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DISSERTAÇÃO DE MESTRADO

PLASMA SANGUÍNEO *Spray Dried* NO PÓS-DESMAME DE LEITÕES EM DIETAS COM ALTA OU BAIXA CONTAMINAÇÃO POR MICOTOXINAS

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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**

Orientador (a): Dr. Diovani Paiano

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como requisito parcial para obtenção do grau de
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RESUMO

Dissertação de Mestrado
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PLASMA SANGUÍNEO *Spray Dried* NO PÓS-DESMAME DE LEITÕES EM DIETAS COM ALTA OU BAIXA CONTAMINAÇÃO POR MICOTOXINAS

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Chapecó, 03 de março de 2017

Foi realizado um experimento para avaliar os efeitos do plasma sanguíneo suíno *spray dried* (PSD) sobre o desempenho e a saúde dos leitões no pós-desmame, desafiados por baixos e altos níveis de micotoxinas. Foram utilizados 56 leitões machos castrados desmamados aos 24±2 dias, com peso de 7,15±0,61 kg, distribuídos em delineamento inteiramente ao acaso em arranjo fatorial 2*2 sem PSD ou com 6% de inclusão de PSD e com baixa (0,95 µg/kg aflatoxinas + 450 µg/kg fumonisinas) ou alta micotoxina (300 µg/kg aflatoxinas + 8.000 µg/kg fumonisinas), com total de quatro tratamentos e sete repetições para o estudo de desempenho, 14 repetições para as variáveis sanguíneas e cinco para análise tecidual do fígado. O experimento teve duração de 15 dias subdivididos em três períodos de cinco dias. Para o desempenho, foram avaliadas as variáveis: consumo de ração, ganho de peso e eficiência alimentar, além de incidência de diarreias e análise de viabilidade econômica do uso do PSD. E como indicadores do *status* de saúde animal, foram analisados: histopatologia do fígado, atividade das enzimas séricas alanina aminotransferase (ALT) e gama glutamil transferase (GGT) e variáveis bioquímicas séricas (proteínas totais, albumina, globulinas, colesterol e triglicerídeos). Ainda, como marcadores de estresse oxidativo foram analisados os níveis séricos de substâncias reativas ao ácido tiubarbitúrico (TBARS), espécies reativas de oxigênio (EROs), e no sangue total catalase (CAT) e superóxido dismutase (SOD), estes quatro também foram analisados no fígado. Para as variáveis de desempenho, não houve interação entre a contaminação de micotoxinas e o uso de PSD, apenas efeito isolado dos fatores. O PSD elevou o consumo de ração e o ganho de peso, e diminuiu a incidência de diarreias, enquanto que as micotoxinas em altas doses reduziram o ganho de peso. O consumo, ganho de peso e eficiência alimentar foram melhores nas dietas com PSD nos períodos de 1-5; 1-10 e 1-15 dias e o PSD apresentou viabilidade econômica até 10 dias após os desmame nas condições testadas. Para as variáveis bioquímicas séricas (proteínas totais, albumina, globulinas, colesterol e triglicerídeos), não houve interação e nem efeito isolado dos fatores, com exceção da ureia, a qual apresentou níveis menores em todos os períodos nas dietas com PSD. No fígado não foram verificadas alterações histopatológicas. Nas variáveisde estresse oxidativo, a alta contaminação por micotoxinas elevou o nível de EROs e TBARS assim como reduziu a atividade de CAT, em contrapartida o PSD evitou o aumento de EROs e TBARS e elevou o nível da enzima antioxidante SOD. O plasma sanguíneo *spray dried* promoveu bom desempenho dos leitões mesmo em situação de alta contaminação por aflatoxinas e fumonisinas, bem como ocasionou melhor aproveitamento da proteína dietética, e inibiu a condição de estresse oxidativo.

Palavras-chave: Aflatoxinas, Alimento Funcional, Fumonisinas, Suínos

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

Spray Dried **PLASMA IN POST-WEANING OF PIGLETS IN DIETS WITH HIGH OR LOW CONTAMINATION BY MYCOTOXINS**
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Chapecó, 03 March 2017

An experiment was conducted to evaluate the effects of Spray Dried Porcine Blood Plasma (SDPP) on the performance and health of piglets in post-weaning, challenged by low and high levels of mycotoxins. Fifty-six weaned and castrated male piglets were used at 24±2 days, body weight 7.15±0.61 kg, distributed in an entirely randomized 2*2 factorial design without SDPP or with a 6% SDPP inclusion level and with low (0.95 µg/kg aflatoxins + 450 µg/kg fumonisins) or high (300 µg/kg aflatoxins + 8,000 µg /kg fumonisins) levels of mycotoxins , with a total of four treatments and seven experimental units for the performance study, 14 experimental units for the blood variables, and five for liver tissue analysis. The experiment lasted 15 days, subdivided into three periods of five days. For performance, we analyzed the variables: feed intake, weight gain and feed efficiency, as well as the diarrhea incidence and the analysis of the economic viability of SDPP use. As indicators of the animal health status, we analyzed liver histopathology, serum enzymes activity of alanine aminotransferase (ALT) and gamma-glutamyl transferase (GGT), and serum biochemical analysis (total protein, albumin, globulins, cholesterol and triglycerides). Moreover, as markers of oxidative stress, we analyzed the serum levels of thiobarbituric acid reactive substances (TBARS), reactive oxygen species (ROS), and total catalase (CAT) and superoxide dismutase (SOD) in the blood. These four were also analyzed in the liver. Regarding the performance variables, there was no interaction between levels of mycotoxins and the use of SDPP, only isolated effects of the factors. SDPP increased feed intake and weight gain, and decreased the diarrhea incidence, while mycotoxins in high doses reduced weight gain. Feed intake, weight gain and feed efficiency performed better in diets with SDPP in the periods of 1-5; 1-10 and 1-15 days. Despite the technical feasibility of the SDPP, current prices did not allow its inclusion in the third period 11-15 days. Considering the total period (1-15 days), the use of SDPP was economically feasible. Regarding the serum biochemical variables (total protein, albumin, globulins, cholesterol and triglycerides), there was neither interaction nor isolated effects of the factors, with the exception of urea, which showed lower levels in all periods in diets with SDPP. Histopathological changes were not observed in the liver. In the variables of oxidative stress, the high contamination by mycotoxins increased the level of ROS and TBARS, as well as reduced CAT activity. In contrast, SDPP prevented the increase of ROS and TBARS and augmented the level of SOD antioxidant enzyme. Spray dried blood plasma fomented good performance of the piglets even in a situation of high levels of aflatoxins and fumonisins, as well as caused better use of dietary protein, and inhibited the condition of oxidative stress.

Keywords: Aflatoxins, Functional Food, Fumonisins, Swine

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INTRODUÇÃO

Conforme dados da USDA (2016) no ano de 2015, foram produzidas cerca de 110,321 milhões de toneladas de carne suína no mundo. No ano de 2016 o Brasil ocupou o quarto lugar no ranking de países produtores de carne suína e o quarto como exportador (ABPA, 2016). O crescimento na produção de carne suína é estimado em de 2,84%/ano até 2019, com crescimento de 1,79%/ano no consumo médio (Brasil, 2016). O aumento na produção intensifica a necessidade de recursos e também de otimizar os recursos disponíveis. Por isso, dentro de cada fase do ciclo da suinocultura, os desafios devem ser estabelecidos para que sejam buscadas soluções pertinentes.

No ciclo de produção de suínos, o desmame é um momento considerado crítico e que requer cuidados específicos, visto às diversas mudanças e desafios, aos quais os leitões são submetidos em um único momento, os quais comprometem o desempenho e elevam a taxa de mortalidade (SILVA et al., 2014). Portanto, devem ser utilizadas estratégias para amenizar os efeitos estressores no desmame sobre a fase, pois a mesma influencia o desempenho subsequente (KUMMER et al., 2009).

Um dos desafios a que muitas vezes os animais são submetidos, a partir da troca da alimentação líquida por sólida, é a exposição à micotoxinas proveniente de grãos e cereais (EULALIO et al., 2015). As micotoxinas são produtos tóxicos de fungos filamentosos, e a toxicidade desses compostos tem efeito não somente para os microorganismos, mas também para os humanos e animais. A ação tóxica pode ser variada, porém como característica geral deprimem o sistema imune e desencadeiam o aparecimento de tumores (PEREIRA; SANTOS, 2011), além de reduzir o consumo de ração, o ganho de peso e a eficiência alimentar (GOBI et al. 2015), alterar parâmetros bioquímicos séricos (DILKIN, 2010) e induzir ao estresse oxidativo (THEUMER et al., 2010).

Em contrapartida, uma das estratégias utilizadas para amenizar os danos causados pelo desmame, são os alimentos funcionais, os quais conferem aos animais, benefícios que vão além do aspecto nutricional, melhoram e incrementam a saúde. O plasma sanguíneo *spray*

dried é considerado um alimento funcional, por reunir alguns atributos benéficos para os leitões, além de fornecer proteína de alta qualidade, é um ingrediente nobre que aperfeiçoa a dieta e dá suporte nutricional para o desempenho do animal (CAMPBELL et al., 2016).

O plasma sanguíneo em pó na dieta de leitões no pós-desmame promove maior consumo de ração (PUJOLS et al., 2016), aumenta o ganho de peso (CASTELO et al., 2015; DALTO et al., 2013; RIGUEIRA et al., 2013), reduz a ativação do sistema imune (CAMPBELL et al., 2008), melhora a imunidade (CAMPBELL et al., 2016), reduz incidência de diarreias (LIMA et al., 2009), atua na barreira intestinal e reduz processos inflamatórios (PEACE, et al., 2011), reduz a quantidade de algumas bactérias entéricas patogênicas, como *E. coli* e S*amonella* (NIEWOLD et al., 2007). O que demonstra ser uma importante estratégia para promover o desempenho de leitões no pós-desmame.

No entanto, seus efeitos frente a um desafio por contaminação de micotoxinas, ainda é pouco estudado, e são poucos os trabalhos científicos que descrevem sua ação em tais condições.

1.1 Desmame de leitões e o desafio por micotoxinas

O desmame de leitões tem por característica uma redução no desempenho, este é o resultado da soma de diversos fatores que causam estresse, como a modificação da dieta, que passa do leite materno para dieta sólida, uso de alimentos para os quais seu metabolismo é imaturo para digerir; o afastamento da matriz; a troca de ambiente, necessidade de ressocialização entre outros (SILVA et al., 2014).

O desmame é um evento que por si só, já é desafiador o suficiente para provocar alterações fisiológicas nos leitões, a ponto de torná-los mais vulneráveis à ocorrência de doenças, conforme confirmado por Sugiharto et al. (2014), que a partir de análises séricas de leitões desmamados, constatou alterações na resposta inflamatória, associadas à ocorrência de diarreias. Logo, pode ser considerado o momento de maior estresse na vida do suíno, em que se observa redução no consumo de alimento e no desempenho, além de aumento da mortalidade e morbidade nas primeiras semanas (KUMMER et al., 2009).

Alguns alimentos possuem elevada importância na alimentação dos suínos a partir da troca da dieta líquida por sólida, como o milho e o farelo de soja, que compõem a dieta dos suínos desde o desmame até o abate. O milho utilizado como a principal fonte energética e o

farelo de soja, como fonte de proteína, podem compor mais de 50% das rações para suínos, mesmo na fase pré-inicial (TRINDADE NETO et al., 2002).

Porém, estes ingredientes muitas vezes são carreadores de micotoxinas, visto que as commodities de grãos e cereais são as principais fontes de concentração de micotoxinas na alimentação animal (BINDER et al., 2007). No Brasil, em um levantamento realizado por Mallmann et al. (2014), referente aos últimos 10 anos, a positividade de aflatoxinas e fumonisinas foram de aproximadamente 42%, e de 83%, respectivamente, em rações para animais de produção.

