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Evaluation of Vomitoxin Control Strategies on Nursery Pig Growth Performance and Blood Measures

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Appreciation is expressed to Hord Family Farms (Bucyrus, OH) for providing the animals and research facilities, and to D. Shawk and P. Hord for technical assistance.

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Evaluation of Vomitoxin Control Strategies on Nursery Pig Growth Performance and Blood Measures¹

Larissa L. Becker, Jordan T. Gebhardt,² Mike D. Tokach, Robert D. Goodband, Joel M. DeRouchey, Jason C. Woodworth, Arnau Vidal,³ and Christos Gougoulias³

Summary

A total of 4,318 pigs (337 \times 1050, PIC; initially 14.3 \pm 0.18 lb) were used in a 35-d growth trial to evaluate mycotoxin control strategies on nursery pig performance and blood measures. Pigs were weaned at approximately 21 d of age and randomly allotted to 1 of 5 dietary treatments. The randomized complete block design was blocking structure including sow farm origin, date of entry into the facility, and average pen BW. A total of 160 pens were used with 80 double-sided 5-hole stainless steel fence line feeders, with feeder serving as the experimental unit. For each feeder, 1 pen contained 27 gilts and 1 pen contained 27 barrows. There were 16 replications per dietary treatment. A common phase 1 diet was fed in pelleted form to all pigs for 7 d prior to treatment diets. Experimental treatments were fed in a single phase and included a 1) low deoxynivalenol (DON) diet; 2) high DON diet; 3) high DON + sodium metabisulfite (SMB); 4) high DON + Technology1; or 5) high DON + Technology1+. Overall (d 0 to 35), pigs fed the high DON diet had reduced (P < 0.001) ADG, ADFI, and final BW compared to the pigs fed the low DON diet. Furthermore, pigs fed the high DON+SMB diet had greater (P < 0.05) ADG, ADFI, and final BW compared to the pigs fed the high DON, high DON+Technology 1, or high DON+Technology1+ diets. An improvement (P < 0.05) in feed efficiency was observed in pigs fed high DON+SMB or high DON+Technology1+ diets compared to the low DON or high DON+Technology1 diets with high DON diets intermediate. Pigs fed high DON+SMB or high DON+Technology1 diets had reduced (P < 0.05) total removals and mortality compared to pigs fed low DON diets with high DON and high DON+Technology1+ intermediate. For economic analysis (d 0 to 35), pigs fed high DON+SMB diets had the greatest (P < 0.05) feed cost, revenue, and IOFC compared to all other treatments. The LC-MS/MS analysis of dried blood spots at the end of the trial revealed that pigs fed high DON or high DON+Technology1 had increased (P < 0.05) DON concentrations in the blood compared to low DON, with high DON+SMB and high DON+Technology1+ intermediate. Interestingly, while not

¹ Appreciation is expressed to Hord Family Farms (Bucyrus, OH) for providing the animals and research facilities, and to D. Shawk and P. Hord for technical assistance.

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statistically significant in this study, the reductions in presence and concentration of other important mycotoxins like fumonisin B1, B2, beta-zearalenol, and the emerging beauvericin—as well as the trends in circulating neutrophil-to-lymphocyte ratio and GGT in blood—when pigs were fed high DON+Technology1+ suggest that other important metabolic processes may be influenced. In summary, pigs fed high DON diets had reduced performance compared to pigs fed low DON diets. In our trial, SMB supplementation to high DON diets led to the greatest improvement in growth performance, but other metabolic changes associated with Technology1+ warrant further investigation.

Introduction

Deoxynivalenol (DON), also known as vomitoxin, is a mycotoxin found in cereal grains and is produced by the Fusarium genus. Pigs are the most susceptible livestock species to DON with exposure to concentrations greater than 1 mg/kg resulting in decreased feed intake and growth. Higher concentrations can result in complete feed refusal and vomiting. The concentration of DON can vary from year to year in cereal grains because of the level of stress the plant experiences during the growing season, such as insect damage, poor soil fertility, and harsh weather conditions.

Due to local supply of feed ingredients, swine producers may have no option but to utilize DON-contaminated cereal grains that contain levels greater than 1 mg/kg, resulting in negative effects on growth. There are several strategies of detoxification available to alleviate DON effects in swine diets. Contaminated feed can be treated chemically, physically, or biologically in which probiotics or enzymes are used to limit DON effects during digestion. Although no DON-detoxifying agents are approved by the U.S. Food and Drug Administration, some products can be beneficial. Therefore, the objective of this study was to determine the effect of in-feed technologies in high DON diets on growth performance, serum chemistry, enzymatic activity, and hematological outcomes in nursery pigs.

Materials and Methods

Animals and diets

The Kansas State University Institutional Animal Care and Use Committee approved the protocol used in this study. This study was conducted at a commercial research facility located in north central Ohio (Bucyrus, OH). A total of 160 pens were used with 80 double-sided 5-hole stainless steel fence line feeders each feeding 2 adjacent pens with feeder serving as the experimental unit. For each feeder, 1 pen contained 27 gilts and 1 pen contained 27 barrows. Each pen was also equipped with a cup waterer to provide *ad libitum* access to feed and water.

Weaned pigs (approximately 21 d of age) originating from three sow farms were placed into the research facility over an 8-d period. A total of 4,318 pigs (337 \times 1050, PIC; initially 14.3 \pm 0.18 lb) were used in a 35-d growth trial. At the time of placement in the nursery facility, pens of pigs were weighed and allotted to 1 of 5 dietary treatments in a randomized complete block design with blocking structure including sow farm

⁴ Eriksen, G.S., and H. Pattersson. 2004. Toxicological evaluation of trichothecenes in animal feed. Anim. Feed Sci. Technol. 112:205-239. doi: 10.1016/j.anifeedsci.2003.08.008

origin, date of entry into the facility, and average pen BW. There were 16 replicates (feeders) per dietary treatment.

