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Robert D. Goodband, Joel M. DeRouchey, and Jordan T. Gebhardt¹*

Summary

A total of 360 barrows (DNA 600 × 241; initially 12.1 ± 0.07 lb) were used in a 38-d growth study to evaluate the effects of including folic acid (Rovimix Folic Acid, DSM, Parsippany, NJ) with or without pharmacological levels of Zn provided by zinc oxide (ZnO) on growth performance and fecal characteristics in nursery pigs. Pigs were weaned at approximately 19 d of age and randomly allotted to 1 of 6 dietary treatments. A total of 72 pens were used with 5 pigs per pen and 12 replications per treatment. Dietary treatments were arranged in a 3×2 factorial with main effects of folic acid (0, 20, or 40 ppm) and ZnO (3,000 ppm of Zn in phase 1, 2,000 ppm in phase 2, or no Zn other than 110 ppm from the trace mineral premix). Diets were corn-soybean meal-based and fed in 2 phases. A common phase 3 diet was fed to all pigs. For the experimental period (d 0 to 24), pigs fed diets with pharmacological levels of Zn had improved ($P \leq 0.030$) d 24 BW, ADG, and ADFI. There was a quadratic ($P < 0.05$) response for d 24 BW, ADG, and ADFI when pigs were fed folic acid with pigs fed 0 or 40 ppm having improved performance compared with pigs fed 20 ppm. For the common period (d 24 to 38), pigs previously fed pharmacological levels of Zn had poorer ($P = 0.028$) feed efficiency compared to pigs previously fed diets without pharmacological Zn. Additionally, a quadratic ($P = 0.008$) response was observed in ADG and ADFI when pigs were previously fed folic acid with pigs fed 0 or 40 ppm having improved performance compared with pigs fed 20 ppm. Overall (d 0 to 38), there was a quadratic ($P < 0.05$) response in final BW, ADG, and ADFI when pigs were fed folic acid, with pigs fed 0 or 40 ppm having improved performance compared to pigs fed 20 ppm. However, no overall differences ($P > 0.10$) were observed when pigs were fed diets with or without pharmacological levels of Zn in phase 1 and 2 diets. Additionally, no statistical differences ($P > 0.10$) in mortality were observed when pigs were fed diets with or without pharmacological levels of Zn although pigs fed no folic acid had numerically the lowest mortality. In conclusion, the addition of folic acid did not improve nursery pig performance with a negative response observed at 20 ppm, regardless of Zn inclusion in the diet.

Introduction

Folic acid is a form of the water-soluble vitamin, B₉, and plays an important role in growth, health, and maintenance of animal metabolism. Specifically, folic acid plays

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a crucial role in protein deposition and tissue synthesis.² Rapidly growing tissues or cells with a high turnover rate are sensitive to folic acid levels, potentially suggesting an increased demand to support growth of young animals.³ Recently, Wang et al.³ conducted research with folic acid levels of 0, 3, 9, or 18 ppm and observed that when folic acid was supplemented to antibiotic free diets, ADG increased linearly ($P = 0.018$). The authors also observed altered metabolism of short-chain fatty acids (SCFAs) in the colon of pigs fed 9 or 18 ppm folic acid with increased ($P < 0.05$) acetic acid and valeric acid concentrations.⁴

Pharmacological levels of Zn from zinc oxide (ZnO) supplementation in nursery pig diets is a common practice in the swine industry because of the reduction in diarrhea and improved growth performance associated with its use. However, there are growing concerns with the use of high levels of Zn in nursery pig diets due to Zn accumulation in the environment and antibiotic resistance.

To our knowledge, no data are available to determine if the response to adding pharmacological levels of Zn is dependent on the level of folic acid supplementation. Therefore, our objective was to determine the effects of including folic acid with or without pharmacological levels of Zn in phase 1 and phase 2 nursery pigs feed on average daily gain, average daily feed intake, feed efficiency, and fecal score and dry matter during the post-weaning period.

