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Non Ruminant Nutrition

Evaluation of dietary mycotoxin control strategies on nursery pig growth performance and blood measures

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ABSTRACT

A total of 4,318 pigs (337 × 1,050, PIC; initially 6.5 ± 0.08 kg) were used in a 35-day study to evaluate dietary 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 in a randomized complete block design with blocking structure including sow farm origin, date of entry into 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 to all pigs in pelleted form for 7 day prior to treatment diets. Experimental treatments were fed from days 7 to 42 after weaning (days 0 to 35 of the study) and included a low deoxynivalenol (DON) diet (1.12 ± 0.623 mg/kg), high DON diet (2.34 ± 1.809 mg/kg), high DON+ 0.50% sodium metabisulfite (SMB), high DON+ one of two mitigating products; 0.30% Technology1, or 0.30% Technology1+. Technology1 and 1+ are comprised of clays, yeast cell wall components, and a blend of plant extracts. Technology1+ also contains SMB. Overall (days 0 to 35), pigs fed high DON had decreased ($P < 0.05$) final BW, ADG, and ADFI compared with low DON. Additionally, pigs fed high DON+SMB had increased ($P < 0.05$) ADG compared with all other treatments. An improvement ($P < 0.05$) in G:F was observed in pigs fed high DON + SMB or high DON + Technology1+ compared with 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 with pigs fed low DON diets with high DON and high DON + Technology1+ intermediate. Liquid chromatography/mass spectrometry analysis of circulating blood collected on day 35 revealed that 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. In summary, pigs fed high DON diets had reduced performance compared with pigs fed low DON. Sodium metabisulfite in high DON diets provided a benefit in growth performance with ADG and G:F exceeding growth performance in the low DON diet while, the improved G:F ratio combined with other immunometabolic changes (gamma glutamyltransferase and creatine kinase) associated with Technology1+ warrant further investigation.

Lay Summary

Mycotoxins are naturally occurring secondary metabolites produced by species of molds that commonly contaminate agricultural crops worldwide. Deoxynivalenol (DON) is a mycotoxin that interferes with protein synthesis, impacts immunity, and can cause organ damage. The current study focused on evaluating dietary mycotoxin control strategies on nursery pig growth performance and blood measures. The results indicated that when pigs were fed high DON diets it resulted in reduced growth performance compared to pigs fed low DON diets. Three mycotoxin control strategies were utilized in this study including sodium metabisulfite (SMB), Technology1, and Technology1+. Sodium metabisulfite supplementation to high DON diets provided a benefit in growth performance with ADG and G:F exceeding growth performance in the low DON diet. Analysis of circulating blood revealed that 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. Furthermore, immunometabolic changes observed when Technology1+ was included warrant additional investigation. In summary, feeding diets with high concentrations of DON reduces growth performance in nursery pigs, but performance can be improved with the inclusion of feed additives and additional research is needed to understand the biological effects of these control strategies.

Key words: deoxynivalenol, mycotoxin, nursery pigs, sodium metabisulfite

INTRODUCTION

Mycotoxins are naturally occurring secondary metabolites produced by species of molds that commonly contaminate agricultural crops worldwide (Alshannaq and Yu, 2017). Mycotoxins are produced from mold growth during different stages of food and feed production. Mold growth can be divided into two categories: field molds and storage molds (Osweiler and Ensley, 2012). Field molds infect crops as parasites and mainly contaminate seeds and plants in the field. Storage molds grow in feedstuffs after harvest during grain storage.

The *Fusarium* species is classified as a field mold and can produce trichothecenes (Bertero et al., 2018). Deoxynivalenol (DON) is a type B trichothecene that interferes with protein

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synthesis, impacts immunity, and can cause organ damage (Ehrlich and Diagle, 1987; Pestka and Smolinski, 2005). Pigs are the most susceptible livestock species to DON with exposure to concentrations greater than 1 mg/kg in feed resulting in decreased feed intake and overall growth (Dänicke et al., 2001). Higher concentrations can result in complete feed refusal and in certain circumstances can cause vomiting (Forsyth et al., 1977; Rotter et al., 1996; Eriksen and Pettersson, 2004).

There are several strategies of detoxification available to alleviate DON effects in swine diets (Weaver et al., 2014; Frobose et al., 2015; Shawk et al., 2019). Contaminated feed can be treated chemically (sodium metabisulfite [SMB] mixed with heat and moisture as well as supplemented in diets; Frobose et al., 2015; Shawk et al., 2019) or biologically in which probiotics or enzymes are used to limit DON effects during digestion (Cheng et al., 2006). It has been well documented that SMB and SMB-based feed additives included in swine diets contaminated with DON result in improved growth performance (Patience et al., 2014; Frobose et al., 2015; Shawk et al., 2019).

