UNIVERSIDADE DO ESTADO DE SANTA CATARINA – UDESC
CENTRO DE EDUCAÇÃO SUPERIOR DO OESTE – CEO
PROGRAMA DE PÓS-GRADUAÇÃO EM ZOOTECNIA

DISSERTAÇÃO DE MESTRADO

Aditivos funcionais como alternativas para minimizar os impactos causados pelas micotoxinas na dieta de poedeiras e leitões

VANESSA DAZUK

CHAPECÓ, 2020

VANESSA DAZUK

ADITIVOS FUNCIONAIS COMO ALTERNATIVAS PARA MINIMIZAR OS IMPACTOS CAUSADOS PELAS MICOTOXINAS NA DIETA DE POEDERIAS E LEITÕES

Dissertação apresentada ao Curso de Mestrado do Programa de Pós-Graduação em Zootecnia, Área de Concentração Ciência e Produção Animal, da Universidade do Estado de Santa Catarina (UDESC), como requisito parcial para obtenção de grau de **Mestre em Zootecnia.**

Orientador: Dr. Aleksandro Schafer da Silva
Co-orientador: Dr. Diovani Paiano
Co-orientador: Marcel Manente Boiago

Chapecó, SC, Brasil

2020

Ficha catalográfica elaborada pelo programa de geração automática da

Biblioteca Setorial do CEO/UDESC,

com os dados fornecidos pelo(a) autor(a)

Dazuk, Vanessa
Aditivos funcionais como alternativas para minimizar os impactos causados pelas micotoxinas na dieta de poederias e leitões / Vanessa Dazuk. -- 2020.
130 p.

Orientador: Aleksandro Schafer da Silva
Coorientador: Diovani Paiano
Coorientador: Marcel Manente Boiago
Dissertação (mestrado) -- Universidade do Estado de Santa Catarina, Centro de Educação Superior do Oeste, Programa de Pós-Graduação em Zootecnia, Chapecó, 2020.

1. Aditivos. 2. Micotoxinas. 3. Poedeiras. 4. Suinos. I. Schafer da Silva, Aleksandro. II. Paiano, Diovani. Manente Boiago, Marcel. III. Universidade do Estado de Santa Catarina, Centro de Educação Superior do Oeste, Programa de Pós-Graduação em Zootecnia. IV. Titulo.

Universidade do Estado de Santa Catarina
UDESC Oeste
Programa de Pós-Graduação em Zootecnia

A Comissão Examinadora, abaixo assinada,
aprova a Dissertação de Mestrado

ADITIVOS FUNCIONAIS COMO ALTERNATIVAS PARA MINIMIZAR OS IMPACTOS CAUSADOS PELAS MICOTOXINAS NA DIETA DE POEDERIAS E LEITÕES

Elaborada por
Vanessa Dazuk

como requisito parcial para obtenção do grau de
Mestre em Zootecnia

Comissão Examinadora:

Dr. Aleksandro Schafer Da Silva (UDESC)

Dra. Ines Andreta (UFRGS)

Dr. Tiago Goulart Petrolli (UNOESC)

Chapecó, 16 de novembro de 2020.

AGRADECIMENTOS

A Deus por sua proteção, amparo e força, por sempre me permitir aprender, evoluir e acreditar sempre em uma força maior.

A minha linda família, que sempre foi a minha base e apoio para tudo, obrigado por sempre acreditarem na minha capacidade, por tudo que sempre fizeram por mim, pelos bons valores.

Ao meu namorado Dirceu, por ser meu companheiro, sempre ao meu lado me ajudando no que fosse preciso, por seu amor e dedicação.

Ao meu orientador professor Aleksandro, pela oportunidade de trabalhar contigo, por ter me acolhido como sua orientada e por estar sempre pronto a me auxiliar, por todos os ensinamentos, aprendi muito contigo e levo com orgulho o seu nome como meu orientador.

Ao meu co-orientador professor Diovani, grata pela oportunidade de ingressar no mestrado e por toda ajuda e ensinamentos.

Professor Marcel Boiago, por sua orientação, dedicação, paciência, na oportunidade que tive em trabalhar com as aves.

Ao grupo de pesquisa GANA, e aos amigos que fiz nesse período do mestrado: Gilneia, Gabizinha, Gabi Campigotto, Davi, Vitor, Bruno, Guilherme, Karol e demais, sempre dispostos a ajudar, companheiros de mates, de boas conversas, sou muito grata por ter conhecido vocês.

Aos meus anjos de quatro patas, Schena, Lilica, Rebeca, Tarzan (que hoje vive na nossa saudade), sempre me presenteando com seu amor e carinho incondicionais.

A UDESC, por seu ensino gratuito e de qualidade, que forma grandes profissionais, e a todos os seus excelentes professores que tive a honra de ser aluna no mestrado.

Muito obrigado!

“Sua tarefa consiste em melhorar-se sempre e cada vez mais. Para isso você nasceu!”

(Chico Xavier)

RESUMO

Dissertação de Mestrado
Programa de Pós-Graduação em Zootecnia
Universidade do Estado de Santa Catarina

ADITIVOS FUNCIONAIS COMO ALTERNATIVAS PARA MINIMIZAR OS IMPACTOS CAUSADOS PELAS MICOTOXINAS NA DIETA DE POEDERIAS E LEITÕES

AUTOR: Vanessa Dazuk
ORIENTADOR: Prof. Dr. Aleksandro Schafer da Silva
Chapecó, 16 de novembro de 2020.

As micotoxinas são metabólitos tóxicos secundários produzidos por fungos filamentosos. As diversas espécies de micotoxinas são consideradas uma preocupação mundial quanto a saúde humana e animal. Na cadeia produtiva animal, as micotoxicoses prejudicam a produtividade dos animais. É preciso buscar alternativas que sejam capazes de minimizar os efeitos tóxicos das micotoxinas, por meio de ingredientes adicionados a alimentação com efeitos hepatoprotetores, de adsorção e inativação das toxinas. Desta forma, o objetivo foi determinar os impactos das micotoxinas aflatoxina B1 (AFLB1), T2 e fumonisina B1 (FB1), na produção avícola e de suínos, assim como verificar os efeitos da adição de biocolina vegetal (BV) e um adsorvente à base de lisado de *Saccharomyces cerevisiae* (LSC) na dieta contaminada desses animais. Para isso, foram realizados três experimentos distintos. No experimento 1, foram utilizadas 64 galinhas poedeiras, divididas em grupo controle T1 (alimentadas com ração basal), grupo T2 (ração basal suplementada com 800 mg/kg de BV), grupo T3 (ração basal contaminada com 2.5 mg/kg de AFLB1) e grupo T4 (ração com 800 mg/kg de BV+ 2.5 mg/kg de AFLAB1). O foco dessa pesquisa foi parâmetros de composição físico-químico de ovos, contagem bacterina total nos ovos (CBT), saúde das aves e desempenho zootécnico. A ingestão de aflatoxina reduziu a taxa de postura das galinhas e a BV não foi capaz de minimizar esse efeito negativo, porém a BV teve efeitos positivos na saúde das galinhas e melhorou a qualidade dos ovos através da ação antioxidante e antimicrobiana. No experimento 2, utilizamos 60 galinhas poedeiras, divididas em grupo NoC (ração basal sem contaminação experimental por micotoxinas, grupo C+ (ração contaminada com 4 ppm de T2 e 20 ppm de fumonisina), grupo C+D500 (ração contaminada com 4 ppm de T2 e 20 ppm de fumonisina + 500 g/ton de LSC, grupo C+D1000 (ração contaminada com 4 ppm de T2 e 20 ppm de fumonisina + 1000 g/ton de LSC e grupo C+D500+U100o (ração contaminada com 4 ppm de T2 e 20 ppm de fumonisina + 500 g/ton de LSC + 1000g/ton Uniwall MOS 25 (ácidos orgânicos, parede celular de levedura e carier mineral). O consumo de ração foi menor nas galinhas que consumiram a ração contaminada pelas micotoxinas. O uso de LSC minimizou os efeitos negativos da micotoxina sobre a taxa de conversão alimentar. As galinhas que ingeriram micotoxinas apresentaram menor resistência e espessura da casca quando comparadas ao NoC. A concentração sérica de espécies reativas de oxigênio foi maior nas galinhas que ingeriram micotoxina apenas no dia

84 em comparação ao NoC. A atividade sérica da glutationa S-transferase foi maior no dia 56 nas galinhas C+D500 e C+D1000 em comparação com outras. Concluímos nesse experimento que o consumo de micotoxinas prejudicou o desempenho e a qualidade dos ovos das galinhas, assim como a adição do lisado de S. cerevisiae e o combinado de outros ingredientes (ácidos orgânicos, a parede celular de leveduras e o transportador mineral) minimizaram alguns efeitos negativos causados por T-2 e FB1. No experimento 3, utilizamos 72 leitões, desmamados com média de 26 dias, divididos em quatro grupos com seis repetições cada. Os tratamentos foram: Afla0Bio0 – (sem aflatoxina e sem biocolina); Afla500Bio0 – (500 ppb de aflatoxina); Afla0Bio800 – 800 mg/kg de biocolina; Afla500Bio800 – 500 ppb de aflatoxina + 800 mg/kg de biocolina. O estudo avaliou o desempenho zootécnico (ganho de peso - GP, consumo de ração – CR e conversão alimentar - CA). Amostras de sangue foram coletadas nos dias 0, 10, 20, 30 e 40 do experimento, assim como foram abatidos 24 animais aos 30 dias do experimento para a coleta de fígado, baço e porção do intestino para análise histopatológica. A suplementação com VB na dose usada, nessa fase de creche, teve efeito positivo nos primeitos 10 dias quando consumida por leitões desafiados com AFLB1, mas de modo geral interferiu no desenvolvimento dos leitões quando consumido. Consumo da aflatoxina aumentou atividade das enzimas AST e ALT no soro, assim como uma maior contagem de neutrófilos, menores níveis de triglicerídeos sérico e variáveis de estresse oxidativo tecidual. Muitos dessas alterações foram evitadas e/ou minimizadas pelo consumo de BV, caracterizada por um potencial antioxidante em animais desafiados com aflatoxina B1. Nesse estudo verificamos efeitos positivos da ingestão de BV sobre a saúde dos leitões, mas negativo sobre o desempenho. De maneira geral, concluímos que as micotoxinas prejudicam o desempenho e saúde animal, e que os aditivos aqui estudados podem ser uma alternativa promissora para minimizar estes impactos negativos na produção animal.
Palavras-chave: Aditivos. Micotoxinas. Poedeiras. Suínos.

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

FUNCTIONAL ADDITIVES AS ALTERNATIVES TO MINIMIZE THE IMPACTS CAUSED BY MYCOTOXINS ON THE DIET OF POEDERIES AND PIGS
AUTHOR: Vanessa Dazuk
ADVISOR: Prof. Dr. Aleksandro Schafer da Silva
Chapecó, 16 november 2020

Mycotoxins are toxic secondary metabolites produced by filamentous fungi. The various species of mycotoxins are considered a worldwide concern in terms of human and animal health. In the animal production chain, mycotoxicosis impair animal productivity. It is necessary to look for alternatives that are able to minimize the toxic effects of mycotoxins, through ingredients added to the diet with hepatoprotective effects, adsorption and inactivation of toxins. Thus, the objective was to determine the impacts of mycotoxins aflatoxin B1 (AFLB1), T2 and fumonisin B1 (FB1), on poultry and swine production, as well as to verify the effects of the addition of vegetable biocolline (BV) and an adsorbent to the base of Saccharomyces cerevisiae (LSC) lysate in the contaminated diet of these animals. For this, three different experiments were carried out. In experiment 1, 64 laying hens were used, divided into control group T1 (fed with basal feed), group T2 (basal feed supplemented with 800 mg / kg BV), group T3 (basal feed contaminated with 2.5 mg / kg of AFLB1) and group T4 (diets with 800 mg / kg of BV + 2.5 mg / kg of AFLAB1). The focus of this research was parameters of physical-chemical composition of eggs, total bacterial egg count (CBT), bird health and zootechnical performance. Ingestion of aflatoxin reduced the laying rate of the chickens and BV was not able to minimize this negative effect, however BV had positive effects on the health of the chickens and improved the quality of the eggs through the antioxidant and antimicrobial action. In experiment 2, we used 60 laying hens, divided into a NoC group (basal feed without experimental mycotoxin contamination, group C + (feed contaminated with 4 ppm of T2 and 20 ppm of fumonisin), group C + D500 (feed contaminated with 4 ppm of T2 and 20 ppm of fumonisin + 500 g / ton of LSC, group C + D1000 (ration contaminated with 4 ppm of T2 and 20 ppm of fumonisin + 1000 g / ton of LSC and group C + D500 + U100o (ration contaminated with 4 ppm of T2 and 20 ppm of fumonisin + 500 g / ton of LSC + 1000g / ton Uniwall MOS 25 (organic acids, yeast cell wall and mineral carier). Feed consumption was lower in chickens that consumed the feed contaminated by mycotoxins The use of LSC minimized the negative effects of mycotoxin on the feed conversion rate Chickens that ingested mycotoxins showed less resistance and thickness of the shell when compared to NoC. The serum concentration of reactive oxygen species was higher in chickens that ingested mycotoxin. only on di to 84 compared to NoC. The serum activity of glutathione S-transferase was higher on day 56 in chickens C + D500 and C + D1000 compared to others. We concluded in this experiment that the consumption of mycotoxins impaired the performance and quality of the eggs of the chickens, as well as the

addition of the S. cerevisiae lysate and the combination of other ingredients (organic acids, the yeast cell wall and the mineral transporter) minimized some negative effects caused by T-2 and FB1. In experiment 3, we used 72 piglets, weaned for an average of 26 days, divided into four groups with six repetitions each. The treatments were: Afla0Bio0 - (without aflatoxin and without biocolina); Afla500Bio0 - (500 ppb of aflatoxin); Afla0Bio800 - 800 mg / kg of biocholine; Afla500Bio800 - 500 ppb of aflatoxin + 800 mg / kg of biocholine. The study evaluated zootechnical performance (weight gain - GP, feed intake - CR and feed conversion - CA). Blood samples were collected on days 0, 10, 20, 30 and 40 of the experiment, as well as 24 animals were slaughtered at 30 days of the experiment for the collection of liver, spleen and intestine portion for histopathological analysis. Supplementation with BV in the dose used, in this daycare phase, had a positive effect in the first 10 days when consumed by piglets challenged with AFLB1, but in general it interfered in the development of the piglets when consumed. Aflatoxin consumption increased the activity of the AST and ALT enzymes in the serum, as well as a higher neutrophil count, lower serum triglyceride levels and tissue oxidative stress variables. Many of these changes were avoided and / or minimized by the consumption of BV, characterized by an antioxidant potential in animals challenged with aflatoxin B1. In this study, we verified positive effects of BV ingestion on piglet health, but negative on performance. In general, we conclude that mycotoxins impair animal performance and health, and that the additives studied here can be a promising alternative to minimize these negative impacts on animal production.

Keywords: Additions. Layers. Pigs. Mycotoxins.

SUMÁRIO

CAPÍTULO I

1. REVISÃO DE LITERATURA

1.1 INTRODUÇÃO

1.1.1 Avicultura e suinocultura

Segundo dados da Associação Brasileira de Proteína Animal (ABPA, 2020), atualmente o Brasil se encontra na posição de maior exportador de carne avícola do mundo, chegando à marca de 4,214 milhões de toneladas no ano de 2019, sendo os três estados do Sul, Paraná, Santa Catarina e Rio Grande do Sul, os responsáveis por mais de 80% da produção nacional, sendo que na suinocultura o cenário também é de alta produtividade chegando à marca de produção de 3,983 milhões de toneladas no mesmo ano, destinados 81% deste volume para mercado interno e 19% para exportações.

No setor de produção de ovos temos um cenário histórico quanto ao consumo interno da produção brasileira, chegando a mais de 99% da produção destinada ao mercado próprio, e um consumo per capita de 230 unidades no ano de 2019 (ABPA, 2020). Com um amplo plantel de matrizes de corte e de postura, a tendência de expansão de mercado consumidor e produtor é contínua.

Pode-se afirmar que os avanços da avicultura e suinocultura brasileiras foram resultados da introdução de inovações nas áreas de genética, nutrição, sanidade e novos equipamentos no sistema criatório, o que possibilitou um ganho significativo na taxa de conversão alimentar, possibilitando um aumento na produtividade, a redução dos custos de produção e por consequência uma redução nos preços dos produtos finais (SCHMIDT & SILVA, 2018).

Com o aumento da produção do setor de proteína animal, aumentam também os desafios, sendo necessário que estes sejam estabelecidos para se buscar soluções pertinentes para cada fase de produção, seja no setor de aves ou de suínos. No ciclo de produção de suínos, o desmame é considerado um período crítico devido aos fatores estressantes que o leitão é submetido nesta fase, sendo este período determinante para o futuro produtivo do animal (EULALIO et al., 2015). Um dos desafios aos quais os animais são submetidos no desmame, é a troca da dieta líquida pela sólida e a possível exposição a agentes tóxicos como as micotoxinas provenientes de grãos e cereais (DILKIN et al., 2010).

A ingestão de alimentos que contenham micotoxinas, pode causar graves efeitos sobre a saúde animal e humana (DIAS, 2018). Em aves de postura as micotoxicoses levam a alterações de órgãos, reduzem a produção e afetam a qualidade dos ovos (SANTURIO, 2000).

1.1.2 Qualidade do ovo

O ovo é um alimento essencial na composição da dieta humana, sendo considerado uma proteína de alto valor biológico, e um alimento nutricionalmente completo. Além disso, é um alimento de baixo custo e acessível para o consumidor de menor poder aquisitivo, sendo que as características físicas e químicas do ovo podem influenciar o seu grau de aceitabilidade no mercado, e, também agregar valor ao produto comercializado (FREITAS at al., 2011).

No entanto, para que todo esse potencial nutritivo do ovo possa ser aproveitado pelo consumidor, é necessário atenção aos fatores que afetam diretamente a sua qualidade. Influências intrínsecas como genética, idade, condição nutricional e sanitária da poedeira, assim como fatores externos tais como o clima e manejo, podem alterar as características dos ovos, resultando em degradação de seus componentes, modificando suas propriedades funcionais e comprometendo sua eficiência como alimento natural ou matéria-prima (OLIVEIRA & OLIVEIRA, 2013).

Segundo ALLEONI & ANTUNES (2001), a qualidade do ovo é medida para descrever as diferenças na produção de ovos frescos, devido a características genéticas, dietas, manejos empregados aos animais e fatores ambientais, aos quais as galinhas são submetidas. Para se avaliar a qualidade do ovo são realizadas análises físico-quimicas e microbiológicas que abrangem a composição, qualidade de casca, qualidade interna e possíveis contaminações por microorganismos. A qualidade do ovo é motivo de preocupação não só para as granjas comerciais, mas também para comerciantes e consumidores, pois além dos aspectos econômicos com perdas do produto, defeitos na qualidade podem significar riscos para a saúde pública (PIRES et al., 2015), entre esses riscos possíveis contaminações por microorganismos como bactérias e micotoxinas (MAZIERO & BERSOT (2010). Resíduos de micotoxinas podem ser encontrados em ovos obtidos de aves alimentadas com rações contendo micotoxinas (OLIVEIRA et al., 2000). Em seu estudo sobre resíduos de aflatoxinas e zearalenona em ovos de galinhas poedeiras, JIA et al., (2016) concluíram que a presença de aflatoxina (123,0 μg/kg) isoladamente ou combinada com zearalenona (260,2 μg/kg) resultou em baixo desempenho de postura e qualidade dos ovos, juntamente com a deposição de resíduos de aflatoxina nos ovos de galinhas.

A contaminação de ovos por bactérias pode se dar por transmissão vertical, quando o trato reprodutor da galinha já está contaminado, ou transmissão horizontal, quando o ovo é contaminado

em contato com o ambiente através de equipamentos e embalagens, ou na passagem pela cloaca e contato com fezes contaminadas (BARCELOS, 2017). Entre as principais bactérias responsáveis por contaminação e comprometimento da qualidade de ovos está a *Escherichia coli*, sendo considerada um dos principais indicadores de contaminação microbiológica dos alimentos (CUPERUS et al., 2016), e um indicador de contaminação fecal, embora possa ser introduzida nos alimentos a partir de fontes não fecais (CARDOSO et al., 2001).

1.1.3 Desafios da fase de creche em suínos

Na produção de suínos, o desmame é considerado uma das fases mais críticas, devido a uma série de mudanças e desafios aos quais os leitões são submetidos em um único momento (SILVA et al., 2014). Portanto, é necessário atenção a este momento crítico para evitar o comprometimento do desempenho produtivo dos suínos, sendo essa fase decisiva para o sucesso da produção suinícola, estando ampla e positivamente relacionada ao desempenho das fases subsequentes (ALVARENGA et al., 2012).

Na suinocultura moderna a prática do desmame precoce traz uma série de desafios aos animais e a indústria, decorrentes, principalmente, da dificuldade de adaptação de leitões jovens ao consumo das dietas sólidas. No desmame, as funções digestivas são ineficazes, o que não permite o aproveitamento eficaz das dietas a base de milho e farelo de soja (ROBLES-HUAYNATE et al., 2013), levando a incidências de diarreias, distúrbios no balanço da microbiota intestinal e riscos de mortalidades (DE ANDRADE et al., 2011).

Um dos desafios a que muitas vezes os animais são submetidos, na mudança das dietas líquidas para as sólidas, é a ingestão de micotoxinas presentes nos grãos e cereais das dietas (FREITAS et al., 2012), ainda, segundo DILKIN (2011), porcas que ingerem aflatoxina B1 podem eliminar aflatoxina M1 pelo leite, intoxicando os leitões lactentes. A ingestão de alimentos que contenham micotoxinas, assim denominadas por serem produtos tóxicos de fungos ambientais que se desenvolvem em alimentos, pode causar graves efeitos sobre a saúde animal (SANTURIO, 2007). As micotoxicoses tem seu grau de severidade influenciado por inúmeros fatores como, espécie animal, sexo, idade, estado de saúde e conforto do animal e quantidade ingerida e acumulada da micotoxina no organismo (DE CASTRO SOUTO et al., 2017)

1.1.4 Micotoxinas e seus efeitos sobre aves e suínos

As micotoxinas são substâncias tóxicas resultantes do metabolismo secundário de diversas linhagens de fungos filamentosos, e estão presentes em todos os lugares, mas predominam em climas tropicais e subtropicais, onde as condições ambientais favorecem o desenvolvimento de fungos (MALLMANN & DILKIN 2011). As principais espécies de fungos que produzem as micotoxinas são *Aspergillus* spp, *Fusarium* spp *e Penicillium* spp., sendo que as micotoxinas podem contaminar alimentos e rações em todos os estágios da cadeia alimentar (GUERRE, 2016).

As micotoxinas produzem efeitos tóxicos tanto em animais quanto em humanos, podendo ser de forma aguda ou crônica (BENNET; KLICH, 2003). O nível de gravidade das micotoxicoses depende da toxicidade da micotoxina, grau de exposição, idade e estado nutricional do individuo, além de que, esses efeitos tóxicos podem ser potencializados pelo sinergismo que pode haver entre as micotoxinas, e as doenças, principalmente as imunossupressoras (HUSSEIN; BRASSEL, 2001; RIBEIRO et al., 2015). Em suínos as micotoxinas induzem vários efeitos tóxicos, inclusive a modulação da resposta imune, aumentando assim a suceptibilidade e gravidade de doenças infecciosas (PIERRON et al., 2016). Este efeito das micotoxinas afeta diretamente a produtividade dos animais, pois durante um processo infeccioso os nutrientes são direcionados para o sistema imunológico ao invés de crescimento e desenvolvimento.

A alimentação de aves e suínos no mundo, com poucas exceções, é consituida basicamente de grãos como o milho, utilizado como fonte de energia nas dietas, e farelo de soja, fonte de aminoácidos digestíveis, que correspondem a até 50% da composição de rações (BERTECHINI, 2012). Porém, apesar da importância, estes ingredientes muitas vezes são carreadores de micotoxinas, visto que as commodities de grãos e cereais são as principais fontes de contaminação por micotoxinas na alimentação dos animais (BAPTISTA et al., 2004), e, segundo WHITLOW (2002), em torno de 25% dos grãos colhidos no mundo estão, possivelmente, contaminados por essas substâncias.

As principais micotoxinas podem ser divididas em três grupos: as aflatoxinas, produzidas por fungos do gênero *Aspergillus* como *A. flavus* e *A. parasiticus;* as ocratoxinas, produzidas pelo *Aspergillus ochraceus* e diversas espécies do gênero *Penicillium,* e as fusariotoxinas, que possuem como principais representantes os tricotecenos, zearalenona e as fumonisinas, produzidas por diversas espécies do gênero *Fusarium* (DILKIN, 2002). Essas toxinas são classificadas de acordo com especificidade junto aos órgãos, apesar de poderem causar danos em mais de um órgão, são consideradas hepatotóxicas, nefrotóxicas, hematotóxicas, neurotóxicas, dematotóxicas, cancerígenas e gastrotóxicas (OKUMA et al., 2018). Os animais que se alimentam com rações previamente contaminadas podem excretar micotoxinas no leite, carne e ovos, e consequentemente,

constituir-se em fonte de contaminação indireta para os humanos, tornando o problema com as micotoxinas uma questão de saúde pública (MAZIERO; BERSOTI, 2010).

A aflatoxina B1 (AFB1), é uma das micotoxinas mais importantes que afetam a produção animal, devido a seus efeitos hepatotóxicos e carcinogênicos (OLIVEIRA et al., 2001). O fígado é o principal órgão afetado pelas aflatoxinas, ocorrendo lesão hepática quando animais e humanos são submetidos a alimentação contendo aflatoxina (BENNET; KLICH, 2003). As aflatoxinas são rapidamente absorvidas pelo trato gastrointestinal dos animais, causando danos significativos ao tecido hepático (FRANCISCATTO et al., 2006), também leva a perda de peso e menor ingestão de alimentos (OLIVEIRA et al., 2001), e afeta parâmetros séricos (KASMANI, et al., 2012). Os padrões de biotransformação da AFB1 variam consideravelmente entre as espécies animais, e mesmo entre indivíduos da mesma espécie, o que poderia justificar os diferentes graus de susceptibilidade à AFB1 observados em cada uma delas (LOPES et al., 2005).

Segundo SANTURIO (2000), em surtos de aflatoxicose no campo, uma das características mais observadas é a má absorção, que se manifesta como partículas de ração mal digeridas na excreta das aves, além da extrema palidez de mucosas e pernas, conhecida como síndrome da ave pálida. Esta palidez de mucosas e pernas pode ser explicada pela ação de alguns compostos como o licopeno e beta-caroteno, que atuam como protetores das células contra a ação tóxica da AFB1 (REDDY et al., 2006), direcionando estas substâncias carotenóides para outra função que não a de pigmentação.

A aflatoxicose em aves de postura leva a uma diminuição na produção de ovos, assim como no tamanho destes, e proporcionalmente diminui o tamanho das gemas sendo isto atribuído a prejuizos da aflatoxina sobre a síntese proteica e lipídica (MALLMANN et al., 2009). A aflatoxina B1 pode ser transmitida tanto para as gemas quanto para as claras dos ovos (SANTURIO, 2000). OLIVEIRA et al. (2000) demonstraram em seu estudo o potencial residual de aflatoxinas em ovos, sendo encontrado 0,16 mg/kg de aflatoxina em ovos de galinhas submetidas a contaminação de 500 mg/kg de alimento. Além disso, parâmetros como a porcentagem de produção e a qualidade de ovos produzidos por galinhas infectadas com aflatoxinas, também são afetados (SANTURIO, 2000; MALLMANN et al., 2009). A resistência da casca dos ovos aumenta quando as aves consomem aflatoxina, devido a produção de casca não ser afetada, e assim, não acompanhar a proporção de redução de gema e clara. Essa espessura maior da casca, pode alterar a eclodibilidade dos ovos, através de reduções nas trocas gasosas entre embrião e ambiente (WASHBURN et al., 1985).

Os suínos são considerados a espécie mais sensível aos efeitos das aflatoxinas, sendo os animais jovens os mais afetados pela aflatoxicose (YU et al., 2005). Assim como nas aves, nos suínos o fígado também é o órgão mais afetado pelas aflatoxinas, pois essa micotoxina tem ação

sobre diversas estruturas do hepatócito, inibindo a síntese de proteínas e a ação de enzimas (SANTURIO, 2007). Em níveis mais elevados de ingestão da toxina, o fígado dos suínos apresenta degeneração gordurosa, além de aspecto friável e hiperêmico (DILKIN, 2002). Os suínos desafiados por aflatoxinas apresentam redução no coeficiente de metabolização da energia e na retenção relativa de nitrogênio, além de outros sinais clínicos como anorexia, imunossupressão e hepatopatias (HAUSCHILD et al., 2006).

Em um estudo de meta-análise com dados de 72 artigos e 7.742 suínos, ANDRETTA et al., (2017), estimaram os impactos produtivos das micotoxinas em suínos, mostrando que animais desafiados apresentam redução de 6% no consumo de ração, 11% no ganho de peso e 4% na eficiência alimentar. As aflatoxinas também estão envolvidas em distúrbios reprodutivos em suínos, sendo o aborto um dos sinais clínicos mais observados em porcas gestantes intoxicadas (DILKIN, 2011).

Juntamente com as aflatoxinas os tricotecenos são considerados um dos grupos que mais causam prejuízos na produção avícola, principalmente devido à imunossupressão dos animais (REIS et al., 2016). Os tricotecenos são produzidos pelos fungos do gênero *Fusarium* sp., e os principais compostos produzidos são: Toxina T2, Deoxynivalenol – DON e Diacetoxyscirpenol – DAS (SANTURIO, 2000). Dentre estes compostos as toxinas T2 e DAS, são consideradas as mais potentes e prejudiciais, pois possuem atividade citotóxica e imunossupressora (PERREIRA; DOS SANTOS, 2011).

Em intoxicações crônicas por T2 em aves de postura, há a redução no consumo de alimentos, ganho de peso, presença de lesões orais, necroses de tecidos linfóides, hematopoiéticos e mucosa oral, além de uma espessura de casca de ovo menor e a ocorrência de peroxidação lipídica, dominuindo assim a concentração da vitamina E nas aves (MALLMANN et al., 2007). Os tricotecenos também tem uma forte capacidade de inibição de síntese proteica (FREIRE et al., 2007), o que em aves de postura pode prejudicar a produção e qualidade de ovos. Segundo YEGANI et al. (2006), os efeitos dos tricotecenos em galinhas incluem a rejeição repentina de ração, redução da produção de ovos e redução da qualidade da casca, com aumento nas porcentagens de mortalidade embrionária e diminuição na eclosão.

O fungo *Fusarium moniliforme* é o responsável pela produção das fumonisinas, sendo a fumonisina B1 a forma molecular mais produzida por ele, e o milho o cereal em que as fumonisinas são mais detectadas (IAMANAKA et al., 2013). Quando se compara os diferentes níveis de toxicidade em diferentes animais, conclui-se que o principal órgão alvo difere em cada espécie, porém órgãos como o fígado e o rim são afetados de forma constante em menor ou maior extensão, classificando a fumonisina como hepatotóxica e nefrotóxica (ENONGENE et al., 2002). Em aves,

os efeitos adversos das fumonisinas caracterizam-se pela redução no desenvolvimento, problemas cardíacos, imunossupressão, degeneração e necrose hepática (MINAMI et al., 2004).

A FB1 exerce um papel importante durante a iniciação do câncer, sendo a indução de danos oxidativos e a peroxidação lipídica eventos iniciais importantes (GELDERBLOM et al., 2001). Segundo SANTURIO (2007), a fumonisina interage com as esfingosinas, estrutura componente dos esfingolipídeos, substâncias com importante funções na integridade da membrana celular. Com a alteração destas funções ocorre a hepatotoxicose e edema pulmonar a partir do aumento da permeabilidade vascular dos pulmões.

1.1.5 Colina vegetal

A colina é uma vitamina do complexo B e é incluída normalmente na dieta de animais na forma de cloreto de colina (DEVLIN, 1998). Diferentemente das outras vitaminas do complexo B, a colina pode ser sintetizada no organismo dos animais em nível hepático (KASPER et al., 2000), sendo exigida em grandes quantidades pelos mesmos. Encontrada tanto em células animais como em células vegetais, a colina pode se apresentar de três formas: colina livre, acetilcolina ou lecitina em fosfolipídios. A colina possui algumas funções básicas no organismo animal, sendo componente essencial da acetilcolina, um neurotransmissor do qual a colina é precursora; fosfatidilcolina, que é um elemento estrutural da membrana celular, na transmissão do impulso nervoso e também na utilização de lipídeos; é precursora da betaina, participando assim da formação da metionina (BERTECHINI, 2012).

A suplementação de cloreto de colina em dietas animais funciona como protetor hepático e auxilia no metabolismo energético através do fígado (BALLOUN, 1956). GUJRAL et al., (2002) em estudo com frangos, concluiram que a suplementação com colina sintética ou de origem vegetal, melhorou a saúde e desempenho dos animais. Suplementos dietéticos de tais compostos, também são uma estratégia nutricional eficaz para diminuir os efeitos adversos de dietas com altas concentrações de energia em animais de produção, que podem levar a síndrome do fígado gorduroso (LEESON; SUMMERS, 2009). Em estudo com frangos de corte (PS et al., 2015), utilizaram produtos lipotrópicos, extrato de lecitina, cloreto de colina e colina vegetal, em dietas de moderada e alta energia, com o objetivo de avaliar desempenho produtivo, atividade de lipídeos e enzimas hepáticas nos animais, o que permitiu concluir que a utilização destes compostos é capaz de reduzir a gordura e melhorar a saúde do fígado.

A deficiência de colina no organismo das aves acarreta o aparecimento de sintomas, como a síndrome do fígado gorduroso e a perose (ZEISEL et al., 1989). A utilização de colina na forma de cloreto de colina é a mais comumente utilizada para suplementações em dietas animais, por ser amplamente disponível no mercado. Porém, apresenta algumas desvantagens, como alta higroscopicidade, característica que dificulta sua utilização em processos de fabricação de rações, levando também a aceleração do processo de oxidação de outras vitaminas da dieta (DEVLIN, 1998). Além disso, aproximadamente 70% desta fonte não é absorvida no intestino sendo convertido em trimetilamina (TMA) pelas bactérias intestinais, um composto tóxico para os animais (MCDOWELL, 2012).

Como alternativa ao uso do cloreto de colina, estudos vêm sendo realizados e demonstram resultados positivos da utilização de colina na forma de colina vegetal. BALDISSERA et al. (2019), utilizando colina vegetal em dietas para tilápia do Nilo, observaram uma melhora significativa nos parâmetros de desempenho zootécnico, um efeito protetor no fígado e melhora do metabolismo energético deste órgão, assim como uma melhoria no estado antioxidante destes animais. Em seu estudo de suplementação de colina vegetal, com ovelhas da raça Lacaune em estado de prenhez ou lactantes, ALBA et al. (2020), encontraram uma melhoria na produção, qualidade e estatus antioxidante do leite, assim como uma melhoria no estado imunológico das ovelhas. PEREIRA FILHO et al. (2015), em estudo de substituição do cloreto de colina por colina na forma de fofatidilcolina na alimentação de frangos de corte, concluiram que a substituição não afetou o desempenho dos animais.

