – 78%	0.20	0.19
L-threonine – 99%	0.05	0.05
Premix of vitamins and minerals[1]	0.20	0.20
Calculated chemical composition	100	100
Energy (kcal/kg)	3050	3150
Brute protein (%)	21.20	19.80
Calcium (%)	0.84	0.76
Available phosphorus (%)	0.40	0.35
Digestible lysine (%)	1.22	1.13
Digestible methionine (%)	0.47	0.45
Digestible methionine + cysteine (%)	0.88	0.83
Digestible threonine (%)	0.79	0.73
Digestible tryptophan (%)	0.21	0.20
Sodium (%)	0.21	0.20

Note: [1] Minimal vitamin and mineral levels per kg of product: vitamin A (5000000 IU); vitamin D3 (1000000 IU); vitamin E (15000 IU); vitamin K3 (1500 mg); vitamin B1 (1500 mg); vitamin B2 (3000 mg); vitamin B6 (2000 mg); vitamin B12 (7000 mcg); folic acid (500 mg); nicotinic acid (15 g); pantothenic acid (7000 mcg); choline (80 g); biotin (100 mg); copper (10 g); iron (50 g); iodine (1000 mg); manganese (80 g); selenium (300 mg); zinc (70 g); minimum humidity (20 g); maximum mineral matter (980 g). Growth promoter (lincomycin, 4,4 mg/ kg of feed); coccidiostatic agent (salinomycin, 64 mg/kg of feed).

Table 2: Growth of broiler chickens fed with experimental feed containing phytogenic blend

Treatment Day 1 to 10					
	FI (g)	WG (g)	FC	BW	Mortality (%)
TC	345	248	1.39	290	2.5
T250	354	257	1.37	299	0.0
T500	346	256	1.35	299	0.0
T1000	345	252	1.37	288	2.5
P	0.944	0.783	0.951	0.516	-
Days 1 to 21					
TC	1350	945	1.43	1002	2.5
T250	1439	999	1.45	1060	2.5
T500	1407	990	1.42	1058	0.0
T1000	1389	982	1.41	1039	2.5
P	0.539	0.714	0.922	0.504	-
Days 1 to 34					
TC	3754	2286	1.64	2450	3.75
T250	3560	2424	1.47	2484	2.5
T500	3588	2375	1.51	2511	2.5
T1000	3687	2305	1.60	2376	2.5
P	0.695	0.225	0.08	0.129	-
Days 22 to 34					
TC	2404[a]	1341	1.79[a]	-	1.25
T250	2124[b]	1425	1.49[b]	-	0.0
T500	2181[b]	1385	1.57[b]	-	2.5
T1000	2298[ab]	1323	1.73[a]	-	0.0
P	**0.046**	0.071	**<0.001**	-	-

Note 1: Feed intake (FI), weight gain (WG), feed conversion (FC), and average body weight (BW). **Note 2:** Different letters ([a, b]) within a column indicate significant differences among Tukey post hoc test treatments. Note 3: Between days 22 to 34 of the experiment, the diet was experimentally contaminated with aflatoxin, simulating a challenging condition—note 4. The treatments were as follows: TC, used as a control for the presence of conventional performance enhancers (80 mg/kg salinomycin + flavomycin 2 mg/kg); T250, T500, and T1000 correspond to the test treatments with phytogenic bend at 250 mg/kg, 500 mg/kg and 1000 mg/kg, respectively.

Table 3. Size of intestinal crypts and villi of 34-day-old broiler chickens fed with an experimental feed containing phytogenic blend.

Treatment	Intestinal villi (µm)	Intestinal crypts (µm)	Villus/crypt ratio
TC	1152 [b]	247.2 [a]	4.66[b]
T250	1410 [a]	223.2 [bc]	6.31[a]
T500	1368 [a]	213.7 [c]	6.40[a]
T1000	1248 [ab]	236.4 [a]	5.70[ab]
P-Value	0.001	0.001	0.001

*Different letters ([a, b]) within the same column indicate significant differences among treatments using the Tukey post hoc test. Note: The treatments were as follows: TC, used as a control for the presence of conventional performance enhancers (80 mg/kg salinomycin + flavomycin 2 mg/kg); T250, T500, and T1000 correspond to the test treatments with phytogenic bend at 250 mg/kg, 500 mg/kg and 1000 mg/kg, respectively.

Table 4: Leukogram in blood and clinical biochemistry and oxidant and antioxidant status in serum of broiler chickens fed with an experimental feed containing phytogenic blend.

Variables	TC	T250	T500	T1000	P-Value
Leukogram					
Leukocytes (x 10^3 µL)	8.88[a]	4.69[b]	4.38[b]	3.85[b]	<0.001
Heterophils (x 10^3 µL)	4.63 [a]	2.54 [b]	2.39 [b]	2.02 [b]	<0.001
Lymphocytes (x 10^3 µL)	3.41 [a]	1.93 [b]	1.85 [b]	1.56 [b]	<0.001
Monocytes (x 10^3 µL)	0.68 [a]	0.11 [b]	0.10 [b]	0.17 [b]	0.015
Eosinophils (x 10^3 µL)	0.16	0.11	0.04	0.10	0.697
Biochemistry					
Total proteins (g/dL)	3.68	3.85	3.76	3.42	0.247
Albumin (g/dL)	1.14	1.20	1.08	1.21	0.596
Globulin (g/dL)	2.54 [a]	2.62 [a]	2.68 [a]	2.21 [b]	0.050
ALT (U/L)	32.7 [a]	18.1[a]	12.7 [b]	14.6 [b]	<0.004
ROS (x 10^3 U DCF/mg of protein)	4.59 [a]	4.22 [b]	4.29 [ab]	4.59 [a]	0.050
PSH (nmol SH/mg protein)	53.0	46.7	55.9	50.3	0.417
NPSH (nmol NPSH/mL)	112.0 [c]	117.2 [bc]	119.9 [ab]	126.9 [a]	<0.001
GST (µmol CDNB/min/mg protein)	159.1	146.9	146.3	153.2	0.142

* Different letters ([a, b, c]) within a line indicate significant differences among treatments using Tukey post-hoc test. **Note:** ROS (reactive oxygen species); GST (glutathione S-transferase); PSH (protein thiols); NPSH (Non-protein thiols). Note: The treatments were as follows: TC, used as a control for the presence of conventional performance enhancers (80 mg/kg salinomycin + flavomycin 2 mg/kg); T250, T500, and T1000 correspond to the test treatments with phytogenic bend at 250 mg/kg, 500 mg/kg and 1000 mg/kg, respectively.

Table 5: Physicochemical composition and oxidant and antioxidant status in the meat of broiler chickens fed with an experimental feed containing phytogenic blend.

Variables	TC	T250	T500	T1000	P-Value
Physicochemical composition					
pH	5.84	5.94	5.88	5.92	0.358
Water retention capacity	0.65	0.63	0.58	0.60	0.141
Cooking loss (%)	12.6	13.1	11.2	10.2	0.051
Lightness (L*)	54.1	56.1	55.9	54.3	0.218
Redness (a*)	-1.58	-1.17	-1.34	-0.59	0.503
Yellowness (b*)	7.88	7.56	7.39	7.85	0.755
Oxidant and antioxidant status					
ROS (x 10^4 U DCF/mg of protein)	3.71[a]	3.55 [a]	2.50 [b]	2.45 [b]	0.011
TBARS (nmol MDA/g tissue)	35.2 [a]	10.9 [b]	10.1 [b]	11.1 [b]	<0.001
PSH (nmol SH/mg protein)	857	839	860	878	0.851
NPSH (nmol NPSH/g tissue)	122.1[c]	138.0 [b]	144.2 [b]	164.8 [a]	<0.001
GST (µmol CDNB/min/mg protein)	195.2	168.0	178.6	180.2	0.562

* Different letters ([a, b, c]) within a line indicate significant differences among treatments using Tukey post-hoc test. **Note:** ROS (reactive oxygen species); GST (glutathione S-transferase); PSH (protein thiols); NPSH (Non-protein thiols). The treatments were as follows: TC, used as a control for the presence of conventional performance enhancers (80 mg/kg salinomycin + flavomycin 2 mg/kg); T250, T500, and T1000 correspond to the test treatments with phytogenic bend at 250 mg/kg, 500 mg/kg and 1000 mg/kg, respectively.

