# The effects of deoxynivalenol-contaminated corn dried distillers grains with solubles in nursery pig diets and potential for mitigation by commercially available feed additives<sup>1,2</sup>

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**ABSTRACT:** Four experiments were conducted to investigate the effects of deoxynivalenol (DON) from naturally contaminated dried distillers grains with solubles (DDGS) and the efficacy of feed additives in nursery pig diets. In Exp. 1, 180 pigs  $(10.3 \pm 0.2 \text{ kg})$ BW) were fed 1 of 5 diets for 21 d. Diets were 1) Positive Control (PC; < 0.5 mg/kg DON), 2) Negative Control (NC; 4 mg/kg DON), 3) NC + 0.10% Biofix (Biomin Inc., Herzogenburg, Austria), 4) NC + 0.15% Cel-can (VAST Inc., Mason City, IA) and 0.50% bentonite clay, and 5) NC + 0.25% Defusion Plus (Cargill Animal Nutrition, Minneapolis, MN). Pigs fed the NC diet had poorer (P < 0.01) ADG than those fed the PC. Pigs fed Defusion Plus had improved (P < 0.03) ADG over those fed NC, whereas pigs fed Biofix or Cel-can with bentonite clay had reduced ADG (P <0.01) compared with those fed PC. In Exp. 2, 340 pigs  $(11.7 \pm 0.1 \text{ kg BW})$  were fed 1 of 8 diets for 21 d. Diets were 1) PC (< 0.5 mg/kg DON), 2) Low NC (1.5 mg/ kg DON), 3) Low NC + 0.15% Biofix, 4) Low NC + 0.30% Biofix, 5) High NC (3.0 mg/kg DON), 6) High NC + 0.30% Biofix, 7) High NC + 0.45% Biofix, and 8) Diet 7 with 5% added water. Increasing the DON level reduced (linear; P < 0.05) ADG, ADFI, and pig BW, and Biofix did not improve performance. In Exp. 3, 1,008 pigs ( $12.5 \pm 0.3$  kg BW) were fed 6 treatments for 24 d. Diets were 1) PC (< 0.5 mg/kg DON), 2) NC (3 mg/kg DON), 3) NC + 0.25% Defusion, 4) NC + 0.50% Defusion, 5) Diet 3 with supplemental nutrients, and 6) Diet 5, pelleted. Pigs fed the NC had decreased (P < 0.01) ADG and ADFI, but adding Defusion improved (linear; P < 0.04) ADG and ADFI over pigs fed NC. Pelleting improved (P < 0.01) both ADG and G:F, resulting in ADG above PC pigs. In Exp. 4, 980 pigs  $(12.0 \pm 0.3 \text{ kg BW})$  were fed 1 of 7 diets in a 28-d trial in a  $2 \times 3 + 1$  factorial arrangement. The 7 treatments were based on 3 diets fed in meal or pellet form: 1) PC (< 0.5 mg/kg DON), 2) NC (3 mg/kg DON), and 3) NC + 0.25% Defusion. Treatment 7 was Diet 3 with supplemental nutrients in pellet form. No interactions were observed between pelleting and Defusion. Pigs fed the NC had decreased (P < 0.01) ADG and ADFI, and pelleting improved (P < 0.01) ADG to PC levels, driven by improved (P < 0.01) G:F. Adding nutrients or Defusion had no effect. Overall, these studies show that Defusion and pelleting can help overcome some of the negative effects of DON, whereas other feed additives and additional nutrients do not.

Key words: deoxynivalenol, detoxifying agents, nursery pigs, pelleting, swine, vomitoxin

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## **INTRODUCTION**

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Deoxynivalenol (**DON**), colloquially known as vomitoxin, is an important mycotoxin in pigs because it occurs frequently and at levels of toxicological relevance in cereal grains. Pigs are the most susceptible livestock species, reacting primarily with decreased feed intake, immune suppression, and, at high concentrations,

<sup>&</sup>lt;sup>2</sup>Appreciation is expressed to Hubbard Feeds Inc. (Mankato, MN) for supplying the deoxynivalenol-contaminated DDGS and New Fashion Pork (Jackson, MN) for providing animals and research facilities.

emesis and complete feed refusal (Forsyth et al., 1977; Rotter et al., 1996a; Eriksen and Pettersson, 2004).

Although the traditional method of diluting DON concentrations during diet formulation is effective (Patterson and Young, 1993), the frequently regional distribution of DON-affected areas may rule out dilution as a viable option; consequently, swine producers often must utilize DON-contaminated grains that elicit negative effects on growth, characterized as > 1 mg/kgin growing pigs (Dänicke et al., 2001). Corn dried distillers grains with solubles (DDGS) also present significant problems because the DON level is generally 3 times greater than the concentration in the original corn source. Several types of detoxification have been proposed to mitigate DON effects. These strategies are typically categorized as technical treatments in which contaminated feed is manipulated chemically, physically, or biologically before feeding or in situ treatments in which adsorbents, probiotics, or enzymes are used to limit DON effects during digestion. Dänicke (2002) summarized that to date, both strategies have proven largely ineffective against DON in both in vitro models (Avantaggiato et al., 2007; Sabater-Vilar et al., 2007) and in vivo growth studies (Friend et al., 1984; Dänicke et al., 2004; Döll et al., 2005).

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. Our experiments were designed to evaluate the effectiveness of 3 commercially available feed additives and pelleting on nursery pig performance when diets contain DON from a naturally contaminated source of DDGS.

## MATERIALS AND METHODS

All experimental procedures and animal care were approved by the Kansas State Institutional Animal Care and Use Committee. All diets were corn-soybean-meal-based, and both a DON-free and naturally contaminated source of corn DDGS were provided by Hubbard Feeds (Mankato, MN). The DDGS were analyzed for mycotoxin concentrations, and the analyzed values were used during diet formulation to incorporate DON into the test diets at desired concentrations. Diets were formulated to meet or exceed all nutrient requirement estimates (NRC, 1998).

In Exp. 1 to 4, several commercial feed additives were tested. Biofix (Biomin Inc., Herzogenburg, Austria) contains both a constituent of yeast cell walls designed to adsorb specific areas of the DON molecule and enzymes purported to degrade the DON molecule. These ingredients have shown effectiveness against aflatoxin and some tricothecenes (Yiannikouris et al., 2004), but previous results against DON have been unsatisfactory. Cel-can (VAST Inc., Mason City, IA) is a mixture of yeast components that supplies fermentation metabolites in combination with bentonite clay aimed at binding and adsorbing mycotoxins. Defusion Plus (Cargill Animal Nutrition, Minneapolis, MN) is a combination of preservatives, antioxidants, amino acids, and direct-fed microbials that is thought to mitigate some of the toxic effects of DON. Defusion (Cargill Animal Nutrition, Minneapolis, MN) was tested in Exp. 3 and 4 and contains a similar ingredient profile to Defusion Plus used in Exp. 1.

## **Experiment** 1

A total of 180 barrows and gilts (Line TR4  $\times$  1050; PIC, Hendersonville, TN), initially  $10.3 \pm 0.2$  kg BW and 34 d of age, were used in a 21-d growth experiment to evaluate the ability of 3 feed additives to ameliorate the negative effects associated with DON contamination in nursery pig diets. Pigs were allotted to pens by initial BW and gender and pens were assigned to 1 of 5 dietary treatments in a randomized complete block design (**RCBD**), with both initial BW and location in the barn serving as blocking factors. There were 6 replicate pens per treatment with 6 pigs per pen. Five diets were formulated to provide the following treatments: 1) Positive Control (PC; < 0.5 mg/kg DON), 2) Negative Control (NC; 4 mg/kg DON), 3) NC + 0.10% Biofix, 4) NC + 0.15% Cel-can and 0.50% bentonite clay, or 5) NC + 0.25% Defusion Plus. Experimental diets were presented in meal form and were fed from d 0 to 21. All diets were formulated to be identical in nutrient composition and contained a total of 17% DDGS (Table 1).

All diets were manufactured at the Kansas State University Animal Science Feed Mill. Samples of each diet were collected from feeders between each weigh day and were blended, subsampled, and sent to the Veterinary Diagnostic Laboratory at North Dakota State University (**NDSU**; Fargo, ND) for 17-component mycotoxin analysis (Table 2). Although all diets were stored under similar conditions, samples of the NC diet and the diet containing Defusion Plus were also collected at the end of the trial to determine if DON levels changed over time. All samples were sent for analysis at the conclusion of the trial.

This experiment was conducted at the Kansas State University Swine Teaching and Research Center. Each pen  $(1.22 \times 1.52 \text{ m})$  contained a 4-hole, dry self-feeder and a nipple waterer to provide ad libitum access to feed and water. Pig weight and feed disappearance were measured on d 0, 3, 7, 10, and 21 of the trial to determine ADG, ADFI, and G:F (Table 3).

