Kansas Agricultural Experiment Station Research Reports

Volume 1
Issue 7 *Swine Day*

Article 36

January 2015

Effects of Potential Detoxifying Agents on Growth Performance and Deoxynivalenol (DON) Urinary Balance Characteristics of Nursery Pigs Fed DON-Contaminated Wheat

H. L. Frobose
Kansas State University, Manhattan, frobose@k-state.edu

E. W. Stephenson
Kansas State University, Manhattan, estephe@k-state.edu

M. D. Tokach
Kansas State University, Manhattan, mtokach@k-state.edu

Effects of Potential Detoxifying Agents on Growth Performance and Deoxynivalenol (DON) Urinary Balance Characteristics of Nursery Pigs Fed DON-Contaminated Wheat

Funding Source

The authors wish to thank NutriQuest (Mason City, IA) for partial financial support.

Authors

H. L. Frobose, E. W. Stephenson, M. D. Tokach, J. M. DeRouchey, S. S. Dritz, R. D. Goodband, J. C. Woodworth, and R. E. Musser

K-STATE Research and Extension

SWINE DAY 2015

KANSAS STATE UNIVERSITY SWINE INDUSTRY DAY 2015

Effects of Potential Detoxifying Agents on Growth Performance and Deoxynivalenol (DON) Urinary Balance Characteristics of Nursery Pigs Fed DON-Contaminated Wheat

H. L. Frobose, E. W. Stephenson, M. D. Tokach, J. M. DeRouchey, S. S. Dritz[1], R. D. Goodband, J. C. Woodworth, and R. E. Musser[2]

Summary

Two experiments were conducted to evaluate the effects of detoxifying agents on the growth performance of nursery pigs fed diets contaminated with deoxynivalenol (DON). Naturally DON-contaminated wheat (6 ppm) replaced noncontaminated wheat in diets to achieve desired dietary DON concentrations. Basal ingredients were tested for mycotoxin and amino acid content prior to diet manufacturing. Diets were pelleted at 180˚F with a 45-s conditioning time.

A total of 238 barrows and gilts (PIC 327 × 1050; initially 29.6 ± 5.6 lb and 42 d of age) were used in a 21-d growth study. Pens of pigs were allotted by BW to 1 of 5 treatments in a completely randomized design with a 2 × 2 + 1 factorial arrangement. The 5 experimental diets included the following components, 1) positive control (PC; <0.5 mg/kg DON); 2) PC + 1.0% Product X (Nutriquest LLC, Mason City, IA); 3) negative control (NC; 4.0 mg/kg DON); 4) NC + 1.0% Product X; and 5) NC + 1.0% sodium metabisulfite (SMB; Samirian Chemicals, Campbell, CA). There were 6 or 7 replicate pens per treatment and 7 pigs per pen. Chemical analysis indicated a low level of fumonisin (<1 ppm) was present but that all DON concentrations matched calculated values. Analyzed DON concentrations were decreased by 92% when pelleted with SMB. Overall (d 0 to 21), a DON × Product X interaction was observed for ADG ($P < 0.05$) and ADFI ($P < 0.10$). Adding Product X to PC diets had no effect on ADG or ADFI; however, when added to NC diets, ADG, and ADFI became worse. As anticipated, DON reduced ($P < 0.001$) ADG, ADFI, and F/G by 24, 16, and 10%, respectively. Deoxynivalenol-associated reductions in ADG were most distinct (50%) during the initial period (0.42 vs. 0.84 lb from d 0 to 7). Adding SMB to NC diets im-

[1] Department of Diagnostic Medicine/Pathobiology, College of Veterinary Medicine, Kansas State University.

[2] The authors wish to thank NutriQuest (Mason City, IA) for partial financial support.

proved ($P < 0.01$) ADG, ADFI, and F/G compared to pigs fed the NC alone, and also improved ($P < 0.02$) ADG and F/G compared to pigs fed PC diets.

A concurrent urinary balance experiment was conducted using diets 3 to 5 from Exp. 1 to evaluate Product X and SMB on DON urinary metabolism. A 10-d adaptation was followed by a 7-d collection using 24 barrows in a randomized complete block design. Pigs fed NC + SMB diet had greater urinary output ($P < 0.05$) than pigs fed NC + Product X, with NC pigs intermediate. Daily DON excretion was lowest ($P < 0.05$) in the NC + SMB pigs. However, as a percentage of daily DON intake, NC + SMB fed pigs excreted more DON than they consumed (164%), greater ($P < 0.001$) than pigs fed the NC (59%) or NC + Product X (48%), and indicative of degradation of DON back to the parent DON molecule. Overall, Product X did not alleviate DON effects on growth nor did it reduce DON absorption and excretion. However, hydrothermally processing DON-contaminated diets with 1.0% SMB restored ADFI and improved F/G. Even so, the urinary balance experiment revealed that some of the converted DON-sulfonate could degrade back to DON under physiological conditions. While SMB appears promising to restore performance in pelleted DON-contaminated diets, additional research needs to address handling and long-term supplementation concerns and to evaluate the stability of the DON-sulfonate conversion.

Keywords: adsorbents, deoxynivalenol, mycotoxins, nursery pigs, sodium metabisulfite

Introduction

Cereal grains are the principal component in swine diets due to the efficiency of cost per calorie provided compared to other ingredients. Nevertheless, fungal infection often occurs in the field and during grain storage, and these fungi leave behind secondary metabolites known as mycotoxins, which can have adverse effects on livestock if ingested in sufficient quantities. The bioavailability of some of the major mycotoxins of concern (e.g. aflatoxins or zearalenone) can be reduced by including inexpensive adsorbent compounds, such as specific clays or hydrated sodium calcium aluminosilicates, in the diet. The inclusion of these compounds results in a reduction in mycotoxin uptake and decreased distribution to the blood and target organs (EFSA, 2009)[3].