Table 6. Profile of fatty acids (g/kg) in the meat of 34-day-old broiler chickens fed with an experimental feed containing phytogenic blend to replace antibiotics.

Variables	T0	T250	T500	T1000	P-value
Fat in meat	7.80 [b]	10.4 [a]	8.15 [b]	6.80 [b]	<0.001
Fatty acid (g/kg)					
Lauric acid (C12:0)	0.13	0.08	0.05	0.14	0.210
Tridecanoic acid (C13:0)	1.39[a]	0.37 [b]	0.02 [c]	0.00 [c]	<0.001
Myristic acid (C14:0)	2.08	2.29	2.00	2.04	0.854
Pentadecyl acid (C15:0)	0.58	0.55	0.46	0.56	0.598
Palmitic acid (C16:0)	212	208	214	222	0.247
Myristoleic acid (C14:1)	0.62 [ab]	0.41 [b]	0.94 [a]	0.80 [a]	0.047
Margaric acid (C17:0)	1.50	1.28	1.13	1.22	0.124
Stearic acid (C18:0)	118 [a]	96.3 [b]	113 [ab]	113 [ab]	0.035
Arachidic acid (C20:0)	1.06	1.30	0.88	0.85	0.051
Heneicosanoic acid (C21:0)	0.55 [a]	0.15 [b]	0.23 [b]	0.26 [b]	0.021
Lignoceric Acid (C24:0)	0.48 [a]	0.33 [a]	0.41 [a]	0.10 [b]	0.005
SFA	339 [a]	311 [b]	333 [ab]	341 [a]	0.032
Palmitoleic acid (C16:1)	9.27 [b]	19.0 [a]	15.1 [ab]	12.8 [ab]	<0.001
Heptadecenoic acid (C17:1)	3.28 [a]	1.04 [b]	0.94 [b]	1.10 [b]	<0.001
Oleic acid trans isomers (C18:1t)	2.32	1.79	1.89	2.04	0.062
Oleic acid (C18:1n9c)	254 [b]	286 [a]	282 [a]	283 [a]	0.021
Trans vacenic (C18:1n7t)	18.4	13.1	16.6	16.1	0.056
Oleic acid (C18:1n9t)	0.24	0.18	0.10	0.29	0.074
Erucic acid (C22:1n9)	39.8 [a]	24.8 [c]	34.7 [ab]	27.0 [bc]	<0.001
Nervonic acid (C24:1n9)	9.40 [a]	6.21 [b]	8.19 [a]	6.18 [b]	0.001
cis-11-Eicosenoic Acid (C20:1n9)	1.78	1.36	1.98	1.62	0.140
MUFA	340 [b]	355 [a]	363 [a]	351 [ab]	0.050
Linoleic acid isomer (C18:2n6- 9c12t)	0.79 [a]	0.97 [a]	0.32 [b]	0.34 [b]	0.025
Linoleic acid isomer (C18:2n6- 9c12c)	283	301	272	279	0.259
ɣ- Linolenic acid (C18:3n6- 6c9c12c)	0.95	0.78	0.84	0.79	0.385
α- Linolenic acid (C18:3n3 - 9c12c15c)	12.7 [b]	16.2 [a]	11.1 [b]	11.27 [b]	0.038
cis-11,14-Eicosadienoic acid (C20:2n6)	6.56 [a]	4.30 [c]	5.38 [ab]	4.46 [bc]	0.003
cis-8,11,14-Eicosatrienoic acid (C20:3n6)	6.18 [a]	4.69 [bc]	5.22 [ab]	4.18 [c]	<0.001
cis-11,14,17-Eicosatrienoic acid (C20:3n3)	0.51 [a]	0.30 [ab]	0.43 [ab]	0.22 [b]	0.010
Arachidonic acid (C20:4n6)	0.12 [ab]	0.21 [a]	0.07 [bc]	0.00 [d]	<0.001
Eicosapentaenoic acid - EPA (C20:5n3)	1.19	0.69	1.00	0.74	0.359
Docosapentaenoic acid - DPA (C22:5n3)	4.71	3.04	4.55	3.22	0.283
Docosahexaenoic acid - DHA (C22:6n3)	3.15	1.62	2.74	1.75	0.072
PUFA	320 [b]	334 [a]	303 [c]	306 [c]	<0.001

Note: * Different letters ([a, b, c]) within a line indicate significant differences among Tukey post-hoc test treatments. The treatments were as follows: TC, used as a control for the presence of

conventional performance enhancers (80 mg/kg salinomycin + flavomycin 2 mg/kg); T250, T500, and T1000 correspond to the test treatments with phytogenic bend at 250 mg/kg, 500 mg/kg and 1000 mg/kg, respectively.

Supplementary Material 1: Fatty acid profile (g/kg) in experimental feed containing phytogenic blend to replace antibiotics.

Fatty acid in feed	Basal diet (g/kg)
Capric acid (C10:0)	0.20
Myristic acid (C14:0)	0.50
Pentadecyl acid (C15:0)	0.20
Palmitic acid (C16:0)	135.5
Palmitoleic acid (C16:1)	0.50
Margaric acid (C17:0)	0.70
Heptadecenoic acid (C17:1)	0.30
Stearic acid (C18:0)	48.6
Oleic acid trans isomers (C18:1t)	0.40
Oleic acid (C18:1n9c)	283.2
Trans vacenic (C18:1n7t)	5.40
Oleic acid (C18:1n9t)	0.10
Linoleic acid isomer (C18:2n6- 9c12t)	0.50
Linoleic acid isomer (C18:2n6- 9t12c)	1.40
Linoleic acid isomer (C18:2n6- 9c12c)	468.7
ɣ- Linolenic acid (C18:3n6- 6c9c12c)	3.80
Arachidic acid (C20:0)	0.90
ɣ- Linolenic acid (C18:3n6- 6c9c12c)	0.80
α- Linolenic acid (C18:3n3 - 9c12c15c)	40.6
cis-11-Eicosenoic acid (C20:1n9)	1.60
Heneicosanoic acid (C21:0)	0.20
cis-11,14-Eicosadienoic acid (C20:2n6)	0.20
cis-8,11,14-Eicosatrienoic acid (C20:3n6)	3.50
Arachidonic acid (C20:4n6)	0.20
Tricosanoic acid (C23:0)	0.50
Lignoceric acid (C24:0)	2.30
Nervonic acid (C24:1n9)	0.10
Docosapentaenoic acid - DPA (C22:5n3)	0.10
Docosahexaenoic acid - DHA (C22:6n3)	0.10
Fat in diet	41.8

Note 1: A diet sample (initial and growth) was collected from each treatment during a 34-day experimental period and from the basal diet. The samples were evaluated, and there was no difference between treatments (P >0.05); therefore, we present only the basal ration results.