Although no feed additives are approved as DON-detoxifying agents by the US Food and Drug Administration, some products have been shown to be beneficial, but more research is needed to compare their effectiveness. Therefore, the objective of this trial was to determine the effect of in-feed technologies in DON-contaminated diets on growth performance, serum chemistry and enzymatic activity, and hematological outcomes in nursery pigs.

MATERIALS AND METHODS

General

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 two adjacent pens 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. Each pen was also equipped with a cup-waterer to provide ad libitum access to feed and water. Feed additions to each individual feeder were made and recorded by an electronic feeding system (Dry Exact; Big Dutchman, Inc. Holland, MI).

Animals and Housing

A total of 4,318 pigs (337 × 1,050, PIC, Hendersonville, TN; initially 6.5 ± 0.08 kg) were used in a 35-day growth study. Pigs were weaned at approximately 21 days of age and randomly allotted to 1 of 5 dietary treatments in a randomized complete block design with blocking structure including sow farm of origin, date of entry into the facility, and average pen BW. A common phase 1 diet was fed in pelleted form to all pigs for approximately 7 days immediately after weaning and prior to treatment diets. The common phase 1 diet was a corn, soybean meal, wheat based diet which also included an enzymatically treated soy product and whey powder and was formulated to meet or exceed NRC (2012) nutrient requirement estimates. Experimental treatments included a low deoxynivalenol (DON) diet (1.12 ± 0.623 mg/kg), a high DON diet (2.34 ± 1.809 mg/kg), high DON + sodium metabisulfite (SMB; Esseco USA, Parsippany, NJ), high DON + Technology1, or high DON + Technology1+. Sodium metabisulfite was included at a rate of 0.50% of the diet (Table 1). Technology1 and Technology1+ (Innovad

Table 1. Diet composition (as-fed basis)[1]

Ingredient, %	6 to 25 kg BW diet[2]
Corn	62.63
Soybean meal, dehulled, solvent extracted	22.02
Soybean meal, expelled	10.75
Limestone	1.10
Monocalcium P	1.20
Salt	0.73
L-Lys-HCl	0.48
DL-Met	0.25
L-Trp	0.05
L-Val	0.15
L-Thr	0.32
Zinc oxide	0.01
Copper sulfate	0.05
Vitamin and trace mineral premix[3]	0.18
Phytase[4]	0.10
Feed additive[5]	+/–
Total	100
Calculated analysis[6]	
Lys	1.35
Ile:Lys	56
Leu:Lys	114
Met:Lys	39
Met and Cys:Lys	60
Thr:Lys	65
Trp:Lys	20.4
Val:Lys	71
His:Lys	37
Total Lys, %	1.49
Net energy, kcal/kg	2,429
SID Lys:NE, g/Mcal	5.56
CP, %	21.4
Ca, %	0.77
STTD P, %	0.52

[1]A common starter pellet was used for approximately 7 days 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.
[2]Low DON diet manufactured with corn containing an average of 2.46 mg/kg DON and high DON diet with an average of 4.17 mg/kg DON.
[3]Premix provided per kg of premix: 1,653,468 IU vitamin A; 551,156 IU vitamin D3; 17,637 IU vitamin E; 1,323 mg vitamin K; 13.2 mg vitamin B12; 22,046 mg niacin; 11,023 mg pantothenic acid; 3,086 mg riboflavin; 3,307 mg thiamin; 77 g Fe from iron sulfate; 88 g Zn from zinc sulfate; 6.6 g Mn from manganese oxide; 9.9 g Cu from copper sulfate; 198 mg I from calcium iodate; and 198 mg Se from sodium selenite.
[4]Quantum Blue 2G (AB Vista, Marlborough, UK) included at 2,000 FTU/kg providedan estimated release of 0.14% STTD P.
[5]Three feed additives were tested in the high DON diet: sodium metabisulfite (SMB; Esseco USA, Parsippany, NJ) inclusion rate of 0.50%, Technology1 (Innovad Global; Essen, Belgium) at 0.30%, and Technology1+ (Innovad Global; Essen, Belgium) at 0.30%. In each diet, additives were included at the expense of corn.
[6]NRC. 2012. Nutrient Requirements of Swine, 11th ed. Natl. Acad. Press, Washington, DC.

Global; Essen, Belgium) were included at 0.30% of the diet. Technology1 and 1+ are comprised of clays, yeast cell wall components, and a blend of plant extracts. Technology1+ also contains SMB. Treatment diets were fed in meal form from days 7 to 42 after weaning (days 0–35 of the study) and were manufactured at the Hord Elevator (Bucyrus, OH). All nutrients met NRC (2012) requirement estimates.

