AMERICAN SOCIETY OF ANIMAL SCIENCE

*Journal of Animal Science*, 2021, Vol. 99, No. 10, 1–8

https://doi.org/10.1093/jas/skab277
Advance Access publication October 2, 2021
**Received:** 27 May 2021 and **Accepted:** 28 September 2021
Toxicology

TOXICOLOGY

# Mycotoxin deactivator improves performance, antioxidant status, and reduces oxidative stress in nursery pigs fed diets containing mycotoxins

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## Abstract

Ingestion of mycotoxins can result in many problems, including decreased growth rates and immune suppression. The present study aimed to evaluate the impact of the supplementation of a mycotoxin deactivator composed by adsorbent clay minerals; inactivated fermentation extracts of *Saccharomyces cerevisiae*; and blend of antioxidants, organic acids, and botanicals in diets containing added mycotoxins for nursery pigs on their performance and antioxidant status. Ninety pigs weaned with 24 d of age (7.12 ± 0.68 kg of BW) were used. Pigs were housed in pens of three animals each according to body weight, litter origin, and sex. The dietary treatments consisted of feeding the pigs with a standard control diet as negative control (NC; mycotoxin levels at accepted regulatory Brazilian Ministry of Agriculture standards; deoxynivalenol (DON): <100 μg/kg; zearalenone (ZEA): <20 μg/kg; fumonisins (FB): <1 mg/kg); the standard diet added with mycotoxins to reach a low contamination level is considered as positive low (PCL−; DON: 900 μg/kg; ZEA: 100 μg/kg; FB: 5,000 μg/kg) without deactivator; a positive low added the deactivator at an inclusion rate of 1 kg/ton (PCL+); the standard diet added with mycotoxins to reach a high contamination level is considered as positive high (PCH−; DON: 4,500 μg/kg; ZEA: 500 μg/kg; FB: 18,000 μg/kg) without the deactivator; and a positive high added the deactivator at an inclusion rate of 5 kg/ton (PCH+). Pigs were individually weighed at the beginning and at the end of each phase and feed intake recorded based on daily pen intake during the experiment. On days 7, 19, 34, and 43 post-weaning, blood samples were drawn for antioxidant analyses. Antioxidant enzymes (glutathione peroxidase [GPx] and total superoxide dismutase [TSOD]), vitamins [Vit A, E, and C], and

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malondialdehyde [MDA]) were evaluated in erythrocyte and plasma samples. Pigs challenged with mycotoxins presented lower performance traits, decrease in the efficiency of central antioxidant systems (↓GPx, ↓TSOD, ↓Vit A, ↓Vit E, and ↓Vit C), and a higher oxidative damage to lipids (↑MDA) when compared with the control and deactivator-associated treatments. Our findings showed that the use of a mycotoxin deactivator can mitigate the negative impacts on performance and oxidative stress when animals are subjected to diets contaminated by different levels of mycotoxins.

**Key words:** deactivator, mycotoxins, nursery, pigs, oxidative stress

**Abbreviations**

| | |
|---|---|
| AFLAs | aflatoxins |
| DON | deoxynivalenol |
| FBs | fumonisins |
| GPx | glutathione peroxidase |
| MDA | malondialdehyde |
| OCHs | ochratoxins |
| TSOD | total superoxide dismutase |
| ZEA | Zearalenone |

## Introduction

Every year the animal production industry suffers from unexplained suboptimal performances in where all other factors such as management and facilities are adequate. These results can be explained by the presence of mycotoxins in diets. Mycotoxins are secondary metabolites of fungi that have toxic properties and can negatively impact animal performance (Binder et al., 2007; Souza et al., 2018), changes in hematological and serum biochemical levels, and negatively affect the immune system (Muller et al., 2018). Furthermore, mycotoxins can cause changes in energy homeostasis linked to the depletion of adenosine triphosphate (ATP) (Baldissera et al., 2018). Intoxication symptoms vary depending on the species ingested and the stage of animal production. The vast majority of mycotoxins are produced by filamentous fungi, including *Aspergillus*, *Fusarium*, and *Penicillum* and are dependent on humidity and temperature variations, as well as harvesting, transport, and storage conditions (Złoch et al., 2020). Commonly, mycotoxins are divided according to their degree of importance, namely, aflatoxins, trichothecins, fumonisins, zearalenone, and ochratoxins (González Pereyra et al., 2020; Yang et al., 2020). Mycotoxin ingestion by animals can result in many problems, including decreased growth rates, liver damage, and immune suppression (Eraslan et al., 2005; Murphy et al., 2006; Souza et al., 2018). The inclusion of mycotoxin binders or deactivators in feed has been used to decrease the intestinal absorption of mycotoxins present in diets (González Pereyra et al., 2020). Mycotoxin binders act as deactivators, as they have the ability to fix the surface of mycotoxins, promoting their excretion along with feces, through the exchange of electrical charges, in this case decreasing the absorption of fungi by the gastrointestinal tract (Fu et al., 2013). In addition, the aspects of purity, particle size, origin and porosity of the binders, as well as the physical properties of the mycotoxin, such as polarity, solubility, and charge distribution should be considered, as they influence the adsorption process (Huwig et al., 2001; Surai et al., 2005). Therefore, the aim of our study was to evaluate the impact of the supplementation of a mycotoxin deactivator in diets containing added mycotoxins for nursery piglets on their performance and antioxidant status.