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2.2 MANUSCRITO I

Protective effects of silymarin in broiler feed contaminated by mycotoxins: growth performance, meat antioxidant status, and fatty acid profiles

Eduardo H. Armanini[a], Marcel M. Boiago[a,b], Bruno Cecere[a], Paulo V. Oliveira[b], Carlos J.S. Teixeira, João V. Strapazzon[a], Nathieli B. Bottari[c], Anielen D. Silva[c], Mateus Fracasso[c], Raquel G. Vendruscolo[d], Roger Wagner[d], Eduardo M. Gloria[e], Vitor W. Horn[f], Ricardo E. Mendes[f], Matheus D. Baldissera[g], Marcelo Vedovatto[h], Aleksandro S. Da Silva[a,b*]

[a] Graduate Program in Animal Science, Universidade do Estado de Santa Catarina (UDESC), Chapecó, SC, Brazil.

[b] Department of Animal Science, UDESC, Chapecó, SC, Brazil.

[c] Graduate Program in Toxicological Biochemistry, Universidade Federal de Santa Maria (UFSM), Santa Maria, Brazil.

[d] Department of Food Science, UFSM, Santa Maria, Brazil.

[e] Department of Microbiology and Parasitology, UFSM, Santa Maria, Brazil.

[g] Department of Physiology and Pharmacology, UFSM, Santa Maria, Brazil.

[h] Department of Animal Science, Universidade do Estado do Mato Grosso do Sul, Brazil.

*Corresponding author: aleksandro.silva@udesc.br (A.S. Da Silva)

Abstract

The aim of this study was to determine whether the inclusion of silymarin in broiler feed was able to mitigate the adverse effects of mycotoxin on growth performance, health status, liver oxidative stress, and fillet fatty acid profiles. A completely randomized design with four treatments, four repetitions, and 15 chicks per repetition was used, with the following groups: a) feed without additives (NoMyc-NoSil); b) feed supplemented with silymarin (NoMyc-Sil); c) feed contaminated with aflatoxin and fumonisin (Myc-NoSil), and d) feed contaminated with mycotoxin and supplemented with silymarin (Myc-Sil). Zootechnical performance, intestinal and liver health, and meat quality were assessed. The consumption of feed contaminated with mycotoxin delayed weight gain and increased feed conversion; however, the addition of

silymarin prevented these adverse effects on the chickens' production systems. ALT levels were higher in Myc-NoSil broilers than in other groups. Intake of silymarin in healthy birds increased serum globulin levels and reduced albumin, ALT, and AST levels. The NoMyc-Sil birds had greater villus heights and crypt depths. Luminosity and water loss by cooking were affected by mycotoxin ingestion, changes that did not occur in the meat of birds that were supplemented with silymarin. The sum of saturated and monounsaturated fatty acids in the meat did not change between treatments, unlike the sum of polyunsaturated fatty acids higher in the meat of birds that consumed silymarin. We conclude that silymarin is a potential additive in broiler feed; it prevents delayed growth performance and improves meat quality.
Keywords: Aflatoxin; fumonisin; natural products; poultry health; oxidative stress.

1. INTRODUCTION

Mycotoxins are a class of toxic secondary metabolites naturally produced by certain mold species. Under specific temperature and humidity conditions, they can be produced pre- or post-harvest (Alshannaq and Yu, 2017). This group includes aflatoxins (AFB), fumonisins, ochratoxins, and trichothecenes; these are the most frequently encountered mycotoxins in agricultural products and foods (Thanushree et al., 2019). Due to their highly resistant nature, mycotoxins tend to remain in the food chain and affect a broad range of agricultural products and foods. Consumption of contaminated cereals with mycotoxins led to the accumulation of these toxins in animal and human organs. This condition causes impaired immunity, endocrine abnormalities, and carcinogenesis, depending on their concentration and co-occurrence (Yu et al., 2018). Aflatoxins are produced principally by *Aspergillus flavus* and *A. parasiticus* and are among the most toxic mycotoxins (Pietro et al., 2015). Chickens are susceptible to aflatoxins; toxicity occurs following long-term intake of contaminated feed. These toxicities include oxidative stress, apoptosis, inflammation, and acute or chronic liver injury (Li et al., 2021). Fumonisins are secondary metabolites compounds produced by *Fusarium verticillioides* and *F. proliferatum* that damage poultry in several ways, including reduction of growth performance, hepatic alterations, and oxidative damage (Dazuk et al., 2020; Sousa et al., 2020). Recent evidence suggested that consumption of mycotoxins-contaminated feed causes impairment of fillet fatty acid profiles via increases in saturated fatty acids and decreases in polyunsaturated fatty acids (PUFA), a condition that negatively impacts human consumers, as observed by Baldissera et al. (2020) in fish feed with ochratoxin A (OTA) and by Mazur-Kusnirek et al. (2019) in broiler chickens fed with a feed naturally contaminated with OTA. For these reasons, strategies have been developed to reduce or prevent mycotoxin toxicity in production animals. An example is plant metabolites as feed additives; these substances have

gained popularity due to their safety, biodegradable nature, and low toxicity (Souza et al., 2019; Ghafarifarsani et al., 2021). One such product is silymarin.

Silymarin is a complex flavonoid isolated from the fruits and seeds of milk thistle (*Silymarin marianum*) that contains a mixture of active flavonolignans and flavonoids; it is historically recognized and used to treat a range of liver diseases (MacDonald-Ramos et al., 2021). Silymarin was initially found in the mountains of the Mediterranean, Asia, and North Africa regions; however, today, it is grown in many parts of the world and is composed principally of six flavonolignans (silybin, isosilybin, silychristin, isosilychristin, silydianin, and silimonin) and other flavonoids (taxifolin, quercetin, dihydrokaempferol, kaempferol, apigenin, naringin, eriodictyol, and chrysoeriol); of these, silybin is the principal active component (MacDonald-Ramos et al., 2021). Recently, Saeed et al. (2019) found that silymarin is a potent hepatoprotective agent for the poultry industry. Based on the hepatotoxic effects of mycotoxins in poultry, we investigated the possible protective effects of silymarin in broilers given feed contaminated by mycotoxins in terms of performance, oxidative stress, liver health, and fatty acid profiles. In a similar study, Egresi et al. (2020) demonstrated that silymarin (0.5 %) over 47 days reduced oxidative stress in livers of ducks given feed containing deoxynivalenol and zearalenone; the authors consider this additive as a compelling approach to protect the liver against mycotoxin-induced liver damage. Similarly, Sakamoto et al. (2018) found that the inclusion of 500 g/ton silymarin in feed for Japanese quails reduced the impairment of growth performance elicited by aflatoxin B1, reducing negative impacts on performance. Based on these findings, in the present study, we determined whether the inclusion of silymarin in broiler feed would mitigate the negative impacts of mycotoxin in terms of growth performance, health status, liver oxidative stress, and fillet fatty acid profiles.

2. Materials and Methods

2.1. Mycotoxin production

Aflatoxins and fumonisins were generated through fermentation in rice, converted to a controlled temperature, and contact-stirred. The material produced was autoclaved with an open valve, dried in an oven with forced ventilation, and fragmented using a 1-mm sieve mill. The concentration of mycotoxins in the material (inoculum) was determined using HPLC (Thorpe et al., 1982).

2.2. Silymarin

Silymarin was purchased from a local drug distributor in the city of Chapecó, SC, Brazil. According to the technical report of the product made available by the company, the silymarin content was 84.16% in the tested commercial product.

2.3. Animals, accommodations, and feed

The experiment took place in the experimental poultry shed at the State University of Santa Catarina, campus in Chapecó, SC, Brazil. For the study, 240 1-day-old Cobb-500 chicks donated by a local Aurora hatchery were used. Immediately after the arrival of the chicks in the experimental house, they were weighed and distributed in a completely randomized design with four treatments, four replicates per treatment, and 15 chicks per repetition. The birds were kept in 1.88-m² pens, with food supply through suspended feeders appropriate for the age of the chicks and the water provided through nipple-type drinkers. Food and water were provided ad libitum. Heating was provided using a gas bell until the 14th day of life because the study was conducted during the Brazilian winter. The light program was followed as presented in the lineage manual.

