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Effects of Increasing Dietary Zinc in Low ABC-4 Diets on Nursery Performance, Fecal Dry Matter, Serum Zinc, and Zinc Excretion

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Summary

With the potential negative impact of pharmacological levels of zinc (typically from zinc oxide; ZnO) on the environment and public health, it's necessary to validate the doses commonly used in commercial swine production and evaluate new nutritional strategies to replace the use of pharmacological levels of Zn. A total of 360 pigs (initially 13.2 ± 1.63 lb) were used to evaluate the effects of increasing dietary Zn and the acid-binding capacity (ABC-4) of the diet on nursery pig performance, fecal dry matter, serum Zn, and Zn excretion. At weaning, pigs were randomly assigned within two body-weight blocks to one of the six dietary treatments in a randomized complete block design. There were five pigs per pen and 12 pens per treatment. Experimental diets were fed for the first 24 d (phases 1 and 2), and then all pigs were fed a common phase 3 diet for 22 d. All diets contained 110 ppm of Zn from $ZnSO_4$ from the trace mineral premix. The control treatment consisted of a low ABC-4 diet (200 and 250 meq/kg from d 0 to 10 and d 10 to 24, respectively). The next four treatments were the control diet with increasing Zn at 500, 1,000, 2,000, and 3,000 ppm in phase 1, and 333, 666, 1,332, and 2,000 ppm in phase 2 using ZnO. The sixth treatment was a high-ABC-4 diet (493 and 470 meq/kg from d 0 to 10 and d 10 to 24, respectively) with 3,000 and 2,000 ppm of added Zn in phases 1 and 2, respectively from ZnO. In all periods, no differences ($P > 0.10$) were observed between the low- and high-ABC-4 diets when 3,000 and 2,000 ppm of Zn were added in phases 1 and 2, respectively. For the experimental period (d 0 to 24), ADG and ADFI increased (linear, $P < 0.05$) as dietary Zn increased, with no difference in F/G. For the overall period, no response ($P > 0.10$) to dietary Zn was observed for any of the performance criteria. For fecal dry matter, no significant effect of dietary Zn was observed at d 10 or 24 ($P > 0.10$). However, at d 10, low-ABC-4 diets had a higher ($P = 0.002$) fecal dry matter than the high-ABC-4 diets at the same dietary level of Zn. Zinc intake, fecal Zn excretion, and Zn absorption increased (quadratic, $P < 0.001$) as dietary Zn concentration increased. A trend was observed (linear, $P = 0.074$) as the apparent total tract digestibility (ATTD) of Zn was increased as dietary Zn concentration increased. Low-ABC-4 diets had higher ($P < 0.05$) Zn intake, absorption, and ATTD of Zn than the high-ABC-4 diet at the same dietary Zn concentration. No difference ($P = 0.921$) was observed for fecal

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Zn excretion between ABC-4 formulation strategies. Day 24 serum-Zn concentration increased (quadratic, $P < 0.001$) as dietary Zn increased and a marginal increase ($P = 0.095$) in favor of the low-ABC-4 diets was observed between the formulation strategies. In conclusion, lowering the dietary ABC-4 capacity of diets containing pharmacological levels of ZnO increased d 10 fecal dry matter, Zn absorption, and the ATTD of Zn. Increasing ZnO in low-ABC-4 diets improved ADG and ADFI during the experimental period. However, this did not translate into overall performance differences.

Introduction

Zinc (Zn) is an essential mineral for swine, playing a key role in various physiological functions such as cellular signaling, digestion, cellular respiration, and nucleic acid metabolism.[2] The requirement of Zn for nursery pigs ranges between 26.6 and 72.4 mg/d.3 However, using pharmacological levels of Zn from zinc oxide (ZnO) in the first two dietary phases post-weaning has been a common strategy in the swine industry to minimize the incidence of post-weaning diarrhea (PWD) and improve pig performance.[4]

The mechanism by which pharmacological levels of Zn reduce the incidence of PWD and enhances performance is not fully understood. Nonetheless, several studies have shown that ZnO provides beneficial effects such as improved nutrient digestibility, antioxidant effects, enhanced intestinal morphology, modulated immune response, increased secretion of some digestive hormones, and antimicrobial activity.[5] Despite these positive effects, the potential negative impact of ZnO on the environment and public health has led some countries to regulate or prohibit its use in swine diets.[6]

