

# The Effects of High-Sulfate Water and Zeolite (Clinoptilolite) on Nursery Pig Performance<sup>1</sup>

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## Summary

A total of 320 nursery pigs (PIC 1050 barrows) were used in a 24-d study to determine the effects of high-sulfate water and dietary natural zeolite on growth performance and fecal consistency of nursery pigs. Eight treatments were arranged as a 2 × 4 factorial with 2 water treatments (control or water with 3,000 ppm sodium sulfate), and 4 dietary zeolite concentrations (0, 0.25, 0.5, and 1.0%). Water treatments remained the same from d 0 to 24 and all diets were fed in 2 phases, with diets containing zeolite having the same inclusion rate in both phases. Phase 1 diets were fed in a pellet form from d 0 to 10 after weaning, with Phase 2 diets fed in meal form from d 10 to 24. Fecal samples were collected on d 5, 9, 16, and 23. These samples were visually assessed and scored on a scale of 1 to 5 to determine consistency of the fecal samples then analyzed for DM.

From d 0 to 10, neither sulfate addition to the water nor zeolite influenced ADG, ADFI, or F/G. Dietary treatment had no effect on fecal consistency; however, pigs drinking control water had a lower ( $P < 0.01$ ) fecal score (fewer visual observations of scours) than pigs drinking high-sodium sulfate water. From d 10 to 24, pigs drinking control water had improved ( $P < 0.01$ ) ADG, ADFI and F/G compared with pigs drinking high-sodium sulfate water. Dietary zeolite increased (linear,  $P < 0.01$ ) ADG and ADFI, but did not affect fecal scores. Similar to Phase 1, pigs drinking control water had lower ( $P < 0.01$ ) fecal scores, indicating less scouring compared with pigs drinking the high-sodium sulfate water. Dry matter analysis indicated that dietary zeolite had no effect on fecal DM, but high-sodium sulfate water decreased ( $P < 0.01$ ) total DM content of fecal samples in both Phase 1 and the first collection in Phase 2, but not on d 23, the final collection.

Overall (d 0 to 24), increasing zeolite increased (linear,  $P < 0.05$ ) ADG and ADFI, but F/G was not affected. Pigs drinking high-sulfate water had decreased ( $P < 0.01$ ) ADG and ADFI and poorer ( $P < 0.01$ ) F/G compared with pigs drinking control water. In conclusion, pigs drinking water with 3,000 ppm sodium sulfate had decreased ADG, ADFI, and poorer F/G from d 10 to 24 and for the overall trial. These pigs also had an increased incidence of scouring as measured by lower fecal DM compared with pigs drinking control water. Although zeolite improved ADG and ADFI, it did not influence fecal consistency.

Key words: nursery pig, sulfate, water, zeolite

<sup>1</sup> The authors would like to thank St. Cloud Mining Co., Truth or Consequences, NM, for providing the zeolite used in this study.

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## Introduction

Zeolites are microporous aluminosilicate minerals composed of alkali and alkaline earth cations along with small amounts of other elements. The zeolite molecules are arranged in 3-dimensional structures that create interconnected channels capable of trapping molecules of proper dimensions similar to a sieve. Zeolite molecules can also bind and release specific molecules by adsorption or ion exchange. In industrial operations, zeolites have been used as detergents because of their ability to bind with water and other molecules. In agriculture, zeolites frequently have been used to reduce odor because of their ability to bind with ammonia. Although research is limited, previous results (Shurson et al., 1984<sup>3</sup>) observed in a study comparing synthetic zeolite and natural zeolite as growth promoters in late nursery phases that the synthetic zeolite (zeolite A) was relatively ineffective as a growth promoter in nursery pig diets. Synthetic zeolite was thought to become disassociated in the acidic environment of the digestive system; however, naturally occurring zeolite (clinoptilolite) was effective in its ammonia-binding capabilities and more stable in the gut, yet when it exceeded 5% of the diet, overall growth performance decreased compared with the control treatment. Perhaps by using its sieving capabilities, natural zeolite can bind with non-nutritive components of the diet and decrease their ability to cross the gut wall, but when inclusion rates become too high, it may bind with required nutrients and decrease pig performance.