## Material and Methods

All methods involving animal handling were realized in accordance with the regulations approved by the Institutional Animal Welfare and Ethics/Protection committee from the Universidade Federal de Minas Gerais (UFMG—CEAU), Brazil, under the protocol no. 349/2019. The study was conducted in the nursery facilities of the Universidade Federal de Minas Gerais/ ICA Swine Production laboratory (UFMG—NEPSUI).

### Animal, housing, experimental design, and diet

A total of 90 piglets (45 barrows and 45 females) belonging to the same commercial genetic line (Topigs Norsvin) were used. Pigs were allotted in a completely randomized block design with three animals per pen according to litter origin, sex, and body weight. Animals entered the experiment with approximately 7.12 ± 0.68 kg of body weight (24 d of age on average) and remained in the trial until 67 d of age on average (total 43 d of trial). The trial consisted of five dietary treatments and six repetitions per treatment. The pen was considered as the experimental unit. The dietary treatments consisted of a standard control diet as a negative control (NC; mycotoxin levels at accepted regulatory Brazilian Ministry of Agriculture standards deoxynivalenol [DON]: <100 μg/kg; zearalenone [ZEA]: <20 μg/kg; fumonisins [FBs]: <1 mg/kg); a standard diet added with mycotoxins to reach a low contamination level is considered as positive low (PCL−; DON: 900 μg/kg; ZEA: 100 μg/kg; FBs: 5,000 μg/kg) without the deactivator; a positive low added the deactivator at an inclusion rate of 1 kg/ton (PCL+); a standard diet added with mycotoxins to reach a high contamination level is considered as positive high (PCH−; DON: 4,500 μg/kg; ZEA: 500 μg/kg; FBs: 18,000 μg/kg) without the deactivator; and a positive high is added the deactivator at an inclusion rate of 5 kg/ton (PCH+). The deactivator (i.e., UNIKEPlus; Adisseo, Dendermonde, Belgium) was composed by adsorbent clay minerals, inactivated fermentation extracts of *Saccharomyces cerevisiae*, blend of antioxidants, preservatives (citric acid, lactic acid, phosphoric acid, and propylene glycol), and botanicals (milk thistle seed—*Silybum marianum*, rosemary leaves—*Rosmarinus officinalis*, licorice root, and boldo leaves). The low level of deactivator inclusion was based on the manufacturers' recommendation and the high-level inclusion was based on the fact that on average mycotoxins inclusion increased 2.5-fold from low to high contamination levels and therefore was decided to double the inclusion level above the level of mycotoxins increase (i.e., 5-fold). Diets were formulated according to the growing phase of piglets: Phase 1 diet, from 24 to 32 d; Phase 2 diet, from 33 to 43 d; Phase 3 diet, from 44 to 57 d; and Phase 4 diet, from 58 to 67 d. Mycotoxins were produced by solid fermentation using known mycotoxin-producing strains of *Fusarium verticillioides* (fumonisins) and *Fusarium graminearum* (des-oxynivalenol and zearalenone). Inoculum of fungal strains were cultured for 7 d in potato dextrose agar

at 25 °C and used to inoculate autoclaved moistened rice. The fermentation was carried in 500 mL erlenmeyer flasks in which 100 g of moistened rice was inoculated with 2 mL spore suspension of fungus (1 × 105 spore/mL) and maintained static at 25 °C for 28 d. Subsequently, the fermented rice was dried, ground, and analyzed for mycotoxins concentration. Adequate levels of inclusion of these materials were used for artificial contamination of feed. All diets (Table 1) were formulated to meet or exceed the nutritional requirements averaged between barrows and gilts according to the recommendations of Brazilian Tables for Poultry and Swine Rostagno H.S. (2017). A sample of each formulated diet was taken at the feed mill for mycotoxin analysis and chemical composition. For mycotoxin analyses, samples of feed were ground to < 0.85 mm and 1 g of the ground material was transferred to a test tube of 50 mL. It was added 10 mL of ultrapure water and 10 mL of acetonitrile/acetic acid (CH3CN:CH3COOH) [99.5:0.5, v/v] and the test tube was placed in a mechanic shaker for 10 min. A mixture of 4 g of MgSO4 and 1 g of NaCl was added and the tube was vigorously hand-shaking for 10 s. The solution was then centrifuged for 15 min at 5,000 × *g*, at 25 °C, and 2.5 mL of supernatant was transferred to a capped glass test tube where 2.5 mL of hexane was added. The solution was shaken for 2 h and then centrifuged at 1,000 × *g*, at 20 °C for 1 min. From lower phase (acetonitrile), 1 mL was withdrawn and dried with nitrogen (N2) stream at 40 °C. The reconstitution was performed with 75 µL of methanol in ultrasonic bath for 10 s and 10 s in the test tube mixer after adding 75 µL of ultrapure water. After centrifugation for 10 min at 14,000 ° *g*, 60 µL was withdrawn and transferred a to vial where 140 µL of ultrapure water was added. Ten microliters was injected in a chromatographic system. Detection and quantification of mycotoxins were performed with high-performance liquid chromatography coupled with tandem mass-spectrometry (LC/MS/MS). Chromatographic separation was carried out using Acqulty UPLC System (Waters, Milford, Massachusetts, EUA) equipped with 100 × 2.1 mm, 1.7 µm Acquity UPLC BEH C18

