

The progression of deoxynivalenol-induced growth suppression in nursery pigs and the potential of an algae-modified montmorillonite clay to mitigate these effects^{1,2}

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ABSTRACT: Two experiments were conducted to characterize the progression of deoxynivalenol (DON)-induced growth suppression and to investigate algae-modified montmorillonite clay (AMMC) as a means to alleviate the effects of DON in nursery pigs. In both experiments, naturally DON-contaminated wheat was used to produce diets with desired DON levels. In Exp. 1, 280 barrows and gilts (10.0 ± 0.2 kg BW) were used in a 28-d experiment arranged in a $2 \times 2 + 1$ factorial design with 8 replicates per treatment. The 5 treatments consisted of 2 positive control (PC) diets with DON below detection limits and with or without 0 or 0.50% AMMC and 3 negative control (NC) diets with 5 mg/kg of DON and containing 0, 0.25, or 0.50% AMMC. No DON \times AMMC interactions were observed. Overall, pigs fed DON had decreased ($P < 0.001$) ADG and final BW regardless of AMMC addition. Feeding DON-contaminated diets elicited the most severe depression ($P < 0.001$) in ADFI and G:F from d 0 to 3, remaining poorer overall ($P < 0.01$) but lessening in severity as exposure time increased. Pigs fed DON diets had greater ($P < 0.05$) within pen BW variation (CV) on d 28. Although the addition of 0.50% AMMC to diets restored ($P < 0.05$) ADFI from d 14 to 21 to levels similar to the PC, no

other differences were observed for AMMC inclusion. In Exp. 2, 360 barrows (11.4 ± 0.2 kg BW) were used in a 21-d experiment with 9 dietary treatments arranged in a 3×3 factorial design with DON and AMMC inclusion as main effects. There were 8 replicate pens per treatment. Treatments consisted of 3 PC diets without DON, 3 low-DON (1.5 mg/kg DON) NC diets, and 3 high-DON (3 mg/kg DON) NC diets with 0, 0.17, or 0.50% AMMC incorporated at each DON level. No DON \times AMMC interactions were observed. As DON level increased, ADG and final BW decreased (quadratic, $P < 0.05$), driven by decreased (quadratic, $P < 0.01$) ADFI and poorer (quadratic; $P < 0.05$) G:F. At both 1.5 and 3 mg/kg DON, reductions in ADG were most marked from d 0 to 7 (15 to 22% lower) and were least distinct from d 14 to 21 (5 to 6% lower). Incorporating AMMC at increasing levels had no effect on ADG, ADFI, G:F, or final BW. Overall, these experiments reinforce DON effects on feed intake but also indicate that the effects of DON on G:F may be more severe than previously thought. Furthermore, some pigs appear to develop tolerance to DON, as effects on ADFI and G:F lessen over time. However, the addition of AMMC did not offset the deleterious effects of DON.

Key words: deoxynivalenol, detoxifying agents, montmorillonite clay, nursery pig, swine, vomitoxin

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INTRODUCTION

Deoxynivalenol (DON) is a member of the type B trichothecenes, which are potent inhibitors of protein synthesis (Rotter et al., 1996). Primarily produced by *Fusarium* fungi, trichothecenes proliferate in cereal grains when flowering coincides with temperate, wet conditions (CAST, 2003). According to a global

survey, DON is the most common mycotoxin in North American feedstuffs, present in 75% of samples at an average of 1.3 mg/kg (Rodrigues and Naehrer, 2012).

Among farm animals, pigs are the most sensitive to DON (Eriksen and Petterson, 2004). While vomiting occurs at high concentrations (Forsyth et al., 1977; Pestka et al., 1987), most reports agree that realistic DON levels (1 to 5 mg/kg) primarily decrease feed intake (Friend et al., 1984; Patience et al., 2014). Deoxynivalenol also reduces intestinal absorption (Grenier and Applegate, 2013) and stimulates and suppresses the immune system (Rotter et al., 1996; Pestka et al., 2004). The severity seems to be dose-dependent, and fluctuations may be related to contradictory feed efficiency effects (Etienne and Waché, 2008). Some reports have observed DON effects lessening over time (Friend et al., 1984; Pollman et al., 1985), but this phenomenon is not well-characterized.

Since environmental conditions dictate DON growth, various methods have been tested to detoxify DON prior to feeding, and feed additives appear to be the most practical (Dänicke, 2002; Awad et al., 2010). Clay minerals used successfully against polar toxins, such as aflatoxins, have poor DON adsorption (Ramos et al., 1996; EFSA, 2009). However, an algae-modified montmorillonite clay (AMMC; Olmix S.A., Brehan, France) has been developed using algal polysaccharides, which enhance the DON adsorptive capacity (Havenaar and Demais, 2006). Therefore, the objectives of the present research were to further characterize the progression of DON-induced suppression of growth and to investigate AMMC as a means to alleviate the effects of DON in nursery pigs.

MATERIALS AND METHODS

All experimental procedures and animal care were approved by the Kansas State Institutional Animal Care and Use Committee. In both experiments, diets were corn–soybean meal based, and sources of both low-DON and naturally DON-contaminated hard red winter wheat were provided by Olmix N.A. (Black River Falls, WI). To maintain consistency and ensure that diets contained the desired levels of DON, basal ingredients (corn and the 2 wheat sources) were analyzed for mycotoxin concentration at North Dakota State University Veterinary Diagnostic Laboratory (NDSU; Fargo, ND) prior to diet formulation and incorporated into test diets to achieve desired DON concentrations. At the manufacturer's request, the DON-contaminated wheat was also analyzed for mycotoxin content at LABOCEA (Ploufragan, France). Due to concerns that high-DON wheat may also have a different AA profile than low-DON wheat, both were analyzed for AA content at the University of Missouri Agricultural Experiment Station Chemical Laboratories (Columbia, MO), and diet formulation was adjusted to account for

the differences. Diets were formulated to meet or exceed all nutrient requirement estimates (NRC, 2012). As recommended by Döll and Dänicke (2003), to evaluate both the specific and unspecific effects of the AMMC feed additive, factorial designs were used in both experiments. The AMMC product is made up primarily of montmorillonite and 10 to 20% algae. According to a chemical analysis conducted at Dairyland Laboratories (St. Cloud, MN), AMMC contained 6.27% CP, 4.5% sugar, 4.3% Ca, 0.94% Na, 0.90% Cl, 0.17% P, and 1.06% K.

Experiment 1

A total of 280 barrows and gilts (PIC 327 × 1050; Hendersonville, TN) were used in a 28-d experiment to determine the effects of DON and AMMC on nursery pig growth. Pigs were initially 10.0 ± 0.2 kg BW and 35 d of age, and there were 8 replicate pens per treatment with 7 pigs in each pen. At weaning, pigs were allotted to pens by initial BW, individual variation in BW, and gender. Pigs were fed a common commercial starter diet for 7 d, at which time they were reweighed and pens were assigned to 1 of 5 treatments in a completely randomized design.

