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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 post weaning piglets challenged with diets contaminated with aflatoxins and fumonisins. Fifty-six male piglets (7.15 ± 0.61 kg) were allocated in four groups: CTL group received a regular diet; SDPP group received a regular diet and 6% SDPP; MYC group received a diet containing 300 µg/kg aflatoxins and 8,000 µg/kg fumonisins; group MYC+SDPP received 300 µg/kg aflatoxins, 8,000 µg/kg fumonisins and 6% SDPP. The animals that received the experimental diet containing mycotoxins (MYC group) had lower weight gain at the end of the experiment compared to the other treatments. Animals receiving SDPP showed decreased urea levels throughout the experiment (P<0.05). Animals from MYC group presented an increased on reactive oxygen species (ROS) and thiobarbituric acid reactive substances (TBARS) levels and decreased catalase activity (P<0.05). In contrast, SDPP prevented the increase of ROS and TBARS and stimulated superoxide dismutase activity (P<0.05). In conclusion, diet contaminated with mycotoxins (group MYC) caused subclinical intoxication in the piglets, as observed by the increase on free radical's production and lipid peroxidation. Conversely, SDPP presented a protective effect, minimizing the effects of oxidative stress caused by aflatoxins and fumonisins ingestion.

Key words: Functional foods, intoxication, mycotoxins, pigs, health.

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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). In this sense, a recent study conducted by Muller et al. (2017) demonstrated that 6% of SDPP in the diet prevents the reduction on leukocyte levels, the negative effects associated with performance and minimize the inflammatory response of piglets intoxicated with aflatoxins and mycotoxins, considering this treatment an interesting approach to modulate the inflammatory process and improve the immune system.

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 containing 300 μg/kg aflatoxins and 8,000 μg/kg fumonisins is able to avoid or minimize the effects caused by mycotoxin intoxication.

MATERIALS AND METHODS

MYCOTOXINS

The mycotoxin production used in this study was previously detailed by Muller et al. (2017). Aflatoxins were obtained by the rice fermentation method, with controlled temperature and constant stirring, and the *Aspergillus parasiticus* strain was used to production of mycotoxin. Fumonisins were obtained from fermentation of corn grains, the *Fusarium verticillioides* strain was used to production of mycotoxin. The mycotoxin contamination levels proposed in our study were achieved in the diets by adding these concentrated mycotoxins. Notwithstanding, control diet 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. The present study was approved by the Ethics Committee of the State University of Santa Catarina, according to approval protocol number 01.34.15.

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 (Table I). 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: CTL (Control diet: 0.95 µg/kg aflatoxins and 450 µg/kg fumonisins); SDPP (control diet: 0.95 µg/kg aflatoxins, 450 µg/kg fumonisins and 6% SDPP); MYC (experimental diet: 300 µg/kg aflatoxins and 8000 µg/kg fumonisins); MYC + SDPP (experimental diet: 300 µg/kg aflatoxins, 8000 µg/kg fumonisins and 6% SDPP). The weight of the litter was scheduled on days 1 and 15 of the experiment, using a digital scale.

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 catalase (CAT) and superoxide dismutase (SOD) activities. 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 thiobarbituric acid reactive substances (TBARS) and reactive oxygen species (ROS) levels, as well as the CAT and SOD activities.

SERUM BIOCHEMISTRY

Serum was used to evaluate alanine aminotransferase (ALT) and gamma-glutamyltransferase (GGT) activities, as well as 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

ROS and 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. (1983). 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% thiobarbituric acid (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 CAT and 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 (1969), 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. Moreover, images of intestinal histopathology were selected to measure villi height and crypt depth (12 per image per blade) from duodenum, jejunum and ileum. The thickness of the mucosa was also measured in the respective reading points according the protocol described by Teixeira et al. (2003). ImageJ software was used for the measurements.

STATISTICAL ANALYSIS

A 2x2 completely randomized design (DIC) was used, with two plasma levels (with or without 6% SDPP inclusion) and contaminated or not 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. One-way ANOVA was performed using repeated measurements to test difference in the parameters over time (considering blocks of groups) $P<0.05$ was considered statistically significant.

RESULTS

No hepatic and intestinal (duodenum and jejunum) lesions were observed in the studied groups (data not showed). No differences were observed between groups ($P>0.05$) for crypt depth, villus height and mucosal thickness in the gut of the piglets (data not shown).

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 MYC diet 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 II). However, serum urea levels (Table II) were lower ($P<0.05$) in piglets that consumed SDPP in the three periods analyzed.

