Evaluating the Effects of an Algae-Modified Montmorillonite Clay in Diets Contaminated with Deoxynivalenol on Nursery Pig Growth Performance[1]

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Summary

A total of 280 nursery pigs (PIC 327 × 1050, initially 21.9 lb and 35 d of age) were used in a 21-d growth trial to evaluate the effects of an algae-modified montmorillonite clay (MMi) on nursery pig performance when fed diets contaminated with deoxynivalenol (DON). Pigs were allotted to pens by weight, and pens were randomly assigned to 1 of 5 dietary treatments arranged in a 2 × 2 + 1 factorial with 7 pigs per pen and 8 pens per treatment. All experimental diets were pelleted. Mycotoxin analyses were conducted on the main ingredients at NDSU[3] and LDA labs[4], and these results were used in diet formulation. Naturally contaminated wheat (10.7 ppm DON) was used to produce diets with approximately 5 ppm DON. The 5 treatments consisted of 2 positive control diets that did not contain DON contamination with or without 0 or 0.50% MMi and 3 negative control diets that were contaminated with 5 ppm of DON and contained 0, 0.25%, or 0.50% MMi. No DON × MMi interactions were observed for the entire study. Overall (d 0 to 21), ADG, ADFI, and d 21 BW decreased ($P < 0.001$) in pigs fed DON-contaminated diets regardless of MMi addition. Feed efficiency was poorer ($P < 0.001$) for pigs fed diets with DON due, primarily to poor feed efficiency in the initial period (d 0 to 7). Pigs fed diets contaminated with DON had greater ($P < 0.05$) BW variation (CV) within pen on d 21. Although the addition of 0.5% MMi to diets restored ($P < 0.02$) ADFI from d 14 to 21, no other treatment differences were observed for MMi inclusion. In conclusion, this study suggests that including MMi will not offset reductions in nursery pig performance caused by high DON levels (> 5 ppm) when diets are fed in pellet form.

Key words: deoxynivalenol, montmorillonite clay, nursery pig, vomitoxin

Introduction

Deoxynivalenol (DON), also known as vomitoxin, develops in cereal grains when excess moisture is present during the flowering stage. It is one of the most detrimental mycotoxins because it occurs frequently in cereal grains, often at levels of toxicological relevance. Pigs are the most DON-susceptible livestock species, with negative effects consisting of decreased feed intake and growth performance, immune suppression, and, at high DON concentrations, vomiting and complete feed refusal.

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Deoxynivalenol presents significant challenges for pork producers because conventionally used detoxification agents such as bentonite clay and activated aluminosilicates provide little to no benefit to DON-contaminated grains. Such adsorbent products have successfully bound mycotoxins with smaller molecular size such as aflatoxin, allowing the toxin to pass through the body without negative effects, but the large diameter of DON prevents it from being adsorbed within the structure of available clay adsorbents. A new algae-modified montmorillonite clay (MMi) product is available that has not been previously studied. This MMi is produced using a patented processing method in which algae polysaccharides are used to expand the layers of the montmorillonite clay. This expansion of the clay layers may provide a substrate for increased adsorption, which could mitigate the negative effects of DON on pig performance; therefore, the aim of this study was to determine the influence of MMi in DON-contaminated feeds on nursery pig growth performance.

Procedures

The Kansas State University Institutional Animal Care and Use Committee approved the protocol used in this experiment. The trial was conducted at the K-State Swine Teaching and Research Center in Manhattan, KS. A total of 280 mixed-sex pigs (PIC 327 × 1050; 21.9 ± 0.2 lb BW and 35 d of age) were used in a 21-d experiment with 8 replicate pens per treatment and 7 pigs in each pen. At weaning, pigs were allotted to pens by initial weight and fed a common diet for 7 d, at which time they were reweighed and pens were assigned to 1 of 5 treatments in a completely randomized design. Each pen contained a 4-hole, dry self-feeder and a nipple waterer to provide ad libitum access to feed and water.

Treatments were arranged in a 2 × 2 + 1 factorial with DON and MMi inclusion as main effects. The experimental treatments consisted of 2 positive control diets that had no DON contamination and contained either 0 or 0.50% MMi and 3 negative control diets formulated to contain 5 ppm DON and either 0, 0.25%, or 0.50% MMi (Table 1). Diets exceeded 2012 NRC[5] nutrient requirements, and apart from the inclusion of DON and MMi were formulated to be identical in nutrient composition.