The experiment groups received the same basal feed (Table 1) formulated according to the nutritional requirements described in the Brazilian poultry tables, based on soybean meal and crushed corn (Rostagno et al., 2017). The treatments were divided as follows; 1) negative control group, in which only the basal feed for birds (NoMyc-NoSil group) was provided; 2) basal feed + silymarin (NoMyc-Sil group), basal feed supplemented with 100 mg of silymarin per kg; 3) positive control group, basal feed contaminated with 0.05 ppm aflatoxin and 20 ppm fumonisin (Myc-NoSil group); 4) mycotoxin + silymarin in the basal feed, at 0.05 ppm aflatoxin and 20 ppm fumonisin + 100 mg silymarin per kg of feed (Myc-Sil group).

2.4. Zootechnical performance

The birds were weighed on days 1, 7, 21, 35, and 41 of life. The consumption of poultry feed (AI) (g/animal/day) was used to calculate the divergence between the feed provided and the surplus feed generated after the determined time. Weight gain (WG) was determined by calculating: (initial weight–final weight)/number of birds per repetition. Feed conversion (FC) was calculated as the amount of food consumed/live weight of the birds.

2.5. Sample collection and slaughter

At the end of the experiment, eight blood samples were collected (day 42) per treatment. The collection was performed using vein branchial puncture, placed subsequently in two tubes, one with anticoagulant for analysis of antioxidant enzymes and blood count, and the other tube

with no anticoagulant to obtain serum. The serum was separated from the blood by centrifugation for 10 minutes at 3500 rpm, stored at –20 ° C until analysis.

On day 42, at the end of the experiment, eight chickens were slaughtered per treatment (two per repetition). Liver and intestine samples were collected for histopathological analysis. The pectoralis major muscle was used to analyze fatty acid profiles, meat quality, and composition.

2.6. Serum biochemistry

Using commercial analysis kits (Analisa®) and a semi-automatic biochemical analyzer BioPlus (Bio-2000®), serum biochemistries and albumin levels were measured. The formula total protein – albumin was used to calculate the globulin concentration.

2.7. Meat quality

2.7.1. Physicochemical composition

Boneless breast muscle was used to analyze the meat. The color (L*, a*, b*) was determined using a Konica Minolta CR-400 colorimeter, in which the bands were measured at three different points in the sample. The parameter L* represents the luminous intensity, and higher values indicate that the meat is lighter; lower values indicate that the meat is darker, the * varies from red (+ to *) to green (- a*) and parameter b* varies from yellow (+ b*) to blue (- b*). The pH of the meat was measured using a pH meter with an electrode. Using the release of water caused by pressure on the muscle, the water retention capacity was determined. For this purpose, 2 grams of the pectoral muscle were placed between filter papers, and a pressure of 10 kg was applied for 5 minutes through an acrylic plate. For the calculation, we considered the exudate weight and the initial muscle weight. As described by Fortuoso et al. (2019), water loss by thawing in the breast sample was evaluated.

2.7.2. Fatty acid and total lipid profile

The lipids were extracted from 3 g of the feed and 2 g of the chicken breast by the mixture of methanol: chloroform described by Bligh and Dyer (1959). The total lipid content was determined by gravimetry after complete evaporation of the organic solvent in an oven with air circulation at 105 °C. The remainder of the organic fraction containing the lipids was subjected to derivatization to obtain fatty acid methyl esters (FAME) (Hartman and Lago, 1973). FAME samples were injected (1 µL) in split mode (20:1) into a Varian 3400CX gas chromatograph (USA). The gas chromatograph was equipped with a flame ionization detector (GC-FID). Analytes were separated in an HP-88 (Agilent Technologies, USA) (100 m × 0.25

mm; 0.20 μm of thickness film) capillary column. The column temperature was held at 50 °C for 1 min, after which temperature was increased to 185 °C at a rate of 15 °C min^{-1}, followed by an increase at 0.5 °C min^{-1} to 190 °C and an increase at 15 °C min^{-1} to 230 °C, where it was held for 10 min. The injector and detector temperature was 250 °C. FAME was identified by comparison with certificate standards FAME Mix 37 (P/N 47885-U), Vaccenic acid methyl ester (P/N 46905-U), and docosapentaenoic methyl ester (P/N 47563-U) (Sigma-Aldrich, USA). Results were expressed as a percentage of the total area of the chromatograms, considering a correction factor (equivalent carbon-chain length) and a conversion factor of an ester to acid (Visentainer, 2012).

2.7.3. Oxidant and antioxidant status

The chest muscle was used for oxidative and antioxidant analysis. The tissue was placed in a 10 mM Tris-HCL solution, pH 7.4 for analysis of reactive oxygen species (ROS), thiobarbituric acid reactive substances (TBARS), glutathione S-transferase (GST), and total thiols. Using a Potter glass homogenizer, the samples were homogenized in a specific buffer solution to produce S1. This homogenate was centrifuged for 10 minutes at 4 °C at 10,000 rpm. ROS levels were determined using the methodology of Halliwell & Gutteridge (2007). TBARS determination was per Jentzsch et al. (1996) and Ohkawa et al. (1978). GST activity was measured as per Habig et al. (1974). Measurement of total thiols was as per Ellman (1969).

2.8. Histopathology and intestinal morphometry

Samples of liver and intestine (jejunum) were collected from eight birds and preserved in 10% formaldehyde. The location of the intestine fragment was standardized as 25 cm from the end of the duodenum. Slides were made using histological sections stained with hematoxylin and eosin to determine villus height and Lieberkühn crypt depths, as per Caruso and Demonte (2005) and as detailed by Galli et al. (2020).

2.9. **Statistical analysis**

The experimental design of this study was one factorial 2 × 2 [concentrate with and without mycotoxin (Myc and NoMyc) and with and without silymarin (Sil and NoSil)]. All data were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC, USA; version 9.4), with Satterthwaite approximation to determine the denominator degrees of freedom for the test of fixed effects. The data of WG, FI, FC were tested for fixed effects of mycotoxin, silymarin, and the interaction and used random effect (mycotoxin × silymarin). The data of serum biochemistry, meat quality, fatty acids, antioxidant profiles were tested for fixed

effects of mycotoxin, silymarin, and the interaction, considering random effects (mycotoxin × silymarin) and animal (pen). Only the BW data were analyzed as repeated measures and were included as fixed effects mycotoxin, silymarin, day, and all possible interactions, considering random effects (mycotoxin × silymarin) and animal (pen). The compound symmetric covariance structure was selected according to the lowest Akaike information criterion. Means were separated using PDIFF, and all results were reported as LSMEANS followed by SEM. Significance was defined when $P \leq 0.05$.

3. Results

3.1 Performance

Results of performance are shown in Table 2. Up to 21 days of the age of the birds, we observed no difference between treatments in WG or FC; however, feed consumption was lower for birds that consumed feed contaminated with mycotoxin during this period. From 1 to 35 days, we observed an effect of mycotoxin and interaction between mycotoxin and silymarin for WG; birds that consumed aflatoxin and fumonisin had less WG than the control group. The Myc-NoSil group had lower feed consumption. On the 42nd day of the production cycle, we observed the lowest WG and feed consumption, associated with greater FC than the other treatments; this finding suggests that the inclusion of silymarin in the poultry feed minimizes the adverse effects on broiler performance. In Figure 1, the interaction between mycotoxin x silymarin x day for bodyweight is shown. Feed containing mycotoxin negatively affected birds' weights, while silymarin in the feed prevented WG delay at the end of the production cycle (days 35 and 42).

