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PROGRAMA DE PÓS-GRADUAÇÃO EM ZOOTECNIA

DISSERTAÇÃO DE MESTRADO

IMPACTOS NEGATIVOS DO CONSUMO DE RAÇÃO CONTAMINADA COM FUMONISINA NA FASE INICIAL DE PRODUÇÃO DE FRANGOS DE CORTE SOBRE O DESEMPENHO, SAÚDE ANIMAL E BENEFÍCIOS DA SUPLEMENTAÇÃO COM FARINHA DE AÇAÍ (*Euterpe oleracea*)

MARCELA CRISTINA SILVEIRA DE SOUSA

CHAPECÓ, 2020

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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**

Orientadora: Prof[a]. Dr[a]Lenita Moura Stefani
Coorientador: Prof. Dr. Aleksandro Schafer da Silva

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Elaborada por
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como requisito parcial para obtenção do grau de
Mestre em Zootecnia

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GRATIDÃO!

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

IMPACTOS NEGATIVOS DO CONSUMO DE RAÇÃO CONTAMINADA COM FUMONISINA NA FASE INICIAL DE PRODUÇÃO DE FRANGOS DE CORTE SOBRE O DESEMPENHO, SAÚDE ANIMAL E BENEFÍCIOS DA SUPLEMENTAÇÃO COM FARINHA DE AÇAÍ (*Euterpe oleracea*)

AUTOR: Marcela Cristina Silveira de Sousa
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Chapecó, 10 de fevereiro 2020

A fumonisina é uma micotoxina produzida pelos fungos do gênero *Fusarium*, com destaque para o *F. verticillioides* isolado comumente no milho, um dos principais ingredientes da dieta animal. Na literatura, estudos com altas doses de fumonisina na dieta mostraram efeitos negativos à saúde e ao desempenho zootécnico das aves, porém são raros os estudos que avaliaram os efeitos dessa micotoxina presente na dieta de frangos de corte em fase inicial de desenvolvimento. Portanto, esse estudo teve como objetivo avaliar se baixas doses de fumonisina na dieta de frangos de corte, em fase inicial, interferem sobre os parâmetros de estresse oxidativo hepático, assim como verificar se o uso de um aditivo natural com propriedade antioxidante seria capaz de minimizar ou evitar essa ocorrência em aves que consumiram ração contaminada com fumonisina. Então, foram realizados dois experimentos. No experimento 1 a finalidade foi avaliar os efeitos hepáticos do consumo de fumonisina na dieta de frangos de corte, machos Cobb 500, em fase inicial. Para isso, oitenta aves de um dia de idade foram divididas em quatro tratamentos, controle (T0); T1, T2, e T3 que receberam alimentação contaminada experimentalmente com fumonisina nas concentrações de 0 ppm, 2,5 ppm, 5 ppm e 10 ppm, respectivamente, a partir do 12° dia de vida. Foram realizadas análises histopatológicas (do pulmão, intestino, fígado e baço), bioquímicas e dos marcadores de estresse oxidativo. Além disso, os animais foram pesados individualmente no início do experimento (dia 12) e nos dias 17 e 21 de idade. Os resultados permitiram concluir que a fumonisina na dieta dos pintinhos causou estresse oxidativo hepático e prejudicou a saúde intestinal, afetando negativamente o ganho de peso. No experimento 2 a finalidade foi verificar se a suplementação da dieta dos pintinhos com farinha de açaí (aditivo natural com propriedade antioxidante) seria capaz de minimizar o estresse oxidativo e garantir bom desempenho as aves. A fim de alcançar esse objetivo, 80 aves de 1 dia de idade foram divididas em quatro tratamentos: controle (TC) - que receberam dieta basal; TCA - dieta basal suplementada com 2% de farinha de açaí; TF - dieta experimentalmente contaminado com fumonisina (10 ppm) e TFA - aves alimentadas com ração experimentalmente contaminada com fumonisina (10 ppm) e suplementada com açaí (2%). O período experimental correspondeu aos primeiros 20 dias de vida dos pintinhos (fase inicial do ciclo de produção). As aves foram pesadas, amostras de sangue e fígado foram coletadas para avaliação de parâmetros indicadores de estresse oxidativo. Os resultados demonstraram que a farinha de açaí não evitou o estresse oxidativo, mas minimizou os efeitos negativos causados

pelo consumo dessa micotoxina; consequentemente observou-se melhora no desempenho destes animais na fase inicial de produção. Por fim, concluímos que a fumonisina demonstrou afetar negativamente a função hepática em pintos em estágio inicial e promoveu o estresse oxidativo hepático, mais evidente após 10 dias de consumo de ração contaminada. Essas mudanças refletem condições fisiológicas e funcionais e baixo desempenho produtivo. No entanto, ração contendo 2% de resíduo de farinha de açaí demonstrou efeitos positivos na ação antioxidante, minimizando em parte os efeitos negativos causados pela FB1. Este fato favoreceu o desempenho dos animais testados. Estes resultados reforçam o potente efeito antioxidante do açaí.

Palavras-chave: Antioxidante. Avicultura. Estresse oxidativo. Micotoxina.

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

NEGATIVE IMPACTS OF FEED CONSUMPTION CONTAMINATED WITH FUMONISIN IN THE INITIAL PHASE OF POULTRY ON PERFORMANCE, ANIMAL HEALTH AND BENEFITS OF SUPPLEMENTING WITH AÇAÍ FLOUR (*Euterpe oleracea*)

AUTHOR: Marcela Cristina Silveira de Sousa
ADVISOR: PhD. Lenita Moura Stefani
Chapecó, February 10, 2020

ABSTRACT

Fumonisin is a type of mycotoxin produced by Fusarium fungi, with emphasis on *F. verticillioides* isolated commonly in corn, one of the main ingredients of the animal diet. In the literature, studies with high doses of fumonisin in the diet presented the negative effects on bird health and animal performance, but studies that evaluate the effects of this mycotoxin on chick, male Cobb 500 diet are still rare. Therefore, this study aimed to evaluate if low doses of fumonisin in the diet of broiler chicken in initial phase could cause hepatic oxidative stress, as well as to verify if the use of a natural additive with antioxidant properties could reduce the oxidative stress of the birds that consumed contaminated feed with fumonisin. Thus, two experiments were performed: experiment 1 evaluated the hepatic effects on fumonisin consumption in the diet of broiler chickens in the early phase. For this, eighty one-day-old broiler chicks were divided into four treatments, control (T0); T1, T2 and T3 and on day 12th received feed experimentally contaminated (0 ppm, 2.5 ppm, 5 ppm and 10 ppm), respectively. Histopathological, biochemical analysis and oxidative stress markers were performed. In addition, the animals were weighed at the beginning of the experiment (day 12) and at 17 and 21 daysof age. The results allowed to conclude that the fumonisin in chicks' diet causes hepatic oxidative stress and impairs intestinal health, affecting negatively weight gain. The experiment 2 was performed to verify whether acai residue flour (natural additive with antioxidant property) supplementation could minimize the oxidative stress and ensure good animal performance. In order to reach this object, eighty one-day-old broiler chicks were divided into four treatments: control (TC) - which received basal diet; TCA - basal diet supplemented with 2% açai flour; TF - experimental diet contaminated with fumonisin (10 ppm) and TFA - poultry fed experimentally with contaminated diet with fumonisin (10 ppm) and supplemented with acai (2%). The trial period corresponds to the first 20 days of the curtain life (initial phase of the production cycle). Birds were weighed, and blood, intestine and liver samples were collected for evaluation of oxidative stress indicators. The results showed that açaí flour did not prevent oxidative stress, but minimized the negative effects caused by the consumption of this mycotoxin; consequently, an improvement in the performance of these animals was observed in the initial production phase. Finally, we conclude that fumonisin has been shown to negatively affect liver function in early-stage chicks and promoted hepatic oxidative stress, more evident after 10 days of consumption of contaminated feed. These changes reflect physiological and functional

conditions and low productive performance. However, a diet containing 2% of açai flour residue demonstrated positive effects on the antioxidant action, partially minimizing the negative effects caused by FB1. This fact favored the performance of the tested animals. These results reinforce the potent antioxidant effect of açai.

Keywords: Antioxidant. Mycotoxin. Oxidative stress. Poultry.

SUMÁRIO

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 "Efeitos de fumonisina na dieta de pintainhos de corte nos primeiros dias de vida sobre saúde e desempenho dos animais", protocolada sob o CEUA nº 5960011018 (ID 000749), 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 10/10/2018.

We certify that the proposal "Effects of fumonisin on the diet of cutting chicks in the first days of life on animal health and performance", utilizing 96 Birds (96 males), protocol number CEUA 5960011018 (ID 000749), 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 10/10/2018.

Finalidade da Proposta: Pesquisa (Acadêmica)

Vigência da Proposta: de 11/2018 a 07/2019 Área: Zootecnia

Origem:	Animais provenientes de estabelecimentos comerciais						
Espécie:	Aves	sexo:	Machos	idade:	1 a 21 dias	N:	96
Linhagem:	Coob500			Peso:	45 a 1000 kg		

Local do experimento: Os animais utilizados neste experimento serão comprados da empresa Globo aves, e mantidos em galpão experimental da Udesc oeste. Setor de avicultura.

Lages, 18 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

CAPÍTULO I

REVISÃO DE LITERATURA

1. Introdução

A cadeia produtiva avícola brasileira possui destaque mundial, tanto em termos de produção quanto exportação e isso se deve a evolução tecnológica pela qual o setor passou ao longo dos anos. O uso da inovação, tecnologia, melhoramento genético, nutrição animal, manejo e ambiência foram fatores importantes e tiveram reflexo imediato na conversão alimentar das aves e no processo produtivo (RIZZI, 1993; PATRICIO et al., 2012; MENDES, 2014).

Considerando os dados de produção divulgados pelo Departamento de Agricultura dos Estados Unidos (USDA/FAS, 2018), observa-se que os Estados Unidos é o país líder mundial, com um volume de 19,4 milhões de toneladas, respondendo por 20% do total mundial. O segundo lugar é ocupado pelo Brasil, com 14%, seguido pela União Europeia e China, com participações no total mundial próximas de 12% cada país. Estes quatro países antes mencionados respondem por cerca de 60% da produção, sendo os restantes 40% da produção mundial efetuada por inúmeros outros países.

No entanto, a manutenção deste nível de produção, requer a superação de desafios diários em todas as etapas do processo. Considerada por muitos, uma das fases mais críticas, a chamada fase inicial de produção compreende, em média, os primeiros 21 dias de vida das aves de corte. Durante essa fase, a ave passa por um rápido desenvolvimento e se prepara para ter o desempenho esperado ao abate. Os primeiros 21 dias são marcados pelo rápido desenvolvimento da ave e também por mudanças fisiológicas importantes, tais como: desenvolvimento do sistema termorregulador; início do desenvolvimento de imunocompetência, além do desenvolvimento de músculos, sistema ósseo e adiposo; portanto, o comprometimento dessa fase de desenvolvimento afeta negativamente o desempenho final do lote (ABREU, 1999). Segundo Araújo (2003) todas as mudanças ocorridas na morfologia intestinal após a eclosão são muito sensíveis às modificações promovidas pela suplementação de nutrientes. Nesse sentido, fatores de estresse como, densidade populacional e/ou contaminantes associados à dieta (micotoxinas) são conhecidos

por afetar negativamente o bem-estar, a saúde e a produtividade das aves comerciais (HENRY et al., 2000; MOSCA et al., 2015; MURUGESAN et al., 2015). A boa adaptação e a funcionalidade dos sistemas digestório e imunológico das aves, dependem totalmente da qualidade das matérias-primas e do valor nutricional da alimentação que elas recebem (ABREU, 1999). Aliando este fato, a todos processos fisiológicos intrínsecos ao desenvolvimento inicial, qualquer dificuldade ou agressão sofrida nesse período implicará diretamente em queda no desempenho final da ave, elevando a mortalidade, os custos de produção e reduzindo o volume de carne produzido, assim como perda de sua qualidade (ABREU, 1999).

Fatores antinutricionais, oxidação, putrefação, toxinas, dentre outras, são características que podem acometer as matérias primas, seja por aspectos naturais de cada ingrediente, falhas no seu processo de produção ou obtenção, ou mesmo devido a uma estocagem malfeita (BUTOLO, 2010; RODRIGUES, 2009; HAMID et. al., 2014; FEKADU GEMEDE, & RETTA, 2014;).

Nesse contexto, a contaminação alimentar pelas micotoxinas tem sido uma questão prioritária da Food and Agriculture Organization of United Nations (FAO) e da Organização Mundial da Saúde (OMS), em função dos impactos toxicológicos significativos na saúde humana e animal. Com o avanço das pesquisas ficou provado que essas substâncias possuem propriedades extremamente tóxicas (ESKOLA et. al., 2019). Assim, cada vez mais, as micotoxinas vêm recebendo espaço no cenário mundial, algumas de maior importância, como as fumonisinas.

Neste trabalho avaliamos o efeito da ação da fumonisina B1 (FB1) sobre parâmetros de estresse oxidativo, saúde e desempenho em frangos de corte na fase inicial de produção, e a capacidade antioxidante da farinha de açaí adicionada à ração de frangos de corte desafiados pela FB1.

1.1. Micotoxinas

Segundo a FAO/OMS, aproximadamente 25% dos alimentos produzidos no mundo estão contaminados com micotoxinas - perdas anuais de um bilhão de dólares. A presença de fungos em alimentos e sua consequente toxicidade são conhecidas há muito tempo, mas a questão tem recebido mais atenção nas últimas décadas. As micotoxinas são substâncias

tóxicas, resultantes do metabolismo secundário de fungos filamentosos, produzidas quando o fungo atinge a maturidade (FREIRE et al., 2007). O termo micotoxina é derivado da palavra grega "*mykes*", que significa fungo, e do latim "*toxican*", que significa toxinas, e é utilizado, principalmente, para fungos de alimentos e de rações (FREIRE et al., 2007; IAMANAKA; OLIVEIRA; TANIWAKI, 2013). Essas substâncias contaminam os produtos agrícolas como milho, soja, e arroz, que são ingredientes básicos na formulação de dietas para nutrição animal.