**Table 1.** Ingredients and analyzed nutrient composition of the experimental diets

| Ingredients | Phase 1 | Phase 2 | Phase 3 | Phase 4 |
|---|---|---|---|---|
| Corn | 31.490 | 37.660 | 48.130 | 59.004 |
| Soybean meal (46%) | 20.000 | 22.000 | 26.000 | 28.000 |
| Pre-Cooked corn | 10.000 | 10.000 | 7.000 | 0 |
| Swine plasma | 5.000 | 3.000 | 0 | 0 |
| Biscuit meal | 5.000 | 5.000 | 5.000 | 5.000 |
| Soybean oil | 3.550 | 3.350 | 3.400 | 3.800 |
| Milk whey | 21.000 | 15.000 | 6.000 | 0 |
| Dicalcium fosfate | 1.694 | 1.901 | 2.090 | 2.025 |
| Calcium carbonate | 0 | 0 | 0.106 | 0.265 |
| Sodium chloride | 0.400 | 0.400 | 0.400 | 0.400 |
| Sweetner[5] | 0.030 | 0.030 | 0.030 | 0.030 |
| Organic Acid blend[3] | 0.200 | 0.000 | 0.000 | 0.000 |
| L-Lysine HCl | 0.415 | 0.475 | 0.587 | 0.511 |
| DL-Methionine | 0.240 | 0.237 | 0.270 | 0.200 |
| L-Threonine | 0.159 | 0.188 | 0.281 | 0.239 |
| L-Tryptophan | 0.092 | 0.096 | 0.090 | 0.067 |
| L-Valine | 0.100 | 0.120 | 0.166 | 0.110 |
| Premix Mineral[1] | 0.100 | 0.100 | 0.100 | 0.100 |
| Premi Vitamin[2] | 0.050 | 0.050 | 0.050 | 0.050 |
| Zinc Oxide 80% | 0.280 | 0.240 | 0.200 | 0.100 |
| Mycotoxin deactivator[4] | Inclusion levels according to treatments | | | |
| Analyzed Nutritional Specs | | | | |
| ME kcal/kg | 3435 | 3412 | 3377 | 3367 |
| Crude Protein. % | 19.48 | 18.76 | 18.04 | 18.27 |
| Total Calcium % | 0.683 | 0.710 | 0.715 | 0.720 |
| Digestible Phosphorus % | 0.439 | 0.430 | 0.400 | 0.360 |
| SID AAS. % | | | | |
| Lysine | 1.35 | 1.30 | 1.26 | 1.20 |
| Methionine + Cysteine | 0.81 | 0.78 | 0.78 | 0.72 |
| Threonine | 0.88 | 0.84 | 0.84 | 0.80 |
| Valine | 0.95 | 0.91 | 0.88 | 0.84 |
| Tryptophan | 0.30 | 0.29 | 0.25 | 0.23 |

[1]Carbo-amino-phospho chelate of Cobalt (Cobalt: 102 mg/kg), Carbo-amino-phospho chelate of Copper (Copper 7.500.00 mg/kg), Carbo-amino-phospho chelate of Chromium (Chromium 100.00 mg/kg), Carbo-amino-phospho chelate of Iron (Iron 52.00 g/kg), Carbo-amino-phospho chelate of Manganese (Manganese 23.00 g/kg), Carbo-amino-phospho chelate of Selenium (Selenium 184.00 mg/kg), Carbo-amino-phospho chelate of Zinc (Zinc 57.50 g/kg), Butylated Toluene Hydroxide (BHT), Calcium Iodine (Iodine 665 mg/kg).
[2]Vitamin A (225.00000 UI/kg), Vitamin D3 (380.0000 UI/kg), Vitamin E (200.000 UI/kg), Vitamin K (10.000 mg/kg), Biotin (1.000 mg/kg), Folic acid (9.000 mg/kg), Nyacin (120.000 mg/kg), Pantotenic acid (60.000 mg/kg), Vitamin B2 (20.000 mg/kg), Vitamin B1 (8.000 mg/kg), Vitamin B6 (12.000 mg/kg) and Vitamin B12 (100.000 mcg/kg).
[3]Mixture of organic acids: Formic Acid/ Acetic Acid/ Propionic Acid/ Lactic Acid/ Citric acid/ Carrier & anticaking.
[4]Composed by adsorbent clay minerals, inactivated fermentation extracts of *Saccharomyces cerevisiae*, blend of antioxidants, preservatives (citric acid, lactic acid, phosphoric acid, and propylene glycol) and botanicals (milk thistle seed—*Silybum marianum*, rosemary leaves—*Rosmarinus officinalis*, licorice root and boldo leaves).
[5]Sweetner composed by a mixture of chemically defined aldehydes, ketones, and esters with sodium saccharin and thaumatin.