Producers, especially those in the upper Midwest, have recently observed increased incidence of scours. Scours typically are associated with health and disease challenge along with the stress that accompanies weaning. Water quality and high-protein diets can also contribute to diarrhea and loose feces. In addition, increased incidence of scouring could be due to high sodium sulfate concentrations within groundwater supplies. Research by Anderson and Stothers (1978)<sup>4</sup> has shown that sulfates act as a natural laxative and can cause an increased occurrence of scours, but without significant detrimental effects on growth performance. Whether sulfates influence performance or not, they lead to increased cost in commercial swine production because producers treat the pigs with antibiotics in an attempt to decrease scour symptoms.

Therefore, our objectives for the study were to evaluate the effects of high-sulfate water on the performance and fecal consistency of newly weaned pigs and to determine whether a natural zeolite (clinoptilolite) could improve fecal consistency and growth of pigs drinking high-sulfate water.

## Procedures

The protocol for this experiment was approved by the Kansas State University Institutional Animal Care and Use Committee. The study was conducted at the K-State Segregated Early Weaning Facility in Manhattan, KS.

A total of 320 nursery pigs (PIC 1050 barrows, initially 11.9 lb, and 21 d of age) were used in a 24-d trial to evaluate the effects of high-sulfate water and dietary zeolite (clinoptilolite) on growth performance. Pigs were weighed and allotted to 1 of 8 treatments

<sup>3</sup> G. C. Shurson, P. K. Ku, E. R. Miller and M. T. Yokoyama. 1984. Effects of Zeolite a or Clinoptilolite in Diets of Growing Swine. *J. Anim. Sci.* 59:1536-1545.

<sup>4</sup> D. M. Anderson and S. C. Stothers. Effects of saline water in sulfates, chlorides and nitrates on the performance of young weanling pigs. *J. Anim. Sci.* 47:900-907.

arranged in a  $2 \times 4$  factorial with main effects of water source (control or water containing 3,000 ppm sodium sulfate) and dietary zeolite (0, 0.25, 0.5, and 1.0%). There were 5 pigs per pen and 8 pens per treatment. Pigs were provided unlimited access to feed and water by way of a 4-hole dry self-feeder and a cup waterer in each pen (5 ft by 5 ft). For the sodium sulfate water treatment, sodium sulfate was mixed in a stock solution and administered in the water supply (Manhattan, KS, municipal water source) of the corresponding pens by a medicator (Dosatron; Dosatron International Inc., Clearwater, FL) at the rate of 1:10 for a calculated inclusion rate of 3,000 ppm of sodium sulfate. All diets were fed in 2 phases (Table 1), and the dietary zeolite concentration was the same in both phases. Phase 1 diets were fed in a pellet form from d 0 to 10 after weaning. Phase 2 diets were fed in a meal form from d 10 to 24. Average daily gain, ADFI, and F/G were determined by weighing pigs and measuring feed disappearance on d 5, 10, 17, and 24.

Chemical composition of the natural zeolite (clinoptilolite) used in the experiment is shown in Table 2. Water samples were collected for both the control water supply with no sodium sulfate and the water treatment with 3,000 ppm sodium sulfate. Samples were analyzed by Servi-Tech Laboratories, Dodge City, KS, and were analyzed for sodium, sulfate, and total dissolved solids (Table 3).

Fecal samples were collected on d 5, 9, 16, and 23. The samples were collected from 3 randomly selected pigs per pen for a total of 24 samples per treatment. Immediately after collection, the samples were individually scored by 5 individuals trained to determine fecal consistency; therefore, 15 consistency scores were made for each pen and an average score was reported for the pen. The scale used for assessing fecal consistency was based on a numerical scale of 1 to 5, where 1 represented a hard, dry fecal pellet, 2 represented a firm formed feces, 3 represented soft moist feces that retained its shape, 4 represented soft unformed feces that assumes the shape of its container, and 5 represented a watery liquid that can be poured. After scoring, samples were analyzed for DM. A 2-stage DM procedure was used. The first stage consisted of drying the complete sample in a 122°F oven for 24 h. Afterward, the samples were allowed to cool, then were ground into a powder. In the second stage, 1 g of the ground sample was placed in a crucible and dried in a 212°F oven for 24 h. The initial DM value was then multiplied by the second to determine a total percentage DM.

Nursery pig growth performance was analyzed as a  $2 \times 4$  factorial with main effects of water treatment and dietary zeolite using the MIXED procedure of SAS (SAS Institute, Inc., Cary, NC). Pen was designated as the experimental unit and contrast statements were used to determine effects of water treatment, linear and quadratic effects of dietary zeolite, and their interactions.