All feed used in this experiment was manufactured in a commercial feed mill that receives loads of corn weekly. The facility uses a rapid test (TotalTox DON; Envirologix, Portland, ME) to evaluate the level of DON in each incoming load of corn and segregates into low and high DON storage bins for segregated use within diets fed to different phases of production. Low and high DON corn samples were collected during the study including 1 set of samples at the beginning of the study and 1 set of samples at the conclusion of the study. Complete feed samples were collected from at least 6 feeders per treatment per feed delivery (approximately 4 to 5 deliveries depending on the treatment) to the research facility. All corn and complete feed samples were submitted for mycotoxin analysis using liquid chromatography with double mass spectrometry (LC–MS/MS) (Activation Laboratories, Tonawanda, NY).

During the experiment, pens of pigs were weighed, and feed disappearance was recorded every 7 days to determine ADG, ADFI, and G:F. Pigs that died or were removed during this study from the inability to overcome sickness or injury were recorded. Removals were defined as any pig taken from a test pen and placed into a hospital pen where they remained for the duration of the study. Any pig that died while in a test pen or a pig that died from a hospital pen was defined at a mortality.

Blood Sampling

One gilt per pen, closest to the average weight of pigs in that pen (80 total), was bled at the end of the study for immunological, hematological, and biochemical analysis. For blood collection, gilts were restrained using a snare and blood was collected from the jugular vein. Blood was collected in tubes without anticoagulant to obtain serum for chemistry and oxidative stress criteria. Blood was allowed to clot before centrifuging for 15 min at 1,500 × *g* to collect serum, and samples were stored at −80 °C until analyzed. Serum samples were sent to 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 Kansas State University 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). Thiobarbituric acid reactive substances assay was a modification of the methods of Yagi (1998) and Aguilar Diaz De Leon and Borges (2020) and samples were run in triplicate in 96-well microplates.

Blood samples were also collected in tubes containing EDTA to obtain whole blood for hematological analysis. Whole blood was sent to Kansas State University Veterinary Diagnostic Laboratory (Manhattan, KS) for complete blood cell counting (CBC). Due to the long transport time from Ohio to Kansas, approximately half of the samples were clotted before analysis could be conducted. As a result, manual leukocyte differentials (Kansas State University Veterinary Diagnostic Laboratory, KS) were performed by a trained veterinary clinical pathologist on the samples that clotted during transportation. Additionally, dried blood spots (DBS) were prepared by placing one drop of blood from each whole blood sample onto a protein saver card (Whatman 903; Cytiva, Marlborough, MA). The DBS cards were sent to University of Ghent, Belgium for mycotoxin biomarker analysis. Complete quantification (ng/mL) or mean peak area ± standard deviation of 36 mycotoxins and their phase I and phase II metabolites was simultaneously performed after extraction and LC–MS/MS analysis of spotted blood volume (approximately 60 µL) on Whatman 903 protein saver cards following the method validated by Lauwers et al. (2019).

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 PCV type 2 titers. Studentized residuals were used to evaluate model assumptions which were reasonably met. Results were considered significant with $P \leq 0.05$ and were considered marginally significant with $P \leq 0.10$.

RESULTS

Analysis of DON in the low DON corn was 2.46 ± 1.86 mg/kg (mean ± standard deviation). Analysis of DON in the high DON corn was 4.17 ± 0.26 mg/kg. Deoxynivalenol concentrations in low DON diets were numerically lower compared to high DON diets with the other treatment diets intermediate (Table 2). Inclusion of SMB to high DON diets resulted in lower DON concentrations compared to Technology1 and 1+. Zearalenone and fumonisin were also detected at low levels in treatment diets.

From days 0 to 35, pigs fed the high DON diet had decreased ($P < 0.05$) ADG, ADFI, and final BW compared to pigs fed the low DON diet (Table 3). Pigs fed the high DON + SMB diet had greater ($P < 0.05$) ADG compared with pigs fed the other treatments. Additionally, pigs fed high DON + Technology1+ had increased ($P < 0.05$) final BW compared with the high DON and similar to 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 pigs fed the low DON or high DON + Technology1 diets with high DON diets intermediate. The differences in ADG and ADFI compared with the high DON diet were mainly driven by the responses in the first 3 weeks of the study (Figs. 1 and 2). The magnitude of difference was greatest for SMB, then low DON, followed by Technology1+ and 1 in comparison to high DON early in the study. The differences between treatments lessened after week 3 for both ADG and ADFI.

