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Evaluation of a Microencapsulated Form of Zinc Oxide on Weanling Pig Growth Performance, Intestinal Morphology, and Zinc Excretion[1]

Payton L. Dahmer, Franco S. Matias-Ferreyra,[2] and Cassandra K. Jones

Summary

A total of 350 pigs (DNA 200 × 400; initially 13.31 ± 0.04 lb BW) were used in a 42-d study with 5 pigs per pen and 12 pens per treatment. At weaning, pigs were randomly allocated to pens and pens were randomly allotted to dietary treatments. Dietary treatments were: 1) negative control (standard nursery diet containing 110 ppm Zn from trace mineral premix); 2) control diet with 3,000 ppm added Zn in the form of ZnO in phase 1 and 2,000 ppm added Zn in the form of ZnO in phase 2 (High-ZnO); 3) control diet with 400 ppm added Zn in the form of ZnO in phases 1 and 2 (Low-ZnO); 4) 3,000 ppm added Zn in the form of microencapsulated ZnO in phase 1 and 2,000 ppm added Zn in the form of microencapsulated ZnO in phase 2 (High-MZnO); and 5) 400 ppm added Zn in the form of microencapsulated ZnO in phases 1 and 2 (Low-MZnO). Pigs were weighed and feed disappearance was determined to evaluate ADG, ADFI, and F/G. On d 10 and d 28, fecal samples from 3 pigs per pen were collected for fecal Zn concentrations. On d 28, 30 pigs (6 pigs per treatment) were euthanized, and small intestinal tissue was collected to evaluate morphology. There was no evidence of differences in ADG, ADFI, or F/G for the entire treatment period (d 0 to d 28; $P > 0.05$). During the common phase 3 (d 28 to 42) pigs fed the negative control, High-MZnO, or Low-MZnO had improved ($P < 0.0001$) ADG compared to pigs fed High- or Low-ZnO, which was driven by an increase in ADFI ($P < 0.0001$). For the entire experiment (d 0 to 42), pigs fed Low-ZnO or High-ZnO had reduced ($P < 0.0001$) ADG compared those fed the negative control. There was no evidence that small intestinal morphology differed significantly between treatments ($P > 0.05$). Finally, a significant treatment × day interaction ($P = 0.04$) was observed for fecal Zn concentrations, where pigs fed High-ZnO had greater fecal Zn levels on d 10 and d 28 compared to pigs fed all other treatments.

Introduction

Zinc oxide (ZnO) can be included at 2,000 to 4,000 ppm in nursery diets to ease the transition of weaning. When fed at these pharmacological levels, ZnO can improve growth performance and limit the colonization of pathogenic bacteria that cause post-

[1] Appreciation is expressed to Vetago SpA, Reggio Emilia, Italy, for supplying the microencapsulated ZnO product for this trial.

[2] Veterinary Diagnostic Laboratory, College of Veterinary Medicine, Kansas State University.

weaning diarrhea.[3,4] A large portion of ZnO is excreted in swine manure and eventually applied to soil as fertilizer, where accumulation of the heavy metal can pose pollution concerns.[5] Several studies have also linked ZnO supplementation to the development of antimicrobial-resistant genes in bacterial pathogens of both animal and human importance, like *E. coli.*[6] This has prompted both consumer and regulatory pressure to limit the use of pharmacological ZnO. Microencapsulation is a proposed strategy to reduce zinc oxide inclusion. This technology allows the zinc particles to be surrounded by a matrix which protects the zinc from degradation in the stomach and allows for a more targeted release along the gastrointestinal tract. Literature suggests that microencapsulation can delay the absorption of nutrients along the gut;[7] however, there is very little data on the efficacy of microencapsulating ZnO and the benefits on nursery pig performance. Thus, the objective of this experiment was to evaluate a microencapsulated form of ZnO compared to conventional ZnO in relation to the growth performance, fecal zinc excretion, and intestinal morphology of weanling pigs.