Treatments were arranged in a $2 \times 2 + 1$ factorial with DON and AMMC inclusion as main effects. Treatments consisted of 2 positive control diets (PC; < 0.5 mg/kg DON), containing either 0 or 0.50% AMMC, and 3 negative control diets (NC), formulated to contain 5 mg/kg DON and either 0, 0.25, or 0.50% AMMC. Apart from the inclusion of DON and AMMC, diets were formulated to be identical in nutrient composition.

Diets were manufactured at the Kansas State University Grain Science Feed Mill. While the stability of AMMC under pelleting conditions is unknown, due to concerns of ingredient segregation, diets were pelleted. A naturally contaminated source of high-DON wheat (10.7 mg/kg DON) was used to provide diets with 5 mg/kg DON. Following final diet manufacturing, diet samples were sent to NDSU for mycotoxin analysis. Only mycotoxins detected above quantitative limits in at least 1 of the experimental diets were reported. Final diets were also sent to the University of Missouri for nutrient analysis.

The trial was conducted at the K-State Swine Teaching and Research Center in Manhattan, KS. Each pen (1.22 by 1.52 m) contained a 4-hole, dry self-feeder and a nipple waterer to provide ad libitum access to feed and water. Pigs were examined daily, and feeders were adjusted to maintain approximately 50% pan coverage. Average daily gain, ADFI, and feed efficiency were determined by weighing pigs and measuring feed disappearance on d 0, 3, 7, 14, 21, and 28. Since pens were initially balanced for within-pen

weight variation, pen CV was also calculated at d 28 to evaluate the effect of DON on pig BW variation.

Experiment 2

A total of 360 barrows (Line 1050; PIC; initially 11.4 ± 0.2 kg and 45 d of age) were used in a 21-d experiment to further characterize the effects of DON and AMMC on nursery pig growth. Pigs were shipped to the facility immediately postweaning and were placed in 2 identical nurseries, each containing 40 pens. Upon arrival, pigs were allotted to pens by BW and fed a common commercial diet for the first 24 d. After pigs reached approximately 12 kg, pens were randomly assigned to 1 of 9 dietary treatments. There were 5 pigs per pen and 8 replicate pens per treatment.

Dietary treatments were arranged in a 3×3 factorial design with DON and AMMC inclusion as the main effects. Treatments consisted of 3 PC diets without DON, 3 low-NC (1.5 mg/kg DON) diets, and 3 high-NC (3 mg/kg DON) diets with 0, 0.17, or 0.50% AMMC incorporated at each level of DON. Diets were manufactured in meal form at the Kansas State University O. H. Kruse Feed Mill in Manhattan, KS.

The 0.17% AMMC inclusion rate was chosen to reflect the manufacturer-recommended feeding level, and the 0.50% inclusion was added to test the ingredient at concentrations known to be effective when similar absorptive clays are added to aflatoxin-contaminated grains (Schell et al., 1993). The AMMC was added at the expense of corn in the diet formulation. Diets exceeded NRC (2012) nutrient requirements, and apart from the inclusion of DON and AMMC, were formulated to be identical in nutrient composition.

Because of a concern that pelleting may have impacted the efficacy of AMMC in Exp. 1, diets were manufactured in meal form at the Kansas State University O. H. Kruse Feed Mill in Manhattan, KS in Exp. 2. A naturally contaminated source of high-DON wheat (6.0 mg/kg DON) was used to provide diets with the desired DON concentrations. Following final diet manufacturing, diet samples were sent to NDSU for mycotoxin analysis. Only mycotoxins detected above quantitative limits in at least 1 of the experimental diets were reported. Final diets were also sent to the University of Missouri for nutrient analysis.

This experiment was conducted at the Kansas State University Segregated Early Weaning Research Facility in Manhattan, KS. Each pen (1.22 by 1.22 m) contained a 4-hole dry self-feeder and a 1-cup waterer to provide ad libitum access to feed and water. Pigs were examined daily, and feeders were adjusted to maintain approximately 50% pan coverage. Average daily gain, ADFI, and G:F were determined

Table 1. Amino acid analysis of hard red winter wheat source, Exp. 1 and 2 (as-fed basis)¹

Item, %	Exp. 1		Exp. 2	
	Low DON ²	High DON	Low DON	High DON
Moisture	9.47	9.83	9.14	10.19
CP	12.86	10.16	11.80	12.20
AA analysis				
Lys	0.40	0.37	0.40	0.44
Ile	0.47	0.36	0.41	0.47
Leu	0.91	0.72	0.84	0.87
Met	0.21	0.16	0.21	0.22
Cys	0.28	0.22	0.27	0.27
Thr	0.37	0.30	0.36	0.38
Trp	0.18	0.12	0.15	0.17
Val	0.62	0.50	0.57	0.47

¹Samples were analyzed for AA profile at the University of Missouri Experiment Station Chemical Laboratories in Columbia, MO.

²DON = deoxynivalenol.

by weighing pigs and measuring feed disappearance on d 0, 3, 7, 14, and 21.

Mycotoxin Analysis

In both experiments, samples of the basal ingredients (corn and 2 wheat sources) and final diets were sent to NDSU for an 18-component mycotoxin analysis. The analysis for trichothecene mycotoxins (DON, 15-acetyldeoxynivalenol, 3-acetyldeoxynivalenol, 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 were analyzed by HPLC. Samples were tested on an as-fed basis, and the practical quantitation limit for trichothecenes was 0.50 mg/kg while the detection limits were 2.0 mg/kg for fumonisins and 20 µg/kg for aflatoxins. In both studies, the high-DON wheat was also sent to LABOCEA where a 43-component toxin screen was performed using liquid chromatography coupled with tandem mass spectrometry techniques. For all toxins, the minimum detection limit at LABOCEA was 10 µg/kg feed.

Statistical Analysis

For both experiments, results were analyzed as a completely randomized design using the MIXED procedure of SAS (SAS Inst., Inc., Cary, NC). The fixed factors in the models included DON level and AMMC inclusion. For Exp. 1, differences were evaluated using pre-planned contrasts, which included 1) the 2-way interaction evaluating the effect of AMMC inclusion at 0.50% compared to none in the PC and NC diets, 2) pigs fed PC vs. NC diets regardless of AMMC inclu-

Table 2. Formulated diet composition, Exp. 1 (as-fed basis)