column (Waters, Milford, Massachusetts, EUA). The column was maintained at 40 °C and the injection volume was 10 μL. The mobile phase consisted of 0.1% formic acid in water(A) and 0.1% formic acid in acetonitrile (B). The acetonitrile (B) concentration was raised gradually from 10% to 90% within 12 min, brought back to the initial conditions at 0.1 min, and allowed to stabilize for 3 min. The mobile phase was delivered at a flow rate of 0.4 mL/min. The LC system was coupled with the Xevo TQS tandem mass spectrometer (Waters, Milford, Massachusetts, EUA), equipped with a turbo-ion electrospray (ESI) ion source. The mass-spectrometer was operated in scheduled multiple reaction monitoring (MRM) in positive mode. The analyses showed the following mycotoxin levels in feed: NC diet; DON: 0 μg/kg; ZEA: 0 μg/kg; FB1: 480 μg/kg; FB2: 142 μg/kg; PCL– and PCL+ diets; DON: 1,704 μg/kg; ZEA: 735 μg/kg; FB1: 5,330 μg/kg; FB2: 1,099 μg/kg; PCH– and PCH+ diets; DON: 3,690 μg/kg; ZEA: 1,168 μg/kg; FB1: 18,799 μg/kg; FB2: 2,912 μg/kg.

## Measurements and collected parameters

Every morning, feed refusals from a tray under the feeding trough were collected when available, and fresh feed was immediately distributed once per day between 0630 and 0730 h. Feed consumption was determined as the difference between feed allowance and the refusals collected over the phase. Thereafter, samples from the refusals were pooled per phase, and then DM content was measured. Samples of the fresh feed were also taken every day and similarly pooled for DM determination and chemical analysis of dietary composition per treatment and phase. The variations in ambient temperature, RH, and photoperiod followed closely the outdoor conditions. Ambient temperature and RH were continuously recorded (1 measurement every 5 min) in the barns, using a data logger connected to a probe (Didai Technology Ltda., Campinas, Brazil) placed at the average height of the piglets in center of the barn.

Piglets were individually weighed at the beginning and at the end of each growing phase during the experiment. For each growing phase, average daily gain of each pig and pen average daily feed intake and gain: feed ratio was calculated. On days 7, 19, 34, and 43 post-weaning, approximately 10 mL of blood samples, from a subsample of 6 pigs/treatment (1 pig per pen, randomly selected on the day of sampling), was collected from the jugular vein into heparinized tubes, centrifuged at 3,000 × *g* for 5 min at room temperature. After centrifugation, plasma and erythrocytes were separated, aliquoted, and stored at −80 °C. Total superoxide dismutase (TSOD) and glutathione peroxidase (GPx) were analyzed in erythrocytes. Malondialdehyde (MDA), vitamin C, vitamin E, and vitamin A were analyzed in plasma. All biochemical analyses were realized at the Laboratory of Oxidative Stress (LEO)/UFRGS, Porto Alegre, RS, Brazil. The method described by Oyanagui (1984) was used for the assay of TSOD activity using a TSOD assay kit A001 (Nanjing Jiancheng Bioengineering Institute). The method is based on the fact that total superoxide dismutase (TSOD) inhibits the generation of nitrite from oxidation of hydroxylamine by superoxide anion ($O2^-$) that is produced by the xanthine/xanthine oxidase system. The activity of TSOD was expressed as units per milliliter and determined by measuring the reduction of optical density of the reaction solution at 550 nm with a spectrophotometer (UV-2000, UNICO Instruments Co. Ltd., Shanghai, China). One unit of SOD was defined as the amount of SOD required to produce 50% inhibition of the rate of nitrite production at 37 °C. The results were normalized for total protein content by the Bradford method, using bovine serum albumin as standard.

Glutathione peroxidase activity was determined using a GPx assay kit A005 (Nanjing Jiancheng Bioengineering Institute; Maral et al., 1977). Glutathione peroxidase is an enzyme that catalyzes glutathione oxidation by oxidizing the reduced tripeptide glutathione (GSH) into oxidized glutathione considering that hydrogen peroxide is used as a substrate of glutathione. Absorbance was recorded at 412 nm. The GPx activity was expressed as units per mg of protein, and 1 unit was defined as a decrease in GSH of 1 mM/min after the decrease in GSH per minute of the nonenzymatic reaction was subtracted. The results were normalized for total protein (Bradford et al., 1976).