A common phase 1 diet was fed in pelleted form to all pigs for 7 d prior to treatment diets. Experimental treatments included a low DON diet, a high DON diet; high DON + sodium metabisulfite (SMB); high DON + Technology1; or high DON + Technology1+. Sodium metabisulfite was included in the diet at 10 lb/ton (Table 1). Technology1 (Innovad Global; Essen, Belgium) was included in diets at 6 lb/ton. Technology1+ (Innovad Global; Essen, Belgium) with SMB included was added to diets at a total product inclusion rate of 6 lb/ton. Treatment diets were fed in one phase and were manufactured at the Hord Elevator (Bucyrus, OH). All diets met the NRC⁵ requirement estimates. Feed samples were collected from at least 6 feeders per treatment per feed delivery to the research facility. Samples were subsampled and sent for mycotoxin analysis (Activation Laboratories, Tonawanda, NY).

Feed additions to each individual feeder were made and recorded by an electronic feeding system (Dry Exact; Big Dutchman, Inc., Holland, MI). Pens of pigs were weighed and feed disappearance was calculated every 7 d until the conclusion of the trial to calculate ADG, ADFI, and feed efficiency. Feed disappearance was measured by using a volumetric regression equation which estimates the quantity of feed remaining in the feeder subtracted by the quantity of feed added to the feeder.

Pigs that died or were removed during this study due to sickness or injury were recorded. Any pig that was removed from a test pen was considered a removal and placed into a hospital pen where they remained for the duration of the study. Mortality is defined as a pig that died while in a test pen or a pig that died from a hospital pen. Total removals and mortality accounted for removals and mortality.

Blood sampling

One average weight gilt of each experimental unit (80 total) was bled on d 28 to 35, depending on the date of entry into the facility, for immunological, hematological, and biochemical analysis. Blood was collected in tubes without anticoagulant to obtain serum for chemistry and oxidative stress parameters. Blood was allowed to clot before centrifuging for 15 min at 1,500 g to collect serum, and samples were stored at -112°F (-80°C) until analyzed. Serum samples were sent to the Iowa State University Veterinary Diagnostic Laboratory (Ames, IA) for antibody titers of porcine circovirus type 2 (PCV2) using an ELISA kit. Serum samples were also sent to the Kansas State Veterinary Diagnostic Laboratory (Manhattan, KS) for serum chemistry analysis. Thiobarbituric acid reactive substances (TBARS) were evaluated on serum samples at Kansas State University swine laboratory (Manhattan, KS). The TBARS assay was a modification of the methods of Yagi (1998)⁶ and Aguilar Diaz De Leon and Borges (2020),⁷ and samples were run in triplicate in 96-well microplates.

⁵ National Research Council. 2012. Nutrient Requirements of Swine: Eleventh Revised Edition. Washington, DC: The National Academies Press. https://doi.org/10.17226/13298.

⁶ Yagi K. Simple assay for the level of total lipid peroxides in serum or plasma. Methods Mol Biol. 1998. 108:101-6. doi: 10.1385/0-89603-472-0:101.

⁷ Aguilar Diaz De Leon, J., Borges, C. R. 2020. Evaluation of oxidative stress in biological samples using the thiobarbituric acid reactive substances assay. J. Vis. Exp. (159), e61122, doi: 10.3791/61122.

Blood samples were also collected in tubes containing an anticoagulant, EDTA to obtain whole blood for hematological analysis. Whole blood samples were sent to the Kansas State Veterinary Diagnostic Laboratory (Manhattan, KS) for complete blood counting (CBC). Due to the long transport time from Ohio to Kansas, approximately half of the samples were clotted before analysis could be conducted. Leukocyte differentials (Kansas State University Veterinary Diagnostic Laboratory, KS) were performed on the samples that clotted during transportation. Dried blood spots (DBS) were prepared by placing one drop of blood from each whole blood sample onto a protein saver card. The DBS cards were sent to University of Ghent, Belgium, for mycotoxin analysis. Complete quantification (ng/mL) or mean peak area \pm standard deviation of 36 mycotoxins and their phase 1 and phase 2 metabolites was simultaneously performed after extraction and liquid chromatography with tandem mass spectrometry (LC-MS/MS) analysis of spotted blood volume (approximately 60 uL) on Whatman 903 protein saver cards.⁸

Economic analysis

For the economic analysis, total feed cost per pig, feed cost per lb of gain, revenue, and income over feed cost (IOFC) were calculated for high and low ingredient prices and market pig price. Feed cost per pig placed was determined by multiplying total feed intake by diet cost. Feed cost per lb of gain was calculated by dividing the total feed cost per pig by the total weight gained. Revenue per pig placed was determined by total gain times the dressing percentage (0.75) and then multiplied by carcass price to convert to a live price. The IOFC was calculated using revenue per pig placed minus feed cost per pig placed. For high ingredient price scenarios, the following prices were used: corn = \$6.00/bushel (\$232.14/ton); soybean meal = \$400/ton; L-Lys HCl = \$0.80/lb; DL-Met = \$2.50/lb; L-Thr = \$1.20/lb; L-Trp = \$5.00/lb; L-Val = \$4.00/lb; SMB = \$0.50/lb; Technology1 at \$1.78/lb; and Technology1+ = \$1.10/lb. For low ingredient price scenarios, the following prices were used: corn = \$3.00/bushel (\$107.14/ton); soybean meal = \$300/ton; L-Lys HCl = \$0.65/lb; DL-Met = \$1.70/lb; L-Thr = \$0.85/lb; L-Trp = \$3.00/lb; L-Val = \$2.50/lb; SMB = \$0.50/lb; Technology1 at \$1.78/lb; and Technology1+ = \$1.10/lb.