These restrictions highlight the need to validate optimum Zn levels and explore alternative nutritional strategies to support nursery performance without pharmacological levels of Zn. Preliminary results suggest that low acid-binding capacity-4 (ABC-4) diets improve nursery performance in the absence of ZnO, but no responses were observed when high pharmacological levels of Zn were used. This suggests there is a relationship between low-ABC-4 diets and dietary ZnO. Therefore, this study aimed to evaluate the effect of increasing dietary Zn in low-ABC-4 diets on nursery performance, fecal dry matter, serum Zn concentration and Zn excretion in feces.

Material and Methods

The Kansas State University Institutional Animal Care and Use Committee approved the protocol used in this experiment. The experiment was conducted in two barns

[2] Kambe, T., Tsuji, T., Hashimoto, A., and N. Itsumura. 2015. The physiological, biochemical, and molecular roles of zinc transporters in zinc homeostasis and metabolism. Physiol. Rev. 95(3):749-84 doi:10.1152/physrev.0035.2014.

[3] NRC. 2012. Nutrient requirements of swine. 11th rev.ed.Natl. Acad.Press, Whashinton, DC.

[4] Poulsen, H.D. 1995. Zinc oxide for weaning pigs. Acta Agric. Scand. A Anim. 45:159-167.

[5] Bonetti, A., Tugnoli, B., Piva, A., and E. Grilli. 2021. Towards zero zinc oxide: Feeding strategies to manage post weaning diarrhea in piglets. Animals 11:642. doi:10.3390/ani11030642.

[6] EU. Commission Implementing Decision of 26.6.2017 Concerning, in the Framework of Article 35 of Directive 2001/82/EC of the European Parliament and of the Council, the Marketing Authorisations for Veterinary Medicinal Products Containing "Zinc Oxide" to Be Administered Orally to Food Producing Species. 2017. Available online: https://ec.europa.eu/health/documents/community-register/2017/20170626136754/dec_136754_en.pdf (accessed on 15 May 2022).

located at the Kansas State University Segregate Early Weaning (SEW) facility in Manhattan, KS. Each pen (4 × 5 ft) was equipped with a 6-hole dry feeder and a cup drinker to provide ad libitum access to feed and water.

Animals and diets

A total of 360 pigs (Line 200 × 400, DNA, Columbus, NE) initially weighing 13.2 ± 1.63 lb were used in a 46-d study. The pigs were weaned at approximately 21 d of age and divided into two body weight (BW) categories, light or heavy. The pigs were then randomly assigned to pens within the BW categories and pens were allotted to one of six dietary treatments. Each pen had five pigs and there were 12 pens per treatment. The two identical barns had an equal representation of dietary treatments and BW categories.

Pigs were fed experimental diets for the first two dietary phases, lasting 10 and 14 d, respectively (Table 1). From d 24 to 45 of the experiment, all pigs were fed a common phase 3 diet. All diets fed in the experimental and common periods contained 110 ppm of added Zn from $ZnSO_4$ provided by the trace mineral premix.

The dietary treatments were comprised of a low-ABC-4 diet, formulated to 200 and 250 meq/kg from d 0 to 10 (phase 1) and d 10 to 24 (phase 2), respectively (control). Crystalline lactose and ME-PRO (Aquatech, Brookings, SD) were used as lactose and protein concentrate sources, along with fumaric acid to lower the ABC-4 value. The next four diets were the control diet with increasing levels of Zn of 500, 1,000, 2,000, and 3,000 ppm in phase 1, and 333, 666, 1,332, and 2,000 ppm in phase 2 using ZnO (containing 72% Zn). The sixth treatment was a high-ABC-4 diet with added Zn levels of 2,000 and 3,000 ppm in phases 1 and 2, respectively. In this diet, crystalline lactose and HP300 (Hamlet Protein, Findlay, OH) were used as lactose and specialty soy sources, respectively, resulting in 493 and 470 meq/kg from d 0 to 10 and d 10 to 24, respectively.