Malondialdehyde (MDA) levels, an index of lipid peroxidation, was measured by high-performance liquid chromatography (HPLC), employing a reversed phase column (SUPELCOSIL LC-18-DB HPLC column; 15 cm × 4.6 mm, 5 μm) and a mobile phase flow rate of 1 mL/min in 30 mmol/L monobasic potassium phosphate (pH 3.6) and methanol (9:1, v/v). The absorbance of the column effluent was monitored at 254 nm (Karatepe, 2004). Under these conditions, the retention time of MDA was approximately 5 min. MDA levels were then calculated from an MDA standard curve.

Vitamin C (ascorbic acid) was measured by HPLC simultaneously with the MDA (Karatepe, 2004). Under the same conditions, the retention time of vitamin C is approximately 3 min. Vitamin C levels were calculated from an ascorbic acid standard curve. Vitamin A (retinol) and vitamin E (alpha-tocopherol) levels were measured simultaneously by HPLC using a 15 × 4.6 mm column (Nucleosil 120 C-18) with a continuous flow of 2 mL/min 93.5:3.5 (v/v) methanol: water. Vitamin A absorbance was monitored at 325 nm. The retention time of vitamin A was approximately 2 min (Barbas et al., 1997). Vitamin A levels were calculated from a retinol standard curve. Vitamin E fluorescence was detected by applying an excitation wavelength of 295 nm and an emission wavelength of 350 nm (Barbas et al., 1997). The retention time of vitamin E was approximately 5 min. Vitamin E levels were calculated from an alpha-tocopherol standard curve.

## Calculations and statistical methods

The maximum, minimum daily average and variance of daily ambient temperatures and relative humidity were calculated and analyzed during the entire experimental period during the nursery phase. Data were submitted to normality test (Shapiro–Wilk) and analyzed using the mixed linear model (PROC MIX procedure) of SAS statistical package (SAS Studio for academics) and differences were considered significant at $P < 0.05$ and tendency at $P < 0.10$. The effects of treatments and initial body weight were considered as a covariate effect and included in the statistical model. Analyses were conducted using the following statistical model:

Yijk = μ + Ai + E(Xijk–X) + eijkl, where μ is the general average, Ai is the treatments, Xijk is the observed value of the covariate "initial body weight," X is the average of the covariate "initial body weight," E is the regression coefficient between the covariate (X) and the response variable (Y), and eijkl is the incidental residual effect of observation.

For the data that did not present normality (metabolic variables), to obtain the homogeneity of the variance, the adjustment of the data was realized by using the sinarc of the square rout. According to the equation (Bolhuis et al., 2005),

$$\mathrm{Sinarc}\sqrt{X} = Y,$$

where

X = data collected,

Y = homogenized data.

The homogenized data were analyzed using a mixed linear model (PROC MIX procedure) of SAS statistical package (SAS Studio for academics) and differences were considered significant at $P < 0.05$ and tendency at $P < 0.10$.

## Results

Average minimum and maximum temperatures and relative humidity levels measured during the experimental period were 23.8 and 31.1 °C, and 49% and 78%, respectively. During the experiment, piglets that presented liquid red or dark brown diarrhea were medicated with an intramuscular injection of tylosin during 3 consecutive days at a dosage of 2.0 mL/piglet. Piglet body weight at entrance did not differ ($P = 0.853$) among the treatments and averaged 7.12 ± 0.04 kg (Table 2).

Treatments influenced the piglets total feed intake throughout the experimental period, whereas piglets exposed to high mycotoxin levels without deactivator presented the lowest feed intakes when compared with low mycotoxin levels without deactivator and control (12.1 vs. 19.9 vs. 24.3 kg, respectively, for PCH– vs. PCL– vs. NC; $P = 0.0001$; Table 1). Average daily feed intake was also influenced ($P = 0.0001$) by the mycotoxin inclusion levels, whereas piglets exposed to high mycotoxin levels without deactivator showed the lowest daily intakes when compared to low mycotoxin levels without deactivator and control (281 vs. 462 vs. 565 g $d^{-1}$, respectively, for PCH- vs. PCL- vs. NC; Table 2).