Statistical analysis

Data were analyzed as a randomized complete block design for one-way ANOVA using the GLIMMIX procedure of SAS OnDemand for Academics (SAS Institute, Inc., Cary, NC). Feeder (2 pens of pigs) was considered the experimental unit. Initial pen average BW, sow farm origin, and date of entry into the facility were used as blocking factors. Treatment was used as the fixed effect. For TBARS assay, microplate was used as a random effect. A \log_2 transformation was used for PCV2 titers. Results were considered significant with $P \le 0.05$ and were considered marginally significant with $P \le 0.10$.

Results and Discussion

Analysis of DON in the low DON corn was 1.1 ppm at the beginning of the study and 3.8 ppm at the end of the study. Analysis of DON in the high DON corn was 4.4 ppm at the beginning of the study and 4.0 ppm at the end of the study. Concentrations of

⁸ Lauwers, M., S. Croubels, B. Letor, C. Gougoulias, and M. Devreese. 2019. Biomarkers for exposure as a tool for efficacy testing of a mycotoxin detoxifier in broiler chickens and pigs. Toxins. 11, 187. doi:10.3390/toxins11040187.

DON, zearalenone, and fumonisin were detected in treatment diets throughout the study (Table 2).

Growth performance

From d 0 to 14, pigs fed the high DON diet had decreased (P < 0.05) ADG, ADFI, and BW compared to the pigs fed the low DON diet (Table 3). Pigs fed the high DON+SMB diet had greater (P < 0.05) ADG, ADFI, and BW compared to the pigs fed the high DON, high DON+Technology1, or high DON+Technology1+ diets. Furthermore, pigs fed high DON+Technology1+ had heavier (P < 0.05) BW compared to high DON and similar to pigs fed low DON. An improvement (P < 0.05) in feed efficiency was observed in pigs fed high DON+SMB compared to the low DON, high DON, or high DON+Technology1, with pigs fed high DON+Technology1+ diets intermediate.

From d 14 to 21, pigs fed the high DON diet had reduced (P < 0.05) ADG, ADFI, and BW compared to the pigs fed the low DON diet. Pigs fed high DON+SMB had greater (P < 0.05) BW, ADG, and ADFI compared to the pigs fed the high DON, high DON+Technology1, or high DON+Technology1+ diets. Similar findings were observed from d 21 to 28 with a statistical difference (P = 0.047) in feed efficiency across treatments, but using a Tukey multiple comparison adjustment no significant pairwise differences were observed.

From d 28 to 35, pigs fed the high DON diet had similar ADG, ADFI, and F/G compared to the low DON diet. Pigs fed the high DON+Technology1 had poorer (P < 0.05) ADG, ADFI, and F/G compared to all other treatments. An improvement (P < 0.05) in feed efficiency was observed for the pigs fed high DON or high DON+Technology1+ compared to pigs fed high DON+Technology1 with low DON and high DON+SMB intermediate.

From d 0 to 35, pigs fed the high DON diet had decreased (P < 0.05) ADG, ADFI, and final BW compared to the pigs fed the low DON diet. Pigs fed the high DON+SMB diet had greater (P < 0.05) ADG, ADFI, and final BW compared to the pigs fed the high DON, high DON+Technology1, or high DON+Technology1+ diets. Additionally, pigs fed high DON+Technology1+ had increased (P < 0.05) ADG and final BW compared to the high DON and similar to the pigs fed low DON diets. An improvement (P < 0.05) in feed efficiency was observed in pigs fed high DON+SMB or high DON+Technology1+ diets compared to the low DON or high DON+Technology1 diets, with high DON diets intermediate.

No differences (P > 0.10) were observed for removals (Table 3). Pigs fed high DON+SMB or high DON+Technology1 had lower (P < 0.05) mortality compared to pigs fed low DON diets with high DON or high DON+Technology1+ intermediate. The same results were observed for total removals and mortality.

For economic analysis, pigs fed high DON+SMB diets had the greatest (P < 0.05) feed cost, revenue, and IOFC compared to all other treatments. Feed cost per lb of gain was greatest (P < 0.05) when pigs were fed high DON+Technology1 diets. Similar findings were observed when using high or low ingredient prices for economic analysis.

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For the blood measurements, no differences (P > 0.10) were observed across treatments for complete blood count or leukocyte differential analyses; however, pigs fed high DON+Technology1+ had numerically the lowest neutrophil:lymphocyte ratio (Table 4). In the serum chemistry analysis, pigs fed high DON+Technology1 diet had a greater (P < 0.05) chloride concentration compared to high DON pigs with low DON, high DON+SMB, or high DON+Technology1+ intermediate (Table 5). Additionally, pigs fed high DON had a greater (P < 0.05) bicarbonate concentration compared to pigs fed high DON+SMB with low DON, high DON+Technology1, or high DON+Technology1+ intermediate. There was a statistical difference (P = 0.038) in anion gap across treatments, but using a Tukey multiple comparison adjustment no significant pairwise differences were observed.

For the dried blood spot card, pigs fed high DON or high DON+Technology1 had increased (P < 0.05) DON concentrations compared to low DON with high DON+SMB and high DON+Technology1+ intermediate (Table 6). A marginally significant difference (P = 0.095) in beta-zearalenol positive samples was observed with the pigs fed high DON diets having the greatest percentage compared to all other treatments. While not statistically different, the presence and concentrations of fumonisin B1 and B2, beauvericin, and beta-zearalenol were numerically reduced in pigs fed high DON+Technology1+ with values similar to pigs fed low DON diets; suggesting that additional research is warranted to further elucidate potential benefits from including this product in diets containing mycotoxin-contaminated grains.

In summary, results of this experiment indicate that pigs fed high DON diets had reduced performance compared to pigs fed low DON diets. These results also indicate that SMB was a suitable mycotoxin control strategy for nursery pig diets with DON concentrations evaluated in this study. The metabolic changes observed in our study by pigs fed high DON+Technology1+ warrant further investigation, especially in diets contaminated with mixed mycotoxins.