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

For blood measurements, no differences ($P > 0.10$) were observed between treatments for complete blood

Table 2. Mycotoxin analysis of corn and complete treatment diets

Item, mg/kg	Low DON	High DON	High DON + SMB[1]	High DON + Technology 1[2]	High DON + Technology 1+[3]
Corn[4]					
DON	2.46 ± 1.86	4.17 ± 0.26	–	–	–
Zearalenone	0.08 ± 0.05	0.10 ± 0.05	–	–	–
Fumonisin	0.15 ± 0.07	ND[6]	–	–	–
Complete treatment diets[5]					
DON	1.12 ± 0.62	2.34 ± 1.81	1.44 ± 0.74	2.20 ± 1.15	1.66 ± 0.56
Zearalenone	0.03 ± 0.04	0.13 ± 0.20	0.09 ± 0.06	0.06 ± 0.04	0.16 ± 0.11
Fumonisin	0.04 ± 0.06	0.08 ± 0.10	0.06 ± 0.09	0.10 ± 0.12	0.08 ± 0.08

[1]Sodium metabisulfite (SMB) inclusion rate of 0.50%.
[2]Technology 1 (Innovad Global; Essen, Belgium) inclusion rate of 0.30%.
[3]Technology 1+ (Innovad Global; Essen, Belgium) inclusion rate of 0.30%.
[4]Low and high DON corn samples were collected during the study including 1 set of samples at the beginning of the study and 1 set of samples at the conclusion of the study. Values in the table represent the average mycotoxin concentration throughout the study± standard deviation.
[5]Feed samples were collected from at least 6 feeders per treatment per feed delivery (approximately 4 to 5 deliveries depending on the treatment) to the research facility and submitted to Activation Laboratories (Tonawanda, NY) for mycotoxin analysis ran in triplicate. Values in the table represent the average mycotoxin concentration throughout the study± standard deviation.
[6]ND = samples did not contain detectable mycotoxin.

Table 3. Evaluation of mycotoxin control strategies on nursery pig growth performance[1]

Item	Low DON[2]	High DON[2]	High DON + SMB[3]	High DON + Technology 1[4]	High DON + Technology 1+[5]	SEM	*P* =
BW, kg							
Day 0	6.5	6.5	6.5	6.5	6.5	0.08	0.432
Day 35	25.3^{a,b}	24.2^{c}	25.7^{a}	23.8^{c}	24.8^{b}	0.23	<0.001
Days 0 to 35							
ADG, g	522^{b}	499^{c,d}	542^{a}	489^{d}	512^{b,c}	6.8	<0.001
ADFI, g	723^{a}	680^{b}	734^{a}	675^{b}	693^{b}	8.8	<0.001
G:F, g/kg	722^{b}	733^{a,b}	738^{a}	724^{b}	738^{a}	3.8	0.001
Removals, %	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^{a,b}	1.203	0.002
Total removals and mortality, %	6.14^{a}	3.69^{a,b}	3.20^{b}	3.22^{b}	4.62^{a,b}	1.368	0.007

[1]A total of 4,318 pigs (initially 6.5 ± 0.08 kg) were used with 54 pigs per replicate and 16 replications per treatment. A common starter pellet was initially used for approximately 7 days 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.
[2] Low DON diet manufactured with corn containing an average of 2.46 mg/kg DON and high DON diet with an average of 4.17 mg/kg DON.
[3] Sodium metabisulfite (SMB) inclusion rate of 0.50%.
[4] Technology 1 (Innovad Global; Essen, Belgium)inclusion rate of 0.30%.
[5] Technology 1+ (Innovad Global; Essen, Belgium)inclusion rate of 0.30%.
[a,b,c,d]Means within a row with different superscripts differ ($P < 0.05$) using a Tukey multiple comparison adjustment.

count or leukocyte differential analyses although the neutrophil:lymphocyte ratio was numerically reduced when Technology1 and 1+ (0.52 and 0.49, respectively, $P = 0.129$) were utilized in high DON diets (Table 4). Similarly, Technology1+ numerically exhibited the lowest level of GGT and the highest level of creatine kinase compared to all other treatments (Table 4). In the serum chemistry analysis, pigs fed the high DON + Technology1 diet had a greater ($P < 0.05$) chloride concentration compared to high DON pigs with others being intermediate (Table 5). Additionally, pigs fed high DON had greater ($P < 0.05$) bicarbonate concentration compared to pigs fed high DON + SMB with others being intermediate. There was a statistical difference ($P = 0.038$) in anion gap between treatments, but using a Tukey multiple comparison adjustment no significant pairwise differences were observed. No statistical differences ($P > 0.10$) were observed for PCV2 titers or TBARS between treatments.

For the circulating blood, pigs fed high DON or high DON + Technology1 had increased ($P < 0.05$) DON concentrations compared with low DON with high DON + SMB and high DON + Technology1+ intermediate (Table 6). A marginally significant difference ($P = 0.095$) in the number of Beta-zearalenol positive samples was observed with pigs fed high DON diets having the greatest percentage compared with all other treatments. While not statistically different, presence and concentrations of fumonisin B1 and B2, beauvericin, and beta-zearalenol were numerically decreased in pigs fed high DON + Technology1+ with values similar to pigs fed low DON diets.