As for the effects of the additive, piglets that were fed the mycotoxin deactivator showed an improved average daily feed intake when compared with corresponding PC treatments (334 vs. 281 kg; 537 vs. 462 g $d^{-1}$, respectively, for PCH+ vs. PCH– and PCL+ vs. PCL–; Table 1). Piglets' body weight development during the entire experiment was influenced by the treatments ($P <$ 0.001), whereas piglets fed high levels of mycotoxins without the deactivator showed reduced growth rates (281 vs. 462 vs. 565 g $d^{-1}$, respectively, for PCH- vs. PCL- vs. NC; $P = 0.0001$; Table 2). As a consequence of the reduced growth rate, the piglets fed high mycotoxin levels without the additive also presented a lower total weight gain and end weight (11.5 vs. 17.2 vs. 18.2 kg; and 18.6 vs. 24.2 vs. 25.3 kg, respectively, for PCH– vs. PCL– vs. NC; $P = 0.0001$; Table 2).

As for the piglets fed the mycotoxin deactivator, animals showed an improved ($P = 0.0001$) daily weight gain (284 vs. 267 g $d^{-1}$; 418 vs. 400 g $d^{-1}$, respectively, for PCH+ vs. PCH– and PCL+ vs. PCL–; Table 1), total weight gain (12.2 vs. 11.5 kg; 18.0 vs. 17.2 kg, respectively, for PCH+ vs. PCH– and PCL+ vs. PCL–; Table 1), and end weight (14.4 vs. 12.1 kg; 23.1 vs. 19.9 kg, respectively, for PCH+ vs. PCH– and PCL+ vs. PCL–; Table 1). Feed conversion was not influenced statically ($P = 0.327$) by the treatments.

Mycotoxin levels and the use of a mycotoxin deactivator influenced or tended to influence antioxidant metabolism variables. Overall total superoxide dismutase (TSOD) activity tended ($P = 0.08$) to be influenced by treatments and was higher in the piglets fed the deactivator followed by the control: 1.58 vs. 1.27 vs. 1.74 vs. 1.11 vs. 1.79 U/mg of protein, respectively, for NC, PCL–, PCL+, PCH–, and PCH+ (Table 3). Overall glutathione peroxidase (GPx) activity was influenced ($P = 0.032$) by treatments and was higher in the PCH+ piglets followed by the NC, PCL+, PCL–, and PCH–: 197 vs. 168 vs. 187 vs. 139 vs. 219 U/mg of protein, respectively, for NC, PCL–, PCL+, PCH–, and PCH+ (Table 3).

Vitamin E levels were significantly different ($P = 0.021$), whereas piglets receiving the deactivator and NC showed the highest values when compared with the PCL– and PCH– piglets (0.569 vs. 0.008 vs. 0.532 vs. 0.007 vs. 0.745 µmol/L vitamin E, respectively, for NC, PCL–, PCL+, PCH–, and PCH+; Table 2). Vitamin A levels were influenced by treatments ($P = 0.046$), whereas piglets from PCL+, PCH+, and NC showed the highest values when compared with the PCL– and PCH– (0.296 vs. 0.267 vs. 0.326 vs. 0.275 vs. 0.306 µmol/L vitamin A, respectively, for NC, PCL–, PCL+, PCH–, and PCH+; Table 3).

## Discussion

Mycotoxins are secondary metabolites produced by fungi of different genera (Blunden et al., 1991; Marin et al., 2013). These metabolites can generate significant economic losses in an animal production system (Kabak et al., 2006). The treatments influenced the piglet's performance, whereas animals challenged with high or low levels of mycotoxins without the deactivator showed a decreased voluntary feed intake, weight gain, and end live weight. In addition, changes in the antioxidant status and serum vitamin A and E levels were observed in challenged piglets. Such effects are considered important indicators of animals that are challenged with dietary contamination by Fusarium mycotoxins. In addition, direct effects such as the inhibition of tissue protein synthesis, nutrient malabsorption,

**Table 2.** Impact of the supplementation of a mycotoxin deactivator in diets containing mycotoxins for piglets from 24 to 67 d of age on overall nursery performance (LS means)

| | Treatments[1] | | | | | Statistics | |
|---|---|---|---|---|---|---|---|
| Variables | NC | PCL– | PCL+ | PCH– | PCH+ | SEM[2] | P value |
| N Repetitions | 6 | 6 | 6 | 6 | 6 | – | – |
| Initial BW, kg | 7.1 | 7.2 | 7.1 | 7.1 | 7.1 | 0.4 | 0.853 |
| Final BW, kg | 25.3a | 24.2b | 25.2[a] | 18.6c | 19.3c | 0.7 | 0.0001 |
| TFI, kg | 24.3a | 19.9b | 23.1c | 12.1d | 14.4e | 0.9 | 0.0001 |
| ADFI, g/d | 565a | 462b | 537c | 281d | 334e | 25 | 0.0001 |
| ADG, g/d | 423a | 400b | 418[a] | 267c | 284d | 11 | 0.0001 |
| TWG, kg | 18.2a | 17.2b | 18.0a | 11.5c | 12.2d | 0.5 | 0.0001 |
| FCR | 1.32 | 1.28 | 1.16 | 1.02 | 1.18 | 0.04 | 0.327 |

[1]NC, negative control; PCL-, low mycotoxin without deactivator; PCL+, low mycotoxin with deactivator; PCH–, high mycotoxin without deactivator; PCH+, high mycotoxin with deactivator. BW – body weight, TFI – Total feed intake, ADFI – average daily feed intake, ADG –average daily weight gain, TWG – Total weight gain, FC – feed conversion ratio.
[2]SEM, standard error of the mean. Letters in different columns indicate statistical difference ($P <0.05$).