As micotoxinas são produzidas através de uma série consecutiva de reações catalisadas por enzimas. Sugere-se que as micotoxinas são formadas quando ocorre acúmulo de precursores metabólicos primários e assim, para evitar esse acúmulo, os fungos desviam o excesso destes precursores para a elaboração de metabólitos secundários, para mantem o primário operando (OKUMA et al., 2018). Atualmente já foram identificadas mais de 400 moléculas de micotoxinas, sendo aflatoxina B1, zearalenona, toxina T-2, desoxinivalenol, ocratoxina A, fumonisina e patulina consideradas de maior relevância para a cadeia produtiva de proteína animal. (YIANNIKOURIS e JOUANY, 2002; OKUMA et al., 2018). A presença de micotoxinas tem causado perdas consideráveis na avicultura em nível mundial (SANTIN et al., 2000).

Essas substâncias merecem atenção, pois oferecem riscos, prejudicando o metabolismo, a saúde e o desempenho dos plantéis. A presença das micotoxinas representa um risco potencial ao desenvolvimento do agronegócio brasileiro. No entanto, desde que foram descobertas, há mais de 50 anos, ainda não foi encontrado um método satisfatório de prevenção da contaminação em produtos agrícolas (IAMANAKA; OLIVEIRA; TANIWAKI, 2013).

No Brasil, a Agência Nacional de Vigilância Sanitária (ANVISA), estabelece os limites máximos para aflatoxinas (AFB1+AFB2+AFG1+AFG2 e AFM1), ocratoxina A (OTA), desoxinivalenol (DON), fumonisinas (FB1 + FB2), patulina (PAT) e zearalenona (ZON) admissíveis em alimentos prontos para oferta ao consumidor e em matérias-primas (ANVISA, 2011 e ANVISA, 2017). Para produtos de origem animal as legislações atuais apresentam limites máximos apenas para aflatoxinas e zearalenona. No entanto, foi instituído o Grupo de Trabalho sobre Micotoxinas em produtos destinados à alimentação animal (BRASIL, 2006), que apresentam duas propostas para Limites Máximos de Tolerância

(LMT) para aflatoxina B1, fumonisinas B1 e B2, zearalenona, DON e ocratoxina A nas rações e concentrados destinados à alimentação animal.

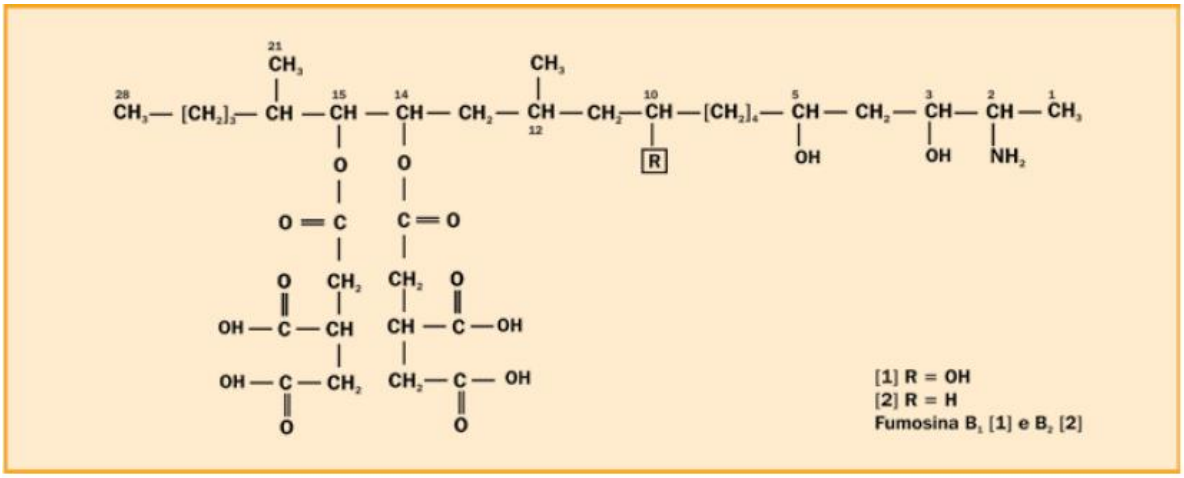
Os efeitos causados pelas micotoxinas em animais e humanos são variados, desde câncer hepatocelular causado por micotoxinas até alterações dérmicas causadas por tricotecenos, além de imunodepressão e inibição de absorção de nutrientes em nível gastrointestinal. (ESCRIVÁ et al.,2015; SAVI & ZENAIDE, 2020).

1.2. Fumonisina

As fumonisinas são produzidas pelos fungos *Fusarium verticillioides***,** *F. proliferatum* e *Alternaria alternata f. sp. lycopersici* (CHEN et al., 1992. RHEEDER et al., 2002). Foram descritas pela primeira vez em 1988 por GERDERBOLM e colaboradores e desde então foram identificados 28 análogos divididos em quatro grupos principais, nomeados como A, B, C e P (RHEEDER et al., 2002).

Mais de dez tipos de fumonisinas foram isoladas e caracterizadas, dividindo-as em quatro grupos, denominados de fumonisinas das séries A, B, C e P (RHEEDER; MARASAS; VISMER, 2002). A fumonisina B1 (FB1) é a forma predominante entre os análogos de fumonisinas (designada como A1, A2, B1, B2, B3 e B4) (NORRED, 1993; VOSS et al., 2007). O nome fumonisina deriva de *Fusarium moniliforme* (atualmente denominado *F. verticillioides*), de onde o primeiro membro desta classe de compostos tóxicos, fumonisina B1(FB1), foi caracterizado. Trata-se de um diester de propano – 12,3-ácido tricarboxílico e 2- amino-12, 16-dimetil-3,5,10,14, 15-pentahidroxieicosano. A esta classe pertence também os outros dois membros, FB2 e FB3, que se distinguem pela ausência de grupos livres de hidroxila (DESJARDINS, 2006).

Figura 1. Estrutura da Fumonisina.



Fonte: Brasil, 2009.

De modo geral, o tempo e temperatura ótimos para a produção de FB1 são sete semanas a 25°C (BRASIL, 2009). Encontradas em todo o mundo, são as principais invasoras dos grãos de milho no campo e estão associadas à contaminação de mais de 90% do milho no Brasil (POZZI et al., 2002; LANZA et al., 2014).

As fumonisinas estão associadas a uma variedade de efeitos adversos à saúde animal. Entre os animais domésticos, os cavalos são as espécies mais sensíveis às fumonisinas (GIANNITTI et al., 2011), responsáveis pela ocorrência de leucoencefalomalácia (LEME), uma intoxicação que acomete o sistema nervoso central dos equinos. O consumo de apenas 10 ppm (partes por milhão) durante 30 dias já pode ocasionar a morte do animal (VINCELLI; PARKER, 2002). Suínos também são afetados pelo consumo de fumonisinas, sendo que o milho contaminado com 5 ppm pode causar anorexia (VOSS; SMITH; HASCHEK, 2007), enquanto que edema pulmonar ocorre pelo consumo de alimentos contaminados em níveis acima de 92 ppm. A morte geralmente ocorre em 48 horas depois de consumir a ração infectada (AHANGARKANI; ROUHI; AZIZI, 2014).

A exposição a micotoxinas pode causar imunotoxicidade e prejudicar a função reprodutiva em animais de produção. Além disso, a exposição de tecidos, como rins, fígado e intestinos, às micotoxinas podem exercer efeitos histopatológicos mudanças que podem interferir no crescimento e sobrevivência dos animais. (YANG et al., 2020).

Sabe-se que as micotoxinas reduzem o desempenho do crescimento e a eficiência alimentar, aumentam a mortalidade e comprometem o sistema imunológico de galinhas (AWAD et al., 2006; YEGANI et al., 2006; SMITH et al., 2012). A fumonisina causa ainda,

nas aves, hepatomegalia, diminuição da absorção intestinal e susceptibilidade a infecções secundárias (LEDOUX et al., 1992). Dietas contaminada com FB1 diminuíram a altura das vilosidades, a relação vilosidade/cripta (RAUBER et al., 2013; ANTONISSEN et al., 2015) e o ganho de peso corporal (LEDOUX et al., 1992) e aumentaram vários sinais clínicos, incluindo necrose hepática, diarréia e raquitismo (BROWN et al., 1992) em aves de criatório. Vacari et al. (2017) ao associarem severidade de lesões de língua em frangos de corte com a qualidade do milho em ração, relatam que a porcentagem de lesões de grau 3 está positivamente correlacionada com a presença de vomitoxina, fumonisina e toxina T-2. Como a fumonisina pode afetar o epitélio intestinal (BOUHET et al., 2004; ANTONISSEN et al., 2014), é conhecido por ser um fator predisponente na enterite necrótica em frangos de corte (ANTONISSEN et al., 2015).

Além disso, no que tange as alterações no metabolismo, sabe-se que ocorrem alterações no metabolismo mitocondrial, na produção de ROS e na desregulação do cálcio estão envolvidas na neurodegeneração (ABRAMOV et al., 2005; BURCHELL et al., 2010). As micotoxinas regulam as vias de sinalização, estresse oxidativo, endoplasmático estresse reticular, apoptose e proliferação em células suína e bovina (YANG et al., 2020).

Para investigar o mecanismo celular da toxicidade do FB1, DOMIJAN & ABRAMOV (2011) realizaram uma série de experimentos em linhas de células neuronais e gliais, tratadas com FB1 em concentração que provavelmente será consumida e constataram que o FB1 inibe o complexo mitocondrial, causando despolarização da membrana mitocondrial, alterações na sinalização de cálcio e produção de ROS. MYBURG et al. (2009) demonstraram que a FB1 tem como alvo específico as mitocôndrias, o núcleo e o nucléolo das células, sugerindo que também é possível que o FB1 exerça seus efeitos biológicos através da ligação a macromoléculas nessas organelas.

Por assemelhar-se estruturalmente às bases esfingóides, o mecanismo de toxicidade e carcinogenicidade da fumonisina B1 consiste na inibição da ceramida sintetase (n-Acil Transferase), uma enzima chave na via de biossíntese dos esfingolipídeos. Essa inibição estimula o aumento da concentração de esfinganina e esfingosina no soro dos animais expostos a essa micotoxina, causando acúmulo de esfinganina e depleção de esfingolipídeos complexos importantes nas funções celulares. A manutenção de baixas concentrações de

esfinganina e esfingosina livres é importante, uma vez que esses compostos possuem uma atividade biológica intrínseca considerável (MINAMI et al., 2004).

1.3. Estresse oxidativo

O estresse oxidativo é definido então como um desequilíbrio entre a produção de radicais livres e o sistema antioxidante, responsável por manter a homeostase do organismo *(*BETTERIDGE, 2000*).*

A oxidação completa de nutrientes para o suprimento de energia biológica é um dos pré-requisitos mais importantes para a formação de formas de vida mais elevadas. No entanto, as células que se beneficiam da respiração oxidativa também sofrem com espécies reativas de oxigênio porque se adaptaram ao oxigênio como fonte de energia (MATSCHKE et al., 2019).

Os pró-oxidantes/radicais livres contêm mais de um elétron não emparelhado, instável e altamente reativo à reação com outras espécies. Em geral, as rotas metabólicas contínuas no sistema humano produzem ROS/radicais livres que atacam especialmente amidos, gorduras, proteínas e ácidos nucleicos. Poucas fontes destinadas ao desenvolvimento de ROS incluem endógenas, por exemplo, mitocôndrias, xantina oxidase (SISEIN, 2001) peroxissomos, inflamação, fagocitose, vias de ácido araquidônico, exercício e lesão por isquemia/reperfusão (HUSSAIN & KUMAR, 2012), íons metálicos livres, explosão respiratória (TAKASHIMA et al., 2012), fumaça de cigarro, solventes industriais e exógenos, como poluentes ambientais e irradiação UltraVioleta.

As células saudáveis equilibram a formação e eliminação de espécies reativas de oxigênio, através de um sistema antioxidante eficiente (MATSCHKE et al., 2019). O sistema de defesa antioxidante é composto pelas vias enzimática e não-enzimática, que incluem as enzimas glutationa-S-transferase (GST), catalase (CAT), glutationa peroxidase (GSH-Px), glutationa (GSH), proteínas e vitaminas. Eles podem desativar os efeitos nocivos de ROS. O corpo, então passa a metabolizar mais enzimas antioxidantes a fim de eliminar ROS (FREI, 1999; AN et al., 2008; AKSU et al., 2016; 2017).

Quando a concentração de espécies reativas não é controlada por mecanismos de defesa endógena, como antioxidantes (tocoferóis, ácido ascórbico e glutationa) ou enzimas

envolvidas na eliminação de radicais de oxigênio (catalase, peroxidase e superóxido dismutase), ocorrem danos oxidativos às proteínas, lipídios e DNA, que podem levar a citotoxicidade, genotoxicidade e até carcinogênese, quando células danificadas (mutadas) podem proliferar. (GAGNÉ, 2014). Portanto, o estresse oxidativo desempenha um papel importante na fisiologia e patologia de várias doenças (MATSCHKE et al., 2019).

1.4. Antioxidantes

Antioxidantes são compostos que atuam inibindo e/ou diminuindo os efeitos do estresse oxidativo, desencadeados pelo excesso de radicais livres e compostos oxidantes. São importantes porque com o combate aos processos oxidativos há menores chances de danos ao DNA e às macromoléculas, amenizando, assim, os danos cumulativos que podem desencadear doenças (SCHAUSS et al., 2006). Esses compostos evitam ou diminuem os danos associados a doenças relacionadas ao estresse oxidativo, neutralizando o efeito deteriorador da ERO e apaziguam os radicais livres desempenhando papel crucial na conservação das melhores funções celulares (KUMARI et al., 2019).