Procedures

Animals and diets

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

A total of 360 barrows (DNA 600 × 241; initially 12.1 ± 0.07 lb) were used in a 38-d growth study to evaluate the effects of including folic acid (Rovimix Folic Acid, DSM, Parsippany, NJ) with or without pharmacological levels of zinc oxide (ZnO) on growth performance and fecal characteristics in nursery pigs. Diets did not contain any antibiotics. Pigs were weaned at approximately 19 d of age and randomly allotted to 1 of 6 dietary treatments. A total of 72 pens were used with 5 pigs per pen and 12 replications per treatment. Dietary treatments were arranged in a 3×2 factorial with main effects of folic acid (0, 20, or 40 ppm) and Zn provided by ZnO (3,000 ppm of Zn in phase 1, 2,000 ppm in phase 2, or no additional Zn above that provided in the trace mineral premix). Experimental diets were corn-soybean meal-based and fed in 2 phases. A

² Hayashi I., K. Sohn, J. Stempak, R. Croxford, and Y. Kim. 2007. Folate deficiency induces cell-specific changes in the steady-state transcript levels of genes involved in folate metabolism and 1-Carbon transfer reactions in human colonic epithelial cells. *J Nutr.* 137(3):607–613. doi: 10.1093/jn/137.3.607.

³ Wang L, X. Tan, H. Wang, Q. Wang, P. Huang, Y. Li, J. Li, J. Huang, H. Yang, and Y. Yin. 2021. Effects of varying dietary folic acid during weaning stress of piglets. *Anim. Nutr.* 7:101-110. doi:10.1016/j.aninu.2020.12.002.

⁴ Wang L, L. Zou, J. Li, Y. Yang, and Y. Yin. Effect of dietary folate level on organ weight, digesta pH, short-chain fatty acid concentration, and intestinal microbiota of weaned piglets. 2021. *J. Anim. Sci.* 99:1-9. doi:10.1093/jas/skab015.

common phase 3 diet was fed to all pigs. All diets contained 110 ppm of Zn provided by the trace mineral premix, but the premixes did not contain folic acid. Pigs were weighed on d 0, 9, 17, 24, 31, and 38 to determine ADG, ADFI, and feed efficiency.

Fecal characteristics

On d 9 and 21 of the experiment, fecal samples were collected from 3 pigs per pen. Samples were collected into individual sealable plastic bags for each pig. Fecal dry matter (DM) was analyzed independently on all 3 samples per pen for both collection days. Fecal DM was determined by drying the fecal sample at 131°F (55°C) for 48 h.

On d 9 and 21, fecal samples were collected in individual sealable plastic bags and fecal scores were assigned to each bagged sample. Fecal scores were assigned based on a 0 to 2 scale, with 0 indicating firm feces, 1 as soft feces, and 2 as diarrhea.

Statistical analysis

Growth performance and SCFA data were analyzed as a completely randomized design for one-way ANOVA. Pen was considered the experimental unit and treatment was used as the fixed effect. Linear and quadratic contrasts were evaluated within increasing folic acid. Fecal dry matter, and fecal score were analyzed as repeated measures representing multiple observations on each pen over time and pen nested within treatment was included in the model as a random intercept to account for subsampling attributed to the multiple observations for each experimental unit on each day. Treatment, day, and the associated interactions were considered fixed effects. For fecal score analysis, data were analyzed as categorical outcomes with a generalized linear mixed model using a multinomial response distribution using a cumulative logit link function. Data were summarized using the FREQ procedure of SAS and reported as percentage of observations within each fecal score category by treatment and day. Results were considered significant with $P \leq 0.05$ and were considered marginally significant with $P \leq 0.10$.

Results and Discussion

Growth performance

For growth performance responses, no folic acid $\times$ pharmacological levels of Zn interactions were observed ($P > 0.10$) throughout the study.

For phase 1 (d 0 to 9), pigs fed pharmacological levels of Zn had increased ($P < 0.01$) d 9 BW, ADG, and ADFI compared to pigs fed diets without added Zn. For pigs fed folic acid, there was a marginally significant quadratic ($P \leq 0.07$) response in d 9 BW and feed efficiency. Pigs fed diets with 20 ppm folic acid had poorer performance compared to pigs fed 0 or 40 ppm.

For phase 2 (d 9 to 24), pigs fed pharmacological levels of Zn had heavier ($P = 0.03$) d 24 BW compared to pigs fed diets without added Zn. Additionally, pigs fed diets with pharmacological levels of Zn tended ($P \leq 0.086$) to have greater ADG and ADFI. For pigs fed folic acid, there was a quadratic ($P \leq 0.05$) response in d 24 BW, ADG, and ADFI observed, with pigs fed 20 ppm having reduced performance compared to pigs fed 0 or 40 ppm.