For fecal scores and fecal DM, repeated measures analysis was conducted using the MIXED procedure of SAS. Pen was the experimental unit and again the fixed effects were water treatment and dietary zeolite. Contrast statements were used to evaluate: (1) linear and quadratic effects of increasing zeolite, (2) linear and quadratic effects over time (collection days), (3) water  $\times$  day interactions, (4) diet  $\times$  day interactions, and (5) water  $\times$  diet  $\times$  day interactions.

## Results and Discussion

During Phase 1 (d 0 to 10), a water source  $\times$  zeolite interaction (linear,  $P < 0.04$ ) was observed for ADFI (Tables 4 and 5), which occurred because ADFI increased as zeolite increased for pigs drinking high-sulfate water, but decreased with increasing zeolite for pigs drinking control water. No other interactions were observed. Sulfate addition to the water and dietary zeolite did not influence ADG, ADFI, or F/G from d 0 to 10 (Table 5).

During Phase 2 (d 10 to 24), increasing zeolite improved (linear,  $P < 0.01$ ) ADG and ADFI, with no effect on F/G. Also, ADG, ADFI, and F/G were poorer ( $P < 0.02$ ) for pigs drinking high-sulfate water compared with those drinking control water.

Overall (d 0 to 24), increasing zeolite increased (linear,  $P < 0.05$ ) ADG and ADFI, but F/G was not affected. Pigs drinking high-sulfate water had decreased ( $P < 0.01$ ) ADG and ADFI and poorer ( $P < 0.01$ ) F/G compared with pigs drinking control water.

A water  $\times$  day interaction ( $P < 0.01$ ) was observed as lower fecal scores over time for pigs drinking high-sodium sulfate water, which indicated their feces became firmer over time. In contrast, fecal consistency scores for the control water group remained consistent throughout the length of the trial.

Dietary zeolite did not influence fecal consistency scores (Tables 6 and 7); however, fecal samples were looser ( $P < 0.01$ ) for pigs drinking high-sodium sulfate water compared with control pigs.

Dietary zeolite had no effect on fecal DM content (Tables 8 and 9) in either Phase 1 or 2, but pigs drinking high-sodium sulfate water had decreased ( $P < 0.01$ ) DM content compared with control pigs. A water  $\times$  day interaction ( $P < 0.01$ ) occurred, which was the result of an increase in fecal DM content of pigs on the sodium sulfate water treatment over time, even though pigs on the control water treatment had consistent DM contents throughout the length of the study.

In conclusion, dietary zeolite appeared to have no impact on the fecal consistency of the pigs drinking high-sodium sulfate water, but the improvement in ADG and ADFI with the addition of zeolite during Phase 2 was interesting and unexpected. Although we are unsure of the biological reason for the improvement in growth performance, it may relate to the sieving properties of zeolite and its contribution to gut microbiology or its ability to bind with anti-nutritional aspects of feed ingredients and reduce their absorption. More research should be conducted to confirm the findings of this study and to determine whether zeolite should be included in nursery pig diets.

As for high-sodium sulfate water, the results from this trial agree with previous research demonstrating its negative effects on fecal consistency. Over time, fecal consistency appears to become better (firmer feces) as pigs adapt to the water; however, our calculated concentration of 3,000 ppm sodium sulfate negatively affected performance.

**Table 1: Diet composition (as-fed basis)**

Item	Phase 1 <sup>1</sup>	Phase 2 <sup>2</sup>
Ingredient, %		
Corn	38.16	57.06
Soybean meal (46.5% CP)	16.99	25.90
Dried distillers grains with solubles	5.00	---
Spray-dried animal plasma	4.00	---
Select menhaden fish meal	---	4.50
Spray-dried blood cells	1.25	---
Spray-dried whey	25.00	10.00
DPS 50 <sup>3</sup>	3.00	---
Monocalcium P (21% P)	0.85	0.38
Limestone	0.85	0.58
Salt	0.30	0.30
Zinc oxide	0.39	0.25
Trace mineral premix	0.15	0.15
Vitamin premix	0.25	0.25
L-Lysine HCl	0.20	0.25
DL-Methionine	0.13	0.13
L-Threonine	0.08	0.11
Phytase <sup>4</sup>	0.13	0.17
Acidifier <sup>5</sup>	0.20	---
Vitamin E, 20,000 IU	0.05	---
Choline chloride 60%	0.04	---
Zeolite (clinoptilolite) <sup>6</sup>	---	0.00
Total	100	100