Procedures

The Kansas State University Institutional Animal Care and Use Committee approved the protocol for this experiment (IACUC #4678). The study was conducted at the Kansas State University Swine Teaching and Research Center in Manhattan, KS.

Animals and diets

A total of 350 pigs (DNA 200 × 400; initially 13.31 ± 0.40 lb BW) were weaned at an average of 21 d of age and used in a 42-d experiment. Weaning was considered d 0 of the trial and at this point pigs were individually weighed and allotted to pens in a completely randomized design. There were 5 pigs per pen and 14 replicate pens per treatment. Each pen (5 × 5 ft) was equipped with a 4-hole dry self-feeder and nipple waterer to supply *ad libitum* access to feed and water. Pens of pigs were randomly allotted to one of five dietary treatments: 1) negative control (standard nursery diet containing 110 ppm Zn from trace mineral premix); 2) control diet with 3,000 ppm added Zn in the form of ZnO in phase 1 and 2,000 ppm added Zn in the form of ZnO in phase 2 (High-ZnO); 3) control diet with 400 ppm added Zn in the form of ZnO in phases 1 and 2 (Low-ZnO); 4) 3,000 ppm added Zn in the form of microencapsulated ZnO in phase 1 and 2,000 ppm added Zn in the form of microencapsulated ZnO in phase 2 (High-MZnO); and 5) 400 ppm added Zn in the form of microencapsulated ZnO in phases 1 and 2 (Low-MZnO). All diets included titanium dioxide at 0.40% as an indigestible marker to determine fecal zinc excretion. Pigs were fed treatment diets from d 0 to d 28 and were then fed a common diet from d 28 to d 42. This allowed for

[3] Hill, G., D. Mahan, S. Carter, G. Cromwell, R. Ewan, R. Harrold, A. Lewis, P. Miller, G. Shurson, and T. Veum. 2001. Effect of pharmacological concentrations of zinc oxide with or without the inclusion of an antibacterial agent on nursery pig performance. Journal of Animal Science 79(4):934-941.

[4] Shelton, N., M. Tokach, J. Nelssen, R. Goodband, S. Dritz, J. DeRouchey, and G. Hill. 2011. Effects of copper sulfate, tri-basic copper chloride, and zinc oxide on weanling pig performance. Journal of Animal Science 89(8):2440-2451.

[5] Poulsen, H. D., and T. Larsen. 1995. Zinc excretion and retention in growing pigs fed increasing levels of zinc oxide. Livestock Production Science 43(3):235-242.

[6] Bonetti, A., B. Tugnoli, A. Piva, and E. Grilli. 2021. Towards Zero Zinc Oxide: Feeding Strategies to Manage Post-Weaning Diarrhea in Piglets. Animals 11(3):642.

[7] Piva, A., V. Pizzamiglio, M. Morlacchini, M. Tedeschi, and G. Piva. 2007. Lipid microencapsulation allows slow release of organic acids and natural identical flavors along the swine intestine. Journal of Animal Science 85(2):486-493.

diets to be fed as part of a standard 3-phase nursery program. Diets were pelleted in phase 1 and fed as meal in phases 2 and 3.

Data collection

All pigs were weighed individually on d 0, 10, 14, 21, and 28, while pens of pigs were weighed using a floor scale on d 35 and 42 to determine ADG. Feeders were individually weighed on each of these days to calculate ADFI on a weekly basis. Fecal samples were collected from the same 3 pigs from every pen on d 10 and 28 to be analyzed for Zn concentration. On d 28, 30 pigs (6 pigs/treatment) were euthanized via captive bolt and transported to the Kansas State University Veterinary Diagnostic Laboratory for necropsy. Serum and small intestinal tissue samples were collected to evaluate circulating Zn levels and intestinal morphology.

Statistical analysis

Data were analyzed as a completely randomized design using the GLIMMIX procedure of SAS (v. 9.4, SAS Institute, Inc., Cary, NC) with pen as the experimental unit for growth performance and Zn excretion data and pig as the experimental units for circulating Zn and intestinal morphology. All comparisons incorporated Tukey-Kramer multiple comparison adjustments. Results were considered significant if $P < 0.05$ and marginally significant if $0.05 < P < 0.10$.