However, according to a 3-year survey of global mycotoxin occurrence (Rodrigues and Naehrer, 2012[4]), the most prevalent (65% of finished feed) mycotoxin in North American feedstuffs is deoxynivalenol (DON), which is known for its feed suppression and immunomodulatory effects in pigs when present in diets at more than 1 ppm. Despite DON's prevalence and known effects, adsorbent compounds have proven largely ineffective against DON in both in vitro models and in vivo growth studies (Dänicke, 2000[5]). Although no DON-detoxifying agents have efficacy claims that are approved by the U.S. Food and Drug Administration, some products are reported to be of benefit in field studies. One such compound is Product X (Nutriquest LLC, Mason City, IA), a

[3] Burel, S. D., M. C. Favrot, J. M. Fremy, C. Massimi, P. Prigent, L. P. Debongnie, and D. Morgavi. Review of mycotoxin-detoxifying agents used as feed additives: mode of action, efficacy and feed/food safety. EFSA-Q-2009-00839, EFSA J. Dec. 8, 2009.

[4] Rodrigues, I. and K. Naehrer. 2012. A three-year survey on the worldwide occurrence of mycotoxins in feedstuffs and feed. Toxins. 4:663-675.

[5] Dänicke, S. 2002. Fusariums toxins in animal nutrition. Lohmann Information 27:29–37.

proprietary blend of adsorbent clays and preservatives. Because nursery pigs are known for their sensitivity to anti-nutritional factors such as mycotoxins, the aim of the experiment was to test the growth performance of nursery pigs fed a naturally DON-contaminated diet in the presence or absence of Product X, and to investigate the effects of DON absorption and excretion using a urinary balance model. Sodium metabisulfite (SMB; $Na_2S_2O_5$), a known biotransforming agent of DON that reacts with DON in the presence of heat and moisture to form a non-toxic DON-sulfonate adduct (DONS; Beyer et al., 2010[6]) and sulfur dioxide gas, was also incorporated into naturally contaminated diets to further evaluate SMB's potential for use in naturally DON-contaminated diets.

Procedures

The Kansas State University Institutional Animal Care and Use Committee approved the protocol used in this experiment. Sources of naturally DON-contaminated hard red winter (HRW) wheat and low-DON HRW wheat were acquired, and an initial 17-component mycotoxin screen was performed at North Dakota State University Veterinary Diagnostic Laboratory (NDSU) using a combination of mass spectrometry, ELISA, and HPLC methods. Based on the analyzed DON concentration, an equal amount of high-DON (6.03 ppm) or low-DON (0.05 ppm DON) wheat was incorporated into experimental diets to achieve desired DON concentrations. Wheat sources were hammermill ground to approximately 600μ, and each source was homogenously blended to minimize any variation in DON concentration between diets. Diets were formulated to meet or exceed NRC (2012) requirements and to be identical in nutrient composition apart from DON concentration and the inclusion of detoxifying agents (Table 1). Diets for the growth performance and urinary balance experiment were manufactured simultaneously at the Kansas State University O. H. Kruse Feed Mill. The 5 experimental diets were: 1) positive control (PC; <0.5 ppm DON), 2) PC + 1.0% Product X, 3) negative control (NC; 4.0 ppm DON), 4) NC + 1.0% Product X, and 5) NC + 1.0% SMB ($Na_2S_2O_5$; Samirian Chemicals, Campbell, CA). Two large batches using the low- or high-DON wheat were initially mixed to ensure consistency in DON concentrations. Each individual diet was then manufactured by subdividing the large batches and incorporating the appropriate detoxifying agent or sand at 1.0% of the final diet.

After mixing complete diets for 2 minutes in a double ribbon mixer, diets were pelleted (CPM Master Model 1000HD; Crawfordsville, IN) at a production rate of 1000 lb/h to maintain a minimum conditioner retention time and temperature of 45 seconds and 180°F, respectively. Diets were manufactured in numeric order to minimize carryover, with a flush between each diet. All personnel involved were required to wear respirators and safety goggles during the pelleting process as sodium metabisulfite releases sulfur dioxide gas in the presence of heat and moisture, which can irritate the eyes and respiratory tract. Samples of each diet were collected both pre- and post-pelleting. Diet samples were stored, frozen, and shipped along with basal ingredient samples to LABOCEA (Ploufragan, France) for a full mycotoxin screen (Table 2) and to Ward Laboratories (Kearney, NE) for chemical analysis (Table 3).

[6] Beyer, M., S. Danicke, D. Rohweder, H.-U. Humpf. 2010. Determination of deoxynivalenol-sulfonate (DONS) in cereals by hydrophilic interaction chromatography coupled to tandem mass spectrometry. Mycotox. Res. 26:109-117.

Growth experiment

A total of 238 barrows and gilts (PIC 327 × 1050; initially 29.6 ± 5.6 lb and 40d of age) were used in a 21-d growth study with 7 replicate pens per treatment and 7 pigs per pen; however, based on limited pen availability, 1 treatment (PC) had 6 replicate pens. Pigs were allotted to pens by initial weight at weaning, and when pigs reached approximately 30 lb, they were reweighed and pen average pig weight was balanced across 1 of 5 treatments in a completely randomized design with a 2 × 2 + 1 factorial arrangement. Deoxynivalenol and Product X inclusion served as main effects with an additional treatment including SMB. Each pen contained a 4-hole, dry self-feeder and a nipple waterer to provide ad libitum access to feed and water. Experimental diets were fed for 21 d with ADG, ADFI, and F/G determined by weighing pigs and measuring feed disappearance on d 7, 14, and 21 (Table 4).