Item AMMC ² :	Positive control (<0.5 mg/kg DON ¹)		Negative control (5.0 mg/kg DON)		
	None	0.50%	None	0.25%	0.50%
Corn	16.90	16.40	16.33	16.20	15.88
Soybean meal, 46.5% CP	30.93	30.98	31.45	31.35	31.45
Hard red winter (HRW) wheat	46.75	46.75			
High-DON ³ HRW wheat			46.75	46.75	46.75
Soybean oil	2.00	2.00	2.00	2.00	2.00
Monocalcium phosphate, 21% P	1.05	1.05	1.05	1.05	1.05
Limestone	1.05	1.00	1.05	1.03	1.00
Salt	0.35	0.35	0.35	0.35	0.35
Vitamin premix with phytase ^{4,5}	0.25	0.25	0.25	0.25	0.25
Trace mineral premix ⁶	0.15	0.15	0.15	0.15	0.15
L-Lys HCl	0.33	0.33	0.33	0.33	0.33
DL-Met	0.10	0.10	0.15	0.15	0.15
L-Thr	0.14	0.14	0.14	0.14	0.14
AMMC		0.50		0.25	0.50
Total	100	100	100	100	100
Calculated analysis					
SID ⁷ AA, %					
Lys	1.28	1.28	1.28	1.28	1.28
Ile:Lys	65	65	62	62	62
Leu:Lys	120	120	115	115	115
Met:Lys	31	31	33	33	33
Met and Cys:Lys	58	58	58	58	58
Thr:Lys	64	64	64	64	64
Trp:Lys	20.7	20.7	18.9	18.9	18.9
Val:Lys	72	72	69	69	69
Total Lys, %	1.42	1.42	1.42	1.42	1.42
ME, kcal/kg	3318	3303	3318	3309	3303
SID Lys:ME, g/Mcal	3.86	3.89	3.87	3.87	3.89
CP, %	22.3	22.3	21.2	21.2	21.3
Ca, %	0.73	0.73	0.73	0.73	0.74
P, %	0.65	0.65	0.66	0.66	0.66
Available P, %	0.48	0.48	0.49	0.48	0.48

¹DON = deoxynivalenol.

²AMMC = algae-modified montmorillonite clay product (Olmix S.A., Brehan, France).

³Analyzed DON concentration in HRW wheat was 10.7 mg/kg.

⁴Provided per kilogram of premix: 4,409,200 IU vitamin A; 551,150 IU vitamin D₃; 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 B₁₂.

⁵Phyzyme 600 (Danisco Animal Nutrition, St. Louis, MO) provided 750 phytase units phytase/kg of diet and 0.13% available P released.

⁶Provided per kilogram of premix: 22.0 g Mn from manganese oxide, 73.4 g Fe from iron sulfate, 73.4 g Zn from zinc sulfate, 11.0 g Cu from copper sulfate, 198 mg I from calcium iodate, and 198 mg Se from sodium selenite.

⁷SID = standardized ileal digestible.

sion, 3) the addition of AMMC (0 vs. 0.50%) in both PC and NC diets, and 4) the linear effects of AMMC inclusion within NC diets alone.

In Exp. 2, the main effects of DON level (0, 1.5, or 3.0 mg/kg) and AMMC inclusion (0, 0.17, or 0.50%) and their 2-way interactions served as fixed effects and barn as a random effect in the model. Preplanned linear and quadratic orthogonal contrasts were used to evalu-

ate the effect of dose. The coefficients for the unequally spaced linear and quadratic contrasts were derived using the IML procedure in SAS. In both experiments, pen was used as the experimental unit, and least squares means were calculated for each independent variable.

Differences were considered significant at *P* values of ≤ 0.05 and marginally significant at *P* values of > 0.05 and ≤ 0.10 .

Table 3. Nutrient analysis of experimental diets, Exp. 1 (as-fed basis)¹

Item, %	Positive control (<0.5 mg/kg DON ²)		Negative control (5.0 mg/kg DON)		
	None	0.50%	None	0.25%	0.50%
AMMC ³ :					
Moisture	10.88	10.80	10.74	10.63	10.76
CP	23.00	23.26	22.29	22.04	22.51
Ether extract	3.16	3.22	2.93	3.08	3.02
Ash	5.03	5.41	5.68	5.82	5.82
AA analysis					
Lys	1.41	1.42	1.41	1.41	1.49
Ile	0.87	0.90	0.85	0.84	0.91
Leu	1.71	1.71	1.66	1.60	1.67
Met	0.43	0.41	0.45	0.41	0.49
Cys	0.38	0.36	0.35	0.32	0.36
Thr	0.90	0.90	0.84	0.86	0.89
Trp	0.30	0.29	0.28	0.30	0.30
Val	0.99	1.01	0.96	0.95	1.02

¹Samples were analyzed at the University of Missouri Agricultural Experiment Station Chemical Laboratories in Columbia, MO.

²DON = deoxynivalenol.

³AMMC = algae-modified montmorillonite clay (Olmix S. A., Brehan, France).

RESULTS

Experiment 1

The AA concentrations (Table 1) of low-DON wheat were generally greater than those of the DON-contaminated wheat, and differences were accounted for in diet formulation (Table 2). Nutrient analyses of experimental diets were consistent with formulated levels, and increased analyzed ash content in AMMC diets correspond with the presence of additional clay (Table 3). Given the analyzed DON concentrations of the low- and high-DON wheat (Table 4), the analyzed DON concentrations of the PC diets accurately reflected formulated levels of <0.5 mg/kg, whereas the NC diets averaged 6.6 mg/kg DON (range 6.4 to 6.7 mg/kg), approximately 20% greater than formulated (Table 5). Although fumonisin B₁ was detected at 2.0 mg/kg in the NC diet without AMMC, analyses confirmed that no other mycotoxins were detected in PC diets above detection limits. Aflatoxin B₁ was detected at low levels (20 and 28 μ g/kg) in 2 of 3 NC diets, but no other mycotoxins were detected in NC diets.

For pig growth performance (Table 6), a 2-way DON \times AMMC interaction was detected from d 4 to 7, where the addition of AMMC improved ($P < 0.05$) ADG and G:F in NC diets but worsened ADG and G:F in PC diets. No other interactions were detected within period or overall, nor were any linear effects detected for increasing the inclusion rate of AMMC.

From d 0 to 3, pigs fed NC diets had decreased ($P < 0.001$) ADG, ADFI, and feed efficiency compared to

Table 4. Mycotoxin analysis of basal ingredients, Exp. 1 (as-fed basis)

Item, mg/kg	Ground corn	Hard red winter wheat	
		Low DON ¹	High DON
NDSU ²			
DON	<0.50	<0.50	10.60 ³
LABOCEA ⁴			
DON	NT ⁵	NT	10.70
15-Acetyl DON	NT	NT	0.12
Zearalenone	NT	NT	0.35
Fumonisin B ₁	NT	NT	0.03

¹DON = deoxynivalenol.

²North Dakota State University (NDSU) Veterinary Diagnostic Laboratory, Fargo, ND. Samples were sent for an 18-component mycotoxin analysis and were analyzed using a variety of mass spectrometry, ELISA, and HPLC methods. Included in the table are mycotoxins found at levels above detection limits (0.5 mg/kg).

³Mean of two duplicate samples sent to NDSU. Individual samples had DON levels of 10.0 and 11.1 mg/kg, respectively.