3.2. Serum biochemistry

Results of serum biochemistry are shown in Table 3. There was an effect of the addition of silymarin in the feed of the chickens on globulin, albumin, ALT, and AST; that is, birds in the NoMyc-Sil group had higher levels of globulin; levels of albumin were lower, similar to levels of AST and ALT that were lower in this treatment than in NoMyc-NoSil. There was also an effect of mycotoxin consumption on uric acid, ALT, and AST levels (Myc-NoSil); that is, these three variables were higher than in the control (NoMyc-NoSil). Interaction between mycotoxin consumption and supplementation with silymarin was found only for ALT level; that is, the addition of silymarin in the feed prevented increased ALT levels (Myc-Sil); it is noteworthy that ALT levels were on average 2.5 times higher in Myc-NoSil birds.

3.3. Meat quality and composition

The physicochemical properties of chicken meat are presented in Table 4. Color (red "A" and yellow "B") did not differ between treatments; this finding differed from the luminosity that showed the effect of mycotoxin consumption and interaction between mycotoxin versus silymarin. Notably, there was greater luminosity in the meat of birds that consumed feed contaminated with a mycotoxin. The consumption of silymarin affected the meat's pH; that is, the intake of the additive raised the pH (NoMyc-Sil). There was an interaction between mycotoxin and silymarin for water loss from cooking; that is, birds that consumed the only mycotoxin (Myc-NoSil) had less loss; but when we used silymarin supplementation in a feed contaminated with aflatoxin and fumonisin (Myc-Sil), this group did not differ from the others. The water retention capacity and shear strength did not differ between treatments.

3.4. Fatty acid profile in meat

The percentage of fat in the meat did not differ between treatments (Table 5). The sum of saturated and monounsaturated fatty acids did not differ between treatments; this finding was different from the sum of polyunsaturated fatty acids (Table 5); we found that the sum of polyunsaturated fatty acids was higher in the two groups that consumed silymarin (NoMyc-Sil and Myc-Sil). Individually, we verified the interaction between mycotoxin and silymarin for SFA C14:0 and C18:0 and monounsaturated fatty acids C16:1 and C22:1n9 ($P < 0.05$). Aa mycotoxin effect was also verified for C17:0 and C18:3n6 fatty acid ($P < 0.05$). A silymarin effect was also observed for fatty acids C14:0, C18:2n6c, and C18:3n3.

3.5. Oxidizing and antioxidant status in meat

Oxidant and antioxidant statuses are shown in Table 6. TBARS levels did not differ between treatments ($P > 0.05$). ROS levels were lower in meat from the group that consumed mycotoxin (Myc-NoSil) than the other treatments. Antioxidant levels (GST and total unions) were higher in this same group (Myc-NoSil) than the other groups.

3.6. Histopathology

There were no histological lesions in the liver and intestines of birds in any group. The intestinal morphometric analysis showed that birds that consumed silymarin had greater villus height and greater crypt depth (Table 7). However, the villus/crypt ratio did not differ between treatments.

4. Discussion

The inclusion of silymarin in poultry feed improved animal health by increasing growth performance, which benefits producers. This compound enhanced meat fatty acid profiles that are impaired by mycotoxin and restored oxidant and antioxidant variables to physiological levels, conditions that benefit animal health and human consumers.

Between days 11 to 35, the presence of mycotoxin on poultry feed impaired WG, and the presence of silymarin did not preserve bird weight. By contrast, between days 11 to 42, silymarin prevented the growth retardation elicited by mycotoxins and prevented the impairment of feed intake and FC, in agreement with findings of Tedesco et al. (2004) in broiler chicks feed with a feed contaminated with 0.8 mg/kg AFB1 and supplemented with 600 mg/kg silymarin. These authors concluded that silymarin protected against the adverse effects of AFB1 on broiler chick performance. Similarly, Alhdary et al. (2017) demonstrated that silymarin prevented adverse effects on WG, feed efficiency, and FC in poultry experimentally given feed containing various sources of aflatoxins. The authors concluded that this compound prevents the adverse effects of aflatoxicosis on performance. Mycotoxins are valuable hepatotoxic compounds. As expected, we observed a significant increase in serum ALT levels in animals given feed containing mycotoxins, as observed by Tarasova et al. (2020) in broilers given feed containing AFB1 and AFB2. Similar results were also reported by Faixová et al. (2010) in broiler chickens given feed contaminated with fumonisins, a condition related to severe liver damage.

Our most important finding was that silymarin prevented the increases in ALT levels, suggesting protective effects on the liver during mycotoxin intoxication. Similar to our observations, Jamshidi et al. (2007) found that 800 mg silymarin/kg feed for 42 days prevented the augmentation of serum ALT levels in broilers given feed containing 1 mg/kg AFB1; their findings suggest the hepatoprotective effects of the compound against mycotoxin-induced liver damage.

Recent evidence suggested that oxidative stress is critical in the pathogenesis of mycotoxin intoxication (Baldissera et al., 2019), especially for poultry Tarasova et al. (2020). In the present study, we observed a significant increase in meat GST activity and NPSH levels in broilers given feed containing mycotoxins; this finding suggests that the enzymatic and non-enzymatic antioxidant defense systems are upregulated to counteract the excessive production of free radicals, a condition that corroborated by decreased meat ROS levels, i.e., the upregulation on antioxidant defense system contributed to reducing the ROS formation and its consequent negative impact on physiology. Excessive ROS might mediate several intracellular

mechanisms that oxidate DNA, proteins, and membrane lipids. The physiological attempt of the body to counteract ROS formation can reduce damage to macromolecules.

Our findings disagree with those of Eraslan et al. (2005), who observed significant increases in serum ROS levels and consequent decreases in serum activities of catalase, superoxide dismutase, and glutathione peroxidase (GPx). This condition exacerbated and contributed to the pathogenesis of intoxication. Another important finding of the present study was that silymarin restored ROS levels and GST activities to values of the control group, suggesting the potent antioxidant capacity of silymarin. Similar to our observations, Yu et al. (2019) demonstrated that silymarin prevented lipid peroxidation and impairment of catalase, superoxide dismutase, and GPx activities in chicken hepatocytes; the authors concluded that silymarin could be a potent alternative to prevent the hepatic damage caused by mycotoxins consumption. An *in vitro* study by Ledur and Santurio (2020) showed that silymarin reduced ROS formation in PK-15 cells exposed to fumonisin B1. This finding also suggested the potent antioxidant activity of silymarin against various mycotoxins. Finally, it is essential to emphasize that neither mycotoxins nor silymarin caused alterations in liver structure, suggesting the safety of silymarin at the tested dose and time of consumption. On the other hand, silymarin increased villus size, which can explain the performance improvement (mainly WG) due to the enhancement of feed absorption.

The consumption of silymarin by chickens altered fatty acid profiles significantly. The increase in the sum of polyunsaturated fatty acids; this finding has not been previously described in the literature. Researchers reported that silymarin in broiler feed caused reduced lipid content in the breast and thigh, and increased muscle resistance to oxidative stress; these findings may be directly related to changes in the fatty acid profiles as we found in the present study (Schiavone et al., 2007). The mechanism involved in the increase of PUFA has not been elucidated; however, we believe that it is an indirect effect, primarily related to the well-known hepatoprotective effect of silymarin (Saeed et al., 2019), which probably alters lipid metabolism at the hepatic level, consequently reflected in the meat. The desired effect in this study was the anti-mycotoxin activity of silymarin; however, this increase in PUFA was a positive result in terms of meat quality for human consumption, as nutritionists recommend consuming omega fatty acids. If it is possible to obtain these benefits in chicken meat rather than dietary supplements, we can positively impact consumer health.