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Table 1. Treatment diet composition (as-fed basis)¹

			High DON	High DON +	High DON +
Ingredient, %	Low DON ²	High DON ²	+ SMB ³	Technology 14	Technology 1+5
Corn	62.63	62.63	62.41	62.30	62.44
Soybean meal, dehulled	22.02	22.02	22.04	22.05	22.04
Soybean meal, expelled	10.75	10.75	10.75	10.75	10.75
Limestone	1.10	1.10	1.10	1.10	1.10
Monocalcium P	1.20	1.20	1.20	1.20	1.20
Salt	0.73	0.73	0.43	0.73	0.60
L-Lys-HCl	0.48	0.48	0.48	0.48	0.48
DL-Met	0.25	0.25	0.25	0.25	0.25
L-Trp	0.05	0.05	0.05	0.05	0.05
L-Val	0.15	0.15	0.15	0.15	0.15
L-Thr	0.32	0.32	0.32	0.32	0.32
Zinc oxide	0.01	0.01	0.01	0.01	0.01
Copper sulfate	0.05	0.05	0.05	0.05	0.05
Vitamin and trace mineral premixes	0.18	0.18	0.18	0.18	0.18
Phytase ⁶	0.10	0.10	0.10	0.10	0.10
Sodium metabisulfite			0.50		
Technology1				0.30	
Technology1+					0.30
Total	100	100	100	100	100

Table 1. Treatment diet composition (as-fed basis)¹

			High DON	High DON +	High DON +
Ingredient, %	Low DON ²	High DON ²	+ SMB ³	Technology 14	Technology 1+5
Calculated analysis					
SID AA, %					
Lys	1.35	1.35	1.35	1.35	1.35
Ile:Lys	56	56	56	56	56
Leu:Lys	114	114	114	114	114
Met:Lys	39	39	39	39	39
Met and Cys:Lys	60	60	60	60	60
Thr:Lys	65	65	65	65	65
Trp:Lys	20.4	20.4	20.4	20.4	20.4
Val:Lys	71	71	71	71	71
His:Lys	37	37	37	37	37
Total Lys, %	1.49	1.49	1.49	1.49	1.49
ME, kcal/lb	1,493	1,493	1,490	1,489	1,491
NE, kcal/lb	1,102	1,102	1,099	1,098	1,099
SID Lys:NE, g/Mcal	5.56	5.56	5.57	5.58	5.57
CP, %	21.4	21.4	21.4	21.4	21.4
Ca, %	0.77	0.77	0.77	0.77	0.77
Na, %	0.32	0.32	0.32	0.32	0.32
Cl, %	0.56	0.56	0.38	0.56	0.49
STTD P, %	0.52	0.52	0.52	0.52	0.52

 $^{^{1}}$ A total of 4,318 pigs (initially 14.3 \pm 0.18 lb) were used with 54 pigs per replicate and 16 replications per treatment. A common starter pellet diet was used for approximately 7 d containing no mycotoxin control products and manufactured with low deoxynivalenol (DON) corn, and treatment diets were formulated in a single phase for the remainder of the study.

²Low DON diet was manufactured with corn containing an average of 2.46 ppm DON and high DON diet with an average of 4.17 ppm DON.

³Sodium metabisulfite (SMB) inclusion at 10 lb/ton.

⁴Technology 1 (Innovad Global; Essen, Belgium) inclusion at 6 lb/ton.

⁵Technology 1+ (Innovad Global; Essen, Belgium) with SMB included with a total product inclusion of 6 lb/ton.

⁶Quantum Blue 2G provided 908 FTU per lb of diet with an expected STTD P release of 0.14%.

Table 2. Mycotoxin analysis of corn and complete treatment diets¹

			High DON	High DON +	High DON +
Item, ppm	Low DON ²	High DON ²	+ SMB ³	Technology 14	Technology 1+5
Corn					
DON	2.46	4.17			
Zearalenone	0.08	0.10			
Fumonisin	0.15	0.00			
Complete treatme	nt diets				
d 0 to 7					
DON	1.55	5.04	2.65	2.98	2.46
Zearalenone	0.07	0.42	0.10	0.05	0.33
Fumonisin	0.00	0.20	0.00	0.10	0.00
d 7 to 14					
DON	1.61		1.36	3.50	1.35
Zearalenone	0.00		0.17	0.11	0.05
Fumonisin	0.00		0.10	0.10	0.00
d 14 to 21					
DON	0.24	1.30	1.17	2.08	1.02
Zearalenone	0.00	0.00	0.00	0.00	0.19
Fumonisin	0.00	0.10	0.00	0.00	0.10
d 21 to 28					
DON	0.69	1.35	0.63	0.49	1.95
Zearalenone	0.03	0.00	0.12	0.04	0.07
Fumonisin	0.10	0.00	0.20	0.00	0.20
d 28 to 35					
DON	1.53	1.66	1.40	1.94	1.50
Zearalenone	0.07	0.10	0.07	0.08	0.16
Fumonisin	0.10	0.00	0.00	0.30	0.10

 $^{^{1}}$ Multiple samples were collected from each diet throughout the study and submitted to Activation Laboratories (Tonawanda, NY) for mycotoxin analysis.

 $^{^{2}}$ Corn samples were collected at the beginning and end of the study. Values represent the average mycotoxin concentration throughout the study.

³Sodium metabisulfite (SMB) inclusion at 10 lb/ton.

⁴Technology 1 (Innovad Global; Essen, Belgium) inclusion at 6 lb/ton.

⁵Technology 1+ (Innovad Global; Essen, Belgium) with SMB included with a total product inclusion of 6 lb/ton.