All dietary treatments were manufactured at the Kansas State University O.H. Kruse Feed Technology Innovation Center in Manhattan, KS. The first phase was fed in pellet form, and the remaining two phases were fed in meal form.

Pigs and feeders were weighed on d 10, 24, and 46 to determine ADG, ADFI, and F/G. Fecal samples were collected via rectal palpation from the same three pigs per pen on d 10 and 24 of the study. These samples were stored at 39.2°F (4°C) until fecal dry matter analysis was conducted. The samples were dried in a forced-air oven for 48 h at 151°F (55°C) for determination of fecal dry matter.

Zinc excretion and serum concentration

A blood sample was taken from 10% of the pigs at placement, and from the middle-weight pig in each pen on d 24. The plasma was obtained through centrifugation, and samples were stored at -20°C until the Zn analysis was conducted

In phase 2, an indigestible marker, titanium dioxide (TiO_2), was added to the diet (0.5%). Following the determination of fecal dry matter, fecal samples were ground and pooled on a per-pen basis, ensuring equal representation from the three pigs in each pen.

The Zn concentration in serum, feed, and dry fecal samples were analyzed using an atomic absorption spectrometer. Before the analysis, the feed and dried feces were digested in 4 M nitric acid at 90°C for 4 h. Plasma samples were diluted in 4M nitric acid at a 1:10 ratio.

The total feces production was estimated using the following equation:

$$Feces, g/d = \frac{Feed\ TiO2,\ \% \times ADFI,\ g/d}{Fecals\ TiO2,\ \%}$$

The daily excretion of Zn was estimated by multiplying the daily feces production by the fecal Zn concentration. Zinc retention was calculated as the difference between Zn intake and excretion in feces.

Apparent total tract digestibility (ATTD) of Zn was estimated using the equation:

$$ATTD\ Zn,\ \% = \frac{Zn\ itake - Zn\ excretion}{Zn\ intake} \times 100$$

Statistical analysis

Data were analyzed as a generalized randomized block design. The lmer function was used from the lme4 package in RStudio [Version 4.0.2 (2020-06-22), R Core Team, R Foundation for Statistical Computing, Vienna, Austria] with pen serving as the experimental unit. For performance data and Zn balance, the model utilized treatment as a fixed effect and BW block and barn as random effects. For fecal dry matter, in addition to the previous model components, the pen was used as a random effect to account for the subsampling associated with multiple individual pigs analyzed from each pen.

The linear and quadratic effects of increasing Zn on performance and fecal dry matter were evaluated for the first five treatments. Contrast coefficients were established based on the Zn concentration fed within a specific dietary phase or the weighted average of Zn concentration when considering multiple dietary phases. Specific contrasts were performed to compare the low- and high-ABC-4 diets at the same Zn concentration (3,000 ppm in phase 1 and 2,000 ppm in phase 2). Treatment differences were considered significant at $P \leq 0.05$ and marginally significant at $0.05 < P \leq 0.10$.

Results and Discussion

In all periods, no differences ($P > 0.10$) were observed between the low- and high-ABC-4 diets with 3,000 then 2,000 ppm of Zn in phases 1 and 2, respectively (Table 2).

Body weight (BW) at d 10 and 24 increased (linear $P \leq 0.015$) as dietary Zn increased. However, no response ($P > 0.10$) to the Zn level previously fed from d 0 to 24 was observed by d 45.

For phase 1 (d 0 to 10), pigs fed increasing Zn had increased ADG (quadratic, $P = 0.004$). There were no differences between Zn levels for ADFI ($P > 0.10$). As a result, F/G improved (linear, $P < 0.001$) as dietary Zn increased.

For phase 2 (d 10 to 24), no response ($P = 0.103$) to Zn level was observed for ADG; however, ADFI increased (linear, $P = 0.008$) as dietary Zn increased. Feed efficiency tended (linear, $P = 0.072$) to worsen as dietary Zn increased.

For the experimental period (d 0 to 24), ADG and ADFI increased (linear, $P < 0.05$) as dietary Zn increased. No response ($P > 0.10$) to Zn level was observed for F/G.