Urinary balance experiment

A balance study was also conducted involving pigs individually housed in stainless-steel metabolism cages (4.9 × 2.0 ft) in an environmentally controlled building. Each cage was equipped with a feeder and a nipple drinker for ad libitum access to water. To determine the effects of Product X and SMB on DON urinary excretion and metabolism, only the 3 NC diets from the growth experiment were included. A total of 24 barrows were used for two replicate groups (12 pigs per group), with 4 pigs per dietary treatment in each group. Pigs were allotted to treatments in a randomized complete block design based on initial BW and location within the experimental room. Pigs were adapted to the diets and to an amount of feed taken up completely by all pigs (3.0 and 3.5 lb for groups 1 and 2, respectively) and to the metabolism cages during a 10-d period. Then, during a 7-d collection period, daily feed intake and urinary output were recorded quantitatively (Table 5). The mean initial BW at the start of the collection period was 93.8 ± 3.7 lb and 114.3 ± 7.8 lb for groups 1 and 2, respectively. Feed allocation was divided into two equal amounts and given twice daily at 0700 and 1500. Due to the low recovery of DON and its primary metabolite de-epoxy-DON (DOM-1) in feces (0.1 to 1.7% of DON intake) in similar studies (Danicke et al., 2007[7], Danicke et al., 2012[8]), fecal DON and fecal DOM-1 were not analyzed in the present experiment. The separation of feces from urine was achieved by using differently sized screens located beneath the slatted floor of the cage and connected to a funnel and urine collection bottle. Each pig's total daily urine output was frozen and then thawed and homogenously mixed at the end of the collection period. A representative aliquot sample was collected and then frozen before being sent for a full mycotoxin screen at LABOCEA (Ploufragan, France) using liquid chromatography-tandem mass spectrometry with a practical quantitation limit of 0.01 ppm.

DON-sulfonate quantification

The primary objective of DONS analysis was to confirm that the decreased analyzed DON in the pelleted NC + SMB diet was due to DON structural modification to

[7] Danicke, S., T. Goyarts, and H. Valenta. 2007. On the specific and unspecific effects of a polymeric glucomannan mycotoxin adsorbent on piglets when fed with uncontaminated or with Fusarium toxins contaminated diets. Arch. Anim. Nutr. 61(4): 266-275.

[8] Danicke, S., H. Valenta, and S. Kersten. 2012. Humic substances failed to prevent the systemic absorption of deoxynivalenol (DON) and its adverse effects on piglets. Mycotoxin Res. 28:253-260.

form DONS, as demonstrated in prior research. An automated electrospray ionization-tandem mass spectrometry (ESI-MS/MS) approach was used, and data acquisition and analysis were carried out as in Beyer et al. (2010). Unfractionated DONS extracts were introduced by continuous infusion into the ESI source on a triple quadrupole MS/MS (4000QTrap, Applied Biosystems, Foster City, CA). An aliquot of 75 µl of extract in methanol/water (3/1 vol/vol) was introduced using an autosampler (LC Mini PAL, CTC Analytics AG, Zwingen, Switzerland) fitted with the required injection loop for the acquisition time and presented to the ESI needle at 30 µl/min. A negative neutral loss scan of 80.9 was used to detect the DON-S molecular ion 377 [M-H]-. The ESI-MS/MS parameters used were: DP -80, EP -10, CE-36, CXP -15, electrospray capillary voltage -4500, collision gas pressure 2 (arbitrary units), interface heater on, source temperature (heated nebulizer) 572°F, curtain gas 20 and both ion source gases 45 (arbitrary units). Seventy-five continuum scans were averaged in multiple channel analyzer mode (MCA).

The background of each spectrum was subtracted, the data were smoothed, and peak areas were integrated using Applied Biosystems Analyst software. For both replicate groups of the urinary balance experiment, samples of each diet (n = 3) were analyzed in triplicate. Peak areas of DONS of the NC+SMB diet were compared to the peak areas of DONS in the NC diet and presented as a ratio.

Statistical analysis

Data collected from both experiments were analyzed using analysis of variance in the MIXED procedure of SAS, version 9.1 (SAS Institute Inc., Cary, NC). In the growth experiment, treatment effects were assessed within each experimental period using pen as the experimental unit. The fixed factors in the model were DON level and the presence or absence of Product X. The pre-planned contrasts in the growth experiment included: 1) interactions between DON and Product X, 2) DON vs. noncontaminated, and 3) the absence or presence of Product X in diets. Finally, two pairwise comparison contrasts were used to evaluate the effects of 1) adding SMB to DON-contaminated diets and 2) NC + SMB versus non-contaminated diets with no detoxifying agents present.

The urinary balance experiment was analyzed as a randomized complete block design with individual pig as the experimental unit. Data from the two replicates were combined and analyzed for replicate × treatment interactions. Due to a lack of significant interactions, the data were combined and analyzed with replicate and block included in the model as random effects. For all data analysis, when a significant overall treatment difference was found, differences among treatments were determined using the PDIFF statement in SAS. Overall significant differences were set at $P < 0.10$ and differences among treatments declared at $P < 0.05$, with tendencies reported when $0.05 \leq P \leq 0.10$.