**Table 1.** Composition of diets, Exp. 1 (as-fed basis)<sup>1</sup>

	Deoxynivaleno	l (DON; 4 mg/kg)
	Positive	Negative
Item	control	control
Corn	51.36	51.36
Soybean meal, 46.5%	28.29	28.29
DDGS	17.00	—
High-DON DDGS <sup>1</sup>		17.00
Monocalcium phosphate, 21% P	0.65	0.65
Limestone	1.20	1.20
Salt	0.35	0.35
Copper sulfate	0.05	0.05
Vitamin premix <sup>2</sup>	0.25	0.25
Trace mineral premix <sup>3</sup>	0.15	0.15
L-Lys HCl	0.40	0.40
DL-Met	0.08	0.08
L-Thr	0.10	0.10
Phytase <sup>4</sup>	0.13	0.13
Feed additive <sup>5</sup>	_	_
Total	100	100
Calculated analysis		
SID <sup>6</sup> amino acids, %		
Lys	1.27	1.27
Ile:Lys	63	63
Met:Lys	32	32
Met and Cys:Lys	59	59
Thr:Lys	63	63
Trp:Lys	17	17
Val:Lys	72	72
Total Lys, %	1.43	1.43
ME, kcal/kg	3,320	3,320
SID Lys:ME, g/Mcal	3.83	3.83
СР, %	22.64	22.64
Ca, %	0.69	0.69
P, %	0.60	0.60
Available P, %	0.42	0.42

<sup>1</sup>Analyzed DON concentration in DDGS was 23.5 mg/kg.

<sup>2</sup>Provided per kilogram of premix: 4,409,200 IU vitamin A; 551,150 IU vitamin D<sub>3</sub>; 17,637 IU vitamin E; 1,764 mg vitamin K; 3,307 mg riboflavin; 11,023 mg pantothenic acid; 19,841 mg niacin; and 15.4 mg vitamin  $B_{12}$ .

<sup>3</sup>Provided per kilogram of premix: 26.5 g Mn from manganese oxide; 110 g Fe from iron sulfate; 110 g Zn from zinc sulfate; 11 g Cu from copper sulfate; 198 mg I from calcium iodate; and 198 mg Se from sodium selenite.

<sup>4</sup>Phyzyme 600 (Danisco Animal Nutrition, St. Louis, MO) provided 750 phytase units phytase/kg and 0.13% available P released.

 $^{5}$ Three feed additives were tested in the Negative Control diet: 0.15% Biofix, 0.25% Defusion Plus, and 0.15% Cel-can with 0.50% bentonite clay. In each diet, additives were included at the expense of corn.

<sup>6</sup>Standardized ileal digestible.

## **Experiment** 2

A total of 340 barrows (Line 1050; PIC, Hendersonville, TN; initially  $11.7 \pm 0.1$  kg BW and 35 d of age) were used in a 21-d growth experiment. Pigs were allotted to pens by BW, and pens were assigned to 1 of 8 treatments in a RCBD, with initial BW location in the barn serving as the blocking factor.

Downloaded from https://academic.oup.com/jas/article-abstract/93/3/1074/4563385 by Kansas State University Libraries user on 01 May 2018 There were 9 replicate pens per treatment with 4 to 5 pigs per pen. All pigs were initially fed common commercial diets for the first 14 d. On d 14 postweaning (d 0 of the experiment), diets comprising the 8 experimental treatments (Table 4) were fed to the pigs. All diets contained 20% corn DDGS. Based on the initial mycotoxin analysis of base ingredients (Table 5), the 8 experimental diets were formulated to contain 1) PC (< 0.5 mg/kg DON), 2) Low NC (1.5 mg/kg DON), 3) Low NC + 0.15% Biofix, 4) Low NC + 0.30% Biofix, 5) High NC (3.0 mg/kg DON), 6) High NC + 0.30% Biofix, 7) High NC + 0.45% Biofix, and 8) High NC + 0.45% Biofix and 5% water added to the diet.

We hypothesized that adding water to diets containing Biofix would enhance the ability of the yeast cell wall constituents to absorb and degrade the mycotoxin molecule; therefore, water was added at 5%, which diluted nutrient concentrations in the remainder of the diet.

This experiment was conducted at the Kansas State University Segregated Early Weaning Research Facility in Manhattan, KS. Each pen  $(1.22 \times 1.22 \text{ m})$  contained a 4-hole, dry self-feeder and 1-cup waterer to provide ad libitum access to feed and water. Base corn, soybean meal, and the 2 sources of DDGS were sent to Romer Labs (Union, MO) and tested for mycotoxin content. These results were used in diet formulation before diets were manufactured at the Kansas State University Animal Science Feed Mill.

After diet manufacturing, each diet was sampled and tested at Romer Labs as well as NDSU Veterinary Diagnostic Laboratory (Table 6). Experimental diets were presented in meal form and were fed from d 0 to 21. Average daily gain, ADFI, and G:F were determined by weighing pigs and measuring feed disappearance on d 4, 7, 14, and 21 of the trial (Table 7).

#### **Experiment 3**

A total of 1,008 barrows and gilts (Fast/PIC  $\times$ Line TR4; Fast Genetics, Saskatoon, SK, Canada; PIC, Hendersonville, TN), initially  $12.5 \pm 0.3$  kg BW, were used in a 24-d growth experiment to evaluate the effect of increased levels of Defusion along with supplemental nutrients and pelleting on the growth performance of nursery pigs fed DON-contaminated diets. Six dietary treatments were arranged in an RCBD, with 6 replicate pens per treatment and average initial pig BW as the blocking factor. Pens were allotted to treatment based on initial pen weight, with an average of 28 pigs per pen (14 barrows, 14 gilts). The experimental diets were formulated (Table 8) to provide the following treatments: 1) PC (<0.5 mg/kg DON), 2) NC (3 mg/kg DON), 3) NC + 0.25% Defusion, 4) NC + 0.50% Defusion, 5) NC + 0.25% Defusion with supplemental nutrients, and

Item,			Composit	e <sup>2</sup>		d 2	213
mg/kg	Positive control	Negative control	Biofix <sup>4</sup>	Cel-can5/bentonite clay	Defusion Plus <sup>6</sup>	Negative control	Defusion Plus <sup>6</sup>
DON	0.8	4.6	4.4	4.3	5.1	6.1	4.6
15-ADON	< 0.5	1.0	1.0	1.0	1.1	1.3	1.0
Total DON	0.8	5.6	5.4	5.3	6.2	7.4	5.6
Fumonisin B <sub>1</sub>	2.0	2.0	2.0	1.0	2.0	2.0	1.0
Zearalenone	< 0.5	0.5	0.5	0.5	0.5	0.6	0.5

**Table 2.** Mycotoxin analysis of diets, Exp. 1 (as-fed basis)<sup>1</sup>

<sup>1</sup>Samples were sent for 17-component mycotoxin analysis at the North Dakota State University Veterinary Diagnostic Laboratory (Fargo, ND). Samples were analyzed using a variety of mass spectrometry, ELISA, and HPLC methods. Included are those mycotoxins found at levels above detection limits. <sup>2</sup>Values are a mean of 6 samples collected on d 2, 5, 8, 12, 14, and 19 that were blended before being analyzed at the conclusion of the experiment.

<sup>3</sup>Collected at the end of the experiment and analyzed in a separate assay from other samples.

<sup>4</sup>Biofix (Biomin Inc., Herzogenburg, Austria).

<sup>5</sup>Cel-can (VAST Inc., Mason City, IA).

<sup>6</sup>Defusion Plus (Cargill Animal Nutrition, Minneapolis, MN).

6) Diet 5, pelleted. Diets 1 to 5 were fed in meal form. For treatments 5 and 6, the added nutrients included supplemental choice white grease (**CWG**), monocalcium phosphate, L-Lys, methionine hydroxy analog, and L-Thr. These supplemental nutrients were added to increase the nutrient density for pigs exposed to DON to potentially offset the known ADFI reductions associated with DON. Treatments 1 to 4 were medicated with chlortetracycline (**CTC**) at 0.22 g/kg, and treatments 5 and 6 with supplemental nutrients were medicated with CTC at 0.24 g/kg. Due the high sodium content in Defusion (23.73% Na), diets with added Defusion contained a lower inclusion rate of salt.