**Table 3.** Impact of the supplementation of a mycotoxin deactivator in diets containing mycotoxins for piglets from 24 to 67 d of age on antioxidant metabolism parameters (LS Means)

| | Treatments[1] | | | | | | Statistics |
|---|---|---|---|---|---|---|---|
| Variables[2] | NC | PCL– | PCL+ | PCH- | PCH+ | SEM[3] | P value |
| N Repetitions | 24 | 24 | 24 | 24 | 24 | – | – |
| TSOD, U/ mg protein | 1.58 | 1.27 | 1.74 | 1.11 | 1.79 | 0.11 | 0.081 |
| GPx, U/ mg protein | 197[a] | 168[d] | 187[c] | 139[e] | 219[a] | 8 | 0.032 |
| MDA, µmol/ L | 4.82 | 5.94 | 5.22 | 6.18 | 5.41 | 0.84 | 0.998 |
| Vitamin C, µmol/ L | 48.56 | 33.20 | 37.98 | 24.59 | 43.28 | 3.91 | 0.237 |
| Vitamin E, µmol/ L | 0.569[b] | 0.008[c] | 0.532[b] | 0.007[c] | 0.745[a] | 0.001 | 0.021 |
| Vitamin A, µmol/ L | 0.296[b] | 0.267[c] | 0.326[a] | 0.275[c] | 0.306[b] | 0.006 | 0.046 |

[1]TSOD, Total superoxide dismutase activity; GPx, Glutathione peroxidase activity; MDA, Malondialdehyde.
[2]NC, negative control; PCL–, low mycotoxin without deactivator; PCL+, low mycotoxin with deactivator; PCH–, high mycotoxin without deactivator; PCH+, high mycotoxin with deactivator.
[3]SEM, standard error of the mean. Letters in different columns indicate statistical difference ($P < 0.05$).

increased lymphoid cellular apoptosis, and higher oxidative damage and cardiovascular toxicity can be evidenced (Genevieve et al., 2000). In this sense, the use of a mycotoxin deactivator can be considered as a viable strategy to attenuate the negative effects caused by mycotoxins in challenged animals, regardless of the level of contamination.

In our study, challenged piglets showed a reduced voluntary feed intake. The underlying mechanisms to this feed intake depression can be explained by the presence of DON as a contaminant. It is well described in the literature that DON is responsible for the elevation of pro-inflammatory cytokines, satiety hormones, and neuroendocrine regulation (Surai et al., 2005; Flannery et al., 2012). In agreement with our findings, previous studies evaluating the effects of dietary contamination with mycotoxins such as DON on the performance of piglets (Kim et al., 2019) and gilts (Kong et al., 2015) also observed significant reductions in voluntary feed intake. In addition to these findings, studies with mice (Flannery et al.., 2012) have shown an increase in serum levels of satiety hormones, after animals were challenged with DON, and observed a lower digestive transit and, therefore, a lower voluntary feed intake.

Fusarium mycotoxins impact by reducing voluntary feed intake and consequently decreasing growth rates and body weight, affecting the overall performance of pigs (Kong et al., 2015). In our study, the reduced growth rates in piglets exposed to mycotoxins can be associated with the depressed voluntary feed intake and also to the effects that mycotoxins have on the oxidative balance, that is, the relationship between reactive oxygen species and antioxidant enzymes. A higher oxidative stress rate could redirect nutrients to control antioxidant activity and maintain homeostasis at the expense of performance. Previous studies (Marin et al., 2013; Kong et al., 2015; Kim et al., 2019) have shown that mycotoxins can cause reduced growth rates in several animal species. The effects of mycotoxins on growth rates of pigs are dependent on age, where young animals, due to the natural effects of the weaning process, are more susceptible to intoxication when compared with animals in the later growing and finishing stages (Kong et al., 2015; Kim et al., 2019). Still according to the later authors, piglets receiving diets contaminated with AFLA (1.800 ppb), DON (1 mg/kg), and FB (12 mg/kg) do not show reduction in body weight development in the first week post-weaning, only after 10 d of exposure to the mycotoxins.