Results and Discussion

Mycotoxin analyses of the high-DON and low-DON wheat at LABOCEA generally matched initial analyses from NDSU showing only minimal co-contamination from other mycotoxins. However, the ground corn used across all diets contained a low level of DON (0.57 ppm) and a high level of fumonisin B_1 (FUM; 8.01 ppm), which is above cautionary levels for swine. Interactive effects between DON and FUM are well

documented (Grenier et al., 2011)[9] and cannot be ruled out completely, but the low inclusion rate (4%) of FUM-contaminated corn in experimental diets makes the impact of any interactive effects likely minimal on experimental outcomes. The analyzed concentration of DON in final diets in general matched anticipated levels, with the NC + SMB diet the only exception (0.35 ppm). To reiterate, all 3 NC diets were initially prepared as a single, large batch to ensure consistent DON levels. That large batch was then split and the appropriate detoxifying agent or sand was incorporated prior to pelleting. The decrease in analyzed DON is likely attributed to the formation of 5-fold greater ($P < 0.01$) ratio of DONS present in the NC + SMB diet compared to the NC alone. DONS is a non-toxic product formed by the reaction between SMB and DON, which is amplified by hydrothermal environmental conditions (Danicke et al. 2005)[10], such as those present in the pelleting conditioner in this study. The presence of low levels of other toxins in experimental diets is most likely inconsequential, as concentrations were all well below cautionary limits for growing swine. Nutrient analyses for CP, Ca, P, and ash content were consistent across experimental diets. Adding 1.0% Product X increased Fe and Mn levels in the diet by approximately 15 and 60% compared to those without. Furthermore, the addition of 1.0% SMB increased dietary S and Na concentrations approximately 2-fold versus other diets.

Growth experiment

From d 0 to 7, a two-way interaction for ADFI was detected in which adding Product X worsened ADG and ADFI ($P < 0.05$) by a greater magnitude in DON-contaminated diets than PC diets. The presence of DON in diets decreased ADG by 52% ($P < 0.001$), driven by 24% lower ADFI ($P < 0.001$) and 56% poorer F/G ($P < 0.01$). However, the addition of SMB to the NC diet markedly improved ADG ($P < 0.001$) and tended to improve ADFI ($P < 0.10$) versus the NC alone. Nevertheless, from d 0 to 7, pigs fed the NC + SMB diet still tended to have decreased ADFI ($P < 0.10$) versus pigs fed the PC.

From d 7 to 14, no DON × Product X interactions were present. The previously observed worsening of F/G for NC-fed pigs was not present and DON's impact on ADFI was less marked than in the initial period. However, pigs fed NC diets still experienced the anorexic effects typically associated with DON, which reduced ADFI ($P < 0.01$) and decreased ADG ($P < 0.01$) relative to pigs fed the PC. Adding Product X to diets had no effect on ADG, ADFI or F/G, but the addition of SMB improved ADG ($P < 0.001$) by 20% compared to the NC, driven primarily by an improvement ($P < 0.001$) in F/G. Pigs fed the NC + SMB diet also exhibited 11% greater F/G ($P < 0.01$) than pigs fed PC diets during the second period.

From d 14 to 21, a tendency for a two-way interaction was detected ($P < 0.10$) for ADG in which Product X inclusion increased ADG in PC diets, but worsened ADG in NC diets. Average daily gain was decreased ($P < 0.001$) for pigs fed the NC, again driven by reduced ADFI ($P < 0.001$) but also by poorer F/G ($P < 0.05$). Product X

[9] Grenier, B., A. P. Loureiro-Bracarense, J. Lucioli, G. D. Pacheco, A. M. Cossalter, W. D. Moll, G. Schatzmayr, and I. P. Oswald. 2011. Individual and combined effects of subclinical doses of deoxynivalenol and fumonisins in piglets. Mol. Nutr. Food Res. 55:761-771.

[10] Dänicke, S., H. Valenta, M. Gareis, H. W. Lucht, and H. von Reichenbach. 2005. On the effects of a hydrothermal treatment of deoxynivalenol (DON)-contaminated wheat in the presence of sodium metabisulfite ($Na_2S_2O_5$) on DON reduction and piglet performance. Anim. Feed Sci. Tech. 118:93–108.

addition tended to worsen F/G ($P < 0.10$), while ADG and ADFI were not affected. SMB supplementation in NC diets improved ADG, ADFI, and F/G ($P < 0.001$) by the greatest magnitude during the third period. Pigs fed the NC + SMB also had increased ADG ($P < 0.05$) compared to pigs fed the PC, driven by an 11% improvement in F/G ($P < 0.01$).

Overall, a two-way interaction was observed for ADG and final BW in which Product X supplementation worsened ADG and final BW ($P < 0.05$) and tended to worsen ADFI ($P < 0.10$) in NC diets but did not affect performance in PC diets. Feeding 4 ppm DON in NC diets decreased ADG (24%; $P < 0.001$) and final BW ($P < 0.001$) during the experimental period, reducing ADFI by 16% and worsening F/G ($P < 0.001$) by 10%. Supplementing 1.0% SMB in the NC diet improved ADG, ADFI, and F/G ($P < 0.01$) over NC alone by 35, 10, and 19%, respectively, resulting in a 12% improvement ($P < 0.001$) in final BW. Unexpectedly, ADG and final BW of pigs fed the NC + SMB diet surpassed even those of pigs fed the noncontaminated PC diet ($P < 0.05$), primarily driven by an 11% improvement in F/G ($P < 0.001$).

These results reiterate the extent to which high-DON diets can negatively impact nursery pig growth performance. The present data agree with findings of Etienne and Waché (2008),[11] who cited a 4.6% decrease in ADFI for every 1 ppm of DON in the diet, and Frobose et al. (2015)[12], who described the feed intake suppression pattern as being the most marked during the initial exposure period and lessening over time. These anorexic effects of DON are most frequently attributed to changes in: metabolism and concentration of brain transmitters such as serotonin in cerebrospinal fluid (Prelusky and Trenholm, 1993)[13], inhibition of small-intestinal motility (Rotter et al., 1996)[14], and development of conditioned taste aversion to DON (Ossenkopp et al., 1994)[15]. The effects are more severe in pigs than other species due to a more rapid absorption and tissue distribution of DON (15 to 30 m) coupled with delayed clearance of DON from cerebrospinal fluid (Prelusky et al., 1990)[16]. Previous reports of the impact of DON on F/G have been more variable (Rotter et al., 1996), but a series of four growth experiments (Frobose et al., 2015) consistently observed depressed F/G during the initial period, which is consistent with the reduction in F/G seen from d 0 to 7 in the growth experiment. This may be associated with wasted feed from pigs sorting feed due to taste aversion and may also be connected with immune system stimulation during the initial

[11] Etienne, M., and Y. Waché. 2008. Biological and physical effects of deoxynivalenol (DON) in the pig. Pages 113–130 in Mycotoxins in Farm Animals. I. Oswald and I. Taranu, ed. Transworld Research Network, Kerala, India.

