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Summary

This experiment was conducted to determine the effect of feeding fumonisin (FUM) contaminated corn on growth performance of 20- to 60-lb nursery pigs. A total of 350 pigs (241 × 600; DNA, Columbus, NE; initially 19.6 lb) were used. Dietary treatments consisted of FUM-contaminated corn blended with relatively FUM-free corn to provide toxin (FB1 + FB2) of 7.2, 14.7, 21.9, 32.7, and 35.1 ppm. Experimental diets were fed in mash form for 28 d. There were 5 pigs per pen and 14 replicates per treatment. After weaning, pigs were fed common diets for 21 days before the experiment started. Then, pens were assigned to treatments in a randomized complete block design with initial weight as the blocking factor. From d 0 to 28, increasing FUM decreased (linear, $P < 0.001$) average daily gain (ADG) and final body weight (BW) and average daily feed intake (ADFI; linear, $P = 0.05$). Feed efficiency (F/G) became poorer as FUM increased (linear, $P = 0.01$). Although tested linear, the greatest reduction in ADG was observed in pigs fed greater than 21.9 ppm of FUM. Increasing FUM increased serum sphinganine (Sa) and sphingosine (So) ratios (linear, $P < 0.001$) on day 14 and 28, which corresponded with the decreased growth performance. Data indicated that the serum Sa:So ratio is a reliable biomarker indicating FUM intoxication. These results suggest that for 20- to 60-lb nursery pigs, diets containing more than 30 ppm of FUM should not be fed, as increasing FUM concentration worsens growth performance and increases serum Sa:So ratio. Furthermore, diets containing greater than 21.9 ppm should be evaluated with caution as further research is warranted to determine the fumonisin concentration between 21.9 and 30 ppm where the negative effects on pig performance are observed.

Introduction

Fumonisin contamination in corn has been an emerging issue in swine feed production. Pigs fed fumonisin-contaminated corn will have reduced growth performance,

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and damage to liver, lungs,³ kidneys,⁴ and gastrointestinal structure.⁵ In severe cases, it may lead to death. Serum sphinganine (Sa) and sphingosine (So) ratio has been used as a biomarker to determine the severity of fumonisin intoxication. Fumonisin disrupts the metabolism of sphingolipid and blocks the synthesis of sphingolipid from Sa and So that results in cell damage and apoptosis. To our knowledge, there are limited data to determine the level of fumonisin that will affect pig growth performance and serum Sa:So. Therefore, the objective of this trial was to determine the effects of feeding corn that was naturally contaminated with fumonisin to 20- to 60-lb nursery pigs.

Procedures

The Kansas State University Institutional Animal Care and Use Committee approved the protocol used in this experiment. This experiment was conducted at the Kansas State University Swine Teaching and Research Center in Manhattan, KS. Each pen (4 × 4 ft) was equipped with a 4-hole, dry self-feeder and a nipple waterer to provide *ad libitum* access to feed and water.

A total of 350 pigs (241 × 600; DNA, Columbus, NE; initially 19.6 lb) were used in a 28-d growth trial. Pigs were weaned at approximately 21 d of age and placed in pens of 5 pigs each based on initial weight and gender. A common phase 1 pelleted diet was fed for 7 d and a common phase 2 mash diet was fed for another 14 d. At d 21 after weaning, which was considered d 0 of the trial, pens of pigs were randomly allotted to treatment in a randomized complete block design with weight as the blocking factor. There were 14 replicate pens per treatment. Pen weights and feed disappearance were measured on d 0, 7, 14, 21, and 28 to determine ADG, ADFI, and F/G. Serum samples were measured on d 0, 14, and 28 to determine serum sphinganine (Sa) and sphingosine (So) ratio. For serum samples, blood was collected from two pigs per treatment and analyzed as a baseline concentration for all treatments on d 0. Blood samples were collected from nine pigs per treatment on d 14 and 28. For each selected pen, the median weight pig was selected and recorded on d 0. The same pig per pen was used in all subsequent serum collections.

All diets were manufactured at the Kansas State University O.H. Kruse Feed Technology Innovation Center in Manhattan, KS. Two diets were formulated using relatively FUM-free corn (control) or FUM-contaminated corn (approximately 50 ppm FUM; Table 1). These two diets were blended at the feed mill to produce three diets with intermediate FUM concentrations. Consequently, five dietary treatments were manufactured with final diets containing 7.2, 14.7, 21.9, 32.7, and 35.1 ppm FUM. All diets met or exceeded the NRC (2012) nutrient requirement estimates.