A fosfatidilcolina possui uma fonte de origem vegetal e apresenta uma maior biodisponibilidade de colina no intestino, quando comparada ao cloreto de colina e não é convertida em trimetilamina pelas bactérias intestinais, assim não sendo tóxica para os animais (DEVLIN, 1998). De acordo com ZEISEL (1990), existe variação na biodisponibilidade e utilização entre os diferentes ésteres de colina, o que justifica a maior eficiência da fosfatidilcolina. Apenas uma parcela da colina ingerida é absorvida intacta, e aproximadamente dois terços é transformado em trimetilamina, responsável por conferir odor de peixe à carne e ovos (COMBS et al., 2016). Porém, a colina ingerida na forma de fosfatidilcolina não está sujeita a esta degradação (ZEISEL, 1990).

Há uma grande tendência na utilização de produtos naturais derivados de plantas, para a proteção do organismo contra agentes tóxicos, infecciosos, derivados da alimentação, do ambiente, e que trazem danos à saúde animal. A biocolina é um extrato vegetal de baixa higroscopicidade, fonte de fosfatidilcolina, á base de *Trachyspermum amni, Citrullus colocynthis, Achyranthus aspera e Azadirachta indica* (FARINA et al., 2017).

1.1.5.1 *Saccharomyces cerevisiae lysate*

Quando há a contaminação de grãos e cereais por micotoxinas, é necessário reduzir os seus efeitos tóxicos, através de métodos físicos, químicos e/ou microbiológicos, além do uso de adsorventes anti-micotoxinas. Os adsorventes são substâncias, que por um efeito de quimio-adsorção, como também por processos enzimáticos e /ou bacterianos impede que o animal que tenha ingerido uma micotoxina o biotransforme em um metabólito, quase sempre, tóxico. O adsorvente passa junto com a toxina pelo trato gastro-intestinal sem que seja absorvido, sendo eliminado posteriormente (MALLMANN; DILKIN, 2011).

Os adsorventes comumente utilizados são a base de aluminosilicatos e bentonita, entre outros que pertencem ao grupo de adsorventes inorgânicos. Porém, sua alta taxa de inclusão e o baixo número de micotoxinas a que se liga, justifica a investigação por outras alternativas de adsorventes para micotoxinas. A classe dos adsorventes orgânicos, como a parede celular de leveduras, vem sendo estudadas como alternativa para a adsorção de micotoxinas. A parede celular de leveduras é um subproduto da indústria alimentar com possibilidade do aproveitamento na alimentação animal, sendo composta por glucanos e mananoligossacarídeos (MOS) e de acordo com sua natureza física e composição química, espera-se que sua superfície celular apresente sítios para adsorção de moléculas (SHETTY; JESPERSEN, 2006).

Os MOS têm um papel importante no metabolismo intestinal, garantindo modificação da microflora, melhoria da integridade intestinal e modulação do sistema imune no lúmem intestinal, além de possuírem propriedade de redução dos efeitos tóxicos oriundos das micotoxinas (CHAUCHEYRAS; DURAND, 2010). PIZZOLITTO et al. (2011), em estudo de ligação da aflatoxina B1 com bactérias do ácido lático e *Saccharomyces cerevisiae*, concluíram que esses microrganismos são capazes de evitar a absorção de aflatoxinas durante seu trânsito gastrointestinal, além de terem propriedades benéficas na saúde intestinal do hospedeiro, mostrando que a utilização de parede de leveduras é altamente promissora para a prevenção de micotoxicoses.

PINHEIRO et al. (2017), avaliaram alguns produtos comerciais á base de leveduras secas de cervejaria (*Saccharomyces cerevisiae*), e probióticos, e sua capacidade de absorção in vitro de AFB1, e encontraram como resultados a eficiência destes produtos para a adsorção de micotoxina. Existem muitos relatos sobre o uso de paredes celulares de levedura fisicamente separadas, obtidas em cervejarias, como aditivo para rações na dieta de aves, ratos, frangos, cavalos, resultando na melhora dos efeitos tóxicos das micotoxinas (SANTIN et al., 2003; RAJU; DEVEGOWDA, 2000; RAYMOND et al., 2003)

1.1.5.2 Betaglucano, carrier mineral, ácidos orgânicos e mananoligossacarídeos (MOS)

Os mananoligossacarídeos (MOS) são consideradas substâncias prebióticas, que levam em sua constituição um derivado complexo de glucomanoproteínas, com ação de inibir a multiplicação de patógenos, o que garante benefícios à saúde (RIBEIRO et al., 2008). As glicomanonas, em condições de pH do aparelho digestivo, são capazes de se ligar seletivamente e inativar as micotoxinas no lúmen intestinal (MADRIGAL-SANTILLÁN et al., 2006). Segundo SANTURIO (2007), um método para colaborar no controle de micotoxinas nos animais, é a utilização de aditivos minerais ou orgânicos na dieta para reduzir a absorção destas pelo trato gastrintestinal destes animais. As argilas naturais são recomendadas para adsorção de aflatoxinas, enquanto adsorventes orgânicos, tendo como componente básico beta-glucanas, podem ser bons adsorventes de aflatoxinas e zearalenona (BUNZEN; HAESE, 2006).

A utilização de ácidos orgânicos como controle de fungos em rações é recomendada (DIXON e HAMILTON, 1981), sendo que o uso de acidificantes durante a armazenagem dos grãos ou nas rações, correspondem a uma grande ferramenta no controle das micotoxinas (FREITAS et al., 1995). ROLL et al. (2010), em seu estudo com frangos de corte submetidos a desafio por aflatoxina via alimentação e suplementação com um adsorvente a base de glucomanano esterificado, concluíram que o adsorvente influenciou positivamente o desempenho, bem-estar e saúde dos animais.

Outra via de adsorção das micotoxinas da dieta é através da utilização de carreadores minerais, amplamente utilizados em dietas de monogástricos devido a sua disponibilidade e eficiência, tendo como principal mecanismo de ação a troca de cargas entre o adsorvente mineral e a micotoxina (HUWIG et al., 2001).

1.1.6 Estresse oxidativo

As micotoxinas são produtos do metabolismo fúngico conhecidas por danos nocivos a saúde humana e dos animais, sendo atribuído a elas enfermidades nos sistemas digestório, urinário, reprodutivo e imune; e o estresse oxidativo, através da peroxidação lipídica, é um importante mediador da toxicidade induzida pelas micotoxinas nesses sistemas (DOI; UETSUKA, 2014). O estresse oxidativo decorre de um desequilíbrio entre a geração de compostos oxidantes e a atuação dos sistemas de defesa antioxidante, sendo que o sistema antioxidante pode ser enzimático ou não enzimático (SIES; STAHL, 1995). O sistema de defesa antioxidante tem o objetivo primordial de

manter o processo oxidativo dentro dos limites fisiológicos e passíveis de regulação, impedindo que os danos oxidativos se amplifiquem, culminados em danos sistêmicos irreparáveis (BARBOSA et al., 2010). A oxidação é parte fundamental do metabolismo celular, no entanto, a produção de espécies reativas ao oxigênio (EROs) eleva-se em lesões teciduais por traumas, infecções, parasitoses, hipóxia e produção de toxinas, devido a ativação da fagocitose, liberação de ferro e cobre ou interrupção da cadeia transportadora de elétrons (FERREIRA; MATSUBARA, 1997).

No entanto, conjuntamente com a produção exacerbada de EROs, enzimas antioxidantes também são sintetizadas com a função de proteção e neutralização dos radicais livres, através da capacidade de doar elétrons e fornecer proteção celular (BIANCHI; ANTUNES, 1999). As principais enzimas antioxidantes responsáveis por minimizar a exacerbação do estresse oxidativo são a glutationa redutase (GsH), superóxido dismutase (SOD), glutationa peroxidase (GPx), glutationa S-transferase e catalase (CAT) (BIRBEN et al., 2012).

Em conjunto com o sistema antioxidante enzimático, tem-se também o sistema antioxidante não-enzimático endógeno, composto pela melatonina, bilirrubina, proteínas de ligação de metal, ácido úrico, poliaminas, entre outros (FERREIRA; ABREU, 2007). Em adição aos efeitos protetores dos antioxidantes endógenos, a inclusão de antioxidantes na dieta é de grande importância (VANNUCCHI et al., 1998). Os alimentos, principalmente as frutas, verduras e legumes, também contêm agentes antioxidantes, tais como as vitaminas C, E e A, a clorofilina, os flavonóides, carotenóides, curcumina e outros que são capazes de restringir a propagação das reações em cadeia e as lesões induzidas pelos radicais livres (BIANCHI; ANTUNES, 1999).

A superóxido dismutase, a catalase e a glutationa peroxidase são enzimas antioxidantes que não apenas desempenham papel fundamental, mas indispensável na capacidade de proteção antioxidante dos sistemas biológicos contra o ataque dos radicais livres. A superóxido dismutase (SOD) é a primeira enzima de desintoxicação e o antioxidante mais poderoso da célula, sendo uma importante enzima antioxidante endógena que atua como um componente do sistema de defesa de primeira linha contra espécies reativas de oxigênio (ROS). Catalisa a dismutação de duas moléculas de ânion superóxido ($*O2$) em peróxido de hidrogênio ($H2O2$) e oxigênio molecular ($O2$) (IGHODARO; AKINLOYE, 2018), evitando dessa forma o acúmulo do $H2O2$, que nessas condições é tóxico para os tecidos ou células do corpo.

A catalase (CAT) é uma enzima antioxidante comum presente em quase todos os tecidos vivos que utilizam oxigênio. A enzima usa ferro ou manganês como co-fator e catalisa a degradação ou redução do peróxido de hidrogênio ($H2O2$) em água e oxigênio molecular, completando o processo de desintoxicação imitado pela SOD (BECKMAN et al., 1988). Juntamente com a catalase a glutationa peroxidase atua impedindo o acúmulo de peróxido de hidrogênio, e os decompõe em

água, desempenhando um papel crucial na inibição do processo de peroxidação lipídica (MUGESH; SINGH, 2000)

1.2 OBJETIVOS

1.2.1 Objetivo geral

Verificar se aditivos funcionais adicionados a dieta tem efeito protetor a saúde para aves e suínos desafiados com micotoxinas, assim como se é capaz de minimizar os efeitos negativos sobre a produção causados pelas micotoxicoses.

1.2.2 Objetivos específicos

- Avaliar se o uso de um adsorvente à base de lisado de *Saccharomyces* cerevisiae (LSC) combinado a outros ingredientes será capaz de minimizar os efeitos negativos das micotoxinas T2 e FB1 sobre saúde, características produtivas e de qualidade dos ovos de poedeiras semipesadas.

- Avaliar se a biocolina vegetal adicionada a alimentação de poedeiras desafiadas com aflatoxina B1, é capaz de minimizar efeitos negativos da micotoxina sobre qualidade de ovos, saúde das aves, e desempenho zootécnico.

- Analisar se o consumo de ração contendo biocolina vegetal na dieta, reduz os níveis oxidativos e aumenta os níveis de antioxidantes a níveis séricos e em ovos

- Verificar se a biocolina vegetal possui capacidade antimicrobiana nos ovos de galinhas desafiadas com *Escherichia colli.*

- Avaliar se a biocolina vegetal possui efeito hepatoprotetor sobre poedeiras semipesadas desafiadas com aflatoxina B1

- Avaliar se a biocolina vegetal possui efeito hepatoprotetor sobre a saúde de leitões desafiados com aflatoxina B1

- Analisar o desempenho zootécnico de leitões desafiados com aflatoxina B1 e suplementados via ração com biocolina vegetal

CAPÍTULO II

2. ARTIGO E MANUSCRITOS

Os resultados desta dissertação são apresentados na forma de um artigo e dois manuscritos, com sua formatação de acordo com as orientações das revistas aos quais foram publicados e submetidos:

Artigo I - **Laying hens fed mycotoxin-contaminated feed produced by *Fusarium* fungi (T-2 toxin and fumonisin B1) and *Saccharomyces cerevisiae* lysate: Impacts on poultry health, productive efficiency, and egg quality**
Publicado: Microbial Pathogenesis

Manuscrito I - **Vegetable biocholine as a hepatoprotectant in laying hens feed with diet contaminated with aflatoxin B_1**
Submetido: World Mycotoxin Journal

Manuscrito II - **Inclusion of vegetable biocholine addictive in piglet feed contaminated with aflatoxin: impact on health and zootechnical performance**
Submetido: Animal Feed Science and Technology

2.1 – ARTIGO I

Laying hens fed mycotoxin-contaminated feed produced by *Fusarium* fungi (T-2 toxin and fumonisin B1) and *Saccharomyces cerevisiae* lysate: Impacts on poultry health, productive efficiency, and egg quality

Vanessa Dazuk[a], Marcel M. Boiago[b, *], Gabriela Rolim[b], Andreia Paravisi[b], Priscilla M. Copetti[c], Bianca P. Bissacotti[c], Vera M. Morsch[c], Marcelo Vedovatto[d], Fabio L. Gazoni[e], Fabrizio Matte[e], Eduardo G. Micotti[f], Aleksandro S. da Silva[b, *]

[a] Graduate Program of Animal Science, Universidade do Estado de Santa Catarina, Chapecó, Brazil.

[b] Department of Animal Science, Universidade do Estado de Santa Catarina, Chapecó, Brazil.

[c] Graduate Program of Toxiciological Biochemistry, Universidade Federal de Santa Maria, Santa Maria, Brazil.

[d] Universidade Estadual de Mato Grosso do Sul, Aquidauana, Brazil.

[e] Vetanco, Brazil.

[f] Escola Superior de Agricultura Luiz de Queiroz (ESALQ), Universidade de São Paulo (USP), São Paulo, Brazil.

Corresponding authors: marcel.boiago@udesc.br; aleksandro_ss@yahoo.com.br

Abstract

Mycotoxins represent substantial challenges to the farming industry. These include toxins produced by *Fusarium* fungi, particularly trichothecenes (toxin T-2) and fumonisin (FB_1). In the present study, we determined the effects of addition on *Saccharomyces cerevisiae* lysate (SCL) added to feed contaminated with T-2 and FB_1 in terms of health, productive efficiency, and egg laying quality. We used 60 Hy-line Brown laying hens, and divided them into five groups with four repetitions per group and three birds per repetition. There was one group with no contamination with toxin (NoC). The four other groups included combinations of mycotoxin (4 ppm T-2, and 20

ppm FB1): A contamination group was used as control (the C+ group), and another two contained 500 g/ton of SCL (Detoxa Plus®) (the C+D500 group) or 1000 g/ton of SCL (the C+D1000 group). Finally, one group received feed containing 500 g/ton of Detoxa Plus® and 1000 g/ton of Uniwall Mos 25® (the C+D500+U1000 group). The experimental period was 84 days, divided into three productive cycles of 28 days each. The NoC hens had greater egg production than the other groups. Hens that consumed feed with SCL had greater egg production than did the C+ group. The NoC hens produced eggs with greater weights than did the C hens; however, C+D1000 and C+D500+U1000 birds produced greater egg weights than did the C+ group. The C+ group produced lower egg masses than did the NoC and C+D500+U1000 groups. The feed intake (FI) was lower in hens that ingested mycotoxin. The use of SCL in feed minimized the negative effects of mycotoxin on feed conversion ratio (FI/dozen). Effects of treatment were detected for feed conversion ratio (kg/kg). The hens that consumed mycotoxin had lower shell resistance and thickness compared to those in the NoC group. The red color of egg yolk was greater in the control groups. There were fluctuations in levels of liver enzymes when birds consumed mycotoxin (sometimes reduced and sometimes increased); nevertheless, the cumulative effect increased the activity of alanine aminotransferase. The serum concentration of reactive oxygen species was greater in hens that ingested mycotoxin only on d 84 compared to the NoC group. Serum glutathione S-transferase activity was greater on d 56 in C+D500 and C+D1000 hens than in the others. We conclude that, in general, the consumption of mycotoxin impaired the performance and quality of the eggs of the hens; the addition of the *S. cerevisiae* lysate and the addition organic acids, yeast cell wall and mineral carrier minimized some of the negative effects caused by T-2 and FB1.

Keywords: Poultry farming; Laying hens; Mycotoxins; Nutrition; Pathogenesis.

1. Introduction

The costs of poultry feed represent the highest percentage of costs in the production chain, around 70% to 80% [1]. Contaminants such as mycotoxins generate substantial losses in the quality of the ingredients and that of the final feed product [2]. Mycotoxins are products of the metabolism of several fungi that proliferate in cereals such as corn, peanuts, wheat, barley, sorghum and rice, all of which are used in animal and human food [3]. Several factors are essential for fungi to produce mycotoxins, particularly high temperature and humidity, long storage times, physical condition of the grains, and the presence of fungi in the grains [4].

Several mycotoxins in foods intended for human and animal consumption have been identified in Brazil [5]. In laying birds, there is substantial contamination with T-2 toxin produced

by fungi of various species of the *Fusarium*; this contamination reduces feed consumption and weight gain in the birds, as well reducing shell thickness and giving rise to lesions in the cavity buccal [6]. Fumonisin B1 (FB1) is also produced by fungi of the genus *Fusarium*; corn and its derivatives are common sites where fumonisins are detected [7].

It is important to note that corn is an important food source for the Brazilian population. In addition, together with soybean meal, it is the main ingredient in animal feed, particularly for swine and poultry [8]. For these reasons, it is essential that the corn have excellent quality, because inferior-quality grains tend to possess anti-nutritional properties that favor the proliferation of fungi, many of which produce mycotoxins [3].

The most efficient way to avoid high concentrations of mycotoxins in food is to prevent the growth of fungi, and to use substances that can be mixed in the feed to adsorb and inactivate the toxins, the so-called adsorbents [9]. Anti-mycotoxins (AAM), adsorbents, and additives are products designed to adsorb, neutralize, or bio-transform mycotoxins, even in the animals' gastrointestinal tracts, thereby reducing the deleterious effects of the toxins [10,11]. Various agents have been tested, including those based on yeasts and organic acids, particularly *Saccharomyces cerevisiae*. Fractions of *S. cerevisiae* cell walls have ample capacity for adsorption to mycotoxins; and several of these strains are considered safe [12,13].

Studies carried out with zearalenone and aflatoxin B1 demonstrated the capacity of adsorption of these toxins by components of the cell wall (PCL) [14,15]. Live strains of yeasts are able to biodegrade mycotoxins, and that there is a capacity to bind toxins in a dose-dependent manner; β-glucans are the primary components involved in this binding [12]. Therefore, in the present study, we determined whether the addition of *S. cerevisiae* lysate (SCL) in feed contaminated with T-2 and FB1 in laying hens would improve bird health, productive efficiency and egg quality.

2. Materials and Methods

2.1. Products: SCL and UNIWALL MOS 25®

The commercial product used in our study was a lysate based on *S. cerevisiae* (SCL) (86%) (Detoxa Plus®, Vetanco do Brasil Importação e Exportação Ltda). Combined with the fungal lysate, we tested a commercial prebiotic based on organic acids, yeast cell walls and a mineral carrier (Uniwall Mos 25®, Vetanco do Brasil Importação e Exportação Ltda). The treatments and doses are described below.

2.2. Mycotoxins

For fumonisin B1 and B2 production, an isolate of *Fusarium verticillioides* was cultured in rice. The fermentation was carried in 500-mL capacity Erlenmeyer flasks, into which 100 g of rice were added. The rice was moistened with tap water (water activity >0.97) and autoclaved at 121 ºC for 1 h. The autoclaved rice was inoculated with 2 mL of a conidial suspension (1 x 10^5 conidia per mL). The conidial suspension was obtained from *F. verticillioides* colonies growing on potato-dextrose agar for 15 days at 25 °C. After inoculation, the flasks were maintained static for 28 days at 25 °C. Subsequently, the ferment was dried and ground to be used to artificially contaminate feeds.

Toxin T-2 production was obtained from maize fermentation by *F. sporotrichioides*. The fermentation was carried out in 500-mL Erlenmeyer flasks to which 100 g of corn were added. The corn was moistened (water activity > 0.97) and autoclaved at 121 °C for 1 h. Subsequently, the corn was inoculated with 2 mL of a conidial suspension (1 x 10^5 conidia per mL). Conidial suspensions were obtained from *F. sporotrichioides* colonies growing on potato-dextrose agar for 15 days at 25 °C. After inoculation, flasks were maintained static for 28 days at 25 °C. Subsequently, the fermented maize was dried and ground to be used to artificially contaminate feed. The concentrations of fumonisin and Toxin T-2 on ground fermented material were measured using HPLC/MS/MS.

2.3. Animals and experimental design

The experiment was carried out in an experimental barn in the city of Chapecó, SC, over 84 days, corresponding to three productive cycles of 28 days each. The feed used was formulated based on corn and soybean meal, according to the nutritional requirements of laying hens [16], detailed in Table 1. We divided 60 Hy-line Brown hens, 25 weeks of age, into five treatments, with four repetitions per treatment and three hens per repetition. The experiment was conducted in a breeding system in cages (0.5 x 0.6 x 0.4 m), equipped with a trough-type feeder and a nipple-type watering device. Food and water were offered ad libitum. The project was approved by the institutional ethics committee, following the recommendations of the Brazilian board of experimentation with the use of animals in research, as well as respecting the current regulations regarding animal welfare.

The treatments were identified as follows: NoC, basal feed without mycotoxin (used as a negative control); C+, feed contaminated with 4 ppm T-2 and 20 ppm FB1 (used as a positive control); C+D500: feed contaminated with 4 ppm of T2 and 20 ppm of FB1 + 500 g/ton of SCL; C+D1000, feed contaminated with 4 ppm of T2 and 20 ppm of FB1 + 1000 g/ton of SCL; and C+D500+U1000: feed contaminated with 4 ppm of T2 and 20 ppm of FB1 + 500 g/ton of SCL +

1000 g/ton Uniwall Mos 25® (organic acids, yeast cell wall and mineral carrier). The dose of mycotoxins described above was calculated based on the concentration of the innocuous compound. The actual concentration was assessed as described in section 2.2.

2.4. Zootechnical performance

Performance in the production phase was evaluated at the end of each 28-day production cycle, in which the following were measured: percentage of egg production, average egg weight, egg mass, feed consumption, and feed conversion (kg of feed/kg of egg produced and kg of feed/dozen eggs produced). The percentage (%) of eggs was obtained by daily counting and collecting eggs from each experimental unit. For average egg weight, we used an analytical balance with precision of ± 0.01 g (model SHIMADZU BL-3200H). Egg mass was calculated in the last three days of each production cycle. The individual weights of all eggs in each experimental plot (cage) were measured, and then the egg mass was calculated using the equation: egg mass = average weight of the eggs (g) x production of the day (%). The feed was stored in buckets, one per repetition of each treatment, with the measurement of consumption performed weekly.

2.5. Sample collection

Blood samples were collected on days 1, 28, 56, and 84 of the experimental period. The birds were manually restrained (n = 8 per group), and blood was drawn from the ulnar vein using a syringe (3 ml) and needle (25/7). The collected blood was allocated to Eppendorf microtubes and centrifuged to separate the serum, and frozen (–20 ° C) until analysis.

2.6. Egg quality analysis

At the end of each 28-day experimental period (days 28, 56 and 84), two eggs were collected per repetition for physical-chemical quality analyses. We measured shell strength (kgf) using a texturometer (Model TA. XT plus, Extralab, Brazil). The albumen and yolk pHs were obtained using a digital pH meter (Model testo 205, Testo, Brazil). Specific gravity and thickness of the dry shell was measured using a digital thickness gauge (Dasqua®) [17]; using these data, we calculated average shell thickness for each egg. To obtain the percentages of yolk, shell, and albumen, the yolks were separated from the albumen, weighed separately, and the shells were washed to completely remove the albumen and placed to dry at room temperature until weighing. Haugh units were calculated as follows: HU = 100 log (average albumen height + 7.57 – 1.7 x egg weight in grams x 0.37) [18]. The yolk index was calculated using the following equation: yolk index = yolk height ÷ yolk width. The yolk color was measured using a DSM colorimetric spectrum

and using a colorimeter (Model CR400, Konica Minolta Sensing Americas, Inc, USA) that assesses luminosity (L*), red intensity (a*) and yellow intensity (b*).

2.7. Serum clinical biochemistry

Serum levels of total proteins, albumin, alkaline phosphatase (AP) and alanine aminotransferase (ALT) were measured using the semi-automatic BioPlus equipment (Bio-2000) and specific commercial kits. Serum globulin levels were calculated as the difference between serum levels of total proteins and albumin.

2.8. Levels of reactive oxygen species and glutathione S-transferase activity

The activity of the enzyme glutathione S-transferase (GST) was analyzed spectrophotometrically at 340 nm as described [19]; the result was expressed as U GST/mg of protein. The activity of the GPx was measured using tert-butyl hydroperoxide as a substrate [20], and the results were expressed as U GPx/mg protein.

The production of reactive oxygen species (ROS) was evaluated by determining 2', 7′-dichlorofluorescein (DCF) in oxidation [21]. DCFH-DA is hydrolyzed by intracellular esterases to form non-fluorescent DCF, which is rapidly oxidized to form highly fluorescent DCF in the presence of ROS. The intensity of the DCF fluorescence correlates with the amount of ROS formed. Fluorescence was measured using excitation and emission wavelengths of 480 and 535 nm, respectively. A calibration curve was generated using standard DCF (0.1–1 μM) and the data was calculated as U DCF/mg protein.

2.9. Statistical analyses

All dependent variables were tested for normality using the Univariate procedure of SAS (SAS Inst. Inc., Cary, NC, USA; version 9.4). Serum concentrations of ROS were not normally distributed and were therefore log-transformed. Then, all data were analyzed using the MIXED procedure of SAS, with Satterthwaite approximation to determine the denominator degrees of freedom for the test of fixed effects. All data were analyzed as repeat measures. Performance and egg quality were tested for fixed effects of treatment, period, and treatment × period, using animals (treatments) as random variables and animals (treatments) as subjects. Serum concentrations of biochemistry variables and GST and ROS were tested for fixed effects of treatment, day, and treatment × day, using cage (treatment) and animal (cage) as random variables and animals (treatments) as subjects. All results obtained on d 0 for each variable were included as covariates in each respective analysis; they were removed from the model when $P > 0.10$. The compound

symmetric covariance structure was selected for serum concentration of ALT and ROS, and a first order autoregressive covariance structure was selected for all other variables. The covariance structures were 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

Effect of treatment versus day, and treatment effect were detected for egg production ($P \leq 0.04$): The NoC hens had greater egg production in all periods compared to C+ hens (Table 3). The hens that consumed feed with SCL had greater egg production in all periods (except for C+1000 hens from d 56 to 84) compared to C+ hens.

Effects of treatment were detected for egg weight ($P = 0.05$), i.e. NoC hens had greater egg weight compared to C+ hens; and C+D1000 and C+D500+U1000 hens had greater egg weight compared to C+ hens. Effect of treatment were detected for egg mass ($P = 0.01$); and the NoC hens had greater values compared to C+ hens and C+D500+U1000 hens had greater egg mass compared to C+ animals.

Effects of treatment versus day, and treatment effect were detected for feed intake (FI) ($P \leq 0.0006$). The NoC hens had greater FI in all periods compared to C+ hens (Table 3). However, from d 0 to d 56, only C+D500 and C+D500+U1000 hens had greater FI. From d 56 to 84, any SCL improved the FI compared to C+ animals.

Effect of treatment versus day, and treatment effect were detected for feed conversion ratio (FI/dozen) ($P = 0.03$). NoC hens had greater values from d 0 to 56, but not from d 56 to 84 compared to C+ hens. All treatment with SCL increased feed conversion from d 0 to 28. Only C+D500 and C+D500+U1000 from d 28 to 56, and any treatment with SCL increased feed conversion ratio from d 56 to 85, compared to C+ animals. Effects of treatment were detected for feed conversion ratio (kg/kg) ($P = 0.05$), i.e. only C+1000 hens had lower values compared to NoC animals.

3.2. Egg quality

Effect of treatment were detected for shell resistance ($P = 0.03$), i.e. hens of C+D500 and C+D500+U1000 treatment had lower values compared to NoC hens. Effect of treatment was detected were detected for shell thickness ($P = 0.04$), i.e. C+D1000 and C+D500+U1000 hens had lower values than did NoC hens (Table 4).

No effects of treatment versus day, and treatment were detected for pH albumin, pH yolk, calculate gravity, shell and yolk percentage, Haugh units, yolk index, and color sub B ($P \geq 0.06$). However, effects of treatment were detected for albumin percentage ($P = 0.05$), and C+D500 hens had greater values compared to NoC hens (Table 4).

Effects of treatment versus day were detected for color sub-spectrum ($P = 0.01$). From d 0 to 28, C+D500+U1000 hens, had greater values than C+ animals. However, from d 28 to 56, NoC hens had greater values compared to C+ hens, and C+D500+U100 hens had greater values compared to C+ hens. No differences were detected for color sub spectrum from d 56 to 84 (Table 4).

Effects of treatment versus day were detected for color sub L ($P = 0.03$), i.e. from d 0 to 28, C+D500 hens had the lowest values compared to the others. However, from d 28 to 56, NoC hens had the lowest values for color sub L compared to other treatments. No differences were detected from d 56 to 84 (Table 4).

Effects of treatment were detected for color sub A ($P = 0.01$), and NoC hens had lowest values compared to others (Table 4).

3.3. Serum biochemistry

Effects of treatment versus day were detected for serum concentration of ALT ($P = 0.01$); i.e. on d 28, C+D1000 hens had greater values compared to C+D500 and C+D500+U1000 hens; and on d 84, NoC animals had lower values compared to other treatments (Table 5).

Effects of treatment were detected for serum concentration of phosphatase ($P = 0.05$), and C and C+D500 animals had lower concentrations compared to the others. On d 84, NoC hens had lower values compared to other treatments (Table 5).

No effects of treatment versus day, and treatment were detected for serum concentration of total protein, albumin, or globulin ($P \geq 0.13$) (Table 5).

3.4. Serum glutathione transferase and reactive oxygen species

Effects of treatment versus day were detected for serum activity of GST ($P = 0.005$), and only on d 28 did C+D500 hens have greater activity compared to others. C+D100 hens had greater activities than NoC, C+ and C+D500+U1000 animals, and the three latter groups did not differ from one another (Figure 1).

Effects of treatment versus day, and treatment were detected for serum concentration of ROS ($P < 0.0001$). NoC animals had lower concentrations only on d 84 compared to the others (Figure 1).

4. Discussion

Egg production was lower in C+ birds, and egg weight and mass were lower in all periods of the study. This can be explained by the negative effects of mycotoxins on protein and lipid synthesis at the blood level of laying hens, as reported previously [7]. When we compared the C+ group with the groups that consumed adsorbent, there were positive effects; that is, there was greater production of eggs as well as greater weight and mass. The beneficial effects of adsorbents as mycotoxin scavengers in birds are widely known [9]. However, in the present study, we used an alternative SCL-based adsorbent that positively modulated the production system using laying hens as an experimental model. The control group and all treatments that received adsorbents had higher feed consumption, that is, the consumption of contaminated feed reduced consumption; conversely, the use of SCL avoided the negative effect that was directly related to low productivity and health. A previous study found that oral lesions in birds poisoned with T2 evolve to necrosis, erosions, and ulcerations at the base of the tongue, on the palate and at the commissure of the beak, reducing feed consumption and weight gain [22]. The role of fumonisins in increasing circulating serotonin has an effect similar to that of trichothecenes in terms of decreasing consumption in production animals [23]. We also observed lower feed conversion per dozen eggs in the positive control (C+) group, which can be explained by the fact that trichothecenes, including T-2, leading to food refusal, reduced feed conversion and diarrhea [24].

Regarding egg quality parameters, higher shell resistance was observed in the NoC and C+ groups, in addition to a greater shell thickness in the NoC group. Another study showed that, in addition to reducing egg production, mycotoxins reduce the size of the eggs, as well as the proportional reduction in the size of the yolks [25]. Regarding the shell, the deposition of calcium in the shell is not affected by the consumption of mycotoxin, thereby providing eggs with greater thickness and shell strength; however, a study reported that greater thickness of the shells can alter hatchability, because it reduces gas exchange between the embryo and the environment [25]. For these reasons, it is important to clarify that greater shell thickness is desirable for commercial laying hens, but undesirable for breeders.

Eggs from the C+D500+U1000 group showed a higher yolk color at various times during the experiment, as well as in the NoC group. A previous study demonstrated that mycotoxicosis in birds led to reduced production of bile salts [7]. Consequently, the absorption of fat and carotenoid pigments was reduced, leading to low pigmentation of the skin and egg yolk, the so-called “pale bird syndrome.”

Increased levels of ALT on day 28 of the experiment in the C+D1000 group was observed; however, at the end of the study, ALT levels increased significantly in all birds that consumed T-2 and FB1. In another study that evaluated the effect of aflatoxin, there were no significant alterations in ALT levels, suggesting that ALT is not a sensitive marker of liver disorders in birds that consumed T-2 and FB1 [26]; this may have been because these mycotoxins did not affect the liver of the chickens at this stage of production. In broiler chickens, our research group recently found that consumption by broilers of feed contaminated with FB1 in the initial production phase (up to 21 days) increased liver enzymes, including ALT, even without histological lesions [27]. In the present study, it is notable that there were lower levels of alkaline phosphatase in birds that ingested mycotoxins; alkaline phosphatase has important functionality at the hepatic level. Lower levels of activity were not expected, and the mechanisms involved are unknown; there was an increase in ALT that characterizes liver damage caused by the cumulative effect of the two mycotoxins consumed for 84 days.

GST activity was higher on day 56 of the experiment in the blood of birds in the group with *S. cerevisiae* lysate at 500 g/ton (C+D500). All treatments of birds that consumed mycotoxin had higher serum levels of ROS at the end of the experiment, suggesting a cumulative effect of mycotoxin. GST acts strongly against the exacerbation of oxidative stress [28]; therefore, increases in its activity can be seen as positive effects in terms of protecting cells, especially in the liver, acting as a detoxification enzyme. The detoxifying action of GST was shown to be important in protecting against oxidative stress and poisoning [29]. In quails that consumed aflatoxin, researchers reported intense oxidative stress, which was minimized by conventional adsorbents [30]; this was not the same results found here with SCL, as ROS levels increased over time due to daily consumption of T-2 and FB1.

In this experiment, the layers did not show any clinical sign of intoxidation; but we found that mycotoxin intake interfered with egg production and quality. We believe that these changes are multifactorial, so we focus on metabolic disorders and oxidative stress. However, this subject has generated preliminary data that merit further research, such as assessing the direct or indirect effects of mycotoxins on hormones involved in oviposition; thus presence of mycotoxins in the egg. In this study, SCL proved to be an alternative, which deserves further tests in order to define a dose.

5. Conclusion

Ingestion of mycotoxins impaired the performance and quality of the eggs of the laying hens; however, the addition of *S. cerevisiae* lysate and the addition organic acids, yeast cell wall and mineral carrier minimized some negative effects caused by mycotoxins T-2 and FB1.

Ethics committee

This work was approved by the Ethics Committee on Animal Use (CEUA) of the State University of Santa Catarina (UDESC), protocol number 3089070619.

References

[1] Bertechini, A. G, 2012. Nutrição de monogástricos. 2. ed., rev. Lavras: Ufla, 373p.

[2] Freire, F.C.O, Vieira, I.G.P, Guedes, M.I.F, Mendes, F.N.P, 2007. Micotoxinas: Importância na Alimentação e na Saúde Humana e Animal. Embrapa Agroindústria Tropical, Fortaleza. Documentos 110, 1.ed.

[3] Prestes, I.D, Rocha, L.O, Nunez, K.V.M, Silva, N.C, 2019. Principais fungos e micotoxinas em grãos de milho e suas consequências. Scientia Agropecuaria 10(4): 559-570.

[4] Pezzini, V, Valduga, E, Cansian, R.L, 2005. Incidência de fungos e micotoxinas em grãos de milho armazenados sob diferentes condições. Rev Inst Adolfo Lutz, 64(1):91-6.

