

## Effect of Zinc Source and Level on Growth Performance, Fecal Dry Matter, and Fecal Zinc Concentration of Nursery Pigs

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### Summary

A total of 2,268 pigs (initially  $12.5 \pm 0.68$  lb) were used to evaluate the effect of zinc (Zn) sources and levels on nursery performance and fecal dry matter. At weaning, pens of pigs were sorted by body weight (BW) and then randomly assigned to one of the seven dietary treatments in a  $3 \times 2 + 1$  arrangement, with main effects of Zn source and level. The Zn sources included zinc oxide (ZnO) and two sources of Zn hydroxychloride. The total dietary Zn levels were set at 1,500 ppm or 1,000 ppm during phase 1 and at 1,000 ppm or 500 ppm during phase 2. An additional treatment was included as a positive control, utilizing 3,000 and 2,000 ppm of Zn from ZnO in phases 1 and 2, respectively. After the experimental period (dietary phases 1 and 2), all pigs were fed a common phase 3 diet for 14 d, which contained 100 ppm of added Zn from ZnSO<sub>4</sub>. On d 7 and 21, fecal samples from three pigs per pen were collected for dry matter analysis. During the experimental phase, common phase, and overall, no significant Zn source  $\times$  level interactions or main effects of Zn source or level were observed. Within the diets provided Zn from ZnO, no differences were observed between treatments for growth performance, mortality, removals, and fecal dry matter. For the d 7 fecal Zn concentration, a significant interaction ( $P = 0.009$ ) between the Zn source  $\times$  level was observed, where a greater ( $P < 0.05$ ) fecal Zn concentration was observed as the dietary Zn inclusion increased when ZnO or Zn hydroxychloride source B was used, but no differences ( $P > 0.10$ ) between levels were observed in the Zn hydroxychloride source A. At d 21, the fecal Zn concentration decreased ( $P < 0.001$ ) as dietary Zn concentration decreased, independent of the Zn source ( $P > 0.10$ ). In conclusion, the source and level of dietary Zn did not significantly impact growth performance, removals, mortality, or fecal dry matter of nursery pigs. However, utilizing lower dietary Zn levels independent of the source resulted in lower fecal Zn concentration.

### Introduction

The use of Zn, particularly from ZnO, during the nursery period has consistently shown benefits in reducing diarrhea and improving performance when included in diets at pharmacological levels. However, the uncontrolled use of ZnO leads to excessive excretion of Zn, resulting in Zn accumulation in soil and water and potential bacterial

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resistance that may ultimately affect public health.<sup>2</sup> Multiple studies have shown that an alternative form of Zn with a higher solubility could maintain adequate performance with lower Zn excretion.<sup>3</sup>

Zinc hydroxychloride is produced through a patented process that involves reacting high-purity forms of Zn with water and hydrochloric acid. This procedure results in hydroxychloride crystals that contain Zn covalently bonded to hydroxyl groups and chloride. The covalent bonds reduce the interaction of Zn with other dietary components, resulting in a higher bioavailability.<sup>4</sup> However, there is limited scientific evidence to determine the impact of this specific chemical form of Zn on nursery performance. Therefore, the objective of this study was to determine the impact of Zn level and source on growth performance and fecal dry matter in nursery pigs.

## 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 independent rooms at a commercial research site operated by Hord Farms West in Pipestone, MN. Each pen (7.5 × 12 ft) was equipped with a six-hole dry feeder and a drinker pan shared by two pens to provide *ad libitum* access to feed and water.

### *Animals and diets*

A total of 2,268 pigs (Line 337 × 1050, PIC, Hendersonville, TN) initially weighing  $12.5 \pm 0.68$  lb were used in a 39-d study with 27 pigs per pen and 12 pens per treatment. At placement, pens were divided into six body weight (BW) blocks and then randomly assigned to one of the seven dietary treatments within each BW block. The two identical rooms had an equal representation of dietary treatments and BW categories.

Pigs were fed experimental diets for the first two dietary phases, lasting 11 and 14 d, respectively. In the remaining 14 d of the experiment, all pigs were fed a common phase 3 diet.

Phase 1 diets were manufactured at Hubbard Feeds in Mankato, MN. Phase 2 and 3 diets were

manufactured at the Hord Farms West feed mill located in Pipestone, MN. The first phase was fed in pellet form, and the remaining two phases were fed in meal form. All diets were formulated to meet or exceed the NRC (2012) requirement estimates for nursery pigs and included 100 ppm of Zn from ZnSO<sub>4</sub> in the trace mineral premix as a basal added Zn level (Table 1).