For the common (d 24 to 46) and overall period (d 0 to 45), no response ($P > 0.10$) to the Zn levels previously fed from d 0 to 24 were observed for ADG, ADFI, and F/G.

No significant effect of dietary Zn was observed for fecal dry matter on d 10 or 24 ($P > 0.10$). However, at the same dietary Zn concentration, pigs fed low-ABC-4 diets had a higher ($P = 0.002$) d 10 fecal dry matter than pigs fed the high-ABC-4 diets.

Zinc intake, fecal Zn excretion, Zn absorption (difference between Zn intake and fecal Zn excretion), and d 24 serum Zn increased (quadratic, $P < 0.05$) as dietary Zn concentration increased, where there was little change observed between 110 and 333 ppm and then a greater response observed between 333 and 2,000 ppm. A trend (linear, $P = 0.074$) in ATTD of Zn was observed with increasing dietary Zn. At of the highest Zn level, pigs fed low-ABC-4 diets had greater ($P < 0.05$) Zn intake, absorption, and ATTD of Zn than pigs fed the high-ABC-4 diet with the same Zn levels. However, no difference between ABC-4 formulation strategy ($P = 0.921$) was observed for fecal Zn excretion, and a marginal increase ($P = 0.094$) in the serum Zn concentration was observed in favor of the low-ABC-4 diets.

In conclusion, increasing the dietary ZnO in low-ABC-4 diets linearly increased the ADG and ADFI during the experimental period. However, this did not translate into overall differences in performance by d 45. Fecal dry matter was not affected by dietary Zn level in the low-ABC-4 diets, but at the same dietary Zn concentration, low-ABC-4 diets increased d 10 fecal dry matter. Zn intake, fecal Zn excretion, Zn absorption, and d-24 plasma Zn quadratically increased as the dietary Zn concentration increased. Lowering the dietary acid-binding capacity increased the ATTD of Zn and the d-24 plasma Zn concentration.

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

	Phase 1		Phase 2		Phase 3
ABC-4	**Low**	**High**	**Low**	**High**	
Ingredient, %					
Corn[2]	52.65	48.81	56.67	54.21	68.06
Soybean meal	14.63	14.66	23.73	23.75	28.12
Crystalline lactose	15.00	15.00	7.50	7.50	---
Microbial enhanced SBM[3]	9.00	---	6.25	---	---
Enzymatically treated SMB[4]	---	13.0	---	9.0	---
Spray-dried bovine plasma	2.5	2.5	---	---	---
Corn oil	2.00	2.00	1.00	1.00	---
Limestone	0.27	0.33	0.35	0.39	0.75
Monocalcium phosphate	1.15	1.05	1.15	1.05	0.85
Salt	0.78	0.78	0.80	0.80	0.60
L-Lys-HCl	0.43	0.43	0.45	0.45	0.55
DL-Met	0.27	0.25	0.26	0.25	0.21
L-Thr	0.21	0.21	0.22	0.23	0.23
L-Trp	0.06	0.04	0.06	0.05	0.05
L-Val	0.09	0.08	0.10	0.10	0.16
Trace mineral premix	0.15	0.15	0.15	0.15	0.15
Vitamin premix	0.25	0.25	0.25	0.25	0.25
Phytase[5]	0.08	0.08	0.08	0.08	0.03
Fumaric acid	0.5	---	0.5	---	---
Zinc oxide	---	0.40	---	0.26	---
TiO2	---	---	0.5	0.5	---
Total	100	100	100	100	100.0

continued

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

	Phase 1		Phase 2		Phase 3
ABC-4	**Low**	**High**	**Low**	**High**	
Calculated analysis					
SID amino acids, %					
Lys	1.35	1.35	1.35	1.35	1.30
Ile:Lys	56	57	58	59	53
Leu:Lys	119	113	119	115	113
Met:Lys	39	38	39	39	36
Met & Cys:Lys	60	61	60	60	57
Thr:Lys	65	65	65	65	63
Trp:Lys	20	20	20	20	19
Val:Lys	70	70	70	70	70
His:Lys	37	36	37	37	35
Total Lys, %	1.51	1.51	1.50	1.50	1.44
NE, kcal/lb	1180	1177	1126	1126	1111
SID Lys:NE, g/Mcal	5.19	5.21	5.44	5.44	5.31
Crude protein, %	20.4	21.0	21.3	21.7	20.0
Lactose, %	15.0	15.0	7.5	7.5	0.0
Ca, %	0.50	0.53	0.55	0.57	0.64
P, %	0.55	0.59	0.59	0.60	0.56
STTD P, %	0.50	0.51	0.49	0.49	0.43
ABC-4, meq/kg	200	441	250	430	---