Table 3. Evaluation of mycotoxin control strategies on nursery pig growth performance and economics¹

•	Low	High	High DON	High DON +	High DON +	OF M	n
Item	DON ²	DON ²	+ SMB ³	Technology 1 ⁴	Technology 1+5	SEM	P =
BW, lb							
d 0	14.3	14.2	14.3	14.3	14.3	0.18	0.432
d 14	24.4^{b}	23.2^{d}	25.3 ^a	$23.4^{c,d}$	$24.0^{\rm b,c}$	0.39	< 0.001
d 21	33.1^{b}	31.5°	34.2ª	31.5°	32.5 ^b	0.43	< 0.001
d 28	43.9^{b}	41.9°	45.0^{a}	41.8°	43.0^{b}	0.50	< 0.001
d 35	55.7 ^{a,b}	53.4°	56.6ª	52.5°	54.6 ^b	0.51	< 0.001
d 0 to 14							
ADG, lb	0.68^{b}	0.62°	0.76^{a}	$0.63^{b,c}$	$0.67^{\rm b,c}$	0.023	< 0.001
ADFI, lb	0.82^{b}	0.75°	0.88^{a}	0.76°	$0.79^{\rm b,c}$	0.021	< 0.001
F/G	1.22a	1.21a	1.15^{b}	1.21 ^a	1.19a,b	0.016	< 0.001
d 14 to 21							
ADG, lb	1.25 ^{a,b}	1.17^{c}	1.27ª	1.15°	$1.20^{\rm b,c}$	0.022	< 0.001
ADFI, lb	1.68^{a}	1.52 ^b	1.66a	$1.52^{\rm b}$	1.58 ^b	0.024	< 0.001
F/G	1.35	1.30	1.31	1.32	1.32	0.017	0.156
d 21 to 28							
ADG, lb	1.52 ^{a,b}	$1.48^{\rm b,c}$	1.54^{a}	1.46°	$1.49^{a,b,c}$	0.020	0.002
ADFI, lb	2.15 ^a	2.03 ^b	2.16 ^a	2.01^{b}	2.05 ^b	0.027	< 0.001
F/G	1.42	1.37	1.41	1.38	1.37	0.013	0.047
d 28 to 35							
ADG, lb	1.69ª	1.64^{a}	1.66ª	1.53 ^b	1.65 ^a	0.237	0.001
ADFI, lb	2.60 ^a	2.51 ^{a,b}	2.56ª	2.43^{b}	2.50 ^{a,b}	0.031	0.001
F/G	$1.54^{a,b}$	1.53 ^b	1.54a,b	1.60^{a}	1.52 ^b	0.018	0.009
d 0 to 35							
ADG, lb	1.15 ^b	$1.10^{\rm c,d}$	1.19ª	$1.08^{ m d}$	$1.13^{\rm b,c}$	0.015	< 0.001
ADFI, lb	1.59ª	1.50^{b}	1.62ª	$1.49^{\rm b}$	1.53 ^b	0.019	< 0.001
F/G	1.39ª	$1.36^{a,b}$	1.36 ^b	1.38^{a}	1.36 ^b	0.007	0.001
Removals, % ⁶	1.27	1.08	1.17	0.98	0.88	0.415	0.923
Mortality, %	4.47^{a}	2.42 ^{a,b}	1.91 ^b	2.08 ^b	$3.43^{\mathrm{a,b}}$	1.203	0.002
Total removals and mortality, %	6.14ª	3.69 ^{a,b}	3.20^{b}	3.22 ^b	4.62 ^{a,b}	1.368	0.007
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Table 3. Evaluation of mycotoxin control strategies on nursery pig growth performance and economics¹

Item	Low DON ²	High DON ²	High DON + SMB ³	High DON + Technology 1 ⁴	High DON + Technology 1+5	SEM	P =
Economics, \$/pig placed							
Low ingredient prices ⁷							
Feed cost	5.72 ^b	5.51 ^b	6.08ª	5.75 ^b	5.74 ^b	0.124	< 0.001
Feed cost/lb gain8	0.149^{b}	$0.149^{\rm b}$	0.150^{b}	0.158 ^a	0.151 ^b	0.0010	< 0.001
Revenue ⁹	17.20 ^b	16.80^{b}	18.30 ^a	16.52 ^b	17.12 ^b	0.385	< 0.001
$IOFC^{10}$	11.48^{b}	11.29 ^{b,c}	12.21 ^a	10.77°	11.38 ^{b,c}	0.266	< 0.001
High ingredient prices ¹¹							
Feed cost	8.78^{b}	8.45^{b}	9.26^{a}	8.67 ^b	8.72 ^b	0.188	< 0.001
Feed cost/lb gain	0.229^{b}	0.227^{b}	0.228^{b}	0.237^{a}	0.229^{b}	0.0014	< 0.001
Revenue ¹²	25.23 ^b	$24.64^{\rm b}$	26.84ª	24.22^{b}	25.11 ^b	0.565	< 0.001
IOFC	16.45 ^b	16.18 ^b	17.57ª	15.55 ^b	16.39 ^b	0.384	< 0.001

 $^{^{1}}$ A total of 4,318 pigs (initially 14.3 \pm 0.18 lb) were used with 54 pigs per replicate and 16 replications per treatment. A common starter pellet diet was used for approximately 7 d containing no mycotoxin control products and manufactured with low deoxynivalenol (DON) corn prior to d 0, and treatment diets were formulated in a single phase for the remainder of the study.

²Low DON diet was manufactured with corn containing an average of 2.46 ppm DON and high DON diet with an average of 4 ppm DON.

³Sodium metabisulfite (SMB) inclusion at 10 lb/ton.

⁴Technology 1 (Innovad Global; Essen, Belgium) inclusion at 6 lb/ton.

⁵Technology 1+ (Innovad Global; Essen, Belgium) with SMB included with a total product inclusion of 6 lb/ton.

⁶Pigs that were removed from a test pen were considered a removal and placed in a hospital pen where they remained alive for the duration of the study.