1.2 Ocorrência de Micotoxinas

As micotoxinas são compostos tóxicos, produzidos como metabólitos secundários por fungos filamentosos, que possuem ação tóxica tanto em animais quanto em humanos, de forma aguda ou crônica (SOARES et al., 2013). As espécies de fungos que produzem micotoxinas pertencem na sua maioria ao gênero *Aspergillus* e à classe *Flavi*. Porém, as mais importantes são *Aspergillus parasiticus, Aspergillus flavus e Aspergillus nomius* (RODRIGUES et al., 2009). No entanto, mesmo a presença de fungos e o bolor aparente, não são indicativos suficientes da presença de micotoxinas (OLDONI et al., 2012).

A umidade, temperatura, substrato e a composição atmosférica são os principais fatores necessários para que ocorra a produção dessas toxinas fúngicas (BAPTISTA et al., 2004), dado que, combinações de temperatura de 29-35 ºC e umidade superior a 15% são favoráveis (RUPOLLO et al., 2006). Portanto, regiões tropicais estão mais suceptíveis ao desenvolvimento de fungos e micotoxinas, por proporcionar melhores condições de desenvolvimento, como calor e umidade em relação às demais (KWIATKOWSKI; ALVES, 2007).

Os grãos e cereais no Brasil apresentam níveis de contaminação variada por micotoxinas, além de combinações variadas de tipos de micotoxinas (MAZIERO; BERSOT, 2010). Eulalio et al. (2015), analisaram 34 matérias-primas, incluindo milho, farelo de soja, farelo de trigo, farinha de carne e derivados de milho e soja, e detectaram uma positividade de 29% de aflatoxinas e 79% de fumonisinas, entre outras micotoxinas detectadas. No mesmo estudo analisaram 13 amostras de rações para suínos, e obtiveram positividade de 69% para aflatoxinas e 85% para fumonisinas.

Segundo levamentamento realizado por Mallmann et al. (2014), referente aos últimos 16 anos, foi observada uma leve diminuição na ocorrência de aflatoxinas no milho, provavelmente por causa das melhorias das condições do armazenamento dos grãos, visto que são micotoxinas relacionadas aos fungos com crescimento no período de armazenamento. Por outro lado, a ocorrência de fumonisinas aumentou, estas são produzidas em sua maioria por fungos de campo. Esse aumento evidencia a complexidade dos controles para evitar a ocorrência desses compostos tóxicos, visto que são necessárias medidas de proporção sistêmica.

As micotoxinas causam tanto perdas diretas de produtos agrícolas, quanto indiretas, as quais são difíceis de mensurar, porém de alta relevância por atingir diversos níveis da cadeia produtiva (IAMANAKA, et al., 2010). Existem centenas de variedades de micotoxinas, das quais as mais comumente encontradas são aflatoxinas, tricotecenos, zearalenonas, fumonisinas, ocratoxinas e patulina. A maioria das micotoxinas é estável ao calor utilizado no processamento de alimentos, de forma que processamentos que utilizam calor, como a peletização, não são eficientes para inativá-las (ABBAS; SHIER, 2009).

1.3 Micotoxinas e seus efeitos sobre os animais

As micotoxinas são conhecidas por causarem danos em alguns órgãos de forma específica, como é o caso das aflatoxinas que atacam principalmente o fígado, as fumonisinas o pulmão, a zearalenona o aparelho reprodutivo, entre outros (FREITAS, et al., 2012). Porém, de forma geral as micotoxinas reduzem o consumo alimentar (GOBI et al., 2015), deprimem o sistema imune (OSWALD et al., 2005) e são cancerígenas e mutagênicas (LAZO; SIERRA, 2008). Ainda, podem ser causadoras de diversas desordens patológicas, principalmente no fígado, rins, sistema endócrino, sistema imunológico e neurológico (FREIRE et al., 2007).

As aflatoxinas são comumente encontradas e altamente tóxicas (LAZO; SIERRA, 2008). Este grupo de toxinas possui pelo menos quatro variedades conhecidas, as quais são: B1, B2, G1 e G2, sendo que a variedade B1 é considerada a mais tóxica e carcinogênica de todas as micotoxinas conhecidas (RICHARD, 2007) além de afetarem negativamente a imunidade dos leitões (RIBEIRO et al. 2008).

Os mecanismos de ação das aflatoxinas resultam em danos principalmente no fígado com ação sobre diversas estruturas dos hepatócitos. Quando no núcleo da célula, inibe a enzima RNA-polimerase, o que afeta a síntese proteica, além de se ligar ao DNA e causar

danos a esta estrutura, que podem resultar em mutações celulares e carcinogênese. Quando na mitocôndria, dificulta a respiração celular e no retículo endoplasmático, atrapalha as funções metabólicas como síntese de proteínas, indução de enzimas e sínteses de fatores envolvidos na coagulação sanguínea, ações estas que podem comprometer a funcionalidade, com impacto nas diversas funções desempenhadas por ele, entre elas o metabolismo das proteínas e dos lipídeos (MALLMANN et al., 1994).

Os efeitos negativos das aflatoxinas foram reportados por Olinda et al. (2016) em um estudo de caso no Nordeste brasileiro, que envolveu 82 suínos de diferentes fases. Os animais consumiram rações contaminadas naturalmente por micotoxinas, com níveis de contaminação aproximados de 1.500 ppb de aflatoxinas e 0,4 ppm de fumonisinas. Os autores verificaram intoxicação aguda, em que os sinais clínicos eram aparentes e incluíam febres, tremores, taquicardia, taquipneia, apatia, perda de peso, fraqueza muscular, urina amarelo citrino e diarreia leve. A atividade das enzimas, utilizadas como marcadores de lesão celular, alanina aminotransferase (ALT), aspartato aminotransferase (AST) e gama glutamil transferase (GGT) estavam elevadas, resultados estes confirmados a partir da análise de fragmentos do fígado, o qual apresentava tamanho aumentado, cor atípica e alterações hepatocelulares de lesões até necrose. Os níveis de proteínas totais, albumina e globulinas estavam baixos, o que demonstrou que ocorreu distúrbio na síntese proteica, e que o fígado teve sua funcionalidade comprometida pela ação das aflatoxinas.

No Brasil até a corrente data não há legislação vigente, para os níveis máximos aceitáveis de micotoxinas na alimentação animal. A portaria 07 de 09/11/1988, que estabelecia (50 ppb) como nível máximo aceitável de aflatoxinas nos alimentos de animais, foi revogada pela Instrução Normativa 30 de 05/08/2009, a qual regulamenta a rotulagem dos alimentos para animais de companhia, mas nada consta sobre micotoxinas. Por causa desta ausência de suporte de informação de forma unificada, os níveis considerados tóxicos ou não, são baseados nos estudos realizados, os quais apresentam níveis e combinações de micotoxinas diversificados.

Em um estudo no qual foi realizado uma intoxicação experimental de suínos por fumonisinas (10 e 30 ppm) os resultados demonstraram que com doses de 10 ppm, mesmo após 28 dias de consumo, os suínos não apresentaram sinais clínicos de intoxicação e as perdas de desempenho não foram significativas. Porém, com 30 ppm, um dos animais foi a óbito por edema pulmonar após 20 dias de consumo, bem como a conversão alimentar e o ganho de peso foram prejudicados (DILKIN et al., 2004).

Efeitos depressivos no consumo, no ganho de peso, bem como na eficiência alimentar, também foram relatados por Weaver et al. (2014) a partir da inclusão de milho contaminado naturalmente por múltiplas micotoxinas na dieta de leitões (250 ppb de aflatoxinas e 6,9 ppm de fumonisinas). Os animais que consumiram dietas contaminadas por três semanas tiveram seu desempenho comprometido.

Em um estudo realizado por Hauschild et al. (2006), com leitões após o desmame, que foram intoxicados com 800 ppb de Aflatoxinas via dieta, foi constatado que o desafio por contaminação desta micotoxina, reduziu o coeficiente de metabolização da energia, pois aumentou a excreção de energia via urina, além de afetar negativamente o balanço de N (nitrogênio), por diminuir a retenção e aumentar a excreção urinária.

As fumonisinas são as micotoxinas de maior incidência no milho (BITENCOURT et al., 2005). Loiseau et al. (2007) estudaram a ação das fumonisinas sobre o intestino delgado, e constataram que essas toxinas apresentam ação seletiva sobre a porção do jejuno, ligando-se aos esfingolipídeos e glicolipídeos das membranas celulares, o que causa lesão e favorece infecções de bactérias patogênicas.

As fumonisinas além de facilitar novas infecções, também agravam processos infecciosos já existentes, constatado por Kovács et al. (2016), a partir de um experimento com suínos com pneumonia, os quais quando submetidos a doses de 20 ppm de fumonisinas tiveram um agravamento do processo infeccioso.

Segundo Santurio (2007), a fumonisina interage com as esfingosinas, estruturas básicas dos esfingolipídeos, que possuem função estrutural na integridade de membrana. A partir da inibição das esfingosinas, provocam hepatotoxicose, além de causarem edema pulmonar a partir do aumento da permeabilidade vascular pulmonar.

Adicionalmente, as aflatoxinas e fumonisinas isoladas ou associadas, induzem ao estresse oxidativo celular com subsequente oxidação de biomoléculas como proteínas, lipídios e DNA, o que pode resultar em lesões genéticas (THEUMER et al., 2010). O estresse oxidativo, consiste no acúmulo de danos oxidativos em diversos níveis celulares que levam ao declínio funcional (ENGERS et al., 2011).

A fim de testar a toxicidade das fumonisinas e seus efeitos nos leitões desmamados, Dilkin et al. (2010) realizaram um estudo com leitões com oito semanas de idade, para os quais administraram via sonda, uma solução salina com aproximadamente 125 ppm de fumonisinas e puderam constatar ocorrência de edema pulmonar, alterações bioquímicas de variáveis sanguíneas como colesterol, atividade de enzima AST (aspartato transaminase) e ALT (alanina aminotransferase).

As micotoxinas podem ainda, alterar o sistema imunomodulador, em um estudo com doses de 0-80 ppm de fumonisinas isoladas e combinadas com zearalenona, deoxinivalenol e nivalenol, frente à proliferação de células do sangue *in vitro* Luongo et al. (2008) verificaram que, as micotoxinas interferiram negativamente na proliferação celular, em especial quando combinadas, as quais proporcionam sinergismo para sua ação tóxica.

A fim de estabelecer uma relação entre consumo de ração e o ganho de peso de animais que consomem micotoxinas, Gobi et al. (2015) realizaram uma meta análise a partir de dados de 85 trabalhos realizados com suínos de alto desempenho, desafiados por contaminação de aflatoxinas, fumonisinas, zearalenona e deoxinivalenol na dieta, de forma isolada e associadas e concluíram que, intoxicação por micotoxinas tanto na forma isolada, quanto associada, comprometem o consumo de ração, como consequência também o ganho de peso e a eficiência alimentar em especial quando combinadas. Conclusão que reforça a hipótese de sinergismo dos efeitos adversos das micotoxinas.

Com o objetivo de investigar as possíveis causas que levam à queda do consumo, ganho de peso e eficiência alimentar dos suínos, Pastorelli et al. (2012) realizaram uma meta análise a partir da compilação de dados de 122 trabalhos feitos com suínos em seis situações diferentes de desafios, sendo uma delas intoxicação por micotoxinas. A partir dos resultados da meta análise, os autores sugeriram que as micotoxicoses interferem negativamente no consumo e no desempenho dos suínos, além de que os animais nessa condição de desafio não se recuperaram ao longo do período experimental.

Mesmo em doses consideradas relativamente baixas, as micotoxinas podem causar efeitos deletérios nos animais. Oswald et al. (2003) administraram via dieta, fumonisinas na dose diária de 0,5 mg/kg de peso corporal, durante seis dias, para leitões desmamados. No último dia de experimento, inocularam os animais via oral com a bactéria *E. Coli e a*pós 24 horas, os animais foram abatidos e mensurado a *E. coli* no intestino. Os autores observaram que os animais intoxicados por fumonisinas, não apresentavam lesões intestinais, todavia continham números maiores de UFC da bactéria patogênica inoculada, tanto no intestino delgado quanto no intestino grosso. Puderam concluir que, as fumonisinas são um fator predisponente para a infecção de bactérias patogênicas oportunistas, bem como desenvolvimento de doenças infecciosas.