**Table 3.** Effect of deoxynivalenol (DON) level and commercial feed additives on nursery pig performance, Exp. 1<sup>1</sup>

			DON (4.0 mg/kg) <sup>2</sup>						
				Cel-can <sup>4</sup> /					
	Positive	Negative		bentonite	Defusion				
Item	control	control	Biofix <sup>3</sup>	clay	Plus <sup>5</sup>	SEM			
d 0 to 21									
ADG, g	585 <sup>a</sup>	419 <sup>c</sup>	409 <sup>c</sup>	418 <sup>c</sup>	469 <sup>b</sup>	22.7			
ADFI, g	895 <sup>a</sup>	719 <sup>b</sup>	687 <sup>b</sup>	699 <sup>b</sup>	739 <sup>b</sup>	29.6			
G:F	0.655 <sup>a</sup>	0.587 <sup>c</sup>	0.597 <sup>bc,x</sup>	0.601 <sup>bc</sup>	0.637 <sup>ab,y</sup>	0.0162			
Pig BW, kg									
d 0	10.31	10.34	10.39	10.37	10.34	0.190			
d 3	11.47	10.72	10.75	10.74	10.76	0.201			
d 7	13.48 <sup>a</sup>	12.23 <sup>b</sup>	12.13 <sup>b</sup>	12.24 <sup>b</sup>	12.16 <sup>b</sup>	0.216			
d 10	17.32 <sup>a</sup>	15.24 <sup>b,x</sup>	15.19 <sup>b,x</sup>	15.54 <sup>b</sup>	16.10 <sup>b,y</sup>	0.320			
d 21	22.60 <sup>a</sup>	19.15 <sup>c</sup>	18.97 <sup>c</sup>	19.15 <sup>c</sup>	20.35 <sup>b</sup>	0.397			

<sup>a,b and x,y</sup>Within a row, means without a common superscript differ P < 0.05 and P < 0.10, respectively.

 $^{1}$ A total of 180 pigs (PIC TR4 × 1,050, initially 10.3 ± 0.2 kg) were used in a 21-d trial with 6 pigs per pen and 9 pens per treatment.

 $^2 \rm The$  analyzed average DON level was 4.6 mg/kg, and the average total DON was 5.6 mg/kg.

<sup>3</sup>Biofix (Biomin Inc., Herzogenburg, Austria).

<sup>4</sup>Cel-can (VAST Inc., Mason City, IA).

<sup>5</sup>Defusion Plus (Cargill Animal Nutrition, Minneapolis, MN).

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Dried distillers grains with solubles originated from a low-DON (0.7 mg/kg DON) and DON-contaminated (15.8 mg/kg DON) source; both were subsampled 10 times, and samples were homogenized before being sent to the NDSU Veterinary Diagnostic Laboratory for a 17-component mycotoxin analysis (Table 9), which was then used for diet formulation. Based on these results, corn DDGS was incorporated at 15.85% to achieve desired DON concentrations. All diets were manufactured at Hubbard Feeds in Mankato, MN. Treatment 6 was pelleted using a CPM 7800 (California Pellet Mill, Crawfordsville, IN) equipped with a 635-mm-thick stainless steel 32-mm pellet die with a conditioning temperature of 54.4°C. Following diet manufacturing, a sample of each diet was collected and analyzed for DON levels using an ELISA test kit (Neogen Inc., 2007) at MVTL Laboratories (New Ulm, MN).

This experiment was conducted at the New Fashion Pork Research Nursery (Buffalo Center, IA). Each pen  $(1.75 \times 4.05 \text{ m})$  contained a 5-hole, dry self-feeder and provided ad libitum access to feed and water. Pig weights and feed disappearance were measured on d 0, 7, 14, and 24 to determine ADG, ADFI, and G:F (Table 10).

## **Experiment** 4

A total of 980 barrows and gilts (Fast/PIC × TR4; Fast Genetics, Saskatoon, SK, Canada; PIC, Hendersonville, TN), initially  $12.0 \pm 0.3$  kg, were used in a 28-d growth experiment. This experiment was designed as a follow-up to Exp. 3 to further evaluate the impact of adding Defusion in combination with pelleting on the growth performance of nursery pigs fed DON-contaminated diets. Seven dietary treatments were arranged in an RCBD with a  $2 \times 3 + 1$  factorial arrangement with 5 replicate pens per treatment, and average initial pig BW was used as the blocking factor. Pens were allotted to treatments based on initial pen

## Table 4. Composition of diets, Exp. 2 (as-fed basis)<sup>1</sup>

		Low-deoxyni	valenol (DON;	1.5 mg/kg) <sup>2</sup>	High-	DON (3.0 m	g/kg) <sup>2</sup>	5% Water <sup>3</sup>
	Positive	Low negative	0.15%	0.30%	High negative	0.30%	0.45%	0.45%
Item	control	control	Biofix	Biofix	control	Biofix	Biofix	Biofix
Ingredient, %								
Corn	49.06	49.06	48.89	48.73	49.06	48.73	48.57	46.16
Soybean meal, 46.5% CP	27.63	27.63	27.65	27.66	27.63	27.66	27.67	26.27
Control DDGS <sup>4</sup> , 29% CP	20.00	10.00	10.00	10.00	_	-	-	_
High-DON DDGS, 28.5% CP	-	10.00	10.00	10.00	20.00	20.00	20.00	19.00
Monocalcium phosphate, 21% P	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.57
Limestone	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.19
Salt	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.33
Copper sulfate	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Vitamin premix <sup>5</sup>	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.24
Trace mineral premix <sup>6</sup>	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.14
L-Lys HCl	0.41	0.41	0.41	0.41	0.41	0.41	0.41	0.39
DL-Met	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
L-Thr	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.07
Phytase <sup>7</sup>	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.12
Biofix <sup>8</sup>	-	_	0.15	0.30	_	0.30	0.45	0.43
Water	-	_	-	_	_	_	-	5.00
Total	100	100	100	100	100	100	100	100
Calculated composition, %								
SID <sup>9</sup> amino acids, %								
Lys	1.27	1.27	1.27	1.27	1.27	1.27	1.27	1.21
Ile:Lys	63	63	63	63	63	63	63	63
Leu:Lys	148	148	148	148	148	148	147	147
Met:Lys	30	30	30	30	30	30	30	30
Met and Cys:Lys	58	58	58	58	58	58	58	58
Thr:Lys	62	62	62	62	62	62	62	62
Trp:Lys	17	17	17	17	17	17	17	17
Val:Lys	72	72	72	72	72	72	72	72
Total Lys, %	1.44	1.44	1.44	1.44	1.44	1.44	1.44	1.37
ME, kcal/kg	3,320	3,320	3,313	3,309	3,320	3,309	3,305	3,139
SID Lys:ME, g/Mcal	3.83	3.83	3.83	3.84	3.83	3.84	3.84	3.84
CP, %	22.9	22.9	22.9	22.9	22.9	22.9	22.9	21.8
Ca, %	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.67
P, %	0.60	0.60	0.60	0.60	0.60	0.60	0.59	0.57
Available P, %	0.42	0.42	0.42	0.42	0.42	0.42	0.42	0.40

<sup>1</sup>Diets were fed for 21 d with d 14 postweaning as d 0 of the experiment. Diets were fed in meal form.

<sup>2</sup>The analyzed average DON content for the low- and high-DON diets was 2.0 and 4.1 mg/kg, respectively.

<sup>3</sup>The 5% water treatment is a duplicate of the high-DON, 0.45% Biofix treatment diluted with 5% water (2.85 mg/kg DON, 0.427% Biofix). <sup>4</sup>Dried distillers grains with solubles.

<sup>5</sup>Provided per kilogram of premix: 4,409,200 IU vitamin A; 551,150 IU vitamin D<sub>3</sub>; 17,637 IU vitamin E; 1,764 mg vitamin K; 3,307 mg riboflavin; 11,023 mg pantothenic acid; 19,841 mg niacin; and 15.4 mg vitamin  $B_{12}$ .

<sup>6</sup>Provided per kilogram of premix: 26.5 g Mn from manganese oxide; 110 g Fe from iron sulfate; 110 g Zn from zinc sulfate; 11 g Cu from copper sulfate; 198 mg I from calcium iodate; and 198 mg Se from sodium selenite.

<sup>7</sup>Phyzyme 600 (Danisco Animal Nutrition, St. Louis, MO) provided 750 phytase units phytase/kg and 0.13% available P released. <sup>8</sup>Biofix (Biomin, Herzogenberg, Austria).

<sup>o</sup>Biolix (Biolilli, Herzogenberg, Aus

<sup>9</sup>Standardized ileal digestible.

weight, with an average of 28 pigs per pen (14 barrows, 14 gilts). Seven experimental treatments were formulated based on 4 experimental diets fed in meal (**M**) or pellet (**P**) form: 1) PC (< 0.5 mg/kg DON), 2) NC (3 mg/kg DON), and 3) NC + 0.25% Defusion. Treatment 7 was Diet 3 (NC + 0.25% Defusion) with supplemental

nutrients fed only in pellet form. The added nutrients included CWG, monocalcium phosphate, L-Lys, methionine hydroxy analog, and L-Thr. These supplemental nutrients were added to increase the nutrient density for pigs exposed to DON to potentially offset the known ADFI reductions associated with DON. Diets 1 to 6

**Table 5.** Mycotoxin analysis of base ingredients, Exp.2 (as-fed basis)

Item, mg/kg	Ground corn	Soybean meal	Control DDGS	High-DON DDGS
Romer Labs <sup>1</sup>				
DON	< 0.2	< 0.2	0.9	8.1
15-ADON	< 0.2	< 0.2	< 0.2	3.4
Total DON <sup>2</sup>	< 0.2	< 0.2	0.9	11.5
NDSU <sup>3</sup>				
DON	-	-	0.7	11.1
15-ADON	-	-	< 0.5	4.7
Total DON <sup>2</sup>	_	-	0.7	15.8

<sup>1</sup>Romer Laboratories (Union, MO). Samples analyzed using a combination of gas chromatography, high-pressure liquid chromatography, and mass spectrometry with a practical quantitation limit of 0.2 mg/kg. Reported value is an average of 2 separate analyses.