[5] MAPA, 2006. Ministério do Estado da Agricultura, Pecuária e Abastecimento. Grupo de Trabalho sobre Micotoxinas em produtos destinados à alimentação animal. Seção 2, pág.5.

[6] Burditt, S.J, Winston, M, Hagler, W, Hamilton, P.B, 1983. Survey of molds and mycotoxins for heir ability to cause feed refusal in chickens. Poultry Science; 62: 2187-91.

[7] Santurio, J.M, 2000. Micotoxinas e micotoxicoses na avicultura. Rev. Bras. Cienc. Avic. vol.2 no.1 Campinas Jan./abr.

[8] Martins, F.A, Ferreira, F.M.D, Ferreira, F.D, Bando, E, Nerilo, S.B, Hirooka, E.Y, Machinski, M.J.R, 2012. Daily intake estima-tes of fumonisins in corn-based food pro--570- ducts in the population of Parana, Brazil. Food Control 26(2): 614-618.

[9] Bunzen, S, Haese, D, 2006. Controle de micotoxinas na alimentação de aves e suínos. Revista Eletrônica Nutritime, v.3, n° 1, p.299-304, janeiro/fevereiro.

[10] Carão, A. C. P, Burbarelli, M. F. C, Polycarpo, G. V, Santos, A. R, Albuquerque, R, Oliveira, C. A. F, 2014. Métodos físicos e químicos de detoxificação de aflatoxinas e redução da contaminação fúngica na cadeia produtiva avícola. Ciência Rural, v. 44, n. 4, p. 699-705. DOI 10.1590/S0103-84782014000400021

[11] Keller, K. M, Oliveira, A. A, Almeida, T. X, Keller, L. A. M, Queiroz, B. D, Nunes, L. M. T, Cavaglieri, L. R, Rosa, C. A. R, 2012. Efeito de parede celular de levedura sobre o desempenho produtivos de frangos de corte intoxicados com Aflatoxina B1. Revista Brasileira de Medicina Veterinária, v. 34, n. 2, p. 101-105.

[12] Armando, M. R, Pizzolitto, R.P, Dogi, C. A, Cristofolini, A, Merkis, C. V, Poloni, C. V, Dalcero, A. M, Cavaglieri, L. R, 2012. Adsorption of ochratoxin A and zearalenone by potential probiotic Saccharomyces cerevisiae strains and its relation with cell wall thickness. Journal of Applied Microbiology, v. 113, p. 256–264.

[13] Jouany, J. P, 2007. Methods for preventing, decontaminating and minimizing the toxicity of mycotoxins in feeds. Animal Feed Science and Technology, v. 137, p. 342–362. https://doi.org/10.1016/j.anifeedsci.2007.06.009

[14] Pinheiro, R. E. E, Pereyra, C. M, Neves, J. A, Calvet, R. M, Santos, J. T. D. O, Lima, C. E, Muratori, M. C. S, 2017. Avaliação in vitro da adsorção de aflatoxina B1 por produtos comerciais utilizados na alimentação animal. Arquivos do Instituto Biológico, 84.

[15] Wang, J. P, Chi, F, Kim, I. H, 2012. Effects of montmorillonite clay growth performance, nutrient digestibility, vulva size, faecal microflora, and oxidative stress in weaning gilts challenged with zearalenone. Animmal Feed Science and Technology, v. 178, p. 158-166. https://doi.org/10.1016/j.anifeedsci.2012.09.004

[16] Rostagno, H.S et al, 2017 Tabelas brasileiras para aves e suínos: composição de alimentos e exigências nutricionais. 4.ed. Viçosa, MG: UFV, DZO.

[17] Freitas, E. R, Sakomura, N.K, Gonzalez, M.M, Barbosa, N.A.A, 2004. Comparação de métodos de determinação da gravidade específica de ovos de poedeiras comerciais. Pesq. agropec. bras., Brasília, v.39, n.5, p.509-512, maio.

[18] Haugh, R. R, 1937. A new method for determining the quality of an egg. US Egg Poultry, 49p.

[19] Giusti, G, Galanti, B, 1984. Colorimetric method, in: H.U. Bergmeyer, (Eds.), Methods of Enzymatic Analysis. 3º Edition, Weinheim, Verlag Chemie, pp. 315–323.

[20] Wendel, A, Fausel, M, Safayhi, H, Tiegs, G, Otter, R, 1984. A novel biologically active seleno-organic compound—II: Activity of PZ 51 in relation to Glutathione Peroxidase. Biochem. Pharmacol. 33, 3241–3245. https://doi.org/10.1016/0006-2952(84)90084-4

[21] Lebel, C.P, Ischiropoulos, H, Bondy, S.C, 1992. Evaluation of the probe 2', 7' – dichlorofluorescin as na indicator of reactive oxygen species formation and oxidative stress. Chem. Res. Toxicol. 5, 227-231. doi: 10.1021/tx00026a012

[22] Santin, E, Maiorka, A, Zanella, I. et al, 2000. Micotoxinas do *Fusarium spp.* na avicultura comercial. Ciênc. Rural. v.31, n.1, p.185- 190.

[23] Reis, J.S, Xavier, E.G, Rossi, P, Lopes, C.N, Dionello, N.J.L, Rutz, F, 2012. Efeitos dos tricotecenos na avicultura e métodos de controle. PUBVET, Londrina, V. 6, N. 23, Ed. 210, Art. 1404.

[24] Dilkan, P, Mallmann, C.A, 2004. Anais do XI Encontro Nacional de Micotoxinas, 30/06 a 02/07 2004. Realizado em Piracicaba – SP, Universidade de São Paulo – Escola Superior de Agricultura Luiz de Queiroz.

[25] Vieira, S.L, 1995. Micotoxinas e produção de ovos. In: I Simpósio Internacional sobre Micotoxinas e Micotoxicoses em Aves; Curitiba, Paraná, Brasil. p.65-80.

[26] Fernandez, A, Verde, M, Gascon, J, Ramos, J, Gomez, D, Luco, F, Chavez, G, 1994. Variations of clinical biochemical parameters of laying hens and broiler chickens fed aflatoxin-containing feed. Avian Pathology 23, 37-47. https://doi.org/10.1080/03079459408418973

[27] Sousa, M.C.S, Galli, G.M, Alba, D.F, Griss, L.G, Gebert, R.R, Souza, C.F, Baldissera, M.D, Gloria, E.M, Mendes, R.E, Zanelato, G.O, Gris, A, Boiago, M.M, Stefani, L.M, Silva, A.S, 2020. Pathogenetic effects of feed intake containing of fumonisin (Fusarium verticillioides) in early broiler chicks. Microbial Pathogenesis. In press, journal pre-proofAvailable online 11 May 2020 Article 104247 https://doi.org/10.1016/j.micpath.2020.104247

[28] Huber, P. C., Almeida, W. P, Fátima, A. D, 2008. Glutationa e enzimas relacionadas: papel biológico e importância em processos patológicos. Química Nova, *31*(5), 1170-1179.

[29] Babbitt, P. C, 2000. Reengineering the glutathione S-transferase scaffold: a rational design strategy pays off. Proceeding National Academy Sciences, Washington, v. 97, n. 19, p. 10293-10300. https://doi.org/10.1073/pnas.97.19.10298

[30] Migliorini, M. J, Da Silva, A. S, Santurio, J. M, Bottari, N. B, Gebert, R. R, Reis, J. H, Volpato, A, Morsch, V.M, Baldissera, M.D, Stefani, L.M, Boiago, M.M, 2017. The Protective effects of an adsorbent against oxidative stress in quails fed aflatoxin-contaminated diet. Acta Scientiae Veterinariae, 45, 1-7.

Table 1: Ingredients and chemical composition of the basic diet offered to chickens

Ingredients	%
Corn	65.70
Soybean flour, 45 %	21.80
Calcitic limestone	8.90
Soybean oil	1.10
Bicalcium phosphate	1.50
DL- Methionine 98%	0.20
NaCl (Table salt)	0.50
Premix*	0.30
TOTAL	100.00
Values calculated according to the centesimal composition of Rostagno (2011)	
Metabolizable energy (Kcal/Kg)	2.848
Crude protein (%)	15.67
Calcium (%)	3.87
Available phosphorus (%)	0.37
Digestible lysine (%)	0.68
Digestible methionine (%)	0.42
Digestible methionine + cystine (%)	0.65
Sodium	0.23

Product composition (kg): vit. A 7,000,000 IU; vit. D3 4,000,000 IU; vit. E 5000 mg; vit. K 1200 mg; vit. B1 360 mg; vit. B2 2000 mg; vit. B6 700 mg; vit. B12 7000 mcg; niacin 7500 mg; biotin 30 mg; pantothenic acid 6000 mg; folic acid 300 mg; iron 1 1000 mg; copper 3000 mg; iodine 204 mg; chlorine 360 mg; promotors grow and efficiency feed 20 mg; coccidiostatic agent 100 g; antifungal agent 2000 mg; antioxidant 10 mg; magnesium 50 g; sulfur 40 g; energy and protein vehicle (q. s. p.) 1,000 g.

Table 2. Actual levels of fuminisin (FB1) and T-2 toxin (T-2) in the diets of laying hens in this study

Treatments	**FB1 (ppm)**	**T-2(ppm)**
NoC	0.59	ND
C	17.10	2.12
C+D500	17.45	2.41
C+D1000	16.85	2.19
C+D500+U1000	17.77	2.32

Note 1: ND – not detected (Limit of quantification 30 ppb). Liquid chromatography with mass spectrometry detection (HPLC – MS/MS).

Table 3. Performance of of laying hens fed with diets containing mycotoxins adsorbents.

Variables	Treatments[1]					SEM	*P*-value	
	NoC	C	C+ D500	C+ D1000	C+ D500+ U1000		Treat	Treat × day
Eggs production, %							0.0001	0.04
d 0 to 28	96.25^{a}	81.84^{c}	86.60^{b}	85.53^{bc}	88.84^{b}	1.70		
d 28 to 56	92.98^{a}	85.56^{b}	84.40^{b}	86.46^{b}	86.55^{b}	1.70		
d 56 to 84	92.46^{a}	78.42^{c}	88.49^{ab}	86.92^{b}	86.81^{b}	1.70		
Egg weight, g	63.86^{a}	57.92^{b}	60.39^{ab}	62.06^{a}	61.19^{a}	1.26	0.05	0.59
Egg mass, g	58.67^{a}	47.64^{c}	51.90^{bc}	51.55^{bc}	52.98^{b}	1.85	0.01	0.84
Feed intake (FI), g							<0.0001	0.0006
d 0 to 28	111.77^{a}	78.15^{c}	94.66^{b}	80.46^{c}	97.04^{b}	2.98		
d 28 to 56	112.51^{a}	84.58^{d}	93.55^{bc}	86.06^{cd}	96.50^{b}	2.98		
d 56 to 84	113.25^{a}	90.99^{b}	92.44^{b}	91.67^{b}	95.95^{b}	2.98		
Feed conversion ratio (FI/dozen)							0.03	0.03
d 0 to 28	1.40^{a}	1.16^{c}	1.29^{b}	1.25^{b}	1.27^{b}	0.03		
d 28 to 56	1.41^{a}	1.26^{d}	1.31b^{c}	1.29^{cd}	1.31^{bc}	0.03		
d 56 to 84	1.42	1.36	1.33	1.33	1.35	0.03		
Feed conversion ratio (kg/kg)	1.91^{a}	1.76^{ab}	1.78^{ab}	1.62^{b}	1.82^{a}	0.06	0.05	0.76

1. Treatments were diets with no contamination (NoC) or contaminated (4 ppm of T2 and 20 ppm of FB1) with mycotoxins (C) and containing 500 (C+D500) or 1000 g/ton of Detoxa Plus® (C+D1000) or containing 500 g/ton of Detoxa Plus® and 1000 g/ton of Uniwall Mos 25® (C+D500+U1000).

$^{a\text{-}c}$Differs ($P \leq 0.05$) between treatments each respective day.

Table 4. Egg quality of laying hens fed with diets containing mycotoxins adsorbents.

Variables	Treatments[1]					SEM	*P*-value	
	NoC	C	C+ D500	C+ D1000	C+ D500+ U1000		Treat	Treat × day
Shell resistance, $\times 10^3$	5302.84^{a}	4959.74^{ab}	4483.13^{b}	4637.33^{ab}	4169.88^{b}	230.11	0.03	0.10
pH albumin	8.29	8.15	8.17	8.21	8.22	0.04	0.24	0.36
pH yolk	5.91	5.91	5.89	5.93	5.88	0.02	0.73	0.34
Calculate gravity	1.12	1.09	1.08	1.09	1.08	0.01	0.38	0.46
Shell thickness, mm	0.38^{a}	0.37^{ab}	0.37^{ab}	0.36^{b}	0.36^{b}	0.005	0.04	0.92
Shell (%)	9.99	9.90	9.42	9.65	9.52	0.20	0.23	0.30
Yolk (%)	26.14	26.88	25.67	26.20	26.37	0.44	0.41	0.06
Albumin (%)	63.68^{b}	63.50^{b}	65.32^{a}	64.19^{ab}	64.42^{ab}	0.45	0.05	0.06
Haugh unit	89.46	91.68	86.34	89.09	88.53	1.23	0.07	0.24
Yolk index	0.47	0.47	0.47	0.46	0.46	0.006	0.50	0.28
Color sub								
Colorimetric fan							0.16	0.01
d 0 to 28	5.25^{ab}	4.75^{b}	5.50^{ab}	5.62^{ab}	5.87^{a}	0.32		
d 28 to 56	5.93^{a}	4.87^{bc}	4.12^{c}	4.62^{bc}	5.37^{ab}	0.32		
d 56 to 84	6.50	6.50	7.12	6.62	7.12	0.32		
L							0.68	0.03
d 0 to 28	62.03^{a}	62.55^{a}	58.94^{b}	62.14^{a}	61.35^{ab}	0.96		
d 28 to 56	58.23^{b}	60.73^{a}	61.48^{a}	59.75^{ab}	61.17^{a}	0.96		
d 56 to 84	59.44	59.91	60.57	60.41	58.54	0.96		
A	-6.55^{a}	-7.03^{ab}	-7.29^{b}	-7.42^{b}	-7.53^{b}	0.20	0.01	0.22
B	44.92	44.83	43.24	44.72	45.03	0.77	0.46	0.61

[1.] Treatments were diets with no contamination (NoC) or contaminated (4 ppm of T2 and 20 ppm of FB1) with mycotoxins (C) and containing 500 (C+D500) or 1000 g/ton of Detoxa Plus® (C+D1000) or containing 500 g/ton of Detoxa Plus® and 1000 g/ton of Uniwall Mos 25® (C+D500+U1000).

[a-c]Differs ($P \leq 0.05$) between treatments each respective day.

Table 5. Serum biochemistry of laying hens fed with diets containing mycotoxins adsorbents.

Variables[1]	Treatments[2]					SEM	*P*-value	
	NoC	C	C+ D500	C+ D1000	C+ D500+ U1000		Treat	Treat × day
ALT (U/L)							0.06	0.01
d 0	24.17	27.74	28.02	29.36	28.57	3.73		
d 28	18.97^{ab}	16.36^{ab}	8.74^{b}	22.19^{a}	11.25^{b}	3.49		
d 56	17.57	9.99	7.88	10.79	9.25	3.73		
d 84	8.28^{b}	41.90^{a}	32.22^{a}	33.87^{a}	34.78^{a}	4.91		
ALP (U/L)	509.68^{a}	343.31^{b}	451.89^{ab}	350.07^{b}	405.64^{ab}	37.28	0.05	0.09
Total protein (mg/dL)	5.06	4.82	5.05	5.20	4.78	0.15	0.33	0.64
Albumin (mg/dL)	1.92	1.85	1.76	1.91	1.71	0.09	0.36	0.13
Globulin (mg/dL)	3.14	3.01	3.29	3.30	3.09	0.15	0.44	0.62

1. ALT, alanine aminotransferase; ALP, Alkaline phosphatase

2. Treatments were diets with no contamination (NoC) or contaminated (4 ppm of T2 and 20 ppm of FB1) with mycotoxins (C) and containing 500 (C+D500) or 1000 g/ton of Detoxa Plus® (C+D1000) or containing 500 g/ton of Detoxa Plus® and 1000 g/ton of Uniwall Mos 25® (C+D500+U1000).

$^{a-c}$Differs ($P \leq 0.05$) between treatments each respective day.

Figure1A

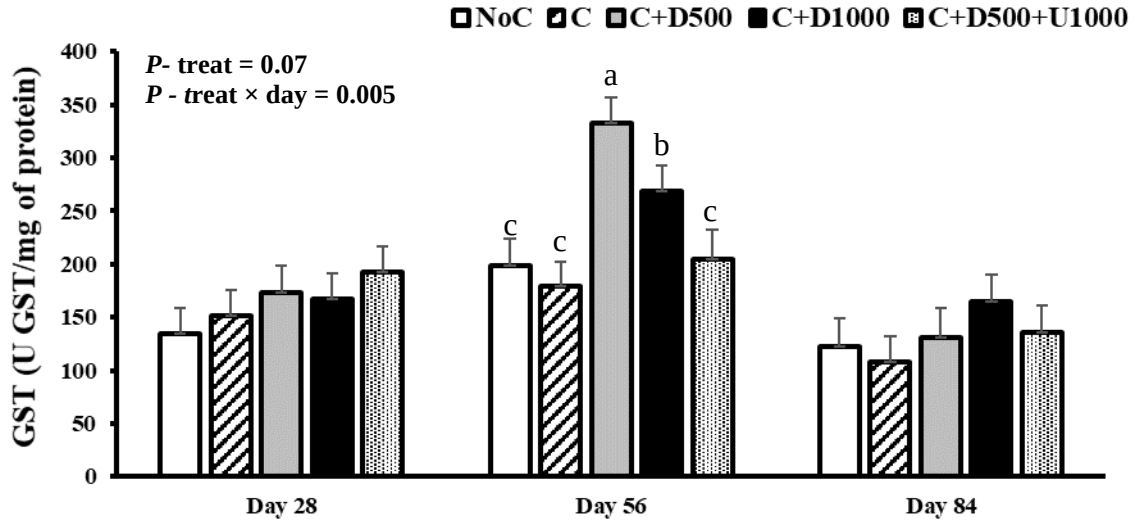


Figure 1 B

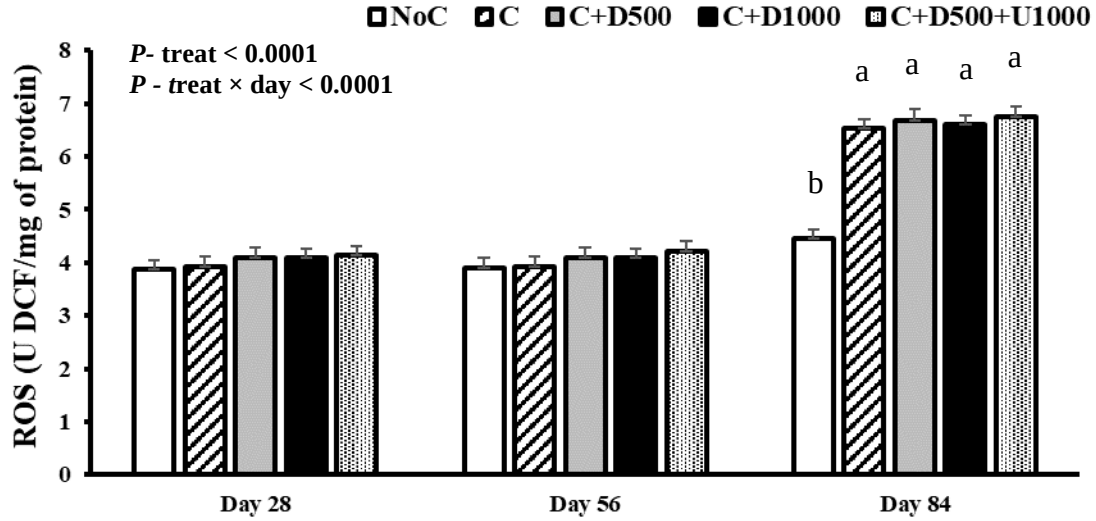


Figure 1. Serum concentration of glutathione transferase (GST) and reactive oxygen species (ROS) of laying hens fed with diets containing mycotoxins adsorbents. Treatments were diets with no contamination (NoC) or contaminated (4 ppm of T2 and 20 ppm of FB1) with mycotoxins (C) and containing 500 (C+D500) or 1000 g/ton of Detoxa Plus® (C+D1000) or containing 500 g/ton of Detoxa Plus® and 1000 g/ton of Uniwall Mos 25® (C+D500+U1000). $^{a-c}$Differs ($P \leq 0.05$) between treatments each respective day. Vertical bars represent the SEM.

2.2 – MANUSCRITO I

Vegetable biocholine as a hepatoprotectant in laying hens feed with diet contaminated with aflatoxin B_1

Biocholine as a hepatoprotectant in laying hens feed against aflatoxin B1

Vanessa Dazuk[1], Marcel M. Boiago[2], Gilneia da Rosa[1], Davi F. Alba[1], Carine F. Souza[3], Matheus D. Baldissera[3], Marcelo Vedovatto[4], Ricardo E. Mendes[5], Janio M. Santurio[6], Guilherme L. Deolindo[2], Aleksandro S. Da Silva[2]

[1]Graduate Program in Animal Science, State University of Santa Catarina (UDESC/CEO) Chapecó, SC, Brazil.

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

[3] Postgraduate Department in Pharmacology, Federal University of Santa Maria (UFSM), Santa Maria, RS, Brazil.

[4] Graduate Program in Animal Science, State University of Mato Grosso do Sul, Aquidauana, MS, Brazil.

[5] Laboratory of Veterinary Pathology, Instituto Federal Catarinense, Concordia, SC, Brazil.

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

Corresponding author: dasilva.aleksandro@gmail.com

Abstract

The aim of the study was to determine whether the addition of vegetable biocholine (VB) in laying hens feed minimizes the effects of daily intake of aflatoxin B1 (AFB1). We allocated Hy-line Brown line laying hens into four groups with four replications/group and four birds/repetition. The treatments were as follows: Afla0Bio0: basal feed without aflatoxin and VB (natural contamination: 0.026 mg AFB1/kg), Afla0Bio800, basal feed supplementation of 800 mg VB/kg (natural contamination: 0.024 mg AFB1/kg); Afla2.5Bio0, basal feed contaminated experimentally with aflatoxin (2.51 mg/kg); Afla2.5Bio800, basal feed contaminated with aflatoxin (2.50 mg/kg) and

supplemented with 800 mg VB/kg. The experiment took place over a period of 42 days, divided into two cycles of 21 days each. Significance was indicated by P≤0.05. The inclusion of aflatoxin reduced egg production after 42 days of consumption of contaminated feed; VB supplementation in the tested dose was insufficient to minimize the negative effects of the toxin on the laying rate. There was a lower percentage of yolk in Afla2Bio0 than in Afla0Bio0, and a higher percentage of albumen and specific gravity in Afla2.5Bio0 than in Afla0Bio0. Ingestion of aflatoxin in the feed increased lipoperoxidation (LPO) and decreased antioxidant capacity in the egg yolk; however, when VB was added, LPO was similar to the control. Lower total bacterial count (TBC) in the eggshell was observed when the birds consumed VB, as well as higher TBC in the eggshell of the birds was challenged with aflatoxin. In the blood of birds that consumed aflatoxin (Afla2.5Bio0) there was an increase in the activity of alkaline phosphatase and a reduction in the activities of glutathione S-transferase and glutathione peroxidase (GPx). In the birds that consumed VB without aflatoxin challenge, we observed that there was a stimulation of GPx activity. We conclude that the consumption of VB had positive effects on the health of the laying hens and improved the quality of the eggs.

Keywords: Poultry farming. Aflatoxin. Choline. Laying hens.

1. Introduction

The presence of mycotoxins in grains and feed and the intake of these foods by farm animals has substantial impact on health and the economy. In laying poultry, aflatoxin is the causative agent of mycotoxicosis, toxic to birds by virtue of its carcinogenic, teratogenic, mutagenic, and immunosuppressive properties (Migliorini et al. 2017). Even in countries with advanced grain production and storage systems, there is great difficulty in controlling mycotoxins, evidenced by the fact that 25% of the world's grains are contaminated with aflatoxin (Bunzen and Haese, 2006)

AFB1 is produced by fungi of the genus *Aspergillus*, which naturally develop in food products such as peanuts, corn, beans, rice, and wheat (Oliveira and Germano, 1997), and according to Food and Drug Administration the maximum aflatoxin level in feed is 20 ppb for immature poultry, 100 ppb for mature poultry and 300 ppb for poultry. The liver is the main organ affected by aflatoxin, and in birds with aflatoxicosis, affected livers become yellowish and friable, with marked fatty infiltration (Santurio, 2000). AFB1 poisoning can damage the birds' antioxidant/oxidant defense systems, inhibiting antioxidant enzymes and increasing the production of free radicals, as reported by Migliorini et al. (2017) in a study with quails. Recently, a study conducted by Khanian et al. (2019) reported that broiler chicks fed with a diet contaminated with AFB1 (200 and 2000

ppb) caused oxidative damage by increasing lipid damage and inhibition of enzymatic and non-enzymatic antioxidant defense systems, all of which contributed to AFB1 induced toxicity.

There is an increasing tendency toward the use of natural products derived from plants in the nutrition of farm animals for protection against toxic and infectious agents that harm animal health (Sutili et al. 2018). Recently, a study conducted by Souza et al. (2018) revealed that dietary supplementation with vegetable biocholine (VB) improved the performance and liver health of Nile tilapia (*Oreochromis niloticus*). Souza et al. (2020a) reported that VB exerted hepatoprotective effects on Nile tilapia given feed containing AFB1, revealing its positive effects on liver performance and health via reduction of free radical's production, lipid damage and protein damage, as well as via increases in the hepatic antioxidant system. In this sense, VB may reduce lipid damage and improve antioxidant capacity; our hypothesis was that diets containing VB would reduce or avoid aflatoxin-induced impairment on antioxidant status. Therefore, the objective of this study was to determine whether VB supplementation would minimize the negative effects of daily consumption of AFB1 on productive efficiency, egg quality, and health of laying hens.

2. Materials and methods

2.1. VB

Vegetable biocoline (VB) (Biocholine Powder®, Technofeed, SP, Brazil) was acquired commercially. The product is produced from plant extracts (*Trachyspermum ammi, Azadichara indica* and *Achyranthes rugas*), and has guarantee levels of 16 g of phosphatidylcholine/kg of extract). VB (800 mg VB/kg of feed) was incorporated in the feed of laying hens based on results published by Souza et al. (2020a).

2.2. Aflatoxin

Aflatoxin was produced by fermentation in rice, converted under constant stirring and controlled temperature. The NRLL 2999 strain of *A. parasiticus* was used according to the method described by West et al. (1973). This fungus isolate is used in the laboratory to produce aflatoxin B1, and we rarely found other types of aflatoxin in the inoculum (i.e., AFB2, AFG1 and AFG2). After autoclaving with a valve opening, the material was dried in a forced ventilation oven and ground in a laboratory mill equipped with a 1-mm sieve. The concentration of aflatoxin was subsequently determined using HPLC (Thorpe et al., 1982). Based on this information, we stipulated a concentration of 3.0 mg AFB1/kg and 0.01 mg AFB2/Kg of feed to challenge the birds. After aflatoxin production, the material was stored for 8 months at room temperature in sealed plastic bags until its use in this experiment.

2.3. Animals and experimental design

The experiment was carried out in the experimental shed in the city of Chapecó, SC, lasting 42 days, which corresponds to two productive cycles of 21 days each. The diet used was formulated based on corn and soybean meal, according to the nutritional requirements of laying hens (Rostagno et al. 2017). The corn used to produce the feed was previously selected using a sieve, in order to remove the compromised grains (broken, with fungus, rotten, among others) and thus to reduce the chance of natural contamination of the feed by mycotoxin.

After production of the feed, the provision of experimental diets was started (called day 1). While an experiment was in progress, the actual measurement of aflatoxin in the diet of men was performed per treatment. The levels of aflatoxin in all diets were measured using HPLC, with immunoaffinity purification, post-column derivatization and fluorescence detection; the methodology is described in detail by Muller et al. (2018). The method presented a limit of quantification (LQ) of 0.5 µg/kg for each aflatoxin (AFB1, AFB2, AFG1 and AFG2).

The analysis showed the actual level of aflatoxin in the diets, values that are lower than the calculated levels: Afla0Bio0 (0.026 mg/kg), Afla0Bio800 (0.024 mg/kg), Afla2.5Bio0 (2.51 mg/kg), and Afla2.5Bio800 (2.50 mg/kg). The AFB2 concentration in the diets was similar and low in the four treatments: Afla0Bio0 (0.008 mg/kg), Afla0Bio800 (0.010 mg/kg), Afla2.5Bio0 (0.009 mg/kg), and Afla2.5Bio800 (0.008 mg/kg). AFG1 and AFG2 were not detected in the experimental diets.

We allocated 64 Hy-line Brown hens, 84 weeks old, into four groups with four repetitions per group and four hens per repetition. The experiment was conducted in a breeding system in cages (0.5 x 0.6 x 0.4 m), equipped with trough-type feeders and nipple-type drinkers. Because the layers were already adapted to each other in the cage, we chose not to exchange hens between cages, in order to avoid stress and fights that occur in these situations. Because each cage with four birds was used as one repetition per group, the following treatments were randomly described inside the shed, in order to minimize any effect of the environment. The treatments were divided as follows: Afla0Bio0 (basal feed without aflatoxin and without biocholine), Afla0Bio800 (basal feed supplemented with 800 mg VB/kg), Afla2.5Bio800 (basal feed contaminated with 2.5 mg aflatoxin/kg); Afla2.5Bio800 (basal feed contaminated with 2.5 mg aflatoxin/kg and supplemented with 800 mg VB/kg of feed).

2.4. Zootechnical performance

Performance in the production phase was evaluated at the end of two 21-day production cycles, and the following variables were considered: percentage of egg production, average egg weight, egg mass, feed consumption, feed conversion (kg of feed/kg of egg produced and kg of feed/dozen eggs produced). The percentage (%) of eggs was obtained by counting and collecting daily eggs from each experimental unit. For the average egg weight, an analytical balance of precision ± 0.01 g (model Shimadzu BL-3200H) was used. Egg mass was calculated in the final three days of each production cycle, with the individual weight of all eggs in each experimental plot (cage) being measured, and then the egg mass was calculated using the equation: egg mass = average weight of the eggs. eggs (g) x production of the day (%). The feed was stored in buckets, one per repetition of each treatment, with the measurement of consumption performed weekly.

2.5 Sample collection, tissue preparation, and protein determination

Blood samples were collected on days 1, 21, and 42 of the experimental periods. The hens were manually contained (n = 6 per group: repetition 1 and 2 were two hens each; and repetitions 3 and 4 was one layer each), with ulnar vein punctured using a syringe (3 mL) and needle (25/7). The collected blood was allocated in Eppendorf microtubes and later centrifuged to separate the serum, and kept frozen (–20 ° C) until analysis.

At the end of the 42-day experimental period, three laying hens per treatment (one per repetition: R1, R2 and R3) were euthanized by cervical dislocation; the methodology was approved by the ethics committee on the use of institutional animals, based on the CONCEA/Brazil regulations. The collection of fragments of liver and intestine (duodenum, jejunum, ileum) was carried out, and these were preserved in 10% formaldehyde. Liver fragments were homogenized in Tris-HCL solution (1:10 v/v), centrifuged at 2000 x *g* for 10 min, and the supernatants were stored in microtubes at - 20 ºC for analysis of oxidative and antioxidant parameters.

Protein concentrations (serum, hepatic tissue, and egg yolk) was determined using the Coomassie Blue method following the methodology described by Read and Northcote (1981) with bovine serum albumin as the standard. These results were used to determine the dilutions and presentation of the results of oxidants and antioxidants.

2.6. Egg analysis

2.6.1. Total bacterial counts and confirmation *Escherichia coli*

One gram of two eggshells per treatment was weighed aseptically and diluted in 9 mL of buffered peptone water in a sterile test tube, homogenized using a vortex shaker giving a 10^{-1} dilution. Then, 200 μL of each sample were inoculated into Petri dishes previously prepared with

standard agar for counting, incubated in a bacteriological oven at 37 ºC for 24 hours and then counted using a colony counter. The results were expressed as colony-forming units per mL (CFU/mL). For confirmation of *Escherichia coli*, an aliquot of the 10^{-1} dilution was seeded in Petri dishes containing methylene blue eosin (MBE) agar and MacConkey agar and incubated at 37 °C for 24 hours. After this period, using a platinum needle, two colonies characteristic for *E. coli* (metallic green colonies on MBE agar and rosettes due to lactose fermentation on MacConkey agar) from each sample were subjected to biochemical tests for the following: urea base agar, TSI agar, SIM medium agar, and Simmons citrate agar, and were incubated again at 37 °C for 24 hours for reading.

2.6.2. Physicochemical parameters

At the end of each 21-day experimental period (days 21 and 42), two eggs were collected per repetition (total 16 eggs per treatment; i.e. 8 eggs per treatment at day 21; and 8 eggs per treatment at day 42) for physicochemical quality analyses as detailed by Galli et al. (2018). In our study, we measured the shell strength (kgf) using a texturometer (Model TA.XT plus, Extralab, Brazil); the albumen and yolk pH obtained using a digital pH meter (Model Testo 205, Testo, Brazil); measured of specific gravity (Freitas et al. 2004); the thickness of the dry shell was measured using a digital thickness gauge (Dasqua®); using these data, the average shell thickness for each egg was calculated. To obtain the percentages of yolk, shell, and albumen, the yolks were separated from the albumen, weighed separately, and the shell was washed to completely remove the albumen and placed to dry at room temperature until weighing. Haugh units were calculated using the equation: HU = 100log (average albumen height + 7.57 - 1.7 x egg weight in gram x 0.37) (Haugh, 1937). The yolk index was calculated using the equation: yolk index = yolk height ÷ yolk width; The yolk color was estimated through the DSM colorimetric fan and using a colorimeter (Model CR400, Konica Minolta Sensing Americas, Inc, USA) that assesses the luminosity (L*), red intensity (a*) and yellow intensity (b*).

2.6.3. Egg yolk oxidant and antioxidant status

The egg yolks (total 16 eggs per treatment; i.e. eight eggs per treatment at day 21; and eight eggs per treatment at day 42) were homogenized (1:20 w v^{-1}) in a medium containing 120 mM potassium chloride and 30 mM buffer phosphate (pH 7.4), and the supernatant fraction obtained was immediately used by dosages.

Total antioxidant capacity against peroxyl radicals (ACAP) was determined according to the method described by Amado et al. (2009) with modification for the eggs yolks samples. This

method consists of finding the antioxidant capacity of tissues using a fluorescent substrate (2′,7′ dichlorofluorescein diacetate - H_2DCF-DA) and the production of peroxyl radicals by thermal decomposition of ABAP (2,2′-azobis (2-methylpropionamidine) dihydrochloride). The fluorescence was determined using a microplate reader (Spectramax I3) at 37 °C (excitation: 485 nm; emission: 530 nm) with readings at every 5 min, during 30 min. The results were expressed as a relative area (the difference between the area with and without ABAP divided by the area without ABAP). The results were express in fluorescence units per mg of protein (FU/mg of protein).

This technique was based on Hermes-Lima et al. (1995) with some modifications by authors, called ferrous oxidation/xylenol orange (FOX) based on the oxidation of Fe (II) under acidic conditions. The Fox method measure lipid peroxides, one of the main products of lipid peroxidation. For lipoperoxidation (LPO) measurements, $FeSO_4$ (1 mM), H_2SO_4 (0.25 M), xylenol orange (1 mM, Sigma) and MilliQ water were sequentially added. Samples or methanol (blanks) were added and incubated for 30 min. Thereafter, absorbance (550 nm) was determined using cumene hydroperoxide (CHP; Sigma) as the standard. Results were expressed as nmol/mL.