[12] Frobose, H. L., E. D. Fruge, M. D. Tokach, E. L. Hansen, J. M. DeRouchey, S. S. Dritz, R. D. Goodband, and J. L. Nelssen. 2015. The effects of deoxynivalenol-contaminated corn dried distillers grains with solubles in nursery pig diets and potential for mitigation by commercially available feed additives. J. Anim. Sci. 93:1074-1088.

[13] Prelusky, D. B., and H. L. Trenholm. 1993. The efficacy of various classes of anti-emetics in preventing deoxynivalenol-induced vomiting in swine. Natural Toxins. 1:296-302.

[14] Rotter, B. A., D. B. Prelusky, and J. J. Pestka. 1996. Toxicology of deoxynivalenol (vomitoxin). J. Toxicol. Environ. Health. 48:1-34.

[15] Ossenkopp, K. P., M. Hirst, and W. A. Rapley. 1994. Deoxynivalenol (vomitoxin)-induced conditioned taste aversion in rats are mediated by the chemosensitive area postrema. Pharmacol. Biochem. Behav. 47:363-367.

[16] Prelusky, D. B., K. E. Hartin, and H. L. Trenholm. 1990. Distribution of deoxynivalenol in cerebral spinal fluid following administration to swine and sheep. J. Environ. Sci. Health. B25:395-413.

exposure, which could increase maintenance requirements. After this initial decrease, F/G of pigs fed DON-contaminated diets was generally similar to those fed the PC diet.

In this study, the addition of Product X at 1.0% in DON-contaminated diets did not alleviate DON's negative effects on nursery pig growth. While Product X did not affect growth when added to the PC diet, intriguingly, when Product X was added to high-DON diets, ADG was suppressed by an additional 11%, mainly driven by 9% lower ADFI. Although the negative DON × Product X interaction was unexpected, adsorbent clays have shown little efficacy in previous research on DON and some adsorbing agents have been reported to be nonselective in that they may affect the utilization of essential nutrients, such as vitamins and minerals (EFSA, 2009).

Pelleting NC diets with 1.0% SMB restored the DON-associated reduction in ADFI, which agrees with previous research (Frobose et al., 2011)[17] and is most likely associated with the greater than ten-fold reduction in analyzed DON levels and the probable conversion to the nontoxic DONS. However, pelleting NC diets with SMB also resulted in consistent improvement in F/G throughout the duration of the experiment versus not only the NC (18%) but also compared to pigs fed the noncontaminated PC diet (11%), which suggests that the benefit may be independent of the DON-contamination of the diet. While the biological mechanism remains unclear, the efficiency benefit is consistent with Danicke et al. (2005) and implies that the hydrothermal treatment of SMB improved the nutrient availability for the animal.

Despite SMB being generally recognized as safe (GRAS status) by the FDA, the release of sulfur dioxide when pelleting diets containing SMB is an additional concern for feed mill employees. It can be irritating to the eyes and respiratory tract and may require the use of protective equipment. Moreover, the addition of 1.0% SMB increased the S and Na concentrations in the diet, and based on results of Til et al. (1972)[18], SMB destroys thiamine and long-term supplementation may result in reductions in growth performance and potentially sulfur-induced thiamine deficiency. As a result, additional research is necessary to determine the minimum SMB level necessary and acceptable feeding duration to minimize feed processing and thiamine deficiency concerns.

Urinary balance experiment

The experimental diets used in the urinary balance experiment were sampled daily within each replicate, and a subsample of each was sent for mycotoxin analysis at LABOCEA. Analyzed DON concentrations were generally similar to those used in the growth study. Daily feed intake was set by the amount of feed consumed daily by NC-fed pigs during the 10-d adaptation period, and no differences in feed disappearance were observed between treatments during the collection period. Pigs fed the NC + SMB diet had the greatest urine output during the collection period, being significantly greater ($P < 0.05$) than pigs fed NC + Product X, with NC pigs intermediate. This additional urinary excretion is likely due to increased water intake due to the elevated dietary Na level when 1.0% SMB was incorporated into the diet.

[17] Frobose, H. L. Swine Day 2011. Report of Progress 1056, pp. 105-113.
[18] Til, H. P., V. J. Feron, A. P. de Groot, and P. van der Wal. 1972. The toxicity of sulphite. II. Short- and long-term feeding studies in pigs. Food Cosmet. Toxicol. 10:463–473.

As calculated from analyzed DON levels, pigs fed NC and NC + Product X treatments consumed a greater amount of DON per d ($P < 0.001$) than pigs fed the NC + SMB diet, since DON conversion to DONS occurred during feed manufacturing when SMB was added before pelleting. The DONS analysis confirmed that DON to DONS conversion was over 5-fold greater ($P < 0.01$) when 1.0% SMB was added to NC diets prior to pelleting versus the NC alone and NC + Product X. Although DONS is known to lack the emetic activity of DON (Young et al., 1987), interestingly, the addition of SMB to NC diets did not reduce the incidence of vomiting. In fact, NC + SMB pigs vomited on 10 occasions as compared to 7 and 3 for the NC and NC + Product X treatments, respectively (data not shown). Still, the daily DON urinary excretion was reduced ($P < 0.001$) for NC + SMB fed pigs versus the NC and NC + Product X, and the excretion of the primary metabolite DOM-1 was also less ($P < 0.05$) in the NC + SMB pigs. However, when expressed as a percentage of daily DON intake, pigs fed the NC + SMB diet excreted more DON than they consumed (164%), which was greater ($P < 0.001$) than pigs fed NC (59%) or the NC + Product X (48%) diet.