Seric TBARS levels (Table III) 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 from MYC group compared to CTL group ($P<0.05$) (Table IV). However, at days 10 and 15 in serum (Table IV) and day 15 in liver (Table V) 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. In SDPP-free diets, hepatic TBARS levels were increased ($P<0.05$) in the presence of mycotoxins (Table V). However, when compared to the treatment with CTL group, TBARS levels were higher ($P<0.05$) when SDPP was added to the diet. At day 15 in liver (Table V) TBARS levels interacted with SDPP and mycotoxin factors ($P<0.05$), i.e. SDPP was able to minimize the negative effects caused by mycotoxin (Table V).

Blood (Table III) and liver (Table V) CAT activities were decreased ($P<0.05$) in the group

TABLE I
Composition of experimental diets. Adapted table of Muller et al. (2017). CTL: control diet; SDPP: control diet and 6% of SDPP; MYC: 300 µg/kg aflatoxins + 8,000 µg/kg fumonisins; MYC+SDPP: 300 µg/kg aflatoxins + 8,000 µg/kg of fumonisins + 6% SDPP.

	CTL	SDPP	MYC	MYC+SDPP
Calculated composition				
Aflatoxins, µg/kg	0.95	0.95	300	300
Fumonisins, µg /kg	450	450	8,000	8,000
Ingredients, %				
Corn	39.43	46.33	33.23	40.11
Soybean meal (45%)	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
Soybean oil	3.40	2.11	3.35	2.08
Vitamin supplement [1]	0.30	0.30	0.30	0.30
Mineral supplement [2]	0.30	0.30	0.30	0.30
Zinc oxide	0.25	0.25	0.25	0.25
Salt	0.37	0.04	0.37	0.04
L-Lysine H-Cl	0.84	0.75	0.86	0.78
DL-Methionine	0.38	0.34	0.38	0.34
L-Threonine	0.45	0.38	0.45	0.38
L-Tryptophan	0.08	0.08	0.08	0.09
L-Isoleucine	0.20	0.29	0.21	0.30
L-Valine	0.58	0.52	0.59	0.52
Spray-dried porcine plasma	-	6.0	-	6.0
Isolated aflatoxins	-	-	0.23	0.23
Isolated fumonisin	-	-	6.91	6.88
Chemical composition				
Metabolizable energy, Mcal/kg	3.40	3.40	3.40	3.40
Crude Protein, %	21.0	21.0	21.0	21.0
Neutral detergent fiber, %	8.98	8.25	8.12	7.39
Acid detergent fiber, %	3.96	3.29	3.66	3.00

[1]Provided the following per kilogram of diet: Vitamin A – 4.167.000 UI, Vitamin D3 – 833.000 UI, Vitamin E – 13.333 mg, Vitamin K3 – 1.000 mg, Vitamin B1 – 1.000 mg, Vitamin B2 – 1.667 mg, Vitamin B6 – 1.000 mg, Vitamin B12 – 8 mg, Niacin – 11.667 mg, Pantothenic Acid – 7.333 mg, Folic acid – 200 mg, Colin – 104 mg, Biotin – 33 mg; [2]Calcium (min. 166 g and max. 203 g), Cobalt – 266.7 mg, Cooper – 66.67 g, Iodine – 600 mg, magnesium – 18.3 g, Selenium – 135 mg, Zinc – 41.67 g, Iron – 66.67 g, 40 mg/kg growth promoter.

TABLE II

Effects of mycotoxin (MY) levels and of the treatment with spray-dried porcine plasma (SDPP) on the activity of alanine aminotransferase (ALT), gamma-glutamyltransferase (GGT), total protein, albumin, globulin, cholesterol, triglycerides and urea in serum of postweaning piglets.

	#Lower levels of mycotoxin		# Higher levels of mycotoxin		Mycotoxin (MY) levels		Presence of SDPP		*P* value		
	Group CLT	Group SDPP	Group MYC	Group MYC+SDPP	Lower	Higher	Without	With	MY	SDPP	MY*SDPP
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	0.56	0.23	0.65
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	0.47	0.74	0.78
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	0.33	0.58	0.65
	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	0.76	0.82	0.45
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	0.73	0.71	0.50
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	0.81	0.73	0.42
	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	0.95	0.84	0.12
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	0.94	0.82	0.58
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	0.90	0.75	0.62
	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	0.84	0.92	0.67
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	0.89	0.85	0.54
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	0.91	0.97	0.59
	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	0.76	0.90	0.29
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	0.80	0.72	0.35
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	0.61	0.83	0.44
	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	0.59	0.89	0.62
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	0.92	0.87	0.84
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	0.95	0.54	0.64

TABLE II (continuation)

	#Lower levels of mycotoxin		# Higher levels of mycotoxin		Mycotoxin (MY) levels		Presence of SDPP		*P* value		
	Group CLT	Group SDPP	Group MYC	Group MYC+SDPP	Lower	Higher	Without	With	MY	SDPP	MY*SDPP
Days											
					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	0.84	0.92	0.79
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	0.68	0.86	0.71
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	0.70	0.84	0.62
					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