2.7 Serum clinical biochemistry

Serum levels of total proteins, albumin, alkaline phosphatase (AP) and alanine aminotransferase (ALT) were measured using the semi-automatic BioPlus equipment (Bio-2000) and specific commercial kits. Serum globulin levels were calculated as the difference between serum levels of total proteins and albumin.

2.8. Oxidant and antioxidant status

Glutathione S-transferase (GST) and glutathione peroxidase (GPx) activities were measured in serum and liver, using a methodology published by Souza et al. (2020a), being expressed as U GPx/mg protein. GST activity was analyzed spectrophotometrically at 340 nm using the method of Habig et al. (1974). GPx activity was measured using tert-butyl hydroperoxide as the substrate (Wendel et al, 1984).

The production of reactive oxygen species (ROS) was evaluated by determining 2', 7′-dichlorofluorescein (DCF) in oxidation (Lebel et al., 1992) in the liver. DCFH-DA is hydrolyzed by intracellular esterases to form non-fluorescent DCF, which is rapidly oxidized to form highly fluorescent DCF in the presence of ROS. The intensity of DCF fluorescence correlates with the amount of ROS formed. Fluorescence was measured using excitation and emission wavelengths of 480 and 535 nm, respectively. A calibration curve was generated using standard DCF (0.1–1 μM).

In the liver, LPO levels were also measured according to the methodology described by Hermes-Lima et al. (1995).

2.9. Histopathology

Liver and intestine fragments (duodenum, jejunum, ileum and cecum) of three hens by treatment were preserved in a formaldehyde solution (10%). Tissue fragments were processed, placed in paraffin blocks, sectioned (sagittal sections - 3 mm thick), and stained with hematoxylin eosin (HE). From each fragment, three slides were produced for reading (triplicate), each evaluation being performed on the entire microscopic area of that slide.

2.10. Determination of minimum inhibitory concentration

The in vitro antibacterial activity of VB was obtained by measuring the minimum inhibitory concentration (MIC) using the broth microdilution method, according to the recommendations described by the Clinical and Laboratory Standards Institute (CLSI, 2014). We selected 3 to 5 colonies per sample of *E. coli*, isolated on MBE agar, inoculated in sterile tubes containing 5 mL of brain heart infusion (BHI) broth and incubated at 37 °C for 24 hours. After BHI became turbid, the bacteria were transferred to saline solution (0.9%) and standardized on the 0.5 MacFarland scale. Then, the inocula were used within 15 minutes. VB was previously diluted in ultra-pure water in the following proportions: 5%, 6%, 7%, 8%, and 9%. Sterilized microplates with 96 U-shaped holes were used, adding 100 µL of each VB dilution, always from the least concentrated to the most concentrated dilution, in each well of the microplate and 10 µL of the microbial inoculum. We placed 10 µL of inoculum plus 100 µL of MH broth and the negative control with only 100 µL of MH broth. The microplates were sealed and incubated in a bacteriological oven at 37 °C for 24 hours, after which 14 µL of 1% 2,3,5-triphenyl tetrazolium chloride solution were aseptically added. Red color indicates that there is bacterial growth. If the original color is maintained, this indicates no growth. Then, the microplates were incubated again for three hours and read.

2.12 Statistical analysis

The experimental design of this study was factorial (2 × 2; feed with and without aflatoxin; feed with and without VB). All dependent variables were tested for normality using Univariate procedure of SAS (SAS Inst. Inc., Cary, NC, USA; version 9.4) and all variables were normally distributed. Then, all data were analyzed using the MIXED procedure of SAS, with Satterthwaite approximation to determine the denominator degrees of freedom for the test of fixed effects. The data of liver variables and total bacterial count in eggshells were not evaluated as repeated

measures, and they were tested for fixed effects (included in the model: aflatoxin, VB, and the interaction aflatoxin × VB) and random effects (included in the model: animal and cage). All other data were analyzed as repeated measures (performance and blood variables). Data of performance, egg quality and blood variables were tested for fixed effects (included in the model: aflatoxin, VB, period or day, and all possible interactions), and random effects (included in the model: animal and cage). All results obtained on d 0 for each variable were included as covariates in each respective analysis, but were removed from the model when $P > 0.10$. 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

During the experiment, no mortality was observed for laying hens, which were apparently healthy (without clinical signs). The inclusion of aflatoxin in laying hen feed reduced ($P = 0.01$) egg production from d 21 to 42, but not from d 0 to 42, compared with no inclusion of aflatoxin (Table 2). No significant effects of VB, or interactions with aflatoxin and periods were detected ($P \geq 0.17$) for egg production (Table 2). No significant effects of aflatoxin, VB, periods, or interactions ($P \geq 0.17$) were detected for egg weight, egg mass, feed intake, or feed conversion (Table 2).

3.2. Egg quality

No significant effects of aflatoxin, VB, periods, or interactions ($P \geq 0.07$) were detected for shell resistance, pH albumin, pH yolk, shell thickness, shell percentage, yolk index, color range, L and A (Table 3). However, the inclusion of aflatoxin in the diet increased ($P \leq 0.05$) the calculate gravity and albumin percentage compared with no inclusion of aflatoxin, and no significant effects of VB or interaction with aflatoxin or period were detected ($P \geq 0.30$) for these variables. Further, the inclusion of aflatoxin in feed decreased ($P = 0.01$) the yolk percentage compared with no inclusion of aflatoxin, and no significant effects of VB or interactions with aflatoxin or period were detected ($P \geq 0.08$) for this variable. In terms of Haugh units, there were effects of aflatoxin × VB ($P = 0.01$), but no individual (aflatoxin or VB) effects or interactions with period ($P \geq 0.08$). The Afla0Bio0 and Afla2.5Bio800 hens had greater Haugh units than did the Afla0Bio800 and Afla2.5Bio0 hens (Figure 1A). Effects of aflatoxin × VB × period ($P = 0.02$) but no individual effects or others interactions ($P \geq 0.16$) were detected for color B. The Afla0Bio0 and

Afla2.5Bio800 hens had greater color B only from d 21 to 42, compared to Afla0Bio800 and Afla2.5Bio800 (Figure 1B).

There were effects of aflatoxin (P = 0.01) but no effects of and VB and interactions ($P \geq$ 0.11) for LPO. The inclusion of aflatoxin in the feed significantly increased LPO levels from d 21 to 42 (Table 3). In terms of ACAP, aflatoxin reduced the ACAP from d 21 to d 42 (P = 0.01); however, there were no effects of VB or interactions ($P \geq 0.11$) between these (Table 3). The inclusion of aflatoxin significantly reduced ACAP levels only from d 21 to 42, compared with no inclusion of aflatoxin (Table 3)

In terms of total bacterial count in eggshells, the inclusion of aflatoxin in the feed significantly increased counts (P = 0.01) and VB decreased them (P = 0.01), compared with no inclusion of aflatoxin, and there was no interaction between aflatoxin × VB (P = 0.99) for this variable (Figure 2).

3.3. Serum biochemistry and oxidants/antioxidants variables

The inclusion of aflatoxin and VB in the feed significantly decrease ($P \leq 0.05$) serum levels of ALT compared with the no inclusion groups, and there were no interactions between aflatoxin, VB and day ($P \geq 0.26$) for serum ALT. The inclusion of aflatoxin in the feed significantly increased (P = 0.01) levels of alkaline phosphatase compared with the no inclusion of aflatoxin group, and interactions between aflatoxin × VB × day were detected (P = 0.05). The Afla2.5Bio800 hens had lower serum concentrations of alkaline phosphatase on d 21 compared to the others, and Afla2.5Bio800 and Afla2.5Bio800 hens had greater concentrations on d 42 compared to Afla0Bio800 hens. The highest concentration was for Afla2.5Bio800 hens (Figure 3). No significant difference ($P \geq 0.11$) was observed between groups with respect to serum total protein levels. The effects of aflatoxin × day were detected (P = 0.04) for serum concentration of albumin and globulin, and the inclusion of aflatoxin in the diet increased the concentration of these variables only on d 21, compared with the no inclusion, and no effects of VB or interactions with aflatoxin and day were detected ($P \geq 0.11$) for serum concentration of albumin and globulin (Table 4).

The inclusion of aflatoxin in the diet significantly reduced (P = 0.05) serum GST activity on d 21, compared with the no inclusion group, and no significant effects of VB or interactions with aflatoxin were detected ($P \geq 0.11$) for GST activity. The inclusion of aflatoxin reduced (P = 0.01) and VB increased (P = 0.05) serum GPx activity on d 21; no interactions between these were detected ($P \geq 0.10$) for GPx (Table 4).

3.4. Liver oxidants/antioxidants variables

The inclusion of aflatoxin in the feed significantly reduced (P = 0.01) the concentration of LPO and GPx and GST activities, and significantly increased (P = 0.01) the concentration of ROS compared with the no inclusion of aflatoxin group. Further, the inclusion of VB in the diet increased (P = 0.01) GPx activity and significantly decreased (P = 0.01) the LPO levels compared with the no inclusion of VB. No significant effects of VB were detected ($P \geq 0.28$) for liver GST activity or ROS levels. Interactions between aflatoxin × VB were detected ($P \leq 0.05$) for GPx and LPO. The Afla0Bio800 hens had greater liver concentrations of GPx than did the others. The Afla2.5Bio0 hens had greater concentrations of LPO than did Afla0Bio800 hens, and Afla2.5Bio800 hens had greater concentrations than did Afla0Bio0 and Afla0Bio800 hens (Figure 4).

3.5. Histopathology

No intestinal damage was observed in hens from all treatments. In the liver, histological changes were observed in all treatments (Figure 5). A predominantly light multifocal mononuclear inflammatory infiltrate was found in all Afla0Bio0 and Afla0Bio800 birds. All Afla2.5Bio0 birds presented moderate-to-severe diffuse macrovacuolar degeneration, and heterophilic (mild multifocal) and mononuclear (moderate multifocal) inflammatory infiltrates. Afla2.5Bio800 birds had mild-to-moderate multifocal macrovacuolar degeneration, and heterophilic (moderate multifocal) and mononuclear (moderate multifocal) inflammatory infiltrates.

3.6. MIC

We found that from the minimum concentration of 5% of VB, there was no bacterial growth for the evaluated isolate (Supplementary material 1).

4. Discussion

The inclusion of aflatoxin in feed significantly decreased egg production, and this reduction deepened over time. These data suggest that the consumption of aflatoxin has a cumulative effect. One hypothesis for this effect is the presence of follicles already in the reproductive tract of birds prior to the consumption of mycotoxin explains this delayed response to decreased production (Vieira, 1995). Another hypothesis is that the amount of AFB1 ingested from d0 to d21 is insufficient to diminish egg production. The disturbances caused by aflatoxin on egg production coincide with the reduction of proteins and lipids in blood levels (Santurio, 2000).

Regarding egg quality parameters, a significant increase in specific gravity and albumen percentage was observed when aflatoxin was included in the feed; however, the percentage of yolk decreased. Santurio (2000) found that, in addition to reducing egg production, aflatoxicosis was

responsible for reducing the size of eggs, and there was a proportional reduction in the size of the yolks due to the damage to protein and lipid synthesis. Rosa and Avila (2000) remarked that specific gravity is a physical measure that approximates the density of the egg, which is basically related to the thickness of the shell, being responsible for variations in the incubation results. The groups Afla0Bio0 and Afla2.5Bio800 groups had a greater Haugh units, suggesting that the eggs of these groups had higher quality, because the HU is a mathematical calculation that considers the weight of the egg with the height of the albumen. In general, larger Haugh units correspond to better egg quality (Alleoni and Antunes, 2001).

Regarding to LPO in the eggs, the AFB1 increased the LPO, and the VB mitigated these harmful effects. Values of ACAP were lower in the group poisoned with aflatoxin, and there were higher ACAP levels in the combined group (Afla2.5Bio800). These data confirm the cytotoxic effects of aflatoxin and a consequent decrease in the antioxidant system caused by the consumption of mycotoxin, as mentioned by Doi and Uetsuka (2014). In addition to being an indication that VB can minimize lipid peroxidation and promote greater activity of the antioxidant system, thereby improving the quality of eggs. Recently, a study conducted by Souza et al. (2020b) revealed that jundiás (*Rhamdia quelen*) given feed containing AFB1 showed a significant increase in the plasma and hepatic levels of ROS and LPO, as we observed in the present study. Souza et al. (2020a) reported that VB reduced or prevented increases in ROS and LPO levels in fish given feed contaminated with aflatoxin, revealing hepaprotective effects. Regarding the time, is possible that aflatoxicosis becomes more severe over the time, suggesting that the time of exposure is an important factor associated with toxicity of AFB1 contaminated diet, and this can be observed by inhibition of GPx activity as observed by Yang et al. (2020) for farm animals.

The total bacterial count (TBC) of the eggs showed very positive results regarding the inclusion of VB in the diet of the birds. When compared to the group that received feed experimentally contaminated with aflatoxin, there was much lower TBC. We associate this positive result with a possible antimicrobial action of the VB compound; however, no analysis of gut microflora was done in the present study to test this hypothesis, and further studies exploring these evaluations may be warranted.

In our study, we observed histological changes in the liver in all treatments, attributable to the advanced age of the birds. The groups that were not subjected to aflatoxin contamination showed few lesions in this organ, whereas the group contaminated with aflatoxin presented severe lesions and severe macrovacuolar degeneration. However, the association of biocoline with aflatoxin, Afla2.5Bio800, showed hepatoprotective effects, minimizing mycotoxin toxicity. The hepatic protection effects of VB are also observed in terms of antioxidant levels in the liver,

increasing the action of the enzyme defense system that also reflects lower lipid lipoperoxidation levels in relation to birds intoxicated by aflatoxin. Our findings regarding liver degeneration and disorders in the antioxidant/oxidant system caused by aflatoxin corroborate the findings of other authors (Wyatt (1991); Amici et al. (2007); Souza et al. (2018). In a recent study with Nile tilapia, Souza et al. (2020a) found that the addition of 800 mg/kg of VB in fish feed minimized the negative effects caused by AFB1, improving antioxidant status.

According to Gonzalez and Silva (2006), most serum alkaline phosphatase is hepatic in origin, where it is present in the cells of the biliary epithelium and in the membranes of hepatocytes; serum levels of the enzyme indicate liver dysfunction. We observed a significant increase in serum level of alkaline phosphatase in the group intoxicated with AFB1, in addition to a decrease in the activity of the antioxidant enzymes GST and GPx. This is explained, according to Santurio (2000), by the fact that AFB1 is immediately bound to albumin and other proteins after being absorbed, and that these proteins spread through tissues, especially the liver, where they transform into toxic metabolites, causing profound changes in the functional properties of the organ, in addition to mycotoxins contributing to the compromise of antioxidant defenses (Marin and Taranu, 2012). Alkaline phosphatase activity was higher in poultry fed with AFB1 on day 42 compared to 21, suggesting an effect of time on AFB1 toxicity, meaning that toxicity is time-dependent. Moreover, it is important to emphasize that several parameters increase or decrease for both intoxicated and non-poisoned birds, suggesting that these variations are physiological and not linked to mycotoxin. The inclusion of aflatoxin and VB in the diet decreased ($P \leq 0.05$) serum ALT concentrations, in comparison with the non-inclusion group. This can be explained by the fact that serum levels peak in proportion to the degree of injury, with the peak occurring three to four days after the injury; however, with return to baseline in up to 14 days (Gonzalez and Silva, 2006). In addition to the egg and liver antioxidant activity, VB stimulated serum antioxidant responses, decreasing phosphatase activity on days 21 and 42 in the Afla2.5Bio800 group, and increasing the GPx enzyme activity in the Afla0Bio800 group.

5. Conclusion

VB supplementation in the diet of laying hens challenged with AFB1 minimized the negative effects of mycotoxin consumption, improving egg quality and bird health. However, VB at the tested dose was not sufficient to minimize the negative effects on egg-laying caused by AFB1.

Ethics committee

This work was approved by the Ethics Committee on Animal Use Research of the State University of Catarina (UDESC), protocol number 9438130319.

Conflict of interest

The authors declare no conflicts of interest.

Acknowledgements

We are grateful to the Program for the Improvement of Higher Education Personnel - Brazil (CAPES) – Finance Code 001.

References

Alleoni, A.C.C, Antunes, A.J, 2001. Unidade Haugh como medida da qualidade de ovos de galinha armazenados sob refrigeração. Scientia Agricola, v.58, n.4, p.681-685, out./dez.

Amado, L.L, Garcia, M.L, Ramos, P.B, Freitas, R.F, Zafalon, B, Ferreira, J.L.R, Yunes, J.S, Monserrat, J.M, 2009. A method to measure total antioxidant capacity against peroxyl radicals in aquatic organisms: Application to evaluate microcystins toxicity. Sci. Total Environ. 407, 2115–2123. https://doi.org/10.1016/j.scitotenv.2008.11.038

Amici, M, Cecarini, V, Pettinari, A, Bonfili, L, Angeletti, M, Barocci, S, Biagetti, M, Fioretti, E, Eleuteri, A.M, 2007. Binding of aflatoxins to the 20S proteasome: effects on enzyme functionality and implications for oxidative stress and apoptosis. J. Biol. Chem. 388, 107–117. https://doi.org/10.1515/BC.2007.012

Bunzen, S, Haese, D, 2006 Controle de micotoxinas na alimentação de aves e suínos. Revista Eletrônica Nutritime, v.3, n° 1, p.299-304, janeiro/fevereiro.

Clinical and Laboratory Standards Institute, 2014. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fourth Informational Supplement. CLSI document M100-S24. Wayne: Clinical and Laboratory Standards Institute.

Doi, K, Uetsuka, K, 2014. Mechanisms of mycotoxin-induced dermal toxicity and tumorigenesis through oxidative stress-related pathways. Journal of Toxicologic Pathology. V.27, p.1-10. https://doi.org/10.1293/tox.2013-0062

U.S. Food and Drug Administration Guidelines for Aflatoxin Level. Acess in https://agriculture.mo.gov/plants/feed/aflatoxin.php.

Freitas, E. R, Sakomura, N.K, Gonzalez, M.M, Barbosa, N.A.A, 2004. Comparação de métodos de determinação da gravidade específica de ovos de poedeiras comerciais. Pesq. agropec. bras., Brasília, v.39, n.5, p.509-512. https://doi.org/10.1590/S0100-204X2004000500014

Galli, G. M, Da Silva, A. S, Biazus, A. H, Reis, J. H, Boiago, M. M, Topazio, J. P, Migliorini, J.M, Guarda, S.N, Moresco, R.N, Ourique, A.F, Santos, C.G, Lopes, L.S, Baldissera, M.D, Stefani, M.L, 2018. Feed addition of curcumin to laying hens showed anticoccidial effect, and improved egg quality and animal health. Research in veterinary science, 118, 101-106. https://doi.org/10.1016/j.rvsc.2018.01.022

Habig, W. H, Pabst, M. J, Jakoby, W. B, 1974. Glutathione S-transferases: the first enzymatic step in mercapturic acid formation. J. Biol. Chem. 249, 7130-7139.

Gonzalez F.H.D, Silva S.C, 2006. Introdução à bioquímica clínica veterinária. 2ª ed. Porto Alegre: Editora da Universidade Federal do Rio Grande do Sul. c. 8, p. 318-337.

Hermes-lima, M, Willmore, W. G, Storey, K. B, 1995. Quantification of lipid peroxidation in tissue extracts based on Fe (III) xylenol orange complex formation. Free Radical Biology and Medicine, *19*(3), 271-280. https://doi.org/10.1016/0891-5849(95)00020-X

Haugh, R. R, 1937. A new method for determining the quality of an egg. US Egg Poultry, 49p.

Lebel, C.P, Ischiropoulos, H, Bondy, S.C, 1992. Evaluation of the probe 2', 7' – dichlorofluorescin as na indicator of reactive oxygen species formation and oxidative stress. Chem. Res. Toxicol. 5, 227-231. doi: 10.1021/tx00026a012

Marin, D.E, Taranu, I, 2012. Overview on aflatoxins and oxidative stress. Toxin Reviews, p.1-12. DOI: 10.3109/15569542.2012.730092. Acesso em: 17 março. 2020

Migliorini, M. J, Da Silva, A. S, Santurio, J. M, Bottari, N. B, Gebert, R. R, Reis, J. H, Volpato, A, Morsch, V.M, Baldissera, M.D, Stefani, L.M, Boiago, M.M, 2017. The Protective effects of an adsorbent against oxidative stress in quails fed aflatoxin-contaminated diet. Acta Scientiae Veterinariae, 45, 1-7.

Muller, L.K.F, Paiano, D, Gugel, J, Lorenzetti, W.R, Santurio, J.M, Tavernari, F.D.C, da Gloria E.M, Baldissera, M.D, Da Silva, A.S, 2018. Post-weaning piglets fed with different levels of fungal mycotoxins and spray-dried porcine plasma have improved weight gain, feed intake and reduced diarrhea incidence. Microbial pathogenesis, v. 117, p. 259-264. doi: 10.1016/j.micpath.2018.02.035.

Oliveira, C.A.F, Germano, P.M.L, 1997. Aflatoxinas: conceito sobre mecanismos de toxidade e seu envolvimento na etiologia do câncer hepático celular. Revista de Saúde Pública, 31 (4): 417-24. https://doi.org/10.1590/S0034-89101997000400011

Read, S. M, NORTHCOTE, D. H, 1981.Minimization of variation in the response to different proteins of the Coomassie blue G dye-binding assay for protein. Analytical biochemistry, v. 116, n. 1, p. 53-64, 1981. https://doi.org/10.1016/0003-2697(81)90321-3

Rosa, P. S, Avila, V. S, 2000. Variáveis relacionadas ao rendimento de incubação de ovos em matrizes de frango de corte. Comunicado Técnico/ 246/ Embrapa Suínos e aves, p. 1-3. Disponível em: https://www.embrapa.br/busca-de-publicacoes/-/publicacao/439552/variaveis-relacionadas-ao-rendimento-da-incubacao-de-ovos-em-matrizes-de-frangos-de-corte. Acessado em: 09 de março de 2020.

Rostagno, H.S et al. Tabelas brasileiras para aves e suínos: composição de alimentos e exigências nutricionais. 4.ed. Viçosa, MG: UFV, DZO, 2017.

Santurio, J.M, 2000. Micotoxinas e micotoxicoses na avicultura. Rev. Bras. Cienc. Avic. vol.2 no.1 Campinas Jan./Abr. https://doi.org/10.1590/S1516-635X2000000100001

Souza, C.F, Baldissera, M.D, Descovi, S.N, Zeppenfeld, C.C, Garzon, L.R, Da Silva, A.S, Stefani, L.M, Baldisserotto, B, 2018. Serum and hepatic oxidative damage induced by a diet contaminated

with fungal mycotoxin on freshwater silver catfish Rhamdia quelen: involvement on disease pathogenesis. Microb. Pathog. 124, 82–86. doi: 10.1016/j.micpath.2018.08.041

Souza, C.F, Baldissera, M. D, Baldisserotto, B, Petrolli, T. G, Da Glória, E. M, Zanette, R. A, Da Silva, A. S, 2020. Dietary vegetable choline improves hepatic health of Nile tilapia (*Oreochromis niloticus*) fed aflatoxin-contaminated diet. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, *227*, 108614. https://doi.org/10.1016/j.cbpc.2019.108614

Sutili F.J, Gatlin, D.M, Heinzmann, B.M, Baldisserotto, B, 2018. Plant essential oils as fish diet additives: benefits on fish health and stability in feed. Reviews in Aquaculture. Volume10, Issue3, Pages 716-726. https://doi.org/10.1111/raq.12197

Thorpe, C.W, Ware, G.M, Pohland, A.E, 1982. Determination of aflatoxins by HPLC with a fluorescence detector and using post column derivatization. In: Proceedings of the Fifth International IUPAC Symposium on Mycotoxins and Phycotoxins. Technical University, Vienna. Eds. W. Pfannhauser, and P. B. Czedic Eysenberg, 52-55.

Vieira S.L, 1995. Micotoxinas e produção de ovos. In: I Simpósio Internacional sobre Micotoxinas e Micotoxicoses em Aves; Curitiba, Paraná, Brasil. p.65-80.

Wendel, A, Fausel, M, Safayhi, H, Tiegs, G, Otter, R, 1984. A novel biologically active seleno-organic compound—II: Activity of PZ 51 in relation to Glutathione Peroxidase. Biochem. Pharmacol. 33, 3241–3245. https://doi.org/10.1016/0006-2952(84)90084-4

Wyatt, R.D. Poultry. In: Smith JE & Hendenson RS, ed. Mycotoxins and Animal Foods. CRC Press, Boca Raton, Fl. 1991. p. 553-605

West, S, Wyatt, R. D, Hamilton, P.B, 1973. Improved yield of aflatoxin by incremental increases of temperature. Applied and Environmental Microbiology 25, 1018-1019.

Table 1: Ingredients and chemical composition of the basic diet offered to laying hens

Ingredients	**Kg**
Corn	47.18
Soybean flour	17.74
Limestone	7.03
Soybean oil	1.53
Bicalcium phosphate	0.74
Premix*	0.22
Table salt	0.38
DL- Methionine	0.13
Calculated chemical composition	**%**
Calcium	4.20
Metabolizable energy (Kcal/kg)	2850
Available phosphate	0.31
Digestible lysine	0.77
Digestible Met+cyst	0.76
Digestible methionine	0.53
Crude protein	16.00
Sodium	0.19
Digestible Threonine	0.62
Digestible Tryptophan	0.18

* Product composition (kg): Vitamin A at 7,000,000 IU; Vitamin D3 at 4,000,000 IU; Vitamin E at 5000 mg; Vitamin K at 1200 mg; Vitamin B1 at 360 mg; Vitamin B2 at 2000 mg; Vitamin B6 at 700 mg; Vitamin B12 at 7000 mcg; niacin 7500 mg; biotin 30 mg; pantothenic acid 6000 mg; folic acid 300 mg; iron 1 1000 mg; copper 3000 mg; iodine 204 mg; chlorine 360 mg; coccidiostatic 100 g; antifungal 2000 mg; antioxidant 10 mg; magnesium 50 g; sulfur 40 g; energy and protein vehicle (q. s. p.) 1,000 g.

Table 2. Mean and standard error of performance of laying hens fed with diets containing aflatoxins and biocholine.

Variables	Aflatoxin[1]		*P*-values[2]			Biocholine[1]		*P*-values[2]		
	Afla0	Afla2.5	Afla	Per	Afla × Per	Bio0	Bio800	Bio	Per	Bio × Per
Egg production, %			0.20	0.01	0.01			0.54	0.01	0.59
d 0 to 21	59.37 (2.95)^{Ax}	64.58 (2.95)^{Ax}				63.69 (2.95)^{Ax}	60.27 (2.95)^{Ax}			
d 21 to 42	60.84 (2.95)^{Ax}	45.68 (2.95)^{By}				53.82 (2.95)^{Bx}	52.71 (2.95)^{Bx}			
Average	55.13 (2.56)^{x}	60.11 (2.56)^{x}				58.76 (2.56)^{x}	56.49 (2.56)^{x}			
Egg weight, g			0.46	0.01	0.42			0.30	0.01	0.24
d 0 to 21	68.90 (1.44)^{Ax}	66.75 (1.44)^{Ax}				69.36 (1.44)^{Ax}	66.29 (1.44)^{Ax}			
d 21 to 42	65.09 (1.44)^{Bx}	64.47 (1.44)^{Bx}				65.20 (1.44)^{Bx}	64.35(1.44)^{Ax}			
Average	66.99 (1.29)^{x}	65.61 (1.29)^{x}				67.28 (1.29)^{x}	65.32 (1.29)^{x}			
Egg mass, g			0.71	0.03	0.24			0.33	0.03	0.73
d 0 to 21	41.01 (2.91)^{Ax}	42.98 (2.91)^{Ax}				44.14 (2.91)^{Ax}	39.85 (2.91)^{Ax}			
d 21 to 42	37.85 (3.13)^{Ax}	33.39 (2.91)^{Bx}				36.84 (3.13)^{Bx}	34.42 (2.91)^{Ax}			
Average	39.44 (2.35)^{x}	38.19 (2.28)^{x}				40.49 (2.35)^{x}	37.14 (2.28)^{x}			
Feed intake (FI), g			0.91	0.01	0.81			0.58	0.01	0.86
d 0 to 21	138.71 (7.56)^{Ax}	139.44 (7.56)^{Ax}				140.71 (7.56)^{Ax}	137.43 (7.56)^{Ax}			
d 21 to 42	91.16 (7.56)^{Bx}	88.53 (7.56)^{Bx}				92.80 (7.56)^{Bx}	86.89 (7.56)^{Bx}			
Average	114.93 (5.70)^{x}	113.98 (5.70)^{x}				116.76^{x}	112.16 (5.70)^{x}			
Feed conversion ratio (kg/dozen)			0.44	0.05	0.17			0.78	0.05	0.45
d 0 to 21	2.80 (0.27)^{Ax}	2.69 (0.27)^{Ax}				2.61 (0.27)^{Ax}	2.88 (0.27)^{Ax}			
d 21 to 42	1.92 (0.27)^{Bx}	2.50 (0.27)^{Ax}				2.26 (0.27)^{Bx}	2.16 (0.27)^{Bx}			
Average	2.35 (0.21)^{x}	2.59 (0.21)^{x}				2.43 (0.21)^{x}	2.52 (0.21)^{x}			
Feed conversion ratio (kg/kg)			0.10	0.57	0.21			0.22	0.57	0.83
d 0 to 21	0.24 (0.05)^{Ax}	0.29 (0.05)^{Ax}				0.22 (0.05)^{Ax}	0.30 (0.05)^{Ax}			
d 21 to 42	0.20 (0.05)^{Ax}	0.37 (0.05)^{Ax}				0.25 (0.05)^{Ax}	0.32 (0.05)^{Ax}			
Average	0.22 (0.04)^{x}	0.32 (0.04)^{x}				0.24 (0.04)^{x}	0.31 (0.04)^{x}			

[1]In a factorial design (2 × 2) was include or not aflatoxin (Afla0 and Afla2.5 for 0 or 2.5 mg of aflatoxin/kg of concentrate, respectively) and also included or not biocholine (Bio0 and Bio800 for 0 or 800 mg of biocholine/kg of concentrate, respectively).
[2]Afla, aflatoxin; Per, period; Bio, biocholine.
[x-y]Differs ($P \leq 0.05$) between treatments (lines).
[A-B]Differs ($P \leq 0.05$) between periods (columns)

Table 3. Mean and standard error of egg quality of laying hens fed with diets containing aflatoxins and biocholine.

Variables	Aflatoxin[1]		*P*-values[2]			Biocholine[1]		*P*-values[2]		
	Afla0	Afla2.5	Afla	Per	Afla × Per	Bio0	Bio800	Bio	Per	Bio × Per
Shell resistance (×10^3)			0.31	0.97	0.46			0.62	0.97	0.32
d 0 to 21	3,669 (318)^{Ax}	4,266 (318)^{Ax}				3,728 (318)^{Ax}	4,207 (318)^{Ax}			
d 21 to 42	3,880 (318)^{Ax}	4,036 (318)^{Ax}				4,018 (342)^{Ax}	3,897 (318)^{Ax}			
Average	3,774 (248)^{x}	4,151 (256)^{x}				3,873 (256)^{x}	4,052 (248)^{Ax}			
pH albumin			0.79	0.01	0.23			0.67	0.01	0.10
d 0 to 21	8.22 (0.08)^{Ax}	8.10 (0.08)^{Bx}				8.21 (0.08)^{Ax}	8.11 (0.08)^{Bx}			
d 21 to 42	8.34 (0.08)^{Ax}	8.41 (0.08)^{Ax}				8.29 (0.08)^{Ax}	8.46 (0.08)^{Ax}			
Average	8.28 (0.06)^{x}	8.26 (0.06)^{x}				8.25 (0.06)^{x}	8.28 (0.06)^{x}			
pH yolk			0.79	0.90	0.39			0.32	0.90	0.12
d 0 to 21	5.99 (0.05)^{Ax}	5.96 (0.05)^{Ax}				5.99 (0.05)^{Ax}	5.96 (0.05)^{Ax}			
d 21 to 42	5.95 (0.05)^{Ax}	6.01 (0.05)^{Ax}				5.91 (0.05)^{Ax}	6.05 (0.05)^{Ax}			
Average	5.97 (0.04)^{x}	5.99 (0.04)^{x}				5.95 (0.04)^{x}	6.00 (0.04)^{x}			
Calculate gravity			0.01	0.08	0.90			0.54	0.08	0.90
d 0 to 21	1.08 (0.01)^{Ax}	1.09 (0.01)^{Ax}				1.08 (0.01)^{Ax}	1.08 (0.01)^{Ax}			
d 21 to 42	1.07 (0.01)^{Ax}	1.08 (0.01)^{Ax}				1.08 (0.01)^{Ax}	1.07 (0.01)^{Ax}			
Average	1.07 (0.01)^{x}	1.09 (0.01)^{y}				1.08 (0.01)^{x}	1.08 (0.01)^{x}			
Shell thickness (mm)			0.89	0.61	0.11			0.40	0.61	0.35
d 0 to 21	0.35 (0.01)^{Ax}	0.37 (0.01)^{Ax}				0.36 (0.01)^{Ax}	0.36 (0.01)^{Ax}			
d 21 to 42	0.36 (0.01)^{Ax}	0.35 (0.01)^{Ax}				0.36 (0.01)^{Ax}	0.34 (0.01)^{Ax}			
Average	0.36 (0.01)^{x}	0.36 (0.01)^{x}				0.36 (0.01)^{x}	0.35 (0.01)^{x}			
Shell (%)			0.29	0.64	0.26			0.80	0.64	0.11
d 0 to 21	8.81 (0.26)^{Ax}	9.40 (0.26)^{Ax}				8.94 (0.26)^{Ax}	9.27 (0.26)^{Ax}			
d 21 to 42	8.97 (0.26)^{Ax}	9.01 (0.26)^{Ax}				9.23 (0.26)^{Ax}	8.75 (0.26)^{Ax}			
Average	8.89 (0.20)^{x}	9.20 (0.20)^{x}				9.08 (0.20)^{x}	9.01 (0.20)^{x}			
Yolk (%)			0.01	0.31	0.48			0.39	0.31	0.08

d 0 to 21	28.14 (0.60)^{Ax}	25.66 (0.60)^{Ax}				27.06 (0.60)^{Ax}	26.74 (0.60)^{Ax}			
d 21 to 42	27.25 (0.60)^{Ax}	25.49 (0.60)^{Ax}				25.60 (0.60)^{Ax}	27.15 (0.60)^{Ax}			
Average	27.70 (0.48)^{x}	25.58 (0.48)^{y}				26.33 (0.48)^{x}	26.94 (0.48)^{x}			
Albumin (%)			0.05	0.21	0.86			0.55	0.21	0.30
d 0 to 21	63.05 (0.71)^{Ax}	64.95 (0.71)^{Ax}				64.00 (0.71)^{Ax}	63.99 (0.71)^{Ax}			
d 21 to 42	63.78 (0.71)^{Ax}	65.50 (0.71)^{Ax}				65.17 (0.71)^{Ax}	64.10 (0.71)^{Ax}			
Average	63.41 (0.60)^{y}	65.22 (0.60)^{x}				64.59 (0.60)^{x}	64.04 (0.60)^{x}			
Haugh unit			0.49	0.06	0.55			0.70	0.06	0.82
d 0 to 21	84.38 (3.57)^{Ax}	83.58 (3.57)^{Ax}				83.59 (3.57)^{Ax}	84.37 (3.57)^{Ax}			
d 21 to 42	79.65 (3.57)^{Ax}	74.87 (3.57)^{Ax}				76.12 (3.57)^{Ax}	78.40(3.57)^{Ax}			
Average	82.02 (2.75)^{x}	79.22 (2.75)^{x}				79.85 (2.75)^{x}	81.38 (2.75)^{x}			
Yolk index			0.32	0.19	0.35			0.16	0.19	0.29
d 0 to 21	0.45 (0.01)^{Ax}	0.45 (0.01)^{Ax}				0.45 (0.01)^{Ax}	0.45 (0.01)^{Ax}			
d 21 to 42	0.45 (0.01)^{Ax}	0.43(0.01)^{Ax}				0.43 (0.01)^{Ax}	0.45 (0.01)^{Ax}			
Average	0.45 (0.01)^{x}	0.44 (0.01)^{x}				0.44 (0.01)^{x}	0.45 (0.01)^{x}			
Color sub										
Range			0.59	0.01	0.18			0.12	0.01	0.18
d 0 to 21	7.06 (0.30)^{Ax}	7.25(0.30)^{Ax}				6.69 (0.30)^{Ax}	7.62 (0.30)^{Ax}			
d 21 to 42	5.19 (0.30)^{Bx}	4.62 (0.30)^{Bx}				4.81 (0.30)^{Bx}	5.00 (0.30)^{Bx}			
Average	6.12 (0.23)^{x}	5.94 (0.23)^{x}				5.75 (0.23)^{x}	6.31 (0.23)^{x}			
L			0.86	0.02	0.11			0.71	0.02	0.11
d 0 to 21	60.94 (0.66)^{Ax}	60.14 (0.66)^{Bx}				61.33 (0.66)^{Ax}	59.75 (0.66)^{Ax}			
d 21 to 42	61.26 (0.66)^{Ax}	62.35 (0.66)^{Ax}				61.33 (0.66)^{Ax}	62.28 (0.66)^{Bx}			
Average	61.10 (0.57)^{x}	61.25 (0.57)^{x}				61.32 (0.57)^{x}	61.02 (0.57)^{x}			
A			0.95	0.95	0.07			0.87	0.95	0.30
d 0 to 21	-7.27 (0.33)^{Ax}	-6.57 (0.33)^{Ax}				-7.02 (0.33)^{Ax}	-6.82 (0.33)^{Ax}			
d 21 to 42	-6.61 (0.33)^{Ax}	-7.26 (0.33)^{Ax}				-6.77 (0.33)^{Ax}	-7.10 (0.33)^{Ax}			
Average	-6.94 (0.27)^{x}	-6.92 (0.27)^{x}				-6.90 (0.27)^{x}	-6.96 (0.27)^{x}			
B			0.53	0.55	0.99			0.98	0.55	0.53
d 0 to 21	46.53 (0.87)^{Ax}	47.10 (0.87)^{Ax}				47.08 (0.87)^{Ax}	46.54 (0.87)^{Ax}			
d 21 to 42	47.07 (0.87)^{Ax}	47.62 (0.87)^{Ax}				47.05 (0.87)^{Ax}	47.64 (0.87)^{Ax}			

Average	46.80 (0.62)^{x}	47.36 (0.62)^{x}				47.07 (0.62)^{x}	47.09 (0.62)^{x}			
LPO[1] (nmol/ml)			0.01	0.01	0.01			0.32	0.01	0.22
d 0 to 21	2,892 (814.3)^{Ax}	2,609 (814.3)^{Bx}				8,566 (814.3)^{Ax}	6,021 (814.3)^{Ax}			
d 21 to 42	5,104 (814.3)^{Ay}	11,696 (814.3)^{Ax}				4,046 (814.3)^{Bx}	3,667 (814.3)^{Ax}			
Average	3,998 (544.7)^{y}	7,152 (544.7)^{x}				4,306 (544.7)^{x}	4,844 (544.7)^{x}			
ACAP[1] (UF/mg protein)			0.50	0.01	0.01			0.18	0.01	0.94
d 0 to 21	2.42 (0.18)^{Ax}	2.44 (0.18)^{Ax}				2.81 (0.18)^{Ax}	3.05 (0.18)^{Ax}			
d 21 to 42	2.37 (0.18)^{Ax}	1.56 (0.18)^{By}				1.86 (0.18)^{Bx}	2.07 (0.18)^{Bx}			
Average	2.39 (0.11)^{x}	2.50 (0.11)^{x}				2.34 (0.11)^{x}	2.56 (0.11)^{x}			

[1] LPO: lipoperoxidation; ACAP: total antioxidant capacity

[2]In a factorial design (2 × 2) was include or not aflatoxin (Afla0 and Afla2.5 for 0 or 2.5 mg of aflatoxin/kg of concentrate, respectively) and also included or not biocholine (Bio0 and Bio800 for 0 or 800 mg of biocholine/kg of concentrate, respectively).