⁴LABOCEA, Ploufragan, France. Samples were analyzed using a 43-component toxin screen using liquid-chromatography coupled with tandem mass spectrometry analysis techniques. Included in the table are mycotoxins found at levels above detection limits (10 μ g/kg).

⁵NT = sample not tested.

pigs fed PC diets. The addition of AMMC to diets had no effect on pig growth. A similar pattern of growth was observed from d 4 to 7, with pigs fed NC diets having decreased ($P < 0.05$) ADG, ADFI, and G:F compared to PC diets, and no differences in growth were detected for AMMC inclusion. From d 7 to 14, pigs fed NC diets continued to have decreased ($P < 0.001$) ADG driven by reduced ($P < 0.05$) feed intake, but no effect of feed efficiency was observed during this period. Once again, the addition of AMMC did not impact pig performance.

From d 14 to 21, ADG and ADFI were decreased ($P < 0.05$) for pigs fed NC diets, but feed efficiency was not affected. The addition of AMMC increased ($P < 0.05$) feed intake, but no effects on ADG or G:F were observed. During the final period (d 21 to 28),

Table 5. Mycotoxin analysis of experimental diets, Exp. 1 (as-fed basis)¹

Item AMMC ³ :	Positive control (<0.5 mg/kg DON ²)		Negative control (5.0 mg/kg DON)		
	None	0.50%	None	0.25%	0.50%
DON ² , mg/kg	<0.5	<0.5	6.6	6.7	6.4
Fumonisin B ₁ , mg/kg	2.0	<2.0	<2.0	<2.0	<2.0
Aflatoxin B ₁ , μ g/kg	<20	<20	20	28	<20

¹North Dakota State University Veterinary Diagnostic Laboratory, Fargo, ND. Samples were sent for 18-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 in at least one diet.

²DON = deoxynivalenol.

³AMMC = algae-modified montmorillonite clay (Olmix S.A., Brehan, France).

Table 6. Effects of deoxynivalenol (DON) and an algae-modified montmorillonite clay (AMMC; Olmix S.A., Brehan, France) on nursery pig performance, Exp. 1¹

Item AMMC:	Positive control (<0.5 mg/kg DON) ²		Negative control (5.0 mg/kg DON) ³			SEM	Probability ^{4,5} , <i>P</i> <	
	None	0.50%	None	0.25%	0.50%		DON	AMMC
d 0 to 3								
ADG, g	178	233	20	24	-1	45.4	0.001	0.511
ADFI, g	410	398	302	315	293	59.0	0.001	0.677
G:F	0.396	0.558	0.038	0.075	0.012	0.109	0.001	0.369
d 4 to 7								
ADG, g	411	340	233	262	261	21.3	0.001	0.141
ADFI, g	497	485	415	436	412	85.0	0.042	0.838
G:F	0.832	0.702	0.596	0.646	0.660	0.083	0.001	0.362
d 7 to 14								
ADG, g	526	530	418	396	458	17.5	0.001	0.178
ADFI, g	704	735	558	583	611	44.6	0.001	0.161
G:F	0.747	0.718	0.743	0.686	0.751	0.032	0.571	0.690
d 14 to 21								
ADG, g	527	586	476	462	483	32.2	0.001	0.138
ADFI, g	814	858	728	734	814	37.3	0.025	0.024
G:F	0.652	0.688	0.660	0.634	0.603	0.024	0.113	0.674
d 21 to 28								
ADG, g	676	633	579	537	559	35.5	0.023	0.383
ADFI, g	1,035	1,022	934	907	1018	36.8	0.166	0.348
G:F	0.656	0.618	0.622	0.590	0.554	0.033	0.156	0.124
d 0 to 28								
ADG, g	533	516	420	403	421	14.2	0.001	0.581
ADFI, g	782	784	686	693	726	23.2	0.003	0.364
G:F	0.683	0.658	0.614	0.585	0.583	0.021	0.002	0.201
Pig BW, kg								
d 0	10.2	10.0	9.90	10.0	10.0	0.09	0.251	0.332
d 28	24.9	24.4	21.7	21.4	21.8	0.41	0.001	0.632
Pen CV, %								
d 0	14.1	13.8	14.2	14.4	14.7	1.00	0.226	0.763
d 28	13.6	12.4	17.1	16.4	14.8	0.015	0.051	0.249

¹A total of 280 barrows and gilts (PIC 327 × 1050; 35 d of age) were used in this 28-d study, with 7 pigs per pen and 8 pens per treatment.

²Formulated levels. A high-DON wheat source was used to produce diets with 5 mg/kg DON.

³Analyzed DON averaged <0.5 and 6.6 mg/kg for positive and negative control diets, respectively.

⁴A 2-way DON × AMMC interaction was detected ($P < 0.01$) from d 4 to 7 where the addition of AMMC improved ADG and G:F in negative control diets, but worsened ADG and G:F in positive control diets. No other interactions were detected within period or overall.

⁵No linear effects ($P > 0.05$) due to AMMC inclusion within DON-contaminated diets were found. AMMC contrast compares diets without AMMC to those containing AMMC at 0.50%.

pigs fed NC diets had decreased ($P < 0.05$) ADG, but no other treatment effects were seen.

Overall (d 0 to 28), pigs fed the NC diets had reduced ADG ($P < 0.001$), driven by poorer ($P < 0.01$) ADFI and G:F, which resulted in decreased ($P < 0.001$) final BW compared to PC-fed pigs. However, the addition of AMMC had no effect. Coefficient of variation of pig BW within pen tended ($P = 0.051$) to be greater in pigs fed NC diets vs. those fed the PC diets.

Experiment 2

Since CP and AA levels were marginally but consistently greater in the DON-contaminated wheat (Table 1),

the soybean meal fraction was increased slightly in PC diets to reflect this difference (Table 7). In the experimental diets, proximate analyses were generally in line with formulated values, and the addition of AMMC was reflected by greater ash contents in those diets (Table 8). Analyzed DON concentrations (Table 9) in the naturally DON-contaminated wheat differed between NDSU (8.4 mg/kg) and LABOCEA (6.0 mg/kg). Low levels of several other mycotoxins were detected in the DON-contaminated wheat source. To ensure that final diet DON levels were adequate to achieve a DON-associated reduction in performance, the analysis from LABOCEA was used as the basis for diet formulation. Analyzed DON in the final diets revealed levels that were within 20% of

Table 7. Formulated diet composition, Exp. 2 (as-fed basis)