Diets were manufactured in pellet form at the K-State Animal Science Feed Mill in Manhattan, KS. All diets were pelleted in an attempt to minimize segregation of dietary ingredients. A naturally contaminated source of high-DON wheat (10.7 ppm DON; Table 2) was used to provide diets with 5 ppm DON. Prior to diet manufacturing, a total of 60 subsamples were collected from both a high-DON and DON-free wheat source. These samples were homogenized and split into replicate samples, which were then sent for mycotoxin analysis at NDSU and LDA Labs. The lab at NDSU conducted an 18-component toxin screen using a combination of mass spectrometry, ELISA, and high-pressure liquid chromatography. LDA Labs performed a 43-component toxin screen using liquid chromatography/mass spectrometry analysis. Due to concerns that high-DON wheat may also have a different amino acid profile than DON-free wheat, both were analyzed for amino acid content (Table 3) at the University of Missouri Agricultural Experiment Station Chemical Laboratories (Columbia, MO), and diet formulation was adjusted to account for the differences. Following final diet manufac-

[5] NRC. 2012. Nutrient Requirements of Swine. 11th ed. Natl. Acad. Press, Washington, DC.

turing, diet samples were sent to NDSU and the University of Missouri for mycotoxin and amino acid analysis, respectively (Table 4).

Average daily gain, ADFI, and F/G were determined by weighing pigs and determining feed disappearance on d 0, 7, 14, and 21 (Table 5). Each pen contained a 4-hole, dry self-feeder and a nipple waterer to provide ad libitum access to feed and water.

Results were analyzed as a completely randomized design. Treatment means were analyzed using the LSMEANS statement and preplanned CONTRAST statements in SAS (SAS Institute, Inc., Cary, NC). The fixed factors in the model included DON level and MMi inclusion. Preplanned contrasts included: (1) the interaction between DON contamination and MMi inclusion, (2) DON contaminated diets vs. non-contaminated diets, (3) 0 vs. 0.5% MMi, and (4) the linear effects of MMi inclusion within DON-contaminated diets. Least squares means were calculated for each independent variable, and means were considered significant at $P < 0.05$.

Results and Discussion

In the present study, the amino acid concentrations of DON-free wheat were generally higher than that of the DON-contaminated wheat. Although DON contamination clearly alters nutrient content,[6] previous research has shown that the alterations are not consistent. This fact reinforces the importance of accounting for these changes in diet formulation to assess the true impact of a mycotoxin contamination on animal performance.

All negative control diets averaged 6.6 ppm DON, approximately 20% higher than targeted concentrations (5 ppm DON). Although fumonisin B_1 was detected at 2.0 ppm in the DON-free diet without MMi, analyses confirmed that no other mycotoxins were detected in positive control diets above the practical quantification limit (PQL; <0.5 ppm). Aflatoxin B_1 was detected at low levels (20 and 28 ppb) in two of three negative control diets, but no other mycotoxins were detected above PQL in DON-contaminated diets. Although the presence of low levels of fumonisin and aflatoxin in several test diets is concerning, according to EFSA (2009[7]) these levels remain below critical concentrations in nursery pig diets.

No DON × MMi interactions were observed for the entire study. As expected, ADFI was reduced ($P < 0.03$) in pigs fed DON-contaminated diets in all periods and for the overall study, with the greatest impact observed in the first 7 d. The almost 24% reduction in ADFI resulted in pigs fed DON-contaminated diets having decreased ($P < 0.001$) ADG and BW for every period and the entire study. Although F/G was poorer ($P < 0.001$) from d 0 to 7 and d 0 to 21, no impact ($P > 0.12$) of DON contamination was observed from d 7 to 14 and d 14 to 21. The approximately 40% poorer F/G during the initial period might correspond with the DON-associated upregulation of immune system function during the initial exposure to the toxin. Finally, pigs fed

[6] Matthaus, K., S. Danicke, W. Vahjen, O. Simon, J. Wang, H. Valenta, K. Meyer, A. Strumpf, H. Ziesenib, and G. Flachowsky. 2004. Progression of mycotoxin and nutrient concentrations in wheat after inoculation with Fusarium culmorum. Arch. Anim. Nutr. 58:19–35.

[7] EFSA, 2009. Pages 141–142 in: Review of mycotoxin-detoxifying agents used as feed additives: mode of action, efficacy and feed/food safety. Scientific report submitted to the European Food Safety Association.

DON-contaminated diets had greater ($P < 0.05$) BW CV within pen on d 21, which indicates more variation in pig weights within a pen.