[2]Afla, aflatoxin; Per, period; Bio, biocholine.

$^{x-y}$Differs ($P \leq 0.05$) between treatments (lines).

$^{A-B}$Differs ($P \leq 0.05$) between periods (columns).

Table 4. Mean and standard error of serum biochemistry and antioxidants enzymes of laying hens fed with diets containing aflatoxins and biocholine.

Variables	**Aflatoxin[1]**		***P*-values[2]**			**Biocholine[1]**		***P*-values[2]**		
	Afla0	**Afla2.5**	**Afla**	**Day**	**Afla × Day**	**Bio0**	**Bio800**	**Bio**	**Day**	**Bio × Day**
ALT[1] (U/L)			0.05	0.02	0.27			0.01	0.02	0.26
d 0	15.08 (2.57)^{Ax}	12.14 (2.86)^{Bx}				16.17 (2.57)^{Bx}	11.06 (2.86)^{Ax}			
d 21	22.21 (2.86)^{Bx}	22.45 (2.98)^{Ax}				28.78 (2.98)^{Ax}	15.88 (2.86)^{Ax}			
d 42	23.40 (3.13)^{Bx}	13.58 (2.97)^{Bx}				20.18 (3.13)^{Bx}	16.81 (2.97)^{Ax}			
Average	20.23 (1.50)^{x}	16.05 (1.55)^{y}				21.72 (1.52)^{x}	14.58 (1.53)^{y}			
Phosphatase (U/L)			0.01	0.04	0.01			0.29	0.02	0.16
d 0	394.6 (44.4)^{Ax}	336.4 (45.7)^{Bx}				332.1 (43.9)^{Ax}	398.9 (46.8)^{Bx}			

d 21	229.0 (49.9)^{Ay}	355.8 (47.4)^{Bx}				326.7 (51.2)^{Ax}	258.1 (48.2)^{Cx}				
d 42	275.5 (52.2)^{Ay}	589.9 (55.1)^{Ax}				364.9 (50.7)^{Ax}	500.5 (56.3)^{Ax}				
Average	299.7 (26.2)^{y}	427.4 (27.0)^{x}				341.2 (27.2)^{x}	385.8 (27.4)^{x}				
Total protein (mg/dL)			0.62	0.07	0.11			0.14	0.07	0.34	
d 0	5.36 (0.38)^{Ax}	4.83 (0.40)^{Ax}				4.89 (0.38)^{Ax}	5.30 (0.40)^{Ax}				
d 21	5.62 (0.38)^{Ax}	6.56 (0.40)^{Ax}				5.56 (0.38)^{Ax}	6.62 (0.40)^{Ax}				
d 42	5.67 (0.38)^{Ax}	4.88 (0.38)^{Ax}				5.39 (0.38)^{Ax}	5.17 (0.40)^{Ax}				
Average	5.55 (0.17)^{x}	5.42 (0.18)^{x}				5.28 (0.17)^{x}	5.69 (0.17)^{x}				
Albumin (mg/dL)			0.47	0.01	0.04			0.11	0.01	0.18	
d 0	1.57 (0.15)^{Bx}	1.36 (0.14)^{Bx}				1.42 (0.15)^{Bx}	1.51 (0.14)^{Bx}				
d 21	2.44 (0.14)^{Ay}	3.01 (0.17)^{Ax}				2.47 (0.16)^{Ax}	2.99 (0.15)^{Ax}				
d 42	1.40 (0.14)^{Bx}	1.30 (0.14)^{Bx}				1.37 (0.15)^{Bx}	1.34 (0.14)^{Bx}				
Average	1.81 (0.08)^{x}	1.89 (0.08)^{x}				1.75 (0.08)^{x}	1.95 (0.08)^{x}				
Globulin (mg/dL)			0.96	0.61	0.05			0.62	0.61	0.10	
d 0	3.86 (0.31)^{Ax}	3.63 (0.33)^{Ax}				3.79 (0.34)^{Ax}	3.70 (0.31)^{Ax}				
d 21	3.14 (0.33)^{Ay}	4.11 (0.36)^{Ax}				3.14 (0.34)^{Ax}	4.12 (0.36)^{Ax}				
d 42	4.30 (0.31)^{Ax}	3.59 (0.30)^{Ax}				4.18 (0.30)^{Ax}	3.71 (0.31)^{Ax}				
Average	3.77 (0.19)^{x}	3.78 (0.19)^{x}				3.70 (0.19)^{x}	3.84 (0.19)^{x}				
GST[1] (U GST/mg protein)			0.05	0.01	0.01			0.32	0.01	0.43	
d 0	6.81 (0.72)^{Ax}	8.60 (0.81)^{Ax}				7.45 (0.85)^{Ax}	7.96 (0.67)^{Bx}				
d 21	5.79 (0.74)^{ABx}	3.20 (1.07)^{By}				5.45 (1.06)^{ABx}	3.56 (0.75)^{Ax}				
d 42	4.66 (0.71)^{Bx}	2.86 (0.99)^{Bx}				3.62 (0.96)^{Bx}	3.90 (0.75)^{Ax}				
Average	5.75 (0.19)^{x}	4.89 (0.36)^{y}				5.50 (0.27)^{x}	5.14 (0.24)^{x}				
GPx[1] (U GPx/mg protein)			0.01	0.08	0.02			0.05	0.08	0.05	
d 0	1.68 (0.66)^{Ax}	1.88 (0.74)^{Ax}				1.88 (0.77)^{Ax}	1.68 (0.61)^{Ax}				
d 21	4.97 (0.77)^{Ax}	0.07 (1.17)^{Ay}				0.65 (1.12)^{Ay}	4.40 (0.82)^{Ax}				
d 42	0.76 (0.71)^{Ax}	0.25 (0.77)^{Ax}				0.51 (0.82)^{Ax}	0.50 (0.65)^{Ax}				
Average	2.47 (0.35)^{x}	0.73 (0.47)^{y}				1.01 (0.47)^{y}	2.19 (0.35)^{y}				

[1]ALT: alanine aminotransferase; GST: glutathione s-transferase; GPx: glutathione peroxidase.

[2]In a factorial design (2 × 2) was include or not aflatoxin (Afla0 and Afla2.5 for 0 or 2.5 mg of aflatoxin/kg of concentrate, respectively) and also included or not biocholine (Bio0 and Bio800 for 0 or 800 mg of biocholine/kg of concentrate, respectively).

[2]Afla, aflatoxin; Per, period; Bio, biocholine.
[x-y]Differs ($P \leq 0.05$) between treatments (lines).
[A-B]Differs ($P \leq 0.05$) between days (columns).

Table 5. Mean and standard error of oxidants/antioxidants in liver of laying hens fed with diets containing aflatoxins and biocholine.

Variables	**Aflatoxin[1]**		***P*-value[2]**	**Biocholine[1]**		***P*-value[2]**
	Afla0	**Afla2.5**	**Afla**	**Bio0**	**Bio800**	**Bio**
GST[1] (U GST/mg of protein)	18.86 (0.71)[x]	13.08 (0.76)[y]	0.01	16.40 (0.76)[x]	15.55 (0.74)[x]	0.45
GPx[1] (U GPx/mg of protein)	1.97 (0.07)[x]	1.08 (0.07)[y]	0.01	1.10 (0.07)[y]	1.95 (0.07)[x]	0.01
ROS[1] (U DCF/mg of protein)	0.55 (0.03)[y]	0.75 (0.04)[x]	0.01	0.68 (0.04)[x]	0.62 (0.04)[x]	0.28
LPO[1] (nmol/g)	585.7 (90.1)[y]	1,421 (93.4)[x]	0.01	1,242 (89.1)[x]	764.4 (93.4)[y]	0.01

[1]GST: glutathione s-transferase; GPx: glutathione peroxidase; ROS: reactive oxygen species; LPO: lipoperoxidation.
[2]In a factorial design (2 × 2) was include or not aflatoxin (Afla0 and Afla2.5 for 0 or 2.5 mg of aflatoxin/kg of concentrate, respectively) and also included or not biocholine (Bio0 and Bio800 for 0 or 800 mg of biocholine/kg of concentrate, respectively).
[2]Afla, aflatoxin; Bio, biocholine.
[x-y]Differs ($P \leq 0.05$) between treatments (lines).

Figure legend

Figure 1. Haugh unit and color B in eggs of laying hens fed with diets containing aflatoxins (Afla) and biocholine (Bio). In a factorial design (2 × 2) was include or not aflatoxin (Afla0 and Afla2.5 for 0 or 2.5 mg of aflatoxin/kg of concentrate, respectively) and also included or not biocholine (Bio0 and Bio800 for 0 or 800 mg of biocholine/kg of concentrate, respectively).

Note: $^{a-b}$Differs ($P \leq 0.05$) between treatments. Vertical bars represent the SEM.

Figure 2. Total bacterial count (TBC) in shell eggs of laying hens fed with diets containing aflatoxins (Afla) and biocholine (Bio). In a factorial design (2 × 2) was include or not aflatoxin (Afla0 and Afla2.5 for 0 or 2.5 mg of aflatoxin/kg of concentrate, respectively) and also included or not biocholine (Bio0 and Bio800 for 0 or 800 mg of biocholine/kg of concentrate, respectively).

Note: $^{a-b}$Differs ($P \leq 0.05$) between treatments. Vertical bars represent the SEM.

Figure 3. Serum phosphatase of laying hens fed with diets containing aflatoxins (Afla) and biocholine (Bio). In a factorial design (2 × 2) was include or not aflatoxin (Afla0 and Afla2.5 for 0 or 2.5 mg of aflatoxin/kg of concentrate, respectively) and also included or not biocholine (Bio0 and Bio800 for 0 or 800 mg of biocholine/kg of concentrate, respectively).

Note: $^{a-b}$Differs ($P \leq 0.05$) between treatments. Vertical bars represent the SEM.

Figure 4. Concentration of glutathione peroxidase (GPx) and lipoperoxidation (LPO) in liver of laying hens fed with diets containing aflatoxins (Afla) and biocholine (Bio). In a factorial design (2 × 2) was include or not aflatoxin (Afla0 and Afla2.5 for 0 or 2.5 mg of aflatoxin/kg of concentrate, respectively) and also included or not biocholine (Bio0 and Bio800 for 0 or 800 mg of biocholine/kg of concentrate, respectively).

Note: $^{a-b}$Differs ($P \leq 0.05$) between treatments. Vertical bars represent the SEM.

Figure 5. Histology (liver and intestine) of laying hens fed with diets containing aflatoxins (Afla) and vegetable biocholine (Bio). Microscopically, in the liver, there was mild to moderate mononuclear inflammatory infiltrates in Afla0Bio0 (A); in liver of hens of Afla0Bio800, there were mild mononuclear inflammatory infiltrates (B); and mild heterophilic inflammatory infiltrate (C); microscopically, in the Afla2Bio0 group, there were moderate mononuclear inflammatory infiltrates in the liver (D), moderate heterophilic inflammatory infiltrates (E) and moderate to severe macrovacuolar degeneration (F); in the Afla2Bio800 group, there were moderate mononuclear inflammatory infiltrates in the liver; (G) and mild to moderate macrovacuolar degeneration (H); intestinal lesions were not observed in the laying hens in this study (I); Note: A factorial design (2 × 2) included or did not include aflatoxin (Afla0 and Afla2.5 for 0 or 2.5 mg of aflatoxin/kg of concentrate, respectively) and also included or did not include biocholine (Bio0 and Bio800 for 0 or 800 mg of biocholine/kg of concentrate, respectively).

Supplementary material 1. Antimicrobial effect using minimum inhibitory concentration for plant biocholine against the bacterium *Escherichia coli.*

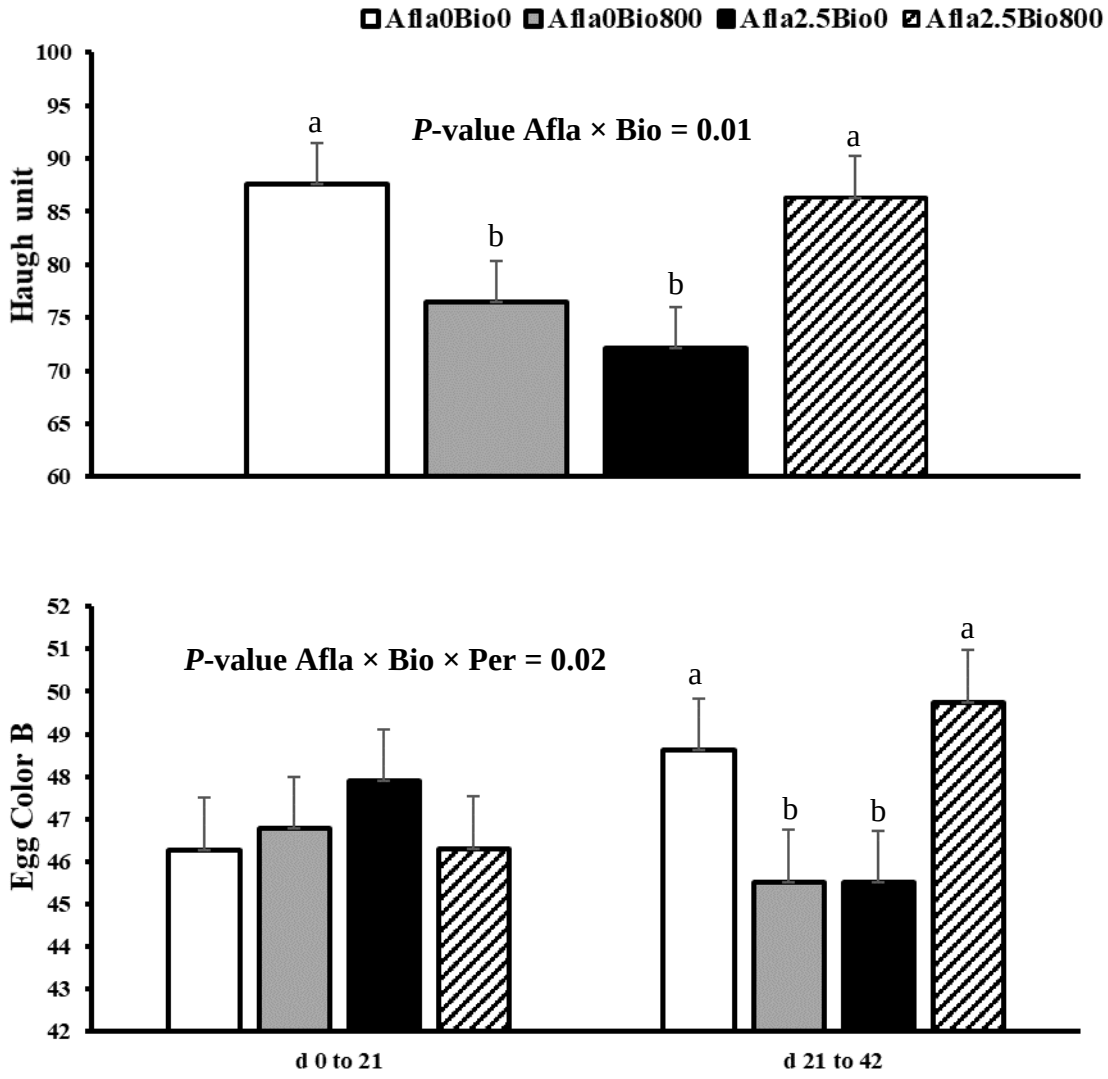
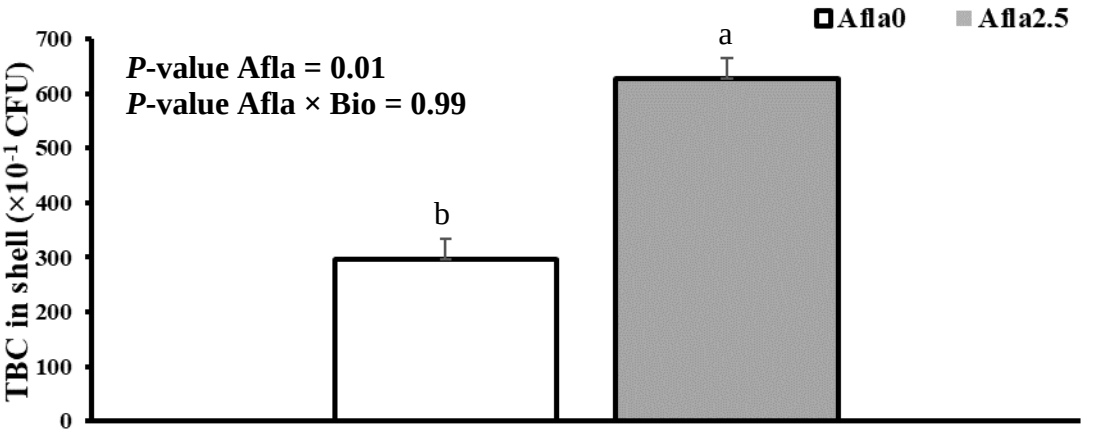

Afla0Bio0
Afla0Bio800
Afla2.5Bio0
Afla2.5Bio800
Haugh unit
100
95
90
85
80
75
70
65
60
a
b
b
a
P-value Afla × Bio = 0.01
Egg Color B
52
51
50
49
48
47
46
45
44
43
42
P-value Afla × Bio × Per = 0.02
a
b
b
a
d 0 to 21
d 21 to 42

Figure 1.


Afla0
Afla2.5
TBC in shell (×10-1 CFU)
700
600
500
400
300
200
100
0
P-value Afla = 0.01
P-value Afla × Bio = 0.99
b
a

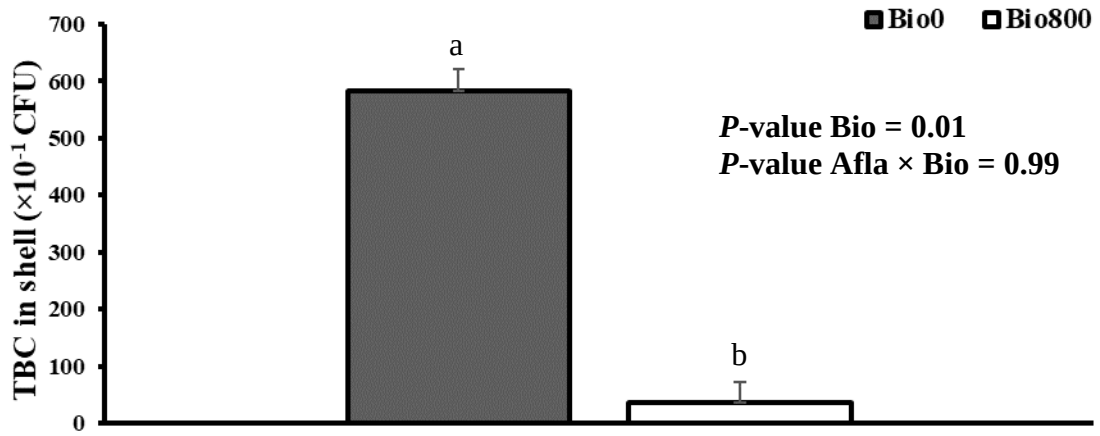


Figure 2.

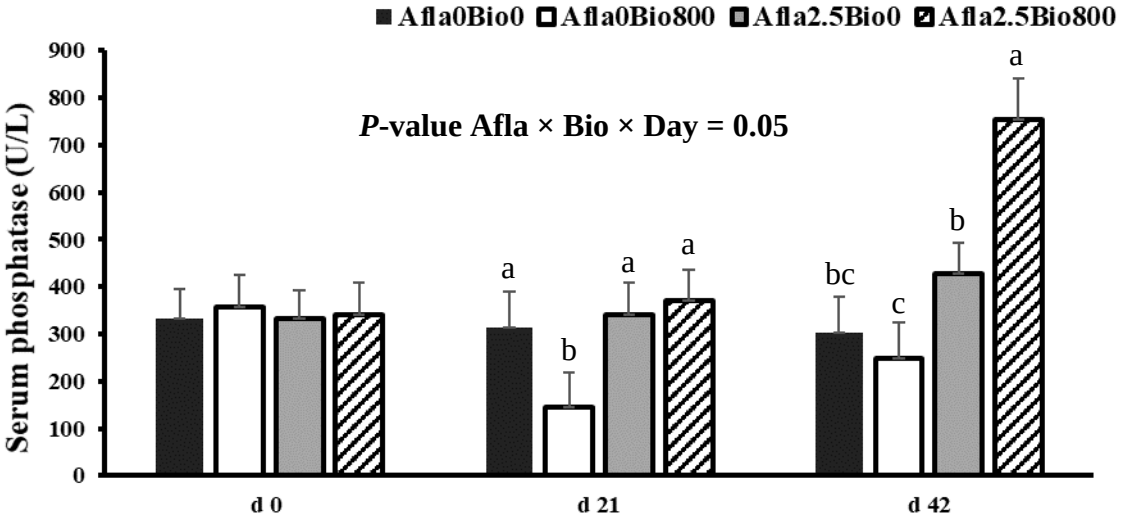
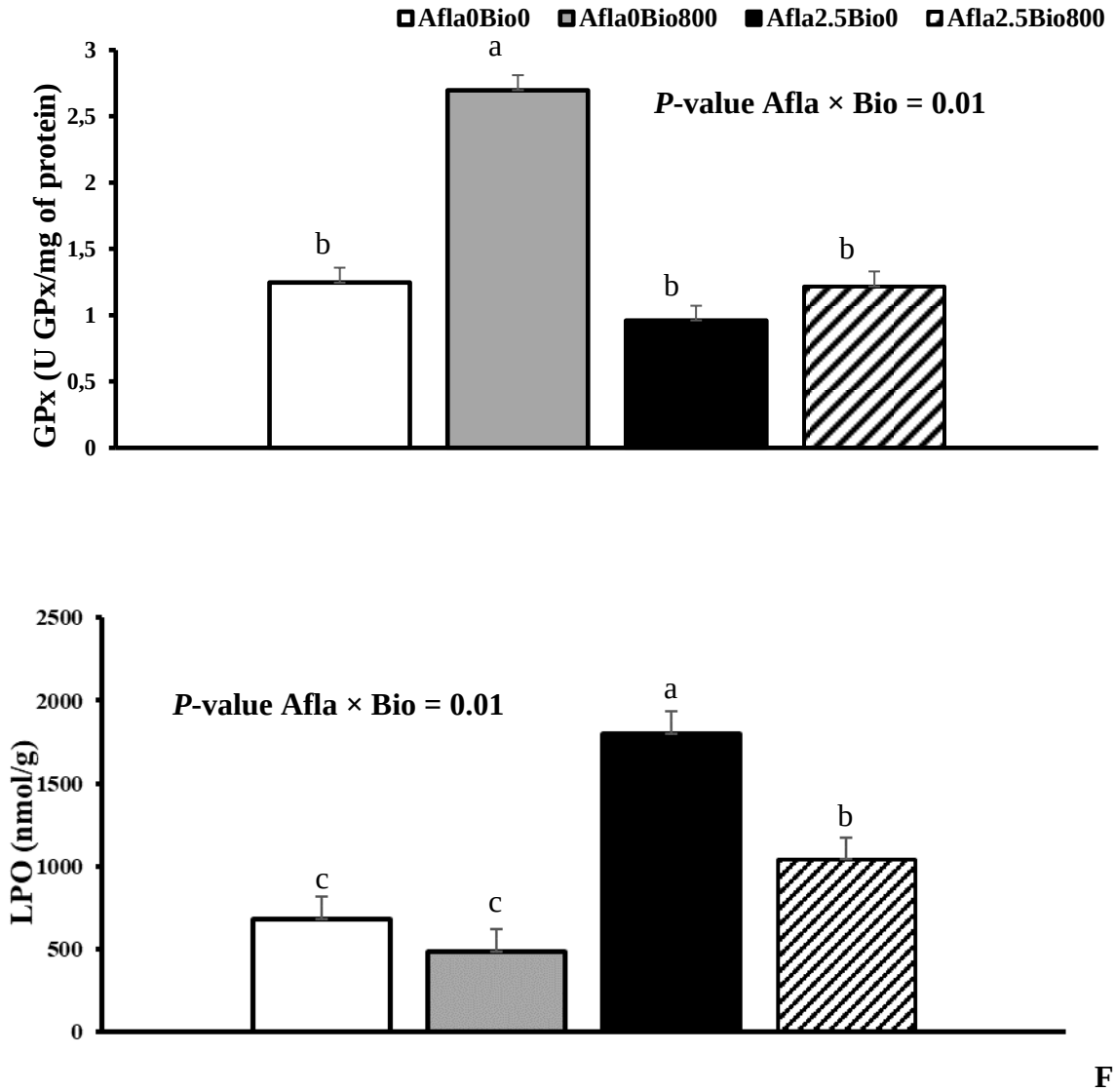


Figure 3.


Afla0Bio0
Afla0Bio800
Afla2.5Bio0
Afla2.5Bio800
GPx (U GPx/mg of protein)
3
2,5
2
1,5
1
0,5
0
a
b
b
b
P-value Afla × Bio = 0.01
LPO (nmol/g)
2500
2000
1500
1000
500
0
c
c
a
b
P-value Afla × Bio = 0.01

F

figure 4.

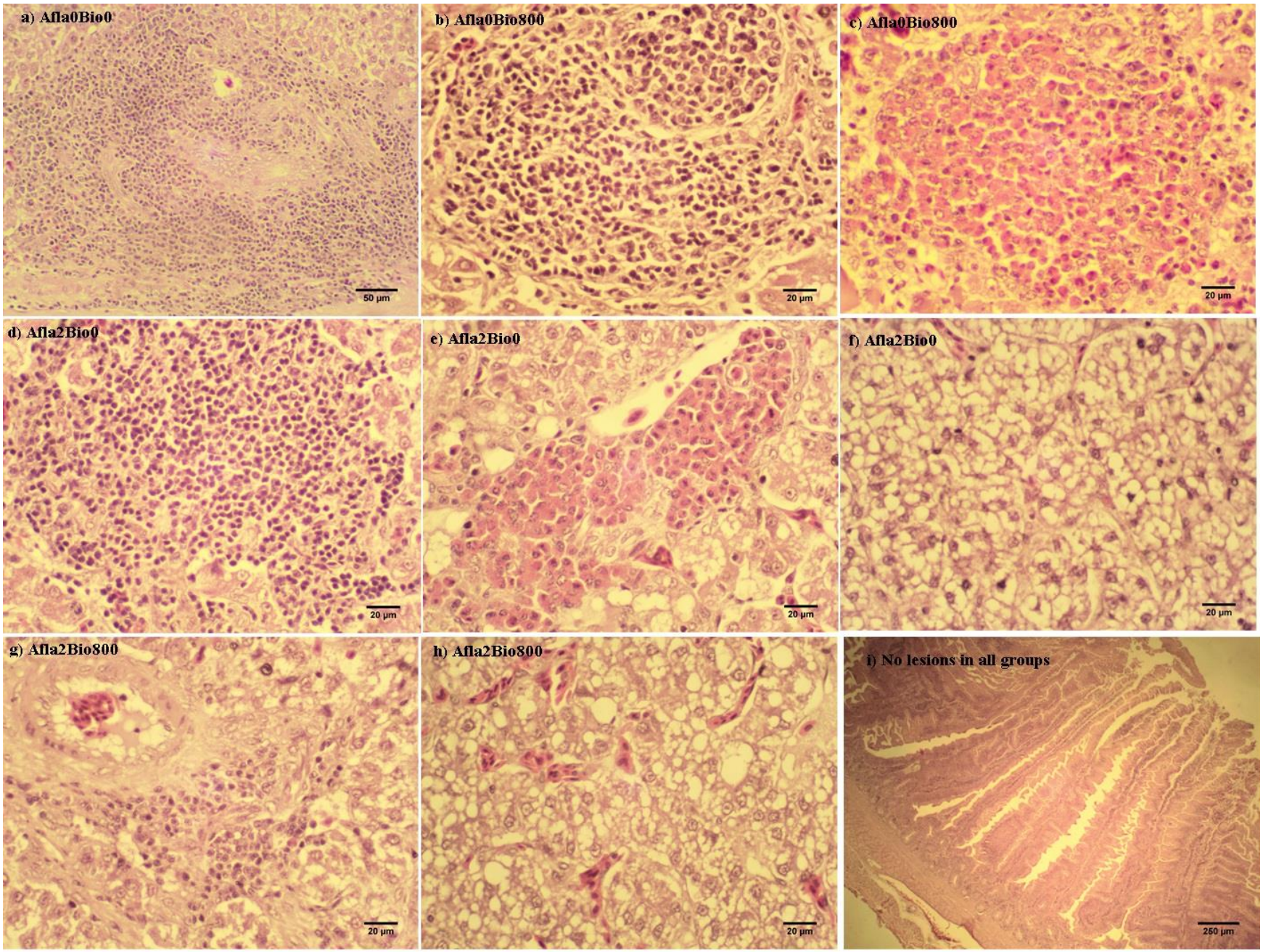
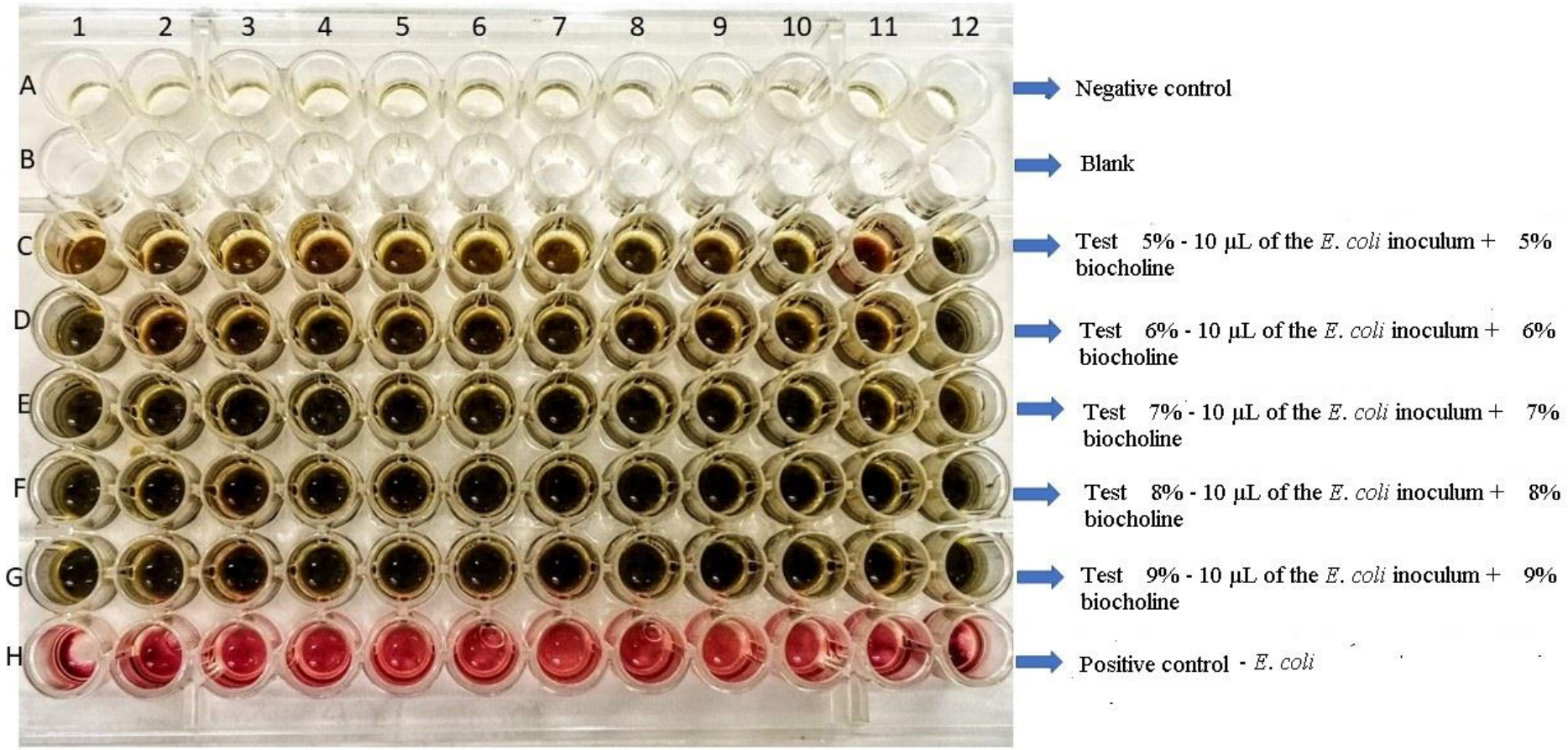

a) Afla0Bio0
50 µm
b) Afla0Bio800
20 µm
c) Afla0Bio800
20 µm
d) Afla2Bio0
20 µm
e) Afla2Bio0
20 µm
f) Afla2Bio0
20 µm
g) Afla2Bio800
20 µm
h) Afla2Bio800
20 µm
i) No lesions in all groups
250 µm

Figure 5



Supplementary material 1

2.3 MANUSCRITO II

De acordo com normas para publicação em: Research Veterinary Science

Inclusion of vegetable biocholine addictive in piglet feed contaminated with aflatoxin B1: impact on health and zootechnical performance

Vanessa Dazuk[1]; Lara Tarasconi[2]; Vitor Molossi[2]; Bruno Cécere[2]; Guilherme L. Deolindo[2]; João V. Strapazzon[1], Nathieli B. Bottari[3], Bianca F. Bissacotti[3], Maria Rosa C. Schetinger[3], Laércio Sareta[4], Ricardo E. Mendes[4], Marcelo Vedovatto[5], Diovani Paiano[2]; Aleksandro Schafer da Silva[2*]

[1] Programa de Pós-graduação em Zootecnia, Universidade do Estado de Santa Catarina (UDESC), Chapecó, Brazil.