<sup>2</sup>Total DON levels as a combination of DON and 15-ADON because these 2 compounds have similar toxicity (Pestka, 1987).

<sup>3</sup>North Dakota State University Veterinary Diagnostic Laboratory (Fargo, ND). Samples were sent for 17-component mycotoxin analysis and analyzed using a variety of mass spectrometry, ELISA, and HPLC methods. Included in the table are mycotoxins found at levels above detection limits.

were medicated with CTC at 0.22 g/kg, whereas treatment 7 with supplemental nutrients used CTC at 0.24 g/ kg. Due the high sodium content in Defusion (23.73% Na), diets with added Defusion contained a lower inclusion level of added salt.

Dried distillers grains with solubles originated from the same source as in Exp. 3 and were analyzed in the same fashion. Based on these results (Table 11), corn DDGS were incorporated into diets at 23.5% to achieve desired DON concentrations (Table 12). All diets were manufactured at Hubbard Feeds in Mankato, MN. Diets were pelleted using a CPM 7800 equipped with a 635-mm-thick stainless steel 32-mm pellet die with conditioning temperatures averaging  $57.0 \pm$  $3.1^{\circ}$ C. Particle sizes averaged 636 µm for the diets fed in meal form. Mycotoxin analyses were conducted at NDSU using a full 17-component toxin screen.

This experiment was conducted at the New Fashion Pork Research Nursery in Buffalo Center, IA. Each pen  $(1.75 \times 4.05 \text{ m})$  contained a 5-hole, dry self-feeder and provided ad libitum access to feed and water. Pig weights and feed disappearance were measured on d 0, 7, 14, and 24 of the trial to determine ADG, ADFI, and G:F (Table 13).

#### Mycotoxin Analysis

In Exp. 1 to 4, samples of the base corn and DDGS were sent to the NDSU Veterinary Diagnostic Laboratory for a 17-component mycotoxin analysis. In Exp. 1, 2, and 4, complete diet samples were also sent to NDSU for analysis. The analysis for tricothecene mycotoxins (DON, 15-acetyldeoxynivalenol [15-ADON], 3-Acetyl DON, nivalenol, and T-2 toxin) along with zearalenone and zearalenol was conducted according to a modified version of Groves et al. (1999) using gas chromatography coupled with mass spectrometry. Aflatoxins and fumonisins are analyzed by HPLC. Samples were tested on an as-fed basis, and the practical quantitation limit for all mycotoxins was 0.5 mg/kg. In Exp. 2, complete diet samples were initially tested for mycotoxin levels at Romer Labs using an HPLC extraction method (Romer Labs Inc.,

Low-DON (1.5 mg/kg) High-DON (3.0 mg/kg) 5% Water<sup>1</sup> Item, Positive 0.30% Biofix mg/kg control Low negative control (NC) 0.15% Biofix High NC 0.30% Biofix 0.45% Biofix 0.45% Biofix Romer Labs<sup>2</sup> DON 0.6 1.5 2.2 1.8 4.4 3.9 3.7 3.7 0.6 0.8 0.7 1.4 1.3 1.2 1.2 15-ADON < 0.2 Total DON 0.6 2.1 3.0 2.5 5.8 5.2 4.9 4.9 NDSU<sup>3</sup> DON 0.7 2.2 2.5 2.2 4.1 4.9 4.1 3.6 15-ADON < 0.5 0.5 0.5 0.9 1.0 0.9 0.5 0.8 Total DON 2.7 5.9 0.7 3.0 2.7 5.0 5.0 4.4 Overall<sup>4</sup> 3.75 1.9 DON 0.6 1.7 2.3 4.3 4.2 3.8 Total DON 3.0 5.4 4.9 0.6 2.3 2.6 5.5 4.7

Table 6. Deoxynivalenol (DON) analysis of experimental diets, Exp. 2

<sup>1</sup>The 5% water treatment is a duplicate of the high-DON, 0.45% Biofix (Biomin, Herzogenberg, Austria) treatment diluted with 5% water (2.85 mg/kg DON, 0.427% Biofix).

<sup>2</sup>Romer Laboratories (Union, MO). Samples analyzed using a combination of gas chromatography, high-pressure liquid chromatography, and mass spectrometry with a practical quantitation limit of 0.2 mg/kg. Reported value is an average of 2 separate analyses.

<sup>3</sup>North Dakota State University Veterinary Diagnostic Laboratory (Fargo, ND). Samples were sent for 17-component mycotoxin analysis and analyzed using a variety of mass spectrometry, ELISA, and HPLC methods. Included in the table are mycotoxins found at levels above detection limits.

<sup>4</sup>Reported value is an average of the 3 analyses conducted (2 analyses at Romer Labs and one at NDSU).

<sup>5</sup>Additional samples were collected at d 0, 7, 14, and 21 and sent to NDSU for DON analysis. Results: d 0 (3.0 mg/kg), d 7 (3.4 mg/kg), d 14 (3.8 mg/kg), and d 21 (3.8 mg/kg).

Table 7. Effects of Biofix and deoxynivalenol (DON) on nursery pig growth performance, Exp. 2<sup>1</sup>

		Low-D	ON (1.5	mg/kg) <sup>2</sup>	H	High-DON (3.0 mg/kg) <sup>2</sup>						I	robabilit	y, P <		
								5% water <sup>3</sup>		DON	effect4	_Low vs.	Biofix lo	w-DON <sup>4</sup>	Biofix hi	gh-DON <sup>4</sup>
	Positive	Low	0.15%	0.30%	High	0.30%	0.45%	0.45%	-			High				
Item	control	NC	Biofix	Biofix	NC	Biofix	Biofix	Biofix	SEM	Lin	Quad	DON <sup>4</sup>	Lin	Quad	Lin	Quad
d 0 to 21																
ADG, g	g 560	564	574	558	530	506	518	517	11.8	0.048	0.159	0.001	0.704	0.316	0.320	0.244
ADFI, g	g 911	896	949	895	850	813	851	861	19.1	0.007	0.449	0.001	0.941	0.014	0.787	0.080
G:F	0.615	0.629	0.607	0.623	0.624	0.623	0.608	0.600	0.007	0.312	0.213	0.768	0.522	0.013	0.113	0.226
Pig BW, k	g															
d 0	11.73	11.63	11.71	11.64	11.63	11.57	11.63	11.58	0.077	0.220	0.453	0.309	0.928	0.279	0.843	0.393
d 4	13.24	13.13	13.24	13.03	12.78	12.57	12.66	12.86	0.139	0.007	0.387	0.001	0.525	0.254	0.347	0.368
d 7	14.57	14.57	14.65	14.45	14.08	13.87	13.96	14.22	0.175	0.021	0.159	0.001	0.554	0.421	0.454	0.473
d 14	18.54	18.92	18.84	18.48	18.33	17.68	18.15	18.26	0.202	0.464	0.049	0.001	0.115	0.567	0.289	0.036
d 21	23.49	23.47	23.77	23.35	22.85	22.19	22.63	22.61	0.255	0.036	0.273	0.001	0.711	0.184	0.288	0.066

<sup>1</sup>A total of 340 pigs (1050, PIC; Hendersonville, TN; initial BW 11.6  $\pm$  0.1 kg) were used in a 21-d trial with 4 to 5 pigs per pen and 9 pens per treatment. <sup>2</sup>The analyzed average DON content for the low- and high-DON diets were 2.0 and 4.1 mg/kg, respectively.

<sup>3</sup>The 5% water treatment is a duplicate of the high-DON, 0.45% Biofix (Biomin, Herzogenberg, Austria) treatment diluted with 5% water (2.85 mg/kg DON, 0.427% Biofix).

<sup>4</sup>Each contrast compared the following treatments: (1) "DON effect" evaluated the linear and quadratic effects of the Positive Control vs. Low- and High-DON NC treatments; (2) "Low vs. High DON" compared the 3 Low-DON treatments to the 3 High-DON treatments; (3) "Biofix Low-DON" and (4) "Biofix High-DON" evaluated the linear and quadratic effects of increasing Biofix levels in Low-DON or High-DON diets, respectively. The effect of adding 5% water to High-DON diets with 0.45% Biofix was not significant (P > 0.202).

2012) with a minimum quantitation limit of 0.15 mg/kg. In Exp. 3, complete diet samples were tested at MVTL Laboratories and tested for DON levels using an ELISA test kit with a range of quantitation between 0.5 and 5.0 mg/kg.