Results and Discussion

Growth performance data are presented in Table 3. Throughout dietary phase 1 (d 0 to d 10) there was no evidence of differences in ADG and ADFI across dietary treatments ($P > 0.05$). However, pigs fed High-MZnO had decreased ($P < 0.01$) feed conversion compared to pigs fed a negative control, while other treatments were intermediate. In the second week post-weaning pigs fed High-ZnO or High-MZnO had increased ($P = 0.01$) ADG compared to those fed Low-MZnO, which was driven by an improvement in feed intake ($P = 0.04$). This response was diminished by week 3, and led to no evidence of differences in ADG, ADFI, or F:G for the entire treatment period (d 0 to d 28; $P > 0.05$). During the common phase 3 (d 28 to 42) pigs fed the negative control, High-MZnO, or Low-MZnO had improved ($P < 0.0001$) ADG compared to pigs fed High- or Low-ZnO, which was driven by an increase in ADFI ($P < 0.0001$). A marginally significant effect of dietary treatment ($P = 0.07$) was observed for feed conversion during this period. For the entire experiment (d 0 to 42), pigs fed Low-ZnO or High-ZnO had reduced ($P < 0.0001$) ADG compared those fed the negative control, which appears to be driven by a trend for decreased ADFI ($P = 0.07$). No evidence of differences ($P > 0.05$) in serum Zn were observed between dietary treatments. Likewise, we saw no indication that small intestinal morphology differed significantly between treatments ($P > 0.05$). A significant treatment × day interaction ($P = 0.04$) was observed for fecal Zn concentrations, where pigs fed High-ZnO had greater fecal Zn levels on d 10 and d 28 compared to pigs fed all other treatments.

In summary, feeding a microencapsulated form of ZnO showed promise to yield similar growth performance to conventional ZnO in the early nursery period; however, there was no improvement compared to a negative control. Microencapsulated ZnO did not appear to impact intestinal morphology or circulating zinc concentrations, however, we did see a reduction in fecal Zn excretion compared to unprotected ZnO. Further

investigation of microencapsulated ZnO is needed to validate its efficacy as a ZnO alternative.

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Table 1. Composition of phase 1 diets (as-fed basis)[1]

	Dietary treatment[2]				
Ingredient, %	**CON**	**High-ZnO**	**Low-ZnO**	**High-MZnO**	**Low-MZnO**
Ground corn	44.62	43.91	44.17	44.13	43.61
Soybean meal, 47.5% CP[3]	17.83	17.83	17.83	17.83	17.83
Spray dried bovine plasma	2.00	2.00	2.00	2.00	2.00
Corn DDGS, 7.5% oil	5.00	5.00	5.00	5.00	5.00
Fish meal	2.50	2.50	2.50	2.50	2.50
Spray dried whey	10.00	10.00	10.00	10.00	10.00
Whey permeate, 80% lactose	10.00	10.00	10.00	10.00	10.00
Choice white grease	1.00	1.00	1.00	1.00	1.00
Calcium carbonate	0.60	0.60	0.60	0.60	0.60
Monocalcium phosphate	0.75	0.75	0.75	0.75	0.75
Sodium chloride	0.30	0.30	0.30	0.30	0.30
L-Lys-HCL	0.45	0.45	0.45	0.45	0.45
DL-Met	0.20	0.20	0.20	0.20	0.20
L-Thr	0.19	0.19	0.19	0.19	0.19
L-Trp	0.03	0.03	0.03	0.03	0.03
L-Val	0.10	0.10	0.10	0.10	0.10
Vitamin premix with phytase[4]	0.25	0.25	0.25	0.25	0.25
Trace mineral premix[5]	0.15	0.15	0.15	0.15	0.15
Choline chloride	0.04	0.04	0.04	0.04	0.04
Enzymatically treated soybean meal[6]	4.00	4.00	4.00	4.00	4.00
Zinc oxide, 72%	-	0.30	0.04	-	-
Zincoret S[7]	-	-		0.08	0.60
Titanium dioxide[8]	0.40	0.40	0.40	0.40	0.40
Total	100.00	100.00	100.00	100.00	100.00

continued

Table 1. Composition of phase 1 diets (as-fed basis)[1]