[2] Departamento de Zootecnia, UDESC, Chapecó, Brazil.

[3] Departamento de Bioquímica e Biologia Molecular, Universidade Federal de Santa Maria, Brazil.

[4] Laboratório de Patologia Veterinária, Instituto Federal Catarinense, Concordia, Brazil.

[5] Departamento de Zootecnia, Universidade Estadual de Mato Grosso do Sul, Brazil.

*Corresponding author: aleksandro_ss@yahoo.com.br

Abstract

The objective of this study was to determine whether the addition of vegetable biocholine (VB) in piglet would minimize negative effects caused by daily aflatoxin intake. We used 72 whole male piglets (7.42 ± 1.27 kg) weaned at an average of 26 days and divided into four groups with six replicates each (2 x 2 factorial). The treatments were identified as Afla0Bio0 - negative control (without aflatoxin and without VB); Afla500Bio0: positive control (500 parts per billion (ppb) of aflatoxin); Afla0Bio800: 800 mg/kg of VB; Afla500Bio800: 500 ppb of aflatoxin + 800 mg/kg of VB. The study evaluated zootechnical performance (weight gain - WG, feed intake - FI and feed conversion - FC), as well as blood samples (days 0, 10, 20, 30 and 40 of the experiment) and tissue (liver, spleen and portion of the intestine). In the first 20 days of the experiment, only the piglets from Afla500Bio0 had less weight gain and less feed consumption; different from the 30th to 40th day when all treatments had lower zootechnical performance compared to the negative control. FC did not differ between treatments. Animals fed with VB had higher carcass yield and greater spleen weight; liver weight was higher in positive control animals. In the liver, higher levels of oxygen-reactive species and lipid peroxidation were observed in Afla500Bio0, associated with greater activity of the enzymes alanine aminotransferase and aspartate aminotransferase, but no histopathological lesion was observed in this organ, as well as in the intestine and spleen. In the intestine there was also oxidative stress, associated with nitrous stress in Afla500Bio0. The VB in the diet was not able to stimulate an enzymatic antioxidant system (catalase, superoxide dismutase and glutathione S-transferase) in the blood and tissues, with the exception of an increase in the GST in the spleen of the Afla500Bio800 animals. The consumption of aflatoxin (Afla500Bio0) increased the neutrophil count, as well as reduced the hematocrit at certain times in this experiment. The results allow us to conclude that the consumption of a diet contaminated with 500 ppb of aflatoxin interferes in the health and performance of piglets in the nursery phase, in a silent way, but capable of generating high economic losses for producers. When VB was added to the piglets' diet in the face of the aflatoxin challenge, it showed hepaprotective potential, however, the dose 800 mg GB/kg of feed with additive is not recommended in this daycare phase.

Keywords: Additions. Nutrition. Mycotoxins. Pigs.

1. Introduction

The nursery phase of swine breeding systems challenging and is critically important to the subsequent phases of the production cycle (Alvarenga et al. 2013). The weaning of piglets is considered critical and that requires specific care on account of the various challenges and changes that the animals are subjected to all at once. The result is often increased mortality rates and delayed performance (Kummer et al. 2009). A possible solution is the exchange of liquid food (sow milk) for a solid food based on ingredients of plant origin such as corn or soybean meal (Tokach et al. 1989). Nevertheless, when this substitution occurs, there is the possibility of exposure to mycotoxins from dietary cereals (Eulalio et al. 2015).

Mycotoxins present in animal feeds are a constant concern in the productive, economic, and health areas of the animal protein chain. According to Dilkin (2002), aflatoxins are present in approximately 38% of pig diets and are responsible for the most significant swine mycotoxicosis, representing an extremely serious condition for swine health. Mycotoxin effects can vary; however, in general, they depress the immune system and lead to the development of tumors (Mallmann et al. 1994; Sharma, 1993), resulting in slower weight gain, digestive disorders, and liver disease (Mallmann and Dilkin, 2011). Immunotoxicity is also reported in pigs subjected to food contaminated with aflatoxin B1 and fumonisin B1, and these mycotoxins exert their toxic effects via various biochemical mechanisms (Liu et al. 2002). The greatest problem surrounding mycotoxicosis derives from losses related to the functionality of organs and systems of animals, implying a decrease in their productive performance (Dilkin, 2002). The liver is most affected by the toxic effects of aflatoxin, resulting in a series of changes in the metabolism of proteins, carbohydrates, and lipids (Santurio, 2007).

For all these reasons, it is critical to search for ingredients and/or additives that, when added to animal feed, minimize the effects of the consumption of mycotoxins. In this sense, natural products derived from plants may help protect animals from toxic agents derived from food and the environment that damage health and performance. Vegetable biocoline (VB) in fish diet had positive results on health and performance (Souza et al. 2018); when these fish were challenged with aflatoxin B1, biocoline has a hepatoprotective effect (Souza et al. 2020). Based on this information, we aimed to determine whether the addition of VB in piglet diet was able to minimize negative effects caused by daily aflatoxin intake, focusing on performance and health.

2. Materials and methods

2.1. Vegetable biocholine (VB)

We used commercially available vegetable biocholine (Biocholine Powder®, Technofeed, SP, Brazil). The product is produced from plant extracts (*Trachyspermum ammi*, *Azadichara indica* and *Achyranthes rugas*), and contains guarantee levels of 16 g of phosphatidylcholine/kg of extract). We used 800 mg VB/kg of feed provided to piglets, based on the results of a study published by Souza et al. (2020).

2.2. Aflatoxin production and analysis

Aflatoxins were produced by the ATCC 13608 strain of Aspergillus flavus during fermentation of converted rice and the follow protocol was used. Erlenmeyer flasks of 500 mL volume were used to receive 100 g of rice. At least 2h before the sterilization 40 mL of distillated water was added to flask and mixed with rice. The sterilization was performed at 121 C during 30 minutes and then the flasks were left to loss temperature before inoculation. The rice was inoculated with 2 mL of 108 spore mL-1 of spore suspension of A. flavus. The incubation was carried out during 21 days at controlled temperature (250Cº) and constant stirring of flasks. After incubation, fermented material was dried in oven at 50Cº and grounded. The concentration of aflatoxin in the inoculum was determined in advance, in order to calculate and determined the amount added in the diets in order to obtain a 500 ppb contamination, a dose already described in the literature for causing delay in the growth of piglets (Schell et al., 1993).

Samples of feed and inoculum were ground to < 0.85 mm material and one gram of the ground material was transferred to test tube of 50 mL. It was added 10 mL of ultrapure water and 10 mL of acetonitrile/acetic acid (CH_3CN:CH_3COOH) [99.5:0.5, v/v] and the test tube was placed in a mechanic shaker for 10 min. A mixture of 4 g of $MgSO_4$ and 1 g of NaCl was added and the tube was vigorously hand-shaking for 10 s. The solution was them centrifuged for 15 min at 5.000 *x g*, at 25 ◦C and 2.5 mL of supernatant was transferred to capped glass test tube where 2.5 mL of hexane was added. The solution was shaken for 2h and then centrifuged at 1.000 *x g*, at 20 ◦C for 1 min. From lower phase (acetonitrile) 1 mL was withdraw and dried with Nitrogen (N_2) stream at 40 ◦C. The reconstitution was performed with 75 µL of methanol in ultrasonic bath for 10s and 10s in test tube mixer after adding 75 µL of ultrapure water. After centrifugation for 10min at 14.000 *x g* 60 µL was withdraw and transferred to vial where 140 µL of ultrapure water was added. Ten microlitres was injected in chromatographic system.

Detection and quantification of aflatoxins were performed with high-performance liquid chromatography coupled with tandem mass-spectrometry (LC/MS/MS). Chromatographic separation

was carried out using Acqulty UPLC System (Waters, Milford, Massachusetts, EUA) equipped with 100 × 2.1 mm, 1.7 µm Acquity UPLC BEH C18 column, (Waters, Milford, Massachusetts, EUA). The column was maintained at 40 ◦C and the injection volume was 10 µL. The mobile phase consisted of 0.1% formic acid in water(A), and 0.1 % formic acid in acetonitrile (B). The acetonitrile (B) concentration was raised gradually from 10 % to 90 % within 12 min, brought back to the initial conditions at 0,1 min, and allowed to stabilize for 3 min. The mobile phase was delivered at a flow rate of 0.4 mL/min. The LC system was coupled with Xevo TQS tandem mass spectrometer (Waters, Milford, Massachusetts, EUA), equipped with a turbo-ion electrospray (ESI) ion source. The mass-spectrometer was operated in scheduled multiple reaction monitoring (MRM) in positive mode. The electrospray ionization and MS/MS conditions are showed in Supplementary Material 1.

2.3. Animals and experimental design

The experiment was carried out in the experimental pig house at the Experimental Farm of the State University of Santa Catarina (FECEO), located in the city of Guatambu, SC, Brazil, over 40 days. The diet was based on corn, soybean meal, and commercial core, according to the nutritional requirements of pigs. For the production of the feed, corn was sieved first in order to select viable grains with less natural contamination by aflatoxins.

We used 72 whole male piglets (7.42 ± 1.27 kg) weaned with an average of 26 days, divided into four groups with six replicates each and three piglets per repetition. The experiment was conducted in a nursery facility consisting of a plastic floor suitable for the phase, troughs with availability of 15 cm trough/animal, and automatic-type drinking troughs with a flow rate of 1/L/min. The installation was heated using an electric heater. The treatments were as follows: Afla0Bio0, negative control (without aflatoxin and without biocholine); Afla500Bio0, positive control (500 ppb of aflatoxin); Afla0Bio800, 800 mg/kg of biocholine; and Afla500Bio800, 500 ppb of aflatoxin + 800 mg/kg of biocholine.

After the analyzes described in section 2.2, we verify that the actual contaminated diets to aflatoxin were as follows: Afla0Bio0 (AFLAB1 = 0.0 ppb; AFLAB2 = 0.0 ppb); Afla500Bio0 (AFLAB1 = 471.8 ppb; AFLAB2 = 8.2 ppb); Afla0Bio800 (AFLAB1 = 0.0 ppb; AFLAB2 = 0.0 ppb); Afla500Bio800 (AFLAB1 = 335.1 ppb; AFLAB2 = 6.4 ppb). AFLAG1 and AFLAG2 not observed in experimental feed.

2.4. Zootechnical performance

The zootechnical performance was evaluated at the end of day 10, 20, 30 and 40 of the experimental periods. During these periods, individual animals and leftover feed were weighed using an electronic scale (model DIGI-TRON UL-5 with column). The rations were stored in individual buckets, one for each repetition. Daily weight gain (ADG) and daily feed intake (ADFI) were measured, from which feed conversion (FCR) was obtained. Daily feed consumption was measured by weighing the feed provided at the beginning of each period and leftovers at the end of each stage, as well as weighing the animals at that time for the DWG. The FC data were calculated as feed consumption/weight gain.

2.5 Sample collection

Blood samples were collected in vacutainer tubes on days 0, 10, 20, 30 and 40 of the experimental periods in tubes containing anticoagulant. First, complete blood counts were performed according to the methodology described below, and a 0.5 mL aliquot of blood was removed for analysis of CAT and SOD activity, stored frozen. Subsequently, blood was centrifuged at 8,000 rpm for 5 minutes, thereby obtaining serum that was allocated in a microtube, and maintained frozen (-20 °C) until biochemical analysis.

On day 32 of the experiment, six animals from each group were slaughtered in a specialized slaughterhouse, according to current legislation of the inspection system. Fragments of the liver, intestine and spleen were collected, and samples were preserved in 10% formaldehyde. A liver fragment was homogenized in saline, centrifuged and the supernatant was removed. These were packed in microtubes and frozen for further analysis of oxidants/antioxidants.

2.6. Hemogram

The hemoglobin, total leukocyte and erythrocyte contents were determined using a commercial kit according to the manufacturer's recommendations. In the sampling, blood smears were made and stained with commercial dye (Rapid Panotype) to perform differential leukocyte counts under a light microscope with a 1000x magnification, as described by Lucas and Jamroz (1961). Hematocrit was measured using microcapillary tubes, centrifuged at 14000 x *g* for 5 min.

2.7. Serum biochemistry

Serum levels of total proteins, albumin, cholesterol, triglycerides, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured using semi-automatic BioPlus equipment (Bio-2000) and specific commercial kits. Serum globulin levels were calculated as the difference between serum levels of total proteins and albumin.

2.8. Oxidizing and antioxidant status

Serum activities of glutathione S-transferase (GST), superoxide dismutase (SOD), and catalase (CAT) were measured. GST activity was measured according to Mannervik and Guthenberg (1981), with modifications. Briefly, GST activity was measured as the rate of formation of dinitrophenyl-S-glutathione at 340 nm in a medium containing 50 mM potassium phosphate at pH 6.5, 1 mM GSH, 1 mM 1-chloro-2, 4-dinitrobenzene (CDNB) as substrate and tissue supernatants (approximately 0.045 mg protein). The results were expressed as U GST/mg protein. The activity of the SOD was measured using the method of Marklund and Marklund (1974) and the results were expressed as nmol SOD/mg of protein. CAT activity was measured using ultraviolet spectrometry, according to the describle method Aebi (1984) and the results were expressed as nmol CAT/mg of protein.

The levels of reactive oxygen species (ROS) in plasma were analyzed by the method described by Halliwell and Gutteridge (2007). The plasma (10 μL) was incubated with 12 μL of dichlorofluorescein (DFC) per mL at 37 ° C for 1 h in the dark. Fluorescence was determined using 488 nm for excitation and 520 nm for emission. The results were expressed as UDCF/mg protein. NOx levels were measured according to the method of Miranda et al. (2001) which indirectly quantifies nitrite/nitrate levels, and the results were expressed as U NOx/mg protein. TBARS values were obtained using the method described by Ohkawa et al. (1978) in tissues and by Jentzsch et al. (1996) in the plasma.

2.9. Organ weight and histopathology

Spleen and liver were weighed during the slaughter process. Then, fragments of liver, intestine and spleen were preserved in a formaldehyde solution (10%). Tissue fragments were processed and placed in paraffin blocks. Then sections were made and stained with hematoxylin eosin (HE).

2.10. Statistical analyses

The experimental design of this study was one factorial 2 × 2 [feed with and without aflatoxin (Afla0 and Afla500) and with (Bio0) and without biocholine (Bio800)]. 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’s degrees of freedom for the test of fixed effects. The data of DWG, DFI, and FC were tested for fixed effects of aflatoxin, biocholine, and the interaction, and as random effect, we included pen (aflatoxin × biocholine). The data of antioxidant response in

liver, spleen, and intestines variables were tested for fixed effects of aflatoxin, biocholine, and the interaction, and as random effects included pen (aflatoxin × biocholine) and animal (pen). All other data were analyzed as repeated measures (body weight and blood variables) and were included as fixed effects aflatoxin, biocholine, day, and all possible interactions, and the random effects included pen (aflatoxin × biocholine) 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. A simple Pearson correlation was evaluated among the antioxidant variables using CORR procedure of SAS to determine the interrelation between these. Significance was defined when $P \leq 0.05$ and tendency when $P > 0.05$ and ≤ 0.10.

3. Results

3.1. Performance

The performance results are presented in Table 2. There was interaction ($P<0.05$) between treatments (AFLA versus BIO) in the first 32 days of the experiment; that is, from day 1 to day 10 and from day 1 to 20, the positive control group (Afla500Bio0) had a lower DWG than did the negative control group (Afla0Bio0); however, the other groups did not differ significantly from both groups. From 1 to 32 days, the negative control (Afla0Bio0) had greater weight gain than the other groups. Between days 21 and 30, weight gain was greater in the animals in the Afla0Bio0 group than in the others, suggesting that supplementation with VB (Afla0Bio800) influenced piglet development. The average body weight of the four groups is shown in Figure 1. In general, the positive control group (Afla500Bio0) had lower body weights at 30 and 40 days compared to Afla0Bio0, with a positive effect of those challenged and supplemented with VB (Afla500Bio800); that is, they had weights statistically similar to the those of the negative control. From 1 to 10, the lowest feed consumption was only in Afla500Bio0 compared to the negative control, different from what was observed between days 1 to 32, while the highest feed consumption was in Afla0Bio0 compared to the others. Between days 21 and 30, the highest DFI was registered in the Afla0Bio0 group in relation to the other treatments; this result is consistent with the greater weight gain of these animals. Feed conversion did not differ significantly between treatments ($P >0.05$).

3.2. Serum biochemistry

Protein and lipid metabolism, as well as liver enzyme activities are presented in Table 3. We found an interaction between day and aflatoxin intake, as well as an effect of aflatoxin and VB in

piglet serum. In general, the inclusion of aflatoxin in the diet increased ($P < 0.01$) levels of ALT and AST on days 20, 30, and 40 when compared to the other treatments (Fig. 2). For total protein, albumin, and globulin, no significant differences were found when comparing groups with or without aflatoxin in any of the evaluated periods ($P > 0.05$). However, when comparing the groups with and without VB, we found lower levels of total proteins and globulins in the Bio800 group on days 10 and 40. Cholesterol levels were not significantly different among any of the compared groups ($P > 0.05$). On day 10, triglyceride levels were higher in the groups without aflatoxin and without VB (Afla0Bio0) when compared with the groups with aflatoxin and with VB (Afla500Bio800).

3.3. Hemogram

Complete blood count results are displayed in Table 4. Counts of eosinophils, lymphocytes, leukocytes and erythrocytes did not differ significantly among groups ($P > 0.05$). Regarding hematocrit, there was an effect of the day on animals that consumed aflatoxin; that is, on days 20 and 30, the hematocrits were higher in those that consumed AflaB1. Piglets in the Afla500 group on the 20^{th} and 30^{th} days had lower neutrophil counts than did the negative control group. On day 10, monocyte counts were lower in the groups of piglets that consumed aflatoxin; on the 20^{th} day, the piglet group that was supplemented with Bio800 showed a higher number of monocytes when compared to the Bio0 group. Piglets in the Afla500 group had higher monocyte counts on day 40 than did the Afla0 group.

3.4. Plasma, blood and tissue antioxidant responses

Serum and whole blood antioxidant responses are shown in Table 5. There were no significant differences ($P > 0.05$) between the groups with respect to GST, SOD, CAT, NOx, ROS, or TBARS. The results of the oxidative and antioxidant status in tissues are shown in Table 6. In the liver, NOx levels were higher in the Afla0Bio0 and Afla500Bio800 groups than in the others. Also in the liver, the levels of ROS and TBARS were higher in piglets in the Afla500Bio0 group. In the spleen, ROS levels were lower in the Afla500Bio0 group. TBARS levels were lower in Afla500Bio800. In the spleen, there was also an effect of the consumption of biocoline on GST activity; that is, greater activity was observed in the animals of the Afla500Bio800 group. In the intestine, an effect of aflatoxin consumption was also observed; that is, intake of aflatoxin increased the activity of GST, as well as the of NOx and ROS (Table 6). Table 7 shows Pearson's correlation coefficients among antioxidant variables in the blood, liver, spleen, and intestines. Significant correlations were observed in the liver and blood between the following variables: TBARS versus GST, ROS versus ROS, and GST versus NOx. In blood and spleen, the following were significant

correlations: ROS versus TBARS, and GST versus TBARS; in the blood and intestine significant correlations were found for TBARS versus TBARS; in the liver and spleen significant correlations were found for NOx versus ROS; in the liver, significant correlations were found for TBARS versus ROS; in liver and intestine significant correlations were found for TBARS versus NOx and ROS versus NOx/ROS/GST; in the spleen and intestine, significant correlations were found for GST versus TBARS; in the intestine, significant correlations were found for NOx versus ROS/GST.

3.5. Carcass yield and liver and spleen weight

Piglets that consumed VB in the diet had higher carcass yields than did the control (Fig. 3a). Liver weight was higher in positive control piglets; that is, animals fed with aflatoxin (Fig. 3b); however, VB supplementation in the diet of piglets challenged with mycotoxin minimized this change ($P > 0.05$). The spleen weight was higher in piglets supplemented only with VB (Fig. 3c).

3.6. Histopathology

No intestinal, hepatic and spleen lesions were observed in any treatment. The intestinal villus/crypt relationship is being evaluated, and the result will be presented on the day of the defense.

4. Discussion

In general, we observed lower weight gain in pigs that consumed aflatoxin in the diet. Furthermore, the consumption of feed with VB in the dose of 800 mg/kg also had a negative effect on weight gain. The negative effect of aflatoxin was expected, because according to the literature, the sensitivity of pigs to this mycotoxin is considered one of the most substantial among animal species (Santurio, 2007). The greatest importance of aflatoxicosis in swine production is silent, as a noticeable clinical picture is not frequently seen, and losses in weight gain have been observed, as was verified in the present study. In a study with diets contaminated with 500 ppb of aflatoxin supplied to weaned piglets, researchers concluded that there was a reduction in the growth rate of the animals (Schell et al. 1993), a result similar to those described by Santurio (2007). In the first 20 days of the nursery phase, VB was able to prevent negative effects of aflatoxicosis on the body weight and feed consumption of piglets (Afla500Bio800), because the weight gain was similar to the animals that did not consume the mycotoxin in the diet experimentally (Afla0Bio0). However, between days 21 to 30, weight gain was lower in the animals of all treatments compared to the control, suggesting that supplementation with VB did not prevent the negative effects caused by aflatoxin, and even interfered with piglet development when used only as an additive (Afla0Bio800).

We believe that the dose of 800 mg VB/kg of feed was high, and was responsible for the negative effect on weight gain. A study conducted in the 1980s highlighted that the supplementation of pigs with choline via diet should avoid excesses when it is desired to obtain maximum performance gains (Southern et al. 1986). Similarly, also in the 1980s, researchers reported reduced gain and efficiency in broilers fed with a hill level only slightly higher than the requirement (Derilo and Balnave 1980). Because VB contains a choline source known as phosphatidylcholine, which needs to be better studied in pigs, and as our study shows that the dose used was not correct, therefore, in future studies it will be necessary to test lower doses in order to calculate the ideal dose capable of enhancing performance, as described in other animal species (Alba et al. 2020; Baldissera et al. 2019; Leal, 2019).

The effects of aflatoxin were also visible when we observed greater serum activity of the enzymes ALT and AST in piglets that had aflatoxin and did not consume VB; however, histologically, no changes were observed. The liver is the main organ affected by aflatoxin, leading to organ damage (Cullen and Newbwene, 1994; Dilkin, 2002; Mallmann, 1994; Santurio, 2007; Zain, 2011). High levels of ALT and AST indicate liver damage, and their values can be induced to changes in cases of fungal and bacterial intoxications (González, 2006). Triglyceride levels were lower in the serum of piglets that received the combination of aflatoxin and biocholine when compared to the negative control; however, there was no concrete explanation for this change. We know that the synthesis of fats occurs in adipose tissue and liver, which are the lipogenic tissues that exist in the body, synthesizing triglycerides that are secreted in the blood stream for use in other tissues (Terao and Ohtsubo, 1991); furthermore, the animals affected by aflatoxicosis undergo important changes in the hepatic metabolism affecting the fat metabolism (Tung et al. 1972), which may explain the lower values of triglycerides in the group of animals subjected to aflatoxin.

There was an effect of aflatoxin on hematocrit; that is, a lower percentage of hematocrit was observed in piglets that consumed mycotoxin. This result was different from that of Muller et al. (2018) when piglets consumed a diet contaminated experimentally with aflatoxin and fumonisin. These authors reported greater hematocrit and hemoglobin concentration (Muller et al. 2018). On days 20 and 40 of the experiment, we recorded higher neutrophil counts and lower monocyte counts in the blood of piglets that consumed aflatoxin (positive control) when compared to the others, suggesting that the intake of VB prevented this alteration in the leukogram. Neutrophils, according to Murphy (2014), are the organism's first line of defense; they act by performing phagocytosis of fungi and bacteria, as well as of dead tissue in inflammatory processes and injuries resulting from aflatoxicosis. In another study by our research group, where piglets were challenged with diets

contaminated with aflatoxin and fumonisin, we found a reduction in total leukocyte counts; however, there was no change in the neutrophil counts (Muller et al. 2018).

Variables indicative of nitrous stress (NOx) and oxidative stress (ROS and TBARS) were elevated in the intestines of piglets in the group exposed to aflatoxin consumption; furthermore, in the livers of these animals, there was a higher concentration of ROS and TBARS associated with the larger size of the liver in these animals. Such changes were not observed in the piglets' blood; however, lower ROS levels were found in the spleen. The formation of free radicals by the organism under normal conditions is inevitable as they are necessary for the cellular respiration process; however, the production of reactive oxygen species is higher in animals when tissue injuries caused by trauma, infections, parasites, toxins, and extreme exercise (Vizzotto, 2017). In the spleen, lower levels of TBARS and greater GST activity in piglets of the Afla500Bio800 group are positive findings for animal health, and may be related to the known antioxidant effect of VB (Baldissera et al. 2019); nevertheless, this effect was modest in our study. When VB was used in diets of Nile Tilapia challenged with aflatoxin B1 (Souza et al. 2020), the results of the antioxidant effect were substantial, unlike what we saw in the present study, where no serum alteration in the oxidant/antioxidant status was found; we believe this related to the subclinical and silent toxicity of this mycotoxin.

We observed correlations among antioxidant variables in blood, liver, spleen, and intestines, in various tissues. This suggests aflatoxin-mediated interference in the functioning of the organs, leading to damage to the health of animals that consume it, as described by other authors (Baldissera et al. 2019; Mallmann et al. 1994; Migliorini et al. 2017; Santurio, 2000; Shane, 1994; Zain, 2011).

5. Conclusion

The consumption of feed contaminated by AFLB1 reduced feed consumption and weight gain in piglets; it also caused subclinical intestinal and hepatic oxidative stress, in addition to increasing the activity of liver enzymes that are biomarkers of liver damage. VB supplementation in piglet diet had no positive effects on performance, but it minimized the negative effects of the food contaminated by aflatoxin B1 only in the first 20 days of the nursery phase. The antioxidant responses to VB were not highlighted in our study; nevertheless, we believe such responses occurred, and the additive probably prevented exacerbated, undesirable oxidative reactions in the animals by increasing levels of free radicals and tissue lipid peroxidation. In general, VB showed hepaprotective potential in the face of the challenge with aflatoxin; however, the dose 800 mg VB/kg of feed with additive is not recommended in the nursery phase.

Ethics committee

The project was approved by the ethics committee on the use of animals in UDESC research, protocol number 8763030419.

References

Aebi H. Catalase in vitro. **Method Enzymology**. 105(1): 121-126, 1984.

Alba, D. F., Favaretto, J. A., Marcon, H., Saldanha, T. F., Leal, K. W., Campigoto, G., ... & Da Silva, A. S. Vegetable biocholine supplementation in pre-and postpartum Lacaune sheep: Effects on animal health, milk production and quality. **Small Ruminant Research**, *190*, 106165, 2020.

Alvarenga, A. L. N., Chiarini-Garcia, H., Cardeal, P. C., Moreira, L. P., Foxcroft, G. R., Fontes, D. O., & Almeida, F. R. C. L. Intra-uterine growth retardation affects birthweight and postnatal development in pigs, impairing muscle accretion, duodenal mucosa morphology and carcass traits. **Reproduction, Fertility and Development,** *25*(2), 387-395, 2013.

Baldissera, M. D., Souza, C. F., Baldisserotto, B., Zimmer, F., Paiano, D., Petrolli, T. G., & Da Silva, A. S. Vegetable choline improves growth performance, energetic metabolism, and antioxidant capacity of fingerling Nile tilapia (Oreochromis niloticus). **Aquaculture**, v. 501, p. 224-229, 2019.

Cullen, John M; Newberne, Paul M. Hepatotoxicidade aguda de aflatoxinas. In: **A toxicologia das aflatoxinas**. Academic Press, p. 3-26, 1994.

Souza, C.F, Baldissera, M. D., Baldisserotto, B., Petrolli, T. G., da Glória, E. M., Zanette, R. A., & Da Silva, A. S. Dietary vegetable choline improves hepatic health of Nile tilapia (Oreochromis niloticus) fed aflatoxin-contaminated diet. **Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology**, *227*, 108614, 2020.

Derilo, Yolanda L.; Balnave, D. The choline and sulphur amino acid requirements of broiler chickens fed on semi-purified diets. **British Poultry Science**, v. 21, n. 6, p. 479-487, 1980.

Dilkin, Paulo. Micotoxicose suína: aspectos preventivos, clínicos e patológicos. **Biológico**, v. 64, n. 2, p. 187-191, 2002.

Eulalio, D. K. et al. Contaminação por micotoxinas em matérias-primas e rações destinadas à suinocultura de minas gerais. In: CONGRESSO ABRAVES, 18., 2015, Campinas. **Anais...** São Paulo: Associação Brasileira de Veterinários Especialistas em Suínos, p.218-220, 2015.

González, F. H. D. **Introdução à Bioquímica Clínica Veterinária.** 2aed. Porto Alegre: UFRGS, 360p, 2006.

Halliwell, B., Gutteridge, J.M.C. **Free radicals in biology and medicine**, 4th edn. Oxford University Press, New York, 2007.

Jentzsch A.M., Bachmann H., Fürst P. & Biesalski H.K. Improved analysis of malondialdehyde in human body fluids. Free Radical Biology & Medicine. 20(2): 251- 256, 1996

Leal, K.W. **Uso de ingredientes e aditivos alternativos na dieta de ovinos: impactos sobre saúde, desempenho zootécnico e qualidade de carne.** Dissertação de mestrado apresentada no programa de pós graduação em zootecnia – UDESC Oeste, Chapecó, 2019.

Migliorini, M. J., Da Silva, A. S., Santurio, J. M., Bottari, N. B., Gebert, R. R., Reis, J. H., ... & Boiago, M. M. The Protective effects of an adsorbent against oxidative stress in quails fed aflatoxin-contaminated diet. **Acta Scientiae Veterinariae,** *45*, 1-7, 2017.

Miranda, Katrina M.; Espey, Michael G.; Wink, David A. A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. **Nitric oxide**, v. 5, n. 1, p. 62-71, 2001.

Murphy, Kenneth. **Imunobiologia de Janeway-8**. Artmed Editora, 2014.

Kummer, R., Gonçalves, M. A. D., Lippke, R. T., Marques, B. M. F., & Mores, T. J. Fatores que influenciam o desempenho dos leitões na fase de creche. **Acta Scientiae Veterinariae,** *37*(1), 195-209, 2009.

Liu, B. H., Yu, F. Y., Chan, M. H., & Yang, Y. L.The effects of mycotoxins, fumonisin B1 and aflatoxin B1, on primary swine alveolar macrophages. **Toxicology and applied pharmacology**, *180*(3), 197-204, 2002.

Lucas, AM., Jamroz, C. **Atlas of avian hematology**. Washington: U.S. Department of Agriculture. 271p, 1961.

Mallmann, C.; Dilkin, Paulo. Mycotoxins and mycotoxicosis in swine. **Translated and edited by G. Zaviezo and D. Zaviezo. Special Nutrients edition. Miami, FL USA**, v. 7, p. 80-81, 2011.

Mallmann, Carlos Augusto; Santurio, Janio Morais; Wentz, Ilmo. Aflatoxinas-Aspectos clínicos e toxicológicos em suínos. **Ciência Rural**, v. 24, n. 3, p. 635-643, 1994.

Mannervik, B., Guthenberg, C. Glutathione transferase (human placenta). **Methods Enzymology** 77, 231–235, 1981.

Marklund, S., Marklund, G. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. **Eur. J. Biochem**. 47, 469–474, 1974

Muller, L. K. F., Paiano, D., Gugel, J., Lorenzetti, W. R., Santurio, J. M., de Castro Tavernari, F., ... & Da Silva, A. S. Post-weaning piglets fed with different levels of fungal mycotoxins and spray-dried porcine plasma have improved weight gain, feed intake and reduced diarrhea incidence. **Microbial pathogenesi***s*, *117*, 259-264, 2018.

Ohkawa H., Ohishi N. & Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. **Analytical Biochemistry**. 95(3): 351-358, 1978.

Santurio, Janio M. Micotoxinas e micotoxicoses nos suínos. **Acta Scientiae Veterinariae**, v. 35, p. S1-S8, 2007.

Santurio, J. M. Micotoxinas e micotoxicoses na avicultura. **Brazilian Journal of Poultry Science**, v. 2, n. 1, p. 01-12, 2000.

Shane, Simon M. Economic issues associated with aflatoxins. In: **The Toxicology of Aflatoxins**. Academic Press, p. 513-527, 1994.

Schell, T. C., Lindemann, M. D., Kornegay, E. T., Blodgett, D. J., & Doerr, J. A. Effectiveness of different types of clay for reducing the detrimental effects of aflatoxin-contaminated diets on performance and serum profiles of weanling pigs. **Journal of animal science,** *71*(5), 1226-1231, 1993.