## Statistical Analysis

For all 4 experiments, data were analyzed as an RCBD using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC) with pen as the experimental unit. In Exp. 1, when treatment effect was a significant source of variation, means were separated using the PDIFF option of SAS. In Exp. 2 to 4, treatment means were analyzed using the LSMEANS statement and preplanned CONTRAST statements in SAS, with block as the random component. In Exp. 2, the fixed factors in the model included DON level and Biofix inclusion. Preplanned contrasts in Exp. 2 included low vs. high DON, the effect of adding 5% water to high-DON diets with 0.45% Biofix, and the linear and quadratic effects of both increasing levels of DON and increasing levels of Biofix in low- and high-DON diets. The coefficients for the unequally spaced linear and quadratic contrasts were derived using the PROC IML procedure in SAS. In Exp. 3, the preplanned contrasts included 1) DON vs. non-contaminated, 2) linear and quadratic effects of levels of Defusion, 3) effect of supplemental nutrients in NC diets with 0.25% Defusion, and 4) diet form (pellet vs. meal) in NC diets containing 0.25% Defusion and supplemental nutrients. For Exp. 4, the

model included pelleting and the inclusion of Defusion as fixed factors. Preplanned contrasts in Exp. 4 were 1) the 2-way interaction between pelleting and adding 0.25% Defusion in NC diets, 2) DON vs. noncontaminated, 3) diet form (pellet vs. meal), 4) the addition of Defusion in NC diets, and 5) the effect of supplemental nutrients in NC diets with 0.25% Defusion (pellet form). In all 4 experiments, least squares means were calculated for each independent variable and mean differences were considered significant at P < 0.05and trends at P < 0.10.

## RESULTS

#### **Experiment** 1

The analyzed dietary DON concentration for the PC diet was 0.8 mg/kg (Table 2). In addition, analyzed dietary DON concentration for the NC, Biofix, Celcan with clay bentonite, and Defusion Plus treatments were 4.6, 4.4, 4.3, and 5.1 mg/kg, respectively. Also, 3-Acetyl DON, 15-ADON, fumonisin  $B_1$ , and zearelenone were detected in diets at or below cautionary dietary limits.

Overall (d 0 to 21), pigs fed the PC diet had greater (P < 0.01) ADG and ADFI than pigs fed DON-contaminated diets (Table 3). The addition of Biofix or Cel-can with bentonite clay had no effect on ADG, ADFI, or G:F in NC diets; however, pigs fed diets containing Defusion Plus had greater ADG (P < 0.03) than pigs fed the NC as well as diets containing Biofix or Cel-can with bentonite

## Table 8. Composition of diets, Exp. 3

			Deox	ynivalenol (3 mg/kg)	
Item	Positive control	Negative control	$NC + 0.25\% DEF^1$	NC + 0.50% DEF <sup>1</sup>	NC + 0.25% DEF <sup>1</sup> + nutrients <sup>2</sup>
Ingredient, %					
Rolled corn	53.31	53.61	53.38	53.15	43.67
Soybean meal, 46.5% CP	26.00	26.00	26.00	26.05	30.50
DDGS <sup>3</sup>	15.85	_	_	_	_
High-DON DDGS <sup>4</sup>	_	15.85	15.85	15.85	15.85
Choice white grease	1.30	1.00	1.10	1.15	5.95
Limestone	1.02	1.02	1.02	1.02	1.01
Monocalcium phosphate, 21% P	0.65	0.65	0.65	0.65	0.80
Salt	0.50	0.50	0.38	0.26	0.38
L-Lys HCl	0.43	0.43	0.43	0.43	0.46
Methionine hydroxy analog	0.11	0.11	0.11	0.11	0.16
L-Thr	0.08	0.08	0.08	0.08	0.10
Trace mineral premix <sup>5</sup>	0.08	0.08	0.08	0.08	0.08
Vitamin premix <sup>6</sup>	0.03	0.03	0.03	0.03	0.03
Medication <sup>7</sup>	0.40	0.40	0.40	0.40	0.44
Mold inhibitor <sup>8</sup>	0.10	0.10	0.10	0.10	0.10
Copper sulfate	0.07	0.07	0.07	0.07	0.07
Selenium, 0.06%	0.05	0.05	0.05	0.05	0.05
Phytase9	0.04	0.04	0.04	0.04	0.04
Defusion	-	-	0.25	0.50	0.25
Total	100	100	100	100	100
Calculated analysis					
SID <sup>10</sup> amino acids, %					
Lys	1.20	1.20	1.20	1.20	1.32
Ile:Lys	60	60	60	60	58
Met:Lys	33	33	33	33	35
Met and Cys:Lys	58	58	58	58	58
Thr:Lys	60	60	60	60	60
Trp:Lys	18	18	18	18	18
Val:Lys	72	72	72	72	70
ME, Kcal/kg	3,307	3,296	3,296	3,296	3,488
СР, %	20.39	20.42	20.40	20.41	21.79
SID Lys:ME, g/Mcal	3.63	3.64	3.64	3.64	3.78
Total Lys, %	1.36	1.36	1.36	1.36	1.50
Ca, %	0.66	0.66	0.66	0.66	0.70
Р%	0.58	0.58	0.58	0.58	0.61
Available P, %	0.30	0.30	0.30	0.30	0.34
Fat, %	4.92	4.63	4.73	4.77	9.29

<sup>1</sup>Defusion (Cargill Animal Nutrition, Minneapolis, MN).

<sup>2</sup>Fed in both meal and pellet form.

<sup>3</sup>Distillers dried grains with solubles.

<sup>4</sup>Analyzed DON concentration in DDGS was 15.8 mg/kg.

<sup>5</sup>Trace mineral premix provided per kg of premix: 13.3 mg of Cu, 1.40 mg of I, 134 mg of Fe, 53.3 mg of Mn, and 160 mg of Zn.

 $^{6}$ Vitamin premix provided per kilogram of premix: 22,046,000 IU of vitamin A, 5,291,000 IU of vitamin D<sub>3</sub>, 97,002 IU of vitamin E, 10,288 mg of vitamin K, 88.2 mg of vitamin B<sub>12</sub>, 79,366 mg of niacin, 61.7 mg of pantothenic acid, and 13,228 mg of riboflavin.

<sup>7</sup>To provide chlortetracycline at 0.22 or 0.24 g/kg.

<sup>8</sup>Ammo Curb (Kemin Industries, Des Moines, IA).

<sup>9</sup>Phyzyme 2500 (Danisco Animal Nutrition, St. Louis, MO) provided 1,000 phytase units/kg, which provided 0.13% available P.

<sup>10</sup>Standardized ileal digestible.

clay. Pigs fed the PC diet had improved G:F (P < 0.03) compared with pigs fed the NC diet and diets containing Biofix or Cel-can with bentonite clay. Pigs fed Defusion Plus had improved G:F (P < 0.04) compared with pigs

fed NC diets and tended to have improved (P < 0.081) G:F compared with pigs fed diets containing Biofix.

At d 3 and 7, pigs fed the PC diet were heavier (P < 0.02) than pigs fed the other diets. At d 10 and d 21, pigs

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Table 9. Mycotoxin analysis of DDGS source and experimental diets, Exp. 3 (as-fed basis)<sup>1</sup>

	DDG	S source <sup>1</sup>		Experimental diets <sup>2</sup>					
Item, mg/kg	Control	High-DON <sup>3</sup>	Positive control	Negative control (NC)	NC + 0.25% DEF <sup>4</sup>	NC + 0.50% DEF <sup>4</sup>	NC + 0.25% DEF and nutrients <sup>5</sup> (meal)	NC + 0.25% DEF and nutrients <sup>5</sup> (pellet)	
DON	0.70	15.80	0.60	2.60	2.00	2.00	2.10	1.10	
15-ADON	< 0.50	3.10	_	-	_	_	-	-	
Zearalenone	< 0.50	1.00	_	-	_	_	-	-	

<sup>1</sup>Dried distillers grains with solubles samples were 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 mg/kg. Included in the table are mycotoxins found above detection limits.

 $^{2}$ Diet samples analyzed for DON using a Neogen (Lansing, MI) Veratox test kit. Positive Control was formulated to contain < 0.5 mg/kg DON and all remaining diets formulated to contain 3 mg/kg DON.

<sup>3</sup>Deoxynivalenol (DON).

<sup>4</sup>Defusion (Cargill Animal Nutrition, Minneapolis, MN).

<sup>5</sup>Supplemental nutrients included an additional 4.45% soybean meal, 4.8% choice white grease, 0.15% monocalcium phosphate, 0.03% L-Lys, 0.05% methionine hydroxyl analog, 0.02% L-Thr, 0.02 g/kg chlortetracycline.

fed the PC diet remained heavier (P < 0.01) than pigs fed other diets; however, at d 10 the pigs fed diets containing Defusion Plus tended (P < 0.073) to be heavier than those fed NC diets or diets containing Biofix. At d 21, pigs fed Defusion Plus diets were heavier (P < 0.05) than pigs fed the remaining treatment diets.