An unforeseen challenge that arose during the execution of this trial was the management of feeders for pigs fed DON-contaminated diets. Although not previously described in the literature, anecdotal visual evidence from this experiment suggests that significant feed sorting may have occurred for pigs consuming DON-contaminated diets despite attempts to manage feeders carefully. The increased variation in pig BW within pens offered the DON-contaminated diets may also suggest that some pigs were more affected by DON than others.

Although the addition of 0.5% MMi to diets restored ($P < 0.02$) ADFI from d 14 to 21, no other differences due to MMi inclusion were detected for any response variable; moreover, within DON-contaminated diets, no ($P > 0.14$) linear effects of added MMi for ADG, ADFI, F/G or pig BW were observed. These data suggest that the processing techniques for the algae-modified montmorillonite clay (MMi) do not significantly increase its adsorption capabilities for DON. Furthermore, the lack of MMi response in the DON-free diet suggests that no other beneficial responses can be expected from including MMi in diets without DON contamination.

In conclusion, this study showed that MMi failed to offset reductions in nursery pig performance caused by high DON levels (>5 ppm) when diets were fed in pelleted form. Future research to define feed-processing effects on the MMi response should be conducted to determine if a better response would be observed with diets fed in meal form.

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

	Positive control (0 ppm DON[1])		Negative control (5 ppm DON)		
Item MMi[2] inclusion:	None	0.50%	None	0.25%	0.50%
Ingredient, %					
Corn	16.90	16.40	16.35	16.25	15.90
Soybean meal (46.5% CP)	30.93	31.00	31.45	31.35	31.45
Hard red winter (HRW) wheat	46.75	46.75	---	---	---
High-DON HRW wheat[3]	---	---	46.75	46.75	46.75
Soybean oil	2.00	2.00	2.00	2.00	2.00
Monocalcium P, 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]	0.25	0.25	0.25	0.25	0.25
Trace mineral premix	0.15	0.15	0.15	0.15	0.15
L-lysine HCl	0.33	0.33	0.33	0.33	0.33
DL-methionine	0.10	0.10	0.15	0.15	0.15
L-threonine	0.14	0.14	0.14	0.14	0.14
MMi montmorillonite clay	---	0.50	---	0.25	0.50
Total	100	100	100	100	100
Calculated composition, %					
Standardized ileal digestible amino acids, %					
Lysine	1.28	1.28	1.28	1.28	1.28
Isoleucine:lysine	65	65	62	62	62
Leucine:lysine	120	120	115	115	115
Methionine:lysine	31	31	33	33	33
Met & Cys:lysine	58	58	58	58	58
Threonine:lysine	64	64	64	64	64
Tryptophan:lysine	20.7	20.7	18.9	18.9	18.9
Valine:lysine	72	72	69	69	69
Total lysine, %	1.42	1.42	1.42	1.42	1.42
ME, kcal/lb	1,505	1,498	1,505	1,501	1,498
SID Lysine: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
DON, ppm	<0.5	<0.5	5.0	5.0	5.0

[1] Deoxynivalenol (DON).
[2] MMi algae-modified montmorillonite clay product (Olmix, Brehan, France).
[3] Analyzed DON concentration in DDGS was 10.7 ppm.
[4] Phyzyme 600 (Danisco Animal Nutrition, St. Louis, MO) provided 750 phytase units phytase/kg and 0.13% available P released.

Table 2. Mycotoxin analysis of basal ingredients

		Hard red winter wheat	
Item, ppm	Ground corn	DON-free[1]	High-DON
NDSU[2]			
DON	<0.50	<0.50	10.60[3]
LDA Labs[4]			
DON	---[5]	---	10.70
15-Acetyl DON	---	---	0.12
Zearalenone	---	---	0.35
Fumonisin B_1	---	---	0.03

[1] Deoxynivalenol (DON).
[2] North 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.
[3] Mean of two duplicate samples sent to NDSU. Individual samples had DON levels of 10.0 and 11.1 ppm, respectively.
[4] LDA Labs, Ploufragan, France. Samples 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.
[5] (---) indicates samples were not tested.

Table 3. Amino acid analysis of basal ingredients (as-fed basis)[1]

	Hard red winter wheat	
Item	DON-free[2]	High-DON
Lysine	0.40	0.37
Isoleucine	0.47	0.36
Leucine	0.91	0.72
Methionine	0.21	0.16
Cysteine	0.28	0.22
Threonine	0.37	0.30
Tryptophan	0.18	0.12
Valine	0.62	0.50

[1] Samples were analyzed at the University of Missouri Agricultural Experiment Station Chemical Laboratories in Columbia, MO.
[2] Deoxynivalenol (DON).