For pigs fed the NC + SMB treatment, DON recovery greater than 100% appears to indicate that some of the DONS was degraded to the parent DON. Recent work by Schwartz et al. (2013)[19] revealed that 3 structurally unique forms of DONS can be formed by the reaction of DON with SMB, dependent on the sulfiting agent and processing conditions present. While DONS-1 and DONS-2 are stable across a broad pH range, DONS-3 can decompose to DON at alkaline pH, such as those in the proximal small intestine. Schwartz-Zimmerman et al. (2014)[20] compared sulfiting agents in a follow-up study and found the predominant form produced by the reaction between DON and SMB to be DONS-3. If the sulfonate formation profile was similar in the present study, this would explain the degradation of a portion of DONS-3 back into DON, which would then be detected as additional DON in the urine. Since the gross DON urine recovery remained only 15% of the DON consumed by pigs fed the NC or NC + Product X diets, the physiological impact from the degradation of DONS back to DON in the digestive tract was likely minimal in this study. Nevertheless, the degradation pattern is important to consider for future research to potentially enhance the efficacy of the reaction with SMB and lower the dietary concentration of SMB needed to alleviate the effects of DON.

The recovery of DON from pigs fed the NC and NC + Product X matches urinary DON recovery rates in previous work. Since urine is the main DON absorption and excretion route, if Product X was able to decrease the uptake of DON, urinary DON excretion would also be decreased. However, in the present study, DON recovery was similar between pigs fed NC or NC + Product X diets, and the lack of a Product X response in urinary metabolism is congruent with the lack of the growth benefit to Product X. Since pigs have limited ability to de-epoxidate DON other than via microbial fermentation in the hindgut, recovery of urinary DOM-1 was minimal (0.2 to 0.9% of DON intake) but consistent with previous work (0 to 1.1%).

[19] Schwartz, H. E., C. Hametner, V. Slavik, O. Greitbauer, G. Bichl, E. Kunz-Vekiru, D. Schatzmayr, and F. Berthiller. 2013. Characterization of three deoxynivalenol sulfonates formed by reaction of deoxynivalenol with sulfur reagents. J. Ag. Food Chem. dx.doi.org/10.1021/jf403438b.

[20] Schwartz-Zimmerman, H.E., G. Weisenberger, C. Unbekannt, S. Hessenberger, D. Schatzmayr, and F. Berthiller. 2014. Reaction of (conjugated) deoxynivalenol with sulphur reagents – novel metabolites, toxicity and application. World Mycotoxin Journal. 7(2):187-197.

In summary, feeding diets contaminated with 4 ppm DON to nursery pigs reduces their growth most severely during the initial exposure period and is primarily associated with feed intake suppression. The addition of Product X neither alleviated the DON-associated effects on pig growth nor reduced DON absorption and urinary excretion compared to pigs fed DON-contaminated diets alone. However, hydrothermally treating DON-contaminated diets with 1.0% SMB restored feed intake and improved F/G markedly. Even so, the urinary balance experiment revealed that a portion of the converted DONS can be degraded back to DON under physiological conditions. Questions remain surrounding processing methods and long-term supplementation effects of SMB, but this research demonstrates that pelleting DON-contaminated diets with SMB can alleviate DON effects on growth. Additional research is also needed to evaluate the effect of sodium metabisulfite on F/G in noncontaminated diets.

Table 1. Composition of experimental diets, Exp. 1 (as-fed basis)

Item	Positive control (PC)	PC + 1.0% Product X[1]	Negative control (NC)	NC + 1.0% Product X[1]	NC + 1.0% SMB[2]
Ingredient, %					
Noncontaminated hard red winter wheat	67.00	67.00	---	---	---
Deoxynivalenol-contaminated wheat, 6 ppm[3]	---	---	67.00	67.00	67.00
Soybean meal, 46.5% CP	24.16	24.16	24.16	24.16	24.16
Corn	4.23	4.23	4.23	4.23	4.23
Limestone	1.40	1.40	1.40	1.40	1.40
Monocalcium phosphate, 21% P	0.60	0.60	0.60	0.60	0.60
Salt	0.35	0.35	0.35	0.35	0.35
L-Lysine-HCl	0.50	0.50	0.50	0.50	0.50
DL-methionine	0.15	0.15	0.15	0.15	0.15
L-threonine	0.20	0.20	0.20	0.20	0.20
L-valine	0.00	0.00	0.00	0.00	0.00
Vitamin premix[4]	0.25	0.25	0.25	0.25	0.25
Trace mineral premix[5]	0.15	0.15	0.15	0.15	0.15
Phytase[6]	0.02	0.02	0.02	0.02	0.02
Product X[1]	---	1.00	---	1.00	---
Sodium metabisulfite[2]	---	---	---	---	1.00
Sand	1.00	---	1.00	---	---
Total	100.00	100.00	100.00	100.00	100.00

continued

Table 1. Composition of experimental diets, Exp. 1 (as-fed basis)

Item	Positive control (PC)	PC + 1.0% Product X[1]	Negative control (NC)	NC + 1.0% Product X[1]	NC + 1.0% SMB[2]
Calculated analysis					
SID[7] amino acids, %					
Lys	1.28	1.28	1.28	1.28	1.28
Ile:lys	59	59	59	59	59
Leu:lys	103	103	103	103	103
Met:lys	33.4	33.4	33.4	33.4	33.4
Met & cys:lys	57.6	57.6	57.6	57.6	57.6
Thr:lys	62.7	62.7	62.7	62.7	62.7
Trp:lys	18.4	18.4	18.4	18.4	18.4
Val:lys	63.9	63.9	63.9	63.9	63.9
Total Lys, %	1.41	1.41	1.41	1.41	1.41
ME, kcal/lb	1,420	1,420	1,420	1,420	1,420
SID Lys:ME, g/Mcal	4.09	4.09	4.09	4.09	4.09
CP, %	20.8	20.8	20.8	20.8	20.8
Ca, %	0.72	0.72	0.72	0.72	0.72
P, %	0.61	0.61	0.61	0.61	0.61
Available P, %	0.42	0.42	0.42	0.42	0.42