*continued*

**Table 1: Diet composition (as-fed basis)**

Item	Phase 1 <sup>1</sup>	Phase 2 <sup>2</sup>
Calculated analysis		
Standardized ileal digestible amino acids, %		
Lysine	1.35	1.30
Isoleucine:lysine	54	61
Leucine:lysine	132	127
Methionine:lysine	30	35
Met & Cys:lysine	57	59
Threonine:lysine	65	63
Tryptophan:lysine	18	17
Valine:lysine	72	68
Total lysine, %	1.51	1.43
CP, %	21.6	21.32
ME, kcal/lb	1,552	1,505
Ca, %	0.75	0.70
P, %	0.73	0.63
Available P, %	0.65	0.47
Na, %	0.75	0.25
K, %	1.07	0.97
Added trace minerals, ppm <sup>7</sup>		
Zn	2,973	1,965
Fe	165	165
Mn	40	40
Cu	17	17
I	0.30	0.30
Se	0.30	0.30

<sup>1</sup>Phase 1 diets were fed from d 0 to 10.

<sup>2</sup>Phase 2 diets were fed from d 10 to 24.

<sup>3</sup>Nutra-Flo Company, Sioux City, IA.

<sup>4</sup>Phyzyme 600, Danisco Animal Nutrition, Carol Stream, IL. Provided 354 and 463 FTU/lb of diet, respectively.

<sup>5</sup>Kem-gest, Kemin Industries Inc., Des Moines, IA.

<sup>6</sup>Zeolite, St. Cloud Mining Company, Truth or Consequences, NM, replaced corn to provide 0, 0.25, 0.50, and 1% zeolite.

<sup>7</sup>Zeolite calculated trace mineral content was added to the calculated trace mineral levels within each respective dietary regimen.

**Table 2: Chemical composition of zeolite (clinoptilolite)<sup>1</sup>**

Element	
Ca, %	2.4
P, %	0.01
K, %	1.2
Na, %	0.1
Zn, ppm	59
Cu, ppm	10
Fe, %	0.6
Mg, %	0.9
Al, %	3.1
Si, %	32.9

<sup>1</sup> Chemical composition performed by used of x-ray fluorescence and conducted at St. Cloud Mining Co., Truth or Consequences, NM.

**Table 3: Analyzed composition of water<sup>1</sup>**

Item, ppm	Control water <sup>2</sup>	3,000 ppm sodium sulfate <sup>3</sup>
Total dissolved solids	321	2,773
Sulfate	84	2,002
Sulfate-sulfur	28	660
Cl	65	49
Na	38	750
Ca	25	26
Mg	12	12
K	6	7
Fe	0.06	0.1
Mn	0.01	0.01
pH	9.1	9

<sup>1</sup> Water samples were analyzed by Servi-Tech Laboratories, Dodge City, KS.

<sup>2</sup> City municipal water, Manhattan, KS.

<sup>3</sup> Calculated mix of 3000 ppm was delivered into water supply at a rate of 1 to 10 by Dosatron medicators (Dosatron International Corp., Clearwater, FL).

**Table 4: Effects of high-sulfate water and dietary zeolite (clinoptilolite) on nursery pig performance<sup>1</sup>**

Zeolite, %:	Water treatment								SEM	P-values		
	Control				3,000 ppm sodium sulfate					Sulfate × zeolite interaction	Sulfate × zeolite	
	0	0.25	0.50	1.0	0	0.25	0.50	1.0			Linear	Quadratic
d 0 to 10												
ADG, lb	0.37	0.36	0.33	0.32	0.28	0.37	0.33	0.32	0.028	0.25	0.17	0.21
ADFI, lb	0.37	0.36	0.33	0.33	0.31	0.36	0.34	0.36	0.019	0.14	0.04	0.34
F/G	1.00	1.00	1.04	1.12	1.32	0.98	1.02	1.12	0.110	0.32	0.24	0.18
d 10 to 24												
ADG, lb	0.78	0.82	0.86	0.90	0.68	0.71	0.78	0.77	0.044	0.92	0.68	0.65
ADFI, lb	1.10	1.15	1.17	1.20	0.98	1.02	1.12	1.12	0.049	0.75	0.49	0.66
F/G	1.42	1.42	1.37	1.33	1.44	1.46	1.45	1.46	0.038	0.51	0.14	0.94
d 0 to 24												
ADG, lb	0.61	0.63	0.62	0.64	0.51	0.57	0.59	0.58	0.028	0.58	0.43	0.25
ADFI, lb	0.79	0.82	0.80	0.82	0.69	0.75	0.79	0.80	0.032	0.23	0.08	0.37
F/G	1.30	1.31	1.29	1.27	1.37	1.32	1.35	1.38	0.026	0.25	0.18	0.30