⁷Corn = \$3.00/bushel (\$107.14/ton); soybean meal = \$300/ton; L-Lys HCl = \$0.65/lb; DL-Met = \$1.70/lb; L-Thr = \$0.85/lb; L-Trp = \$3.00/lb; L-Val = \$2.50/lb; SMB = \$0.50/lb; Technology1 at \$1.78/lb; and Technology1+ = \$1.10/lb.

⁸Feed cost/lb gain = total feed cost per pen ÷ total gain per pen.

⁹Revenue = (total gain/pig placed \times 0.75) \times \$0.60.

 $^{^{10}}$ Income over feed cost = revenue – feed cost.

 $^{^{11}}$ Corn = \$6.00/bushel (\$232.14/ton); soybean meal = \$400/ton; L-Lys HCl = \$0.80/lb; DL-Met = \$2.50/lb; L-Thr = \$1.20/lb; L-Trp = \$5.00/lb; L-Val = \$4.00/lb; SMB = \$0.50/lb; Technology1 at \$1.78/lb; and Technology1+ = \$1.10/lb.

 $^{^{12}}$ Revenue = (total gain/pig placed × 0.75) × \$0.88.

 $^{^{}a,b,c,d}$ Means within a row with different superscripts differ (P < 0.05) using a Tukey multiple comparison adjustment.

Table 4. Evaluation of mycotoxin control strategies on nursery pig blood parameters¹

Low	High	High DON	High DON +	High DON +		
DON ²	DON ²	+ SMB ³	Technology 14	Technology 1+5	SEM	P =
5	6	6	9	5		
5.73	5.77	6.08	5.75	5.89	0.190	0.592
10.87	10.93	11.53	11.18	11.08	0.346	0.585
10.09	10.28	10.51	10.26	10.24	0.326	0.901
36.71	37.51	38.88	37.12	37.54	1.321	0.744
34.68	35.35	37.38	35.12	35.55	1.254	0.483
64.62	64.80	64.17	64.44	63.34	1.861	0.982
19.14	19.03	19.02	19.47	18.78	0.526	0.862
29.64	29.38	29.68	30.24	29.64	0.292	0.152
27.46	27.57	27.07	27.77	27.46	0.338	0.541
18.19	17.35	17.82	17.84	18.47	0.650	0.663
20.14	20.02	19.65	19.68	19.34	1.882	0.998
7.51	7.53	7.65	6.06	5.78	1.273	0.496
7.51	7.53	7.65	6.06	5.78	1.273	0.496
10.08	11.45	10.92	12.79	12.48	1.433	0.566
0.66	1.16	1.42	0.86	1.10	0.326	0.432
0.12	0.12	0.10	0.15	0.14	0.073	0.967
5.77	5.87	5.90	5.85	5.68	0.191	0.879
311.83	285.96	309.47	221.02	254.64	46.297	0.382
0.89	0.68	0.69	0.50	0.50	0.147	0.209
	5 5.73 10.87 10.09 36.71 34.68 64.62 19.14 29.64 27.46 18.19 20.14 7.51 7.51 10.08 0.66 0.12 5.77 311.83	DON² DON² 5 6 5.73 5.77 10.87 10.93 10.09 10.28 36.71 37.51 34.68 35.35 64.62 64.80 19.14 19.03 29.64 29.38 27.46 27.57 18.19 17.35 20.14 20.02 7.51 7.53 10.08 11.45 0.66 1.16 0.12 0.12 5.77 5.87 311.83 285.96	DON² DON² + SMB³ 5 6 6 5.73 5.77 6.08 10.87 10.93 11.53 10.09 10.28 10.51 36.71 37.51 38.88 34.68 35.35 37.38 64.62 64.80 64.17 19.14 19.03 19.02 29.64 29.38 29.68 27.46 27.57 27.07 18.19 17.35 17.82 20.14 20.02 19.65 7.51 7.53 7.65 7.51 7.53 7.65 10.08 11.45 10.92 0.66 1.16 1.42 0.12 0.10 5.77 5.87 5.90 311.83 285.96 309.47	DON² DON² + SMB³ Technology 1⁴ 5 6 6 9 5.73 5.77 6.08 5.75 10.87 10.93 11.53 11.18 10.09 10.28 10.51 10.26 36.71 37.51 38.88 37.12 34.68 35.35 37.38 35.12 64.62 64.80 64.17 64.44 19.14 19.03 19.02 19.47 29.64 29.38 29.68 30.24 27.46 27.57 27.07 27.77 18.19 17.35 17.82 17.84 20.14 20.02 19.65 19.68 7.51 7.53 7.65 6.06 10.08 11.45 10.92 12.79 0.66 1.16 1.42 0.86 0.12 0.12 0.10 0.15 5.77 5.87 5.90 5.85 311.83 285.96 309.47	DON2 DON2 + SMB3 Technology 14 Technology 1+5 5 6 6 9 5 5.73 5.77 6.08 5.75 5.89 10.87 10.93 11.53 11.18 11.08 10.09 10.28 10.51 10.26 10.24 36.71 37.51 38.88 37.12 37.54 34.68 35.35 37.38 35.12 35.55 64.62 64.80 64.17 64.44 63.34 19.14 19.03 19.02 19.47 18.78 29.64 29.38 29.68 30.24 29.64 27.46 27.57 27.07 27.77 27.46 18.19 17.35 17.82 17.84 18.47 20.14 20.02 19.65 19.68 19.34 7.51 7.53 7.65 6.06 5.78 10.08 11.45 10.92 12.79 12.48 0.66 1.16<	DON² DON² + SMB³ Technology 1⁴ Technology 1+⁵ SEM 5 6 6 9 5 5.73 5.77 6.08 5.75 5.89 0.190 10.87 10.93 11.53 11.18 11.08 0.346 10.09 10.28 10.51 10.26 10.24 0.326 36.71 37.51 38.88 37.12 37.54 1.321 34.68 35.35 37.38 35.12 35.55 1.254 64.62 64.80 64.17 64.44 63.34 1.861 19.14 19.03 19.02 19.47 18.78 0.526 29.64 29.38 29.68 30.24 29.64 0.292 27.46 27.57 27.07 27.77 27.46 0.338 18.19 17.35 17.82 17.84 18.47 0.650 20.14 20.02 19.65 19.68 19.34 1.882