	Dietary treatment[2]				
Ingredient, %	**CON**	**High-ZnO**	**Low-ZnO**	**High-MZnO**	**Low-MZnO**
Calculated analysis					
Standardized ileal digestible (SID) amino acids					
Lysine	1.40	1.40	1.40	1.40	1.40
Ile:Lys	56	56	56	56	56
Leu:Lys	116	116	116	117	116
Met:Lys	36	36	36	36	36
Met and Cys:Lys	58	58	58	58	58
Thr:Lys	64	64	64	64	64
Trp:Lys	19.1	19.1	19.1	19.2	19.1
Val:Lys	70	70	70	71	70
ME, kcal/lb	1,548	1,543	1,547	1,547	1,548
NE kcal/lb	1,156	1,152	1,155	1,154	1,156
SID lysine:NE, g/mcal	4.10	4.12	4.10	4.10	4.10
CP, %	21.7	21.7	21.7	21.9	21.7
Ca, %	0.71	0.71	0.71	0.71	0.71
STTD P, %	0.46	0.46	0.46	0.46	0.46

[1]Phase 1 diets were fed from d 0 to 10 and were manufactured at the Kansas State University O.H. Kruse Feed Technology Innovation Center (Manhattan, KS).

[2]Dietary treatments were: negative control basal diet with no added ZnO (CON); basal diet with 3,000 ppm added Zn from ZnO in phase 1 and 2,000 ppm added Zn from ZnO in phase 2 (High-ZnO); basal diet with 400 ppm added Zn from ZnO in phases 1 and 2 (Low-ZnO); basal diet with 3,000 ppm added Zn from microencapsulated ZnO in phase 1 and 2,000 ppm added Zn from microencapsulated ZnO in phase 2 (High-MZnO); and basal diet with 400 ppm added Zn from microencapsulated ZnO in phases 1 and 2 (Low-MZnO).

[3]CP = crude protein.

[4]Provided per lb of premix: 750,000 IU vitamin A; 300,000 IU vitamin D; 8,000 IU vitamin E; 600 mg vitamin K; 6 mg vitamin B_{12}; 9,000 mg niacin; 5,000 mg pantothenic acid; 1,500 mg riboflavin.

[5]Provided per lb of premix: 110 ppm Zn from zinc sulfate; 110 ppm Fe from iron sulfate; 10 ppm Mn from manganese oxide; 5 ppm Cu from copper sulfate; 0.3 ppm I from calcium iodate; 0.3 Se from sodium selenite.

[6]HP300 (Hamlet Protein, Findlay, OH).

[7]Zincoret S (Vetagro SpA, Reggio Emilia, Italy).

[8]Titanium dioxide (Brenntag Specialties, Inc., South Plainfield, NJ) was included at 0.4% in all diets as an indigestible marker for fecal Zn excretion.

Table 2. Composition of phase 2 diets (as-fed basis)[1]