Um grande número de antioxidantes naturais e sintéticos foram encontrados. Antioxidantes endógenos podem ser enzimáticos ou não enzimáticos (AGUILAR et al., 2016). Antioxidantes enzimáticos endógenos consistem em glutationa peroxidase, superóxido dismutase e catalase, enquanto antioxidantes não enzimáticos são ácido úrico, ácido lipóico, bilirrubina, glutationa e melatonina. Antioxidantes exógenos são carotenóides, vitamina E, A e C, flavonóides naturais ou diferentes outros compostos. A vitamina C é um antioxidante solúvel em água que atua junto com a vitamina E para proteger os lipídios da peroxidação (ASÁ et al., 2019).

1.5. Açaí

O açaí (*Euterpe oleracea*) é um dos mais importantes produtos do extrativismo nacional e um dos principais responsáveis por dar visibilidade à biodiversidade da Floresta Amazônica. O açaizeiro ocorre espontaneamente no Brasil, nos Estados do Amapá, Maranhão, Pará (CALZAVARA 1972; CAVALCANTE, 1991), Tocantins (BALICK, 1986) e no Mato Grosso (Macedo, 1995). Além das fronteiras brasileiras, é encontrado na Guiana,

Guiana Francesa, Suriname, Venezuela (CALZAVARA 1972; ROOSMALEN, 1985; CAVALCANTE, 1991) e Colômbia (BALICK, 1986).

Segundo o relatório mensal da CONAB (jul/19), o estado do Pará é o maior produtor, com produção anual de mais de 1,3 milhão toneladas, em uma área superior a 219 mil hectares. Em seguida está o Amazonas (52 mil toneladas) e Roraima (com 3,5 mil). Apesar de o açaí ser um produto originário das terras amazônicas brasileiras, os EUA são o país com o maior processamento e exportação de produtos à base de açaí. A porção destinada ao consumo humano do fruto da palmeira é a polpa, que constitui cerca de 30% da massa total do fruto em relação aos 68% ocupados pela semente ou caroço (YUYAMA et al., 2011).

Considerando o valor total produzido, 90% desse volume correspondem a resíduos gerados após o processamento agroindustrial da fruta para produção de celulose. Esses resíduos são compostos basicamente de sementes e fibras ligadas, que têm potencial como material lignocelulósico renovável (TEIXEIRA et al. 2002).

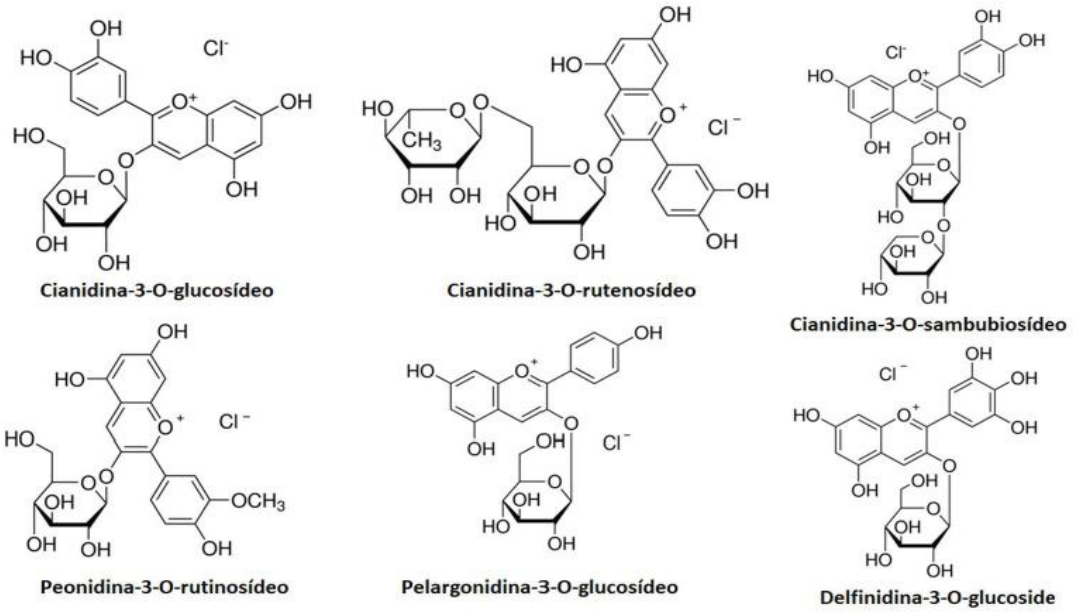
O uso da semente de açaí despertou o interesse também do setor da produção pecuária, para a alimentação animal. Pesquisadores observaram que, a inclusão de sementes de açaí como substituto do feno aumenta a ingestão e o ganho de peso diário em ovelhas. (GOMES et al. 2012). Arruda et al. (2018) ao analisar o potencial uso de sementes de açaí como ingrediente alternativo, na alimentação de frangos até os 28 dias, demonstraram que o farelo de açaí pode ser incluído em proporções de até 10% nas dietas de frangos sem prejudicar o desempenho. O nível de inclusão de 10% mostrou-se economicamente igual ao tratamento controle, o que demonstra ser uma alternativa viável para a produção de frangos de crescimento lento, e a redução dos impactos negativos gerados por resíduos no meio ambiente.

Estudos mostram que o consumo de alimentos ricos em polifenóis, principalmente os da classe dos flavonoides, tem sido relacionado com o baixo risco no desenvolvimento de diversas doenças, devido às propriedades antioxidantes presentes no alimento. Dentro desse contexto, um fruto que vem ganhando destaque, entre esses alimentos, é o açaí (*Euterpe oleracea* Mart.), por apresentar em sua composição quantidades significativas de um grupo dos flavonoides, conhecido como antocianinas (LIMA et al., 2012; LIN et al., 2007). O que o torna uma das cinco frutas com maior potencial antioxidante, apresentando diversas propriedades − anti-inflamatória e farmacológica – associadas ao combate de doenças

desencadeadas pela presença excessiva deespécies reativas de oxigênio (radicas livres) (KANG et al., 2010; POZO-INSFRAN et al., 2006; PACHECO-PALENCIA et al., 2008).

Os flavonoides são uma grande classe de compostos polifenólicos de baixa massa molecular que possuem um esqueleto de 15 carbonos constituído por dois anéis de benzeno, ligados através de um anel de pirano heterocíclico. Essa classe de polifenóis foi classificado em seis subgrupos: flavonas, flavonóis, flavanonas, flavan-3-ols, isoflavonas e antocianidinas (GOMES et al., 2016).

Figura 2. Estrutura química das Antocianinas encontradas no Açaí (*Euterpe oleacea*).



Fonte: Cedrim, P. C. A. S. et al.,2018.

No açaí, a classe das antocianinas desperta maior interesse devido à sua prevalência (POZO-INSFRAN et al., 2006). Cesar et al (2014) relataram que o açaí apresentou maior poder antioxidante do que outros frutos ricos em antocianina, como mirtilos e amoras, e observaram que a cianidina-3-glucosídeo (1040 mg/L de polpa) era a antocianina em maior quantidade na polpa do fruto. Felssner et al (2016) ao analisarem o caroço de açaí, encontraram a seguinte composição nutricional, na matéria seca: Proteína Bruta 4,23ér%; Extrato Etéreo 3,18%; Matéria Mineral 6,79%; Fibra Bruta 28,3% e Fibras Digestíveis Totais 88,97%. Este ingrediente apresentou elevada capacidade antioxidante, vista pelo percentual de descoloração do radical DPPH pelos antioxidantes, os quais foram de 49,8%; 93,4% e 66,8% para os extratos etéreo, etanólico e aquoso, respectivamente, indicando alto teor de

compostos antioxidantes solúveis em álcool e água. Nesta análise, os resultados de descoloração foram muito superiores ao BHT (padrão; 19,35%). O caroço de açaí apresentou 5,95% de polifenóis totais, 3,43% de taninos e 2,94% de antocianinas.

Fortuoso et al. (2019) ao suplementar a dieta de galinhas poedeiras com farinha de açaí observaram capacidade antioxidante estimulada, o que proporcionava benefícios à saúde, resultando em maiores taxas de produção, além de melhores qualidade. Além disso, com a redução da peroxidação lipídica do ovo, à qualidade dos ovos foi preservada, aumentando sua vida útil.

1.6. Objetivo

Esse estudo teve como objetivo avaliar se baixas doses de fumonisina na dieta de frangos de corte em fase inicial de desenvolvimento causam estresse oxidativo hepático e afetam o desempenho dessas aves, assim como verificar se o uso de um aditivo natural com propriedade antioxidante seria capaz de minimizar ou evitar o extresse oxidativo das aves que consumiram ração contaminada com fumonisina.

CAPÍTULO II
MANUSCRITOS

Os resultados desta dissertação são apresentados na forma de dois manuscritos, com formatação de acordo com as orientações das revistas aos quais foram submetidos.

MANUSCRITO 1

Submetido a *Microbial Pathogenesis*

Pathogenetic effects of feed intake containing low-dose of fumonisin (*Fusarium verticillioides*) in early broiler chicks

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Abstract

Fusarium verticillioides is often responsible for contamination of poultry feed with the mycotoxin fumonisin. The objective of the study was to determine whether low-dose fumonisin-contaminated feed in the early phase of broiler chicks causes oxidative imbalances and interferes with weight gain. One-day-old male Cobb 500 broiler chicks (n=80) were divided into four treatments of 20 birds each, all of which were fed basal feed until the 11th day of age. From day 12, some birds were challenged with fumonisin in the feed: Control (T0) continued receiving the basal ration; treatments T1, T2, and T3 were given feed experimentally contaminated with fumonisin at concentrations of 2.5 ppm, 5 ppm and 10 ppm, respectively. After the 5th (day 17) and 10th (day 21) days, ten birds from each treatment were euthanized for blood and tissue collection to measure histopathological, biochemical and oxidative stress markers. All animals were weighed individually at the beginning of the experiment (day 12), and at 17 and 21 days of age. Birds that ingested 10 ppm of fumonisin (T3) had lower ($P<0.05$) weight

gain compared to those in T0. At 21 days, the body weights of the T1, T2 and T3 chicks were 1.3%, 8.97% and 18.7% lower, respectively, than those of T0. No histological lesions in the livers were observed for any treatment; however, higher levels of reactive oxygen species (ROS: day 21) and lipoperoxidation (LPO: days 17 and 21) were observed, associated with lower liver activity of the enzymes superoxide dismutase (SOD: day 21), glutathione peroxidase (GPx: day 17 and 21) and glutathione S-transferase (GST: day 21) when birds consumed 5 or 10 ppm of fumonisin. In serum, LPO levels and SOD and GPx activities were lower for groups consuming high doses of fumonisin in the diet (T2 and T3); ROS levels and GST activity were higher in these birds. Birds that consumed fumonisin-containing diets had lower levels of alanine aminotransferase, total protein and albumin (T3); as well as lower serum glucose levels (days 17 and 21), uric acid and triglycerides (day 21) in T3 than in T0. At 21 days, there were smaller crypt sizes and intestinal villi in birds that consumed high levels of fumonisin. These results suggest that low-dose fumonisin in chick diet causes hepatic oxidative stress and impairs intestinal health, consequently negatively affecting weight gain.

Keywords: hepatotoxicity, mycotoxins, oxidative stress, performance, poultry farming, pathogenesis.

1. Introduction

Fumonisins are secondary metabolic compounds produced by *Fusarium verticillioides* and *F. proliferatum*. They constitute a group of mycotoxins that contaminate corn and its derivatives, alone and in combination with other mycotoxins

[1]. Fifteen fumonisin variants have been identified [2]; however, the main fumonisins that negatively impact animal production are B1 (FB1), B2 (FB2) and B3 (FB3) [3]. The high incidence of fumonisin in Brazilian corn is a cause of concern; a study from the 1990s reported 100 and 97.4% of fumonisin contamination with B1 (FB1) and B2 (FB2), respectively [4]. To avoid this problem, a prophylactic approach can be taken during cultivation and grain management, including harvestin at appropriate times, drying at appropriate temperatures and proper storage conditions.

Mycotoxins are known to reduce animal performance, growth and feed efficiency, to increase mortality and to compromise the immune system of birds [5, 6, 7]. In horses, consumption of fumonisin-contaminated corn may cause leukoencephalomalacia; in pigs, ingestion of fumonisin causes pulmonary edema syndrome (PEP) and hydrothorax [8]. Studies have shown that exposure of experimental and production animal models to FB1 can cause nephrotoxicity and hepatotoxicity, combined with hypercholesterolemia [2]. In humans, epidemiological studies in the Transkei, South Africa and Central China regions correlated the high rate of esophageal and liver cancer with intake of highly fumonisin-contaminated corn [8, 9]. In broiler chicks, negative effects caused by FB1 were described by Rauber et al. [10], who found decreased food intake and weight gain, as well as liver and kidney weight gain; histological lesions were found in the livers of the chicks that consumed the toxin. FB1 has also been reported as an immunosuppressive agent in chicks when present in feed above 200 mg FB1/kg [11]. Mycotoxins, including fumonisin, are known to cause direct effects on the animal when consumed daily at levels considered

high for the species, in addition to causing indirect effects such as oxidative stress [12, 13].