[1] A proprietary combination of adsorbent clays and preservatives (Nutriquest LLC, Mason City, IA).
[2] Sodium metabisulfite ($Na_2S_2O_5$; Samirian Chemicals, Campbell, CA).
[3] Basal ingredient sample sent to the North Dakota State University Veterinary Diagnostic Laboratory (Fargo, ND) for a full 17-component toxin screen. Samples were analyzed using a variety of mass spectrometry, ELISA, and HPLC methods with a practical quantitation limit of 0.5 ppm.
[4] Provided per lb of premix: 2,000,000 IU of vitamin A; 250,000 IU of vitamin D_3; 8,000 IU of vitamin E; 800 mg of vitamin K; 7 mg of vitamin B_{12}; 9,000 mg of niacin; 5,000 mg of pantothenic acid; and 1,500 mg of riboflavin.
[5] Provided per lb of premix: 12 g Mn from manganese oxide, 50 g Fe from iron sulfate, 50 g Zn from zinc sulfate, 5 g Cu from copper sulfate, 90 mg I from calcium iodate, and 90 mg Se from sodium selenite.
[6] HiPhos 2700 (DSM Nutritional Products LLC, Parsippany, NJ, USA) contains 1,228,503 phytase units/lb premix.
[7] Standardized ileal digestible.

Table 2. Mycotoxin analysis of basal ingredients and experimental diets, Exp. 1 (as-fed basis)[1]

	Basal ingredients			Experimental diets[2]				
Item	Ground corn	High DON HRW wheat[3]	Low DON HRW wheat	Positive control (PC)	PC + 1.0% Product X[4]	Negative control (NC)	NC + 1.0% Product X[4]	NC + 1.0% SMB[5]
Mycotoxin, ppm								
Deoxynivalenol (DON)	0.57	5.70	0.05	0.04	0.06	4.10	4.23	0.35
De-epoxy-DON	---	0.02	---	---	---	0.02	0.02	---
15-Acetyl DON	0.05	0.17	---	---	---	0.11	0.13	0.04
3-Acetyl DON	0.01	0.06	---	---	---	0.03	0.03	----
Zearalenone	0.10	0.02	---	---	---	0.01	0.03	0.03
Fumonisin B_1	8.01	0.27	0.38	0.93	0.59	0.63	0.70	0.67
Fumonisin B_2	1.05	0.09	0.13	0.28	0.15	0.15	0.17	0.20
Fumonisin B_3	0.66	0.03	0.05	0.31	0.16	0.23	0.20	0.18
Monoliformine	0.26	---	---	---	---	---	---	---
Ergot alkaloids[7]	---	0.20	---	---	---	0.16	0.15	0.13

[1] Basal ingredient and experimental diet samples were sent to LABOCEA (Ploufragan, France) for a 40-component toxin screen. Samples were analyzed using liquid chromatography-tandem mass spectrometry with a practical quantitation limit of 0.01 ppm.

[2] Positive control diets formulated to contain <0.5 ppm DON, and all remaining diets formulated to contain 4.0 ppm DON. All diets were pelleted at 185˚F with a minimum conditioner retention time of 45 sec.

[3] Hard red winter (HRW) wheat analyzed for deoxynivalenol (DON) concentration (6.0 ppm) prior to diet formulation.

[4] A proprietary blend of absorbent clays and preservatives (Nutriquest LLC, Mason City, IA).

[5] Sodium metabisulfite ($Na_2S_2O_5$; Samirian Chemicals, Campbell, CA).

[6] A sample was collected after dietary ingredients were mixed into the batch, but prior to the conditioning and pelleting process.

[7] Reported as the sum of the ergot alkaloid compounds ergocornin, ergocristin, ergocryptin, ergometrin, ergosin, and ergotamine.

Table 3. Chemical analysis of diets, as-fed basis[1]

Item	Positive control (PC)	PC + 1.0% Product X[2]	Negative control (NC)	NC + 1.0% Product X[2]	NC + 1.0% SMB[3]
DM, %	89.59	89.23	89.55	89.71	89.16
CP, %	22.5	22.4	22.0	22.4	22.2
Ca, %	0.80	0.80	0.82	0.77	0.76
P, %	0.54	0.51	0.55	0.57	0.58
S, %	0.23	0.24	0.24	0.24	0.46
Na, %	0.12	0.14	0.12	0.15	0.32
K, %	0.85	0.83	0.85	0.92	0.92
Mg, %	0.17	0.17	0.17	0.19	0.18
Zn, ppm	105.7	91.6	126.5	107.0	109.2
Fe, ppm	282.0	320.0	270.0	314.0	233.0
Mn, ppm	63.0	106.0	65.0	102.0	68.0
Cu, ppm	19.6	21.9	20.2	18.1	22.5
Ash, %	5.3	5.0	5.3	5.2	5.1

[1] Dietary samples were collected post-pelleting and sent for chemical analysis at Ward Laboratories (Kearney, NE).
[2] A proprietary blend of absorbent clays and preservatives (Nutriquest LLC, Mason City, IA).
[3] Sodium metabisulfite ($Na_2S_2O_5$; Samirian Chemicals, Campbell, CA).