As micotoxinas afetam os suínos de forma multifatorial, e estes apresentam certa sensibilidade aos seus efeitos tóxicos, e quando expostos a níveis altos destes contaminantes, podem ter seu desenvolvimento e desempenho comprometidos (DILKIN, 2004).

1.4 Plasma sanguíneo *spray dried* (PSD)

O plasma *spray dried* (PSD) é produzido a partir da técnica *spray drying,* na qual o sangue é centrifugado, e a parte líquida ou plasma sanguíneo, é desidratado através da aspersão por um roteador ou por bicos aspersores, em que as gotículas caem em uma câmara de calor com temperaturas de 200-240 ºC. A secagem (30 a 90 segundo) resulta em um pó fino de cor amarronzada (POLO et al., 2010). Os extratos resultantes desse processo apresentam maior estabilidade físico-química (OLIVEIRA; PETROVICK, 2010), e mantém parcialmente integros e ativos componentes do plasma, como as imunoglobulinas que representam aproximadamente 20% do total da proteína bruta deste ingrediente, que podem ser utilizados na defesa do organismo frente a agentes infecciosos (PIERCE et al., 2005).

A proteína do plasma foi testada por Thomaz et al. (2009), em substituição a proteína do leite em pó na dieta de leitões desmamados. E não foram observadas perdas no ganho a partir da substituição, além de que o PSD promoveu menor ativação do sistema imune, resultado provavelmente associado às imunoglobulinas ativas.

1.5 Mecanismos de ação do plasma *spray dried* (PSD)

Quando o PSD é adicionado às dietas como fonte proteica, substitui parcialmente fontes de proteína de origem vegetal, como o farelo de soja. Os principais problemas com o uso do farelo de soja nas fases pré-iniciais são os fatores antinutricionais, que não são totalmente desativados no processamento da soja, de forma que o seu uso reduz o aproveitamento da proteína nesta fase. Além de provocarem irritabilidade da mucosa intestinal, redução da capacidade absortiva e aumento da incidência de diarreias (BENEVIDES et al., 2011). Por outro lado, o PSD é considerado uma fonte de proteína de alto valor biológico, pois além da boa palatabilidade, apresenta alta digestibilidade e não contém fatores antinutricionais (CAMPBELL, et al., 2008).

A partir de testes com níveis crescentes de plasma para leitões desmamados aos 28 dias, Lopes et al. (2009) constataram que a melhor inclusão seria de 5,6%, porém que o plasma a partir da segunda semana após o desmame não apresentou mais efeito benéfico. Resposta semelhante foi relatada por Lora Graña et al. (2010), os quais testaram os efeitos do plasma para leitões desmamados aos 21 dias e concluíram que a inclusão de PSD na dieta, em especial nas duas primeiras semanas, proporcionou melhora na palatabilidade, alta

digestibilidade da proteína, efeitos de regeneração da parede intestinal que resultou em melhora na saúde e no desempenho dos leitões.

Segundo Remus et al. (2013), o benefício proporcionado pelo PSD sobre o desempenho dos leitões ocorre intensamente na primeira semana pós-desmame. Com o desenvolvimento do leitão, ocorre redução dos benefícios do uso deste ingrediente, sendo que apartir dos 35 dias de idade os efeitos já não significativos.

Resultados positivos a partir do uso do plasma, também foram descritos em um estudo realizado por Dalto et al. (2011), no qual os autores relataram que a utilização de 10 e 20 g/animal/dia de PSD na dieta para leitões recém-desmamados, proporcionou melhor aproveitamento da proteína pelos leitões mais leves, melhor recuperação de leitoes leves e maior viabilidade econômica. Todavia, a intensidade dos resultados benéficos do PSD, depende dos desafios aos que os animais são expostos, sendo que quanto menor o desafio, menor também a expressividade dos benefícios proporcionados por este ingrediente (BUDIÑO et al., 2010).

Com objetivo de avaliar a morfologia intestinal e a quantidade de *E. Coli* no intestino de leitões desmamados aos 28 frente ao consumo de PSD (5 e 7,5% de inclusão), Barbosa et al. (2013), verificaram aumento no consumo de ração e o no ganho de peso, com redução da incidência de unidades formadoras colônias (UFC) de *E. coli* e que a quantidade de *E. coli* no intestino estava diretamente ligada à ocorrência de diarreias.

Da mesma forma Barbosa et al. (2012) forneceram PSD na dieta de leitões experimentalmente desafiados por *E. coli* no pós-desmame, e verificaram redução desta bactéria, com consequente redução da ocorrência de diarreias. Corroborado pelo trabalho de Niewold et al. (2007), no qual citam que o PSD possui anticorpos específicos para *Escherichia Coli* F4. Pois em situação de infecção experimental por *E. coli* F4 (0149K91), a excreção das UFC desta bactéria patogênica, foi reduzida a partir do consumo de 8% de PSD na dieta, além de aumentar o consumo e ganho de peso diário.

Porém, resultados diferentes quanto à ação do PSD sobre *E. coli* foram relatados por Van Djik (2002), a partir de um estudo com infecção experimental de *E. Coli* (0139:K82 LT), no qual não encontraram efeito do PSD (8% de inclusão na dieta) sobre a excreção dessa bactéria, bem como na incidência de diarreias, apesar de constatar efeito sobre o desempenho (consumo e ganho de peso). Há que considerar que as cepas utilizadas nos trabalhos mencionados, são diferentes, o que sugere que o PSD tem imunoglobulinas de ação sobre *E. coli*, porém sobre algumas cepas específicas.

Outro fator a ser considerado é redução à ativação do sistema imune com o uso do PSD, desta forma, os nutrientes que seriam utilizados na resposta imune (em especial na produção de imunoglobulinas), ficam disponíveis para a síntese de tecidos, o que pode aumentar o desempenho dos leitões (NOFRARIAS et al., 2006). Esse é provavelmente um dos principais motivos do uso do PSD na dieta melhorar a produtividade, pois torna disponível para crescimento, desenvolvimento ou desempenho nutrientes que seriam utilizados na resposta inflamatória (CAMPBEL et al., 2008).

O PSD também pode ser considerado uma alternativa ao uso de antimicrobianos para leitões no pós-desmame, por reduzir a adesão de bactérias patogênicas à parede intestinal, reduzir a resposta pró-inflamatória a partir da redução de citocinas, melhorar o consumo de ração e o ganho de peso, o que resulta em uma maior eficiência alimentar (TORRALORDONA, 2010).

Além de reduzir os processos inflamatórios, a partir da redução da produção de citocinas pró-inflamatórias, Gao et al. (2014) constataram que o PSD também tem ação sobre o estresse oxidativo, no intestino e nos níveis sanguíneos com aumento da quantidade de antioxidantes enzimáticos como a catalase (CAT) e redução de malondialdeído (produto da peroxidação lipídica).

Dalto et al. (2013) testaram o fornecimento 10 e 20 g de PSD/animal/dia, para leitões desmamados, separados em grupos de mais leves e mais pesados, por 10 dias. O PSD nos dois tratamentos favoreceu a maturação dos órgãos linfóides, visualizado a partir da elevação dos níveis de igA (imunoglobulina A). Especificamente para a dose de 20 g/dia, proporcionou melhorias na morfologia intestinal. Além, de igualar o desempenho dos animais leves aos pesados.

Resultados pertinentes quanto aos benefícios do PSD em leitões desmamados, também foram relatados por Weaver et al. (2014) frente à intoxicação por múltiplas micotoxinas provenientes de milho naturalmente contaminado. Na primeira fase (12 dias) com inclusão de 6% de PSD e na segunda fase (21 dias) um dos tratamentos com 6% e outro com 3%. Para a segunda fase os animais foram divididos, de modo que de um mesmo tratamento da primeira fase, agora originasse dois grupos, um para ser desafiado por micotoxinas e outro não. A partir do confronto entre os tratamentos, concluiram que os animais que receberam PSD na primeira fase, quando desafiados por micotoxinas na fase subsequente, mesmo sem continuar a consumir PSD, tiveram melhor desempenho, visualizado a partir do maior consumo e ganho de peso. Os resultados evidenciaram que o PSD não só proporcionou melhor desempenho enquanto consumido, como melhor condicionou os animais para desafios futuros.

Um fator que deve considerado no processo decisório de incluir um ingrediente alternativo na dieta, é a viabilidade econômica, Muniz et al. (2001) a partir de uma avaliação econômica do uso de 5% e 3% de PSD na primeira e segunda semana pós-desmame respectivamente, para leitões desmamados aos 20 dias de idade, consideraram o ganho de peso e o índice de eficiência econômica e concluíram que o PSD tornou as rações mais econômicamente viáveis por proporcionar maiores ganhos em especial para os leitões de baixo peso. A viabilidade econômica do PSD no pós-desmame, também foi avaliada por Dalto et al. (2011), para animais que consumiram 20 g de PSD por dia com resultados positivos para a inclusão na fase de creche.

Os resultados obtidos na literatura indicam que o uso PSD apresenta versatilidade quanto à sua aplicação, visto que este foi testado em diversas situações. No entanto há que considerar que quando é fornecido o PSD na dieta de leitões, a intensidade da resposta proprocionada dependerá da combinação de diferenes fatores, como a composição da dieta, o nível de inclusão de PSD, o *status* de saúde do animal e o nível de desafio ambiental (VAN DIJK et al., 2001).

2 - CAPÍTULO II
MANUSCRITOS

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

Journal of Animal Phisiology and Animal Nutrition e
Research in Veterinary Science

2.1 – MANUSCRITO I

Plasma sanguíneo *spray dried* em dietas com alta ou baixa contaminação por micotoxinas para leitões no pós-desmame sobre o desempenho e viabilidade econômica

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Spray dried plasma in diets with high or low levels of mycotoxins for piglets in post-weaning on performance and economic viability

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Abstract

Mycotoxins are a problem in pig farming especially in post-weaning, given the physiological immaturity of weaned piglets, so that ingredients, such as spray dried porcine plasma (SDPP), can be an alternative to minimize it. This study was carried out in order to evaluate the effects of the use of SDPP for piglets in post-weaning, fed with diets with low or high levels of mycotoxin contamination. Fifty-six weaned castrated piglets were used at 24±2 days, body weight 7.15±0.61 kg, allotted in an entirely randomized 2*2 factorial design without SDPP or with a 6% SDPP and with low (0.95 µg/kg aflatoxins + 450 µg /kg fumonisins) or high (300 µg/kg aflatoxins + 8,000 µg /kg fumonisins) level of mycotoxin, with a total of four treatments, seven experimental units with two piglets. The experimental period consisted of 15 days, subdivided into three periods of five days, in which feed intake, weight gain, feed efficiency, days with diarrhea, and economic feasibility of SDPP use were evaluated. There was no interaction between the contamination by mycotoxins and the use of SDPP for the variables studied. Feed intake intake, weight gain and feed efficiency were better in diets with SDPP in the periods of 1-5; 1-10 and 1-15 days. In the periods 1-10 and 1-15, the treatments with SDPP showed lower diarrhea incidence. The high dose of mycotoxins reduced weight gain in 11-15 days. Despite the technical feasibility of the SDPP, current prices did not allow its inclusion in the third period 11-15 days. Considering the total period (1-15 days), the use of SDPP was economically feasible. Spray dried plasma fostered better weight gain, feed intake and reduced the diarrhea incidence in piglets post weaning, and its use is recommended in situations of low or high levels of mycotoxins.

Keywords: Aflatoxins, Functional Food, Nursery, Fumonisins, Swine

Introduction

In industrial pig farming, the weaning period is considered critical, since it gathers several stress factors for the piglets simultaneously, such as removal from their mothers, grouping with unknown individuals, change of environment and the sudden change of liquid for solid feeding (Pinheiro, 2014). The sum of these factors leads to a decrease in feed intake, weight gain and increase the diarrhea incidence (Campbell et al., 2013).