5. Conclusion

The addition of silymarin in feed contaminated with aflatoxin and fumonisin prevents adverse effects on growth performance, probably due to a hepatoprotective effect.

Consumption of silymarin by birds also improved meat quality, particularly by preventing adverse effects caused by the mycotoxin related to luminosity and water loss by cooking. Finally, the meat of the birds that consumed the silymarin had higher levels of PUFA.

Ethics Committee

The ethics committee at the State University of Santa Catarina approved the project using animals in research, protocol number 7562021219.

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Table 1: Ingredients and basal feed used for all experimental groups.

Ingredients (kg/ton)	Age (days)		
	1–21	22–35	36–45
Corn	551.51	580.05	621.35
Soybean meal	373.01	337.00	298.22
Soy oil	39,05	49.19	49.70
Bicalcium phosphate	12.71	13.00	112,25
Calcitic limestone	11.42	9.28	8.00
Iodized salt	4.86	4.23	3.95
DL-Methionine – 99%	2.91	2.82	2.50
L-lysine – 78%	2.03	1.95	2.63
L-threonine – 99%	0.50	0.48	0.40
Premix of vitamins and minerals[1]	2.00	2.00	2.00
Vitamin E in powder (mg/kg)	200	200	200
Calculated chemical composition	100	100	100
Energy (kcal/kg)	3050	3150	3200
Crude protein (%)	21.20	19.80	18.40
Calcium (%)	0.84	0.76	0.66
Available phosphorus (%)	0.40	0.35	0.31
Digestible lysine (%)	1.22	1.13	1.06
Digestible methionine (%)	0.47	0.45	0.42
Digestible methionine + cysteine (%)	0.88	0.83	0.77
Digestible threonine (%)	0.79	0.73	0.69
Digestible tryptophan (%)	0.21	0.20	0.19
Sodium (%)	0.21	0.20	0.19

[1] Minimal vitamin and mineral levels per kg of product: vitamin A (5.000.000 UI); vitamin D3 (1.000.000 IU); vitamin E (15.000 UI); vitamin K3 (1.500 mg); vitamin B1 (1.500 mg); vitamin B2 (3.000 mg); vitamin B6 (2.000 mg); vitamin B12 (7.000 mcg); folic acid (500 mg); nicotinic acid (15 g); pantothenic acid (7000 mcg); choline (80 g); biotin (100 mg); Copper (10 g); iron (50 g); iodine (1.000 mg); manganese (80 g); selenium (300 mg); zinc (70 g); minimum humidity (20 g); maximum mineral matter (980 g)., 10 mg/ kg of feed); coccidiostat (salinomycin, 64 mg/kg of feed).

Table 2. Growth performance of poultry fed with feed containing mycotoxins and silymarin

Variables[1]	Combined treatments[2]				SEM	*P*-values[3]		
	NoMyc-NoSil	NoMyc-Sil	Myc-NoSil	Myc-Sil		Myc × Sil	Myc	Sil
WG								
d 1 to 7	0.12	0.12	0.12	0.11	0.01	0.31	0.25	0.78
d 1 to 21	0.87	0.84	0.86	0.85	0.02	0.75	0.86	0.37
d 1 to 35	2.52^{a}	2.42^{ab}	2.28^{b}	2.43^{ab}	0.05	**0.05**	**0.05**	0.71
d 11 to 42	3.29^{a}	3.15^{a}	2.90^{b}	3.19^{a}	0.06	**<0.01**	**0.01**	0.25
FI								
d 1 to 7	0.16^{ab}	0.17^{a}	0.15^{b}	0.14^{c}	0.004	**0.02**	**<0.01**	0.98
d 1 to 21	1.26	1.25	1.19	1.18	0.03	0.95	**0.04**	0.76
d 1 to 35	3.64	3.62	3.45	3.54	0.08	0.46	**0.05**	0.64
d 1 to 42	5.22^{a}	5.11^{a}	4.90^{b}	5.12^{a}	0.07	**0.04**	**0.05**	0.46
FC								
d 1 to 7	1.34	1.39	1.26	1.21	0.03	0.10	**<0.01**	0.91
d 1 to 21	1.45	1.49	1.38	1.39	0.03	0.76	**0.04**	0.53
d 1 to 35	1.46	1.50	1.51	1.46	0.04	0.18	0.82	0.18
d 1 to 42	1.59^{b}	1.62^{b}	1.69^{a}	1.61^{b}	0.02	**<0.01**	**0.02**	0.15

[1] Weight gain (WG), feed intake (FI), and feed conversion (FC).
[2]In a factorial design (2 × 2) included or not mycotoxin (Myc and NoMyc); and also included or not silymarin (Sil and NoSil).
[3]Myc, mycotoxin; Sil, silymarin.
[4]Mycotoxin: 0,5 and 20 ppm of aflatoxin and fumonisin per kg of concentrate, respectively. Also, 100 mg of silymarin/kg of concentrate was used.
[5] Difference ($P \leq 0.05$) between treatments was some lines; (effects of Myc × Sil).

Table 3. Serum biochemistry of poultry fed with feed containing mycotoxins and silymarin

Variables[1]	Combined treatments[2]				SEM	*P*-values[3]		
	NoMyc-NoSil	NoMyc-Sil	Myc-NoSil	Myc-Sil		Myc × Sil	Myc	Sil
Total protein	3.84	3.69	3.75	3.75	0.20	0.72	0.95	0.72
Globulin	1.51	1.71	1.35	1.77	0.15	0.47	0.75	**0.05**
Albumin	2.33	1.98	2.40	1.96	0.13	0.77	0.77	**<0.01**
Uric acid	4.26	3.80	5.69	6.03	0.63	0.51	**<0.01**	0.92
Cholesterol	109	112	138	125	10.5	0.48	0.05	0.63
ALT	2.87[b]	2.75[b]	9.00[a]	1.75[b]	0.71	**<0.01**	**<0.01**	**<0.01**
AST	286	251	401	354	21.8	0.78	**<0.01**	**0.05**

[1] Total protein (g/dL), globulin (g/dL), albumin (g/dL), uric acid (mg/dL), cholesterol (mg/dL), alanine aminotransferase (ALT – U/L), and aspartate aminotransferase (AST – U/L).
[2]In a factorial design (2 × 2) included or not mycotoxin (Myc and NoMyc, respectively); and also included or not silymarin (Sil and NoSil, respectively).
[3]Myc, mycotoxin; Sil, Silymarin.
[4]Mycotoxin: 0,5 and 20 ppm of aflatoxin and fumonisin per kg of concentrate, respectively. Also, 100 mg of silymarin/kg of concentrate was used.
[5] Difference ($P \leq 0.05$) between treatments was some lines; (effects of Myc × Sil).

Table 4. Meat quality of poultry fed with feed containing mycotoxins and silymarin

Variables[1]	Combined treatments[2]				SEM	*P*-values[3]		
	NoMyc-NoSil	NoMyc-Sil	Myc-NoSil	Myc-Sil		Myc × Sil	Myc	Sil
Cor A	-0.51	-1.08	-1.49	-1.27	0.58	0.51	0.33	0.77
Cor B	12.9	12.0	11.8	11.2	1.24	0.90	0.44	0.55
Cor L	48.5^{c}	50.6^{bc}	55.5^{a}	53.2^{ab}	0.92	**0.03**	**<0.01**	0.97
pH	5.80	5.95	5.76	5.85	0.06	0.57	0.27	**0.05**
WHC	0.42	0.36	0.41	0.44	0.04	0.27	0.41	0.73
WLC	23.1^{a}	23.2^{a}	17.6^{b}	21.4^{a}	0.75	**0.03**	**<0.01**	**0.02**
SF	2.53	3.36	3.34	2.79	0.52	0.20	0.82	0.80

[1]Water retention capacity (WHC), Water loss by cooking (WLC), and shear force (SF).
[2]In a factorial design (2 × 2) included or not mycotoxin (Myc and NoMyc, respectively); and also included or not silymarin (Sil and NoSil, respectively).
[3]Myc, mycotoxin; Sil, Silymarin.
[4]Mycotoxin: 0,5 and 20 ppm of aflatoxin and fumonisin per kg of concentrate, respectively. Also, 100 mg of silymarin/kg of concentrate was used.
[5] Difference ($P \leq 0.05$) between treatments was some lines; (effects of Myc × Sil).