³ Zomborszky-Kovacs, M., F. Vetesi, P. Horn, I. Repa and F. Kovacs. 2002. Effects of Prolonged Exposure to Low-Dose Fumonisin B1 in Pigs. *J. Vet. Med. B* 49, 197–201.

⁴ Colvin, B.M., A.J. Cooley and R.W. Beaver. 1993. Fumonisin toxicosis in swine: clinical and pathologic findings. *J. Vet. Diagn. Invest.* 1993 Apr, 5(2):232-41.

⁵ Bouhet, S., E. Hourcade, N. Loiseau, A. Fikry, S. Martinez, M. Roselli, P. Galtier, E. Mengheri and I.P. Oswald. 2004. The mycotoxin fumonisin B1 alters the proliferation and the barrier function of porcine intestinal epithelial cells. *Toxicol Sci.* 2004 Jan, 77(1):165-71.

Representative diet samples were obtained from every fifth bag of feed manufactured. Samples were analyzed for dry matter, crude protein, calcium, phosphorus, neutral detergent fiber, and fat (Ward Laboratories, Inc., Kearney, NE; Table 2). Diet samples and FUM-contaminated corn samples were analyzed for mycotoxin levels at North Dakota State University Veterinary Diagnostic Laboratory (Fargo, ND) with reported FUM being the sum of fumonisin B1 plus fumonisin B2 (Table 3). All other mycotoxins except FUM were below detectable levels.

Data were analyzed as a randomized complete block design with block as a random effect and pen as the experimental unit. For every response, 2 analytical models were constructed by using homogenous variance and heterogenous variance models weighted by treatment. After comparing the 2 models, 1 model was selected based on ANOVA test ($P \leq 0.05$), and used in determining linear and quadratic effects, and pairwise comparisons among treatments. Polynomial contrasts were constructed to evaluate the linear and quadratic effects of increasing FUM on ADG, ADFI, F/G, BW, and serum Sa:So ratio. Contrast coefficients were adjusted for unequally spaced treatments. Pairwise comparisons were conducted on treatment means using a Tukey adjustment to prevent inflation of Type I error due to multiple comparisons. Data were analyzed using R program.⁶ Results were considered significant at $P \leq 0.05$ and marginally significant at $0.05 < P \leq 0.10$.

Results and Discussions

Chemical analysis (Table 2) for dry matter, crude protein, calcium, phosphorus, neutral detergent fiber, and fat was within formulated range and similar between treatments.

From d 0 to 28, feeding pigs diets containing increasing FUM decreased (linear, $P < 0.001$) ADG and d 28 BW, ADFI (linear, $P = 0.05$), and worsened (linear, $P = 0.01$) F/G (Table 4). The effect of increasing FUM on ADG, ADFI, and F/G by week is shown in Figures 1, 2, and 3, respectively. Increasing FUM linearly ($P < 0.05$) decreased ADG from d 7 to 14 and d 21 to 28 (Figure 1), and linearly ($P < 0.05$) worsened F/G from d 0 to 7 and d 7 to 14. The decrease in ADG resulted in pigs fed 32.7 and 35.1 ppm FUM being 3.4 and 2.8 lb lighter ($P < 0.05$) than pigs fed 7.2 ppm FUM on d 28. There was no statistical evidence for d 28 BW differences between diets containing 7.2 to 21.9 ppm fumonisin.

Serum Sphinganine (Sa) and Sphingosine (So) Ratio

On d 14, feeding pigs increasing FUM increased (linear, $P < 0.001$) serum Sa:So ratio from 0.47 to 1.40. Pigs fed 14.7, 21.9, 32.7, and 35.1 ppm of FUM had higher ($P < 0.05$) Sa:So ratio than pigs fed 7.2 ppm of FUM. On d 28, feeding pigs increasing FUM increased (linear, $P < 0.001$) serum Sa:So ratio with pigs fed 21.9, 32.7, and 35.1 ppm of FUM having higher ($P < 0.05$) Sa:So ratio than pigs fed 7.2 ppm of FUM. Pigs fed 32.7, and 35.1 ppm FUM had higher ($P < 0.05$) Sa:So ratio than pigs fed lower FUM concentration.

⁶ R Core Team. 2018. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>.

In conclusion, increasing dietary FUM concentration from 7.2 to 35.1 ppm for 28 d worsened growth performance (BW, ADG, ADFI, and F/G), and increased serum Sa:So ratio from 0.47 to 1.40 on d 14 and 0.55 to 1.58 on d 28. By correlating growth performance result with serum Sa:So ratio, there was a threshold of approximately 20 to 30 ppm of dietary FUM that significantly decreased growth performance and increased serum Sa:So ratio. Therefore, serum Sa:So ratio has the potential to be used as a reliable biomarker for FUM intoxication, and diets that contain more than 30 ppm of FUM should not be fed to 20- to 60-lb nursery pigs. Furthermore, diets containing greater than 21.9 ppm should be evaluated with caution as further research is warranted to determine the FUM concentration between 21.9 and 30 ppm where the negative effects on pig performance are observed.