Southern, L. L., Brown, D. R., Werner, D. D., & Fox, M. C. Excess supplemental choline for swine. **Journal of Animal Science**, *62*(4), 992-996, 1986.

Souza, C.F, Baldissera, M.D, Descovi, S.N, Zeppenfeld, C.C, Garzon, L.R, da Silva, A.S, Stefani, L.M, Baldisserotto, B. Serum and hepatic oxidative damage induced by a diet contaminated with fungal mycotoxin on freshwater silver catfish Rhamdia quelen: involvement on disease pathogenesis. **Microb. Pathog.** 124, 82–86. doi: 10.1016/j.micpath.2018.08.041, 2018.

Souza, C.F, Baldissera, M. D, Baldisserotto, B, Petrolli, T. G, Da Glória, E. M, Zanette, R. A, Da Silva, A. S. Dietary vegetable choline improves hepatic health of Nile tilapia (*Oreochromis niloticus*) fed aflatoxin-contaminated diet. **Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology,** *227*, 108614, 2020. https://doi.org/10.1016/j.cbpc.2019.108614

Sharma, Raghubir P. Immunotoxicity of mycotoxins. **Journal of Dairy Science**, v. 76, n. 3, p. 892-897, 1993.

Terao, K.; Ohtsubo, K. Atividades biológicas das micotoxinas: micotoxicose de campo e experimental. **Micotoxinas e alimentos de origem animal**, p. 455-488, 1991.

Thorpe, C.W, Ware, G.M, Pohland, A.E. Determination of aflatoxins by HPLC with a fluorescence detector and using post column derivatization. In: **Proceedings of the Fifth International IUPAC Symposium on Mycotoxins and Phycotoxins. Technical University**, Vienna. Eds. W. Pfannhauser, and P. B. Czedic Eysenberg, 52-55, 1982.

Tokach, M. D.; Nelssen, J. L.; Allee, G. L. Effect of protein and (or) carbohydrate fractions of dried whey on performance and nutrient digestibility of early weaned pigs. **Journal of Animal Science**, v. 67, n. 5, p. 1307-1312, 1989.

Tung, Hsi-Tang; Donaldson, W. E.; Hamilton, P. B. Altered lipid transport during aflatoxicosis. **Toxicology and applied pharmacology**, v. 22, n. 1, p. 97-104, 1972.

Vizzotto, E. Radicais livres e mecanismos de proteção antioxidante. **Subject of Biochemical Fundamentals of Metabolic Disorders. Programa de Pós-Graduação em Ciências Veterinárias, Universidade Federal do Rio Grande do Sul**, 2017.

Zain, Mohamed E. Impact of mycotoxins on humans and animals. **Journal of Saudi chemical society**, v. 15, n. 2, p. 129-144, 2011.

Table 1. Ingredients and nutritional composition of diets

Ingredients (g/kg)	Pre-initial I	Pre-initial II	Initial I
Ground corn, 7.8% CP	400	500	650
Soybean flour, 46% CP	100	250	300
Pre-initial core I [1]	500	-	-
Pre-initial core II [2]	-	250	-
Initial core I [3]	-	-	50
Calculated composition*			
Crude protein, (g/kg)	20.2	20.3	19.9
Metabolizable energy, Mcal/kg	3.52	3.43	3.36
Calcium (g/kg)	6.80	7.02	7.01
Available phosphorus, (g/kg)	3.39	3.56	3.38
Digestible lysine, (g/kg)	14.5	13.5	12.8
Digestible methionine, (g/kg)	5.85	5.14	4.78
Digestible Threonine, (g/kg)	1.16	1.08	1.02

[1] Minimum guarantee levels/kg of product; Crude Protein 210 g; Ether extract 55 g; Calcium 10 g; Phosphorus 7 g; Sodium 6 g; Co 1.6 mg; Cu 300 mg; Fe 300 mg; I 3.6 mg; Mn 110 mg; If 0.8 mg; Zn 5.4 g; Cr 0.6 mg; Vit. At 29,000 IU; Vit. D3 6,000 IU; Vit. E 160 IU; Vit. K3 7 mg; Vit. B1 7

mg; Vit. B2 11 mg; Vit. B6 7 mg; Vit. B12 140 µg; Folic acid 1.4 mg; Nicotinic acid 81 mg; Pantothenic acid 51 mg; Choline 1.9 g; Biotin 0.2 mg; Lysine 22 g; Methionine 8,000 mg; Phytase 1,000 FTU; Xylanase 3,000 EPU; *S. cerevisiae* 4.8 x 10^9; *L. acidophilus* 5.5 x10^7; *B. bifidum* 3.9 x 10^7; *B. amyloliquefaciens* 1.2 x 10^8. Maximum levels/kg of product: Humidity 90 g; Crude Fiber 20 g; Ca 14 g; ash 400 g.

[2] Minimum guarantee levels/Kg of product: Crude Protein 160 g; Ethereal Extract 50 g; Calcium 18 g; Phosphorus 10 g; Sodium 10 g; Co 3.2 mg; Cu 600 mg; Fe 600 mg; I 7.2 mg; Mn 220 mg; If 1.6 mg; Zn 10 g; Cr 1.2 mg; Vit. At 58,000 IU; Vit. D3 12,000 IU; Vit. And 320 IU; Vit. K3 14 mg; Vit. B1 14 mg; Vit. B2 23 mg; Vit. B6 14 mg; Vit. 280 mcg B12; Folic acid (min) 2.8 mg; Nicotinic acid (min) 163 mg; Pantothenic acid 102 mg; Choline 2,120 mg; Biotin 0.4 mg; Lysine 25 g; Methionine 10 g; Phytase 2,000 FTU; Xylanase 6,000 EPU. Maximum levels/kg of the product: Humidity 50 g; Crude Fiber 30 g; Ca 25 g; Ashes 450 g.

[3] Minimum guarantee levels / Kg of product: Calcium 90 g; Phosphorus 20 g; Sodium 35 g; Cu 1000 mg; Fe 1000 mg; I 20 mg; Mn 500 mg; If 8 mg; Zn 15 g; Vit. At 180,000 IU; Vit. D3 36,000 IU; Vit. And 400 IU; Vit. K3 60 mg; Vit. B1 28 mg; Vit. B2 80 mg; Vit. B6 30 mg; Vit. 360 mcg B12; Folic acid 8 mg; Nicotinic acid 600 mg; Pantothenic acid 320 mg; Choline 3,120 mg; Biotin 2 mg; Lysine 40 g; Methionine 40 g; Threonine 5,500 mg; Zinc bacitracin 900 mg. Maximum levels/kg of product: Humidity 20 g; Ashes 730 g; Ca 160 g.

* Values calculated based on the nutritional matrix proposed by Rostagno et al. (2017) and in the nutritional composition of the core.

Table 2. Performance of piglets fed with diets containing aflatoxins and biocholine.

Variables[1]	Treatments[2]				SEM	*P*-values[3]		
	Afla0Bio0	Afla500Bio0	Afla0Bio800	Afla500Bio800		Afla × Bio	Afla	Bio
DWG								
d 1 to 10	0.172[a]	0.130[b]	0.156[ab]	0.180[a]	0.01	<0.01	0.40	0.16
d 1 to 20	0.335[a]	0.281[b]	0.293[ab]	0.306[ab]	0.01	0.05	0.29	0.64
d 1 to 30	0.410[a]	0.332[b]	0.353[b]	0.360[b]	0.02	0.03	0.05	0.43
d 11 to 20	0.497	0.432	0.430	0.431	0.03	0.28	0.30	0.28
d 21 to 30	0.536[a]	0.416[b]	0.453[b]	0.451[b]	0.02	0.02	0.01	0.30
d 31 to 41	0.651	0.639	0.535	0.633	0.04	0.21	0.32	0.17
DFI								
d 1 to 10	0.295[a]	0.245[b]	0.258[ab]	0.293[a]	0.02	0.04	0.71	0.77
d 1 to 20	0.465	0.408	0.412	0.437	0.03	0.14	0.56	0.65
d 1 to 30	0.662[a]	0.583[b]	0.553[b]	0.580[b]	0.04	0.05	0.22	0.39
d 11 to 20	0.633	0.572	0.558	0.583	0.04	0.27	0.63	0.41
d 21 to 30	0.998[a]	0.760[b]	0.795[b]	0.815[b]	0.07	0.05	0.13	0.30
d 31 to 41	1.181[a]	1.050[b]	1.017[b]	1.072[ab]	0.06	0.07	0.49	0.21
CA								
d 1 to 10	1.703	1.915	1.683	1.645	0.10	0.23	0.40	0.17
d 1 to 20	1.383	1.472	1.402	1.437	0.06	0.63	0.28	0.88

d 1 to 30	1.605	1.627	1.570	1.611	0.06	0.87	0.59	0.67
d 11 to 20	1.273	1.345	1.301	1.357	0.06	0.88	0.29	0.74
d 21 to 30	1.835	1.818	1.762	1.818	0.10	0.73	0.85	0.73
d 31 to 41	1.820	1.673	1.932	1.707	0.09	0.68	0.06	0.45

[1]DWG: daily weight gain; DFI: feed consumption; CA: food conversion

[2]In a factorial design (2 × 2) was include or not aflatoxin (Afla0 and Afla500 for 0 or 500 ppb of aflatoxin/kg of concentrate, respectively) and also included or not biocholine (Bio0 and Bio800 for 0 or 800 mg of biocholine/kg of concentrate, respectively).

[3]Afla, aflatoxin; Bio, biocholine.

[a-b]Differs ($P \leq 0.05$) between treatments (lines).

Table 3. Serum biochemistry of piglets fed with diets containing aflatoxins and biocholine.

Variables[1]	Aflatoxin[2]		SEM	*P*-values[3]		Biocholine[2]		SEM	*P*-values[3]	
	Afla0	Afla500		Afla × Day	Afla	Bio0	Bio800		Bio× Day	Bio
Total protein (mg/dL)				0.87	0.26				0.02	0.01
d 1	5.10	4.72	0.33			5.29	4.23	0.33		
d 10	5.08	4.70	0.33			5.58[a]	4.20[b]	0.33		
d 20	6.44	6.07	0.24			6.39	6.13	0.24		
d 30	6.03	5.88	0.24			5.86	6.06	0.24		
d 40	6.22	6.28	0.24			6.27	6.23	0.24		
Average	5.77	5.53	0.15			5.93[a]	5.37[b]	0.15		
Albumin (mg/dL)				0.28	0.30				0.38	0.43
d 1	3.00	2.82	0.18			2.77	3.05	0.18		
d 10	2.99	2.83	0.18			2.78	3.05	0.18		
d 20	2.80	2.98	0.13			2.86	2.92	0.13		
d 30	3.13	3.43	0.13			3.41	3.16	0.13		
d 40	2.71	3.05	0.13			2.88	2.88	0.13		
Average	2.93	3.02	0.07			2.94	3.01	0.07		
Globulin (mg/dL)				0.95	0.20				<0.01	<0.01
d 1	2.03	1.83	0.36			1.83	1.02	0.36		

d 10	2.01	1.82	0.35			2.83[a]	1.01[b]	0.35		
d 20	3.64	3.09	0.26			3.53	3.20	0.26		
d 30	2.90	2.45	0.26			2.45	2.90	0.26		
d 40	3.51	3.23	0.26			3.39	3.35	0.26		
Average	2.82	2.49	0.18			3.01[a]	2.29[b]	0.18		
Cholesterol (mg/dL)				0.65	0.61				0.55	0.76
d 1	66.32	72.81	4.80			66.84	72.29	4.80		
d 10	66.44	72.58	4.77			66.90	72.12	4.77		
d 20	57.75	57.92	3.46			56.92	58.75	3.46		
d 30	56.92	53.00	3.46			55.93	54.00	3.46		
d 40	68.00	67.75	3.46			70.58	65.17	3.46		
Average	63.09	64.81	2.37			63.43	64.47	2.37		
Triglycerides (mg/dL)				<0.01	0.61				<0.01	0.64
d 1	150.98	105.27	17.62			151.35	103.90	17.81		
d 10	200.98[a]	105.27[b]	17.62			202.35[a]	103.90[b]	17.81		
d 20	48.39	54.44	17.62			52.93	59.90	17.81		
d 30	66.23	111.27	17.62			65.93	111.56	17.81		
d 40	56.06	55.11	17.62			56.93	54.23	17.81		
Average	98.53	86.27	12.79			98.10	86.70	13.04		
ALT (U/L)				<0.01	<0.01				0.15	<0.01

d 1	21.26	21.57	1.62			21.45	21.39	1.62		
d 10	21.26	21.57	1.62			21.45	21.39	1.62		
d 20	26.76[b]	35.74[a]	1.62			33.45	29.05	1.62		
d 30	27.43[b]	42.57[a]	1.62			37.95	32.05	1.62		
d 40	32.26	34.24	1.62			36.45	30.05	1.62		
Average	25.79b	31.14a	0.74			30.15[a]	26.79[b]	0.74		
AST (U/L)				<0.01	<0.01				0.23	0.05
d 1	44.12	43.54	2.66			44.22	43.45	2.66		
d 10	44.12	43.54	2.66			44.22	43.45	2.66		
d 20	39.96[b]	56.38[a]	2.66			47.72	48.61	2.66		
d 30	41.96[b]	64.88[a]	2.66			58.72	48.11	2.66		
d 40	41.79[b]	53.21[a]	2.66			49.22	45.78	2.66		
Average	42.40[a]	52.31[b]	1.03			48.82[a]	45.88[b]	1.03		

[1]ALT: alanine aminotransferase; AST: Aspartate aminotransferase.

[2]In a factorial design (2 × 2) was include or not aflatoxin (Afla0 and Afla500 for 0 or 500 ppb of aflatoxin/kg of concentrate, respectively) and also included or not biocholine (Bio0 and Bio800 for 0 or 800 mg of biocholine/kg of concentrate, respectively).

[3]Afla, aflatoxin; Bio, biocholine.

[a-b]Differs ($P \leq 0.05$) between treatments (lines).

Table 4. Hemogram of piglets fed with diets containing aflatoxins and biocholine.

Variables	Aflatoxin[1]		SEM	*P*-values[2]		Biocholine[1]		SEM	*P*-values[2]	
	Afla0	Afla500		Afla × Day	Afla	Bio0	Bio800		Bio× Day	Bio
Erythrocytes (x10^6 µL)				0.77	0.49				0.82	0.89
d 1	6.68	6.80	0.39			6.85	6.63	0.39		
d 10	6.67	6.78	0.39			6.83	6.62	0.39		
d 20	6.019	6.62	0.27			6.13	6.51	0.27		
d 30	5.49	5.52	0.27			5.45	5.56	0.27		
d 40	6.37	6.26	0.27			6.42	6.22	0.27		
Average	6.25	6.40	0.15			6.34	6.31	0.15		
Hematocrit (%)				<0.01	0.19				0.84	0.75
d 1	38.74	36.51	1.29			37.55	37.69	1.25		
d 10	38.74	36.51	1.29			37.55	37.69	1.25		
d 20	36.41[b]	41.43[a]	1.29			38.55	39.28	1.25		
d 30	36.91[b]	43.59[a]	1.29			40.55	39.94	1.25		
d 40	33.69	34.48	1.29			35.08	33.08	1.25		
Average	36.90	38.50	0.75			37.85	37.54	0.69		
Hemoglobin (g/dL)				0.52	0.31				0.96	0.42
d 1	10.70	10.64	0.47			10.71	10.63	0.47		

d 10	10.76	10.60	0.46			10.73	10.63	0.46		
d 20	8.46	8.80	0.33			8.68	8.57	0.33		
d 30	7.81	8.13	0.33			8.17	7.77	0.33		
d 40	13.21	14.27	0.33			14.00	13.47	0.33		
Average	10.19	10.49	0.21			10.46	10.22	0.22		
Leucocytes ($x10^3/\mu L$)				0.52	0.31				0.96	0.42
d 1	10.70	10.64	0.47			10.71	10.63	0.47		
d 10	10.76	10.60	0.46			10.73	10.63	0.46		
d 20	8.46	8.80	0.33			8.68	8.57	0.33		
d 30	7.81	8.13	0.33			8.17	7.77	0.33		
d 40	13.21	14.27	0.33			14.00	13.47	0.33		
Average	10.19	10.49	0.21			10.46	10.21	0.21		
Neutrophils ($x10^3/\mu L$)				0.05	0.24				0.60	0.39
d 1	5.32	4.99	5.32			5.16	5.16	0.56		
d 10	5.30	4.96	5.30			5.16	5.10	0.55		
d 20	5.72^a	4.61^b	5.72			4.88	5.45	0.40		
d 30	5.03^a	3.81^b	5.03			3.88	4.96	0.40		
d 40	4.82	5.50	4.82			5.10	5.22	0.40		
Average	5.24	4.78	0.27			4.84	5.18	0.27		
Lymphocytes ($x10^3/\mu L$)				0.33	0.79				0.69	0.54

d 1	4.11	4.71	0.65			4.50	4.31	0.65		
d 10	4.06	4.62	0.65			4.33	4.34	0.64		
d 20	8.36	7.04	0.46			7.36	8.04	0.46		
d 30	7.07	6.77	0.46			6.47	7.38	0.46		
d 40	4.82	4.76	0.46			4.88	4.71	0.46		
Average	5.68	5.58	0.28			5.51	5.76	0.27		
Monocytes ($x10^3/\mu L$)				<0.01	0.93				<0.01	0.85
d 1	0.22	0.18	0.02			0.17	0.16	0.03		
d 10	0.25^a	0.18^b	0.02			0.27^a	0.16^b	0.03		
d 20	0.23	0.29	0.02			0.22^b	0.30^a	0.03		
d 30	0.13	0.17	0.02			0.12	0.19	0.03		
d 40	0.08^b	0.14^a	0.02			0.09	0.14	0.03		
Average	0.19	0.19	0.01			0.19	0.19	0.02		
Eosinophils ($x10^3/\mu L$)				0.12	0.74				0.27	0.58
d 1	0.24	0.25	0.05			0.23	0.26	0.05		
d 10	0.24	0.25	0.05			0.23	0.26	0.05		
d 20	0.33	0.46	0.05			0.37	0.42	0.05		
d 30	0.37	0.24	0.05			0.36	0.25	0.05		
d 40	0.29	0.34	0.05			0.37	0.26	0.05		
Average	0.29	0.31	0.02			0.31	0.29	0.02		

[2]In a factorial design (2 × 2) was include or not aflatoxin (Afla0 and Afla500 for 0 or 500 ppb of aflatoxin/kg of concentrate, respectively) and also included or not biocholine (Bio0 and Bio800 for 0 or 800 mg of biocholine/kg of concentrate, respectively).

[2]Afla, aflatoxin; Bio, biocholine.

[a-b]Differs ($P \leq 0.05$) between treatments (lines).

Table 5. Serum or blood antioxidant response of piglets fed with diets containing aflatoxins and biocholine.

Variables[1]	**Aflatoxin**[2]		**SEM**	***P*-values**[3]		**Biocholine**[2]		**SEM**	***P*-values**[3]	
	Afla0	**Afla500**		**Afla × Day**	**Afla**	**Bio0**	**Bio800**		**Bio× Day**	**Bio**
GST in serum (U GST/mg of protein)				0.75	0.92				0.81	0.81
d 1	79.7	76.4	30.4			83.3	72.9	30.4		
d 10	88.4	83.8	16.7			86.9	85.3	16.7		
d 20	106.3	110.4	15.5			104.5	112.1	15.5		
d 30	115.8	106.2	15.5			107.4	114.6	15.5		
d 40	214.3	235.1	15.5			235.1	214.3	15.5		
Average	120.9	122.4	10.9			123.4	119.8	10.1		
SOD in blood (nmol SOD/mf of protein)				0.76	0.39				0.73	0.45
d 1	6.52	5.56	1.09			6.10	5.97	1.09		
d 10	6.74	5.68	1.09			6.09	6.33	1.08		
d 20	6.19	5.02	0.69			5.65	5.56	0.69		

d 30	4.71	4.89	0.72			5.10	4.51	0.69		
d 40	7.35	7.70	0.69			8.40	6.65	0.69		
Average	6.30	5.77	0.44			6.27	5.81	0.43		
CAT in blood (nmol CAT/mg of protein)				0.11	0.93				0.94	0.74
d 1	18.9	12.6	8.95			16.2	15.3	2.70		
d 10	15.6	18.8	15.6			16.1	18.3	2.69		
d 20	20.2	20.8	20.2			19.8	21.2	2.69		
d 30	14.5	17.1	14.7			15.3	16.3	2.69		
d 40	14.2	14.9	14.2			15.3	13.8	2.69		
Average	16.7	16.8	1.06			16.5	17.0	0.95		
NOx in serum (U NOx/mg of protein)				0.13	0.11				0.88	0.97
d 1	0.59	0.29	0.59			0.32	0.57	0.45		
d 10	0.52	0.30	0.52			0.42	0.40	0.28		
d 20	0.61	0.34	0.61			0.37	0.59	0.28		
d 30	0.76	1.35	0.76			1.07	1.05	0.28		
d 40	0.47	0.37	0.47			0.61	0.24	0.31		
Average	0.59	0.53	0.14			0.56	0.57	0.14		
ROS in serum (U DCF/mg of protein)				0.20	0.74				0.16	0.92
d 1	276.4	260.9	32.2			268.3	269.0	32.2		
d 10	331.5	335.9	20.3			324.8	342.6	20.3		

d 20	258.2	252.5	20.3			283.3	227.4	20.3		
d 30	266.2	278.3	20.3			250.4	294.1	20.3		
d 40	266.4	295.4	20.3			280.5	281.4	20.3		
Average	279.7	284.6	10.6			281.4	282.9	10.2		
TBARS in serum (nmol MDA/mL)				0.77	0.67				0.11	0.16
d 1	20.1	21.3	1.28			19.4	22.0	1.40		
d 10	11.6	10.5	1.68			11.3	10.9	1.62		
d 20	13.2	11.3	1.28			12.5	12.0	1.40		
d 30	9.95	9.79	1.28			9.31	10.4	1.40		
d 40	9.15	9.27	1.28			8.92	11.5	1.40		
Average	12.8	12.4	0.60			12.3	13.9	0.77		

[1]GST: glutathione s-transferase; SOD: superoxide dismutase; CAT; catalase; NOx: nitric oxide; ROS: reactive oxygen species; TBARS: Thiobarbituric acid reactive substances

[2]In a factorial design (2 × 2) was include or not aflatoxin (Afla0 and Afla500 for 0 or 500 ppb of aflatoxin/kg of concentrate, respectively) and also included or not biocholine (Bio0 and Bio800 for 0 or 800 mg of biocholine/kg of concentrate, respectively).

[3]Afla, aflatoxin; Bio, biocholine.

[a-b]Differs ($P \leq 0.05$) between treatments (lines).

Table 6. Liver, spleen and intestine antioxidant concentration of piglets fed with diets containing aflatoxins and biocholine.

Variables[1]	Combined treatments[2]				SEM	*P*-values[3]		
	Afla0Bio0	Afla500Bio0	Afla0Bio800	Afla500Bio800		Afla × Bio	Afla*	Bio+
Liver								
GST (U GST/mg of protein)	2440	1522	2141	2022	292	0.10	0.05	0.75
NOX (U NOx/mg of protein)	0.75[a]	0.52[b]	0.43[b]	0.70[a]	0.07	<0.01	0.75	0.32
ROS (U DCF/mg of protein)	647[b]	1426[a]	594[b]	726[b]	84.8	<0.01	<0.01	<0.01
TBARS (nmol MDA/mL)	53.0[b]	65.2[a]	44.1[b]	53.2[b]	4.31	0.04	0.03	0.03
Spleen								
GST (U GST/mg of protein)	987	942	380	443	115	0.65	0.94	<0.01
NOX (U NOx/mg of protein)	0.59	0.62	0.65	0.59	0.08	0.57	0.77	0.86
ROS (U DCF/mg of protein)	802[a]	595[b]	772[a]	615[ab]	65.6	0.05	0.14	0.24
TBARS (nmol MDA/mL)	48.0[a]	53.0[a]	47.2[a]	34.2[b]	4.61	0.05	0.41	0.05
Intestines								
GST (U GST/mg of protein)	292	1029	519	926	156	0.32	<0.01	0.70
NOX (U NOx/mg of protein)	0.36	0.94	0.38	0.74	0.13	0.38	<0.01	0.44
ROS (U DCF/mg of protein)	456	1541	483	115	219	0.33	<0.01	0.39
TBARS (nmol MDA/mL)	16.7	18.1	15.3	16.9	2.01	0.95	0.47	0.56

[1]GST: glutathione s-transferase; NOx: nitric oxide; ROS: reactive oxygen species; TBARS: Thiobarbituric acid reactive substances

[2]In a factorial design (2 × 2) was include or not aflatoxin (Afla0 and Afla500 for 0 or 500 mg of aflatoxin/kg of concentrate, respectively) and also included or not biocholine (Bio0 and Bio800 for 0 or 800 mg of biocholine/kg of concentrate, respectively).

[3] Afla, aflatoxin; Bio, biocholine: [a-b]Differs ($P \leq 0.05$) between four treatments referent to interaction Afla versus Bio (lines).

Note: *Afla0Bio0 versus Afla500Bio0; + Afla0Bio800 versus Afla500Bio800.

Table 7. Pearson coefficients correlations[1, 2] among antioxidant variables in blood, liver, spleen and intestines of piglets fed with diets containing aflatoxins and biocholine.

		Blood				**Liver**				**Spleen**				**Intestines**			
		NOx	**TBARS**	**ROS**	**GST**	**NOx**	**TBARS**	**ROS**	**GST**	**NOx**	**TBARS**	**ROS**	**GST**	**NOx**	**TBARS**	**ROS**	**GST**
Blood	**NOx**	-	0.30	0.10	**0.35****	**-0.33****	-0.19	-0.22	-0.16	0.30	0.15	-0.10	-0.26	-0.14	-0.01	0.02	0.19
	TBARS		-	-0.03	**0.59***	**-0.36****	-0.09	-0.20	**0.60***	0.05	-0.24	-0.10	-0.30	-0.26	**-0.41***	-0.13	-0.08
	ROS			-	**0.37****	-0.10	-0.23	**-0.41***	-0.10	-0.18	**-0.40***	-0.04	-0.07	-0.14	-0.03	-0.12	-0.05
	GST				-	**-0.40***	-0.15	-0.22	0.18	0.04	**-0.43***	**-0.37****	-0.27	-0.10	-0.25	0.12	0.22
Liver	**NOx**					-	0.22	-0.06	0.02	-0.16	0.01	**0.52***	0.02	-0.07	-0.01	-0.07	-0.06
	TBARS						-	**0.59***	-0.10	0.21	0.19	-0.06	0.08	**0.55***	0.09	**0.38****	0.25
	ROS							-	-0.25	0.13	0.33	-0.10	0.23	**0.50***	0.01	**0.52***	**0.40***
	GST								-	-0.11	-0.28	0.19	0.01	-0.28	**-0.33**	**-0.34****	**-0.35***
Spleen	**NOx**									-	0.11	-0.10	-0.24	0.28	-0.16	0.03	-0.03
	TBARS										-	0.29	0.20	-0.07	0.19	0.08	0.08
	ROS											-	0.33	-0.19	0.04	0.02	-0.02
	GST												-	0.11	**0.41***	0.13	-0.01
Intestines	**NOx**													-	0.28	**0.46***	**0.36***
	TBARS														-	-0.01	0.02
	ROS															-	**0.94**

[1]Upper row = correlation coefficients [** tendency to differ ($P \leq 0.10$) and * differ ($P \leq 0.05$)]; lower row, between parenthesis = *P*-values.

[2]n = 24

[3]GST: glutathione s-transferase (U GST/mg of protein); NOx: nitric oxide (U NOx/mg of protein); ROS: reactive oxygen species (U DCF/mg of protein); TBARS: Thiobarbituric acid reactive substances (nmol MDA/mL)

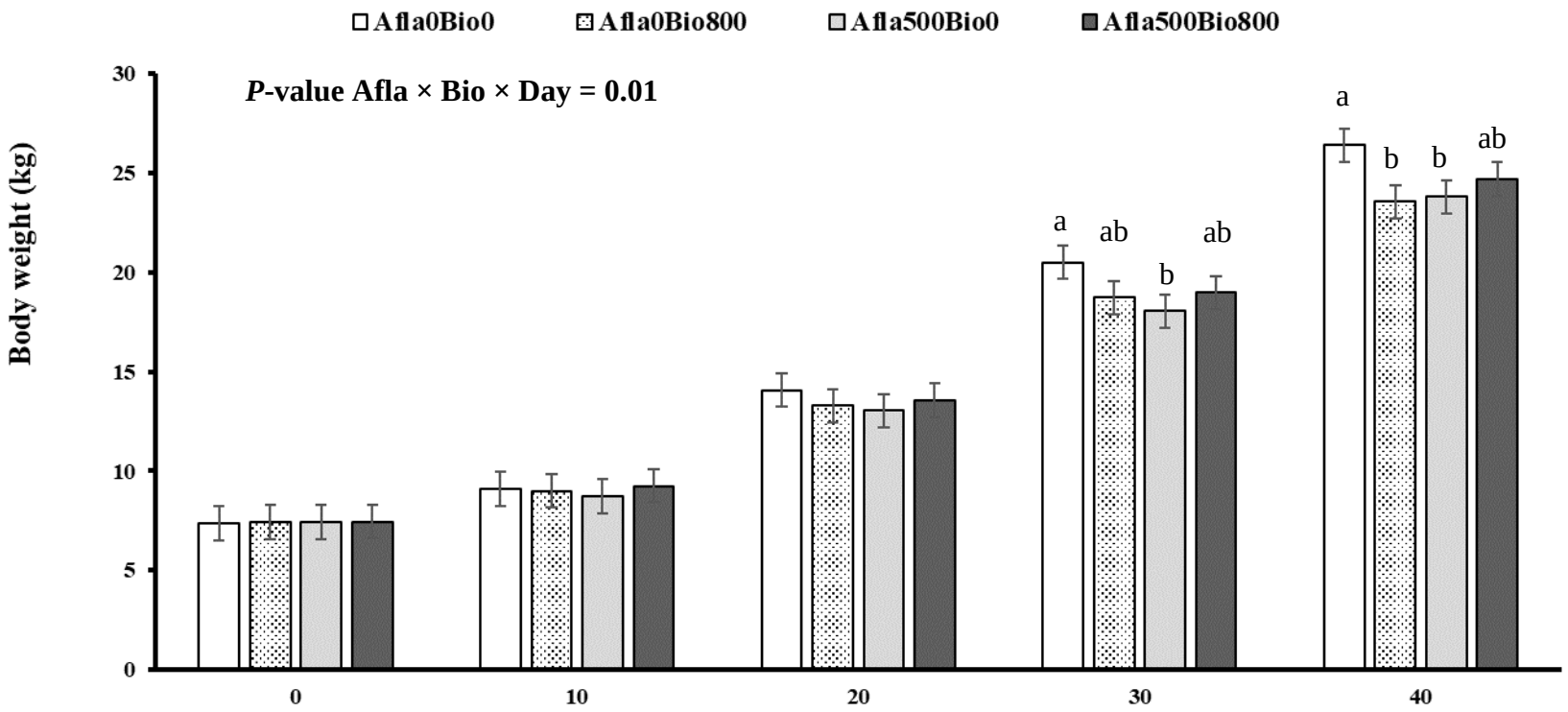


Figure 1. Growth of piglets fed with diets containing aflatoxins (Afla) and biocholine (Bio). In a factorial design (2 × 2) was include or not aflatoxin (Afla0 and Afla500 for 0 or 500 ppb of aflatoxin/kg of concentrate, respectively) and also included or not biocholine (Bio0 and Bio800 for 0 or 800 mg of biocholine/kg of concentrate, respectively).

[a-b]Differs ($P \leq 0.05$) between treatments. Vertical bars represent the SEM.

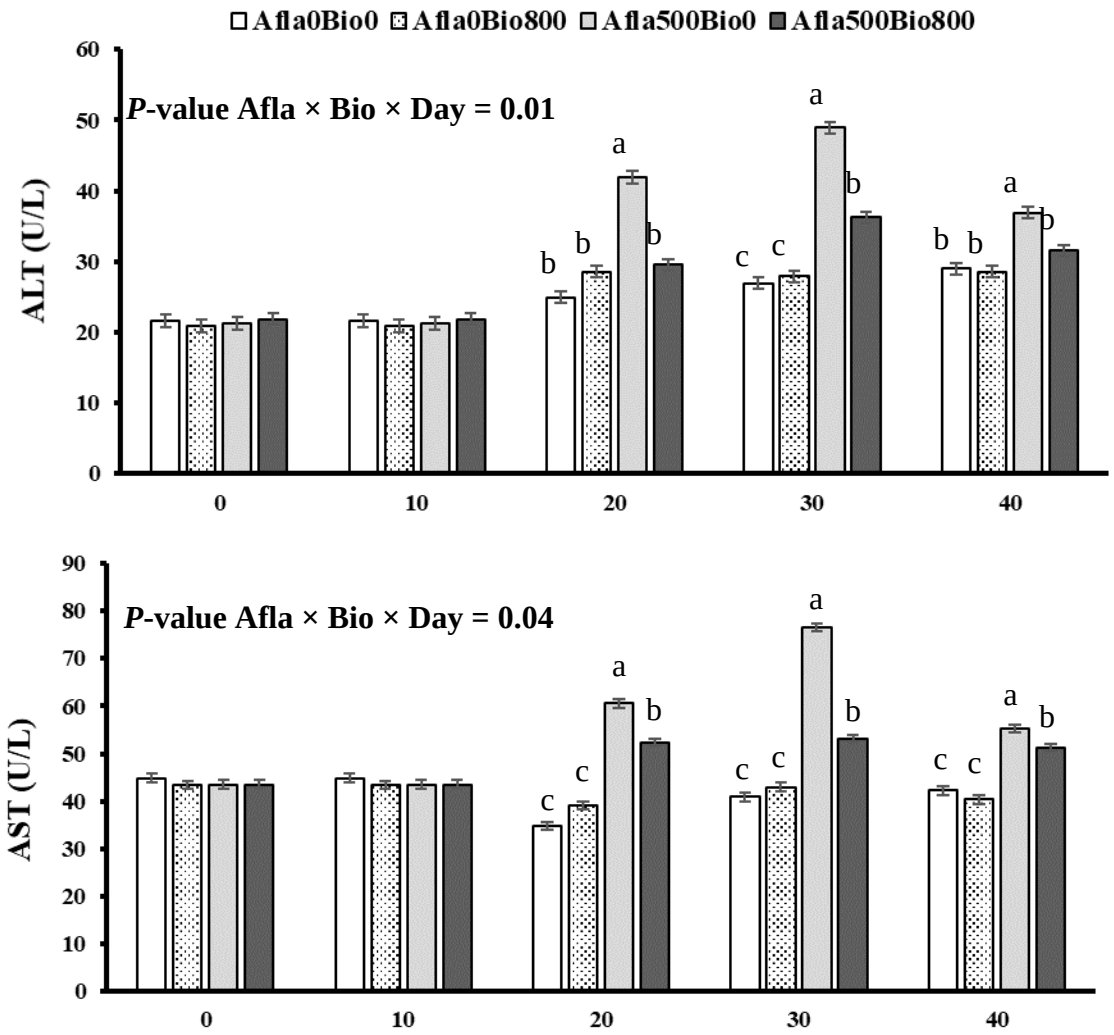


Figure 2. Activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST of piglets fed with diets containing aflatoxins (Afla) and biocholine (Bio). In a factorial design (2 × 2) was include or not aflatoxin (Afla0 and Afla500 for 0 or 500 ppb of aflatoxin/kg of concentrate, respectively) and also included or not biocholine (Bio0 and Bio800 for 0 or 800 mg of biocholine/kg of concentrate, respectively).
[a-c]Differs ($P \leq 0.05$) between treatments. Vertical bars represent the SEM.