The change of feeding entails the inclusion of ingredients in the diet of the piglets that will be a part of their feed throughout the whole cycle, such as corn and soybean meal – relevant ingredients that together represent over 50% of the production cost (ICP Suíno-Embrapa, 2016). However, these ingredients of high dietary importance often turn out to be carriers of mycotoxins (Daga et al., 2015).

Mycotoxins are secondary metabolites of some varieties of filamentous fungi, mainly *Aspergillus*, *Fusarium* and *Penicillium*, produced with the objective of making the environment less competitive, giving the fungi an advantage to enjoy the nutrients available (Oliveira et al., 2015). However, what makes mycotoxins worrisome is that its toxicity extends to animals and human beings with health damages (Rocha, et al., 2014).

There are over 400 types of mycotoxins, with particular attention to a small group, due to their toxicity, among them aflatoxins, fumonisins, zearalenone, trichothecenes and ochratoxins (Cardoso Filho et al., 2015). Mycotoxins may have specific action according to their variety, but generally compromise protein synthesis, are immunosuppressive and carcinogenic (Zain, 2011), which results in economic damage to pig raising (Castro et al., 2015). Additionally, the combination of more than one mycotoxin in the same food has a synergistic effect, so that it may enhance its harmful effects (Oliveira et al., 2016).

By contrast, some functional food can be used in the post-weaning phase in order to promote performance and productivity, such is the case of Spray Dried Porcine Plasma (SDPP) (Adewole et al., 2016). SDPP is considered a functional food, due to its characteristics that provide benefits, as well as its nutritional aspect. SDPP is considered a high quality source of protein, with the presence of active immunoglobulin with good palatability to piglets. All these attributes make SDPP a possible tool to promote piglet performance after weaning, with increased productivity and immune system functions (Hu et al., 2014). The benefits of using SDPP in piglet feeding during the nursery phase are quite significant, which has lead authors such as Cromwell (2009) to classify it as one of the major discoveries regarding swine nutrition in the last 100 years.

Facing the challenge of mycotoxins and considering SDPP a beneficial alternative to use in this phase, adding to the low volume of studies that assess the combination of these elements, this study was conducted with the goal of evaluating the effects of the use of spray dried porcine plasma in diets for weaned piglets, contaminated with low or high levels of aflatoxins and fumonisins.

Material and methods

The present study, protocol number 01.34.15, was approved by the Ethics Committee of the State University of Santa Catarina (UDESC).

Animals and Facilities

The experiment was carried out in a pig production teaching unit (27°12'S52°37'W) in May. For this intent, fifty-six castrated male piglets, crossbreed commercial line, were used, with an initial weigh of 7.15±0.61 kg, weaned at 24±2 days, acquired from pig producing units of the region. They were distributed in a 2x2 factorial design, without plasma or with a 6% SDPP inclusion in the diets and with low (0.95 µg/kg aflatoxins + 450 µg /kg fumonisins) or high (300 µg/kg aflatoxins + 8,000 µg /kg fumonisins) levels of mycotoxins, with a total of four treatments, seven experimental units with two piglets.

The piglets were housed in experimental pens of 1.2 x 0.5 m, with slatted plastic floor, equipped with a rounded bottom tube feeder and a cup-type drinker. Feeds and water were *ad libitum*. Feeding was manual, with seven feedings per day, in order to avoid waste, stimulate consumption and additionally visualize the diarrhea incidence.

The nursery room was equipped with double curtains and automated system for the activation of heaters, programmed for activation with a minimum temperature of 23°C and shutdown at 25°C. To record room temperature, minimum and maximum thermometers were used, of dry bulb and wet bulb, with two daily records (8:00 AM and 4:00 PM), from which the minimum temperatures 22.1°C±1.9°C, maximum temperatures 25.8°C±1.1°C and relative humidity 77.4±6.7% were calculated.

Experimental diets

The experimental feeds consisted of four different mash diets (Table 01), produced from corn and soybean meal, according to the minimum nutritional recommendations for the post-weaning phase (Rostagno et al., 2011). The amino acid composition of the corn and

soybean meal used was estimated according to the crude protein analysis and the corn and soybean meal amino acid profile proposed by Rostagno et al. (2011). All diets were formulated based on the concept of ideal protein, with correction up to the sixth limiting amino acid.

The feeds made up to the four treatments and differed among themselves by the level of aflatoxins and fumonisins and by the addition or not of 6% of SDPP, being TA (0.95 µg/kg aflatoxins + 0.45 mg/kg fumonisins); TB (0.95 µg/kg aflatoxins + 0.45 mg/kg fumonisins + 6% SDPP); TC (300 µg/kg aflatoxins + 8.0 mg/kg fumonisins); and TD (300 µg/kg aflatoxins + 8.0 mg/kg fumonisins + 6% SDPP), with seven experimental units (seven pens with two piglets). The level of aflatoxins and fumonisins in diets A and B was due to the inclusion of naturally contaminated corn and soybean meal obtained from local trade, and in diets C and D by the inclusion of naturally contaminated corn and soybean meal plus the addition of isolated aflatoxins and fumonisins.

Mycotoxins

The aflatoxins were produced by fermentation in rice, converted under constant agitation and at controlled temperature. The strain NRLL 2999 of *Aspergillus parasiticus* was used according to the method described by West et al. (1973). After autoclaving with an open valve, the material was dried in a forced ventilation oven and grounded in a laboratory mill equipped with a 1 mm sieve. The concentration of aflatoxins was later determined by HPLC (Thorpe et al., 1982). After fermentation, a total of 130,000 µg of aflatoxins/kg of rice with approximately 83% of aflatoxin B1, 9.5% of aflatoxin B2, 3.4% of aflatoxin G1 and 4.2% of aflatoxin G2 was obtained.

Fumonisins were produced from the fermentation of samples of corn grains. For this purpose, 1 kg of corn samples were conditioned in plastic containers of 4 L capacity, added with distilled water sufficient to reach water activity (WA) $\geq$ 0.95 and autoclaved for 1 hour by 1 ATM and 125ºC. Autoclaved corn was inoculated with an isolate of *Fusarium verticillioides*, producer of fumonisins, and left to incubate at 25°C for 30 days, subsequently subjected to the same procedures previously described for rice and was Obtained fumonisin levels averaged 110,000 µg /kg.

The amount of contaminated material added to the diets with high levels of mycotoxins was calculated based on the level of contamination present in the sources of aflatoxins and fumonisins and their desired level in the final diet. Meanwhile, the low level derived from the naturally contaminated corn and soybean meal, as previously described.

Performance variables

Feed intake, weight gain and feed efficiency (gain/consumption) were assessed every five days (periods) in three sequential periods from weaning 1-5, 6-10 and 11-15 days, as well as the respective combinations 1-10 and 1-15 days. These were obtained through the weighing of the animals on the first day of each experiment period (before feeding) and at the end of the experiment. On the same days, feed intake was accounted to calculate the feed intake, for which the feed waste was considered. In a complementary way, the diarrhea incidence was measured, obtained from observation along the feeding. When diarrhea was observed, a positive occurrence was recorded in the experimental unit for that day, without specific identification of which piglet or of intensity. At the end of each five-day period, the total number of days with diarrhea per pen was calculated in the respective periods.

Economic analyses

The first economic analysis was the calculation of the bio-economic indices, elaborated based on the results of weight gain and feed intake, obtained for the diets with and without plasma. The economic bio-indices were used for the elaboration of inequalities of prediction of the maximum price for the SDPP to make its use feasible, according to the expression adapted from Guidoni et al. (1997)'s:

$$MPSDPP \leq \left[PRP(Gain_i - Gain_0) - \sum_{J \neq L=1}^{N} P_j \left(C_{ji} \times FI_i - C_{jo} \times FI_0 \right) \right] / (C_{li} \times FI_i)$$

in which MPSDPP = maximum price of SDPP so that the diet where it will be used has the same economic efficiency as the diet with zero inclusion SDPP; PRP = price per kg of piglet; $Gain_i$ = average weight gain of piglets from the treatment with i level of SDPP (6%); $Gain_o$ = average weight gain of piglets from the treatment without SDPP; P_j = price of the other ingredients in each diet; C_{ji} = percentage of the ingredient j in the diet i; FI_i = average total feed intake per animal inherent to the diet i; C_{jo} = percentage of the ingredient j in the diet without SDPP; FI_o = average total feed intake per animal on the diet without SDPP; C_{li} = percentage of the SDPP in the diet i.

The second analysis, aiming to estimate the economic viability of the use of SDPP, consisted of the multiplication of the bio-indices obtained from the previous stage by the prices of the ingredients from local trade (December 2016): R$ 9,66 for kg of piglet (equivalent to three times the price of the finished pig kg); R$ 0,76 for common corn; R$ 4,48

for whey; R$ 1,36 for soybean meal 45% CP; R$ 1,68 for sugar; R$ 1,68 for dicalcium phosphate; R$ 0,13 for calcitic limestone; R$ 3,19 for soybean oil; R$ 12,84 for micromineral supplement; R$ 8,45 for vitamin supplement; R$ 5,00 for zinc Oxide; R$ 0,36 for common salt; R$ 6 for L-lysine; R$ 11,56 for DL-methionine; R$ 7,20 for L-threonine; R$ 31,05 for L-tryptophan; R$ 36,79 for L-valine and an estimated cost of R$ 80 for the kg of L-isoleucine, respectively.

Experimental design

The experimental design used was a 2*2 factorial design, with two factors (low or high levels of mycotoxins and without or with 6% SDPP), what totaled four treatments with seven experimental units with two piglets.

The values obtained were previously analyzed as for the normality of errors using the Kolmogorov-smirnov test and presented normality (P>0.05). Subsequently, the performance variables and the diarrhea incidence were submitted to the analysis of variance and to the F-test using the SAEG statistical package, for the performance variables the initial weights were considered. The following statistical model was used: $Y_{ijk}= \mu + A_i + B_j + (AB) + e_{ijk}$ where Y = value of the variable tested under the i-th level of factor A and j-th level of factor B; μ = overall mean associated with all observations; A_i = effect of the i-th level of factor A (low or high levels of mycotoxin); B_j = effect of the j-th level of factor B (without or with inclusion of 6% of SDPP); $A_i * B_j$ = effect of the A and B interaction; e_{ijk} = random error associated with all observations and P <0.05 was adopted as difference and values of P>0.05 and P<0.10 were considered as trend according to Xu et al. (2016).

Results

There was no interaction between mycotoxins and SDPP for any of the variables evaluated (P>0.05), suggesting that the deleterious effects of mycotoxins and/or SDPP benefits were independent.

Performance

The individual feed intake was higher (P<0.05) in diets with SDPP in all periods evaluated. The difference in consumption among the animals that consumed SDPP was about 0.5 kg/animal per period, totaling about 1.5 kg of feed intake improve in the total period (Table 2).

Weight gain and feed efficiency were higher (P<0.05) in the diets with SDPP in the periods of 1-5, 1-10 and 1-15 (Table 2). Individual weight gain in the total period was about 1.0 kg bigger for the treatment with SDPP. The last period (11-15 days), when evaluated separately from the others, showed no effect of the SDPP on weight gain (P>0.05) and a tendency of worsening intake was observed (P=0.06) for diets high on mycotoxins. Mycotoxins did not influence (P>0.05) the diarrhea incidence (Table 3). By contrast, the use of SDPP in diets decreased (P<0.05) the diarrhea incidence in the periods 1-10 and 1-15. The incidence of days with diarrhea in the total period (1-15 days) was about 2.5 times lower in the treatment with 6% SDPP.

Economic analyses

In the bio-indices generated (Table 4), the factor that most influenced the maximum price SDPP can cost, in the first periods (1-5 and 6-10 days), was the piglet bio-index, followed by corn, whey powder and soybean meal. In the third period, corn was the ingredient with the most relevant bio-index on SDPP cost to enable its use. The maximum estimated price for SDPP to be economically viable should be lower than R$ 78,76, 62,94 and 42,37, respectively, for the periods 1-5, 1-10 and 1-15 days. Considering separately phases 6-10 and 11-15 days, the cost of the plasma should be lower than R$ 55, 97 and 11,63 respectively. The cost of the SDPP charged in the experimental period was 18,00 R$/kg, in a way that the simulation indicated the feasibility of using the SDPP. Considering the third experimental period of 11-15 days, the price charged did not make the use of plasma economically feasible.