For the experimental period (d 0 to 24), pigs fed diets with pharmacological levels of Zn had improved ($P \leq 0.03$) d 24 BW, ADG, and F/G. When pigs were fed folic acid there was a quadratic ($P \leq 0.02$) response in d 24 BW, ADG, and ADFI with pigs fed 0 or 40 ppm having improved performance compared with pigs fed 20 ppm.

During the common period (d 24 to 38), pigs previously fed pharmacological levels of Zn had poorer ($P = 0.028$) feed efficiency compared to pigs previously fed diets without added Zn. Additionally, pigs previously fed pharmacological levels of Zn tended ($P = 0.096$) to have decreased ADG compared to pigs previously fed no added Zn. A quadratic ($P = 0.008$) response was observed for ADG and ADFI when pigs were previously fed folic acid with pigs fed 0 or 40 ppm having improved performance compared with pigs fed 20 ppm.

Overall (d 0 to 38), there was a quadratic ($P \leq 0.003$) response in final BW, ADG, and ADFI when pigs were fed folic acid in phase 1 and 2 diets with pigs fed 0 or 40 ppm having improved performance compared to pigs fed 20 ppm. No differences ($P > 0.10$) were observed when pigs were fed diets with or without pharmacological levels of Zn in phases 1 and 2. Additionally, no statistical differences ($P > 0.10$) were observed in mortality across all dietary treatments. However, numerical differences were observed. Pigs fed no added folic acid had 0% mortality, compared to pigs fed 20 or 40 ppm that had 7.5% or 10% mortality, regardless of Zn addition.

Fecal analysis

For analysis of fecal scores, no folic acid $\times$ pharmacological levels of Zn $\times$ day interactions or main effect of folic acid was observed ($P > 0.10$). There was a marginally significant pharmacological levels of Zn $\times$ day interaction ($P = 0.064$) where pigs fed diets with no added Zn had a higher frequency of diarrhea on d 9 compared to d 24 (Figure 1). Additionally, a main effect of pharmacological levels of Zn was observed where pigs fed diets with added Zn had reduced ($P < 0.001$; Figure 2) frequency of diarrhea presence.

In conclusion, the addition of folic acid did not improve nursery pig performance with a negative response observed at 20 ppm, with or without pharmacological levels of Zn in the diet. Additionally, pigs fed pharmacological levels of Zn had improved growth performance in the experimental period, but no improvement was observed in the common period, resulting in no overall benefit.

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

Ingredient, %	Phase 1²	Phase 2³	Phase 3⁴
Corn	44.97	49.90	64.24
Soybean meal, dehulled	17.34	23.55	31.78
Bovine blood plasma	2.00	---	---
DDGS	5.00	7.50	---
Fish meal	2.50	---	---
Whey powder	10.00	---	---
Whey permeate	10.00	10.00	---
Microbial-enhanced soy protein ⁵	4.00	4.00	---
Choice white grease	1.00	1.00	---
Calcium carbonate	0.45	0.75	0.90
Monocalcium P	0.80	1.00	1.00
Salt	0.30	0.50	0.60
L-Lys-HCl	0.40	0.55	0.50
DL-Met	0.18	0.22	0.21
L-Thr	0.17	0.22	0.21
L-Trp	0.02	0.04	0.04
L-Val	0.08	0.12	0.13
Vitamin and trace mineral premixes ⁶	0.40	0.40	0.40
Zinc oxide ⁷	+/-	+/-	---
Folic acid ⁸	+/-	+/-	---
Total	100	100	100

continued

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

Ingredient, %	Phase 1²	Phase 2³	Phase 3⁴
Calculated analysis			
SID AA, %			
Lys	1.35	1.35	1.35
Ile:Lys	57	57	56
Leu:Lys	120	118	115
Met:Lys	35	37	36
Met and Cys:Lys	58	58	58
Thr:Lys	64	63	63
Trp:Lys	19.2	19.3	19.4
Val:Lys	70	70	70
Total Lys, %	1.51	1.51	1.50
NE, kcal/lb	1,158	1,134	1,097
SID Lys:ME, g/Mcal	3.95	4.00	4.12
CP, %	21.5	21.5	21.3
Ca, %	0.66	0.66	0.71
STTD P, %	0.58	0.51	0.47

¹Dietary treatments were arranged in a 3 × 2 factorial with main effects of folic acid and pharmacological levels of Zn.