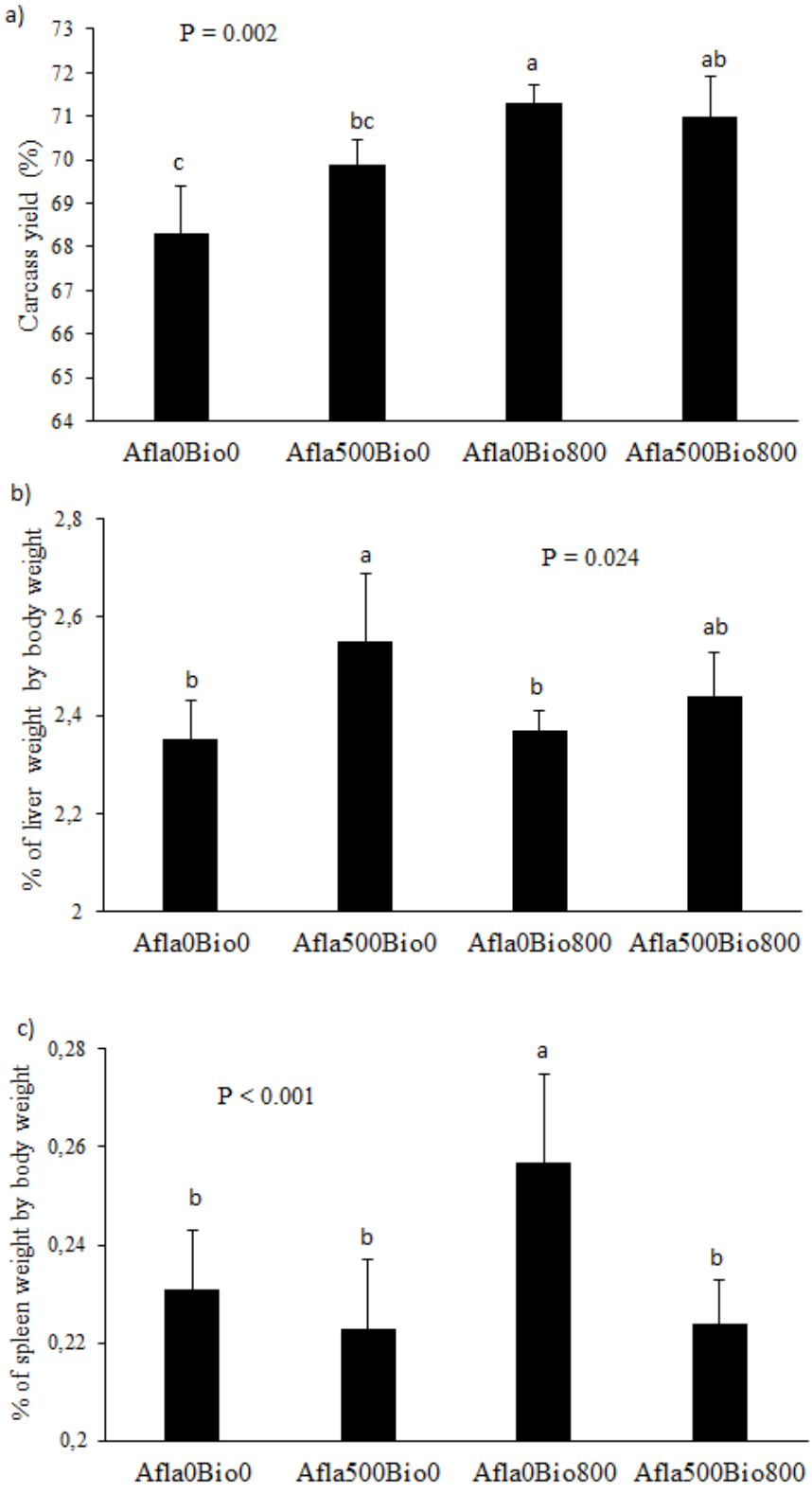


Figure 3: Carcass yield (a), liver weight percentage compared to body weight (b) and spleen weight percentage related to body weight (c) of piglets that consumed aflatoxin and

were supplemented with vegetable biocholine. Treatment effect ($p < 0.05$) was observed and differences were illustrated by different letters on the same graph.

Supplementary Material 1. The electrospray ionization and MS/MS conditions used to determinate aflatoxin levels in diets.

Analyte	MRM Transition	Dwell Time (s)	Cone Voltage (V)	Collision Energy (eV)
Aflatoxin B1	313.2>285,2	0.01	50	23
	313.2>241.2			40
Aflatoxin B2	315.2>287.2	0.01	50	26
	315.2>259.2			28
Aflatoxin G1	329.2>243.2	0.01	40	25
	392.2>283.2			25
Aflatoxin G2	331.2>245.2	0.01	45	30
	331.2>257.2			30

3 – CONSIDERAÇÕES FINAIS

A ingestão de dietas contaminadas por micotoxinas por poedeiras e leitões, leva a perdas de desempenho e afeta a saúde destes animais. O impacto econômico ocasionado pelas micotoxinas na produção animal se inicia na produção dos grãos utilizados para a alimentação destes animais, o que leva ao aumento dos custos de produção destes e perdas significativas muitas vezes silenciosas, continuando até o final da cadeia produtiva ocasionando baixas de desempenho animal, chegando até a mortalidades quando o grau de contaminação é elevado. Além disso, pesquisas tem chamado a atenção para a presença de resíduos de micotoxinas em produtos de origem animal, e caso essa cadeia de contaminação das micotoxinas não seja evitada, a consequência é a ingestão destes resíduos por parte dos consumidores finais

No manuscrito I, a utilização da biocolina vegetal para poedeiras via dieta proporcionou efeitos benéficos na saúde dos animais, com efeito hepatoprotetor e antioxidante, efeitos já conhecidos nessas condições, assim como nosso grupo de pesquisa também reportou esses efeitos positivos quando do desafio por aflatoxina em peixes. De modo geral, muitos desses efeitos já foram descritos em suínos e poedeiras em nosso estudo, sendo alguns resultados altamente significativos principalmente quando relacionado a saúde dos animais. Além disso, a biocolina na dieta das poedeiras apresentou um efeito antimicrobiano, reduzindo a presença de *Escherichia coli* em ovos, podendo ser utilizada como aditivo na prevenção e controle desta infecção em granjas comerciais. O consumo de BV minimizou os danos ao fígado causados pela toxicidade por micotoxinas. O consumo de BV não minimizou o efeito negativo da toxina na produção de ovos, mas teve efeitos positivos na saúde das galinhas e melhorou a qualidade dos ovos (ação antioxidante e antimicrobiana).

No manuscrito II , em suínos, a suplementação com BV (800 mg/kg) na fase de creche, interferiu no desenvolvimento dos leitões, afetando de forma negativa o ganho de peso, o que abre portas para um estudo de doses, pois para leitões nessa fase, a biocolina na atual dose avaliada não é indicada. Nos primeiros 10 dias de creche, a BV consumida por leitões desafiados com AFLB1 mantiveram o ganho de peso. A ingestão de BV também demonstrou um potencial antioxidante frente ao desafio da aflatoxina B1 via dieta de

leitões, assim como um efeito hepatoprotetor. O uso desta fonte natural de colina deve ser considerado, visto a tendência de um mercado que busca alternativas naturais em substituição às formas sintéticas dos aditivos na alimentação animal, mas cabe lembrar que esse produto comercial testado tem formulado a base de extrato de plantas, contendo outros componentes além da fosfatidilcolina que podem estar relacionados aos efeitos positivos na saúde dos leitões e negativos sobre o crescimento observado aqui.

No artigo I, utilizamos dietas contaminadas com as micotoxinas FB1 e T2 fornecidas a poedeiras e avaliamos o uso de adsorventes a base de lisado de *S. cerevisiae*, ácidos orgânicos, parede celular de levedura e carrier mineral. Quando comparamos o grupo controle positivo com os grupos que consumiram adsorvente, houve resultados positivos, ou seja, houve maior produção de ovos, bem como maior peso e massa nos grupos que receberam os adsorventes, além de um maior consumo de ração nesses grupos. De forma geral concluímos nesses estudo que a ingestão de micotoxinas prejudicou o desempenho e a qualidade dos ovos de galinhas poedeiras; no entanto, a adição de lisado de S. cerevisiae e a adição de ácidos orgânicos, parede celular de levedura e transportador mineral minimizou alguns efeitos negativos causados pelas micotoxinas T-2 e FB1. Neste estudo, o SCL mostrou-se uma alternativa, que merece mais testes para definir uma dose.

A utilização de aditivos funcionais e tecnológicos na nutrição animal, assim como alternativos no combate aos efeitos negativos das micotoxinas na cadeia de proteína animal é de fundamental importância para que possamos garantir a produtividade, e aos consumidores a qualidade e segurança alimentar dos produtos provenientes da produção animal. Os estudos ainda são preliminares, sendo necessário esclarecer mecanismos de ação, assim como definir as doses ideiais, porém os resultados são promissores e podem ser úteis para nutricionistas e pesquisadores.

REFERÊNCIAS

ABPA. ASSOCIAÇÃO BRASILEIRA DE PROTEINA ANIMAL. **Relatorio anual 2020. Brasília**. Disponível em: <https://abpa-br.org/abpa-lanca-relatorio-anual-2020/>, acesso em 08 de julho de 2020.

ALBA, DAVI F. et al. Vegetable biocholine supplementation in pre-and postpartum Lacaune sheep: Effects on animal health, milk production and quality. **Small Ruminant Research**, p. 106165, 2020.

ALLEONI, A. C. C.; ANTUNES, A. J. Unidade Haugh como medida da qualidade de ovóide galinha armazenados sob refrigeração. **Scientia Agrícola**, v.58, n.4, p.681-85, 2001 http://dx.doi.org/10.1590/S0103-90162001000400005

ALVARENGA, A. L. N.; CHIARINI-GARCIA, H.; CARDEAL, P. C.; MOREIRA, L. P.; FOXCROFT, G. R.; FONTES, D. O.; ALMEIDA, F. R. C. L. Intra-uterine growth retardation affects birthweight and post natal development in pigs, impairing muscle accretion, duodenal mucosa morphology and carcass traits. **Reproduction, fertility and development**, Sidney, v. 25, n. 2, p. 387-395, 2012.

ANDRETTA, INES et al. Situação brasileira da ocorrência de micotoxinas em alimentos para suínos e meta-análise do impacto produtivo. **Anais do X SINSUI – Simpósio Internacional de Suinocultura,** Porto Alegre, RS, p. 108-118, 2017.

BAPTISTA, ANTONIO SAMPAIO; HORII, JORGE; BAPTISTA, APARECIDO SAMPAIO. Fatores físico-químicos e biológicos ligados à produção de micotoxinas. **Boletim do Centro de Pesquisa de Processamento de Alimentos,** v. 22, n. 1, 2004.

BARBOSA, KIRIAQUE BARRA FERREIRA et al. Estresse oxidativo: conceito, implicações e fatores modulatórios. **Revista de nutrição,** v. 23, n. 4, p. 629-643, 2010.

BARCELOS, D.; De VITOR, M. C.; MUNHOZ, A. M. Identificação bacteriana em isolados de cascas de ovos expostos em comércio popular na região de Guarulhos. **Revista Saúde**, v. 11, n. 12, 2017.

BALDISSERA, M. D.; SOUZA, C. F.; BALDISSEROTTO, B.; ZIMMER, F.; PAIANO, D.; PETROLLI, T. G.; DA SILVA, A. S. Vegetable choline improves growth performance, energetic metabolism, and antioxidant capacity of fingerling Nile tilapia (Oreochromis niloticus). **Aquaculture**, v. 501, p. 224–229, 2019.

BALLOUN, S. L. Choline and tallow in breeder hen diets. **Poultry Science**, v. 35, n. 3, p. 737-738, 1956.

BAPTISTA, ANTONIO SAMPAIO et al. The capacity of manno-oligosaccharides, thermolysed yeast and active yeast to attenuate aflatoxicosis. **World Journal of Microbiology and Biotechnology,** v. 20, n. 5, p. 475-481, 2004.

BECKMAN, JOSEPH S. et al. Superoxide dismutase and catalase conjugated to polyethylene glycol increases endothelial enzyme activity and oxidant resistance. **Journal of Biological Chemistry**, v. 263, n. 14, p. 6884-6892, 1988.

BENNETT, J. W.; KLICH, M. Mycotoxins. **Clin.Microbiol.** Rev. v.16, n.3, p. 497–516, 2003.

BERTECHINI, A. G. **Nutrição de monogástricos**. 2. ed., rev. Lavras: Ufla, 373p, 2012.

BIANCHI, MARIA DE LOURDES PIRES; ANTUNES, LUSÂNIA MARIA GREGGI. Radicais livres e os principais antioxidantes da dieta. **Rev Nutr**, v. 12, n. 2, p. 123-30, 1999.

BIRBEN, ESRA et al. Oxidative stress and antioxidant defense. **World Allergy Organization Journal**, v. 5, n. 1, p. 9-19, 2012.

BÜNZEN, SILVANO; HAESE, DOUGLAS. Controle de micotoxinas na alimentação de aves e suínos. **Revista Eletrônica Nutritime**, v. 3, n. 1, p. 299-304, 2006.

CARDOSO, A. L. S. P. et al. Pesquisa de coliformes totais e coliformes fecais analisados em ovos comerciais no laboratório de patologia avícola de descalvado. **Arquivos do Instituto Biológico**, v. 68, n. 1, p. 19-22, 2001.

CHAUCHEYRAS-DURAND, F.; DURAND, H. **Probiotics in animal nutrition and health. Beneficial microbes**, v. 1, n. 1, p. 3-9, 2010.

COMBS JR, GERALD F.; MCCLUNG, JAMES P. The vitamins: fundamental aspects in nutrition and health. **Academic press**, 2016.

CUPERUS, TRYNTSJE et al. Protective effect of in ovo treatment with the chicken cathelicidin analog D-CATH-2 against avian pathogenic E. coli. **Scientific reports**, v. 6, p. 26622, 2016 https://doi.org/10.1038/srep26622

DIXON, RICHARD C.; HAMILTON, PAT B. Evaluation of some organic acids as mold inhibitors by measuring CO2 production from feed and ingredients. **Poultry Science**, v. 60, n. 10, p. 2182-2188, 1981.

DEVLIN, THOMAS M. **Manual de bioquímica: com correlações clínicas**. Editora Blucher, 1998.

DE ANDRADE, CARLA et al. Levedura hidrolisada como fonte de nucleotídeos para leitões recém-desmamados**. R. Bras. Zootec**, v. 40, n. 4, p. 788-796, 2011.

DE CASTRO SOUTO, POLLYANA CRISTINA MAGGIO et al. Principais micotoxicoses em suínos. **Veterinária e Zootecnia**, v. 24, n. 3, p. 480-494, 2017.

DIAS, ANDERSON SILVA. Micotoxinas em produtos de origem animal. **Revista Científica de Medicina Veterinária**, n. 30, 2018.

DILKIN, P., DIREITO, G., SIMAS, M.M.S., MALLMANN, C.A., CORREA, B. Toxicokinetics and toxicological effects of single oral dose of fumonisin B1 containing Fusarium verticillioides culture material in weaned piglets. **Chemico-Biological Interactions** 185, 157-162, 2010.

DILKIN, PAULO. Efeitos das micotoxinas na reprodução de suínos. **IV Simpósio Brasil Sul de Suinocultura, Chapecó, SC**, p. 57-67, 2011.

DILKIN, PAULO. Micotoxicose suína: aspectos preventivos, clínicos e patológicos. **Biológico**, v. 64, n. 2, p. 187-191, 2002.

DOI, KUNIO; UETSUKA, KOJI. Mechanisms of mycotoxin-induced dermal toxicity and tumorigenesis through oxidative stress-related pathways. **Journal of toxicologic pathology**, v. 27, n. 1, p. 1-10, 2014.

ENONGENE, E. N.; SHARMA, R. P.; BHANDARI, N.; MILLER, J. D.; MEREDITH, F. I.; VOSS, K. A.; RILEY, R.T. Persistence and reversibility of the elevation in free sphingoid bases induced by fumonisin inhibition of ceramide synthase. **Toxicological Sciences**, Orlando, v.67, p.173-181, 2002.

EULALIO, D. K. et al. Contaminação por micotoxinas em matérias-primas e rações destinadas à suinocultura de minas gerais. In: CONGRESSO ABRAVES, 18., 2015, Campinas. **Anais...** São Paulo: Associação Brasileira de Veterinários Especialistas em Suínos, 2015. p.218-220.

FARINA, GIOVANI et al. Performance of broilers fed different dietary choline sources and levels. **Ciência Animal Brasileira**, v. 18, 2017.

FERREIRA, ISABEL CFR; ABREU, RUI. Stress oxidativo, antioxidantes e fitoquímicos. **Bioanálise,** p. 32-39, 2007.

FERREIRA, A. L. A.; MATSUBARA, L. S. Radicais livres: conceitos, doenças relacionadas, sistema de defesa e estresse oxidativo. **Revista da associação médica brasileira,** v. 43, n. 1, p. 61-68, 1997.

FILHO, D. F. L.; PEREIRA, D. C. O.; POSSAMAI, E. Dietary supplementation of alternative methionine and choline sources in the organic broiler production in Brazil. **Braz. J. Poul. Sci**., v. 17, p. 489-496, 2015

FRANCISCATO C., LOPES S.T.A., SANTURIO J.M., WOLKMER P., MACIEL R.M., PAULA M.T., GARMATZ B.C. & COSTA M.M. Concentrações séricas de minerais e funções hepatica e renal de frangos intoxicados com aflatoxina e tratados com montmorilonita sódica. **Pesquisa Agropecuária Brasileira.** 41(8): 1573-1577, 2006

FREIRE, FRANCISCO DAS CHAGAS OLIVEIRA et al. Micotoxinas: importância na alimentação e na saúde humana e animal. Fortaleza: **Embrapa Agroindústria Tropical**, v. 48, 2007.

FREITAS, L. W. et al. Aspectos qualitativos de ovos comerciais submetidos a diferentes condições de armazenamento. **Revista Agrarian,** *4*(11), 66–72, 2011 https://doi.org/http://ojs.ufgd.edu.br/index.php/agrarian/article/view/998

FREITAS, B.V.; MOTA, M.M.; DEL SANTO, T.A. et al. Mycotoxicoses in Swine: a Review. **Journal of Animal Production Advances,** Wilmington, v.2, p.174-181, abr. 2012.

FREITAS, THIAGO STELLA DE et al. Efeito da aplicação de ácidos orgânicos sobre o desenvolvimento fúngico e as alterações do valor nutritivo do milho com alto teor de umidade. **Salão de Iniciação Científica (7.: 1995: Porto Alegre). Livro de resumos. Porto Alegre: UFRGS, 1995.**, 1995.

GUERRE, PHILIPPE. Worldwide mycotoxins exposure in pig and poultry feed formulations. **Toxins**, v. 8, n. 12, p. 350, 2016.

GUJRAL, DEEPAK et al. Effect of herbal liver stimulants on efficacy of feed utilization in commercial broiler chicken**. Indian Journal of Animal Research**, v. 36, n. 1, p. 43-45, 2002.

GELDERBLOM, W. C. et al. Fumonisin-induced hepatocarcinogenesis: mechanisms related to cancer initiation and promotion. **Environmental Health Perspectives**, v. 109, n. suppl 2, p. 291-300, 2001.

HAUSCHILD, LUCIANO et al. Digestibilidade de dietas e balanços metabólicos de suínos alimentados com dietas contendo aflatoxinas. **Ciência Rural**, v. 36, n. 5, p. 1570-1575, 2006.

HUSSEIN, HUSSEIN S.; BRASEL, JEFFREY M. Toxicity, metabolism, and impact of mycotoxins on humans and animals. **Toxicology**, v. 167, n. 2, p. 101-134, 2001.

HUWIG, A. et al. Mycotoxin detoxication of animal feed by different adsorbents. **Toxicology Letters,** v.122, n.2, p. 179-188, 2001.

IAMANAKA, BEATRIZ THIE; OLIVEIRA, IDJANE SANTANA; TANIWAKI, MARTA HIROMI. Micotoxinas em alimentos. **Anais da Academia Pernambucana de Ciência Agronômica**, v. 7, p. 138-161, 2013.

IGHODARO, O. M.; AKINLOYE, O. A. First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental

role in the entire antioxidant defence grid. **Alexandria journal of medicine,** v. 54, n. 4, p. 287-293, 2018.

JIA, RU et al. The toxic effects of combined aflatoxins and zearalenone in naturally contaminated diets on laying performance, egg quality and mycotoxins residues in eggs of layers and the protective effect of Bacillus subtilis biodegradation product. **Food and Chemical Toxicology**, v. 90, p. 142-150, 2016.

KASMANI F.B., TORSHIZI M.A.K. ALLAMEH A. & SHARIATMADARI F. A novel aflatoxin-binding Bacillus probiotic: Performance, serum biochemistry, and immunological parameters in Japanese quail. **Poultry Science**. 91(12): 1846-1853, 2012.

KASPER, CRAIG S.; WHITE, M. RANDALL; BROWN, PAUL B. Choline is required by tilapia when methionine is not in excess. **The Journal of nutrition**, v. 130, n. 2, p. 238-242, 2000.

LEESON, STEVEN; SUMMERS, JOHN D. Commercial poultry nutrition. **Nottingham University Press,** 2009.

LOPES, PAULO RODINEI SOARES et al. Crescimento e alterações no fígado e na carcaça de alevinos de jundiá alimentados com dietas com aflatoxinas. **Pesquisa agropecuária brasileira**, v. 40, n. 10, p. 1029-1034, 2005.

MADRIGAL-SANTILLÁN, E. et al. Antigenotoxic effect of Saccharomyces cerevisiae on the damage produced in mice fed with aflatoxin B1 contaminated corn. **Food and Chemical Toxicolog y**, Sto Tomás, v. 44, p. 2058-2063, 2006.

MALLMANN, C.; DILKIN, PAULO. Mycotoxins and mycotoxicosis in swine. **Translated and edited by G. Zaviezo and D. Zaviezo. Special Nutrients edition. Miami, FL USA**, v. 7, p. 80-81, 2011.

MALLMANN, C. A.; DILKIN, P.; RAUBER, R. H. Micotoxinas e micotoxicoses na avicultura. **Doenças das Aves**. 2ª ed. FACTA, Campinas, SP, p. 821-832, 2009.

MALLMANN, CARLOS AUGUSTO et al. Micotoxinas en ingredientes para alimento balanceado de aves. **In: XX Congreso Latinoamericano de Avicultura**. Porto Alegre, Brasil. 2007.

MAZIERO, MAIKE TAÍS; BERSOT, L. DOS S. Micotoxinas em alimentos produzidos no Brasil. **Revista brasileira de produtos agroindustriais**, v. 12, n. 1, p. 89-99, 2010.

MCDOWELL, LEE RUSSELL. **Vitamins in animal nutrition: comparative aspects to human nutrition**. Elsevier, 2012.

MINAMI, LUCIANA et al. Fumonisinas: Efeitos toxicológicos, mecanismo de ação e biomarcadores para avaliação da exposição. **Cienc Agrar**, v. 25, p. 207-24, 2004.

MUGESH, GOVINDASAMY; SINGH, HARKESH B. Synthetic organoselenium compounds as antioxidants: glutathione peroxidase activity. **Chemical Society Reviews**, v. 29, n. 5, p. 347-357, 2000.

OLIVEIRA, D. D.; OLIVEIRA, B. L. **Qualidade e tecnologia de ovos**. Lavras: Editora Ufla, 223 p., 2013.

OLIVEIRA IV, CARLOS AUGUSTO FERNANDES et al. Aflatoxin B1 residues in eggs of laying hens fed a diet containing different levels of the mycotoxin. **Food Additives & Contaminants**, v. 17, n. 6, p. 459-462, 2000.

OLIVEIRA, C.A.F, ALBUQUERQUE, R, CORREA, B, KOBASHIGAWA, E, REIS, T.A, FAGUNDES, A.C.A, LIMA, F.R. Produção e qualidade dos ovos de poedeiras submetidas à intoxicação prolongada com aflatoxina b1. **Arq. Inst. Biol**., São Paulo, v.68, n.2, p.1-4, jul./dez, 2001.

OKUMA, TARA A.; HUYNH, THU P.; HELLBERG, ROSALEE S. Use of enzyme-linked immunosorbent assay to screen for aflatoxins, ochratoxin A, and deoxynivalenol in dry pet foods. **Mycotoxin research**, v. 34, n. 1, p. 69-75, 2018.

PEREIRA, KELLY CRISTINA; DOS SANTOS, CARLOS FERNANDO. Micotoxinas e seu potencial carcinogênico. **Ensaios e Ciência**, v. 15, n. 4, 2011.

PIERRON, ALIX; ALASSANE-KPEMBI, IMOURANA; OSWALD, ISABELLE P. Impact of mycotoxin on immune response and consequences for pig health. **Animal Nutrition**, v. 2, n. 2, p. 63-68, 2016.

PINHEIRO, RAIZZA EVELINE ESCÓRCIO et al. Avaliação in vitro da adsorção de aflatoxina B1 por produtos comerciais utilizados na alimentação animal. **Arquivos do Instituto Biológico**, v. 84, 2017.

PIRES, F. M.; PIRES, S. F.; ANDRADE, C. L.; CARVALHO, D. P.; BARBOSA, A. F. C.; MARQUES, M. R. Fatores que afetam a qualidade dos ovos de poedeiras comerciais: armazenamento, idade, poedeira. **Nutritime Revista Eletrônica**, on-line, Viçosa, v. 12, n. 6, p. 4379-4385, 2015.

PIZZOLITTO, ROMINA P. et al. Binding of aflatoxin B1 to lactic acid bacteria and Saccharomyces cerevisiae in vitro: a useful model to determine the most efficient microorganism. **INTECH Open Access Publisher**, 2011.

PS, CHETHEN et al. Effects of lipotropic products on productive performance, liver lipid and enzymes activity in broiler chickens. **Poultry Science Journal,** v. 3, n. 2, p. 113-120, 2015.

RAYMOND, S. L.; SMITH, T. K.; SWAMY, H. V. L. N. Effects of feeding a blend of grains naturally contaminated with Fusarium mycotoxins on feed intake, serum chemistry, and hematology of horses, and the efficacy of a polymeric glucomannan mycotoxin adsorbent. **Journal of animal science**, v. 81, n. 9, p. 2123-2130, 2003.

RAJU, M. V. L. N.; DEVEGOWDA, G. Influence of esterified-glucomannan on performance and organ morphology, serum biochemistry and haematology in broilers exposed to individual and combined mycotoxicosis (aflatoxin, ochratoxin and T-2 toxin). **British poultry science**, v. 41, n. 5, p. 640-650, 2000.

REDDY, LALINI; ODHAV, BHARTI; BHOOLA, KANTI. Aflatoxin B1-induced toxicity in HepG2 cells inhibited by carotenoids: morphology, apoptosis and DNA damage. **Biological chemistry**, v. 387, n. 1, p. 87-93, 2006.

REIS, JANAÍNA SCAGLIONI et al. Efeitos dos tricotecenos na avicultura e métodos de controle. **Pubvet,** v. 6, p. Art. 1399-1404, 2016.

ROBLES-HUAYNATE, Rizal Alcides et al. Efeito da adição de probiótico em dietas de leitões desmamados sobre as características do sistema digestório e de desempenho. **Revista Brasileira de Saúde e Produção Animal**, v. 14, n. 1, 2013.

ROLL, V. F. B. et al. Hematologia de frangos alimentados com dietas contendo aflatoxinas e adsorvente de toxinas. **Archivos de zootecnia**, v. 59, n. 225, p. 93-101, 2010.

RIBEIRO, C.L.N; BARRETO, S.L.T; HANNAS, M.I. Micotoxinas encontradas em rações e alimentos utilizados na produção comercial de aves no brasil. **Nutritime Revista Eletrônica,** on-line, Viçosa, v.12, n1, p. 3910-3924, 2015.

RIBEIRO, R. P.; FLEMMING, J. S.; BACILA, A. R. Uso de leveduras (Saccharomyces cerevisae), parede celular de leveduras (SSCW), ácidos orgânicos e avilamicina na alimentação de frangos de corte. **Archives of Veterinary Science**, v. 13, n. 3, 2008.

SANTIN, ELIZABETH et al. Evaluation of the efficacy of Saccharomyces cerevisiae cell wall to ameliorate the toxic effects of aflatoxin in broilers. **International Journal of Poultry Science,** v. 2, n. 5, p. 341-344, 2003.

SANTURIO, J.M. Micotoxinas e micotoxicoses na avicultura. **Rev. Bras. Cienc. Avic.** vol.2 no.1 Campinas Jan./Abr, 2000.

SANTURIO, Janio M. Micotoxinas e micotoxicoses nos Suínos**. Acta Scientiae Veterinariae**, v. 35, p. S1-S8, 2007.

SHETTY, PRATHAPKUMAR HALADY; JESPERSEN, LENE. Saccharomyces cerevisiae and lactic acid bacteria as potential mycotoxin decontaminating agents. **Trends in food science & technology**, v. 17, n. 2, p. 48-55, 2006.

SCHMIDT, N. S.; SILVA, C. L. D. Pesquisa e desenvolvimento na cadeia produtiva de frangos de corte no Brasil. **Revista de Economia e Sociologia Rural**, 56(3), 467-482, 2018. http://dx.doi.org/10.1590/1234-56781806-94790560307

SIES, HELMUT; STAHL, WILHELM. Vitamins E and C, beta-carotene, and other carotenoids as antioxidants. **The American journal of clinical nutrition,** v. 62, n. 6, p. 1315S-1321S, 1995.

SILVA, G. A. et al. Impacto do desmame no comportamento e bem-estar de leitões: revisão de literatura. **Veterinária em Foco**, Canoas, v. 12, p. 32–48, jul./dez. 2014.

SOARES, C. et al. Microbiologia Fungos produtores de micotoxinas. **Portuguese Society for Microbiology Magazine**, Lisboa, v. 1, p. 1–9, jul. 2013.

VANNUCCHI, HELIO et al. Papel dos nutrientes na peroxidação lipídica e no sistema de defesa antioxidante. **Medicina (Ribeirão Preto Online**), v. 31, n. 1, p. 31-44, 1998.

ZEISEL, S. H.; DACOSTA, K. A., YOUSSEF, M.; HENSEY, S. Conversion of dietary choline to trimethylamine and dimethylamine in rats: dose-response relationship. **Journal Nutrition**., p. 119:800-804, 1989.

ZEISEL, STEVEN H. Choline deficiency. **The Journal of nutritional biochemistry**, v. 1, n. 7, p. 332-349, 1990.

YEGANI, MOJTABA et al. Effects of feeding grains naturally contaminated with Fusarium mycotoxins on performance and metabolism of broiler breeders. **Poultry science**, v. 85, n. 9, p. 1541-1549, 2006.

YU, JIUJIANG et al. Aspergillus flavus genomics: gateway to human and animal health, food safety, and crop resistance to diseases. **Revista iberoamericana de micología**, v. 22, n. 4, p. 194-202, 2005.

WASHBURN, K. W. et al. Effects and mechanism of aflatoxin on variation in shell strength. **Poultry Science**, v. 64, n. 7, p. 1302-1305, 1985.

WHITLOW, L. W.; HAGLER, W. M.; DIAZ, D. E. Mycotoxins in feeds. **Feedstuffs**, v. 74, n. 28, p. 1-10, 2002.

CARTA COMITÊ DE ÉTICA

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 "Utilização de um adsorvente de micotoxinas composto por parede de levedura em rações para poedeiras semipesadas.", protocolada sob o CEUA nº 3089070619 (ID 000963), sob a responsabilidade de **Marcel Manente Boiago** - 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 18/07/2019.

We certify that the proposal "Utilization of a mycotoxin adsorbent composed of yeast wall in diets for semi heavy hens. ", utilizing 60 Birds (60 females), protocol number CEUA 3089070619 (ID 000963), under the responsibility of **Marcel Manente Boiago** - 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 07/18/2019.

Finalidade da Proposta: Pesquisa (Acadêmica)

Vigência da Proposta: de 07/2019 a 06/2020 Área: Zootecnia

Origem:	Animais provenientes de outros projetos						
Espécie:	Aves	sexo:	Fêmeas	idade:	25 a 35 semanas	N:	60
Linhagem:	Hy Line			Peso:	1600 a 2000 g		

Local do experimento: A pesquisa será conduzida no setor de avicultura e no Laboratório de Nutrição Animal do Departamento de Zootecnia da UDESC Oeste Campus Chapecó.

Lages, 02 de novembro de 2020

José Cristani
Coordenador da Comissão de Ética no Uso de Animais
Universidade do Estado de Santa Catarina

Pedro Volkmer de Castilhos
Vice-Coordenador da Comissão de Ética no Uso de Animais
Universidade do Estado de Santa Catarina

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 "Adição de colina vegetal na alimentação de galinhas poedeiras no terço final de produção", protocolada sob o CEUA nº 9438130319 (ID 000865), 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 12/04/2019.

We certify that the proposal "Addition of vegetable choline to feed laying hens in the final third of production", utilizing 140 Birds (140 females), protocol number CEUA 9438130319 (ID 000865), 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 04/12/2019.

Finalidade da Proposta: Pesquisa (Acadêmica)

Vigência da Proposta: de 04/2019 a 12/2019 Área: Zootecnia

Origem:	Aviário do setor de avicultura CAV/UDESC						
Espécie:	Aves	sexo:	Fêmeas	idade:	70 a 71 semanas	N:	140
Linhagem:	galinhas poedeiras			Peso:	2500 a 3000 g		

Local do experimento: O experimento vai ocorrer no setor de avicultura localizado na Universidade do Estado de Santa Catarina (UDESC), em gaiolas de galinhas com capacidade de 7 animais/cada. As análises das amostras de ovos serão realizadas no Laboratório de Nutrição Animal (LANA) do departamento de Zootecnia da Universidade do Estado de Santa Catarina (UDESC). Em relação às análises bioquímicas serão realizadas no Laboratório de Anatomia Animal da mesma instituição.

Lages, 02 de novembro de 2020

José Cristani
Coordenador da Comissão de Ética no Uso de Animais
Universidade do Estado de Santa Catarina

Pedro Volkmer de Castilhos
Vice-Coordenador da Comissão de Ética no Uso de Animais
Universidade do Estado de Santa Catarina

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 "Adição de colina vegetal na dieta de leitões alimentados com ração experimentalmente contaminada com aflatoxina ", protocolada sob o CEUA nº 8763030419 (ID 000881), 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 12/04/2019.

We certify that the proposal "Addition of vegetable choline in the diet of piglets fed experimentally contaminated feed with aflatoxin", utilizing 48 Swines (48 males), protocol number CEUA 8763030419 (ID 000881), 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 04/12/2019.

Finalidade da Proposta: Pesquisa (Acadêmica)

Vigência da Proposta: de 05/2019 a 04/2020 Área: Zootecnia

Origem:	Animais de proprietários						
Espécie:	Suínos	sexo:	Machos	idade:	21 a 50 dias	N:	48
Linhagem:	não definido			Peso:	6 a 22 kg		

Local do experimento: O estudo será realizado em uma unidade experimental no interior da cidade de Xaxim, Oeste do estado de Santa Catarina, Brasil.

Lages, 07 de novembro de 2019

Ubirajara Maciel da Costa
Coordenador da Comissão de Ética no Uso de Animais
Universidade do Estado de Santa Catarina

em aberto
Vice-Coordenador da Comissão de Ética no Uso de Animais
Universidade do Estado de Santa Catarina