Note: There was no interaction and no isolated effect of the factors for any of the biochemical variables evaluated ($P > 0.05$, $n=14$ per group). Groups formed: CTL: control diet; SDPP: control diet and 6% of SDPP; MYC: 300 µg/kg aflatoxins and 8,000 µg/kg fumonisins; MYC+SDPP: 300 µg/kg aflatoxins, 8,000 µg/kg of fumonisins and 6% SDPP. # Lower levels of mycotoxin (Groups CTL and SDPP = 0.95 µg/kg aflatoxins and 450 µg/kg fumonisins) and Higher levels of mycotoxin (Groups MYC and MYC+SDPP = 300 µg/kg aflatoxins and 8,000 µg/kg fumonisins). *Interaction between variables. Values followed by different lowercase letters (^{a},^{b}) in the line differ ($P < 0.05$).

TABLE III

Results of thiobarbituric acid reactive species (TBARS) levels in serum and catalase (CAT) and superoxide dismutase (SOD) activities in blood of piglets fed with mycotoxin (MY) diet and supplemented with spray-dried porcine plasma (SDPP).

	# Lower levels of mycotoxin		# Higher levels of mycotoxin		Mycotoxins (MY) levels		Presence of SDPP		*P* value		
	Group CLT	Group SDPP	Group MYC	Group MYC+SDPP	Lower	Higher	Without	With	MY	SDPP	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

Note: Values followed by different lowercase letters ([a],[b]) in the line differ ($P<0.05$; $n=14$ per group) for the F test. Groups formed: CTL: control diet; SDPP: control diet and 6% of SDPP; MYC: 300 µg/kg aflatoxins and 8,000 µg/kg fumonisins; MYC+SDPP: 300 µg/kg aflatoxins, 8,000 µg/kg of fumonisins and 6% SDPP. # Lower levels of mycotoxin (Groups CTL and SDPP = 0.95 µg/kg aflatoxins and 450 µg/kg fumonisins) and Higher levels of mycotoxin (Groups MYC and MYC+SDPP = 300 µg/kg aflatoxins and 8,000 µg/kg fumonisins). *Interaction between variables.

MYC 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 V), i.e., SOD activity was increased in animals treated with SDPP and receiving a MYC group (P<0.05).

The animals that received the MYC group had lower weight gain at the end of the experiment (day 15) compared to the other treatments (P<0.05). Numerically, animals receiving SDPP had higher final weight and weight gain at days 15 of experiment (Table VI). At day 15 to weight and day 1-15 to weight gain have interacted of SDPP and mycotoxin factors (P<0.05), i.e. SDPP was able to minimize the negative effects caused by mycotoxin for growth (Table VI).

Over time (days 5 to 10; and 5 to 15; and 10 to 15), no significant difference was observed (P>0.05) to serum biochemistry (ALT, GGT, urea, triglycerides, cholesterol, total protein, albumin and globulin), serum and liver oxidants (TBARS and ROS) and antioxidant enzymes in the blood and liver (CAT and SOD). Over time (days 5 to 15), a significant difference was observed for the four groups regarding body weight (P<0.001), that is, there was an increase in the weight of the animals that were in the growth phase.

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 parts-per-billion (ppm)), fumonisins (6.9 parts-per-million (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 respiratory rate, increased 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 and alkaline phosphatase (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.

The results of this study clearly demonstrated the beneficial effects of SDPP supplementation. Body weight gain significantly improved in the animals that receive SDPP diet during the experiment. From a nutritive perspective, this improvement in the growth performance may be due to nutrient utilization, a high quality protein with a high amino acid profile that can support weight gain and rapid muscle growth. Moreover, according to Ermer et al. (1994), the SDPP has a good diet palatability improving the immuno-competence of animals.

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 a diet contaminated with mycotoxins, 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 Torrallardona (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 (IL-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

TABLE IV
Levels of reactive oxygen species (ROS) in serum of piglets fed with mycotoxin diet and supplemented with spray-dried porcine plasma (SDPP). Note: At days 10 and 15 serum 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.

Serum ROS (U DCFA/µL)				
	[#]Lower levels of mycotoxin	[#]Higher levels of mycotoxin	*Means*	*P*
Day 5				
Without SDPP	1.325±234	2.451±1.183	1.888±1.053	0.43
With SDPP	1.346±200	1.702±453	1.524±376	0.26
Means	1.335±207	2.076±968		0.04
P	0.89	0.19	0.31	
Day 10				
Without SDPP	1.315±272	2.255±750	1.785±908	0.01
With SDPP	1.418±461	1.614±578	1.516±510	0.54
Means	1.366±396	1.940±873		0.35
P	0.47	0.02	0.79	
Day 15				
Without SDPP	1.389±196	2.393±339	1.891±698	0.001
With SDPP	1.381±416	1.447±471	1.414±439	0.85
Means	1.385±331	1.920±871		0.15
P	0.92	0.001	0.65	

Note: Different to $P<0.05$ ($n=14$ per group) by the F test. [#]Lower levels of mycotoxin (Groups CTL and SDPP = 0.95 µg/kg aflatoxins and 450 µg/kg fumonisins) and Higher levels of mycotoxin (Groups MYC and MYC+SDPP = 300 µg/kg aflatoxins and 8,000 µg/kg fumonisins).