In our study, independent of the mycotoxin levels, piglets that received the deactivator showed improved performance traits. Our findings are in agreement with studies (Huwig et al., 2001; Kabak et al., 2006; Kim et al., 2019) that evaluated the use of binders in mycotoxin challenged animals. According to one of these later studies (Kim et al., 2019), a positive recovery can be observed in the period between 34 and 41 d of the offsetting of the exposure to the mycotoxins in piglets. Mycotoxin binders have been studied to mitigate mycotoxicosis by promoting the reduction of the mycotoxin bioavailability, and consequently, by reducing the inflammatory response, all of which can contribute to improve intestinal health, and prevent oxidative stress (Chaytor et al., 2011; Weaver et al., 2013 and 2014; Sun et al., 2015). The use of yeast cell wall and algae-based carbohydrate binders have demonstrated that their β-D-glucans composition and tridimensional structure are able to chemically adsorb mycotoxins (Yiannikouris et al., 2004a, 2004b, 2004c, and 2006), followed by a reduced absorption of mycotoxins in the small intestine (Yiannikouris et al., 2013), decreased accumulation of mycotoxins in specific organs, and increased metabolic clearance (Firmin et al., 2010), therefore protecting vital organs against mycotoxin exposure.

In our study, lower mycotoxin contamination levels associated with the inclusion of the deactivator at baseline levels (i.e., 1 kg/ ton) improved TSOD and GPx enzyme activity and decreased oxidative damage to lipids (↓MDA), thus favoring the metabolic response against oxidative stress. Nevertheless, including a higher level of deactivator (i.e., 5 kg/ ton) in diets with high mycotoxin levels showed not be enough to avoid an increase in oxidative stress. This statement can be corroborated by the observed decrease in TSOD and GPx and an increase in MDA levels. In agreement with our findings, a study (Duan et al., 2013) evaluating low and basal levels of inclusion of adsorbents in diets for weaned piglets reported an improvement in the antioxidant capacity of the animals, protecting them from severe oxidative damage. Different from the previous findings, a study (Xing et al., 2020) investigating different levels of inclusion of Galacto-oligosaccharide (GOS), from 500 to 2000 mg/ kg, reported that there was no increase in the levels of TSOD and GPx, but the authors did observe a reduction in the levels of MDA in all treatments when compared with standard treatment without the inclusion of GOS. Evaluating the effects of diets contaminated with DON (3.1 ppm) in weaned piglets (Van Le Thanh et al., 2016), the authors observed an increase in TSOD levels; however, the levels of GPx and MDA remained unchanged when compared with the standard. In another study with rats (Sun et al., 2015), the inclusion of an organic adsorbent in diets contaminated with aflatoxins did not affect the TNF-a, IgG and MDA concentration. Harper et al. (2010) and Meissonnier et al. (2008)

at high levels of dietary aflatoxin contamination observed no differences in the MDA levels in piglets during the nursery phase. The difference between these later studies and ours could be related to the fact that we tested the association of three mycotoxins at higher levels and they only evaluated one. Altogether, these findings indicate the possible synergistic effect among toxins on the piglet metabolism, representing a major threat to the animal's health, welfare, and performance (Weaver et al., 2013 and 2014; Pierron et al., 2016).

In our study, piglets challenged without the deactivator showed significant vitamin A and E reductions. Literature has described the impacts of aflatoxins damage to DNA, leading to cell death and the formation of tumors (Kanora et al., 2009). They are the most acutely toxic of all the mycotoxins for pigs, causing extensive liver damage (Hamilton P.B., 1977). Feeding pigs diets contaminated with aflatoxin may exacerbate vitamin A and vitamin E deficiency, as well as reduce immune function (Hamilton et al., 1974). In this sense, our findings indicate that the use of a mycotoxin deactivator can be efficient to reduce the negative impacts of aflatoxins on vitamin A and E depletions.

In conclusion, mycotoxins have a strong negative impact on piglet performance during nursery phase, affecting negatively voluntary feed intake, growth performance, and antioxidant metabolism. The results of our study indicate that the consumption of 1,700 μg/kg DON, 735 μg/kg ZEA, 5,330 μg/kg FB1, and 1,099 FB2 μg/kg reduced ADG by 5% and ADFI by 18%; and the consumption of 3,690 μg/kg DON, 1,168 μg/kg ZEA, 18,799 μg/kg FB1 and 2,912 μg/kg FB2 reduced ADG by 37% and ADFI by 50%. The higher effect of these mycotoxins on voluntary feed intake may have been the main reason for the reduction in growth performance. The addition of a deactivator at two different levels of inclusion (i.e., 1 or 5 kg/ ton), depending on the contamination level, resulted in varying benefits to piglets. The deactivator at a low level of contamination did provide ability to improve the performance of piglets. This additive also improved the antioxidant balance of these animals. While at a high level of contamination, even increasing inclusion by 5-fold was not enough to reduce the impacts of mycotoxins on feed intake, growth, and antioxidants status. In general, the use of the deactivator showed benefits for reducing the effects of mycotoxins on nursery piglets. It is important to state that these responses may vary under different mycotoxin concentrations, types, and mixtures.

## Acknowledgments

We gratefully acknowledge Mr. Elton Prates (swine production unit technician) for his technical assistance during the trial execution.

## Conflict of interest statement

The authors declare no real or perceived conflicts of interest.

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