Table 4. Mycotoxin analysis of experimental diets (as-fed basis)[1]

	Positive control (0 ppm DON[2])		Negative control (5 ppm DON)		
Item MMi inclusion:[3]	None	0.50%	None	0.25%	0.50%
DON, ppm	<0.5	<0.5	6.6	6.7	6.4
Fumonisin $B_{1,}$ ppm	2.0	<2.0	<2.0	<2.0	<2.0
Aflatoxin B_1, ppb	<20	<20	20	28	<20

[1] North 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.
[2] Deoxynivalenol (DON).
[3] MMi algae-modified montmorillonite clay product (Olmix, Brehan, France).

Table 5. The effects of deoxynivalenol (DON) and an algae-modified montmorillonite clay (MMi) on nursery pig performance[1,2]

	Positive control (DON 0 ppm)[3]		Negative control (DON 5 ppm)[4]				Probability, $P <$[5]	
Item MMi:[2]	None	0.50%	None	0.25%	0.50%	SEM	DON	MMi
d 0 to 7								
ADG, lb	0.68[b]	0.65[b]	0.31[a]	0.35[a]	0.33[a]	0.07	0.001	0.77
ADFI, lb	1.01[b]	0.99[b]	0.81[a]	0.85[ab]	0.80[a]	0.16	0.01	0.74
F/G	1.57[a]	1.58[a]	2.69[b]	2.54[b]	2.51[b]	0.19	0.001	0.68
d 7 to 14								
ADG, lb	1.16[b]	1.17[b]	0.92[a]	0.87[a]	1.01[a]	0.04	0.001	0.18
ADFI, lb	1.55[b]	1.62[b]	1.23[a]	1.29[a]	1.35[a]	0.10	0.001	0.16
F/G	1.35	1.40	1.35	1.48	1.35	0.06	0.71	0.60
d 14 to 21								
ADG, lb	1.16[b]	1.29[b]	1.05[ab]	1.02[a]	1.07[ab]	0.07	0.001	0.14
ADFI, lb	1.79[b]	1.89[b]	1.61[a]	1.62[a]	1.80[b]	0.08	0.03	0.02
F/G	1.55[ab]	1.46[a]	1.52[ab]	1.60[ab]	1.69[b]	0.06	0.12	0.54
d 0 to 21								
ADG, lb	1.00[b]	1.03[b]	0.76[a]	0.74[a]	0.80[a]	0.05	0.001	0.18
ADFI, lb	1.45[b]	1.50[b]	1.21[a]	1.26[a]	1.31[ab]	0.11	0.001	0.16
F/G	1.46[a]	1.46[a]	1.61[b]	1.67[b]	1.65[b]	0.05	0.001	0.67
Pig BW, lb								
d 0	21.9	21.9	21.9	22.0	22.0	0.20	0.25	0.33
d 7	26.7[b]	26.4[b]	24.0[a]	24.4[a]	24.2[a]	0.54	0.001	0.84
d 14	34.7[b]	34.6[b]	30.5[a]	30.6[a]	31.2[a]	0.76	0.001	0.47
d 21	42.8[b]	43.6[b]	37.7[a]	37.7[a]	38.6[a]	1.20	0.001	0.16
Pen CV, %								
d 0	14.1	13.8	14.2	14.4	14.7	1.00	0.23	0.76
d 21	13.6[ab]	12.1[a]	16.6[b]	15.2[ab]	14.7[ab]	0.015	0.05	0.22

[a, b] Values without a common superscript are statistically different ($P < 0.05$).

[1] A total of 280 pigs (PIC 327 × 1050; 35 d of age) were used in this 21-d study, with 7 pigs per pen and 8 pens per treatment.

[2] MMi (Olmix S.A., Brehan, France).

[3] Formulated mycotoxin levels. Deoxynivalenol (DON)-contaminated wheat was used to produce diets with 5 ppm DON.

[4] Analyzed DON levels averaged 6.6 ppm for negative control diets.

[5] No interactions were detected ($P > 0.05$) between mycotoxin level and inclusion of MMi, and no linear effects ($P > 0.05$) due to MMi inclusion within DON contaminated diets were found. 'MMi' contrast compares diets without MMi to those containing MMi at 0.50%.