Discussion

Performance

The increase of feed intake treatments with 6% inclusion of SDPP may have resulted from an improvement in the palatability, associated with better piglet health status. The increase of intake was also detected by Gattás et al. (2008), who observed a linear increase in feed intake and weight gain of piglets weaned early at 14 days, as they augmented the inclusion of SDPP in the diet. There was better gain from the inclusion of 6.6% in the first 14 days after weaning, after what it remained constant and did not provide gains 29 days post weaning.

Weaning is a sufficiently stressful factor to suppress feed intake by piglets (Li and Patience, 2016) and other factors can aggravate it, such is the case of vaccine administration.

Facing this fact, in order to test the stimulating effect of SDPP on consumption, Pujols et al. (2016) introduced SDPP in the diet of weaned piglets along with a vaccination program, and analyzed feed intake, morbidity and mortality of the animals in the later stages. They observed that the animals that consumed SDPP in their diet showed increased intake and body weight, with higher feed efficiency, lower morbidity and mortality rates in the subsequent phases. The authors associated that with a better conditioning of the animals due to the greater intake and body weight, as well as the improvement of their intestinal health, provided by the SDPP.

The greater weight gain in diets with SDPP is likely associated with the sum of the following factors: partial substitution of the source of vegetal protein from soybean meal and its respective anti-nutritional factors for an animal source of protein, with better nutritional potential and improved feed intake and palatability, as well as the presence of functional components, such as active immunoglobulin, which improved the immunological status of the piglets.

Furthermore, SDPP was studied by Jeong et al. (2016), who verified higher apparent ileal digestibility of N (nitrogen) and energy for SDPP when compared to soybean meal for weaned piglets. Additionally, soybean meal, even after processing, presents anti-nutritional factors such as trypsin, stachyose and raffinose inhibitors, which may decrease protein utilization in newly weaned animals, as well as cause irritability of the intestinal mucosa, with reduction of their absorptive capacity and increased diarrhea. On the other hand, SDPP, besides presenting bigger protein digestibility and does not have known anti-nutritional factors (Barbosa et al., 2012).

In the same line of studies, Perez-Bosque et al. (2016) assessed the administration of enterotoxins and SDPP, and concluded that plasma has anti-inflammatory mechanisms that include the reduction of the adhesion of pathogenic molecules to the intestinal mucosa and the reduction of leukocyte activation. This provides a better balance of the intestinal mucosa when in an inflammatory process so that nutrients that would be used in the immune response are available for productive purposes, with consequent improvement in the piglets performance.

Similarly, Tran et al. (2014), aiming to study SDPP mechanisms of action on the intestinal morphology, carried out two parallel studies, one *in vivo* and another *in vitro*. The animals that received SDPP showed greater intake and growth in the first week post-weaning, and the beneficial effects of SDPP were also observed in the *in vitro* experiment, with the increased proliferation of epithelial enterocytes in the jejunum. Based on the hypothesis that

SDPP improves intestinal health, promoting greater absorption of nutrients, added to the fact of SDPP's better protein quality, we can assume that the piglet will perform better even in more challenging situations, as observed in treatments with high mycotoxins level and SDPP and absence of interactions.

In isolation, there was no difference (P>0.05) in weight gain in the period of 11-15 days (Table 2). Similar results were observed by Barbosa et al. (2007), with absence of SDPP effect over piglets intake at 29-35 days. The absence of a positive response in the performance of the piglets from the tenth day of SDPP consumption is probably because at this moment piglets are more matured, with higher production of digestive enzymes than in the first and second weeks after weaning. This allows both a better use of nutrients from vegetal sources, and the development of the immune system and the increase of their active immunity (Formigoni and Fontes, 2014). This hypothesis is reinforced by the lower diarrhea incidence in the third stage of all treatments, probably due to the maturation of the digestive system.

Similarly, Remus et al. (2013) point out that the beneficial effects of plasma on performance decrease as the animal develops, and the greater benefit of SDPP on performance occurs in the first week after weaning, and from 35 days of age the beneficial effects are no longer relevant. It is necessary to consider that the level of challenges to which the animal is subjected in health care is a determinant factor for the benefits of plasma to stand out, so that the greater the challenge the more intense the effects of SDPP on piglets at weaning will be (Budiño et al., 2016).

The harmful effect of mycotoxins in the third period (11-15 days) seen in this study was also observed by Weaver et al. (2014) who, when testing the effects of SDPP in a challenging situation by mycotoxins, observed a reduction in intake and weight gain, and, in contrast, the beneficial effects on these variables from the use of SDPP. The absence of effects of high contamination of mycotoxins in the first two stages, only with a tendency to worsen intake (P=0.06) in the third stage, may be associated with the cumulative effect the mycotoxins had on piglets in the previous stages. This means that the level and/or time of exposure were not sufficiently high to have effects on performance variable studied until the third stage. Likewise, Sun et al. (2015) found no effect on growth performance after a study about piglets in nursery, which received a dose of 20 μg/kg of aflatoxins for 35 days.

Feed efficiency was higher in diets with SDPP (1-5, 1-10 and 1-15 days). The higher feed efficiency from the consumption of SDPP is probably associated to the plasma's beneficial effects on gain, as previously mentioned. However, when analyzed in isolation, in the last period (11-15 days) there was a trend (P=0.06) of inversion, with better feed

efficiency in the diet without SDPP, which we believe may be associated to the recovery and rehydration of the animals affected by diarrhea in the previous phases and not necessarily to effects caused by the diet. This was based on the frequency of days with diarrhea that in the third period decreased more intensely for the treatment without plasma, which decreased by about 1.1 days (1.9 days to 0.7 days), whereas for the treatment with SDPP the reduction was about 0.3 days (0.4 days to 0.1 days). At the same time, the piglet maturation (intestinal and immunological) occurs naturally, as previously mentioned, which may have resulted in a trend for better effects on the FE of this period in diets without SDPP.

The incidence of days with diarrhea in piglets (Table 3) that did not received diet with 6% SDPP) were about 3.6 times bigger ($P>0.05$) than diets with SDPP) for the whole period. Post-weaning diarrhea may originate from multifactorial causes, ranging from infectious aspects to nutritional imbalance, and plasma, for its multifactorial benefits (better palatability, greater digestibility, active immunoglobulin, among others) was sufficient to minimize ($P<0.05$) the occurrence of diarrhea in the total period. We believe that the absence of positive plasma effects ($P>0.05$) on diarrhea in the first phase (1-5 days) may be associated with the short evaluation period (five days), which is close to the incubation period of diarrhea-causing agents associated with disinfection and depopulation carried out before the work, which favored their occurrence in the subsequent phases.

This hypothesis, referring to the benefits of plasma on diarrhea reduction, is reinforced by allegations made by Lima et al. (2009) that diets drafted in a balanced way, with high digestibility ingredients can diminish the occurrence of diarrhea.

As previously discussed, one of the main elements of SDPP are active immunoglobulin that can be easily absorbed intact within the first twelve hours of the piglet's life, with a reduction after this period, and cease completely around 48 hours, after the closure of the intestinal wall (Heim et al., 2011). In spite of that, studies have shown that they may have some local action on the intestine, resulting in greater intestinal health, better integrity of the mucosa and reduced pathogen permeability, with a lower diarrhea incidence (Petchow et al., 2014), corroborating with the results obtained.

Immunoglobulin present in the plasma have activity on pathogenic bacteria such as *Salmonella* and *E. Coli*, one of the most common causes of infectious diarrhea in piglets (Heddegard, 2016). The increase in immunity from SDPP consumption has also been confirmed by Campbell et al. (2016) from the exposure of animals to infectious diseases, which presented better performance, even when challenged. All these factors combined can explain the smaller diarrhea incidence in animals with SDPP consumption.

Economic analyses

The highest values of the bio-index for piglet gain obtained in the first two periods (1-5 and 6-10 days) compared to the others were expected, since several authors evinced that the benefits of plasma are more intense in the first days after weaning, reducing over time (Remus et al., 2013). Consequently, in the evaluation of the 3rd period (11-15 days) in isolation, where the plasma's benefits were smaller, corn, the ingredient of greater inclusion, became the main bio-index to determine the maximum price SDPP could cost.

The simulation of maximum SDPP price indicated that its use was economically feasible (1-5, 1-10 and 1-15 days). Although it was economically feasible in the third period (11-15 days), for promoting higher feed intake, its estimated price was higher than the market price for plasma in the period (R$ 18,00), which made it economically inefficient during this period. The economic feasibility of this ingredient in post-weaning was also verified by Dalto et al. (2011), who obtained results favorable to the use of SDPP and its benefits influenced the whole nursery period. However, there is a need for further studies on the economic break-even point of SDPP use.

We should note that the beneficial effects of plasma in the studied phase might not be reduced only to it, but also result in benefits for later stages, for better preparation of the piglets for the subsequent phases. As described by Weaver et al. (2014), which may enable its use in phases further away from weaning date, so that all results observed, especially those about economic feasibility, may be underestimated. Another noteworthy fact is that for this work a high degree of prophylaxis was envisioned. In field situations, often more challenging, the benefits obtained on the economic sphere could be greater. Thus, further studies are necessary to assess performance and economic viability of plasma use in the pre-initial phase, considering the probable benefits over later phases in different challenging situations.

The addition of SDPP has promoted an increase in intake and weight gain, as well as reduced diarrhea incidence in piglets in a situation of low or high challenge by aflatoxins and fumonisins contamination. Mycotoxins reduced gain in the third period. In isolation, SDPP did not present economic feasibility in the third period (11-15), but presented economic feasibility in the periods 1-5, 1-10 days and total period (1-15 days).

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Conflicts of interest

The authors declare that there is no conflict of interest.

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Table 1- Composition of experimental diets.

Itens, %	Low mycotoxins		High mycotoxins	
	Without SDPP	With SDPP	Without SDPP	With SDPP
Spray-dried porcine plasma	-	6.0	-	6.0
Isolated aflatoxins	-	-	0.23	0.23
Isolated fumonisin	-	-	6.91	6.88
Grinded corn	39.43	46.33	33.23	40.11
Soybean meal (45% CP)	31.38	20.25	30.43	19.31
Whey powder	15.0	15.0	15.0	15.0
Sugarcane	5.0	5.0	5.0	5.0
Dicalcium phosphate	1.29	1.23	1.30	1.24
Limestone	0.74	0.82	0.74	0.84
Soy oil	3.40	2.11	3.35	2.08
Vitamin suplement [1]	0.30	0.30	0.30	0.30
Mineral suplement [2]	0.30	0.30	0.30	0.30
ZnO	0.25	0.25	0.25	0.25
Salt	0.37	0.04	0.37	0.04
L-Lys H-Cl	0.84	0.75	0.86	0.78
DL-Met	0.38	0.34	0.38	0.34
L-Thr	0.45	0.38	0.45	0.38
L-Trp	0.08	0.08	0.08	0.09
L-Val	0.58	0.52	0.59	0.52
L-Ile	0.20	0.29	0.21	0.30
Calculated composition				
Aflatoxins, µg/kg	0.95	0.95	300	300
Fumonisins, µg /kg	450	450	8,000	8,000
Ca, %	0.85	0.85	0.85	0.85
Available P, %	0.43	0.43	0.43	0.43
Na, %	0.28	0.28	0.28	0.28
Metabolible energy, Mcal/kg	3.40	3.40	3.40	3.40
Crude Protein, %	21.0	21.0	21.0	21.0
Lis digestible, %	1.65	1.65	1.65	1.65
Met+ Cis digestible, %	0.91	0.91	0.91	0.91
Trp digestible, %	0.30	0.30	0.30	0.30
Thr digestible, %	1.11	1.11	1.11	1.11
Val digestible, %	1.37	1.37	1.37	1.37
Ile digestible, %	0.91	0.91	0.91	0.91
Neutral detergente fiber, %	8.98	8.25	8.12	7.39
Acid detergente fiber, %	3.96	3.29	3.66	3.00

[1]Provided the following per kilogram of diet: Vit. A – 4.167.000 UI, Vit. D3 – 833.000 UI, Vit. E – 13.333 mg, Vit. K3 – 1.000 mg, Vit. B1 – 1.000 mg, Vit. B2 – 1.667 mg, Vit. B6 – 1.000 mg, Vit. B12 – 8 mg, Niacin – 11.667 mg, Pantothenic Acid – 7.333 mg, Folic acid – 200 mg, Colin – 104 mg, Biotin – 33 mg; [2]Ca - (min. 166 g e max. 203 g), Co – 266,7 mg, Cu – 66,67 g, I – 600 mg, Mn – 18,33 g, Se – 135 mg, Zn-41,67 g, Fe-66,67 g. * 40 mg/kg growth promoter.