Oxidative stress is defined as an imbalance between free radical production and the antioxidant system that is responsible for maintaining the homeostasis of the organism [14]. This imbalance may be due to decreased levels of antioxidant actives such as tocopherols, ascorbic acid and glutathione [15], decreased activity of enzymes involved in the elimination of oxygen free radicals such as glutathione peroxidase (GPx) and superoxide dismutase (SOD), as well as increased production of reactive species [16]. When the concentration of reactive species is not controlled by internal defense mechanisms, oxidative damage occurs in proteins, lipids and DNA, leading to cytotoxicity, genotoxicity and carcinogenesis [15], all of which affect animal health and consequent productive performance. Oxidant and antioxidant status is altered when birds ingest mycotoxins in the diet; nevertheless, in the early stages of chicks, studies involving these analyses are rare. It is also in the early stages that poultry farmers and field technicians have attributed poor zootechnical performance to intake of foods contaminated with mycotoxin, including fumonisin (personal communication). Nevertheless, because these are field observations, more detailed and controlled investigations are needed. Therefore, the objective was to determine whether low-dose fumonisin-contaminated feed in the early phase of broiler chicks causes oxidative imbalance and interferes with weight gain.

2. Materials and Methods

The experiment was conducted in a 10-m^2 experimental shed with a 3.5-m ceiling divided into four boxes, with an area of 1 m^2 each, equipped with fixed troughs in the center of each box and on the sides with fans to control the internal temperature. The birds were heated using a gas bell lamp. Artificial illumination of the house was provided for 24 hours during the initial days, followed by the lighting regimen according to age as prescribed in the pedigree manual.

2.1 Fumonisin contamination

Level of contamination by fumonisins in chicks feed was measured by adding contaminated material produced from the fermentation of corn. Corn samples (1kg) were stored into plastic bags Distilled water was added to it to reach water activity ≥ 0.95. This was autoclaved for 1 h at 1 ATM and 125 °C. Autoclaved corn was inoculated with *F. verticillioides* isolates and was incubated at 25 °C for 30 days. Thereafter, the corn was dried at 65 °C for 48 h, milled and analyzed to determine the fumonisin concentration. The amount of contaminated material added to the diet was calculated based on the level of contamination present and the level of fumonisin desired in the final diet. The inoculum used for these studies contained 96.4% of fumonisin B1.

2.2 Animals and experimental design

We obtained 80 male one-day-old Cobb 500 broiler chicks. The birds were weighed, randomly allocated into four treatments of 20 chicks each, and housed in wood shaving boxes. The boxes measured 0.82 cm in height × 1.75 cm in length. The

boxes received 24 h of light through incandescent bulbs during the first 7 days of life. Changes regarding room temperature and light program during the experimental period were adjusted according to the recommendations in the Cobb 500® Lineage Management Handbook.

All birds received basal feed (Table 1) until the 21st day of life, i.e., throughout the experimental period. The composition of the feed based on corn and soybean was calculated according to the Brazilian poultry table [17]. The chicks received feed and water *ad libitum* during the entire experiment.

The treatments were as follows: control (T0), birds that received basal feed; treatments T1, T2 and T3 consisted of chicks that consumed basal feed, experimentally contaminated with 2.5 ppm, 5 ppm and 10 ppm of fumonisin, respectively. The first 11 days corresponded to the adaptation period (basal feed only, without fuminisin). Experimentally contaminated feed were taken between at days 12 to 21 of life. This period of exposure to fumonisin was based on field data, when poultry farmers reported problems in the production system associated with low doses of mycotoxin. Doses were also defined based on field findings, i.e. levels of feed collected from batches of birds that demonstrated poor zootechnical performance in the early phase (Micotoxin Laboratory, São Paulo University, Brazil). The strategy employed in this study was to simulate a situation of natural field contamination by low-dose fumonisin. Therefore, we tested oral challenges with fumonisin.

The levels of fumonisins in all feeds were measured using high performance liquid chromatography. The extraction and purification procedures was performed according to the Fumonitest WB column guide (VICAM Science Technology), using a

chemical reaction procedure according to EN 16006 (European Committee for Standardization, 2011). The analysis showed that true levels of fumonisin were similar to the calculated values, i.e., 0.26 ppm (T0), 2.74 ppm (T1), 5.27 ppm (T2) and 10.3 ppm (T3) of fumonisin.

2.3 Weighing and feed intake

The birds were weighed on an electronic scale individually on arrival (day 1), on the day they started consuming the fumonisin diet (day 12), and on days 17 and 21 of age (5 and 10 days after the start of the experiment). Each bird was considered an experimental unit to determine weight. The feed was given ad libitum, and intake was measured during the 21 days of the experiment.

2.4 Sample collection

After the 5^{th} and 10^{th} days of experiment (days 17 and 21 of age, respectively), ten birds from each treatment were anesthetized using halothane. Blood (5 ml) was collected by cardiac puncture, followed by cervical displacement euthanasia as recommended by the Research Ethics Committee. Next, a necropsy procedure was performed and lung, intestines, liver and spleen fragments were collected. The organs were used for histopathological analysis; liver was used for biochemical analysis of cell injury biomarkers.

For the evaluation of oxidant/antioxidant parameters, livers was kept refrigerated after collection (box with ice) until preparation that occurred within 1 hour after material collection. Livers were homogenized in 10 volumes (1:10, w/v) of 30

mM sodium phosphate buffer, pH 7.4, containing 120 mM KCl. Homogenates were centrifuged at 850 × *g* for 10 min at 4 °C and the supernatants collected was frozen (-80 ºC).

2.5. Sample processing

2.5.1 Analysis of oxidant/antioxidant status

Serum and hepatic ROS levels were determined using the DCF oxidation method described by LeBel et al. [18]. Fluorescence was measured using excitation and emission wavelengths of 485 and 538 nm, respectively. A calibration curve was established using 2',7'-dichlorofluorescein (DCF; 0.1 nM to 1 µM), and results were expressed as U DCF/mg of protein.

The methodology of lipid peroxidation (LPO) was based on Hermes-Lima et al. [19] with some modifications by the authors, called Fox based on the oxidation of Fe (II) under acidic conditions. The Fox method measures lipid peroxides, among the principal products of lipid peroxidation. For 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 and cumene hydroperoxide (CHP; Sigma) was employed as a standard. LPO was expressed as nmol/mL.

The activity of superoxide dismutase (SOD) was determined according to the auto-oxidation principle of pyrogallol, inhibited in the presence of SOD. The optical density change was determined kinetically for two minutes at 420 nm, at ten second

intervals according to methodology described by Beutler [20]. Activity was expressed as U SOD/mg of protein.

The activity of glutathione peroxidase (GPx) was measured indirectly by monitoring the oxidation rate of NADPH at 340 nm using cumene hydroperoxide (CuOOH), according to Wendel [21]. The enzymatic activity was expressed as U GPx/mg protein.

The activity of GST was measured according to Mannervik and Guthenberg [22] with slight modifications. GST activity was measured by the rate of formation of dinitrophenyl-S-glutathione at 340 nm in a medium containing 50 mM potassium phosphate, pH 6.5, 1 mM GSH, 1 mM 1-chloro-2,4-dinitrobenzene (CDNB) as substrate, and tissue supernatants (approximately 0.045 mg of protein). The results were calculated and expressed as U GST/mg protein.

2.5.2 Serum biochemistry

Blood was placed in test tubes without anticoagulant and centrifuged at 1,500 rpm for 10 minutes. The extracted serum was placed in *Eppendorf* tubes for subsequent measurement of glucose, uric acid, total proteins, albumin, cholesterol, triglycerides, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) (Bio-2000 BioPlus®) using commercial kits (Analisa®). Globulin values were calculated as the difference between total protein and albumin.

2.6. Histopathology

Gut, liver, spleen and lung fragments were stored in 10% formalin for two days, transferred to 70% alcohol and stored until sample processing. The material was fixed in paraffin and then cuts were made at 3-mm intervals. The slides were mounted and stained with hematoxylin and eosin (H&E).

To determine villus length and crypt depth, the methodology described by Caruso and Demonte [23] was followed, where histological images of the slides were captured using a Digital Electronic Eyepiece Camera Video microcamera, coupled to a TNB-Biological Trinocular Microscope (41T-PL OPTON) and a specific program for capturing histological images (ImageJ, National Institutes of Health). ImageJ is a computer program built on Java for image processing; it enables display, editing, analysis, and processing in addition to providing ease of saving and printing 8-, 16-, and 32-bit images.

To determine villus length, a straight line was drawn from the tip of the villus to the upper portion of the crypt, and to determine the depth of the crypt, another line was drawn from the base of the crypt to its upper portion. From the previous calibration of the program with distance known as a ruler photo with a minimum distance of 10 µm, this provided the measurements of the villi and crypts that were taken in eight different villi and crypts.

2.7 Statistical analysis

All parameters were tested for normality using the Shapiro-Wilk test. Skewness, kurtosis and homogeneity were evaluated using the Levene test, with log

transformation when needed. One-way ANOVA was performed to compare groups, followed by the post-hoc test (Tukey test) to assess the accuracy of the data. Significant difference was set at $P < 0.05$.

3. Results

3.1. Body weight and feed intake

Chick body weight is shown in Fig. 1. There was no significant difference between groups on days 1, 12 and 17, as opposed to day 21, when there were significantly lower ($P < 0.05$) body weights in T3 (10 ppm) than in T0 (control). At 21 days, body weights of the T1, T2 and T3 treatment chicks were 1.3%, 8.97% and 18.7% lower, respectively, than those of T0.

Feed intake during the 21 days of the experiment was similar between groups, that is, 1,297 g/chick (T0), 1,310 g/chick (T1), 1,302 g/chick (T2) and 1,292 g/chick (T3). During the experimental period, there was no chick mortality associated with any treatment.

3.2 Oxidant and antioxidant status

Serum antioxidant/oxidant markers are displayed in Table 2. ROS levels showed no significant ($P > 0.05$) changes between groups when assessed on day 17. However, serum ROS levels were significantly higher ($P < 0.05$) groups T1, T2, and T3 than in the control group on day 21. LPO levels showed no significant ($P > 0.05$) changes between groups when assessed on day 17; however, they were significantly lower ($P < 0.05$) in group T3 than in T0 on day 21. SOD activity did not change

significantly ($P > 0.05$) between groups when evaluated on day 17; however, it was significantly lower ($P < 0.05$) in group T3 than in T0 on day 21. On day 17, GPx activity was significantly lower ($P < 0.05$) in group T1 than in T0; activity was significantly higher ($P < 0.05$) in T3 than in T0, while it was significantly lower ($P < 0.05$) in all groups compared to T0 on day 21. GST activity did not differ significantly ($P > 0.05$) on day 17; however, it was higher in group T3 than in T0 on the 21st day ($P < 0.05$).

Antioxidant/oxidant markers in the liver are displayed in Table 3. At 17 days, there were no significant differences between groups ($P > 0.05$) with respect to ROS, SOD or GST; however, LPO levels were higher ($P < 0.05$) in the livers of birds in T2 and T3 than in those of T0; GPx activity was lower ($P < 0.05$) at 17 days in chicks that consumed FB1 (T1). At 21 days of age, ROS levels were significantly higher ($P < 0.05$) in groups T2 and T3 than in T0, while LPO levels were higher ($P < 0.05$) in group T3. SOD activity was significantly lower ($P < 0.05$) in T2 and T3 than in T0 on day 21, whereas GPx activity was significantly lower ($P < 0.05$) in all groups than in T0. Finally, GST activity was significantly lower in group T3 than in T0 on day 21 ($P < 0.05$).

3.3. Serum biochemistry

Serum clinical biochemistry results are displayed in Table 4. ALT and protein levels were significantly lower ($P < 0.05$) at day 21 in group T3 than in T0. Glucose levels were significantly lower ($P < 0.05$) on days 17 and 21 in group T3 than in T0. Triglyceride and uric acid levels were significantly lower ($P < 0.05$) on day 21 in group

T3 than in T0. No significant differences ($P > 0.05$) were observed AST, cholesterol and globulin levels on days 17 and 21 between groups.

3.4. Histology

For all treatments, no histological lesions were observed in the intestine, liver, lung, or spleen of the chicks (Figure 2). The data suggest that these doses of fumonisin in chick feed did not cause histopathological changes/injuries.

However, when measuring crypt size and villus, differences were found between treatments (Table 5). On day 17, there were no differences between groups for crypt size and villi ($P > 0.05$); however, it was found greater relationship villus/crypt ratios when birds ingested high FB1 doses in the diet (group T3) ($P < 0.05$; Table 5). At day 21, villus size (group T3) and crypt size (groups T1, T2 and T3) were significantly smaller ($P < 0.05$) than those of control (Table 5).

4. Discussion

Some of the most relevant factors in broiler production are feed conversion and weight gain. Despite the small number of birds in this study, it is possible to state that fumonisin affected weight gain in broilers in the initial phase. The 10 mg/kg dose of FB1 in the diet did not cause mortality; however physiological conditions such as liver function was compromised at the cellular level and with functional consequences. Carbohydrate, protein and lipid metabolism were affected, may interfering with weight gain. Negative impacts on performance of fumonisin-fed chicks are not new: In the 1990s, researchers demonstrated that FB1 caused decreased weight gain in the early-

stages of chick's life [24]. Butkeraitis et al. [25] observed a reduction in food intake and decreased body weight in quails fed with 50 and 250 mg FB1/kg of feed. Therefore, we believe that this experimental design was adequate since it was able to cause subclinical toxicity associated with metabolic and functional disorders in the birds, allowing us to investigate the relationship between oxidative and antioxidant status in the liver.

After 10 days of dietary intake of FB1, smaller crypt size and villus were observed, suggesting alterations on intestinal health given the importance of these structures in nutrient absorption. Fumonisin is known to cause villus atrophy and goblet cell hyperplasia in the jejunum of birds [26]. The effect of FB1 on villus height and crypt depth has been shown to be small [27]. We believe that the change in crypt size and villus is directly related to lower levels of glucose, triglycerides and serum uric acid, because these variables are directly affected by the absorption process.