Table 4. Effects of detoxifying agents on growth performance of nursery pigs fed deoxynivalenol (DON)-contaminated wheat[1]

	Positive control (PC)[2]		Negative control (NC; 4.0 ppm DON)[2]				Probability, $P <$[3]				
Item	PC	1.0% Product X[4]	NC	1.0% Product X[4]	1.0% SMB[5]	SEM	DON × Product X	DON	Product X	SMB vs. PC	SMB vs. NC
d 0 to 7											
ADG, lb	0.84	0.83	0.51	0.33	0.89	0.038	0.024	0.001	0.012	0.324	0.001
ADFI, lb	1.42	1.42	1.18	0.97	1.30	0.045	0.022	0.001	0.019	0.054	0.055
F/G	1.70	1.72	2.33	3.67	1.47	0.464	0.144	0.006	0.132	0.712	0.165
d 7 to 14											
ADG, lb	1.18	1.16	1.06	1.00	1.27	0.044	0.596	0.003	0.326	0.119	0.001
ADFI, lb	1.83	1.85	1.68	1.56	1.80	0.071	0.320	0.003	0.414	0.696	0.221
F/G	1.55	1.60	1.59	1.55	1.41	0.037	0.275	0.941	0.847	0.009	0.001
d 14 to 21											
ADG, lb	1.28	1.39	1.10	1.07	1.43	0.044	0.091	0.001	0.364	0.023	0.001
ADFI, lb	2.03	2.06	1.79	1.70	2.01	0.043	0.144	0.001	0.511	0.738	0.001
F/G	1.59	1.49	1.63	1.60	1.41	0.404	0.443	0.056	0.089	0.003	0.001
d 0 to 21											
ADG, lb	1.10	1.12	0.89	0.80	1.20	0.029	0.045	0.001	0.257	0.020	0.001
ADFI, lb	1.76	1.77	1.55	1.41	1.70	0.040	0.056	0.001	0.113	0.291	0.007
F/G	1.60	1.58	1.74	1.77	1.42	0.034	0.432	0.001	0.942	0.001	0.001
Pig BW, lb											
d 0	29.6	29.6	29.6	29.6	29.6	0.31	0.999	0.966	0.968	0.999	0.976
d 7	35.5	35.4	33.2	31.9	35.8	0.33	0.066	0.001	0.043	0.429	0.001
d 14	43.7	44.4	41.0	38.9	44.7	0.59	0.020	0.001	0.256	0.213	0.001
d 21	52.6	53.4	48.7	46.4	54.7	0.66	0.022	0.001	0.237	0.027	0.001

[1] A total of 238 barrows and gilts (PIC 327 × 1050; initially 29.6 ± 5.6 lb and 42 d of age) were used in a 21-d experiment with 6 or 7 replicate pens per treatment and 7 pigs per pen. All diets were fed in pelleted form.

[2] Positive control (PC) and negative control (NC) diets formulated to contain <0.5 ppm and 4.0 ppm DON, respectively.

[3] Each contrast compared the following treatments: 1) "DON × Product X" evaluated the two-way interaction between DON and adding 1.0% Product X; 2) "DON" compared PC to NC, excluding only the sodium metabisulfite (SMB) treatment; 3) "Product X" compared diets with Product X (2 and 4) to diets without (Diets 1 and 3); and 4) "SMB vs. PC" and "SMB vs. NC" compared the NC diet with 1.0% SMB to pigs fed the NC or PC diets without detoxifying agents, respectively.

[4] A proprietary blend of absorbent clays and preservatives (Nutriquest LLC, Mason City, IA).

[5] Sodium metabisulfite ($Na_2S_2O_5$; Samirian Chemicals, Campbell, CA).

Table 5. Urinary excretion of deoxynivalenol (DON) and its metabolite de-epoxy-DON (DOM-1) of piglets fed DON-contaminated diets with or without detoxifying agents[1]

	Negative Control (4.0 ppm DON)				
Item Detoxifying agent:	None	1.0% Product X[2]	1.0% SMB[3]	SEM	Probability $P <$
Analyzed DON, ppm[4]	4.28	4.63	0.22		
Analyzed DON-S, ppm[5]	1.00	0.73	5.67	1.318	0.004
ADFI, lb	3.22	3.22	3.25	0.10	0.970
Urine output, L	20.5^{ab}	18.2^{a}	26.3^{b}	2.16	0.043
DON consumption, mg/d	6.21^{b}	6.79^{b}	0.32^{a}	0.223	0.001
Excretion in urine, mg/d					
DON	3.65^{b}	3.29^{b}	0.52^{a}	0.164	0.001
DOM-1	0.54^{b}	0.39^{ab}	0.18^{a}	0.103	0.068
Excretion in urine [% of DON intake]					
DON	58.7^{a}	48.2^{a}	164.4^{b}	6.80	0.001
DOM-1	0.24^{a}	0.21^{a}	0.87^{b}	0.037	0.001

[1] A total of 24 barrows (PIC 327 × 1050; 93.8 ± 3.7 lb and 114.3 ± 7.8 lb at the onset of the collection period for replicate 1 and 2, respectively) over two replicate groups (n=12) were used in a 17-d experiment with 8 pigs per treatment. The collection period (d 11 to 17) is shown above. All diets were fed in pelleted form.

[2] A proprietary blend of absorbent clays and preservatives (Nutriquest LLC, Mason City, IA).

[3] Sodium metabisulfite ($Na_2S_2O_5$; Samirian Chemicals, Campbell, CA).

[4] Analyzed at LABOCEA (Ploufragan, France) using liquid chromatography-tandem mass spectrometry with a practical quantitation limit of 0.01 ppm. The average of two replicate groups is reported.

[5] Analyzed at the Kansas State University Lipidomics Laboratory using liquid chromatography-tandem mass spectrometry. Peak areas of DONS of the NC + SMB diet were compared to the peak areas of DONS in NC diet and presented as a ratio.

[a,b] Means without a common superscript differ $P < 0.05$.