#### **Experiment** 2

After diet sampling, the analyzed DON concentrations from Romer Labs were greater and more variable between diets than expected, so the samples were analyzed a second time. Technicians at Romer Labs indicated that their analysis procedures are less accurate when DON concentrations exceed 5 mg/kg (such as with the high-DON DDGS used in the diets). A separate set of ingredient and diet samples were then sent to the NDSU Veterinary Diagnostic Laboratory for comparative analysis. The NDSU results for the contaminated DDGS were approximately 50% greater (15.8 mg/kg) than the results reported by Romer Labs (10.1, 12.1 mg/kg), which explains why the test diets formulated to contain 1.5 (low-DON) and 3.0 mg/kg (high-DON) actually averaged approximately 2.0 and 4.1 mg/kg, respectively. Based on variability between labs and analyses, an average level of DON and Total DON for each diet was calculated representing the mean of the 3 separate analyses (Table 4).

For the overall trial period (d 0 to 21), increasing DON from 1.5 to 3.0 ppm decreased (linear; P < 0.05) ADG and ADFI, which was primarily due to a decrease in ADFI when DON levels increased from 1.5 to 3.0 mg/kg. Concentration of DON did not influence G:F. Within DON-contaminated diets, pigs fed high-DON diets had poorer (P < 0.01) ADG than those fed low-

**Table 10.** Effects of Defusion (DEF) in combination with supplemental nutrients and pelleting on growth performance of nursery pigs fed deoxynivalenol (DON)-contaminated diets, Exp. 3<sup>1</sup>

		DON (3.0 mg/kg) <sup>2</sup>							Pro	bability,	P <	
Item	Positive control	Negative control (NC)	NC + 0.25%1 DEF <sup>3</sup>	NC + 0.50% DEF <sup>3</sup>	NC + $0.25\%$ DEF <sup>3</sup> and nutrients (meal)	$NC + 0.25\% DEF^3$ and nutrients (pellet)	SEM	DON	Defu Linear	ision Quad	Added nutrients <sup>4</sup>	Pellet vs. meal
d 0 to 24												
ADG, g	617	575	597	612	583	637	9.5	0.003	0.009	0.710	0.285	0.001
ADFI, g	s 892	850	863	883	798	818	13.8	0.012	0.044	0.796	0.001	0.207
G:F	0.692	0.676	0.692	0.693	0.731	0.780	0.009	0.242	0.204	0.504	0.006	0.001
Pig weights	s, kg											
d 0	12.44	12.59	12.53	12.48	12.57	12.58	0.27	0.509	0.611	1.000	0.856	0.969
d 7	15.83	15.48	15.58	15.67	15.74	16.08	0.27	0.169	0.443	0.990	0.517	0.171
d 14	20.18	19.73	19.96	20.30	20.03	20.56	0.33	0.139	0.064	0.819	0.801	0.084
d 24	27.38	26.65	26.99	27.33	26.69	27.87	0.40	0.076	0.098	0.986	0.459	0.006

<sup>1</sup>A total of 1008 barrows and gilts (Fast/PIC  $\times$  TR4; initially 12.5  $\pm$  0.3 kg BW) were used in a 24-d growth experiment with 28 pigs per pen and 6 replicate pens per treatment.

<sup>2</sup>The analyzed average DON content for the Negative Control diets was 2.0 mg/kg.

<sup>3</sup>Defusion (Cargill Animal Nutrition, Minneapolis, MN).

<sup>4</sup>Each contrast compared the following treatments: (1) "DON" compared Positive Control to Negative Control; (2) "Defusion" compared the linear and quadratic effects of adding 0, 0.25%, or 0.50% Defusion to Negative Control diets; (3) "Added Nutrients" compared NC + 0.25% Defusion with and without nutrients, both in meal form; and (4) "Pellet vs. Meal" evaluated treatments 6 and 7 where NC + 0.25% Defusion with nutrients was fed in meal vs. pellet form.

**Table 11.** Mycotoxin analysis of diets, Exp. 4 (as-fed basis)<sup>1</sup>

	DDG	S source <sup>2</sup>					Experimen	tal diets <sup>2,3</sup>	
Item,			Positive	e control	Negative	e control	NC + 0.25	5% defusion <sup>5</sup>	NC + 0.25% defusion and nutrients <sup>6</sup>
mg/kg	Control	High-DON <sup>4</sup>	Meal	Pellet	Meal	Pellet	Meal	Pellet	Pellet
DON	0.60	11.70	< 0.5	0.50	3.30	3.30	2.90	1.70	_
15-ADON	< 0.50	2.40	< 0.5	< 0.5	0.80	0.70	0.70	0.60	_
Total DON	0.60	14.10	< 0.5	0.50	4.10	4.00	3.60	2.30	_
Zearalenone	< 0.50	1.70	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	_

<sup>1</sup>A total of 980 barrows and gilts (Fast/PIC  $\times$  TR4; initially 12.0  $\pm$  0.3 kg) were used in a 28-d growth experiment with 28 pigs per pen and 5 replicate pens per treatment.

<sup>2</sup>Dried distillers grains with solubles and diet samples were 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 mg/kg. Included in the table are mycotoxins found above detection limits.

<sup>3</sup>Positive Control diet formulated to contain < 0.5 mg/kg DON and all remaining diets formulated to contain 3 mg/kg DON.

<sup>4</sup>Deoxynivalenol (DON).

<sup>5</sup>Defusion (Cargill Animal Nutrition, Minneapolis, MN).

<sup>6</sup>Supplemental nutrients included additional soybean meal, choice white grease, minerals, synthetic amino acids, and medication. Diet samples for mycotoxin analysis were not available.

DON diets, which was driven by a reduction in ADFI (P < 0.01) because G:F was not affected. Within low-DON diets, ADFI increased (quadratic, P < 0.01) with the addition of 0.15% Biofix, but at 0.30% Biofix, ADFI dropped to a level similar to the low NC. Conversely, G:F worsened (quadratic, P < 0.01) when 0.15% Biofix was added, which recovered when 0.30% Biofix was added. This fluctuation in ADFI and G:F explains why adding 0.15% or 0.30% Biofix did not influence ADG in low-DON diets. Within high-DON diets, no differences were observed in ADG or G:F when pigs were fed 0.30% or 0.45% Biofix, although pigs fed increasing levels of Biofix tended (quadratic, P = 0.082) to have lower ADFI than pigs fed the high NC diet. The addition of 5% water to high-DON diets with 0.45% Biofix did not influence ADG, ADFI, or G:F.

Pigs fed increasing levels of DON weighed less (linear, P < 0.02) at d 4 and d 7 compared with the PC pigs, with similar responses on d 14 (quadratic, P < 0.05) and on d 21 (linear, P < 0.04). Within DON-contaminated diets, pigs fed high-DON levels had lower BW (P < 0.01) than pigs fed diets containing low-DON concentrations. When Biofix concentrations were increased in high-DON diets, pig BW decreased (quadratic, P < 0.04) at d 14, and those pigs tended to weigh less (quadratic, P = 0.069) at d 21, but increasing Biofix in low-DON diets did not affect pig BW at any time point. Adding 5% water to high-DON diets with 0.45% Biofix did not affect pig BW at any of the weigh periods.

#### **Experiment 3**

Analyzed DON concentration for the PC diet was 0.6 mg/kg, below critical concentrations of DON in nursery pig diets (> 1 mg/kg; Dänicke et al., 2001). The analyzed dietary DON concentrations for NC, NC

+ 0.25% Defusion, NC + 0.50% Defusion, and NC + 0.25% Defusion with increased nutrients (meal form) were lower than formulated levels (3 mg/kg) at 2.6, 2.0, 2.0, and 2.1 mg/kg, respectively. When the NC + 0.25% Defusion with increased nutrients was pelleted, analyzed DON levels decreased to 1.1 mg/kg.

Overall (d 0 to 24), pigs fed NC diets containing DON had decreased (P < 0.01) ADG compared with those fed PC diets, which was driven by a decrease (P < 0.01) in ADFI, with G:F remaining unaffected (Table 10). Within NC diets, increasing Defusion improved (linear, P < 0.01) ADG by increasing (linear, P < 0.04) ADFI, but did not influence G:F. Adding supplemental nutrients to DON-contaminated diets with 0.25% Defusion fed in meal form improved (P < 0.01) G:F, but due to the simultaneous reduction (P < 0.01) in ADFI, ADG did not differ. Pelleting DON-contaminated diets containing 0.25% Defusion improved (P < 0.01) ADG, driven by a typical pelleting response in which G:F was improved (P < 0.01) and ADFI was unaffected.

Regarding pig BW, no differences were observed on d 7 across treatments. Additionally, feeding 3 mg/kg DON did not affect pig BW on d 14, but pigs fed DONcontaminated diets tended (P=0.077) to weigh less at the conclusion of the trial on d 24. Within DON-contaminated diets, increasing Defusion tended to increase (linear; P =0.098) pig BW at d 14 and 24. Although adding supplemental nutrients to DON-contaminated diets with 0.25% Defusion did not alter pig BW, pelleting the diet improved (P < 0.01) final BW on d 21 within treatments where supplemental nutrients were fed.