Table 4. Evaluation of mycotoxin control strategies on nursery pig blood parameters¹

Item	Low DON ²	High DON ²	High DON + SMB ³	High DON + Technology 1 ⁴	High DON + Technology 1+5	SEM	P =
Leukocyte differential ⁷							
Sample count, n	15	16	16	16	16		
Segmented neutrophil, %	39.05	33.40	34.16	31.97	30.02	2.845	0.211
Band neutrophil, %	0.15	0.00	0.13	0.06	0.19	0.099	0.581
Total neutrophil, %	39.19	33.40	34.29	32.03	30.21	2.871	0.218
Lymphocyte, %	57.73	61.78	59.48	63.92	64.74	3.016	0.369
Monocyte, %	2.43	4.10	5.01	3.51	4.06	0.709	0.120
Eosinophil, %	0.45	0.59	1.07	0.55	0.70	0.236	0.368
Basophil,%	0.08	0.00	0.18	0.07	0.13	0.076	0.491
Neutrophil:Lymphocyte ratio	0.79	0.60	0.66	0.52	0.49	0.092	0.129

 $^{^1}$ A total of 4,318 pigs (initially 14.3 \pm 0.18 lb) were used with 54 pigs per replicate and 16 replications per treatment. A common starter pellet diet was used for approximately 7 d containing no mycotoxin control products and manufactured with low deoxynivalenol (DON) corn, and treatment diets were formulated in a single phase for the remainder of the study. Blood samples were collected from 1 average weight gilt of each experimental unit at the end of the study.

²Low DON diet was manufactured with corn containing an average of 2.46 ppm DON and high DON diet with an average of 4.17 ppm DON.

³Sodium metabisulfite (SMB) inclusion at 10 lb/ton.

⁴Technology 1 (Innovad Global; Essen, Belgium) inclusion at 6 lb/ton.

⁵Technology 1+ (Innovad Global; Essen, Belgium) with SMB included with a total product inclusion of 6 lb/ton.

⁶Banded neutrophil concentration was 0 K/uL for all 31 samples. Basophil concentration was non-detectable for 28 of the 31 samples. Count of samples with detectable levels were 1, 0, 2, 0, 0 across treatments low DON, high DON+SMB, high DON+Technology 1, high DON+Technology 1+, respectively. Detectable levels were 0.3 K/uL for treatment low DON and 0.2 K/uL for treatment high DON+SMB.

Metamyelocyte and myelocyte were non-detectable for all 79 samples. Nucleated erythrocytes per 100 WBC were non-detectable for 70 of the 79 samples. Count of samples with detectable levels were 2, 3, 2, 0, 2 across treatments low DON, high DON+SMB, high DON+Technology1, high DON+Technology1+, respectively.

 $^{^{}a,b,c,d}$ Means within a row with different superscripts differ (P < 0.05) using a Tukey multiple comparison adjustment.

Table 5. Evaluation of mycotoxin control strategies on nursery pig serum parameters¹

Item	Low DON ²	High DON ²	High DON + SMB ³	High DON + Technology 1 ⁴	High DON + Technology 1+5	SEM	<i>P</i> =
Serum chemistry ⁶							
Glucose, mg/dL	107.88	105.38	105.25	110.7	109.50	2.365	0.366
Urea nitrogen, mg/dL	8.69	9.44	8.81	9.63	7.88	0.694	0.415
Creatinine, mg/dL	0.59	0.62	0.69	0.67	0.63	0.052	0.717
Protein total, g/dL	4.91	4.86	5.06	4.83	4.92	0.077	0.243
Albumin, g/dL	3.84	3.76	3.86	3.71	3.83	0.075	0.551
Globulin calculated, g/dL	1.07	1.10	1.20	1.12	1.09	0.055	0.515
Calcium total, mg/dL	11.31	10.96	11.10	11.04	11.39	0.131	0.104
Phosphorus, mg/dL	11.73	11.31	11.53	11.35	11.87	0.233	0.289
Sodium, mmol/L	142.75	142.00	142.81	141.75	142.62	0.634	0.531
Potassium, mmol/L	6.41	6.26	6.55	6.59	6.63	0.285	0.880
Sodium:Potassium	22.75	23.25	22.38	23.00	22.19	0.937	0.928
Chloride, mmol/L	100.13^{ab}	99.81 ^b	101.00^{ab}	101.25 ^a	100.13^{ab}	0.380	0.007
Bicarbonate, mmol/L	26.63^{ab}	27.69ª	24.94^{b}	25.94^{ab}	26.50^{ab}	0.558	0.016
Anion gap calculated, mmol/L	23.56	22.00	24.75	22.05	23.81	0.740	0.038
Aspartate transaminase P5P, U/L	68.00	70.75	56.38	61.53	75.00	7.072	0.345
Alkaline phosphatase, U/L	268.06	275.31	252.44	264.44	275.31	11.559	0.584
Gamma glutamyltrans- ferase, U/L	60.06	70.44	59.56	71.88	57.63	5.538	0.213
Creatine kinase, U/L	2,077	2,559	1,881	2,767	3,338	472.9	0.168
Porcine circovirus type 2 titer							
Log ₂ titer	9.03	9.42	9.24	8.93	8.58	0.235	0.108
TBARS, μM MDA ⁷	13.07	12.30	12.56	12.86	13.66	0.960	0.567

 $^{^{1}}$ A total of 4,318 pigs (initially 14.3 \pm 0.18 lb) were used with 54 pigs per replicate and 16 replications per treatment. A common starter pellet diet was used for approximately 7 d containing no mycotoxin control products and manufactured with low deoxynivalenol (DON) corn, and treatment diets were formulated in a single phase for the remainder of the study. Serum samples were collected from 1 average weight gilt of each experimental unit at the end of the study. 2 Low DON diet was manufactured with corn containing an average of 2.46 ppm DON and high DON diet with an average of 4.17 ppm DON.