Oliveira et al. [28] reported that the livers of birds fed 50 and 250 mg/kg FB1 showed moderate bile duct hyperplasia and multiple focal necrosis areas; we did not observe such lesions in the current study. AST is thought to be a sensitive enzyme that increases its activity in cases of FB1 poisoning [29], even though it is a non-specific marker of liver damage [30]. In the present study, enzyme levels did not significantly change, consistent with the histopathology; that is, no lesions were found to justify alteration in AST levels. ALT activity was significantly lower in birds that consumed higher levels of FB1; however, there is no exact explanation for this finding; we believe that loss of liver function may be related because there is reduction in serum albumin levels (the reference value for species is approximately 1.51 mg/dL), the main

protein produced by the liver. Results similar to those of our study for total protein and albumin have been reported in FB1-fed chicks [31].

Oxidative stress is understood as the imbalance between oxidizing and antioxidant compounds, with excessive generation of free radicals or their insufficient speed of removal. Such processes promote the oxidation of biomolecules, with consequent loss of their biological functions and/or homeostatic imbalance, the manifestation of which is potential oxidative damage to cells and tissues [16]. FB1 is cytotoxic and inhibits protein and DNA synthesis, promotes oxidative stress, induces DNA fragmentation and disrupts the cell cycle [32, 33].

In the present study, higher serum ROS levels were found in T2 and T3 groups at 21 days than in the control group; ROS and LPO levels in the liver were greater as well. Both findings suggest increased levels of reactive species and lipid damage. Baily et al. [34] found that consumption of 5, 15 and 45 mg FB1/kg diet for 12 days caused serum lipid damage; however, there were no lesions in duck liver and kidney, as observed in the present study. By contrast, Poersch et al. [12] reported severe lipid alterations in the livers of chickens fed 100 mg FB1/kg diet for 21 days, corroborating our observations. FB1-induced changes in phospholipid and fatty acid biosynthesis may represent important effects of fumonisins in terms of disrupting the integrity and function of hepatocyte membranes [35] that would explain oxidative imbalance and alterations on antioxidant system. SOD is an important antioxidant enzyme that eliminates excess oxygen free radicals by catalytic conversion of hydroxyl radicals to hydrogen peroxide that are subsequently broken down by CAT into non-toxic oxygen and water [36]. Birds that ingested FB1 had lower tissue SOD activity, reducing the

resistance of cells to oxidative damage. Reductions in serum activity of antioxidant enzymes SOD and CAT and increased SOD activity in the liver have been reported by Fu et al. [37] as indicative of aflatoxin poisoning in piglets fed with 5.3 and 372.8 μg/kg feed; no indicator of oxidation was analyzed. The glutathione (GSH) system catalyzes the dismutation of hydrogen peroxide in water and oxygen. Glutathione operates in cycles between its oxidized and reduced form [38]. GST, on the other hand, has is highly specific for reduced glutathione (GSH) [39]. We found lower GPx and GST glutathione peroxidase activity in the T3 group than in the control group at 21 days. This may suggest the consumption of these antioxidant enzymes in an attempt to avoid oxidative damage to cells and macromolecules, as noted by Poersch et al. [12] in chickens fed 100 mg FB1/kg feed for 21 days.

It is important to remember the cumulative effect of fumonisins that is related to the duration of exposure and sensitivity of the animal [40, 41]. Hou et al. [42] also demonstrated a decrease in antioxidant enzyme when mice consumed mycotoxin-contaminated foods, as did Yuan et al. [43] when evaluating the action of FB1 in porcine endothelial cells. By analyzing antioxidant/oxidant markers, it is possible to observe oxidative imbalance that may contribute to liver alterations during FB1 poisoning, because the increase in oxidative stress is thought to be an important component of the toxic effects involved in mycotoxin poisoning.

Conclusion

Contamination with low-dose fumonisin in the feed of broiler chicks during the early-stages of life manifested as liver alterations, even in the absence of visible lesions. Nevertheless, the chicks suffered heterogeneous oxidative stress associated with loss of function, manifested as reduced serum albumin levels. Birds that ingested fumonisin at dose 10 ppm for 10 days (between 12 and 21 days of life) had approximately 18% lower body weights than controls.

Conflict of Interest:

The authors declare that they have no conflicts of interest.

Ethics committee

All procedures this project were approved by the *Comitê de Ética do Uso de Animais na Pesquisa* (CEUA) of the *Universidade do Estado de Santa Catarina*, under protocol number 5960011018.

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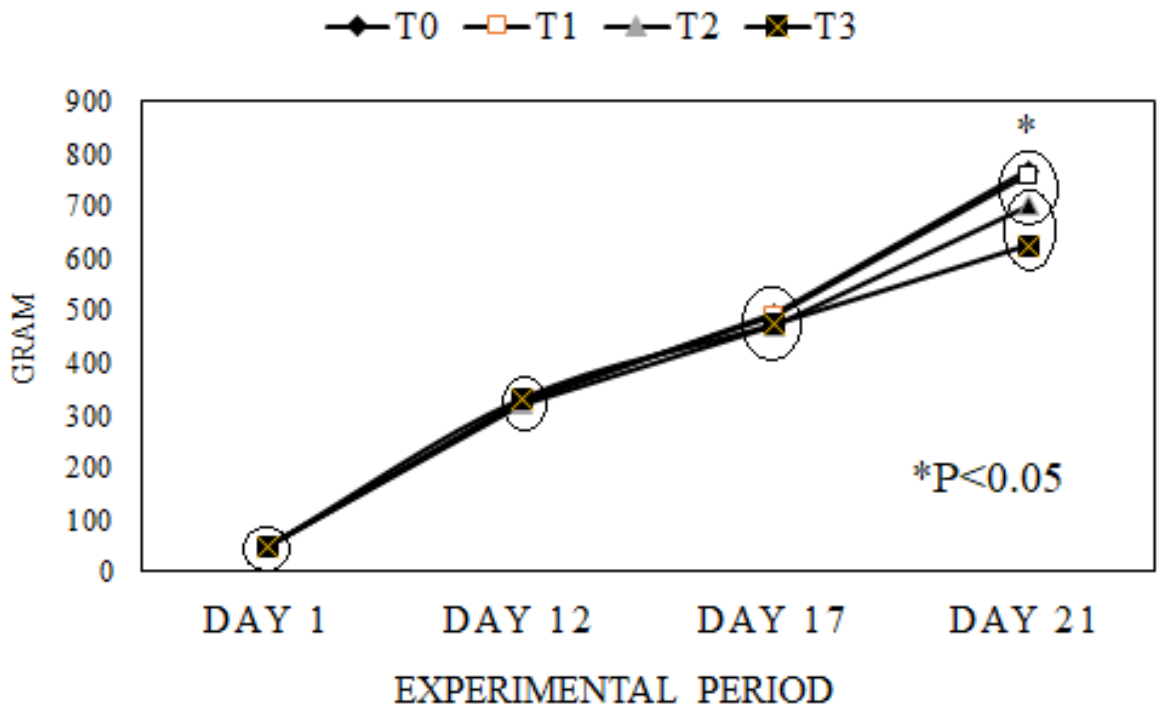

Figure 1. Body weight of chicks fed diets contaminated with various levels of fumonisin at days 1, 12, 17 and 21 of life. *Indicates significant difference between groups outside the same cycle.

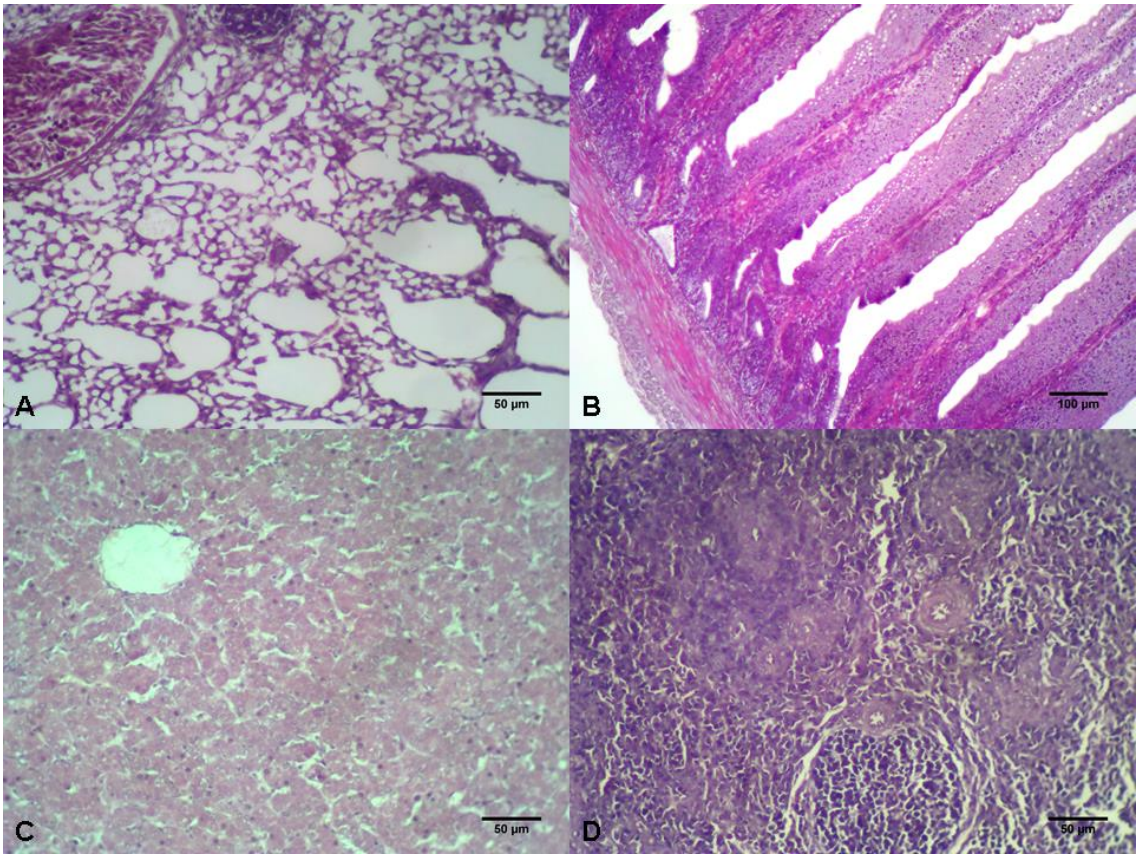

Figure 2: Histological images of chicks fed various doses of fumonisin in the first phase of the production cycle. **A) Lung:** Absence of lesions (HE, 200x). **B) Intestine:** Absence of lesions (HE, 100x). **C) Liver:** Absence of lesions (HE, 200x). **D) Spleen:** Absence of lesions (HE, 200x).

Table 1: Ingredients and calculated composition of the basal diet provided to chicks.

Ingredients (%)	Age: 1 – 21
Corn	58.3
Soybean meal	35.9
Soy oil	1.01
Bicalcium phosphate	1.99
Calcitic limestone	0.94
Iodized salt	0.53
DL-Methionine	0.37
Lysine	0.40
Threonine	0.14
Premix of vitamins and minerals [1]	0.30
Calculated chemical composition	
Energy (Mcal/kg)	2.96
Crude protein (%)	22.1
Calcium (%)	0.94
Available phosphorus (%)	0.47
Digestible lysine (%)	1.36
Chloride (%)	0.20
Digestible methionine + cysteine (%)	0.96
Digestible threonine (%)	0.88
Digestible tryptophan (%)	0.21
Sodium (%)	0.22
Linoleic acid	1.89

[1]Vitamin premix for chickens/kg: vitamin A (5000000 UI), vitamin D3 (1000000 UI), vitamin E (15000 UI), vitamin K3 (1500 mg), vitamin B1 (1500 mg), vitamin B2 (3000 mg), vitamin B6 (2000 mg), vitamin B12 (7000 mcg), folic acid (500 mg), nicotinic acid (15 g), pantothenic acid (7000 mcg), choline (80 g), biotin (100 mg), max humidity (40 g), max mineral matter (500 g). Micromineral premix: cooper (10 g), iron (50 g), iodine (1000 mcg), manganese (80 g), selenium (300 mg), zinc (70 g), maximum humidity (20 g), mineral matter (980 g).

Table 2. Levels of reactive oxygen species, lipid peroxidation and the antioxidant enzymes superoxide dismutase, glutathione peroxidase and glutathione transferase in serum at 17 and 21 days of life in chicks fed diets contaminated with various levels of fumonisin.

Variable	Days of life	T0	T1	T2	T3
ROS (U DCF/mg protein)	17	0.57 (0.21) [a]	0.52 (0.16) [a]	0.62 (0.30) [a]	0.47 (0.07) [a]
	21*	0.43 (0.07) [c]	0.59 (0.12) [b]	0.73 (0.18) [ab]	0.91 (0.22) [a]
LPO (nmol/mL)	17	615.0 (302)[a]	625.6 (317)[a]	516.2 (285)[a]	520.4 (232)[a]
	21*	499.0 (230)[a]	415.6 (185) [ab]	415.2 (157) [ab]	327.8 (100) [b]
SOD (U SOD/mg protein)	17	3.52 (1.0) [a]	3.24 (0.35) [a]	2.81 (1.16) [a]	2.44 (0.61) [a]
	21*	2.97 (0.5) [a]	3.0 (0.23) [a]	2.62 (0.22) [ab]	2.10 (0.43) [b]
GPx (U GPx/mg protein)	17*	5.74 (2.1) [a]	2.0 (1.16) [c]	4.44 (1.15) [ab]	3.43 (1.95) [bc]
	21*	7.57 (2.17) [a]	3.56 (1.18) [b]	3.72 (0.93) [b]	2.0 (1.03) [c]
GST (U GST/mg protein)	17	6.78 (0.4) [a]	7.17 (1.7) [a]	6.66 (2.45) [a]	6.52 (1.44) [a]
	21*	6.82 (1.3) [b]	6.11 (2.3) [b]	8.47 (1.41) [ab]	9.66 (1.3) [a]

Note: results presented as mean and standard deviation. *$P \leq 0.05$ on the same line shows differences between groups, and differences are represented by different letters (a e b).