Dietary treatments were arranged in a 3 × 2 + 1 factorial, with the main effects of Zn source and level. The Zn sources included ZnO and two sources of Zn hydroxychloride (Hydroxy Zn, SAM Nutrition, Bloomington, MN, and Intellibond Z, Selko, Indianap-

<sup>2</sup> Duan, M., Gu, J., Wang, X., Li, Y., Zhang, R., Hu, T., & Zhou, B. 2019. Factors that affect the occurrence and distribution of antibiotic resistance genes in soils from livestock and poultry farms. *Ecotoxicol. Environ. Saf* 180: 114-122. doi:10.1016/j.ecoenv.2019.05.005.

<sup>3</sup> Case, C. L., and M. S. Carlson. 2002. Effect of feeding organic and inorganic sources of additional zinc on growth performance and zinc balance in nursery pigs. *J. Anim. Sci.* 80:1917-1924. doi:10.2527/2002.8071917x.

<sup>4</sup> Leisure, N. J., C. C. Jackson, M. Huang, T. B. Moore, and F. A. Steward. 2014. Micronutrient supplement. US Pat. No. 8,802,180 B2.

olis, IN). The total dietary Zn levels were set at 1,500 ppm or 1,000 ppm during phase 1 and at 1,000 ppm or 500 ppm during phase 2. An additional treatment was included as a positive control, utilizing 3,000 and 2,000 ppm of Zn from ZnO in phases 1 and 2, respectively.

Pigs and feeders were weighed weekly from d 11 to 39 to determine ADG, ADFI, and F/G. Fecal samples were collected via rectal palpation from three pigs per pen on d 7 and 21 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).

The mineral concentration in feed and dry fecal samples was analyzed using an inductive coupled plasma (ICP) analyzer at the K-State Research and Extension Soil Testing Lab. Before the analysis, the feed and dried feces were ashed and then digested in 4 M nitric acid at 194°F (90°C) for 4 h. A feed sample per dietary treatment and phase was analyzed for the main chemical components (dry matter, crude protein, fat, ash, and acid detergent fiber) at the Midwest Laboratories (Omaha, NE) following AOAC methodologies.

Pigs removed from the experimental pens due to health problems or slow growth were ear-tagged with a consecutive number and placed in independent pens within each room, where they received special care. Removed animals did not receive the same experimental diet they had received prior to removal due to logistical constraints. At the end of the experiment, removals were counted to determine total mortality (pen + removals mortality).

Due to multiple reports of *E. coli* in other nurseries filled with piglets from the same sow farm origins, all the piglets were vaccinated against *E. coli* in the second week post placement, utilizing Entero Vac (Arco Laboratories, Jewell, Iowa).

### *Statistical analysis*

Data were analyzed as a randomized complete 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 as the experimental unit. For performance data, 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.

Specific contrasts were conducted to compare the effect of Zn source, levels, and their interaction on the response variables (performance and fecal dry matter). Additionally, linear and quadratic responses to the ZnO level were evaluated. Contrast coefficients were established based on the Zn concentration fed within a specific dietary phase or the weighted average of Zn concentration based on feed intake when considering multiple dietary phases.

Mortality and removals were analyzed using a binomial distribution. The proportion of dead or removed pigs over the initial pigs placed per pen was used as the response variable for mortality and removals. Results were considered significant with  $P \leq 0.05$  and marginally significant with  $P \leq 0.10$ .

## Results and Discussion

The proximate analysis and mineral concentration of experimental diets were consistent with formulated values considering analytical variation (Table 2).

For growth performance, mortality, removals, mortality and removals, total mortality, and fecal dry matter, no significant interactions or main effects ( $P > 0.10$ ) were observed between the Zn sources and level. Furthermore, there were no linear or quadratic responses ( $P > 0.10$ ) based on ZnO level for growth performance or survivability.

For fecal Zn concentration, on d 7, a significant ( $P = 0.009$ ) Zn source  $\times$  level interaction was observed, where a greater ( $P < 0.05$ ) fecal Zn concentration was observed as the dietary Zn inclusion increased when ZnO or Zn hydroxychloride source B was used, but no differences ( $P > 0.10$ ) between levels were observed in the Zn hydroxychloride source A. At d 21, no significant interaction ( $P = 0.748$ ) between Zn source  $\times$  level was observed. For the main effects, there was no significant impact ( $P > 0.10$ ) of the Zn source on d 7 or 21 fecal Zn concentration. However, a marginal effect of Zn level was detected at d 7 ( $P = 0.065$ ), and a significant effect was observed at d 21 ( $P < 0.001$ ), where fecal Zn concentration decreased as dietary Zn levels decreased in both cases. Furthermore, fecal Zn concentration linearly decreased ( $P = 0.001$ ) as ZnO levels were reduced.