#### **Experiment** 4

Analyzed total DON was reported as a combination of DON and 15-ADON, which has been shown

Table	12.	Com	position	of diet	s. Exp.	4
			000101011		o, 2p.	

			Deoxynivalenol (3	3 mg/kg)
Item	Positive control	Negative control	NC + 0.25% fefusion <sup>1</sup>	NC + 0.25% fefusion and nutrients <sup>2</sup>
Ingredient, %				
Rolled corn	46.28	46.14	45.99	36.55
Soybean meal, 46.5% CP	25.45	25.50	25.50	30.00
DDGS <sup>3</sup>	23.50	23.50	23.50	23.50
Pork fat	1.40	1.35	1.40	6.00
Limestone	1.19	1.05	1.04	1.00
Monocalcium phosphate, 21% P	0.45	0.60	0.60	0.70
Salt	0.46	0.46	0.31	0.31
L-Lys HCl	0.41	0.44	0.44	0.48
Methionine hydroxy analog	0.06	0.13	0.13	0.30
L-Thr	0.04	0.08	0.08	0.10
Trace mineral premix <sup>4</sup>	0.08	0.08	0.08	0.08
Vitamin premix <sup>5</sup>	0.03	0.03	0.03	0.03
Medication <sup>6</sup>	0.40	0.40	0.40	0.44
Mold inhibitor <sup>7</sup>	0.10	0.10	0.10	0.10
Copper sulfate	0.07	0.07	0.07	0.07
Selenium, 0.06%	0.05	0.05	0.05	0.05
Phytase <sup>8</sup>	0.04	0.04	0.04	0.04
Defusion	_	-	0.25	0.25
Total	100	100	100	100
Calculated analysis				
SID <sup>9</sup> amino acids, %				
Lys	1.20	1.20	1.20	1.32
Ile:Lys	55	55	55	55
Met:Lys	28	28	28	28
Met and Cys:Lys	58	58	58	58
Thr:Lys	60	60	60	60
Trp:Lys	18	18	18	18
Val:Lys	65	65	65	65
ME, Kcal/kg	3,304	3,304	3,304	3,492
СР, %	21.63	21.53	21.52	22.92
SID Lys:ME, g/Mcal	3.63	3.63	3.63	3.78
Total Lys, %	1.38	1.39	1.39	1.52
Ca, %	0.69	0.67	0.67	0.70
Р%	0.58	0.58	0.58	0.61
Available P, %	0.30	0.30	0.30	0.33
Fat, %	5.54	5.57	5.62	9.94

<sup>1</sup>Defusion (Cargill Animal Nutrition, Minneapolis, MN).

<sup>2</sup>Fed in both meal and pellet form.

<sup>3</sup>Distillers dried grains with solubles.

<sup>4</sup>Trace mineral premix provided per kilogram of premix: 13.3 mg of Cu, 1.40 mg of I, 134 mg of Fe, 53.3 mg of Mn, and 160 mg of Zn.

<sup>5</sup>Vitamin premix provided per kilogram of premix: 22,046,000 IU of vitamin A, 5,291,000 IU of vitamin D<sub>3</sub>, 97,002 IU of vitamin E, 10,288 mg of vitamin K, 88.2 mg of vitamin B<sub>12</sub>, 79,366 mg of niacin, 61.7 mg of pantothenic acid, and 13,228 mg of riboflavin.

 $^{6}\mbox{To}$  provide chlortetracycline at 0.22 or 0.24 g/kg.

<sup>7</sup>Ammo Curb (Kemin Industries, Des Moines, IA).

<sup>8</sup>Phyzyme 2500 (Danisco Animal Nutrition, St. Louis, MO) supplied 1,000 phytase units/kg, which provided 0.13% available P release.
<sup>9</sup>Standardized ileal digestible.

to have similar toxicity in the pig (Pestka, 1987). In PC diets, DON concentrations were < 0.5 mg/kg and 0.5 mg/kg for the meal and pelleted forms, respectively. Negative Control DON levels were analyzed to be 3.3 mg/kg in both meal and pelleted form. When 0.25% Defusion was added to NC diets, DON levels

were 2.9 mg/kg in meal diets and 1.7 mg/kg in pelleted diets. Diet samples from the NC diet with 0.25% Defusion where supplemental nutrients were added were not available for mycotoxin analysis.

Overall (d 0 to 28), no interactions occurred between the main effects of pelleting and adding Defusion

		(	,		,	1						
					DON	$(3.0 \text{ mg/kg})^2$		Probability, <i>P</i> <				
	Positive control		Negative control		NC + 0.25% defusion <sup>3</sup>		NC + 0.25% defusion <sup>3</sup> and nutrients		4	Pellet vs.		Added
Item	Meal	Pellet	Meal	Pellet	Meal	Pellet	Pellet	SEM	DON <sup>4</sup>	meal <sup>4</sup>	Defusion	<sup>4</sup> nutrients <sup>4</sup>
d 0 to 28												
ADG, g	641	666	589	649	603	655	654	10.6	0.095	0.001	0.351	0.943
ADFI, g	995	988	912	950	934	938	937	16.0	0.003	0.349	0.725	0.931
G:F	0.645	0.674	0.646	0.684	0.646	0.698	0.699	0.007	0.495	0.001	0.309	0.980
Pig BW,	kg											
d 0	11.93	12.01	11.98	12.08	11.94	12.03	11.93	0.29	0.559	0.295	0.668	0.520
d 7	15.38	15.65	15.05	15.17	14.94	15.47	15.48	0.29	0.032	0.050	0.593	0.958
d 14	19.69	19.97	18.92	19.41	18.56	19.87	19.24	0.39	0.049	0.015	0.875	0.185
d 21	24.66	25.33	23.97	25.01	24.04	25.34	25.02	0.41	0.071	0.001	0.455	0.410
d 28	29.88	30.64	28.46	30.37	28.82	30.58	30.25	0.45	0.012	0.001	0.367	0.458

**Table 13.** Effects of pelleting, Defusion, and supplemental nutrients on growth performance of nursery pigs fed deoxynivalenol (DON)-contaminated diets, Exp.  $4^1$ 

<sup>1</sup>A total of 980 barrows and gilts (Fast/PIC × TR4; initially  $12.0 \pm 0.3$  kg) were used in a 28-d growth experiment with 28 pigs per pen and 5 replicate pens per treatment.

<sup>2</sup>In Negative Control diets, the analyzed DON and Total DON levels were 2.8 and 3.5 mg/kg, respectively.

<sup>3</sup>Defusion (Cargill Animal Nutrition, Minneapolis, MN).

<sup>4</sup>Each contrast compared the following treatments: (1) "DON" compared Positive Control to Negative Control (NC) in both meal and pellet form; (2) "Pellet vs. Meal" compared final diet form in the first 6 treatments; (3) "Defusion" compared NC to NC + 0.25% Defusion in both meal and pellet form; and (4) "Added Nutrients" compared treatments 6 and 7, where nutrients were added to diet specifications. The interaction between pelleting and adding 0.25% Defusion was not significant (P > 0.223), so it is not included in the table.

to DON-contaminated diets. Pigs fed NC diets had reduced (P < 0.01) ADG and ADFI compared with those fed the PC, but they did not differ in G:F. Pelleting the diet increased (P < 0.01) ADG, stimulated by an improvement (P < 0.01) in G:F, but no difference was observed in ADFI compared with diets fed in meal form. The addition of 0.25% Defusion to DON-contaminated nursery pig diets did not affect ADG, ADFI, or G:F; furthermore, adding supplemental nutrients to DONcontaminated pelleted diets with 0.25% Defusion did not influence nursery pig performance.

For pig BW, pigs fed diets containing 3 mg/kg DON weighed less (P < 0.05) at d 7, 14, and 28 and tended to weigh less (**b**) at d 21 than those fed PC diets. Additionally, pigs fed pelleted diets were heavier (P < 0.05) each week than those fed diets in meal form. Finally, neither Defusion nor supplemental nutrients influenced pig BW at any of the weekly time points during the experiment.

#### DISCUSSION

Although the informal name "vomitoxin" implies that feeding DON commonly results in vomiting, very few studies confirm this (Etienne and Waché, 2008). Deoxynivalenol concentrations as high as 20 mg/kg can elicit emesis in young pigs (Young et al., 1983), but during "Fusarium years," when DON contamination of cereal crops is widespread, typical DON concentrations are much lower, with regionally affected corn

averaging 3.1 mg/kg (Côté et al., 1984). For DON concentrations below 20 mg/kg in cereal grains, Etienne and Waché (2008) described a 4% reduction in ADFI and 7% reduction in ADG for each additional mg/kg of DON when final diet concentrations exceed 1 mg/kg. In the current trials, DON was incorporated into NC diets using naturally DON-contaminated DDGS, for which DON-related reductions in growth performance had not been previously quantified. In Exp. 2, when low DON levels (1.5 mg/kg) were tested, neither ADFI (564 vs. 560 g) nor ADG (896 vs. 911 g were different from pigs fed PC diets, whereas in pigs fed DON levels above 1.5 mg/kg in Exp. 1 to 4, ADFI was reduced by 4% and ADG by 5% for each additional mg/kg of DON in the final diet. In Exp. 2, even feeding high-DON diets (4.4 mg/kg) caused growth reductions that were not as marked as in the other 3 experiments for unknown reasons. The variation in DON effects both within and between experiments may be associated with the presence of low levels of other DON metabolites (e.g., 15-ADON, 3-Acetyl DON) in naturally contaminated feedstuffs, which can further exacerbate DON effects (EFSA, 2009). Finally, it is also noteworthy that in Exp. 2 only castrated males were used, whereas the other 3 experiments used both castrated males and gilts. Although less significant effects were seen in Exp. 2, if sex-related, these results would contrast with Côté et al. (1985), who reported lower and more variable weight gains in castrated males than in gilts fed the same DON-contaminated diets.