²Phase 1 diets were fed from d 0 to 9 (12 to 15 lb).

³Phase 2 diets were fed from d 9 to 24 (15 to 27 lb).

⁴A common phase 3 diet was fed to all pigs from d 24 to 38 (27 to 44 lb).

⁵ME-PRO, Prairie AquaTech, Brookings SD.

⁶Ronozyme HiPhos (DSM, Parsippany, NJ) included at 1,250 FTU/kg provided an estimated release of 0.13% STTD P.

⁷Zinc oxide was included in the diet at the expense of corn to provide 3,000 ppm of Zn in phase 1 and 2,000 ppm in phase 2 in the treatment groups containing pharmacological levels of Zn from ZnO.

⁸Rovimix Folic Acid (DSM, Parsippany, NJ) included at the expense of corn at 0.0025% or 0.005% of the diet for the respective treatments to provide 0, 20, or 40 ppm folic acid.

Table 2. The effects of folic acid and zinc oxide on nursery pig growth performance¹

Folic acid, ppm ³ :	No pharmacological levels of Zn			Pharmacological levels of Zn ²			SEM	<i>P</i> =		
	0	20	40	0	20	40		Zn	Folic acid	
									Linear	Quadratic
BW, lb										
d 0	12.1	12.1	12.1	12.1	12.1	12.1	0.07	0.771	0.878	0.837
d 9	15.3	15.0	15.4	15.8	15.4	15.7	0.21	0.011	0.888	0.072
d 24	26.4	25.1	26.5	27.2	26.4	27.3	0.52	0.030	0.825	0.019
d 38	44.6	41.2	44.6	44.3	42.7	44.0	0.90	0.812	0.878	0.003
d 0 to 9 (phase 1)										
ADG, lb	0.35	0.33	0.37	0.41	0.38	0.40	0.022	0.007	0.889	0.111
ADFI, lb	0.39	0.38	0.41	0.46	0.44	0.46	0.023	0.004	0.645	0.315
F/G	1.12	1.20	1.12	1.11	1.16	1.13	0.032	0.796	0.662	0.069
d 9 to 24 (phase 2)										
ADG, lb	0.74	0.69	0.72	0.76	0.73	0.77	0.029	0.086	0.902	0.049
ADFI, lb	1.01	0.91	0.99	1.03	0.98	1.05	0.033	0.067	0.975	0.012
F/G	1.37	1.37	1.37	1.36	1.35	1.37	0.025	0.755	0.844	0.698
d 0 to 24 (experimental period)										
ADG, lb	0.60	0.53	0.59	0.63	0.59	0.63	0.021	0.016	0.808	0.015
ADFI, lb	0.78	0.70	0.77	0.81	0.77	0.82	0.026	0.019	0.896	0.009
F/G	1.31	1.32	1.31	1.30	1.30	1.31	0.018	0.568	0.803	0.942
d 24 to 38 (common period)										
ADG, lb	1.30	1.15	1.30	1.22	1.17	1.19	0.040	0.096	0.649	0.008
ADFI, lb	1.84	1.65	1.83	1.78	1.72	1.75	0.049	0.573	0.647	0.008
F/G	1.42	1.44	1.42	1.46	1.49	1.47	0.027	0.028	0.953	0.452
d 0 to 38										
ADG, lb	0.86	0.75	0.84	0.85	0.79	0.83	0.025	0.761	0.497	0.002
ADFI, lb	1.17	1.04	1.15	1.17	1.10	1.15	0.033	0.439	0.522	0.002
F/G	1.37	1.38	1.37	1.38	1.39	1.39	0.013	0.154	0.867	0.522
Mortality, %	0.0	10.0	5.0	0.0	10.0	10.0	3.87	0.999	0.969	0.967
Fecal DM, %										

¹A total of 360 pigs (initially 12.1 ± 0.07 lb) were used with 5 pigs per pen and 12 replications per treatment. Dietary treatments were arranged in a 3 × 2 factorial with main effects of folic acid and zinc oxide. No folic acid × zinc oxide interactions were observed (*P* > 0.10). Fecal samples were collected individually from 3 pigs per pen (216 pigs total) on d 9 and 24.

²Zinc oxide was included in the diet to provide 3,000 ppm of Zn in phase 1 and 2,000 ppm in phase 2.