[1] Phases 1, 2, and 3 were fed from d 0 to 10, 10 to 24, and 24 to 46, respectively.
[2] In the low-ABC-4 diets, ZnO (72% Zn) was added at the expense of corn to form the experimental diets. For phase 1, the added ZnO levels were 0, 0.05, 0.12, 0.26, and 0.40%. For phase 2, the added ZnO levels were 0, 0.03, 0.08, 0.17, and 0.26%. In both cases, these values correspond to treatments 1 through 5, respectively.
[3] ME-PRO (Aquatech, Brookings, SD).
[4] HP 300 (Hamlet protein, Findlay, OH).
[5] Ronozyme Hiphos 2700 (dsm-firmenich, Parsippany,NJ) provided an estimated release of 0.14% STTD P in phases 1 and 2 and 0.12% STTD P in phase 3.

Table 2. Effect of dietary Zn and ABC-4 value of the diet on nursery performance and fecal dry matter[1]

	Dietary Zn, ppm[2]							*P* =		
Phase 1:	110	500	1,000	2,000	3,000	3,000				
Phase 2:	110	333	666	1,332	2,000	2,000		Zn level[4]		
ABC-4[3]:			Low			High	SEM	Linear	Quadratic	ABC-4[5]
BW, lb										
d 0	13.2	13.2	13.2	13.2	13.2	13.2	1.63	0.845	0.957	0.998
d 10	16.9	17.1	17.1	17.2	17.6	17.7	1.86	0.006	0.695	0.676
d 24	29.6	29.6	29.7	30.0	30.9	30.7	2.66	0.015	0.424	0.675
d 45	62.8	62.5	63.1	62.9	63.7	63.3	3.76	0.381	0.788	0.730
Phase 1 (d 0 to 10)										
ADG, lb	0.38	0.39	0.40	0.41	0.45	0.46	0.038	0.004	0.004	0.627
ADFI, lb	0.43	0.43	0.44	0.44	0.45	0.47	0.031	0.348	0.855	0.383
F/G	1.15	1.13	1.12	1.09	1.02	1.03	0.042	< 0.001	0.577	0.838
Phase 2 (d 10 to 24)										
ADG, lb	0.91	0.89	0.90	0.91	0.95	0.92	0.062	0.103	0.311	0.420
ADFI, lb	1.19	1.18	1.24	1.23	1.30	1.23	0.080	0.008	0.793	0.104
F/G	1.32	1.33	1.38	1.36	1.37	1.33	0.021	0.072	0.229	0.189
Experimental period (d 0 to 24)										
ADG, lb	0.69	0.68	0.69	0.69	0.74	0.73	0.046	0.023	0.286	0.689
ADFI, lb	0.88	0.87	0.91	0.89	0.94	0.91	0.056	0.016	0.686	0.254
F/G	1.28	1.28	1.31	1.29	1.28	1.25	0.020	0.879	0.198	0.186
Common phase (d 24 to 46)[6]										
ADG, lb	1.51	1.50	1.52	1.50	1.49	1.48	0.053	0.540	0.817	0.834
ADFI, lb	2.23	2.25	2.27	2.27	2.24	2.21	0.093	0.856	0.471	0.555
F/G	1.48	1.50	1.49	1.52	1.51	1.49	0.016	0.192	0.375	0.463
Overall (d 0 to 46)										
ADG, lb	1.08	1.07	1.09	1.07	1.10	1.09	0.048	0.463	0.609	0.736
ADFI, lb	1.52	1.53	1.56	1.54	1.57	1.53	0.073	0.284	0.842	0.383
F/G	1.41	1.43	1.43	1.44	1.43	1.41	0.010	0.418	0.104	0.179
Fecal dry matter, %[7]										
d 10	26.67	27.11	27.65	27.73	27.64	24.78	0.628	0.263	0.389	0.002
d 24	27.11	27.22	27.60	27.09	27.37	26.35	0.601	0.895	0.878	0.222