Table 3. Levels of reactive oxygen species, lipid peroxidation and the antioxidant enzymes superoxide dismutase, glutathione peroxidase and glutathione transferase in livers at 17 and 21 days of age in chicks fed diets contaminated with various fumonisin levels.

Variable	Days of life	T0	T1	T2	T3
ROS (U DCF/mg protein)	17	0.94 (0.3)[a]	0.85 (0.1) [a]	1.05 (0.15) [a]	0.82 (0.18) [a]
	21*	0.97 (0.3) [b]	1.41 (0.28) [ab]	1.48 (0.21) [a]	1.97 (0.31) [a]
LPO (nmol/mL)	17*	341 (82) [b]	339 (109) [b]	591 (180) [a]	586 (171) [a]
	21*	375 (165) [b]	517 (105) [ab]	477 (133) [ab]	718 (259) [a]
SOD (U SOD/mg protein)	17	2.89 (0.43) [a]	2.25 (0.8) [a]	2.43 (0.4) [a]	2.48 (0.37) [a]
	21*	2.96 (0.4) [a]	3.64 (0.61) [a]	2.07 (0.31) [b]	2.07 (0.22) [b]
GPx (U GPx/mg protein)	17*	7.99 (3.08) [a]	4.20 (1.04) [b]	4.32 (1.9) [ab]	4.58 (2.04) [ab]
	21*	6.1 (1.62) [a]	3.15 (2.1) [bc]	3.50 (1.25) [b]	1.01 (0.5) [c]
GST (U GST/mg protein)	17	71.1 (25) [a]	68.9 (13) [a]	80.2 (39) [a]	59.4 (16) [a]
	21*	82.7 (14) [a]	66.6 (15) [ab]	73.4 (14) [ab]	54.9 (12) [b]

Note: results presented in mean and standard deviation. $P \leq 0.05$ on the same line shows differences between groups (*), and differences are represented by different letters.

Table 4. Biochemical and enzymatic variables in serum at 17 and 21 days of age in chicks given feed contaminated with different levels of fumonisin.

Variable	Days of life	T0	T1	T2	T3
ALT (U/L)	17	26.2 (8.2)[a]	17.1 (11.0)[a]	19.5 (7.4)[a]	18.6 (5.3)[a]
	21*	18.8 (6.2)[a]	14.1 (4.7)[ab]	13.5 (8.0)[ab]	8.6 (2.1)[b]
AST (U/L)	17	143 (44)[a]	161 (44)[a]	161 (22)[a]	166 (24)[a]
	21	173.8 (38)[a]	197.4 (46)[a]	187.8 (49)[a]	157.2 (29)[a]
Total protein (g/dL)	17	2.31 (0.37)[a]	2.46 (0.5)[a]	2.48 (0.37)[a]	2.38 (0.4)[a]
	21*	2.5 (0.2)[a]	2.48 (0.34)[a]	2.33 (0.10)[ab]	2.11 (0.2)[b]
Albumin (g/dL)	17	1.08 (0.13)[a]	1.0 (0.08)[a]	0.96 (0.2)[a]	1.01 (0.17)[a]
	21*	1.1 (0.08)[a]	1.03 (0.08)[a]	0.91 (0.16)[ab]	0.86 (0.11)[b]
Globulin (g/dL)	17	1.22 (0.3)[a]	1.66 (0.9)[a]	1.9 (0.6)[a]	1.36 (0.3)[a]
	21	1.4 (0.2)[a]	1.45 (0.28)[a]	1.41 (0.16)[a]	1.4 (0.4)[a]
Glucose (mg/dL)	17*	265 (48)[a]	250 (45)[ab]	231 (14)[ab]	204 (16)[b]
	21*	253 (31)[a]	238 (26)[ab]	236 (18)[ab]	220 (12)[b]
Triglycerides (mg/dL)	17	74 (21)[a]	72 (15)[a]	55 (10)[a]	48 (7.5)[a]
	21*	75 (30)[a]	58 (27)[ab]	48 (20)[ab]	39 (11)[b]
Cholesterol (mg/dL)	17	89 (10)[a]	74 (14)[a]	79 (15)[a]	90 (8.7)[a]
	21	92 (12)[a]	94 (18)[a]	95 (11)[a]	91 (11)[a]
Uric acid (mg/dL)	17	4.6 (1.7)[a]	5.6 (3.8)[a]	6.3 (1.6)[a]	5.54 (1.3)[a]
	21*	8.0 (2.7)[a]	6.7 (2.1)[a]	5.7 (1.7)[ab]	3.8 (1.2)[b]

Note: results presented in mean and standard deviation. $P \leq 0.05$ on the same line shows differences between groups (*), and the differences are represented by different letters.

Table 5: Villus height, crypt depth and villus/crypt ratio in chicks fed diets contaminated with various levels of fumonisin: T0 (0 mg/kg), T1 (2.5 mg/kg), T2 (5 mg/kg) and T2 (10 mg/kg).

Variable	**Days of life**	**T0**	**T1**	**T2**	**T3**
Villus height	17	933.3 (285)	1246.8 (102)	1154.2 (260)	1235.8 (222)
(µm)	21*	1211.8 (238)[a]	859.7 (224)[ab]	880.8 (111)[ab]	822.8 (41.0)[b]
Crypt depth	17	169.0 (33.6)	182.2 (12.0)	162.4 (36.6)	159.7 (30.4)
(µm)	21*	178.7 (25.4)[a]	117.7 (37.0)[b]	128.4 (25.5)[b]	137.2 (21.7)[b]
Villus/crypt Ratio	17*	5.52 (2.72)[b]	6.84 (0.71)[ab]	7.11 (1.41)[ab]	7.74 (1.57)[a]
	21	6.78 (1.20)	7.30 (1.83)	6.86 (1.49)	6.00 (1.09)

* Significant difference between groups ($P < 0.05$). Differences between groups are shown with different letters in the same line (a and b).

MANUSCRITO 2

Submetido a *Microbial Pathogenesis*

Fumonisin-(*Fusarium verticillioides*)-contaminated feed causes hepatic oxidative stress and negatively affects broiler performance in the early stage: Does supplementation with açai flour residues (*Euterpe oleracea*) minimize these problems?

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ABSTRACT

Fusarium verticillioides is often responsible for contamination of poultry feed with the mycotoxin fumonisin. The aim of this study was to determine whether oxidative stress caused by intake of fumonisin-contaminated feed affects broiler performance at an early stage of development, as well as to test whether the addition of açai residue flour to contaminated feed would minimize these negative effects of redox metabolism. Birds were divided into four groups, with four repetitions of five animals each: control (TC) - birds that received basal feed; TCA treatment - basal feed supplemented with 2% açai flour; TF treatment - feed experimentally contaminated with fumonisin (10 ppm); TFA treatment – fumonisin-contaminated feed (10 ppm) and supplemented with açai fluor (2%). The experiment lasted 20 days, that is, the first 20 days of the chicks' lives. At the end of the experiment, the birds were weighed, and blood, intestine and liver samples were collected. The TCA and TFA had greater body weights and weight gain than did TF. Further, TCA and TFA had lower feed conversion than did TF. Açai flour intake (TCA and TFA) stimulated albumin synthesis and reduced serum AST activity. Nitrate/nitrite (NOx) levels were higher in serum of fumonisin-challenged (TF) birds than in groups; NOx levels were also higher in the livers of all test groups (TF, TCA and TFA) than in TC. Serum glutathione S-transferase (GST) activity was lower in fumonisin-consuming groups (TF and TFA); this was different from what occurred in the liver, that is, higher GST activity in TF and lower activity in TFA than in TC. Catalase activity (CAT) was also higher in the fumonisin-challenged groups (TF and TFA) and the groups supplemented with açai flour (TCA) than in TC. Serum reactive species (RS) and TBARS (lipid peroxidation) levels in the liver were lower in birds supplemented with açai flour and exposed to fumonisin. These data suggest that the addition

of açai flour in the feed of early chickens improves animal performance and minimizes the effects of hepatic oxidative stress in birds fed fumonisin-contaminated feed.

Keywords: Açai flour residue, fumonisin, antioxidant, broiler, mycotoxin, oxidative stress, pathogenesis.

1. INTRODUCTION

A number of studies have documented the damage caused by mycotoxins in terms of bird health and production due to reduced weight gain and feed efficiency, as well as increased mortality and compromised immune systems [1, 2, 3]. Weibking et al. [4] described negative effects caused by fumonisin in broiler chicks, and Muller et al. [5] documented the oxidative stress resulting from daily high-level fumonisin intake. The imbalance between free radicals and antioxidant defense systems damages DNA, proteins and lipids [6].

Several approaches have been attempted to decrease mycotoxin exposure in humans and animals. Studies have shown that natural antioxidant substances mitigate and/or prevent the toxic effects of mycotoxins [7]. Based on this information, there has been an intense search for natural antioxidant substances and additives that reduce oxidative processes and levels of circulating and tissue free radicals [8]. Foods rich in polyphenols, thought to have high antioxidant power, especially of the anthocyanin class, are increasingly being used to prevent metabolic syndrome-related diseases [9].

One fruit in particular that presents this property is açai (*Euterpe oleracea*). Brazil is the largest producer of açai and the foreign market has been investing in importation for uses in both the food and pharmaceutical industries [10]. A recent study showed that açai flour, a

byproduct of açai fruit, when added to laying hen feed showed high antioxidant potential [11]. The high content of polyphenols in açai makes it one of the five fruits with the highest antioxidant potential, showing several properties, including anti-inflammatory and pharmacological effects that are associated with treatment of diseases that generate large numbers of reactive oxygen species (ROS) [12, 13, 14]. Chromatographic analysis of açai pulp demonstrated the presence of significant amounts of polyphenols, especially the anthocyanins cyanidine 3-glucoside and cyanidine 3-rutinoside [15, 16]. These anthocyanins modulate lipid metabolism and improve the parameters evaluated in metabolic syndrome [9].

Given this background, it can be predicted that the antioxidant effects of açai will minimize or neutralize the oxidative stress triggered by mycotoxin [17, 18], as well as consequent performance losses. Therefore, the objective of this research was to determine whether oxidative stress caused by intake of fumonisin-contaminated feed affects broiler performance at an early stage of development, as well as to test whether the addition of açai residue flour to contaminated feed would minimize these negative effects of redox metabolism.

2. MATERIAL AND METHODS

2.1 Açai flour residue

The açai flour residue (AFR) used in this study was purchased from a natural products company (Essential, Chapecó, Southern Brazil). The flour was obtained by milling the pits and dried fruit residues (pulp and peel). Total phenolic and antioxidant activity in AFR were measured [11].

2.2 Animals, and experimental design

Male broiler chicks (n=80), 1-day-old Cobb 500 were divided into four treatments with four repetitions of five birds each and housed in wood shaving litter. They received feed and water *ad libitum* throughout the study. The composition of the feed based on corn and soybean was calculated according to the Brazilian Poultry Table (Table 1) [19]. The treatments were as follows: the negative control (TC), birds that received basal diet; TCA, birds supplemented with 2% of açai residue flour; TF, the positive control, feed contaminated with fumonisin (10 ppm); TFA, supplemented with açai residue flour (2%) and feed with fumonisin (10 ppm). The 2% dose of açai residue flour was based on the study by Fortuoso et al. [11]; while the fumonisin dose (10 ppm) was chosen based on the results of Lee et al. [20].

The experimental period lasted 20 days, corresponding to the first 20 days of chick´s life. Açai flour residue was used to feed birds throughout the experimental period; fumonisin was added to the feed at day 11 of the experiment.

Feed and açai flour residue samples were collected, frozen and analyzed for bromatological composition. Dry matter (DM), ash, ether extract (EE), crude protein (CP), and neutral detergent fiber (NDF) analyses were performed [21]. Total phenolic and antioxidant activity in diets also were evaluated [11]

2.3 Growth performance

Birds were weighed to calculate weight gain and average daily weight gain at three time points (days 1, 10 and 20) using a digital balance. Feed intake was also measured during the experimental period and at the end and used to calculate feed conversion.

2.4 Sampling

On day 20, eight birds from each treatment (two by repetition) were anesthetized with isoflurane in a plastic chamber followed by cervical dislocation. Blood and liver were collected for histopathological analysis and measurement of biochemical and oxidative stress markers as detailed below.

Tissues were placed in 10 mM Tris-HCl pH 7.4 solution and gently homogenized in a glass potter homogenizer in specific buffer. The homogenate was centrifuged at 10,000 *g* at 4 °C for 10 min to produce supernatants that were stored at –80 °C until use. Analyses of TBARS (thiobarbituric acid reactive substances), RS (reactive species), SOD (superoxide dismutase), CAT (catalase), NOx (nitrite/nitrate) and GST (glutathione S-transferase) were determined at fixed protein concentrations using the Coomassie blue method according to Bradford [22] with bovine albumin as the standard.

2.5 Serum clinical biochemistry

Serum levels of total protein, albumin, uric acid, triglycerides and cholesterol, as well as activity of alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were measured on a semi-automated analyzer (BioPlus 2000®) using commercial kits (Analisa®, Brazil) following the manufacturer's recommendations. Globulin levels were obtained using the formula (total protein – albumin).

2.6 Oxidant/antioxidant status

Liver lipid peroxidation were determined as TBARS levels, measured by the absorbance of red product at 532 nm according to the method described by Ohkawa et al. [23] and expressed as nmol MDA/mg of protein.

Levels of reactive species (RS) were determined using the indicator 2'-7'-dichlorofluorescein (DCFH) according to the methodology adapted from Halliwell and Gutteridge [24]. Serum or hepatic homogenate (1 mg/mL) samples were incubated in the presence of dark DCFH for 1 h until and the amount of reactive species was measured by fluorescence at excitation length at 488 nm and emission at 525 nm. Results were expressed as U DCF/mg protein.