	Dietary treatment[2]				
Ingredient, %	**CON**	**High-ZnO**	**Low-ZnO**	**High-MZnO**	**Low-MZnO**
Ground corn	49.31	49.16	49.27	48.91	49.23
Soybean meal, 47.5% CP[3]	24.60	24.60	24.60	24.60	24.60
Corn DDGS, 7.5% oil	7.50	7.50	7.50	7.50	7.50
Spray dried whey	10.00	10.00	10.00	10.00	10.00
Choice white grease	1.00	1.00	1.00	1.00	1.00
Calcium carbonate	0.85	0.85	0.85	0.85	0.85
Monocalcium phosphate	0.85	0.85	0.85	0.85	0.85
Sodium chloride	0.50	0.50	0.50	0.50	0.50
L-Lys-HCL	0.55	0.55	0.55	0.55	0.55
DL-Met	0.22	0.22	0.22	0.22	0.22
L-Thr	0.20	0.20	0.20	0.20	0.20
L-Trp	0.04	0.04	0.04	0.04	0.04
L-Val	0.08	0.08	0.08	0.08	0.08
Vitamin premix with phytase[4]	0.25	0.25	0.25	0.25	0.25
Trace mineral premix[5]	0.15	0.15	0.15	0.15	0.15
Enzymatically treated soybean meal[6]	3.50	3.50	3.50	3.50	3.50
Zinc oxide, 72%	-	0.30	0.04	-	-
Zincoret S[7]	-	-	-	0.40	0.08
Titanium dioxide[8]	0.40	0.40	0.40	0.40	0.40
Total	100.00	100.00	100.00	100.00	100.00

continued

Table 2. Composition of phase 2 diets (as-fed basis)[1]

	Dietary treatment[2]				
Ingredient, %	**CON**	**High-ZnO**	**Low-ZnO**	**High-MZnO**	**Low-MZnO**
Calculated analysis					
Standardized ileal digestible (SID) amino acids					
Lysine	1.35	1.35	1.35	1.35	1.35
Ile:Lys	0.56	0.56	0.56	0.56	0.56
Leu:Lys	119	119	119	119	119
Met:Lys	37	37	37	37	37
Met and Cys:Lys	58	58	58	58	58
Thr:Lys	63	63	63	63	63
Trp:Lys	18.9	18.9	18.9	18.9	18.9
Val:Lys	67	67	67	67	67
ME, kcal/lb	1,524	1,520	1,524	1,524	1,524
NE kcal/lb	1,134	1,131	1,134	1,134	1,134
SID lysine:NE, g/mcal	4.02	4.03	4.02	4.02	4.02
CP, %	21.2	21.2	21.2	21.2	21.2
Ca, %	0.70	0.70	0.70	0.70	0.70
STTD P, %	0.40	0.40	0.40	0.40	0.40

[1]Phase 2 diets were fed from d 10 to 28 and were manufactured by Hubbard Feeds (Beloit, KS).

[2]Dietary treatments were: negative control basal diet with no added ZnO (CON); basal diet with 3,000 ppm added Zn from ZnO in phase 1 and 2,000 ppm added Zn from ZnO in phase 2 (High-ZnO); basal diet with 400 ppm added Zn from ZnO in phases 1 and 2 (Low-ZnO); basal diet with 3,000 ppm added Zn from microencapsulated ZnO in phase 1 and 2,000 ppm added Zn from microencapsulated ZnO in phase 2 (High-MZnO); and basal diet with 400 ppm added Zn from microencapsulated ZnO in phases 1 and 2 (Low-MZnO).

[3]CP = crude protein.

[4]Provided per lb of premix: 750,000 IU vitamin A; 300,000 IU vitamin D; 8,000 IU vitamin E; 600 mg vitamin K; 6 mg vitamin B_{12}; 9,000 mg niacin; 5,000 mg pantothenic acid; 1,500 mg riboflavin.

[5]Provided per lb of premix: 110 ppm Zn from zinc sulfate; 110 ppm Fe from iron sulfate; 10 ppm Mn from manganese oxide; 5 ppm Cu from copper sulfate; 0.3 ppm I from calcium iodate; 0.3 Se from sodium selenite.

[6]HP300 (Hamlet Protein, Findlay, OH).

[7]Zincoret S (Vetagro SpA, Reggio Emilia, Italy).

[8]Titanium dioxide (Brenntag Specialties, Inc., South Plainfield, NJ) was included at 0.4% in all diets as an indigestible marker for fecal Zn excretion.