Item AMMC ² :	Positive control (<0.5 mg/kg DON ¹)			Low negative control (1.5 mg/kg DON)			High negative control (3.0 mg/kg DON)		
	None	0.17%	0.50%	None	0.17%	0.50%	None	0.17%	0.50%
Corn	15.07	14.89	14.53	15.35	15.17	14.81	15.63	15.45	15.09
Soybean meal, 46.5% CP	31.58	31.60	31.62	31.25	31.26	31.29	30.92	30.93	30.96
Hard red winter (HRW) wheat	50.00	50.00	50.00	25.00	25.00	25.00			
High-DON ³ HRW wheat				25.00	25.00	25.00	50.00	50.00	50.00
Monocalcium phosphate, 21% P	1.05	1.05	1.05	1.05	1.05	1.05	1.05	1.05	1.05
Limestone	1.00	1.00	1.00	1.05	1.05	1.05	1.10	1.10	1.10
Salt	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
L-Lys HCl	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33
DL-Met	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
L-Thr	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13
Vitamin premix with phytase ^{4,5}	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Trace mineral premix ⁶	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
AMMC		0.17	0.50		0.17	0.50		0.17	0.50
Total	100	100	100	100	100	100	100	100	100
Calculated analysis									
SID ⁷ AA, %									
Lys	1.28	1.28	1.28	1.28	1.28	1.28	1.28	1.28	1.28
Ile:Lys	64	64	64	65	65	65	65	65	65
Leu:Lys	118	118	117	118	118	118	117	117	117
Met:Lys	31	31	31	31	31	31	31	31	31
Met and Cys:Lys	57	57	57	57	57	57	57	57	57
Thr:Lys	63	63	63	63	63	63	63	63	63
Trp:Lys	21.2	21.2	21.2	20.7	20.7	20.7	20.3	20.3	20.2
Val:Lys	68	68	68	69	69	69	70	70	70
Total Lys, %	1.43	1.43	1.43	1.43	1.43	1.43	1.43	1.43	1.43
ME, kcal/kg	3,183	3,179	3,165	3,181	3,177	3,165	3,181	3,175	3,164
SID Lys:ME, g/Mcal	4.02	4.03	4.04	4.02	4.03	4.04	4.02	4.03	4.05
CP, %	22.7	22.7	22.6	22.6	22.6	22.6	22.6	22.6	22.6
Ca, %	0.68	0.68	0.68	0.69	0.69	0.69	0.71	0.71	0.71
P, %	0.68	0.68	0.68	0.69	0.69	0.69	0.71	0.71	0.70
Available P, %	0.50	0.50	0.50	0.51	0.51	0.51	0.51	0.51	0.51

¹DON = deoxynivalenol.

²AMMC = algae-modified montmorillonite clay product (Olmix S.A., Brehan, France).

³Analyzed DON concentration in HRW wheat was 6.0 mg/kg at LDA Laboratories (Ploufragan, France).

⁴Provided per kilogram of premix: 4,409,200 IU vitamin A; 551,150 IU vitamin D₃; 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 B₁₂.

⁵Phyzyme 600 (Danisco Animal Nutrition, St. Louis, MO) provided 750 phytase units phytase/kg and 0.13% available P released.

⁶Provided per kilogram of premix: 22.0 g Mn from manganese oxide, 73.4 g Fe from iron sulfate, 73.4 g Zn from zinc sulfate, 11.0 g Cu from copper sulfate, 198 mg I from calcium iodate, and 198 mg Se from sodium selenite.

⁷Standardized ileal digestible.

the targeted DON level, averaging 1.7 and 3.2 mg/kg for the 1.5 and 3.0 mg/kg targets, respectively (Table 10).

A DON × AMMC interaction (linear, $P < 0.05$) was observed from d 0 to 7, where increasing AMMC improved ADG in PC and low-NC diets but decreased ADG in high-NC diets (Table 11). The interaction for ADG appeared to be driven by a tendency for a G:F interaction (linear, $P < 0.05$) in which increasing AMMC inclusion worsened feed efficiency in high-NC diets, whereas feed efficiency remained similar in pigs fed PC and low-NC diets regardless of AMMC inclusion. Furthermore, a tendency for a DON × AMMC interaction for feed ef-

iciency (quadratic, $P = 0.073$) was observed from d 14 to 21, where increasing AMMC in PC and low-NC diets worsened feed efficiency, whereas in high-NC diets, increasing AMMC initially improved but subsequently worsened G:F at the 0.50% inclusion rate.

For the main effects of DON and AMMC on growth performance (Table 12), from d 0 to 3, ADG, ADFI, and G:F decreased (linear, $P < 0.001$) with increasing DON concentrations. From d 4 to 7, increasing DON level progressively worsened ADG ($P < 0.05$), driven not by ADFI but as a consequence of poorer ($P < 0.05$) G:F. From d 7 to 14, ADG decreased (linear, $P < 0.001$)

Table 8. Nutrient analysis of experimental diets, Exp. 2 (as-fed basis)¹

Item, %	Positive control (<0.5 mg/kg DON ²)			Low negative control (1.5 mg/kg DON)			High negative control (3.0 mg/kg DON)		
	None	0.17%	0.50%	None	0.17%	0.50%	None	0.17%	0.50%
AMMC ³ :									
Moisture	9.77	9.60	9.93	10.04	9.77	9.89	9.97	9.66	9.75
CP	24.9	23.8	24.2	23.4	23.2	23.3	23.5	23.7	23.5
ADF	2.6	2.4	2.1	2.7	3.5	2.2	2.5	2.6	2.6
NDF	7.6	7.0	7.5	7.6	8.0	7.4	6.8	7.2	7.2
Ether extract	2.4	2.6	2.5	2.6	2.9	2.6	2.6	2.8	2.7
Ash	5.14	5.31	5.53	5.53	5.61	5.57	5.65	5.73	5.96
Ca	0.71	0.81	0.82	0.86	0.83	0.76	0.87	0.83	0.89
P	0.74	0.67	0.68	0.69	0.69	0.71	0.69	0.71	0.74

¹Samples were analyzed at the University of Missouri Agricultural Experiment Station Chemical Laboratories in Columbia, MO.

²DON = deoxynivalenol.

³AMMC = algae-modified montmorillonite clay (Olmix S.A., Brehan, France).

as DON increased in the diet. This growth reduction was influenced primarily by progressively poorer (quadratic, $P < 0.001$) G:F as ADFI decreased (quadratic, $P < 0.001$) and then recovered with increasing DON concentrations. From d 14 to 21, increasing DON level tended to decrease (linear, $P = 0.087$) ADG, and increasing AMMC level tended to reduce (linear, $P = 0.094$) feed efficiency. Overall (d 0 to 21), increasing DON concentration in nursery pig diets progressively worsened (linear, $P < 0.001$) ADG and final BW, governed predominantly by a decrease (linear, $P < 0.001$) in feed efficiency with poorer ADFI (quadratic, $P < 0.001$) as a

contributing influence. The addition of AMMC had no effect on overall ADG, ADFI, G:F, or final BW.