<sup>1</sup>A total of 320 weanling pigs (PIC 1050 barrows, initial BW of 11.9 lb and 21 d of age) were used with 5 pigs per pen and 8 pens per treatment.

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**Table 5: Main effects of high-sulfate water and dietary zeolite (clinoptilolite) on nursery pig performance<sup>1</sup>**

Item	Water treatment			Zeolite, %					Zeolite <i>P</i> -values		
	Control	3,000 ppm sodium sulfate	SEM	0	0.25	0.50	1.0	SEM	Sulfate	Linear	Quadratic
d 0 to 10											
ADG, lb	0.34	0.33	0.014	0.32	0.37	0.33	0.32	0.020	0.40	0.51	0.31
ADFI, lb	0.35	0.34	0.010	0.34	0.36	0.33	0.35	0.014	0.62	0.90	0.90
F/G	1.04	1.11	0.055	1.16	0.99	1.03	1.12	0.078	0.36	0.97	0.12
d 10 to 24											
ADG, lb	0.84	0.74	0.029	0.73	0.77	0.82	0.84	0.035	<0.01	<0.01	0.39
ADFI, lb	1.15	1.06	0.033	1.04	1.09	1.14	1.16	0.039	<0.01	<0.01	0.21
F/G	1.38	1.45	0.019	1.43	1.44	1.41	1.40	0.027	0.02	0.32	0.86
d 0 to 24											
ADG, lb	0.63	0.56	0.017	0.56	0.60	0.61	0.61	0.021	<0.01	0.05	0.20
ADFI, lb	0.81	0.76	0.022	0.74	0.78	0.80	0.81	0.026	0.01	0.02	0.23
F/G	1.29	1.35	0.013	1.34	1.31	1.32	1.33	0.019	<0.01	0.85	0.43

<sup>1</sup> A total of 320 weanling pigs (PIC 1050 barrows, initial BW of 11.9 lb and 21 d of age) were used with 5 pigs per pen and 8 pens per treatment.

**Table 6: The interactions of high-sulfate water and dietary zeolite (clinoptilolite) on fecal consistency<sup>1,2,3,4</sup>**

Zeolite %	Water treatment								SEM	P-values		
	Control				3,000 ppm sodium sulfate					Sulfate × zeolite		
	0%	0.25%	0.50%	1.0%	0%	0.25%	0.50%	1.0%		Linear	Quadratic	
Day of collection												
d 5	3.4	3.3	3.0	3.2	4.1	4.1	4.1	4.1	0.13	0.58	0.26	
d 9	3.4	3.3	3.4	3.3	4.0	4.0	4.4	4.0	0.13	0.68	0.12	
d 16	3.3	3.0	3.3	3.2	3.6	3.9	3.5	3.5	0.13	0.44	0.72	
d 23	3.3	3.2	3.5	3.2	3.7	3.6	3.6	3.4	0.13	0.50	0.53	
Mean	3.4	3.2	3.3	3.2	3.9	3.9	3.9	3.8	0.07	0.23	0.80	

<sup>1</sup> A total of 792 fecal samples were collected (192 per collection day; fecal samples were collected on d 5, 9, 16, and 23). Three samples were taken per pen and were scored by 5 trained individuals; those 15 scores were then averaged and reported as pen means for each collection day.

<sup>2</sup> Three samples were collected randomly from 3 pigs per pen, and samples were scored on a numerical scale from 1 to 5.

<sup>3</sup> Scoring scale guidelines: 1 = dry firm pellet, 2 = firm formed stool, 3 = soft stool that retains shape, 4 = soft unformed stool that takes shape of container, 5 = watery liquid that can be poured.

<sup>4</sup> Water × diet × day interaction ( $P = 0.18$ ).