³Sodium metabisulfite (SMB) inclusion at 10 lb/ton.

⁴Technology 1 (Innovad Global; Essen, Belgium) inclusion at 6 lb/ton.

⁵Technology 1+ (Innovad Global; Essen, Belgium) with SMB included with a total product inclusion of 6 lb/ton.

⁶Sorbitol dehydrogenase (SDH) below detection limit of 0.3 U/L for 73 of 80 samples. Count of samples with detectable SDH levels were 2, 1, 2, 1, 1 for treatments low DON, high DON+SMB, high DON+Technology1, high DON+Technology1+, respectively. Total and direct bilirubin were below detection limit of 0.2 mg/dL for all 80 samples.

⁷TBARS = Thiobarbituric acid reactive substances. μM of MDA (malondialdehyde) equivalent.

 $^{^{}a,b,c,d}$ Means within a row with different superscripts differ (P < 0.05) using a Tukey multiple comparison adjustment.

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Table 6. Evaluation of the systemic exposure of nursery pigs at the end of the study (d 35) to mycotoxins and their phase 1 and phase 2 metabolites with the method of dried blood spot under different control strategies¹

_	Low	High	High DON	U	High DON +		
Item	DON ²	DON ²	+ SMB ³	Technology 14	Technology 1+5	SEM	<i>P</i> =
DON							
Concentration, ng/mL	7.44^{b}	10.00^{a}	$8.04^{\mathrm{a,b}}$	9.78^{a}	$9.31^{a,b}$	0.596	0.006
Positive, %	77.4	100.0	77.4	94.9	100.0	12.42	0.703
Count of samples LOD < result < LOQ, <i>n</i>	0	0	0	0	0		
DON glucuronide							
Peak area	1,004	1,330	987	1,363	1,209	154.9	0.156
Positive, %	38.3	78.5	28.7	78.5	59.4	20.07	0.155
Count of samples LOD < result < LOQ, n	0	0	0	0	0		
DOM-1							
Concentration, ng/mL		3.59	2.82		3.58	2.454	0.904
Positive, %	34.8	42.2	64.8	34.8	64.8	14.79	0.339
Count of samples LOD < result < LOQ, n	6	6	5	6	8		
Fumonisin B1							
Concentration, ng/mL	4.83	7.52	4.84	5.43	4.67	1.852	0.526
Positive, %	43.2	63.5	56.8	43.2	43.2	13.39	0.719
Count of samples LOD < result < LOQ, n	4	4	6	3	3		
Fumonisin B2							
Concentration, ng/mL		146.4	146.4	43.4		141.90	0.860
Positive, %	9.5	15.2	21.4	15.2		11.91	0.926
Count of samples LOD < result < LOQ, n	2	1	3	2	0		

Table 6. Evaluation of the systemic exposure of nursery pigs at the end of the study (d 35) to mycotoxins and their phase 1 and phase 2 metabolites with the method of dried blood spot under different control strategies¹

Item	Low DON ²	High DON ²	High DON + SMB ³		High DON + Technology 1+5	SEM	<i>P</i> =
Beauvericin							
Peak area	1,782	3,050	2,835	2,346	2,047	649.6	0.410
Positive, %	56.3	56.3	37.5	50.0	56.3	12.55	0.831
Count of samples LOD < result < LOQ, n	0	0	0	0	0		
Beta-zearalenol							
Concentration, ng/mL	7.93	6.41	10.32	6.08	7.95	2.10	0.266
Positive, %	59.1	94.1	68.1	83.4	40.4	18.05	0.095
Count of samples LOD < result < LOQ, n	3	8	6	7	5		

¹A total of 4,318 pigs (initially 14.3 ± 0.18 lb) were used with 54 pigs per replicate and 16 replications per treatment. A common starter pellet diet was used for approximately 7 d containing no mycotoxin control products and manufactured with low deoxynivalenol (DON) corn, and treatment diets were formulated in a single phase for the remainder of the study. Dried blood spots (DBS) were prepared by placing one drop of blood from each whole blood sample onto a protein saver card. The DBS cards were sent to the University of Ghent, Belgium, for mycotoxin analysis. Complete quantification (ng/mL) or mean peak area ± standard deviation of 36 mycotoxins and their phase 1 and phase 2 metabolites was simultaneously performed after extraction and liquid chromatography with tandem mass spectrometry (LC-MS/MS) analysis of spotted blood volume (approximately 60 uL) on Whatman 903 protein saver cards. Concentration is reported by treatment for all samples with a sample result > limit of quantification (LOQ). Positive samples are reported as percentage of samples within treatment with result > limit of detection (LOD). Samples with a result of trace detection of metabolite (LOD < sample result < LOQ) are reported as count by treatment. One sample had trace levels of fumonisin B3 from the high DON treatment.

²Low DON diet was manufactured with corn containing an average of 2.46 ppm DON and high DON diet with an average of 4.17 ppm DON.

³Sodium metabisulfite (SMB) inclusion at 10 lb/ton.

⁴Technology 1 (Innovad Global; Essen, Belgium) inclusion at 6 lb/ton.

⁵Technology 1+ (Innovad Global; Essen, Belgium) with SMB included with a total product inclusion of 6 lb/ton.

 $^{^{}a,b,c,d}$ Means within a row with different superscripts differ (P < 0.05) using a Tukey multiple comparison adjustment.