Table 5. Fatty acids profile of meat from poultry fed with feed containing mycotoxins and silymarin

Variables[1]	Combined treatments[2]				SEM	*P*-values[3]		
	NoMyc-NoSil	NoMyc-Sil	Myc-NoSil	Myc-Sil		Myc × Sil	Myc	Sil
Fat (%)	1.10	1.09	1.12	1.45	0.19	0.40	0.33	0.43
Perfil de AG								
C14:0	0.46[a]	0.30[b]	0.33[b]	0.32[b]	0.04	**0.05**	0.18	**0.05**
C14:1	0.16	0.17	0.20	0.17	0.02	0.29	0.45	0.69
C15:0	0.11	0.09	0.08	0.08	0.01	0.39	0.26	0.35
C16:0	25.48	23.14	23.69	22.79	1.16	0.07	0.20	0.35
C16:1	2.26[a]	1.35[b]	1.32[b]	1.62[b]	0.25	**0.03**	0.20	0.24
C17:0	0.17	0.20	0.23	0.21	0.02	0.14	**0.05**	0.68
C18:0	9.40[b]	10.1[b]	11.6[a]	9.97[b]	0.62	**0.05**	0.12	0.48
C18:1n9t	0.25	0.21	0.22	0.20	0.02	0.64	0.48	0.15
C18:1n9c	27.68	27.53	27.15	29.02	1.29	0.44	0.72	0.51
Vaccenic	2.11	2.01	2.08	1.74	0.18	0.51	0.40	0.23
C18:2n6c	26.1	28.8	26.6	28.5	0.85	0.29	0.48	**0.04**
C18:3n6	0.11	0.12	0.14	0.13	0.01	0.35	**0.02**	0.90
C20:0	0.16	0.16	0.21	0.18	0.02	0.32	0.09	0.54
C18:3n3	1.45	1.73	1.43	1.73	1.16	0.97	0.93	**0.05**
C20:1n9	0.25	0.21	0.20	0.24	0.02	0.14	0.78	0.93
C20:2n6	0.37	0.41	0.45	0.46	0.05	0.73	0.24	0.60
C20:3n6	0.41	0.40	0.38	0.38	0.04	0.88	0,52	0.98
C22:1n9	2.51[ab]	2.89[a]	2.90[a]	1.78[b]	0.42	**0.04**	0.41	0.40
C20:5n3	0.10	0.09	0.09	0.06	0.01	0.71	0.20	0.15
C22:5n3	0.30	0.34	0.38	0.25	0.05	0.15	0.88	0.40
C22:6n3	0.22	0.25	0.30	0.18	0.05	0.11	0.98	0.34
AGS	36.6	33.8	36.2	33.5	1.35	0.68	0.53	0.13
AGMS	35.2	33.8	34.1	34.8	1.03	0.32	0.95	0.32
AGPS	28.2[b]	32.4[a]	29.8[b]	31.7[a]	0.78	**0.05**	0.25	**<0.01**

[1] myristic acid (C14:0); pentadecanoic acid (C15:0), palmitic acid (C16:0), palmitoleic acid (C16:1), heptadecanoic acid (C17:0), stearic acid (C18:0), cis-9-octadecenoic acid (oleic acid) (C18:1n9c), trans-9-octadecenoic acid (oleic acid) (C18:1n9t), linoleic acid (C18:2n6), alpha-linolenic acid (C18:3n3), arachidic acid (C20:0), alpha-linolenic acid (C18:3n3), cis-11-eicosenoic acid (C20:1n9), is-11,14-eicosadienoic acid (C20:2n6), cis-8,11,14-eicosatrienoic acid (C20:3n6), eicosapentaenoic acid (C20:5n3), docosapentaenoic acid (C22:5n3) and docosahexaenoic acid (C22:6n3).

[2]In a factorial design (2 × 2) included or not mycotoxin (Myc and NoMyc, respectively); and also included or not silymarin (Sil and NoSil, respectively).

[3]Myc, mycotoxin; Sil, Silymarin.

[4]Mycotoxin: 0,5 and 20 ppm of aflatoxin and fumonisin per kg of concentrate, respectively. Also, 100 mg of silymarin/kg of concentrate was used.

[5] Difference ($P \leq 0.05$) between treatments was some lines; (effects of Myc × Sil).

Table 6. Antioxidant profile from the meat of poultry fed with feed containing mycotoxins and silymarin.

	Combined treatments[2]				SEM	*P*-values[3]		
Variables[1]	**NoMyc-NoSil**	**NoMyc-Sil**	**Myc-NoSil**	**Myc-Sil**		**Myc × Sil**	**Myc**	**Sil**
TBARS	15.59	15.91	13.73	16.12	0.97	0.30	0.41	0.18
ROS	2770[a]	2913[a]	812[b]	2662[a]	236	**<0.01**	**<0.01**	**<0.01**
GST	34.6[b]	36.3[b]	57.8[a]	39.9[b]	4.29	**0.04**	**<0.01**	**0.05**
Total thiols	0.74[c]	0.80[bc]	2.13[a]	1.15[b]	0.15	**<0.01**	**<0.01**	**<0.01**

[1]Thiobarbituric acid reactive substances (TBARS), reactive oxygen species (ROS), glutathione S-transferase (GST), and total thiols.
[2]In a factorial design (2 × 2) included or not mycotoxin (Myc and NoMyc for xx or 0 mg of mycotoxin/kg of concentrate, respectively) and also included or not silymarin (Sil and NoSil for 100 or 0 mg of silymarin/kg of concentrate, respectively).
[3]Myc, mycotoxin; Sil, Silymarin.
[a-c]Differs ($P \leq 0.05$) between combined treatments (lines; effects of Myc × Sil).

Table 7. Villi, crypt and relation villi/crypt in the intestine of poultry fed with feed containing mycotoxins and silymarin.

	Combined treatments[2]				**SEM**	***P*-values[3]**		
Variables[1]	**NoMyc-NoSil**	**NoMyc-Sil**	**Myc-NoSil**	**Myc-Sil**		**Myc × Sil**	**Myc**	**Sil**
Villi	1113.8[b]	1420.6[a]	1081[b]	1079.1[b]	28.6	**0.05**	0.63	**<0.01**
Crypt	224.8[b]	307.2[a]	233.4[b]	243.8[b]	14.3	**0.02**	0.51	**<0.01**
Villi/crypt	4.95	4.62	4.63	4.43	1.39	0.06	0.71	0.68

[1]In a factorial design (2 × 2) included or not mycotoxin (Myc and NoMyc, respectively); and also included or not silymarin (Sil and NoSil, respectively).
[2]Myc, mycotoxin; Sil, Silymarin.
[3]Mycotoxin: 0,5 and 20 ppm of aflatoxin and fumonisin per kg of concentrate, respectively. Also, 10.0 mg of silymarin/kg of concentrate was used.
[4] Difference ($P \leq 0.05$) between treatments was some lines; (effects of Myc × Sil).