DISCUSSION

The origin of the DON used in studies appears to be important (Eriksen and Petterson, 2004; Etienne and Waché, 2008). At equivalent DON levels, purified sources of DON elicited less severe growth depression compared to naturally contaminated DON sources, even when no other mycotoxins were detected (Trenholm et al., 1994). While this difference is yet to be explained, proposed hypotheses include the presence of other fungal components in naturally contaminated grains that contribute to DON toxicity, differential rates or degree of DON absorption, and potential undervaluation of DON due to the difficulty of analyzing toxin in a complex grain matrix (Etienne and Waché, 2008; Pestka, 2010). Finally, given that potential detoxifying agents are designed to prevent the effects of naturally contaminated feedstuffs, wheat predominately contaminated with DON was identified and used to incorporate into test diets at desired concentrations. Low concentrations of aflatoxin and fumonisin were also detected in Exp. 1 diets but at concentrations well below safe levels, determined as less than 200 $\mu\text{g}/\text{kg}$ for aflatoxin and 5 mg/kg for fumonisins (Thaler and Reese, 2010). Therefore, while multi-toxin interactive effects cannot be totally excluded, in the present study, mycotoxin analyses indicate that the observed growth responses were primarily due to DON.

Although rarely accounted for when testing DON-detoxifying agents, *Fusarium* pathogens are also known to alter the nutrient content and digestibility of the affected grain. Mätthaus et al. (2004) reported greater CP and ash contents and smaller kernels in wheat inoculated with *Fusarium culmorum*. Thanh et al. (2015) also observed increased analyzed N concentrations in diets containing DON-contaminated wheat. However, Dänicke et

Table 9. Mycotoxin analysis of basal ingredients, Exp. 2 (as-fed basis)

Item, mg/kg	Ground corn	Hard red winter wheat	
		Low DON ¹	High DON
NDSU ²			
DON	<0.50	<0.50	8.40
LABOCEA ³			
DON	NT ⁴	NT	6.03
De-epoxy DON	NT	NT	0.02
15- <i>O</i> -Acetyl DON	NT	NT	0.07
3-Acetyl DON	NT	NT	0.03
Zearalenone	NT	NT	0.02
HT-2 Toxin	NT	NT	0.02
Ergocryptine	NT	NT	0.08
Ergosine	NT	NT	0.02
Tenuazonic acid	NT	NT	0.05

¹DON = deoxynivalenol.

²North Dakota State University Veterinary Diagnostic Laboratory, Fargo, ND. Samples were sent for an 18-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.

³LABOCEA (Ploufragan, France). Samples were analyzed using a 43-component toxin screen using liquid chromatography/mass spectrometry analysis methods. Included in the table are mycotoxins found at levels above detection limits.

⁴NT = samples not tested.

Table 10. Mycotoxin analysis of experimental diets, Exp. 2 (as-fed basis)¹

Item	Positive control (<0.5 mg/kg DON ²)			Low negative control (1.5 mg/kg DON)			High negative control (3.0 mg/kg DON)		
	None	0.17%	0.50%	None	0.17%	0.50%	None	0.17%	0.50%
AMMC ³ :	None	0.17%	0.50%	None	0.17%	0.50%	None	0.17%	0.50%
DON, mg/kg	<0.5	<0.5	<0.5	1.7	1.8	1.7	3.4	2.7	3.5

¹Diet samples were analyzed at North Dakota State University Veterinary Diagnostic Laboratory, Fargo, ND. An 18-component mycotoxin analysis was conducted using a variety of mass spectrometry, ELISA, and HPLC methods. Included in the table are mycotoxins found at levels above detection limits.

²DON = deoxynivalenol.

³AMMC = algae-modified montmorillonite clay (Olmix S. A., Brehan, France).

al. (2004b) reported no differences in CP concentrations between wheat sources. In the present study, the high-DON wheat source was generally lower in CP and AA content in Exp. 1 and greater in AA content in Exp. 2. While *Fusarium*-induced fluctuations in nutrient content appear inconsistent, they highlight the need to account for differences during diet formulation so that the mycotoxin-specific effects and efficacy of detoxifying agents can be interpreted accurately. Contamination with *Fusarium* can also impact nutrient digestibility. In a pig growth study by Thanh et al. (2015), pigs fed DON-contaminated diets (4.6 mg/kg) had reduced DM, energy, and fat digestibility. However, this conflicts with previous reports where feeding DON-contaminated diets had no impact (Dänicke et al., 2004b) or even increased total tract nutrient digestibility in feed-restricted pigs (Dänicke et al., 2004a). Authors attributed these fluctuations in digestibility to variations in the grain varieties used, production of cell wall-degrading enzymes by *Fusarium* fungi, and DON-induced changes in intestinal absorption capacity (Bracarense et al., 2012).

Unlike some other mycotoxins, the effects of DON on tissue composition and blood metabolites are negligible and well-characterized (Swamy et al., 2002; Madson et al., 2014). Accordingly, these analyses were not measured in the present study. From a growth perspective, in the present studies, feeding diets containing approximately 1.7 or 3.2 mg/kg DON in Exp. 2 and 6.6 mg/kg DON in Exp. 1 decreased ADG by 10, 13, and 20%, respectively, compared to controls. A pair of meta-analyses (Dänicke, 2002; Etienne and Waché, 2008) both calculated that once dietary DON exceeds 1 mg/kg, BW gain decreases by approximately 7% for each additional mg of DON. In the present study, pigs fed low levels of DON in Exp. 2 generally followed these predictive equations, but the effects of feeding 6.6 mg/kg DON in Exp. 1 were not as severe as projected.

While it is known that DON is more rapidly and efficiently absorbed (55%) in pigs compared to other species and that pigs have limited ability to metabolize DON into less toxic forms (Prelusky et al., 1988; Goyarts and Dänicke, 2006; Wu et al., 2010), the variability in toxicity between individual pigs is not well characterized. During Exp. 1, within pen CV in BW in-

creased when pigs were fed DON-contaminated diets. This observation indicates that some pigs may be more sensitive to DON than others, may develop a tolerance to DON more rapidly, or may have a greater ability to metabolize DON. To our knowledge, the effects of DON on BW variation between similarly treated pigs have not been previously reported, but future studies should attempt to clarify this observation. Unfortunately, pens of pigs in Exp. 2 were not initially balanced for BW variation, and thus changes over time could not be evaluated.

In most pig growth studies with DON, decreased feed intake is the most commonly observed effect. This reduction in intake appears to be primarily associated with altered neuroendocrine signaling in the digestive and central nervous systems of the pig, particularly via elevated levels of serotonin (Prelusky, 1994; Rotter et al., 1996). Known to reduce intestinal motility and gastric emptying in rodents, this serotonergic effect is likely to impact pigs in a similar fashion (Fioramonti et al., 1993). However, Ossenkopp et al. (1994) also demonstrated that DON causes conditioned taste aversion in rats, mediated by the area postrema of the brain, which is likely to contribute to the anorexic effects of DON.