Table 2- Effects of level of mycotoxin and of the treatment with spray-dried porcine plasma (SDPP) on piglet performance.

	Low mycotoxins		High mycotoxins		Mycotoxins		SDPP		P values =			
	Without SDPP	With SDPP	Without SDPP	With SDPP	Low	High	Without	With	Mycotoxins	SDPP	M*S	CV %
Body weight, kg												
Initial	7.13	7.15	7.11	7.19	7.14	7.15	7.12	7.17	0.94	0.62	0.77	3.65
5° day	7.37	8.24	7.35	8.36	7.81	7.86	7.36	8.30	-	-	-	-
10° day	8.58	10.30	8.47	10.40	9.44	9.43	8.52	10.35	-	-	-	-
15° day	10.71	12.90	10.30	12.39	11.81	11.35	10.51	12.65	-	-	-	-
Feed intake, kg/piglet/period												
1-5 days	1.20	1.62	1.12	1.66	1.41	1.39	1.16 a	1.64 b	ns	0.01	ns	28.33
6-10 days	1.71	2.24	1.52	2.20	1.98	1.86	1.61 a	2.22 b	ns	<0.01	ns	19.60
11-15 days	2.59	3.10	2.12	2.75	2.84	2.43	2.35 a	2.93 b	0.06	0.03	ns	21.45
1-10 days	2.91	3.86	2.64	3.85	3.38	3.25	2.77 a	3.86 b	ns	<0.01	ns	21.54
1-15 days	5.49	6.95	4.75	6.61	6.22	5.68	5.12 a	6.78 b	0.23	0.01	ns	20.70
Weight gain, kg/piglet/period												
1-5 days	0.24	1.09	0.24	1.17	0.67	0.71	0.24 a	1.13 b	ns	<0.01	ns	83.32
6-10 days	1.21	2.06	1.12	2.04	1.64	1.58	1.16 a	2.05 b	ns	<0.01	ns	35.77
11-15 days	2.13	2.60	1.84	1.99	2.37 a	1.91 b	1.98	2.29	0.03	0.15	ns	23.57
1-10 days	1.45	3.15	1.36	3.21	2.30	2.29	1.40 a	3.18 b	ns	<0.01	ns	41.32
1-15 days	3.58	5.75	3.20	5.20	4.67	4.20	3.39 a	5.48 b	0.32	<0.01	ns	29.14
Feed efficiency (weight gain/feed intake)												
1-5 days	0.05	0.64	-0.07	0.67	0.34	0.30	-0.01 a	0.65 b	ns	<0.01	ns	138.33
6-10 days	0.69	0.92	0.61	0.91	0.81	0.76	0.65 a	0.98 b	ns	0.01	ns	32.85
11-15 days	0.85	0.84	0.89	0.72	0.85	0.80	0.87	0.78	0.32	0.06	0.07	12.57
1-10 days	0.46	0.81	0.33	0.81	0.63	0.57	0.39 a	0.81 b	ns	<0.01	ns	47.11
1-15 days	0.65	0.83	0.60	0.78	0.74	0.69	0.63 a	0.80 b	0.30	<0.01	ns	18.33

* Values followed by different lowercase letters in the line differ (P <0.05) for the F test; ns = not significant; CV = coefficient of variation.

Table 3- Effects of levels of mycotoxins and of the treatment with spray-dried porcine plasma (SDPP) on incidence of piglet diarrhea.

	Low mycotoxins		High mycotoxins		Mycotoxins		SDPP		P values=			
	Without SDPP	With SDPP	Without SDPP	With SDPP	Low	High	Without	With	Mycotoxins	SDPP	M*S	CV %
1-5 days	1.3	0.4	0.7	0.4	0.9	0.6	1.0	0.4	0.47	0.15	ns	143.6
6-10 days	1.9	0.6	1.9	0.3	1.2	1.1	1.9 a	0.4 b	ns	<0.01	ns	106.2
11-15 days	0.7	0.1	0.7	0.1	0.4	0.4	0.7	0.1	ns	0.06	ns	181.8
1-10 days	3.1	1.0	2.6	0.7	2.1	1.6	2.9 a	0.9 b	ns	0.02	ns	112.3
1-15 days	3.9	1.1	3.3	0.9	2.5	2.1	3.6 a	1.0 b	ns	0.01	ns	110.4

* Values followed by different lowercase letters in the line differ (P <0.05) for the F test; ns = not significant; CV = coefficient of variation.

Table 4 - Estimated bio-economical indexes for determining the maximum SDPP price for use in piglet diets.

	Periods (days after weaning)				
Bio-econ. indexes for kg*	1-5	6-10	11-15	1-10	1-15
Piglet	9.077	6.648	1.764	7.680	5.128
Grinded corn	-3.082	-2.961	-2.453	-3.970	-2.771
Whey powder	-0.728	-0.682	-0.491	-0.925	-0.611
Soybean meal (45% CP)	0.355	0.449	0.842	0.539	0.596
Sugarcane	-0.243	-0.227	-0.164	-0.308	-0.204
Dicalcium phosphate	-0.052	-0.048	-0.032	-0.066	-0.042
Limestone	-0.051	-0.049	-0.039	-0.066	-0.045
Soy oil	0.050	0.060	0.103	0.074	0.076
Vitamin suplement	-0.015	-0.014	-0.010	-0.018	-0.012
Mineral suplement	-0.015	-0.014	-0.010	-0.018	-0.012
ZnO	-0.012	-0.011	-0.008	-0.015	-0.010
Salt	0.037	0.038	0.043	0.050	0.040
L-Lys H-Cl	-0.027	-0.024	-0.013	-0.033	-0.020
DL-Met	-0.012	-0.011	-0.006	-0.015	-0.009
L-Thr	-0.010	-0.009	-0.003	-0.013	-0.007
L-Trp	-0.004	-0.004	-0.003	-0.005	-0.004
L-Val	-0.017	-0.016	-0.008	-0.022	-0.013
L-Ile	-0.024	-0.024	-0.021	-0.032	-0.023
Maximum SDPP price estimated to be economically feasible, R$/kg.					
Maximum price **	78.76	55.97	11.63	62.94	42.37

*Economic Bio-indices obtained according Guidoni et al. (1994) to be multiplied by the prices of the ingredients to estimate the maximum price of SDPP so that the supplemented diets have the same economic efficiency of the diets without SDPP. **Estimated maximum price for the SDPP to be economically feasible in the piglets diets according to the prices obtained in December 2016, in which the SDPP cost was R$ 18.00.

3.2 –MANUSCRITO II

Efeito do plasma sanguíneo *spray dried* sobre a saúde de leitões desmamados e desafiados por dieta contaminada por aflatoxinas e fumonisinas

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Spray-dried porcine plasma added to diets contaminated with aflatoxins and fumonisins shows beneficial effects to piglet health

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Abstract

This study was aimed to analyze the effects of spray-dried porcine plasma (SDPP) on the health of postweaning piglets challenged with diets contaminated with aflatoxins and fumonisins. For this study, 56 male castrated piglets were randomly distributed in a 2x2 factorial arrangement, without SDPP or with 6% SDPP in the diet and with low (0.95 µg/kg aflatoxins + 450 µg/kg fumonisins) or high (300 µg/kg aflatoxins + 8,000 µg/kg fumonisins) mycotoxins levels, with 14 replicates for biochemical serum and oxidative stress analyzes. Blood was collected at days 5, 10 and 15 of experiment to evaluate the activity of the enzymes alanine aminotransferase (ALT) and gamma-glutamyltransferase (GGT) and the levels of total proteins, albumin, globulins, cholesterol and triglycerides. Cell injury biomarkers such as thiobarbituric acid reactive species (TBARS), reactive oxygen species (ROS), catalase (CAT) and superoxide dismutase (SOD) were analyzed in the blood and liver of the animals. Similarly, ALT and GGT activities and protein and lipid metabolism did not differ among the groups. Animals receiving SDPP showed decreased urea levels throughout the experiment ($p<0.05$). Diets containing high mycotoxin contamination increased ROS and TBARS levels and decreased CAT activity ($p<0.05$). In contrast, SDPP prevented the increase of ROS and TBARS and stimulated SOD activity ($p<0.05$). In conclusion, high mycotoxin levels caused subclinical intoxication in the piglets, as observed by the increase in cell injury biomarkers. Conversely, SDPP presented a protective effect, minimizing the effects of oxidative stress caused by aflatoxins and fumonisins ingestion.

Keywords: Functional foods, intoxication, mycotoxins, piglets.

Introduction

Mycotoxins are toxic compounds produced by filamentous fungi that challenge pigs in the most diverse production cycle stages (Freitas et al., 2012). However, at weaning, piglets are more vulnerable to functional disorders, given the stresses of the various changes at this stage (Sugiharto et al., 2014). At high doses, mycotoxins can trigger problems of various orders, such as altering enzyme activity (Dilkin et al., 2010), serum biochemical variables, histology and functionality of some organs (Olinda et al., 2016), inducing oxidative stress (Fu et al., 2013) and compromising animal consumption and performance (Pastorelli et al., 2012).

Many strategies are used to mitigate the harmful effects of weaning, including the use of functional foods. Among the functional foods used for piglets, we highlight the spray-dried porcine plasma (SDPP), which is considered a high-quality protein capable to improve the palatability and feed consumption (Pujols et al., 2016). SDPP has anti-inflammatory properties due to the presence of active immunoglobulins that act on the intestinal barrier and prevent adhesion of pathogenic bacteria to the wall of the intestine (Hedegaard et al., 2016). In addition, dietary SDPP enhances immunity (Campbell et al., 2016) and reduces the activation of the immune system (Campbell et al., 2008). The benefits of SDPP in piglet weaning are considered as one of the most important discoveries in pig nutrition in the last 100 years (Crowmell, 2009).

The impacts of SDPP supplementation on the health of pigs fed diets containing mycotoxins have been scarcely explored. Therefore, this study aimed to verify whether the addition of SDPP to postweaning piglet diets contaminated with low and high levels of aflatoxins and fumonisins is able to avoid or minimize the effects caused by mycotoxin intoxication.

Material and methods

Mycotoxins

Aflatoxins were obtained by the rice fermentation method, with controlled temperature and constant stirring. The *Aspergillus parasiticus* strain NRLL 2999 was used, according to the methodology of West et al. (1973). The rice was autoclaved, dried with hot air and milled. A level of 130,000 µg/kg aflatoxins/kg rice containing 83% aflatoxin B1, 9.5% aflatoxin B2, 3.4% aflatoxin G1 and 4.2% aflatoxin G2 was quantified by HPLC (Thorpe et al., 1982).

Fumonisins were obtained from fermentation of corn grains. Samples were prepared by adding 1 kg of corn to plastic Erlenmeyer flasks containing distilled water (water activity ≥ 0.95). Flasks were autoclaved at 125°C/1 h, inoculated with *Fusarium verticillioides* and incubated at 25°C for 30 days. Fumonisin levels obtained were 110,000 µg/kg corn.

The high mycotoxin contamination levels proposed in our study were achieved in the diets by adding these concentrated mycotoxins. Notwithstanding, low contamination levels were obtained by using naturally contaminated soybean and corn meals.