In conclusion, the source and level of dietary Zn did not significantly impact the overall growth performance, fecal dry matter, removals, or mortality. However, utilizing lower levels of dietary Zn, independent of the source, resulted in lower fecal Zn concentration.

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**Table 1. Diet composition (as-fed basis)<sup>1</sup>**

<b>Item</b>	<b>Phase 1</b>	<b>Phase 2</b>	<b>Phase 3</b>
Corn <sup>2</sup>	43.53	58.60	56.55
Soybean meal	16.68	28.81	29.95
Corn DDGS	5.00	7.50	10.00
Spray-dried whey	25.00	---	---
Microbial enhanced SBM <sup>3</sup>	5.00	---	---
Corn oil	1.00	---	---
Limestone	0.45	0.77	0.72
Monocalcium phosphate	1.10	1.35	0.70
Salt	0.15	0.45	0.29
L-Lys-HCl	0.48	---	---
DL-Met	0.26	0.24	0.18
L-Thr	0.19	---	---
L-Trp	0.03	0.02	---
L-Val	0.12	0.13	0.05
Thr <sup>4</sup>	---	0.30	0.21
Liquid Lysine, 55%	---	0.80	0.64
Trace mineral premix <sup>5</sup>	0.15	0.15	---
Vitamin premix	0.25	0.25	---
Vitamin + trace mineral premix <sup>5</sup>	---	---	0.13
Choline chloride 60%	0.04	---	---
Phytase <sup>6</sup>	0.08	0.08	0.04
Sodium metabisulfite	0.50	---	---
Feed Aid <sup>7</sup>	---	0.55	0.55
Total	100.0	100.0	100.0

*continued*

**Table 1. Diet composition (as-fed basis)<sup>1</sup>**

Item	Phase 1	Phase 2	Phase 3
Calculated analysis			
SID amino acids, %			
Lys	1.35	1.35	1.30
Ile:Lys	57	55	60
Leu:Lys	119	120	132
Met:Lys	39	39	37
Met & Cys:Lys	60	60	60
Thr:Lys	65	65	65
Trp:Lys	20	20	21
Val:Lys	70	69	70
His:Lys	34	37	40
Total Lys, %	1.49	1.51	1.47
ME, kcal/lb	1521	1470	1486
NE, kcal/lb	1143	1079	1086
SID Lys:NE, g/Mcal	5.36	5.68	5.43
Crude protein, %	20.43	21.82	22.68
Ca, %	0.71	0.76	0.63
P, %	0.69	0.69	0.57
STTD P, %	0.62	0.56	0.44

<sup>1</sup> Phases 1, 2, and 3 were fed for 11, 14, and 14 d, respectively.

<sup>2</sup> The total dietary Zn was adjusted according to the experimental treatments using ZnO (72% Zn), Hydroxy Zn (58% Zn; SAM Nutrition, Bloomington, MN), or Intellibond Z (55% Zn; Selko, Indianapolis, IN) at the expense of corn.

<sup>3</sup> ME-PRO (Aquatech, Brookings, SD).

<sup>4</sup> Thr Pro (CJ America).

<sup>5</sup> The premixes provided 100 ppm of Zn from ZnSO<sub>4</sub> in all the dietary treatments and phases.

<sup>6</sup> Phases 1 and 2: Ronozyme Hiphos 2700 (dsm-firmenich, Parsippany, NJ) provided an estimated release of 0.14% STTD P. Phase 3: Optiphos Plus 2500 G (Huvepharma, Sofia, Bulgaria) provided an estimated release of 0.13% STTD P.

<sup>7</sup> Elanco Animal Health, Greenfield, IN.