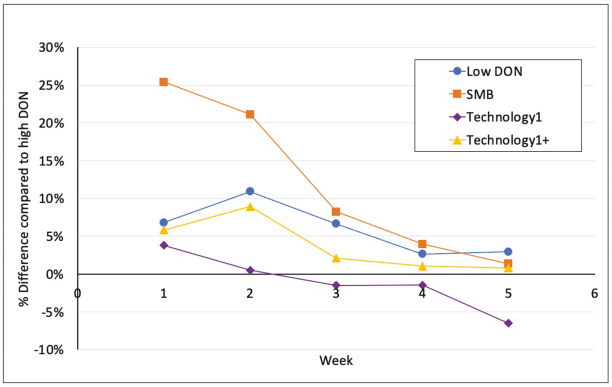

Figure 1. Average daily gain by week relative to high DON treatment (horizontal axis).

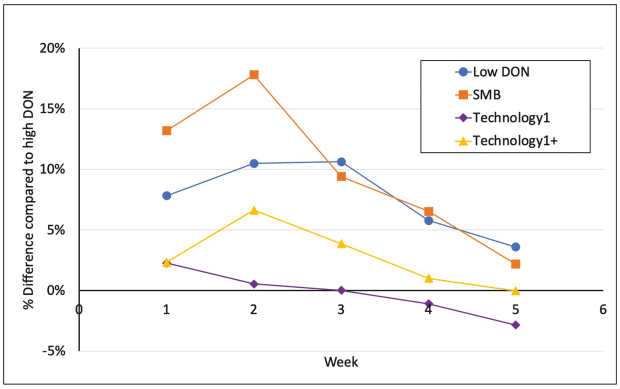

Figure 2. Average daily feed intake by week relative to high DON treatment (horizontal axis).

DISCUSSION

Deoxynivalenol is one of the most common mycotoxins found in cereal grains such as corn, wheat, and barley (Yazar and Omurtag, 2008). This mycotoxin is highly water soluble and is effectively absorbed in the upper gastrointestinal tract (Dänicke et al., 2004; Goyarts et al., 2006; Broekaert et al., 2017). It has been documented that DON ingestion results in reduced feed intake and body weight gain with pigs being the most susceptible livestock species, especially young pigs (Larivière et al., 2021). A meta-analysis conducted by Andretta et al. (2012) indicated a reduction of 0.28% in body weight gain for each mg/kg of DON in the diet. The data reported herein showed a greater impact from DON as we observed a reduction in body weight gain of 3.6% for each mg/kg of DON in the diet. Furthermore, Patience et al. (2014) observed that pigs fed diets with 4 mg/kg DON had a 6% reduction of ADG and ADFI in comparison to pigs fed diets with less than 1.5 mg/kg DON, which calculates to 2.4% reduction in ADG for each mg/kg of DON in the diet. In our study, we observed a 4% and 6% reduction in ADG and ADFI, respectively, when pigs were fed diets with an average of 2.34 mg/kg compared with pigs fed 1.12 mg/kg, with a large portion of the reduction driven by the observations early in the study. Similarly, Shawk et al. (2019) also observed a larger reduction in body weight gain early in the experiment. Similar to Frobose et al. (2015), we observed differences in feed efficiency in diets naturally contaminated with DON. The reduction in feed efficiency may be due to pigs wasting feed by sorting through the feed creating wastage. Furthermore, previous research by Pestka et al. (2004) demonstrated an upregulation of immune criteria including cytokines, chemokines, and inflammatory genes caused by DON. While we did not detect any direct evidence of immune system stimulation based on the measurements used in the current study, it could be hypothesized that the reduction in feed efficiency may be associated with stimulation of the immune system during the initial exposure of DON which could result in increased maintenance requirements in the pig. This hypothesis would need to be explored further to understand the mechanism leading to the observed difference in feed efficiency.

Although not feeding mycotoxin contaminated grains is the ideal way to avoid the harmful effects of mycotoxins, the use of contaminated feed may be unavoidable. Moreover, a recent long-term longitudinal study in broiler chickens, spanning 18 successive life-cycles, demonstrated that even low doses of multiple mycotoxins can cause a negative impact on livestock (Kolawole et al., 2020). As a result, feed additives with mycotoxin mitigation properties can be used to reduce the toxic effects within the animal. The present study evaluated 3 mitigation strategies (SMB, Technology1 and Technology1+) thought to reduce the negative effects of DON on pig performance. Utilizing feed additives in mycotoxin contaminated grains has been well documented (Frobose et al., 2015; Shawk et al., 2019; Larivière et al., 2021). To the best of our knowledge, there is currently no research available that documents the effects of Technology1 or 1+ added to mycotoxin contaminated pig diets.