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

Item	7.2 ppm	14.7 ppm	21.9 ppm	32.7 ppm	35.1 ppm
Ingredients, %					
Corn	64.70	53.34	41.98	19.25	--
Fumonisin corn ²	--	11.36	22.73	45.45	64.70
Soybean meal	28.00	28.00	28.00	28.00	28.00
Soy oil	3.00	3.00	3.00	3.00	3.00
Monocalcium phosphate	0.85	0.85	0.85	0.85	0.85
Calcium carbonate	0.75	0.75	0.75	0.75	0.75
Sodium chloride	0.60	0.60	0.60	0.60	0.60
L-Lysine HCl	0.55	0.55	0.55	0.55	0.55
DL-Methionine	0.21	0.21	0.21	0.21	0.21
L-Threonine	0.23	0.23	0.23	0.23	0.23
L-Tryptophan	0.06	0.06	0.06	0.06	0.06
L-Valine	0.16	0.16	0.16	0.16	0.16
Vitamin premix	0.25	0.25	0.25	0.25	0.25
Trace mineral premix	0.15	0.15	0.15	0.15	0.15
Phytase ³	0.08	0.08	0.08	0.08	0.08
Total	100	100	100	100	100
Standard ileal digestible (SID) amino acids, %					
Lysine	1.30	1.30	1.30	1.30	1.30
Isoleucine:lysine	53	53	53	53	53
Leucine:lysine	111	111	111	111	111
Methionine:lysine	36	36	36	36	36
Met and cystine:lysine	56	56	56	56	56
Threonine:lysine	63	63	63	63	63
Tryptophan:lysine	20.0	20.0	20.0	20.0	20.0
Valine:lysine	69	69	69	69	69
Histidine:lysine	35	35	35	35	35
Net energy, kcal/lb	1,151	1,151	1,151	1,151	1,151
Crude protein, %	19.8	19.8	19.8	19.8	19.8
Calcium, %	0.61	0.61	0.61	0.61	0.61
STTD P, ⁴ %	0.44	0.44	0.44	0.44	0.44

¹Diet mycotoxin concentration was analyzed at North Dakota State University Veterinary Diagnostic Laboratory (Fargo, ND) by LC/MS/MS assay. All mycotoxins except fumonisin were below detectable level.

²Approximately 50 ppm of fumonisin.

³Ronozyme HiPhos 2700 (DSM Nutritional Products, Basel, Switzerland) provided 306 FTU per lb of feed and an expected P release of 0.10%.

⁴STTD P = standardized total tract digestible phosphorus.

Table 2. Chemical analysis of diets (as-fed basis)^{1,2}

Item	Fumonisin concentration (ppm)				
	7.2	14.7	21.9	32.7	35.1
Proximate analysis, %					
Dry matter	87.54	87.56	87.72	87.92	88.14
Crude protein	19.3	19.3	19.8	19.7	19.8
Calcium	0.73	0.65	0.57	0.67	0.63
Phosphorus	0.51	0.49	0.50	0.51	0.53
Neutral detergent fiber	6.5	5.6	6.6	6.8	6.3
Fat (oil)	5.1	4.9	4.8	5.0	5.1

¹A representative sample of each diet was collected from every fifth bag of feed manufactured for each treatment, homogenized, and submitted for proximate analysis (Ward Laboratories, Inc., Kearney, NE).

²Diet mycotoxin concentration was analyzed at North Dakota State University Veterinary Diagnostic Laboratory (Fargo, ND) by LC/MS/MS assay. All mycotoxins except fumonisin were below detectable level.

Table 3. Dietary mycotoxin level (as-fed basis, ppb)^{1,2}

Item	7.2 ppm	14.7 ppm	21.9 ppm	32.7 ppm	35.1 ppm
Aflatoxin B1	< 20	< 20	< 20	< 20	< 20
Aflatoxin B2	< 20	< 20	< 20	< 20	< 20
Aflatoxin G1	< 20	< 20	< 20	< 20	< 20
Aflatoxin G2	< 20	< 20	< 20	< 20	< 20
Fumonisin B1	5,680	11,709	17,349	25,207	27,459
Fumonisin B2	1,487	2,955	4,507	7,461	7,537
HT-2 Toxin	< 200	< 200	< 200	< 200	< 200
T-2 Toxin	< 20	< 20	< 20	< 20	< 20
Ochratoxin	< 20	< 20	< 20	< 20	< 20
Sterigmatocystin	< 20	< 20	< 20	< 20	< 20
Zearalenone	< 100	< 100	< 100	< 100	< 100
Vomitoxin	< 200	< 200	< 200	< 200	< 200

¹A representative sample of each diet was collected from every fifth bag of feed manufactured for each treatment.