**Table 2. Analyzed composition of experimental diets (as-fed basis)<sup>1,2</sup>**

Source:	Dietary Zn, ppm						
	ZnO		Zn hydroxychloride <sup>3</sup>				
			A		B		
<b>Phase 1:</b>	<b>3,000</b>	<b>1,500</b>	<b>1,000</b>	<b>1,500</b>	<b>1,000</b>	<b>1,500</b>	<b>1,000</b>
<b>Phase 2:</b>	<b>2,000</b>	<b>1,000</b>	<b>500</b>	<b>1,000</b>	<b>500</b>	<b>1,000</b>	<b>500</b>
Phase 1							
DM, %	88.13	87.92	88.55	88.84	89.00	87.78	87.66
CP, %	19.6	20.10	19.20	19.60	18.10	19.70	19.40
Fat, %	4.86	4.63	4.32	5.14	4.92	4.83	4.92
Ash, %	5.52	6.08	5.79	6.18	6.05	5.92	5.85
ADF, %	1.70	1.80	1.80	2.80	2.90	2.90	3.40
P, %	0.63	0.66	0.68	0.67	0.65	0.64	0.66
Ca, %	0.83	0.85	0.77	0.81	0.74	0.71	0.79
Microminerals, ppm							
Zn	2,675	1,562	1,138	1,453	1,882	1,866	1,244
Mg	1,631	1,605	1,531	1,557	1,547	1,488	1,513
Na	4,067	3,439	3,480	3,437	3,583	3,500	3,463
K	11,817	11,743	11,072	10,889	11,328	10,909	10,563
Cu	34	28	28	29	37	24	25
Fe	427	339	309	282	326	302	293
Mn	106	70	58	60	54	69	53
Phase 2							
DM, %	85.92	86.24	85.71	86.29	86.69	86.89	86.96
CP, %	20.60	22.50	20.4	20.20	19.2	20.60	20.80
Fat, %	3.75	2.64	2.74	2.42	3.08	2.95	2.94
Ash, %	5.48	5.41	5.45	5.82	5.37	5.18	5.55
ADF, %	3.40	4.20	4.0	3.60	3.7	4.00	3.70
P, %	0.60	0.64	0.62	0.70	0.70	0.63	0.70
Ca, %	0.51	0.56	0.63	0.83	0.88	0.58	0.78
Microminerals, ppm							
Zn	1,425	883	439	1,014	635	982	599
Mg	1,831	1,835	1,667	1,705	1,670	1,749	1,783
Na	2,333	2,830	3,732	4,640	3,283	2,648	3,740
K	9,732	9,615	8,681	8,327	7,558	7,972	7,932
Cu	33	19	8	12	59	20	15
Fe	307	279	272	337	354	286	296
Mn	50	43	47	50	63	64	55

<sup>1</sup> For proximate analysis, the values represent the mean of one sample per phase. For mineral analysis, the value represents the mean of two samples. Phase 1 was fed for 11 d in a pellet form and phase 2 for 14 d in a mash form.

<sup>2</sup> After phase 2, a common phase 3 was fed to all experimental units for 14 d. The analyzed composition of this diet was DM: 85.71, CP: 21.80, fat: 3.20, ash: 4.33, and NDF: 4.00. All values are expressed as a % of the final feed on an as-fed basis.

<sup>3</sup> Source A: Hydroxy Zn, SAM Nutrition, Bloomington, MN - Source B: Intellibond Z, Selko, Indianapolis, IN.

**Table 3. Effect of Zn sources and levels on nursery performance, fecal dry matter, and fecal zinc concentration<sup>1</sup>**

Item	Dietary Zn, ppm								SEM	<i>P</i> <sup>3,4</sup>		
	Source :		ZnO		Zn hydroxychloride <sup>2</sup>					Source × Level	Source	Level
	Phase 1:	Phase 2:	3,000	1,500	A		B					
		2,000	1,000	1,000	1,500	1,000	1,500	1,000				
BW, lb												
d 0		12.4	12.4	12.5	12.5	12.4	12.4	12.6	0.68	0.617	0.778	0.703
d 11		15.1	15.1	15.6	15.2	15.0	15.1	15.4	0.43	0.512	0.674	0.501
d 25		29.0	28.3	28.5	28.8	27.6	28.1	28.3	0.70	0.203	0.998	0.536
d 39		48.1	47.2	48.0	48.0	46.9	47.0	47.3	1.00	0.316	0.659	0.980
Period 1 (d 0 to 11)												
ADG, lb		0.25	0.25	0.28	0.25	0.24	0.24	0.25	0.037	0.370	0.333	0.832
ADFI, lb		0.34	0.33	0.34	0.35	0.34	0.34	0.34	0.014	0.895	0.734	0.702
F/G		1.43	1.46	1.24	1.54	1.48	1.54	1.37	0.216	0.588	0.624	0.082
Period 2 (d 11 to 25)												
ADG, lb		0.91	0.88	0.87	0.90	0.83	0.89	0.85	0.041	0.382	0.901	0.023
ADFI, lb		1.15	1.12	1.12	1.15	1.07	1.13	1.09	0.034	0.399	0.883	0.073
F/G		1.27	1.27	1.28	1.27	1.29	1.28	1.29	0.036	0.942	0.952	0.444
Experimental period (d 0 to 25)												
ADG, lb		0.60	0.59	0.60	0.60	0.56	0.59	0.58	0.048	0.516	0.703	0.356
ADFI, lb		0.77	0.76	0.76	0.77	0.73	0.77	0.75	0.032	0.693	0.794	0.164
F/G		1.30	1.29	1.27	1.31	1.32	1.31	1.30	0.057	0.559	0.728	0.440
Period 3 (d 24 to 39)												
ADG, lb		1.36	1.35	1.38	1.37	1.37	1.35	1.36	0.025	0.931	0.400	0.303
ADFI, lb		1.96	1.93	2.00	2.01	1.98	1.99	1.98	0.054	0.743	0.767	0.885
F/G		1.45	1.43	1.44	1.47	1.44	1.48	1.46	0.039	0.823	0.692	0.482