Glutathione S-transferase (GST) activity was measured spectrophotometrically at 340 nm using the method of Habig et al. [25]. The mixture contained test sample, 0.1 M potassium phosphate buffer (pH 7.4), 100 mM GSH and 100 mM CDNB, which was used as substrate. Enzyme activity was expressed as µmol/CDNB/mg protein.

Hepatic superoxide dismutase (SOD) activity was based on inhibition of the O_2^- reaction using adrenaline as a substrate, as described by McCord and Fridovich [26]. SOD activity was determined by measuring the rate of adrenochrome formation observed at 480 nm in a reaction medium containing 50 mM glycine-NaOH, pH 10 and 1 mM adrenaline. Results were expressed as units of (IU) SOD/mg protein.

Hepatic catalase (CAT) activity was measured according to the modified method of Nelson and Kiesow [27]. This assay involves the change in absorbance at 240 nm for 2 min due to the decomposition of hydrogen peroxide-dependent catalase (H_2O_2). Results were expressed as nmol/mg protein.

For the determination of nitrite/nitrate (NOx) ratio, an aliquot (200 µL of samples) was homogenized in 200 mM Zn_2SO_4 and acetonitrile (96%, HPLC grade). Thereafter, homogenates were centrifuged at 16,000 *g* for 20 min at 4 °C and the supernatants were separated for nitrite/nitrate content analysis as previously described by Miranda et al. [28]. The resulting pellet was suspended in NaOH (6 M) for protein determination and results were expressed as µmol/mg protein.

2.7 Parasitological examination

The floating-centrifuge technique with a saturated sugar solution was used to quantify the number of eggs, oocyst and cyst per gram of feces. One gram of feces was homogenized in 15 mL of saturated sugar solution, then filtered and placed in cone tubes with glass laminates on the surface. Then the tubes were centrifuged for 5 min (2500 rpm), after this step was complete, the laminate was transferred to a glass slide where there was a drop of lugol solution. In a light microscope (10x), the number of oocysts per gram of feces was counted.

2.8 Histopathology

Liver and intestine fragments were fixed in 10% formalin for two days, after which time the material was transferred to 70% alcohol where it was stored until sample processing. Samples were processed routinely, embedded in paraffin and cut in sections ranging from 3 to 4 µm of thickness. Tissue sections were stained with hematoxylin and eosin (H&E). To determine villus length and crypt depth, the methodology described by Caruso and Demonte [29] was followed, where histological images of the slides were captured using a Digital

Electronic Eyepiece Camera Video micro-camera, coupled to a TNB-Biological Trinocular Microscope (41T-PL OPTON) and a specific program for capturing histological images. To determine villus length, a straight line was drawn from the tip of the villus to the upper portion of the crypt, and to determine the depth of the crypt, another line was drawn from the base of the crypt to its upper portion, using ImageJ.

2.9 Statistical analyses

The results were tested for normality using the Shapiro-Wilk test and were tested for homogeneity using the Levene test. Data that did not show normal distribution were log-transformed (levels of LPO, RS, leukogram and biochemical variables (AST and cholesterol)). Subsequently, the data were analyzed using two-way analysis of variance (ANOVA) followed by Tukey post hoc tests. The data were analyzed using the Package ggplot2. Significant differences were defined as $P < 0.05$. The statistical analyses were performed using R-language, v.2.15.1 (R Development Core Team 2012). Results were expressed as mean and standard deviation.

3. RESULTS

3.1 Chemical composition of the diet and açai characterization

The table 1 shows ingredients used to formulate the base concentrate of broiler chickens and chemical composition of the diet. As can be observed, the feed was formulated using major compounds such as corn and soy in addition to amino acids and vitamins. The table 2 shows the compounds found in acai flour residue.

3.2 Growth performance

Table 3 shows the results of animal performance. Greatest body weight at 10 days of experiment was recorded for birds in the groups that consumed açai flour residue. At the end of the experiment, TF had significantly lower body weights than the TC, TCA and TFA groups. The birds in the TCA and TFA groups consumed larger amounts of feed than did the TF. In terms of weight gain, the TCA and TFA groups showed higher values during the initial phase than did the TF group. Lower feed conversion was observed in the TCA and TFA groups (Table 3).

3.3 Serum biochemistry

Serum biochemical results are presented in Table 4. Higher albumin levels were observed in birds in the TCA and TFA groups than in the TF group. Globulin levels were higher in fumonisin-fed birds (TF) than in TC or TFA. AST activity in the TC, TCA and TFA groups were lower than that of the TF. Uric acid levels were lower in birds that consumed açai flour (TCA and TFA) than in the other groups. Total levels and ALT activity did not differ between treatments.

3.4 Serum oxidant/antioxidant status

Serum oxidant and antioxidant status markers are displayed in Table 4. NOx levels were higher in fumonisin-challenged (TF) birds than in the other groups. GST activity was lower and TBARS levels higher in the serum of fumonisin-challenged birds (TF and TFA). ROS levels were higher in TF than in TC and TCA.

3.5 Hepatic oxidant/antioxidant status

Table 5 displays the hepatic oxidant and antioxidant profiles. GST activity differed among all treatments, with highest activity in the liver of TF birds and lowest activity for TFA birds. CAT activity and NOx levels were higher in livers of the three test groups (TF, TCA, TFA) than in (TC). Levels of TBARS and hepatic ROS were lower in chicks not challenged with fumonisin and consuming dietary açai flour (TCA). Liver SOD activity did not differ between groups (Table 5).

3.6 Histopathology

Macroscopically and microscopically no lesions were observed in the liver and intestine of any bird in the study. Intestinal morphometry data are displayed in Table 6. TF birds had smaller intestinal villus size than did TC. Crypt size was smaller in all test groups (TF, TCA and TFA) than in control (TC). Chicks from the TFA group had higher villus/crypt ratios than did the other groups, just as the TF birds had lower villus/crypt ratios (Table 6).

3.7 Parasitological examination

No parasitic gastrointestinal infection was observed during the experimental period in all birds: the parasitological examinations were negative for parasite eggs, cysts and oocysts.

4. DISCUSSION

Mycotoxins are widely found in bird feed and once absorbed it may cause cellular damage manifesting as nephrotoxicity, cytotoxicity and hepatotoxicity [17,18]. In this study, our hypothesis was that supplementation with açai, a natural antioxidant, to fumonisin-

contaminated feed would minimize the negative effects on animal performance and on metabolic and oxidative disorders in chicks. Initially our data revealed a possible improvement in performance of fed-açai flour (2%) animals, even those challenged with fumonisin (10 ppm). The formulation of açai flour (2%) appeared to be well-accepted by the animals with increased intake rates and consequently, body weights. These results may be related to the high nutritional value of açai, as well as the nutritional components found in flour that stimulate nutrient absorption and protein biosynthesis.

Studies using other natural compounds such as cayenne (*Capsicum annum*) and turmeric (*Curcuma longa*) at various concentrations in broiler feed [30] showed that the feed conversion rate was better in poultry fed with 400 g of saffron and 200 g of cayenne. Turmeric extract has been shown to improve feed utilization by stimulating protein synthesis through the poultry enzyme system [31], resulting in better digestion, increased nutrient metabolism and greater weight gain [32]. We believe that similar mechanisms occurred in this study when the birds consumed feed supplemented with açai flour, a nutritious feed and with desirable biological properties.

At least 15 fumonisin-related compounds have been identified to date; however, fumosin B1 (FB1) is the most worrying on account of toxicity and widespread occurrence [33]. *In vitro* [34, 35] and *in vivo* studies [36, 37] revealed the potential of FB1 to induce oxidative stress with consequent generation of ROS. The liver and kidneys are the main targets of FB1 toxicity in almost all animal species already evaluated [38]; these data corroborate our findings in chicks that ingested FB1 at 10 to 20 days of age.

Antioxidants, mainly of natural origin, have been used effectively against the toxic effects of several mycotoxins [7]. Natural alternatives are sought after for treatment of

diseases and metabolic disorders, and there is demand on the part of consumers for healthier, residue-free foods. Increasing the intake of exogenous antioxidants inhibits the damage caused by oxidative stress by inhibiting the initiation or propagation of the oxidative chain reaction, including free radical scavengers, singlet oxygen inhibitors and reducing agents [39].

Fortuoso et al. [11] tested supplementation with açai flour in laying hens feed, and reported potent effects on antioxidant capacity, with higher production rates, egg quality, reduced lipid peroxidation in egg and increased shelf life. Similarly, in this study, we observed the antioxidant effects of açai flour on broiler diets in the initial production phase, which minimized the oxidative stress caused by FB1 intake.

We found that albumin levels did not differed in TF when compared to TC, suggesting the functional of the liver by the action of fumonisin, did not affect protein synthesis and protein catabolism. Supplementation with açai flour promoted higher serum albumin levels in the TCA and TFA groups than in the TF group, consequently increasing performance (Table 3). Protein clearance is essential because oxidized proteins form aggregates and cross-links resulting in mitochondrial toxicity, as globulin levels were shown to be greater in the TF group than in the control (Table 3), possibly indicating immune system involvement in challenged animals.

Açai flour showed serum and cytoprotective stimulatory effects manifested as lower AST levels in the TCA and TFA groups than in the control group (Table 4). This result suggests a hepatoprotective effect of açai flour in this contamination model. The liver is the oxidative center of many metabolic and detox reactions. Hepatocytes have a high density of mitochondria; therefore, the risk of oxidative insult is greater [40]. Our data suggest that FB1

contamination disrupted the turnover sphingolipid biosynthesis and altered plasma membrane composition. Fumonisin exposure has been associated with hepatic and systemic defects as well as with the induction of oxidative stress leading to lipid and protein damage [35, 41, 42]. These damages were minimized by the consumption of açai flour in the current study.

We observed greater serum lipid peroxidation in fumonisin-challenged chicks than in the control group. Similarly, there was a greater release of reactive species and nitrite and nitrate into the extracellular medium of TF birds than in controls. These findings support the notion that these birds were in a situation of hepatic and systemic oxidative stress. One consequence of uncontrolled production of oxygen-reactive species is lipid peroxidation that produces byproducts such as MDA [43]. Ingestion of FB1 increased extracellular MDA-TBA levels contributing to oxidative stress, attributable to an imbalance between endogenous reactive species production and cellular antioxidant capacity, leading to electron transport chain imbalances, a well-known mechanism. A previous study suggested that oxidative stress caused by exposure to FB1 disrupted mitochondrial respiration, inhibiting electron transport chain complex I, increasing the generation of RS [44] In response, the antioxidant defense system is stimulated to detoxify and neutralize the effects of intracellular RS excess and plasma membrane decomposition [45]. Nevertheless, the role of the resulting antioxidant response in the context of FB1 toxicology has not been well established [35, 46, 47]. This is why researchers have been looking at alternatives to minimize the effects of FB1 by supplementing with antioxidants such as the açai flour used here.

The first line of defense against oxidative stress in cells is the induction of antioxidants that eliminate RS and dampen oxidative damage to macromolecules [45]. Regarding antioxidant status indicators, we found deficits in serum GST activity serum, but

not hepatic activity. Glutathione is an important intracellular antioxidant in hepatocytes that protects against oxidative damage and is involved in detoxification of foreign substances such as fumonisin [48]. Therefore, our findings suggest a greater need for enzymatic action of GST in the liver as a compensatory effect of damage caused by FB1 contamination in challenged birds. By contrast, the association of açai flour promoted increased serum GST activity probably in order to directly erase hydroxyl radicals and other oxygen-centered free radicals. When we observe the activity of GST in hepatocytes, enzyme activity was increased and this effect was reduced by the association of açai flour and fumonisin-contaminated feed in birds of the TFA group compared to the TF group.

An alternative enzymatic mechanism for hydrogen peroxide detoxification was investigated in hepatocytes. Birds that consumed fumonisin-containing feed (TF group) had higher CAT activity. The reason for this increase is explained by the greater need for decomposition of the final product of SOD, H_2O_2. Feed rich in açai flour enhanced the activity of hepatic CAT, promoting a potent local antioxidant effect.

In summary, our hypothesis was confirmed that the phytochemical composition of açai would protect against oxidative damage caused by FB1 by stimulation and/or direct antioxidant action. We believe that this mechanism may have contributed to the preservation of crypts and villi. The components present in açai flour neutralized the fumonisin present in the feed. These results open the way for a better understanding of the effects of daily intake of açai by broiler chicks, suggesting that flour is a positive modulator of intestinal health even when consumed by challenged birds (TFA) it showed higher villus/crypt ratios. Açai is a viable option as a nutritional additive in açai-producing regions, where the volume of waste is large due to little usage.

The addition of açai flour to the chicken diet cause numerically increase the levels of crude protein, ash, ether extract and fiber, as well as an increase in total phenolic compound diets and increased antioxidant capacity. In general, individually or in combination, these results may have a role in the positive response of açai flour supplementation on hepatic oxidative stress caused by FB1. It is known that phenolic compounds have antioxidant effects; just as açai flour when consumed by birds has beneficial effects on the production and stimulation of the antioxidant system [11].

5. Conclusion

Fumonisin ingestion induced oxidative stress in young chicks manifested by reduced hepatic functionality, elevated extracellular RS and lipid peroxidation. Feed containing 2% açai flour residue minimized the negative effects caused by FB1 in part, and consequently favored better performance of chicks, even those consuming fumonisin. In summary, these results demonstrate the potent antioxidant effect of açai flour when used in the feed of early-stage broilers, despite fumonisin contamination.

Conflicts of interest. The authors declare that they have no conflicts of interest with the contents of this article.