Previous reports indicate that unless DON levels exceed 1 mg/kg, the effects on pig growth are minimal; however, each additional milligram per kilogram of DON is predicted to decrease ADFI by 4 to 5% (reviewed by Dänicke [2002] and Etienne and Waché [2008]). In the present experiments, the effects of DON on feed intake were often less severe. In Exp. 1, feeding 6.6 mg/kg DON only reduced ADFI by 10%. In Exp. 2, feeding 1.7 mg/kg DON elicited an 8% decrease in ADFI, which is consistent with the prediction equation, but interestingly, ADFI was only reduced by 2% at the greater DON concentration of 3.2 mg/kg. In Exp. 1, ADFI remained suppressed throughout the study but to a much lesser extent during the last 2 wk compared to the initial 2 wk (6 vs. 22%). In Exp. 2, lower DON levels resulted in negligible feed intake effects after the initial exposure period. These results are consistent with earlier reports, where feed intake was often restored after 7 to 14 d if diets contained less than 3 mg/kg DON (Lun et al., 1985; Grosjean et al., 2002; Rempe et al., 2013). These observations also support the hypothesis

Table 11. Interactive effects of an algae-modified montmorillonite clay (AMMC) on growth performance of nursery pigs fed diets contaminated with low levels of deoxynivalenol (DON), Exp. 2^{1,2}

Item	Positive control (<0.5 mg/kg DON)			Low negative control ³ (1.5 mg/kg DON)			High negative control ³ (3.0 mg/kg DON)			SEM	Probability, <i>P</i> <	
	None	0.17%	0.50%	None	0.17%	0.50%	None	0.17%	0.50%		DON × AMMC	Linear
d 0 to 3												
ADG, g	409	387	418	325	280	346	275	292	198	34.8	0.049	0.124
ADFI, g	629	615	631	549	539	580	519	526	531	27.3	0.862	0.691
G:F	0.650	0.630	0.657	0.590	0.513	0.597	0.524	0.555	0.370	0.042	0.019	0.069
d 4 to 7												
ADG, g	426	394	435	363	382	383	389	367	360	28.0	0.446	0.397
ADFI, g	508	502	528	540	513	490	544	540	499	34.5	0.233	0.815
G:F	0.865	0.801	0.849	0.667	0.765	0.808	0.728	0.683	0.715	0.065	0.996	0.301
d 7 to 14												
ADG, g	576	572	599	537	490	527	484	481	506	32.2	0.987	0.315
ADFI, g	832	885	924	760	722	767	908	820	882	53.7	0.258	0.843
G:F	0.70	0.646	0.651	0.710	0.689	0.700	0.547	0.596	0.583	0.031	0.272	0.722
d 14 to 21												
ADG, g	672	688	652	667	624	603	639	643	641	20.9	0.527	0.277
ADFI, g	963	955	975	931	933	920	980	919	970	84.9	0.851	0.424
G:F	0.71	0.728	0.680	0.726	0.678	0.663	0.658	0.705	0.664	0.064	0.493	0.073
d 0 to 21												
ADG, g	556	550	559	517	484	499	488	486	479	14.6	0.618	0.268
ADFI, g	785	797	824	745	726	739	807	758	788	25.7	0.300	0.884
G:F	0.71	0.691	0.681	0.696	0.669	0.678	0.609	0.643	0.608	0.025	0.559	0.177
Pig BW, kg												
d 0	11.4	11.4	11.4	11.4	11.4	11.4	11.4	11.4	11.4	0.24	0.965	0.996
d 21	23.1	23.0	23.2	22.3	21.6	21.9	21.7	21.6	21.5	0.48	0.740	0.488

¹A total of 360 barrows (PIC 1050; initially 45 d of age) were used in a 21-d experiment with 8 pens per treatment and 5 pigs per pen. All diets were fed in meal form.

²Algae-modified montmorillonite clay (AMMC; Olmix S. A., Brehan, France).

³Denotes formulated levels. High-DON wheat (6.0 mg/kg) was used to incorporate DON into diets at desired concentrations.

that pigs develop some degree of adaptation to DON after the initial exposure period (Dersjant-Li et al., 2003). Development of tolerance to the anorectic effects of DON is congruent with observations that DON-induced taste aversion diminishes with time, which is common among anorexic compounds dependent on serotonergic mechanisms (reviewed by Rotter et al. [1996]).

While the effects of DON on feed intake are well-characterized, DON-induced changes in feed efficiency are multidimensional and less understood. At the cellular level, DON causes cell death via apoptosis and inhibits protein synthesis by obstructing translation at the ribosomal level, leading to ribotoxic stress syndrome (reviewed by Pestka [2010]). These effects are known to have the greatest impact on rapidly dividing cells, such as epithelial and immune cells in the gastrointestinal tract (GIT; Van De Walle et al., 2010). Thus, DON contamination causes compromised barrier function by decreasing the expression of tight junction proteins (Van De Walle et al., 2010; Pinton et al., 2012) and can increase the susceptibility of the GIT to bacterial infections (Grenier and Applegate, 2013). Exposure to DON also decreases the

rate of epithelial cell division, resulting in flattened intestinal villi and reducing the absorptive surface area for nutrient uptake (Bracarense et al., 2012). Combined with DON-induced leukocyte apoptosis, which suppresses immune function (Pestka et al., 2004), these effects are likely to contribute to growth retardation. Conceivably, these effects indicate that the toxicity of DON might be dramatically greater if exposure were to occur alongside bacterial infection. Nonetheless, the modulation of these digestive and immune functions by DON does not always affect animal growth parameters (Grenier and Applegate, 2013).

Feeding moderate levels of DON (3.5 to 6.6 mg DON) for extended periods (95 to 115 d) during the grow-finish phase consistently worsened feed efficiency in 3 experiments (Bergsjø et al., 1993; Dänicke et al., 2004a; Patience et al., 2014). However, in short-term studies on young pigs, the effects of DON on feed efficiency appear more transitory. In several growth studies, overall G:F was not affected by DON exposure (Friend et al., 1984; Pollman et al., 1985; Grosjean et al., 2002). Nonetheless, when reported by phase, pigs regularly have poorer G:F during the initial period (Pollman et al., 1985;

Table 12. Main effects of deoxynivalenol (DON) and algae-modified montmorillonite clay (AMMC; Olmix S. A., Brehan, France) on nursery pig performance, Exp. 2¹