*continued*

**Table 3. Effect of Zn sources and levels on nursery performance, fecal dry matter, and fecal zinc concentration<sup>1</sup>**

Item	Dietary Zn, ppm								SEM	<i>P</i> = <sup>3,4</sup>		
	Source :	Zn hydroxychloride <sup>2</sup>								Source × Level	Source	Level
		Phase 1:	ZnO	A		B						
Phase 2:	3,000	1,500	1,000	1,500	1,000	1,500	1,000	500				
Overall (d 0 to 39)												
ADG, lb		0.86	0.85	0.87	0.86	0.84	0.85	0.85	0.040	0.752	0.902	0.848
ADFI, lb		1.18	1.16	1.19	1.19	1.16	1.19	1.17	0.033	0.852	0.849	0.586
F/G		1.37	1.37	1.36	1.40	1.39	1.40	1.39	0.043	0.819	0.845	0.359
Mortality, %		0.93	0.31	0.00	0.31	0.31	0.93	0.62	0.532	0.809	0.286	0.999
Removals, %		11.29	8.92	8.33	11.58	9.51	7.45	9.21	3.044	0.228	0.164	0.890
Removals + mortality, %		12.24	9.27	8.67	12.24	9.86	8.38	9.86	3.645	0.246	0.246	0.762
Total mortality, % <sup>5</sup>		2.75	2.22	1.49	2.75	1.74	1.49	1.99	1.450	0.289	0.508	0.498
Fecal dry matter, % <sup>6</sup>												
d 7		23.3	22.2	22.7	23.6	25.8	24.0	23.6	1.70	0.425	0.609	0.581
d 21		19.5	18.6	18.6	18.9	19.5	18.4	20.0	1.50	0.534	0.994	0.229
Fecal Zn, ppm DM basis												
d 7 <sup>7</sup>		11,339	6,535	4,734	5,879	7,014	6,988	4,899	2,286.2	0.009	0.404	0.065
d 21		8,699	5,400	3,297	4,868	2,864	5,101	2,988	166.4	0.748	0.293	<0.001

<sup>1</sup> A total of 2,268 pigs (Line 337 × 1050, PIC, Hendersonville, TN) initially weighing 12.5 ± 0.68 lb were used in a 39-d growth study with 27 pigs per pen and 12 pens per treatment. Treatments were assigned in a 3 × 2 factorial with the main effects of Zn source and levels. An additional treatment was included as a positive control (pharmacological levels of ZnO in phases 1 and 2).

<sup>2</sup> Source A: Hydroxy Zn, SAM Nutrition, Bloomington, MN - Source B: Intellibond Z, Selko, Indianapolis, IN

<sup>3</sup> *P-value* associated with source and level compares the three sources (ZnO and Zn hydroxychloride A and B) at 1,500 or 1,000 ppm in phase 1 and 1,000 or 500 ppm in phase 2.

<sup>4</sup> Linear and quadratic effects of the ZnO level were tested. No significant responses (*P* > 0.10) were observed for any period or performance parameters, except for fecal Zn concentration, where at d 7 and 21 fecal Zn concentration linearly decreases (*P* < 0.001) as dietary Zn concentration decreases.

<sup>5</sup> Total mortality = mortality in pen + removals mortality.

<sup>6</sup> Three pigs per pen were sampled at the end of the second and third post-placement week; the weighted average days on feed were 7 and 21, respectively.

<sup>7</sup> For the significant interaction (*P* = 0.009) between the Zn source × level, a significant difference (*P* < 0.05) between levels was observed when ZnO or Zn hydroxychloride source B was used, but no differences (*P* > 0.10) between levels were observed in the Zn hydroxychloride source A treatment.