Table 3. Effects of feeding microencapsulated zinc oxide (ZnO) on nursery pig growth performance[1]

	Dietary treatment[2]						
Item	CON	High-ZnO	Low-ZnO	High-MZnO	Low-MZnO	SEM	*P*-value
BW, lb							
d 0	13.23	13.35	13.22	13.38	13.38	0.18	0.943
d 10	16.04	16.21	16.08	16.2	16.52	0.26	0.716
d 28	31.36	30.64	30.68	32.19	32.24	0.65	0.243
d 42	52.00[a]	47.18[b]	45.80[b]	52.13[a]	53.75[a]	1.05	< 0.0001
Phase 1 (d 0 to 10)							
ADG	0.29	0.29	0.29	0.28	0.32	0.01	0.233
ADFI	0.42	0.47	0.45	0.45	0.46	0.02	0.317
F/G	1.45[a]	1.62[ab]	1.55[ab]	1.61[b]	1.44[ab]	0.03	0.008
Phase 2 (d 10 to 28)							
ADG	0.80	0.80	0.79	0.83	0.81	0.02	0.808
ADFI	1.22	1.28	1.16	1.17	1.12	0.05	0.266
F/G	1.53	1.60	1.47	1.41	1.38	0.04	0.278
Overall treatment (d 0 to 28)							
ADG	0.70	0.67	0.64	0.68	0.70	0.03	0.375
ADFI	1.05	1.03	0.98	0.99	0.94	0.05	0.529
F/G	1.50	1.54	1.53	1.46	1.34	0.04	0.365
Phase 3 (d 28 to 42)							
ADG	1.48[a]	1.24[b]	1.13[b]	1.46[a]	1.46[a]	0.04	< 0.0001
ADFI	2.03[a]	1.66[b]	1.70[b]	2.08[a]	2.19[a]	0.07	< 0.0001
F/G	1.37	1.34	1.50	1.42	1.50	0.03	0.065
Overall experiment (d 0 to 42)							
ADG	0.97[a]	0.87[bc]	0.80[c]	0.94[ab]	0.96[ab]	0.03	< 0.0001
ADFI	1.37	1.28	1.18	1.35	1.35	0.05	0.065
F/G	1.41	1.47	1.48	1.44	1.41	0.02	0.853

[1] A total of 350 pigs (DNA 200 × 400) were used in a 42-day experiment with 5 pigs per pen and 12 pens per treatment.
[2]Dietary treatments were: negative control basal diet with no added ZnO (CON); basal diet with 3,000 ppm added Zn from ZnO in phase 1 and 2,000 ppm added Zn from ZnO in phase 2 (High-ZnO); basal diet with 400 ppm added Zn from ZnO in phases 1 and 2 (Low-ZnO); basal diet with 3,000 ppm added Zn from microencapsulated ZnO in phase 1 and 2,000 ppm added Zn from microencapsulated ZnO in phase 2 (High-MZnO); and basal diet with 400 ppm added Zn from microencapsulated ZnO in phases 1 and 2 (Low-MZnO).

Table 4. Effect of feeding microencapsulated zinc oxide (ZnO) on weanling pig fecal Zn excretion[1]

	Dietary treatment[2]						
Sampling day	CON	High-ZnO	Low-ZnO	High-MZnO	Low-MZnO	SEM	Treatment × day, *P* =
d 10	2,260[cde]	9,960[a]	4,560[bc]	5,830[b]	6,960[b]	629.5	0.035
d 28	624[e]	9,413[a]	1,791[de]	2,659[cd]	6045[b]		

[1]A total of 350 pigs (DNA 200 × 400) were used in a 42-day experiment with 5 pigs per pen and 12 pens per treatment.
[2]Dietary treatments were: negative control basal diet with no added ZnO (CON); basal diet with 3,000 ppm added Zn from ZnO in phase 1 and 2,000 ppm added Zn from ZnO in phase 2 (High-ZnO); basal diet with 400 ppm added Zn from ZnO in phases 1 and 2 (Low-ZnO); basal diet with 3,000 ppm added Zn from microencapsulated ZnO in phase 1 and 2,000 ppm added Zn from microencapsulated ZnO in phase 2 (High-MZnO); and basal diet with 400 ppm added Zn from microencapsulated ZnO in phases 1 and 2 (Low-MZnO).