Item	Formulated DON ² , mg/kg				SEM	AMMC, %			SEM	Probability, <i>P</i> <			
	<0.5	1.5	3.0	None		0.17%	0.50%	DON		AMMC			
								Linear		Quad	Linear	Quad	
d 0 to 3													
ADG, g	405	317	255	27.9	337	320	321	27.9	0.001	0.480	0.503	0.537	
ADFI, g	625	556	525	23.0	565	560	581	23.0	0.001	0.144	0.222	0.411	
G:F	0.646	0.567	0.483	0.028	0.588	0.566	0.541	0.028	0.001	0.938	0.144	0.817	
d 4 to 7													
ADG, g	418	376	372	16.2	393	380.9	392.6	16.2	0.047	0.342	0.915	0.557	
ADFI, g	513	514	528	24.9	531	518	506	24.9	0.536	0.774	0.312	0.840	
G:F	0.838	0.747	0.709	0.040	0.753	0.750	0.790	0.040	0.015	0.549	0.425	0.727	
d 7 to 14													
ADG, g	582	518	490	27.3	532	514	544	27.3	0.001	0.218	0.329	0.148	
ADFI, g	880	750	870	41.3	833	809	858	41.3	0.764	0.001	0.352	0.293	
G:F	0.665	0.700	0.575	0.018	0.652	0.644	0.645	0.018	0.001	0.001	0.809	0.800	
d 14 to 21													
ADG, g	671	631	641	12.0	659	652	632	12.0	0.087	0.103	0.103	0.935	
ADFI, g	965	928	956	80.6	958	936	955	80.6	0.754	0.166	0.949	0.370	
G:F	0.705	0.689	0.676	0.061	0.697	0.704	0.669	0.061	0.124	0.940	0.094	0.356	
d 0 to 21													
ADG, g	555	500	484	9.0	520	507	513	9.0	0.001	0.053	0.644	0.292	
ADFI, g	802	737	784	17.3	779	760	784	17.3	0.357	0.001	0.629	0.233	
G:F	0.694	0.681	0.620	0.021	0.672	0.668	0.656	0.021	0.001	0.039	0.206	0.889	
Pig BW, kg													
d 0	11.4	11.4	11.4	0.14	11.4	11.4	11.4	0.14	0.999	0.979	0.968	0.998	
d 21	23.1	21.9	21.6	0.28	22.3	22.0	22.2	0.28	0.001	0.220	0.789	0.510	

¹A total of 360 barrows (PIC 1050; initially 45 d of age) were used in a 21-d experiment with 24 replicate pens per treatment and 5 pigs per pen. All diets were fed in meal form.

²Denotes formulated levels. High-DON wheat (6.0 mg/kg) was used to incorporate DON into diets at desired concentrations.

Frobose et al., 2015). This is consistent with the poorer G:F reported in experiments with shorter durations (5 to 9 d; He et al., 1993; Li et al., 2011). Similar observations were observed in both of the present experiments, with severely reduced G:F during the first 3 d of DON exposure, lessening slightly by d 7, and no longer present thereafter. This initial DON-induced feed efficiency depression still had a more marked negative effect on overall ADG than the impact of DON on feed intake. In Exp. 1, it is likely that the greater DON levels fed (6.6 mg/kg) contributed to the poorer feed efficiency observed, likely mediated by previously described effects, such as suppressed immune and GIT function. However, in Exp. 2, lower levels of DON were fed (1.7 and 3.2 mg/kg) and yet the effects of DON on G:F were just as severe (11%) as in Exp. 1. Health challenge may have contributed to the more marked effect of DON on feed efficiency, as pigs in Exp. 2 were concomitantly affected by influenza, which originated from the source sow farm. Moreover, in both experiments, the authors observed that pigs fed DON-contaminated diets required frequent adjustment of feeders to maintain the predetermined 50% pan coverage, with DON-fed pigs being more likely to sort through the

feed leading to complete feed pan coverage. This would be congruent with earlier reports that the illness-inducing effects of DON can induce conditioned taste aversion that lessens over time (Osweiler et al., 1990; Ossenkopp et al., 1994). This observation requires additional investigation, but feed wastage during this period would also contribute to poorer feed efficiency. Finally, one may question whether pigs exposed to DON in field conditions are consistently exposed to DON levels great enough to allow tolerance to develop. In large-scale commercial situations, pigs are more likely to be fed diets containing multiple sources of cereal grains and, therefore, may be exposed to DON intermittently rather than continuously as has been provided in almost all experiments testing DON effects. Currently, it is unknown whether the severity of growth effects may differ when pigs are intermittently exposed to DON compared to continuous DON exposure.

Some technical treatments applied prior to feeding contaminated grains are known to partially or completely detoxify DON (e.g., physical separation, inactivation by heat/microbes, and ozone or ammonia gas treatment); however, these methods have been too labor- and cost-intensive to merit widespread commercial adoption or

have failed to meet government regulations (McKenzie et al., 1997; Döll and Dänicke, 2004; Young et al., 2007; Li et al., 2011). Supplementing contaminated diets with detoxifying agents is widely regarded as a more practical approach; however, currently-available feed additives have generally failed to alleviate the effects of DON (Ramos et al., 1996; Huwig et al., 2001; Awad et al., 2010). While previous attempts to use mineral adsorbing agents on nonpolar mycotoxins such as DON have been ineffective (Döll and Dänicke, 2004; Döll et al., 2005), the use of AMMC had not been previously tested *in vivo*.

Through a patented process (Amadeite; Olmix S.A., Brehan, France), the structure of the montmorillonite is modified using ulvans extracted from green seaweed (Lahaye and Robic, 2007). These water-soluble polysaccharides act as pillars between layers and result in a 10-fold increase of the inter-laminar space. This transformation enhanced the DON adsorptive capacity of the algae-modified montmorillonite clay (AMMC; Olmix S.A., Brehan, France) by 40% in a gastrointestinal model at low inclusion rates (0.1%; Havenaar and Demais, 2006). Nevertheless, regardless of the concentration of AMMC used and the level of DON in the diet, AMMC failed to alleviate DON-induced growth suppression in both experiments. The lack of an AMMC response in Exp. 2, when diets were fed in meal form, indicates that the heat and pressure present during pelleting was unlikely responsible for the lack of a response to AMMC in Exp. 1. Since factorial designs were used in both studies, we were able to demonstrate that AMMC supplementation also elicited no negative effects on toxin-free PC pigs. This is of note since a review by Döll and Dänicke (2003) revealed that potential detoxifying agents were actually more likely to decrease rather than improve performance in DON-contaminated diets. In many past *in vivo* studies, potential detoxifying agents have only been tested in the DON-exposed group and not added to the toxin-free diet, failing to demonstrate any unspecific effects the agent may have in toxin-free control pigs. This inadequate experimental design limits interpretation of results when testing potential detoxifying agents.

In the present study, fluctuations in nutrient content in DON-contaminated vs. toxin-free wheat reiterate the importance of accounting for these differences in studies assessing the impact of DON and potential detoxifying agents. Though DON contamination resulted in similar overall growth reductions to those seen in previous reports, these experiments indicate that the effects of DON on feed efficiency may be more severe than previously thought. Time-dependent changes observed for feed intake and efficiency also appear to be important in understanding how swine producers should address future DON contamination situations. Depending on the growth stage and DON level in the diet, pigs

appear to develop some tolerance to DON. However, the impact of intermittent, repeated exposure to DON deserves additional attention, as it may actually be more often observed in field situations. Despite novel processing methods, algae-modified montmorillonite clay was ineffective at preventing the adverse effects of DON on nursery pig growth performance.

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