²Diet mycotoxin concentration was analyzed at North Dakota State University Veterinary Diagnostic Laboratory (Fargo, ND) by LC/MS/MS assay. All mycotoxins except fumonisin were below detectable level.

Table 4. Effect of fumonisin concentration on 20- to 60-lb nursery pig growth performance^{1,2}

Item	Fumonisin concentration (ppm)					SEM	Probability, <	
	7.2	14.7	21.9	32.7	35.1		Linear	Quadratic
BW, lb								
d 0	19.7	19.6	19.6	19.7	19.5	-- ³	0.60	0.79
d 28	62.0 ^a	61.0 ^{ab}	61.2 ^{ab}	59.2 ^b	58.6 ^b	0.94	< 0.001	0.41
d 0 to 28								
ADG, lb	1.49 ^a	1.47 ^{ab}	1.49 ^a	1.41 ^{ab}	1.40 ^b	0.02	< 0.001	0.18
ADFI, lb	2.24	2.19	2.23	2.15	2.16	0.04	0.05	0.77
F/G	1.50	1.49	1.50	1.52	1.55	0.02	0.01	0.12

^{a,b}Means within a row with different superscripts differ ($P < 0.05$).

¹A total of 350 pigs (241 × 600; DNA, Columbus, NE; initially 19.6 lb) were used in a 28-d experiment with 5 pigs per pen and 14 pens per treatment.

²ADG = average daily gain. ADFI = average daily feed intake. F/G = feed efficiency.

³Heterogenous variance: 7.2 ppm (0.42), 14.7 ppm (0.42), 21.9 ppm (0.40), 32.7 ppm (0.47), and 35.1 ppm (0.41).

Table 5. Effect of fumonisin concentration on serum sphinganine (Sa) and sphingosine (So) ratio^{1,2,3}

Item	Fumonisin concentration (ppm)					SEM	Probability, <	
	7.2	14.7	21.9	32.7	35.1		Linear	Quadratic
d 14								
Sa:So	0.47 ^c	0.84 ^b	1.00 ^b	1.14 ^{ab}	1.40 ^a	0.09	< 0.001	0.36
d 28								
Sa:So	0.55 ^c	0.77 ^{bc}	0.93 ^b	1.42 ^a	1.58 ^a	-- ⁴	< 0.001	0.14

^{a,b,c}Means within a row with different superscripts differ ($P < 0.05$).

¹A total of 350 pigs (241 × 600; DNA, Columbus, NE; initially 19.6 lb) were used in a 28-d experiment with 5 pigs per pen and 14 pens per treatment.

²Two pigs per treatment were sampled and analyzed as baseline for all treatments on d 0 (Sa: So = 0.22); 9 pigs per treatment were sampled and analyzed on d 14 and 28.

³Serum Sa:So ratio was analyzed at University of Missouri Veterinary Medical Diagnostic Laboratory (Columbia, MO) by HPLC.

⁴Heterogenous variance: 7.2 ppm (0.03), 14.7 ppm (0.07), 21.9 ppm (0.08), 32.7 ppm (0.07), and 35.1 ppm (0.15).