Animals and conditions

Fifty-six commercial line castrated piglets, weighing 7.2 ± 0.61 kg were weaned at 24 ± 2 days. The piglets were housed in pairs in metal cages measuring 1.2 x 0.5 m, with plastic leaked floor, equipped with manual feeders and troughs. Room temperature was set at 23-25 °C and was controlled by automated electric heaters.

Experimental design

Four isonutritive diets were formulated according to minimum nutritional requirements recommended by Rostagno et al. (2011), with maize and soybean meals as the main ingredients of the diet. The four diets corresponded to the four treatments provided to the groups of postweaning animals, differing by the level of contamination of aflatoxins and fumonisins and by the addition or not of 6% SDPP, as follows: Group GA (0.95 µg/kg aflatoxins and 450 µg/kg fumonisins); Group GB (0.95 µg/kg aflatoxins, 450 µg/kg fumonisins and 6% SDPP); Group GC (300 µg/kg aflatoxins and 8,000 µg/kg fumonisins); Group GD (300 µg/kg aflatoxins, 8,000 µg/kg fumonisins and 6% SDPP).

Sample collection

The experimental period comprised the first 15 days after weaning. Blood samples were collected using vacutainer tubes at days 5, 10 and 15 after diet consumption. Subsequently, serum was obtained by centrifugation at 8,000 rpm for 10 minutes. Blood was also collected in tubes with sodium citrate for analysis of CAT and SOD. Whole blood and serum were kept at -20 °C until analysis.

At day 15 of experiment, five animals per group were euthanized. Liver fragments were removed and fixed in 10% formalin buffer for histopathological analysis. Liver fragments were also collected and homogenized in 10 mM Tris-HCl buffer (pH 7.4) for analysis of TBARS, ROS, SOD and CAT.

Serum biochemistry

Serum was used to evaluated alanine aminotransferase (ALT) and gamma-glutamyltransferase (GGT) activity and total protein, albumin, globulins, urea, cholesterol and triglyceride levels. Analyzes were performed using commercial kits (Analisa®), following the manufacturer's instructions in a semiautomatic biochemical analyzer (Bioplus 2000®). Globulin levels were calculated as the difference between total proteins and albumin.

Levels of free radicals and lipid peroxidation in serum and liver

Reactive oxygen species (ROS) and thiobarbituric acid reactive species (TBARS) levels were measured to determine free radicals and lipid peroxidation in the serum and liver of the piglets, respectively. ROS levels were determined in serum and in liver homogenates according to the method described by Ali et al. (1992). Samples were diluted 1:10 (v:v) in 10 mM Tris-Hcl, pH 7.4, and 5 µl of dichlorofluorescein diacetate (DCFH-DA) were added according the methodology described by Bass et al. (1993). The results were expressed in U DCFA/µL. TBARS levels in serum were analyzed according to the method described by Jentzsch et al. (1996) and expressed in nmol malondialdehyde (MDA)/mL. Liver fragments were homogenized in 50 mM Tris-Hcl, pH 7, and centrifuged at 2,500 rpm for 15 min. The supernatant (S1, 200 µL) was incubated at 95 °C for 60 min in acidic medium with 8.1% sodium dodecyl sulfate, 0.5 mL acetic acid buffer (500 mM, pH 3.4) and 0.6% TBA. TBARS levels were measured at 532 nm according to the method of Ohkawa et al. (1978). The results were expressed in nmol MDA/mg of protein.

Antioxidant enzymes

The activity of the antioxidant enzymes catalase (CAT) and superoxide dismutase (SOD) was analyzed in whole blood and in hepatic homogenates. CAT activity was measured according to the method described by Nelson and Kiesow (1972), and expressed in nmol CAT/mg protein. SOD activity was quantified according to the technique described by McCord and Fridovich (1976), and expressed in U SOD/mg of protein.

Histopathological analysis

Fragments of the right medial lobe of the liver and of the intestine (duodenum and jejunum) were collected and fixed in 10% buffered formalin solution. Samples were routinely processed and stained with hematoxylin and eosin (H&E) for histopathological analysis.

Statistical analysis

A 2x2 completely randomized design (DIC) was used, with two plasma levels (with or without 6% SDPP inclusion) and high or low contamination of aflatoxins and fumonisins, with a total of four treatments with 14 replicates for blood variables, five for liver tissue analysis and one animal per experimental unit. The data were submitted to Shapiro-Wilk and Kolmogorov-Smirnov normality tests and the residues were transformed when necessary to meet the normality assumption. Thereafter, variables were submitted to analysis of variance using the statistical package SAS 9.2, according to the mathematical model: $Y_{ijk} = \mu + A_i + B_j + (AB) + e_{ijk}$ where: Y = response variable; μ = the overall mean of the experiment for the variable (overall mean associated with all observations); A_i = effect of the i-th plasma level; B_j = effect of j-th mycotoxin level; A_i*B_j = interaction effect A x B; e_{ijk} = random error. $P<0.05$ was considered statistically significant.

Results

The treatments evaluated did not promote histopathological alterations in the liver and intestine. Moreover, no differences were observed in the activities of the liver enzymes ALT and GGT ($P>0.05$). The high mycotoxin intoxication also did not affect liver functions related to protein synthesis and lipid metabolism, as the biochemical parameters indicative of these activities such as total proteins, albumin, cholesterol and triglycerides were not altered ($P>0.05$). Globulin levels were also not influenced ($P>0.05$) by the treatments (Table 1). However, serum urea and TBARS levels (Table 2) were lower ($P<0.05$) in piglets that consumed SDPP in the three periods analyzed.

At day 5 of treatment, serum ROS levels were increased in pigs that received high mycotoxins levels ($P<0.05$) (Table 3). However, at days 10 and 15 serum and liver ROS levels interacted with SDPP and mycotoxin factors ($P<0.05$), i.e., SDPP was able to neutralize the mycotoxin-induced increase of ROS levels (Table 3). TBARS levels in serum were lower in the animals that consumed SDPP ($P<0.05$; Table 3). In SDPP-free diets, hepatic TBARS levels were increased ($P<0.05$) in the presence of mycotoxins (Table 4). However, when compared to the treatment with low mycotoxin levels, TBARS levels were higher ($P<0.05$) when SDPP was added to the diet.

Blood (Table 2) and liver (Table 4) CAT activities were decreased ($P<0.05$) in the treatment with high mycotoxins levels at day 15 of experiment. Blood SOD activity was increased at day 10 in the diet with SDPP ($P<0.05$). Conversely, an interaction was observed

in liver SOD activity (Table 4), i.e., SOD activity was increased in animals treated with SDPP and receiving high mycotoxins levels ($P<0.05$).

Discussion

The lack of clinical signs in the piglets of this study is likely to be due to the low mycotoxin levels and mainly to the short experimental course that were not sufficient to cause severe intoxication, to damage the liver and to alter biochemical variables related to health and liver function. Subclinical intoxication similar to observed in our study was obtained by Weaver et al. (2014), who reported that diets containing aflatoxins (250 ppb), fumonisins (6.9 ppm) and SDPP fed to animals for three weeks did not change biochemical variables, total proteins, albumin, globulins and cholesterol levels and ALT activity.

Fu et al. (2013) fed piglets for 42 days with diets containing 5 and 373 ppb of aflatoxins and did not observe effects on the activity of ALT and GGT enzymes and on serum levels of total proteins, urea and albumin. Dilkin et al. (2010) challenged 25 kg pigs with a dose of 125 ppm fumonisins administered to fasted animals by using an esophageal catheter. Clinical signs observed included lethargy, increased of respiratory rate, increased of cardiac frequency, lateral decubitus as the preferential position and reduction of food and water consumption. However, no histopathological changes were observed in the liver, as well in the activities of ALT, ALP and in the levels of total protein and albumin. We believe that these variables are not good markers to identify mycotoxin poisoning in piglets at the doses or at the time evaluated in our study, differing from a severe intoxication (Olinda et al., 2016). Therefore, more sensitive markers are needed.

Consumption of SDPP reduced serum urea levels. Urea is considered an indicator of the quality of dietary protein. Dalto et al. (2011) reported a reduction in plasma urea levels with the use of SDPP for piglets at 35 days of age with consumption of 20 g of SDPP per day. The same results were found by Weaver et al. (2014) who observed lower levels of circulating urea in animals that consumed plasma after weaning.

The elevation of blood SOD activity at day 15 after SDPP treatment is likely to be a compensatory effect related to the reduction of CAT caused by consumption of the high mycotoxin level diet, which also increased ROS levels. According to Oliveira et al. (2009), this probably can be explained by an increase in the production of antioxidants due to generation of ROS, which was observed in our study.

A reduction of the antioxidant enzymes SOD and CAT in the blood and an increase of SOD in the liver were reported by Fu et al. (2013) as indicative of aflatoxin (372.8 ppb)

intoxication, but no oxidation indicators were analyzed. Induction of mycotoxin-associated oxidative stress was also found by Theumer et al. (2010) with experimental doses of 40 ppb aflatoxins and 100 ppm fumonisins administered to rats. The authors also reported an increase of TBARS levels and CAT and SOD activities. Biomarkers of genetic damage were also evaluated by Theumer et al. (2010) who found a direct correlation of DNA damage with oxidative stress markers. Therefore, the authors suggested that mycotoxins induce indirectly genotoxicity-mediated oxidative stress.

The potential of SDPP to counteract oxidative stress observed in our study corroborates the findings of Gao (2014) in an experiment with piglets aging 3 to 21 days. The author reported that the addition of 10% SDPP to diets reduced serum TBARS levels and elevated CAT activity in the intestinal mucosa. According to Torralordona (2010), SDPP diet acts reducing proinflammatory cytokines by impairing the adhesion of pathogens to the intestinal wall, since one of the attributes of SDPP is the presence of specific active immunoglobulins for some enteric pathogenic bacteria. According to Soares et al. (2015), ROS can induce the production of proinflammatory cytokines, leading again to ROS production, therefore triggering a vicious circle between oxidative stress and inflammation, which would have a negative effect on animal production.

According to literature, the anti-inflammatory effect of SDPP exists (Campbell et al., 2008), since SDPP stimulates the production of interleukin-10, which has anti-inflammatory properties. Perez-Bosque et al. (2016) found an increase in the amount of IL-10 and a reduction of proinflammatory cytokines when 8% SDPP was added to the diet of laboratory rats, concluding that SDPP promotes activation of the immune system by reducing inflammatory response. Furthermore, Campbell et al. (2016) reinforce that SDPP prevents lesions of pathogenic bacteria in the intestinal wall, and thereby reduces the activation of the immune system and the production of proinflammatory cytokines by being a food that boosts the immune system and promotes health and performance of the animals.

The likely mechanism of action of plasma to reduce oxidative stress is associated with the presence of active immunoglobulins, which may have conferred protective action to the intestinal wall, as well as stimulating the production of anti-inflammatory and reducing proinflammatory cytokines, since proinflammatory cytokines lead to oxidative stress.

Oxidative stress has a negative impact on the performance of pigs, and the detection of this imbalance and the factors that lead to this condition is important to reestablish balance and promote the health of the pigs (Bezerra et al., 2015). The mechanisms of the antioxidant effect of SDPP have not been elucidated. However, based on the results of the present study it

can be suggested that SDPP provided a protective effect and avoided the condition of oxidative stress caused by the ingestion of diets with high levels of aflatoxins and fumonisins.

Conclusion

Mycotoxins caused subclinical intoxication in the piglets and altered high-sensitivity cell lesion biomarkers, which characterize a situation of oxidative stress. In contrast, SDPP showed a cellular protective effect and avoided the exacerbation of oxidative reactions. Additionally, treatment stimulated the activity of the antioxidant enzyme SOD. The addition of SDPP to diets also provided better utilization of dietary protein by postweaning piglets with reduction of plasma urea.

Interest conflicts

The authors declare that there is no conflict of interest.

Ethics Committee

The present study was approved by the Ethics Committee of the State University of Santa Catarina, according to approval protocol number 01.34.15.

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Table 1. Effects of mycotoxin levels and of the treatment with spray-dried porcine plasma (SDPP) on the activity of alanine aminotransferase (ALT), gamma-glutamyltransferase (GGT) and biochemical variables in serum of postweaning piglets.