[1] A total of 360 pigs (Line 241 × 400, DNA, Columbus, NE; initially 13.2 ± 1.63 lb) were used in a 46-d growth study with five pigs per pen and 12 pens per treatment.
[2] 110 ppm of Zn from $ZnSO_4$ were added in all dietary phases. ZnO (72% of Zn) was used as a Zn source to reach the level of the different experimental treatments.
[3] Low-ABC-4 diets were formulated to 200 and 250 meq/kg from d 0 to 10 and d 10 to 24 in their basal level (without ZnO inclusion), respectively. However, the ABC-4 capacity of these diets increased with the dietary Zn level. The high-ABC-4 diets contained 441 and 430 meq/kg from d 0 to 10 and d 10 to 24, respectively.
[4] Linear and quadratic effects of the Zn level were evaluated in the low-ABC-4 diets. Contrast coefficients were established based on the Zn concentrations fed within a specific dietary phase or the weighted average of Zn concentration when considering multiple dietary phases.
[5] Compares treatments with 3,000 and 2,000 ppm of Zn in phases 1 and 2, respectively, under the two formulation strategies (low and high ABC-4).
[6] All experimental groups were fed with the same common diet.
[7] Same three pigs per pen were sampled on days 10 and 24.

Table 3. Effect of dietary Zn and ABC-4 value on Zn balance at d 24, dry matter-basis[1]

	Dietary Zn, ppm[2]							*P* =		
Phase 1:	**110**	**500**	**1,000**	**2,000**	**3,000**	**3,000**				
Phase 2:	**110**	**333**	**666**	**1,332**	**2,000**	**2,000**		**Zn level[4]**		
ABC-4:[3]			**Low**			**High**	**SEM**	**Linear**	**Quadratic**	**ABC-4[5]**
Zn intake, mg/d	159.2	183.1	398.1	698.4	1,147.3	1,056.4	40.75	< 0.001	< 0.001	< 0.001
Fecal excretion of Zn, mg/d	125.9	154.3	307.7	578.1	875.6	878.1	33.16	< 0.001	< 0.001	0.921
Difference, mg/d[6]	33.3	28.7	90.4	120.3	271.7	178.3	20.86	< 0.001	< 0.001	< 0.001
ATTD of Zn, %	20.5	15.7	22.8	17.3	23.7	16.7	2.81	0.074	0.148	0.002
Serum Zn, mg/L										
d 0[7]			0.81 ± 0.10				---	---	---	---
d 24	0.67	0.70	0.91	1.27	2.05	1.83	0.098	< 0.001	0.012	0.094

[1] A total of 360 pigs (Line 241 × 400, DNA, Columbus, NE. initially 13.2 ± 1.63 lb.) were used with five pigs per pen and 12 pens per treatment.
[2] 110 ppm of Zn from $ZnSO_4$ were added in all dietary phases. ZnO (72% of Zn) was used as a Zn source to reach the level of the different experimental treatments. Dietary analyzed Zn values were 294, 342, 707, 1,251, 1,951, and 1,899 ppm for treatments 1 to 6, respectively.
[3] Low ABC-4 diets were formulated to 200 and 250 meq/kg from d 0 to 10 and d 10 to 24 in their basal level (without ZnO inclusion), respectively. However, the ABC-4 capacity of these diets increased with the dietary Zn level. The high-ABC-4 diets contained 441 and 430 meq/kg from d 0 to 10 and d 10 to 24, respectively.
[4] Linear and quadratic effects of the Zn level were evaluated in the low-ABC-4 diets. Contrast coefficients were established based on the Zn concentrations fed within a specific dietary phase or the weighted average of Zn concentration when considering multiple dietary phases.
[5] Compares treatments with 2,000 ppm of Zn under the two formulation strategies (low and high ABC-4).
[6] Difference = Zn intake – Fecal Zn excretion.
[7] At weaning, a blood sample from 10% of the pigs was collected, and serum Zn analyzed.