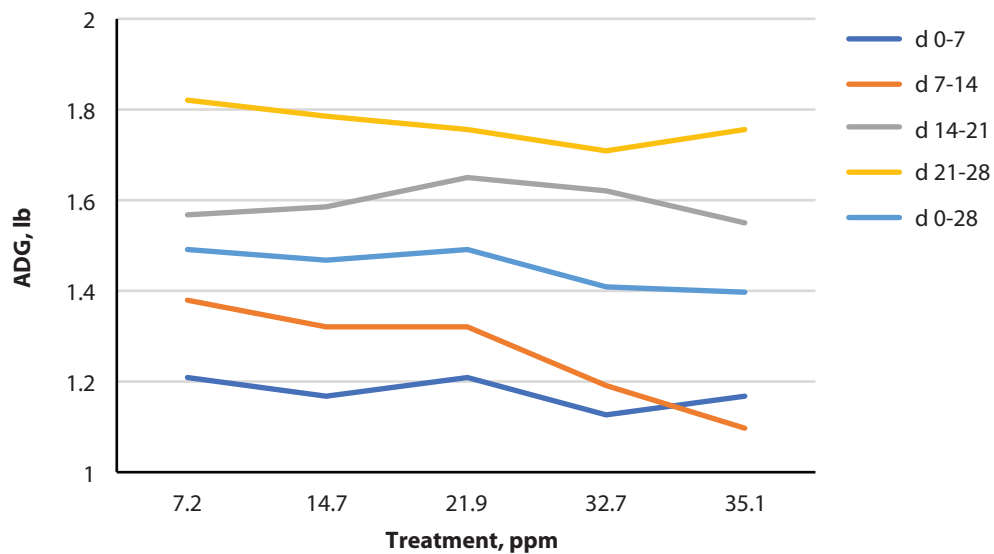


Figure 1. Average daily gain (ADG) of each growth period.

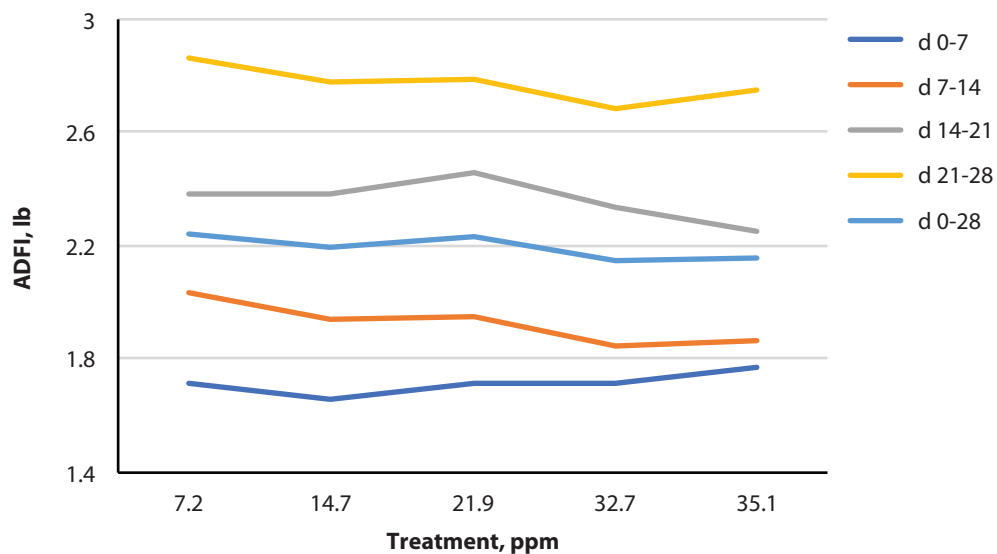


Figure 2. Average daily feed intake (ADFI) of each growth period.

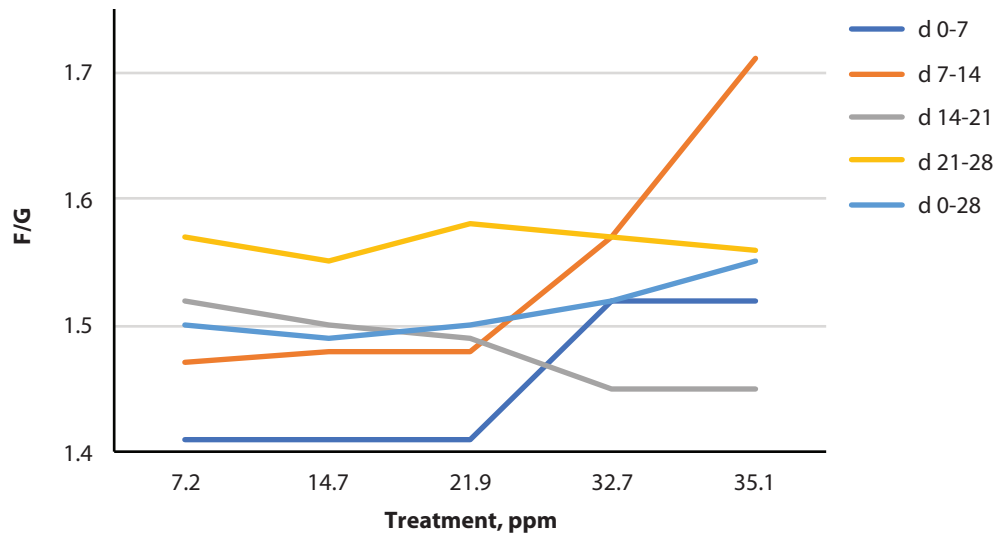


Figure 3. Feed efficiency (F/G) of each growth period.