TABLE V
Results of reactive oxygen species analysis (ROS), thiobarbituric acid reactive species (TBARS), catalase (CAT) and superoxide dismutase (SOD) in the liver of piglets fed with mycotoxin diet and supplemented with spray-dried porcine plasma (SDPP).

	#Lower levels of mycotoxin	#Higher levels of mycotoxin	*Means*	*P*
	ROS (U DCFA/µL)			
Without SDPP	579±150	1.708±449	1.148±669	0.001
With SDPP	644±189	970±325	807±304	0.75
Means	612±132	1.339±537		0.05
P	0.80	0.030	0.24	
	TBARS (nmol MDA/mL)			
Without SDPP	13.05±1.53	22.70±2.76	17.85±5.50	0.001
With SDPP	16.39±2.32	20.22±4.38	18.30±4.19	0.14
Means	14.72±1.54	21.46±3.69		0.02
P	0.04	0.74	0.87	
	CAT (nmol CAT/mg protein)			
Without SDPP	22.91±1.35	21.32±2.27	22.11 ±1.84	0.45
With SDPP	23.35±2.08	20.83±1.33	22.09 ±2.11	0.24
Means	23.13 ± 1.69	21.08 ± 1.84		0.05
P	0.85	0.82	0.92	
	SOD (UI SOD/mg protein)			
Without SDPP	14.76 ± 1.86	20.35 ± 5.26	17.56±4.56	0.06
With SDPP	14.76 ± 1.87	31.14 ± 7.09	22.95 ±9.92	0.001
Means	14.76±1.55	25.75±8.40		0.07
P	0.98	0.03	0.14	

Note: Different to $P<0.05$ ($n=5$ per group) by the F test. #Lower levels of mycotoxin (Groups CTL and SDPP = 0.95 µg/kg aflatoxins and 450 µg/kg fumonisins) and Higher levels of mycotoxin (Groups MYC and MYC+SDPP = 300 µg/kg aflatoxins and 8,000 µg/kg fumonisins).

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. Also, the levels of immunoglobulin IgA in the intestine is considered an indicator to evaluate the intestinal mucosa immunity, where high levels of IgA are released during an inflammatory process, as observed by Tran et al. (2014) in weaning piglets fed with a diet containing mycotoxins. In this sense, these same authors revealed that treatment with SDPP reduced the intestinal IgA levels, which demonstrated the anti-inflammatory effects of SDPP. According to these authors, this response can contribute to improvement of immune and antioxidant systems, as observed in this present study. 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

TABLE VI
Weight and weight gain of piglets of fed with mycotoxin diet and supplemented with spray-dried porcine plasma (SDPP).

	[#]Lower levels of mycotoxin	[#]Higher levels of mycotoxin	*Means*	*P*
	Weight (kg): Day 1			
Without SDPP	7.33±0.4	7.30±0.6	7.31±0.5	0.91
With SDPP	7.28±0.5	7.35±0.6	7.31±0.5	0.90
Means	7.30±0.4	7.32±0.6		0.86
P	0.51	0.88	0.97	
	Weight (kg): Day 15			
Without SDPP	12.8 ±1.12	11.2 ±0.86	12.0±0.9	0.05
With SDPP	13.6 ±1.13	13.7±1.22 [A]	13.6±1.1	0.93
Means	13.2 ±1.1	12.4±1.0		0.17
P	0.38	0.03	0.12	
	Weight gain (kg): Day 1-15			
Without SDPP	5.3±1.20	3.9±0.91	4.6±1.0	0.01
With SDPP	6.3 ±0.82	6.3±0.86	6.3±0.8	0.98
Means	5.8±1.0	5.1±0.8		0.57
P	0.29	0.001	0.001	

Note: Different to $P<0.05$ (n=14 per group) by the F test. [#]Lower levels of mycotoxin (Groups CTL and SDPP = 0.95 µg/kg aflatoxins and 450 µg/kg fumonisins) and Higher levels of mycotoxin (Groups MYC and MYC+SDPP = 300 µg/kg aflatoxins and 8,000 µg/kg fumonisins).

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 containing 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. In addition, the use of SDPP as a supplement stimulated the performance of the animals, which had greater weight gain at the end of the study. Therefore, SDPP had beneficial effects for the production system and health of the litters, protecting against the negative effects of the mycotoxin present in the diet.

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