	Group GA	**Group GB**	**Group GC**	**Group GD**				
	Low mycotoxin		**High mycotoxin**		**Mycotoxin**		**SDPP**	
	Without SDPP	**With SDPP**	**Without SDPP**	**With SDPP**	**Low**	**High**	**Without**	**With**
Days	ALT (U/L)							
5	30.43±7.54	35.13±6.54	28.81±9.12	32.89±10.30	32.78±7.30	30.85±9.84	29.62±8.32	34.01±8.83
10	33.18±6.11	33.06±8.82	29.88±13.32	32.89±7.96	33.12±7.95	31.38±11.11	31.53±10.72	32.98±8.46
15	31.90±6.21	32.77±8.51	29.96±9.48	33.14±5.68	32.34±7.54	31.55±8.01	30.93±7.99	32.96±7.21
	GGT (U/L)							
5	33.29±10.19	33.81±11.41	30.94±7.29	34.79±10.06	33.55±10.68	32.86±8.76	32.11±8.69	34.30±10.63
10	32.68±7.95	32.94±9.86	31.63±11.03	37.18±12.94	32.81±8.86	34.40±12.31	32.15±9.55	35.06±11.82
15	32.10±9.16	33.06±10.45	32.02±5.88	34.79±7.26	32.58±9.77	33.40±6.60	32.06±7.51	33.92±9.11
	Total proteins (g/dL)							
5	5.53±1.05	5.41±0.75	5.81±0.95	5.78±1.12	2.82±0.89	2.82±1.01	5.67±0.99	5.59±0.95
10	5.36±1.08	5.41±0.61	5.35±1.40	5.43±1.12	5.39±0.87	5.39±1.26	5.36±1.26	5.42±0.91
15	5.23±0.49	5.25±1.12	5.38±1.50	5.48±1.57	5.24±0.88	5.43±1.50	5.31±1.19	5.37±1.36
	Albumin (g/dL)							
5	2.41±0.57	2.62±0.47	2.76±0.70	2.52±0.69	2.51±0.52	2.64±0.69	2.59±0.66	2.57±0.57
10	2.54±0.47	2.74±0.56	2.69±0.79	2.64±0.56	2.64±0.52	2.67±0.69	2.61±0.66	2.69±0.55
15	2.54±0.51	2.57±0.53	2.63±0.52	2.56±0.59	2.56±0.53	2.60±0.54	2.58±0.51	2.57±0.55
	Globulins (g/dL)							
5	3.12±1.40	2.79±0.77	3.05±0.97	3.26±1.10	2.95±1.10	3.15±1.02	3.09±1.16	3.02±0.96
10	2.81±0.88	2.68±0.66	2.66±0.86	3.26±0.82	2.74±0.76	2.96±0.83	2.73±0.86	2.97±0.74
15	2.68±0.71	2.68±1.32	2.75±1.67	2.91±1.95	2.68±1.08	2.83±1.78	2.72±1.35	2.80±1.64
	Cholesterol (mg/dL)							
5	50.14±11.90	49.94±9.29	54.36±12.63	52.93±18.87	50.04±10.40	53.64±15.77	52.25±12.23	51.43±14.37
10	52.68±7.42	52.45±12.49	52.70±16.42	53.61±12.53	52.57±10.18	53.15±14.57	52.69±12.97	53.03±12.29
15	52.07±18.94	54.02±21.15	51.15±15.46	55.24±13.06	53.05±20.05	53.20±15.15	51.61±16.92	54.63±17.71
	Triglycerides (mg/dL)							
5	45.29±18.59	46.93±17.34	49.54±22.32	48.17±16.04	46.11±17.66	48.85±19.17	47.41±20.18	47.55±16.43
10	41.07±12.67	45.87±23.88	47.21±18.61	45.27±13.19	43.47±19.59	46.24±15.98	44.14±16.30	45.57±19.34
15	41.52±18.47	44.13±21.95	46.52±25.62	47.95±28.32	42.83±20.07	47.24±26.72	44.02±22.24	46.04±25.42

* There was no interaction and no isolated effect of the factors for any of the biochemical variables evaluated ($P > 0.05$).

Table 2. Results of thiobarbituric acid reactive species (TBARS) and urea levels in serum and catalase (CAT) and superoxide dismutase (SOD) activities in blood.

	Group GA	**Group GB**	**Group GC**	**Group GD**							
	Low mycotoxins		**High mycotoxins**		**Mycotoxins**		**SDPP**		**P value**		
	Without SDPP	**With SDPP**	**Without SDPP**	**With SDPP**	**Low**	**High**	**Without**	**With**	**Mycotoxins**	**Plasma**	**MY*SDPP**
Days	TBARS (nmol MDA/mL)										
5	8.13±2.79	5.71±1.92	10.79±4.15	6.14±2.31	6.92±2.55	8.46±4.04	9.46±3.76 [b]	5.93±2.07 [a]	0.16	<0.01	0.31
10	7.42±2.23	5.71±1.13	7.85±0.90	5.64±1.81	6.57±1.69	6.74±1.39	7.64±1.79 [b]	5.67±1.49 [a]	0.48	0.01	0.98
15	6.69±0.52	5.45±1.23	9.19±3.25	5.68±2.30	6.07±0.97	7.43±4.15	7.94±4.17 [b]	5.57±1.83 [a]	0.21	<0.01	0.22
	CAT (nmol CAT/mg protein)										
5	13.29±2.19	14.71±3.25	17.32±7.40	15.34±1.58	14.00±2.84	16.33±5.27	15.30±5.97	15.02±2.49	0.17	0.86	0.30
10	16.43±6.35	15.37±5.04	14.76±5.36	13.80±1.26	15.91±5.71	14.28±3.76	15.61±6.75	14.58±4.04	0.15	0.85	0.66
15	15.93±5.36	14.60±4.18	12.10±2.48	12.47±1.01	15.26a±4.61 [a]	12.29b±2.41 [b]	14.01±5.62	13.54±3.38	<0.01	0.65	0.42
	SOD (UI SOD/mg protein)										
5	0.86±0.33	0.95±0.58	1.07±0.71	0.88±0.46	0.90±0.48	0.98±0.59	0.97±0.57	0.91±0.51	0.79	0.75	0.94
10	0.67±0.33	0.82±0.21	0.81±0.16	0.89±0.38	0.75±0.21	0.85±0.34	0.74±0.15 b	0.86±0.31 [a]	0.28	0.04	0.95
15	0.61±0.15	0.67±0.14	0.71±0.28	0.77±0.22	0.64±0.15	0.74±0.24	0.66±0.23	0.72±0.20	0.32	0.25	0.35
	Urea (mg/dL)										
5	32.79±21.16	18.25±9.36	33.00±17.92	16.93±6.93	25.52±17.33	24.96±15.94	32.89±19.15 [b]	17.59±8.20 [a]	0.89	<0.01	0.84
10	25.89±9.22	19.72±8.62	28.84±14.13	18.86±13.55	22.81±8.82	23.85±13.77	27.37±12.24 [b]	19.29±10.99 [a]	0.47	<0.01	0.23
15	24.79±14.21	17.58±6.76	26.69±10.68	16.98±7.31	21.18±11.67	21.83±10.23	25.74±12.23 [b]	17.28± 6.90 [a]	0.92	<0.01	0.87

* Values followed by different lowercase letters in the line differ (P <0.05) for the F test.

Table 3. Levels of reactive oxygen species (ROS) in serum.

Serum ROS (DCFA/µL)			
	Low mycotoxins	High mycotoxins	Means
	Day 5		
Without SDPP	1.325±234	2.451±1.183	1.888±1.053
With SDPP	1.346±200	1.702±453	1.524±376
Means	1.335±207 [b]	2.076±968 [a]	
	Day 10		
Without SDPP	1.315±272 [b]	2.255±750 [Aa]	1.785±908
With SDPP	1.418±461	1.614±578 [B]	1.516±510
Means	1.366±396	1.940±873	
	Day 15		
Without SDPP	1.389±196 [b]	2.393±339 [Aa]	1.891±698
With SDPP	1.381±416	1.447±471 [B]	1.414±439
Means	1.385±331	1.920±871	

*Values followed by different lowercase letters in the row and different capitals in the column differ ($P < 0.05$) by the F test.

Table 4. Results of reactive oxygen species analysis (ROS), thiobarbituric acid reactive species (TBARS), catalase (CAT) and superoxide dismutase (SOD) in the liver of piglets.

	Low Mycotoxins	High Mycotoxins	*Means*
	ROS (U DCFA/µL)		
Without SDPP	579±150[b]	1.708±449 [Aa]	1.148±669
With SDPP	644±189	970±325 [B]	807±304
Means	612±132	1.339±537	
	TBARS (nmol MDA/mL)		
Without SDPP	13.05±1.53 [Bb]	22.70±2.76 [a]	17.85±5.50
With SDPP	16.39±2.32 [A]	20.22±4.38	18.30±4.19
Means	14.72±1.54	21.46±3.69	
	CAT (nmol CAT/mg protein)		
Without SDPP	22.91±1.35	21.32±2.27	22.11 ±1.84
With SDPP	23.35±2.08	20.83±1.33	22.09 ±2.11
Means	23.13 ± 1.69 [a]	21.08 ± 1.84 [b]	
	SOD (UI SOD/mg protein)		
Without SDPP	14.76 ± 1.86	20.35 ± 5.26 [B]	17.56±4.56
With SDPP	14.76 ± 1.87 [b]	31.14 ± 7.09 [Aa]	22.95 ±9.92
Means	14.76±1.55	25.75±8.40	

*Values followed by different lowercase letters in the row and different capitals in the column differ ($P<0.05$) by the F test.

3 – CONSIDERAÇOES FINAIS

Os resultados encontrados nos estudos realizados evidenciam os efeitos prejudiciais das micotoxinas para os suínos no pós-desmame, visto que em dados momentos afetou negativamente o ganho de peso dos leitões (manuscrito 01), além de induzir ao estresse oxidativo (manuscrito 02). Em contra partida, o plasma sanguíneo *spray dried* (PSD), confirmou sua aplicabilidade como alimento funcional, com aumentou no consumo e ganho de ração em todo o período experimental, (1-15 dias), além de diminuir a incidência de diarreias e apresentar viabilidade econômica (manuscrito 01). Os efeitos benéficos também foram visualizados no *status* de saúde dos leitões, no qual o PSD demonstrou efeito protetor nos parâmetros de estresse oxidativo, além de confirmar a melhora do aproveitamento da proteína dietética. Mesmo um desafio de alta contaminação por micotoxinas na forma associada, não neutralizaram os efeitos benéficos do PSD, os quais foram visualizados de forma multifatorial, em que o PSD mesmo em condições de desafio, promoveu tanto o desempenho quanto a saúde dos animais. Há que considerar, que os benefícios se somam, pois um animal com um *status* de saúde melhor, terá melhor desempenho. Novos estudos podem ser realizados a fim de aprofundar o conhecimento dos efeitos do uso do plasma como alimento funcional para leitões no pós-desmame, em situação de desafio por micotoxinas.

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Cetea UDESC
Comitê de Ética em Experimentação Animal
Centro de Ciências Agroveterinárias

CARTA DE APROVAÇÃO

O(s) projeto(s) abaixo relacionado(s):

Protocolo: 01.34.15
Título: Plasma sanguineo em pó como atenuante dos efeitos prejudiciais das aflatoxinas e fumonisina em leitões.
Coordenador/Pesquisador: Diovani Paiano

Foi(ram) analisado(s) pelo Comitê de Ética em Experimentação Animal da UDESC (CETEA/UDESC) em reunião de 05 de maio de 20016, tendo sido **APROVADO(S)** em seus aspectos éticos e metodológicos, para utilização de animais em pesquisa, de acordo com as diretrizes e normas nacionais e internacionais, especialmente a Lei 11.794 de 08 de novembro de 2008 que disciplina a criação e utilização de animais em atividades de ensino e pesquisa no Brasil.

Lages, 06 de maio de 2016.

Prof. Ubirajara Maciel da Costa
Coordenador do CETEA/UDESC

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