In diets naturally contaminated with DON, the biological mechanism of SMB is suggested to be due to the chemical alteration of DON to a nontoxic DON-sulfonate adduct form (Young et al., 1987; Frobose et al., 2015). We observed evidence of the potential bioconversion of DON by SMB with diets containing SMB having lower DON concentrations. Previous research indicated improvements in energy and protein utilization in broilers fed sorghum-based diets that were steam pelleted with SMB (Selle et al., 2013, 2014; Truong et al., 2016). The positive effect of SMB on growth is thought to be due to the oxidative–reductive depolymerization of starch polysaccharides and the reduction of disulfide cross-linkages in proteins resulting in improved starch and protein availability (Truong et al., 2016). Furthermore, SMB has the ability to reduce trypsin inhibitors in soybean meal by the reduction of disulfide cross-linkages (Sessa and Ghantous, 1987; Wang et al., 2009). Therefore, the improved performance (feed intake, ADG, and feed efficiency) observed in our study could be explained by the biological effects of SMB and/or the chemical alteration of DON by SMB.

Technology1 and 1+ contain a variety of ingredients including clays aiming toward mycotoxin binding effects, yeast cell wall components focusing on mycotoxin binding and immunomodulatory effects, and a blend of plant extracts for antioxidant and liver enhancement effects. Yeast cell wall is composed primarily of polysaccharides including beta-glucans which can give adsorptive capacities for mycotoxins through hydrogen and ionic bonding (Jouany et al., 2005). They can also provide positive immunomodulatory effects, in particular, of the innate immunity as reviewed recently by Han et al. (2020) and De Marco et al. (2020). In vitro, beta-glucan has been shown to adsorb mycotoxins as aflatoxin B1, DON, and ochratoxin A (Weaver et al., 2014). The

Table 4. Evaluation of mycotoxin control strategies on nursery pig blood criteria[1]

Item	Low DON[2]	High DON[2]	High DON + SMB[3]	High DON + Technology 1[4]	High DON + Technology 1+[5]	SEM	*P* =
Complete blood count[6]							
Sample count, *n*	5	6	6	9	5	–	–
Erythrocyte concentration, M/μL	5.73	5.77	6.08	5.75	5.89	0.190	0.592
Hemoglobin, g/dL	10.87	10.93	11.53	11.18	11.08	0.346	0.585
Cellular hemoglobin, g/dL	10.09	10.28	10.51	10.26	10.24	0.326	0.901
Hematocrit spun, %	34.68	35.35	37.38	35.12	35.55	1.254	0.483
Mean cell volume, fL	64.62	64.80	64.17	64.44	63.34	1.861	0.982
Mean cell hemoglobin, pg	19.14	19.03	19.02	19.47	18.78	0.526	0.862
Mean cell hemoglobin concentration, g/dL	29.64	29.38	29.68	30.24	29.64	0.292	0.152
Cell hemoglobin concentration mean g/dL	27.46	27.57	27.07	27.77	27.46	0.338	0.541
RBC distribution width, %	18.19	17.35	17.82	17.84	18.47	0.650	0.663
Leukocyte count, K/μL	20.14	20.02	19.65	19.68	19.34	1.882	0.998
Segmented neutrophil concentration, K/μL	7.51	7.53	7.65	6.06	5.78	1.273	0.496
Total neutrophils, K/μL	7.51	7.53	7.65	6.06	5.78	1.273	0.496
Lymphocyte concentration, K/μL	10.08	11.45	10.92	12.79	12.48	1.433	0.566
Monocyte concentration, K/μL	0.66	1.16	1.42	0.86	1.10	0.326	0.432
Eosinophil concentration, K/μL	0.12	0.12	0.10	0.15	0.14	0.073	0.967
Plasma protein, g/dL	5.77	5.87	5.90	5.85	5.68	0.191	0.879
Fibrinogen, mg/dL	311.83	285.96	309.47	221.02	254.64	46.297	0.382
Neutrophil:lymphocyte ratio	0.89	0.68	0.69	0.50	0.50	0.147	0.209
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 6.5 ± 0.08 kg) were used with 54 pigs per replicate and 16 replications per treatment. A common starter pellet was initially used for approximately 7 days 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.
[2] Low DON diet manufactured with corn containing an average of 2.46 mg/kg DON and high DON diet with an average of 4.17 mg/kg DON.
[3] Sodium metabisulfite (SMB) inclusion rate of 0.50%.
[4] Technology 1 (Innovad Global; Essen, Belgium) inclusion rate of 0.30%.
[5] Technology 1+ (Innovad Global; Essen, Belgium) inclusion rate of 0.30%.
[6]Banded neutrophil concentration was 0 K/μL 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, high DON+SMB, high DON + Technology1, high DON + Technology1+, respectively. Detectable levels were 0.3 K/μL for treatment low DON and 0.2 K/μL for high DON+SMB.
[7]Metamyelocyte and myelocyte were non-detectable for all 79 samples. Nucleated erythrocytes per 100 WBC was 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, 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.

yeast extract also contains mannan oligosaccharides which have been linked not only with the enhancement of the intestinal integrity and the digestive and absorptive function of the gut in the post-weaning period in pigs but also with the improvement of the immune response and enhancement in disease resistance by promoting antigen presentation (Halas and Nochta, 2012; Duan et al., 2016).