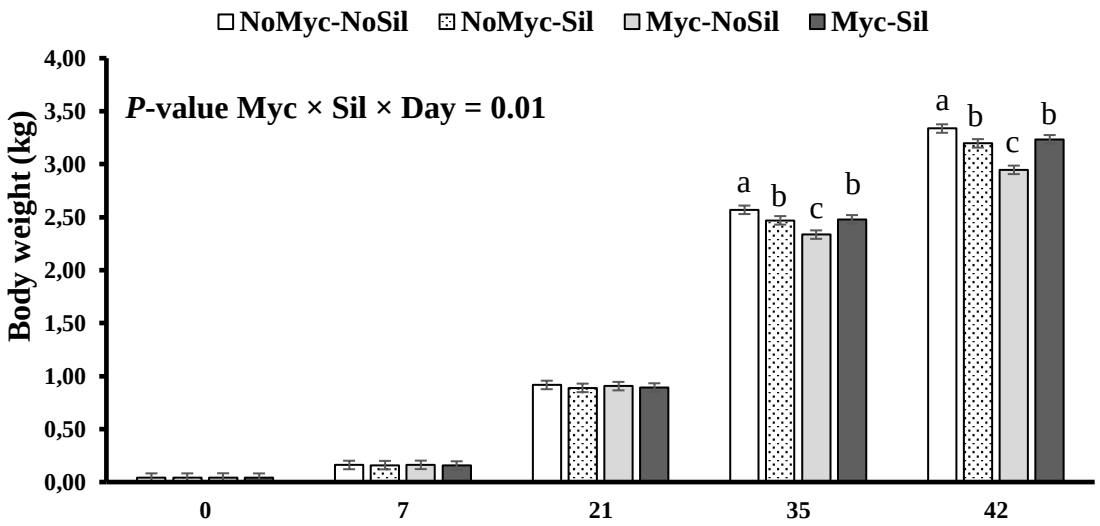

Figure 1. Growth of poultry fed with feed containing mycotoxins (Myc) and Silymarin (Sil). In a factorial design (2 × 2) was include or not mycotoxin (Myc and NoMyc for xx or 0 mg of mycotoxin/kg of concentrate, respectively) and also included or not silymarin (Sil and NoSil for 100 or 0 mg of silymarin/kg of concentrate, respectively).
[a-b]Differs ($P \leq 0.05$) between treatments. Vertical bars represent the SEM.

Material Supplementary 1. Profile fatty acids in the broiler based feed.

Compound[1]	**Mean**	**Standard deviation**	**Coefficient of variation**
C14:0	0.10	0.01	8.14
C15:0	0.03	0.00	2.90
C16:0	14.90	0.70	4.73
C16:1	0.11	0.01	5.86
C17:0	0.12	0.00	3.77
C18:0	4.48	0.25	5.65
C18:1n9c	25.98	0.17	0.66
Vaccenic	0.91	0.02	2.67
C18:2n6c	48.28	0.75	1.55
C18:3n6	0.39	0.03	8.88
C18:3n3	3.97	0.18	4.46
C20:1n9	0.21	0.01	7.14
C21:0	0.03	0.00	3.78
C22:0	0.35	0.03	8.11
C23:0	0.05	0.00	6.09
C24:0	0.10	0.01	8.75
Fat	6.71	0.17	2.57

[1] Fatty acids are: myristic acid (C14:0); pentadecanoic acid (C15:0), palmitic acid (C16:0), palmitoleic acid (C16:1), heptadecanoic acid (C17:0), stearic acid (C18:0), cis-9-octadecenoic acid (oleic acid) (C18:1n9c), linoleic acid (C8: 2n6), alpha-linolenic acid (C18:3n3), gamma-linolenic acid (C18:3n6), cis-11-eicosenoic acid (C20:1n9), heneicosanoic acid (C21:0); behenic (C22:0); tricosanoic acid (C23:0); tetracosanoic acid (C24:0). Note: Food samples were collected from the three production phases (1 to 21, 22 to 35, and 36 to 42), frozen and at the end of the experiment, the three samples were mixed, and a pool was used for the profile analysis of fatty acids and lipids in the feed consumed.

3 CONSIDERAÇÕES FINAIS

Podemos concluir que a adição do blend fitogênico pode ser uma alternativa de substituição dos antimicrobianos convencionais utilizados na avicultura de corte, o qual não compromete a saúde intestinal, qualidadade da carne e o desempenho zootécnico das aves. A suplementação do blend fitogênico possui efeitos antioxidantes e antimicrobianos, e ajuda a reduzir os efeitos negativos causados pela oxidação na produção de frangos de corte.

A suplementação com 250 g/kg do composto fitogênico aumenta os níveis de gordura na carne de peito, reduz os ácidos graxos saturados e aumenta os ácidos graxos insaturados, todos desejáveis e interessantes do ponto de vista da saúde do consumidor. O composto fitogênico minimizou os efeitos negativos na saúde e no desempenho dos frangos ocasionados pela aflatoxicose.

A adição de silimarina na ração de frangos de corte contaminadas com aflatoxinas e fumonisinas evita efeitos adversos no desempenho do crescimento, provavelmente devido a um efeito hepatoprotetor. A suplementação de silimarina melhorou a qualidade da carne, principalmente ao prevenir os efeitos adsersos causados pelas micotoxinas relacionados à luminosidade e perda de água por cozimento. A carne das aves suplementadas com silimarina apresetou níveis mais elevados de PUFA.

A utilização do blend fitogênico e da silimarina, apresentaram diminuição dos efeitos negativos ocasionados pela contaminação das micotoxinas nas rações de frangos de corte, sendo potenciais compostos fitogênicos disponíveis para o combate das micotoxicoses e das perdas econômicas ocasionadas no setor avícola.

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ANEXOS

UDESC UNIVERSIDADE DO ESTADO DE SANTA CATARINA | LAGES CENTRO DE CIÊNCIAS AGROVETERINÁRIAS

Comissão de Ética no Uso de Animais

CERTIFICADO

Certificamos que a proposta intitulada "Aditivos como alternativa para minimizar os efeitos negativos em frangos de corte que consumiram ração contaminadas com aflatoxinas e fumonisinas", protocolada sob o CEUA nº 7562021219 (ID 001093), sob a responsabilidade de **Aleksandro Schafer da Silva** - que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica ou ensino - está de acordo com os preceitos da Lei 11.794 de 8 de outubro de 2008, com o Decreto 6.899 de 15 de julho de 2009, bem como com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi **aprovada** pela Comissão de Ética no Uso de Animais da Universidade do Estado de Santa Catarina (CEUA/UDESC) na reunião de 06/12/2019.

We certify that the proposal "Additives as an alternative to minimize the negative effects on broilers that consumed aflatoxins and fumonisins contaminated feed", utilizing 880 Birds (880 males), protocol number CEUA 7562021219 (ID 001093), under the responsibility of **Aleksandro Schafer da Silva** - which involves the production, maintenance and/or use of animals belonging to the phylum Chordata, subphylum Vertebrata (except human beings), for scientific research purposes or teaching - is in accordance with Law 11.794 of October 8, 2008, Decree 6899 of July 15, 2009, as well as with the rules issued by the National Council for Control of Animal Experimentation (CONCEA), and was **approved** by the Ethic Committee on Animal Use of the University of Santa Catarina State (CEUA/UDESC) in the meeting of 12/06/2019.

Finalidade da Proposta: Pesquisa (Acadêmica)

Vigência da Proposta: de 12/2019 a 12/2020 Área: Zootecnia

Origem:	Animais provenientes de estabelecimentos comerciais						
Espécie:	Aves	sexo:	Machos	idade:	1 a 42 dias	N:	880
Linhagem:	a definir			Peso:	40 a 3500 g		