Ethics Committee. This project was approved by the Committee for the Use of Animals in Research (CEUA) of the Universidade do Estado de Santa Catarina (UDESC) under protocol number 2990090519.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author contributions

Silveira, Galli, Gloria and Da Silva conceived and designed research; Galli, Silveira, Alba, Bottari, Lopes, and Leal performed data analysis; Stefani, Schetinger, Mendes and Morsch made critical contribution to the discussion. All authors revised the manuscript. All authors read and approved the final manuscript.

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Table 1: Ingredients used to formulate the basal concentrate of broiler chickens and chemical composition of the diet.

Ingredients	**Basal concentration (kg)**
Corn	58.3
Soybean meal	35.9
Soy oil	1.01
Bicalcium phosphate	1.99
Calcitic limestone	0.94
Iodized salt	0.53
DL-Methionine	0.37
Lysine	0.40
Threonine	0.14
Premix of vitamins and minerals [1]	0.30
Total	**100 kg**
Calculated chemical composition	
Energy (Mcal/kg)	2.96
Crude protein (g/kg)	221
Calcium (g/kg)	9.40
Available phosphorus (g/kg)	4.70
Digestible lysine (g/kg)	13.6
Chloride (g/kg)	2.00
Digestible methionine + cysteine (g/kg)	9.60
Digestible threonine (g/kg)	8.80
Digestible tryptophan (g/kg)	2.10
Sodium (g/kg)	2.20
Linoleic acid (g/kg)	18.9

Chemical composition	TC: Control	TCA: Control + açai flour	TF: Fumonisin	TFA: Fumonisin + açai flour
Dry matter (g/kg)	921	917	919	920
Ash (g/kg)	45.6	46.7	45.9	46.6
Crude protein (g/kg)	220	229	221	228
Ether extract (g/kg)	40.2	41.9	40.7	41.1
NDF (g/kg)	8.47	8.66	8.52	8.62
Total phenolic	nd	2.98	nd	2.84
IC50	941	241	847	209

Note 1: Vitamin premix for chickens/kg: vitamin A (5000000 UI), vitamin D3 (1000000 UI), vitamin E (15000 UI), vitamin K3 (1500 mg), vitamin B1 (1500 mg), vitamin B2 (3000 mg), vitamin B6 (2000 mg), vitamin B12 (7000 mcg), folic acid (500 mg), nicotinic acid (15 g), pantothenic acid (7000 mcg), choline (80 g), biotin (100 mg), max humidity (40 g), max mineral matter (500 g). Trace minerals premix: cooper (10 g), iron (50 g), iodine (1000 mcg), manganese (80 g), selenium (300 mg), zinc (70 g), maximum humidity (20 g), mineral matter (980 g).
Note 2: Note: nd - not detected.

Note 3: Total phenolic compounds (mg/100 g dry matter) and antioxidant activity against the DPPH - IC50 (µg/mL). Importantly, the lower the IC50 value, the greater the antioxidant activity in diet.

Table 2: Chemical composition, total phenolic compounds and antioxidant activity against the DPPH (IC50) of the açai flour residue.

Ingredients	Açai flour residue
Chemistry composition	
Dry matter (g/kg)	974.1
Crude protein (g/kg)	94.2
Ether extract (g/kg)	69.8
Ash (g/kg)	26.4
NDF (g/kg)	689.5
Phenolic and antioxidants	
Total phenolic (mg/100 g dry matter)	114.7
IC50[1]	84.6

[1]Note: Importantly, the lower the IC50 value, the greater the antioxidant activity.

Table 3. Bird performance: control (TC) - birds that received basal feed; TCA treatment - basal feed supplemented with 2% açai flour; TF treatment – feed experimentally contaminated with fumonisin (10 ppm); TFA treatment – feed experimentally contaminated with fumonisin (10 ppm) and supplemented with açai (2%).

Groups	Body weight (g)	Feed intake (g)	Weight gain[1] (g)	Daily weight gain (g)	Feed conversion
DAY 10					
TC	219.0^{b}	204.4^{b}	179.5^{b}	17.9^{b}	1.13^{ab}
TF	221.3^{b}	217.5^{ab}	186.5^{ab}	18.6^{ab}	1.16^{a}
TCA	234.6^{a}	215.3^{ab}	195.2^{ab}	19.5^{ab}	1.10^{b}
TFA	238.4^{a}	220.4^{a}	198.8^{a}	19.8^{a}	1.10^{b}
P-value	**0.021***	**0.050***	**0.013***	**0.010***	**0.012***
CV (%)	**4.85**	**8.96**	**3.75**	**3.41**	**2.74**
DAY 15					
TC	434.3^{a}	499.7^{a}	394.8^{a}	26.3^{a}	1.26^{a}
TF	400.2	423.2^{b}	360.8^{b}	24.0^{b}	1.17^{ab}
TCA	428.5^{a}	428.3^{b}	389.5^{a}	25.9^{a}	1.09^{b}
TFA	425.5^{a}	486.5^{a}	386.0^{a}	25.7^{a}	1.26^{a}
P-value	**0.044***	**0.004***	**0.042***	**0.034***	**0.015***
CV (%)	**6.42**	**3.71**	**9.74**	**4.58**	**2.36**
DAY 20					
TC	600.2^{a}	679.1^{b}	560.5^{a}	28.0^{a}	1.21^{b}
TF	569.6^{b}	664.1^{c}	530.1^{c}	26.5^{b}	1.25^{b}
TCA	586.5^{a}	678.7^{b}	547.7^{b}	27.4^{a}	1.23^{b}
TFA	585.8^{a}	740.9^{a}	543.8^{b}	27.2^{ab}	1.36^{a}
P-value	**0.002***	**0.018***	**0.024***	**0.008***	**0.003***
CV (%)	**5.51**	**12.7**	**5.44**	**3.96**	**2.87**

Note: [1]Weight gain at the three time points of the experiment (Days 10, 15 and 21) was calculated by subtracting body weight from weight on the first day of life. (TC: 39.5 g; TCA: 39.5 g; TF: 38.7 g; TFA: 41.9 g).

Table 4: Mean ± standard deviation of clinical biochemistry and status oxidant/antioxidant in serum of birds.

Variable	TC: Control	TF: Fumonisin	TCA: Control + açai flour	TFA: Fumonisin + açai flour	P
Clinical biochemistry					
Uric acid (mg/dL)	8.64 (2.18)[a]	9.28 (2.25)[a]	5.24 (1.23)[b]	6.75 (1.08)[b]	0.034*
Total protein (g/dL)	2.59 (0.27)	2.83 (0.35)	2.79 (0.43)	2.76 (0.21)	0.396
Albumin (g/dL)	1.19 (0.17)[ab]	1.10 (0.10)[b]	1.28 (0.18)[a]	1.33 (0.21)[a]	0.021*
Globulin (g/dL)	1.40 (0.20)[b]	1.67 (0.36)[a]	1.51 (0.37)[ab]	1.44 (0.19)[b]	0.050*
AST (U/L)	174.8 (16.7)[b]	203.4 (43.3)[a]	179.2 (20.3)[b]	164.5 (20.4)[b]	0.043*
ALT (U/L)	6.67 (1.05)	6.90 (2.07)	5.71 (1.71)	5.88 (1.73)	0.298
Oxidant and antioxidant status					
GST (µmol/CDNB/ min)	928 (111)[a]	567 (154)[b]	984 (72.1)[a]	433 (64.5)[b]	< 0.001*
ROS (x10 U DCFA/ mg of protein)	6.10 (0.74)[b]	7.40 (0.65)[a]	5.90 (0.55)[b]	6.40 (1.06)[ab]	0.012*
TBARS (mmol MDA/mL)	17.7 (3.70)[b]	30.8 (5.25)[a]	20.4 (1.35)[b]	26.3 (3.30)[a]	< 0.001*
NOx (µmol/mg of Protein	15.0 (3.38)[b]	21.3 (3.99)[a]	16.3 (3.19)[b]	14.4 (3.00)[b]	< 0.001*

Note: *P < 0.05 indicates significant difference between groups indicate by different letters (a, b) in the same line. The treatments were: control (TC) - birds that received basal feed; TCA treatment - basal feed supplemented with 2% açai flour; TF treatment - feed experimentally contaminated with fumonisin (10 ppm); TFA treatment – feed contaminated with fumonisin (10 ppm) and supplemented with açai (2%).

Table 5: Mean ± standard deviation of status oxidant/antioxidant in liver of birds.

Variable	TC: Control	TF: Fumonisin	TCA: Control + açai flour	TFA: Fumonisin + açai flour	P
Status oxidant and antioxidant					
GST (µmol/Cdnb/ min)	181 (39.6)^{c}	272 (46.1)^{a}	224 (36.8)^{b}	109.1 (12.6)^{d}	< 0.001*
ROS (x10 U DCFA/ mg of protein)	8.30 (0.6)^{a}	8.30 (0.74)^{a}	7.0 (1.05)^{b}	7.30 (1.20)^{ab}	0.050*
TBARS (mmol MDA/mg of protein)	84.0 (7.0)^{a}	87.0 (10.4)^{a}	68.7 (8.8)^{b}	78.8 (20.1)^{ab}	0.042*
NOx (µmol/mg of protein	3.90 (1.96)^{b}	16.4 (3.32)^{a}	11.0 (5.77)^{a}	16.6 (4.11)^{a}	< 0.001*
SOD (U SOD/ mg of protein	1.10 (0.13)	0.90 (0.24)	0.91 (0.07)	0.90 (0.03)	0.794
CAT (nmol CAT/ mg of protein	2.60 (0.60)^{c}	3.20 (0.41)^{b}	3.70 (1.30)^{ab}	4.20 (0.91)^{a}	< 0.001*

Note: *P <0.05 indicates significant difference between groups indicate by different letters (a, b) in the same line. The treatments were: control (TC) - birds that received basal feed; TCA treatment - basal feed supplemented with 2% açai flour; TF treatment - feed experimentally contaminated with fumonisin (10 ppm); TFA treatment – feed contaminated with fumonisin (10 ppm) and supplemented with açai (2%).

Table 6. Crypt and intestinal villi morphometry of broiler chickens in the initial phase of production fed with fumonisin-contaminated feed supplemented with açai flour.

Groups	**Villus height (µm)**	**Crypt depth (µm)**	**Villus/crypt ratio**
TC	1068.4 (100.7)[ab]	192.0 (15.6)[a]	5.56 (0.52)[b]
TF	803.8 (22.3)[c]	166.0 (11.0)[b]	4.84 (0.32)[c]
TCA	977.2 (22.1)[b]	173.7 (7.5)[b]	5.62 (0.19)[b]
TFA	1042.2 (34.5)[a]	163.5 (17.5)[b]	6.37 (0.24)[a]
P-value	< 0.001*	<0.001*	0.002*

Note: *P <0.05 indicates significant difference between groups indicate by different letters (a, b) in the same line. The treatments were: control (TC) - birds that received basal feed; TCA treatment - basal feed supplemented with 2% açai flour; TF treatment – feed experimentally contaminated with fumonisin (10 ppm); TFA treatment - feed contaminated with fumonisin (10 ppm) and supplemented with açai (2%).

2. CONSIDERAÇÕES FINAIS

A fumonisina demonstrou afetar negativamente a função hepática em pintos em estágio inicial por promover estresse oxidativo hepático, mais evidente após 10 dias de consumo de ração contaminada. Essas mudanças refletem condições fisiológicas e funcionais e baixo desempenho produtivo dos pintos. No entanto, ração contendo 2% de resíduo de farinha de açaí, demonstrou efeitos positivos na ação antioxidante, minimizando em parte os efeitos negativos causados pela FB1. Este fato favoreceu o desempenho dos animais testados. Estes resultados reforçam sobre o potente efeito antioxidante do açaí, demonstrando sua ação quando usado na alimentação de frangos de corte em estágio inicial, apesar de desafiados pela contaminação com fumonisina.

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4. ANEXOS

5.1. Anexo 1

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 "Efeitos de fumonisina na dieta de pintainhos de corte nos primeiros dias de vida sobre saúde e desempenho dos animais", protocolada sob o CEUA nº 5960011018 (ID 000749), 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 10/10/2018.

We certify that the proposal "Effects of fumonisin on the diet of cutting chicks in the first days of life on animal health and performance", utilizing 96 Birds (96 males), protocol number CEUA 5960011018 (ID 000749), 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 10/10/2018.

Finalidade da Proposta: Pesquisa (Acadêmica)

Vigência da Proposta: de 11/2018 a 07/2019 Área: Zootecnia

Origem:	Animais provenientes de estabelecimentos comerciais						
Espécie:	Aves	sexo:	Machos	idade:	1 a 21 dias	N:	96
Linhagem:	Coob500			Peso:	45 a 1000 kg		

Local do experimento: Os animais utilizados neste experimento serão comprados da empresa Globo aves, e mantidos em galpão experimental da Udesc oeste. Setor de avicultura.

Lages, 18 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

5.2. Anexo 2

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 "Influencia da adição de farinha de açaí na dieta de pintainhos de corte sobre desempenho e proteção hepática quando desafiados com contaminação do alimento por fumonisina", protocolada sob o CEUA nº 2990090519 (ID 000959), 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 18/07/2019.

We certify that the proposal "Influence of the addition of açaí flour on the diet of cutting chicks on performance and hepatic protection when challenged with fumonisin contamination of the food", utilizing 80 Birds (80 males), protocol number CEUA 2990090519 (ID 000959), 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 07/18/2019.

Finalidade da Proposta: Pesquisa (Acadêmica)

Vigência da Proposta: de 05/2019 a 08/2019 Área: Zootecnia

Origem:	Animais provenientes de estabelecimentos comerciais						
Espécie:	Aves	sexo:	Machos	idade:	1 a 21 dias	N:	80
Linhagem:	Coob500			Peso:	45 a 900 g		

Local do experimento: Setor de avicultura da UDESC Oeste

Lages, 18 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