Furthermore, Technology1+ contains an additional agent of SMB. In our study, when pigs were fed Technology1+, we observed heavier final body weights and improved gain

Table 5. Evaluation of mycotoxin control strategies on nursery pig serum criteria[1]

Item	Low DON[2]	High DON[2]	High DON + SMB[3]	High DON + Technology 1[4]	High DON + Technology 1+[5]	SEM	*P* =
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}	27.69^{a}	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 glutamyltransferase, U/L	60.06	70.44	59.56	71.88	57.63	5.538	0.213
Creatine kinase, U/L	2,077.3	2,559.2	1,881.4	2,766.8	3,338.1	472.89	0.168
Porcine circovirus type 2 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 6.5 ± 0.08 kg) were used with 54 pigs per replicate and 16 replications per treatment. A common starter pellet was initially used for approximately 7 days 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 manufactured with corn containing an average of 2.46 mg/kg DON and high DON diet with an average of 4.17 mg/kg DON.
[3] Sodium metabisulfite (SMB) inclusion rate of 0.50%.
[4] Technology 1 (Innovad Global; Essen, Belgium) inclusion rate of 0.30%.
[5] Technology 1+ (Innovad Global; Essen, Belgium) inclusion rate of 0.30%.
[6] Sorbitol 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, 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.
[7]TBARS = 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.

compared to pigs fed high DON or Technology1 diets. This suggests the improved performance observed with Technology1+ could be associated to the addition of SMB. Frobose et al. (2015) also observed heavier final body weights and improved gain when utilizing a feed additive containing SMB.

To further understand how mycotoxins, specifically DON, impact the pig, our study evaluated the effects on immunity, oxidative stress, and organ health. An indicator of immune system function is complete blood count including neutrophils, monocytes, lymphocytes, and eosinophils. Similar to our experiment, Pinton et al. (2008) did not observe any major effect on hematological parameters in pigs fed 2.2 to 2.5 mg/kg DON, in comparison to pigs fed control diets without any mycotoxins and the same was observed by Chaytor et al. (2011) when pigs were fed chronically with combinations of low levels of aflatoxin (0.06 to 0.18 mg/kg) and DON (0.3 to 0.9 mg/kg). Additionally, while not statistically significant, the differences in the neutrophil:lymphocyte ratio in our experiment suggest that other important metabolic processes may be influenced when Technology1 and 1+ are utilized in high DON diets. The neutrophil:lymphocyte ratio has been proposed as a sensitive biomarker of immune response in pigs, whereby under "physiologic" stress the number of neutrophils increases, while the number of lymphocytes decreases (Widowski et al., 1989; Sutherland et al., 2009). As previously mentioned, no statistically significant differences in complete blood counts were observed between treatments, but future research should further explore the effect of high DON and various control strategies on concentrations of circulating white blood cells.

Malondialdehyde was used as an indicator of lipid peroxidation (oxidative damage). We observed lipid peroxidation did not differ between low and high DON diets,

Table 6. Evaluation of mycotoxin control strategies on detection of mycotoxins and metabolites in blood of nursery pigs[1]

Item	Low DON[2]	High DON[2]	High DON + SMB[3]	High DON + Technology 1[4]	High DON + Technology 1+[5]	SEM	*P* =
DON							
Concentration, ng/mL	7.44[b]	10.00[a]	8.04[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, *n*	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	—	—
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	—	—

[1]A total of 4,318 pigs (initially 6.5 ± 0.08 kg) were used with 54 pigs per replicate and 16 replications per treatment. A common starter pellet was initially used for approximately 7 days 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. DBS cards were sent to University of Ghent, Belgium for mycotoxin analysis. Complete quantification (ng/mL) or mean peak area ± standard deviation of 36 mycotoxins and their phase I and phase II metabolites was simultaneously performed after extraction and LC–MS/MS analysis of spotted blood volume (approximately 60 µL) 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 B3from the High DON treatment.
[2]Low DON diet manufactured with corn containing an average of 2.46 mg/kg DON and high DON diet with an average of 4.17 mg/kg DON.
[3]Sodium metabisulfite (SMB) inclusion rate of 0.50%.
[4]Technology 1 (Innovad Global; Essen, Belgium) inclusionrate of 0.30%.
[5]Technology 1+ (Innovad Global; Essen, Belgium) inclusion rate of 0.30%.
[a,b,c,d] Means within a row with different superscripts differ ($P < 0.05$) using a Tukey multiple comparison adjustment.

and diets with mycotoxin mitigation strategies, as determined by a single (end point) measurement. Few studies have determined the ability of DON to cause oxidative stress, especially in pigs (Weaver et al., 2014).