In all 4 experiments, high DON levels affected pig growth most markedly during the initial period, reducing ADG in the NC diet to approximately 33% and 76% of pigs fed the PC diet on d 3 (126 vs. 386 g/d; Exp. 1) and d 4 (288 vs. 377 g/d; Exp. 2). Reductions in performance due to DON continued throughout the entire trial in Exp. 1 and 4, which used diet treatments with greater concentrations of DON than Exp. 2 and 3. In Exp. 2 and 3, after the decrease in performance during the initial period, pigs fed low-DON NC diets performed similarly to pigs fed the PC. This result agrees with a review of the dose-dependent DON effects on feed intake by Etienne and Waché (2008), who suggested that the suppression of feed intake usually persists for the duration of the experiment when DON concentrations exceed 3 mg/kg (Côté et al., 1985; Lun et al., 1985), but normal intake usually recovers after 1 to 2 wk for lower DON concentrations (Grosjean et al., 2002).

Although DON effects were primarily driven by decreased feed intake, feed efficiency was also depressed during the initial period in all 4 experiments. After the initial decrease, feed efficiency for pigs fed DON-contaminated diets was generally similar to those fed the PC diet. This reduction in G:F may be associated with wasted feed from pigs sorting through the feed. Additionally, it may be connected with immune system stimulation during the initial exposure to low to moderate levels of DON, which could increase the maintenance requirement of the pig. Pestka et al. (2004) described a DON-stimulated upregulation of the expression of cytokines, chemokines, and inflammatory genes, and high doses of DON promote leukocyte apoptosis with concurrent immune system suppression (Pestka et al., 2004; Oswald, 2007).

When the 3 commercially available feed additives were tested at their recommended levels in diets containing an average of 4.6 mg/kg DON in Exp. 1, the growth performance of pigs fed these products remained lower than pigs fed the PC (0.8 mg/kg DON). Adding 0.25% Defusion Plus increased ADG by 12% compared with NC, driven by an 9% improvement in G:F and a numeric increase (3%) in ADFI. This result agrees with reports by Mahan (2010) and Patience (2011), who also saw improvements in ADG when adding a similar product, Defusion, to pigs fed DONcontaminated diets. In contrast, Biofix and Cel-can were ineffective against dietary DON at the inclusion rate used in the present experiments. Whether rates greater than 0.10% of Biofix would be more effective is unclear, but in a study by Mahan (2010), feeding 0.40% Biofix did not affect the performance of nursery pigs fed diets containing 2 or 7 mg/kg DON.

In Exp. 2, Biofix was tested at varying levels in nursery pig diets containing an average of 2.0 and 4.1

mg/kg DON, respectively. Although adding 0.15% Biofix improved ADFI in low-DON diets, in this experiment, pigs fed the low-DON diets (2.0 mg/kg) did not differ in overall growth performance compared with the PC; moreover, adding 0.30% or 0.45% Biofix to high-DON diets tended to reduce pig weights, although similar levels of Biofix in previous research had also shown no improvements in performance due to Biofix (Mahan, 2010). Biofix was theorized to be more effective in diets containing added moisture by potentially enhancing the microbial activity of the Saccharomyces cerevisiae yeast culture. In the present experiment, adding 5% water to a diet containing 0.45% Biofix had no effect on growth performance. Heat and a stale odor also built up over time in this diet, which may have contributed to the lack of response. Based on the accumulation pattern of DON during grain storage described by Langseth et al. (1993), samples from this diet were collected on d 0, 7, 14, and 21 and analyzed to evaluate any change in DON level, which increased slightly (3.0 to 3.8 mg/kg) during the experimental period. Overall, the results of Exp. 1 and 2 reaffirm previous in vivo research, where the use of enzymes and adsorbent materials aimed at binding to the DON molecule in the gastrointestinal tract were largely ineffective (Dänicke et al., 2004; Awad et al., 2010).

In Exp. 3, adding Defusion to diets containing 2 mg/kg DON improved ADG by approximately 4% and 6% when 0.25% or 0.50% Defusion was added, respectively, which agrees with previous research in which Defusion responses were primarily driven by ADFI improvements (Mahan 2010; Patience 2011). Overall, pigs fed 0.50% Defusion performed similarly to pigs fed PC diets; furthermore, pelleting the same diet elicited a typical pelleting response, improving ADG by 7.5%, primarily through enhanced feed efficiency, thus surpassing the growth performance of pigs fed PC diets (637 vs. 617 g/day). Although adding Defusion to diets with low-DON improved growth performance in Exp. 3, when DON levels were elevated to approximately 3 mg/ kg in Exp. 4, Defusion had no effect on overall growth performance. Even so, pelleting DON-contaminated diets with and without Defusion increased ADG by over 9% in Exp. 4, offsetting the approximate 7% reduction caused by feeding diets containing 3 mg/kg DON. In both experiments, supplementing additional nutrients in DON-contaminated diets containing 0.25% Defusion had no effect on ADG. No interactions occurred between pelleting and adding Defusion on pig growth performance, but it is notable that pelleting diets containing Defusion resulted in analyzed DON levels 91% and 71% lower for Exp. 3 (2.2 vs. 1.1 mg/kg) and Exp. 4 (2.9 vs. 1.7 mg/kg), respectively.

Defusion contains a variety of ingredients, including preservatives, organic acids, fermentation products, and

supplemental vitamins and amino acids; consequently, many possible mechanisms operating independently or in some combination could improve growth performance of pigs fed DON-contaminated diets. One specific ingredient in Defusion is sodium metabisulfite, which, when mixed in combination with heat and moisture, chemically alters the structure of DON to a nontoxic DONsulfonate adduct form (Young et al., 1987). Therefore, the pelleting process in the presence of Defusion in Exp. 3 and 4 may have provided the hydrothermal action necessary to convert DON and reduce analyzed DON levels, which may be partly responsible for increased feed intake. Defusion-related improvements in feed efficiency as well as the growth performance improvements when diets were fed in meal form cannot be explained by sodium metabisulfite alone, which suggests that additional ingredients in Defusion might also have an effect. Defusion contains L-Trp, which may play a role because increasing the Trp:Lys ratio above 0.18 has shown some improvement in feed conversion (Vinyeta et al., 2010); however, a study by Rotter et al. (1996b) showed no evidence that supplemental tryptophan could modulate DON toxicity. Regarding fermentation products, a recent study by Li et al. (2011) reported improvements in pig growth in DON-contaminated diets when Bacillus sp. LS 100 was isolated from chicken digesta and mixed into the diet, suggesting that the addition of Bacillus cultures to Defusion may be able to initiate some microbial detoxification of DON.

The variation in response to Defusion seen in the present study also may be attributed to the fact that in Exp. 1, Defusion Plus, which contains additional flow agents, was used, whereas in Exp. 3 and 4, the standard Defusion product was used. In Exp. 1, adding Defusion Plus at an inclusion rate of 0.25% resulted in increased ADG, driven primarily by an improvement in G:F. Defusion was used at 0.25% in Exp. 3 and 4 and did not improve ADG, but the addition of 0.50% Defusion in Exp. 3 did increase ADG. In this case, the improvement in ADG was driven primarily by improvements in ADFI. The variability in DON or the presence of low levels of other mycotoxins between experiments may have influenced the response to Defusion, but it may also indicate that the ingredient differences between products may also influence nursery pig growth performance.

Deoxynivalenol from naturally contaminated corn DDGS caused reductions in performance in pigs similar to those other researchers have found with DON from other cereal grains. The use of adsorbent materials (e.g., Biofix and Cel-can with bentonite clay) in DON-contaminated diets was ineffective in the present study; nevertheless, Defusion showed promise, because it improved nursery pig growth performance in 2 of 3 experiments. The addition of 0.25% to 0.50% Defusion may lessen the impact of DON in nursery pig diets when dietary DON levels are below 5 mg/ kg, although the mechanism by which performance is improved remains unclear. Finally, the present experiments infer that when feeding DON-contaminated diets, pelleting the diet can provide a predictable improvement in ADG and G:F, which may serve as a way to mitigate DON-related reductions in performance.

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