**Table 7: Main effects of high-sulfate water and dietary zeolite (clinoptilolite) on fecal consistency scores<sup>1,2,3,4</sup>**

Item	Water treatment			Zeolite					Sulfate	Zeolite P-values		
	Control	3,000 ppm sodium sulfate	SEM	0%	0.25%	0.50%	1.0%	SEM		Linear	Quadratic	
Day of collection												
d 5	3.2	4.1	0.07	3.7	3.7	3.6	3.7	0.09	<0.01	0.55	0.38	
d 9	3.4	4.1	0.07	3.7	3.7	3.9	3.6	0.09	<0.01	0.74	0.18	
d 16	3.2	3.6	0.07	3.5	3.4	3.4	3.4	0.09	<0.01	0.37	0.79	
d 23	3.3	3.6	0.07	3.5	3.4	3.5	3.3	0.09	<0.01	0.25	0.64	
Mean	3.3	3.8	0.04	3.6	3.5	3.6	3.5	0.05	<0.01	0.14	0.75	

<sup>1</sup> A total of 792 fecal samples were collected (192 per collection day; fecal samples were collected on d 5, 9, 16, and 23). Three samples were taken per pen and were scored by 5 trained individuals; those 15 scores were then averaged and reported as pen means for each collection day.

<sup>2</sup> Three samples were collected randomly from 3 pigs per pen, and samples were scored on a numerical scale from 1 to 5.

<sup>3</sup> Scoring scale guidelines: 1 = dry firm pellet, 2 = firm formed stool, 3 = soft stool that retains shape, 4 = soft unformed stool that takes shape of container, 5 = watery liquid that can be poured.

<sup>4</sup> Day main effect ( $P \leq 0.01$ ).

**Table 8: The interactions of high-sulfate water and dietary zeolite (clinoptilolite) on fecal dry matter, %<sup>1,2,3</sup>**

Zeolite %	Water treatment								SEM	P-values	
	Control				3,000 ppm sodium sulfate					Sulfate × zeolite	
	0%	0.25%	0.50%	1.0%	0%	0.25%	0.50%	1.0%		Linear	Quadratic
Day of collection											
d 5	21.4	21.0	23.5	23.1	13.5	12.7	14.0	13.2	0.01	0.41	0.87
d 9	23.9	25.0	25.2	26.2	19.0	18.0	17.0	19.8	0.01	0.64	0.24
d 16	25.6	26.4	24.6	26.0	25.6	20.9	24.4	23.7	0.01	0.85	0.61
d 23	24.6	25.8	21.9	25.7	21.9	23.9	24.3	24.6	0.01	0.43	0.14
Mean	23.9	24.6	23.8	25.3	20.0	18.9	19.9	20.4	0.01	0.73	0.86

<sup>1</sup> A total of 792 fecal samples were collected (192 per collection day).

<sup>2</sup> Three samples were collected randomly from 3 pigs per pen, and samples were dried using a 2-stage drying method.

<sup>3</sup> Water × diet × day interaction ( $P = 0.41$ )

**Table 9: The main effects of high-sulfate water and dietary zeolite (clinoptilolite) on fecal DM, %<sup>1,2,3</sup>**

Item	Water treatment			Zeolite					SEM	sulfate	Zeolite P-values	
	Control	3,000 ppm sodium sulfate	SEM	0%	0.25%	0.50%	1.0%	Linear			Quadratic	
Day of collection												
d 5	22.3	13.4	0.01	17.4	16.9	18.8	18.2	0.01	<0.01	0.39	0.71	
d 9	25.1	18.5	0.01	21.4	21.5	21.1	23.0	0.01	<0.01	0.22	0.39	
d 16	25.6	23.7	0.01	25.6	23.7	24.5	24.9	0.01	0.04	0.88	0.29	
d 23	24.5	23.7	0.01	23.2	24.9	23.1	25.2	0.01	0.39	0.27	0.72	
Mean	24.4	19.8	<0.01	21.9	21.7	21.9	22.8	0.01	<0.01	0.13	0.34	

<sup>1</sup> A total of 792 fecal samples were collected (192 per collection day; fecal samples were collected on d 5, 9, 16, and 23).

<sup>2</sup> Three samples were collected randomly from 3 pigs per pen, and samples were dried using a 2-stage drying method.

<sup>3</sup> Day main effect ( $P < 0.01$ ).