Another important measure evaluated in this study was the effect of DON on organ health, specifically the enzymes gamma glutamyltransferase (GGT) and creatine kinase (CK). Although not statistically significant, pigs fed Technology1+ had numerically reduced GGT and higher levels of CK. Gamma glutamyltransferase plays a significant role in helping the liver metabolize drugs and other toxins (Harvey et al., 1989). Previous research has reported elevated serum GGT activity in diets contaminated with fumonisins (175 and 101 mg/kg) for 14 days (Motelin et al., 1994). Higher serum GGT levels have recently been independently associated with increased burden of subclinical inflammation across

several metabolic states in humans (Ali et al., 2016). Similarly, increased levels of CK have been associated with reduced inflammation in a general population in a large human cohort study (Bekkelund and Johnsen, 2018). Creatine kinase activity provides a temporal and spatial buffer of cell energetic homeostasis (Schlattner et al., 2006) via the provision of adenosine triphosphate (ATP) for the proper function of ATPases enzymes (e.g., Na^+, K^+, and H^+-ATPase pumps) (Saks, 2008). Baldissera et al. (2018) confirmed the involvement of reduced CK serum levels in the impairment of energetic homeostasis in pigs fed a diet co-contaminated with mycotoxins (0.3 mg/kg of aflatoxins and 8 mg/kg of fumonisins), which was also accompanied by reduced body weight. Similarly, Chaytor et al. (2011) reported numerically reduced CK activity in pigs under chronic exposure of DON- and aflatoxin-contaminated diets and, Holanda and Kim (2020) under chronic exposure of DON. While differences in GGT and CK were not observed in the current study, previous publications would indicate that these enzymes would be important measurements to investigate further.

Naturally, pigs have a wide range of normal levels of biological measures (Bollen et al., 2010). Although chloride, bicarbonate, and anion gap levels were statistically different between treatments, the values were not outside of the suggested reasonable range and were similar to values observed by Weaver et al. (2014). Other research have shown that consumption of DON or DON- and aflatoxin-contaminated diet did not significantly affect biochemical blood parameters whereas, the impact was more profound towards the global and the specific immune response of the pigs (Pinton et al., 2008; Chaytor et al., 2011). To the best of our knowledge, there is no research available reporting the effect of high DON, SMB, or other DON-detoxifying agents on bicarbonate and anion gap levels in pigs and warrants further investigation.

One of the limitations of the current study is blood measurements were only collected at a single point in time at the end of the experimental feeding period. Thus, these measures may not be a clear indicator of the metabolic changes that occurred over the span of this experiment. The impact of DON levels on immune criteria and oxidative stress warrants further research with measurements collected periodically throughout the study. Additionally, all treatment groups were continually exposed to levels of DON and other mycotoxins, so it is difficult to establish a true understanding of the effect of the presence of DON and other mycotoxins on these biological measurements compared to animals provided diets completely devoid of mycotoxins.

The combination of several mycotoxins in naturally contaminated feed can result in synergistic and negative additive effects (Alassane-Kpembi et al., 2015). Therefore, it is important to be able to analyze multiple mycotoxins with a highly sensitive method such as liquid chromatography–tandem mass spectrometry (LC–MS/MS), and preferably through a minimally invasive technique such as the dried blood spot (DBS) sampling method used in this study. The mycotoxin analysis via the DBS method offers several advantages such as ease of blood collection and the low blood volume needed, which improves its use even in small animals. Dried blood spot collection only requires one drop of blood placed on a Whatman 903 protein saver card. When utilizing DBS cards in our study as a practical means to sample blood, we observed reductions in the presence and concentration of DON, fumonisin B1 and B2, beta-zearalenone, and beauvericin when Technology1+ was added to high DON diets, indicating that other metabolic processes may be influenced when mycotoxin detoxifying feed additives including yeast cell wall components and plant extracts aiming to support liver function, are used. As indicated by Lauwers et al. (2019), DBS cards have the ability to detect several different mycotoxin biomarkers. The DBS cards were able to further detect multiple mycotoxin biomarkers including beta-zearalenol and beauvericin, compared to the mycotoxin analysis utilized for the feed samples. Beauvericin is an emerging *Fusarium* mycotoxin with large prevalence in feed, even more than DON in some cases (Khoshal et al., 2019). Moreover, beauvericin could potentially have an impact in livestock because it has been identified as a highly toxic compound, similar to DON, for swine intestinal cells (Khoshal et al., 2019).

In summary, results of this experiment indicate that pigs fed high DON diets had reduced growth performance compared to pigs fed low DON diets. These results also indicated that SMB inclusion in high DON diets provided a benefit in growth performance with ADG and G:F exceeding growth performance in the low DON diet. The immunometabolic changes observed in our study by pigs fed high DON + Technology1+ warrant further investigation.

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Conflict of interest statement

The authors declare no conflict of interest; however, Arnau Vidal and Christos Gougoulias are employees of Innovad NV/SA (Essen, Belgium) who contributed financial support and the test products for this project.

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