³Rovimix Folic Acid (DSM, Parsippany, NJ).

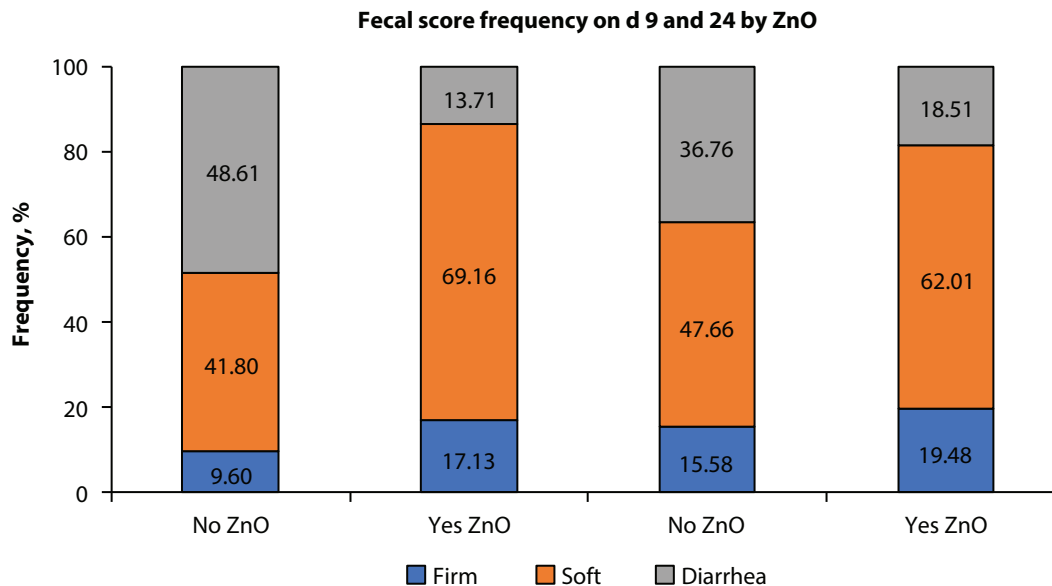


Figure 1. Fecal score frequency on d 9 and 24 by main effect of pharmacological levels of Zn. Zn $\times$ day, $P = 0.064$.

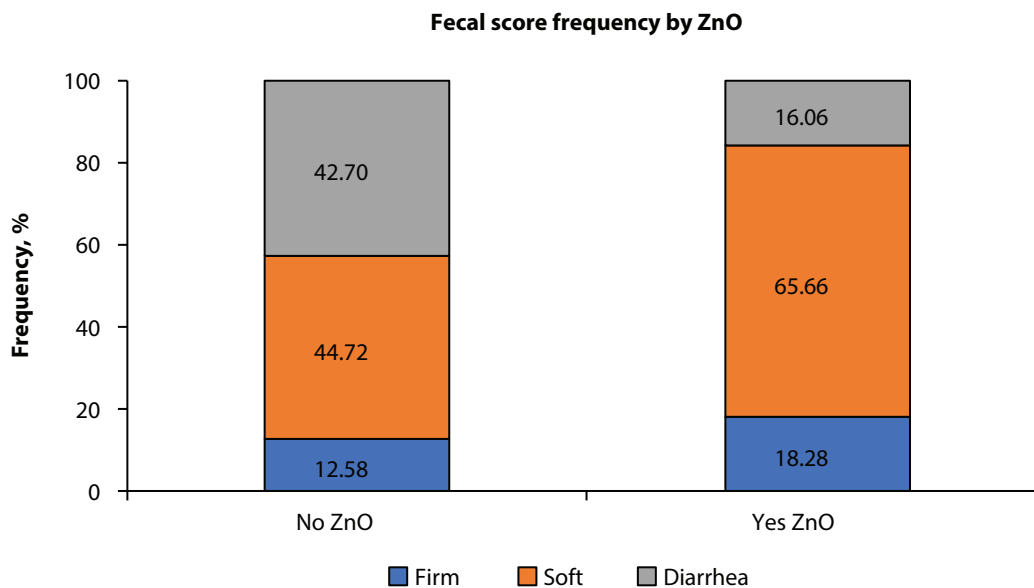


Figure 2. Fecal score frequency by main effect of pharmacological levels of Zn, $P < 0.001$.