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Summary

A total of 360 weanling pigs (DNA 241 × 600; initially 12.0 ± 0.07 lb) were used in a 42-d growth trial to determine the effects of dietary crude protein and pharmacological levels of Zn (from ZnO) on nursery pig performance and fecal dry matter. Pigs were balanced for gender, stratified into three weight categories, and randomly allotted to pens. Pens of pigs were then assigned to one of six dietary treatments in a generalized randomized block design with five pigs per pen and 12 replicate pens per treatment. The treatments were in a 3 × 2 factorial arrangement with main effects of crude protein (low, medium, high) and Zn (110 or 3,000 mg/kg). Crude protein (CP) levels were low (20.5% in phase 1; 20% in phase 2), medium (22% in phase 1; 21.5% in phase 2), and high (23.5% in phase 1; 23% in phase 2). All basal diets contained 110 mg/kg Zn from $ZnSO_4$ and ZnO, which was added to reach 3,000 mg/kg where applicable. Diets were fed in three phases: phase 1 (d 0 to 10), phase 2 (d 10 to 25), and a common diet was fed to all pigs during phase 3 (d 25 to 42). During phase 1 (d 0 to 10), there was a quadratic CP × Zn interaction ($P = 0.035$) observed for F/G, where increasing CP in low-added Zn diets did not influence F/G. However, when CP increased in diets containing 3,000 mg/kg added Zn, a quadratic response was observed ($P = 0.035$) with the medium CP treatment having poorer F/G compared to the low and high CP diets. During phase 1, increasing CP decreased (linear, $P \leq 0.047$) ADG and ADFI, but there was no effect on F/G. During phase 2 (d 10 to 25), increasing CP tended to decrease ADFI (linear, $P = 0.063$), and F/G was improved (quadratic, $P = 0.005$) for pigs fed the medium CP diet. Pigs fed diets with added Zn had increased ($P \leq 0.033$) ADG and ADFI, with no effect on F/G. For the experimental period (d 0 to 25), ADFI decreased (linear, $P = 0.011$) and ADG tended (linear, $P = 0.100$) to decrease as dietary CP increased. Feed efficiency tended (quadratic, $P = 0.052$) to be improved for pigs fed the medium CP diets. Pigs fed pharmacological levels of Zn had greater ADG, ADFI, and improved F/G ($P \leq 0.002$) compared with those fed basal levels of Zn. During the common phase (d 25 to 42), pigs previously fed the medium CP diets had the lowest (quadratic, $P = 0.018$) ADG, and increasing the CP level worsened F/G (linear, $P = 0.045$; quadratic, $P = 0.051$). Pigs previously fed pharmacological levels of Zn tended ($P = 0.052$) to have decreased ADG, with no differences for ADFI or F/G. Overall (d 0 to 42), pigs fed the medium CP diets tended (quadratic, $P \leq 0.098$)

to have decreased ADG and ADFI compared with those fed low or high CP diets. No differences were observed in F/G based on CP level. Pigs fed pharmacological levels of Zn in the experimental phase tended ($P = 0.066$) to have greater overall ADG, with no evidence of a difference for ADFI or F/G. A Zn × day interaction was observed ($P = 0.015$) for fecal DM, where pigs fed pharmacological levels of Zn had increased fecal DM on d 10 ($P < 0.001$) and d 25 ($P = 0.024$), but the magnitude of improvement was greater on d 10. In summary, there was minimal evidence of interactions between dietary CP and Zn level. In phase 1, pigs had greater ADFI and ADG with low CP diets, which also tended to be observed in the overall trial period. Pharmacological levels of Zn resulted in improvements in ADG, ADFI, and F/G during the experimental period. During the common period, pigs previously fed pharmacological Zn had lower ADG, which resulted in only a tendency for improved ADG for the overall trial period, with no overall difference in ADFI or F/G.

Introduction

Weaning stress driven by dietary, environmental, and management changes can be challenging for young pigs. Zinc oxide (ZnO), when included at pharmacological levels (2,000 to 3,000 mg/kg), has been shown to mitigate the post-weaning growth lag by reducing post-weaning diarrhea (PWD) and enhancing growth performance.[1] However, concerns have arisen regarding this practice, and alternative strategies are actively being investigated and developed, such as lowering dietary crude protein (CP) levels, dietary acidifiers, and fiber, among other strategies. After weaning, pigs experience reduced feed intake but remain in a protein-dependent phase of growth. Thus, meeting the metabolic demand for amino acids is important to maximize protein deposition. However, high CP levels can result in more nitrogen reaching the large intestine, promoting the proliferation of pathogenic bacteria and increasing the risk of PWD.[2] It has been hypothesized that the amount of nitrogen in the hindgut may influence the efficacy of Zn. Currently, limited information exists regarding the interaction between dietary CP levels and Zn. The objective of this study was to improve our understanding of this relationship to develop nutritional strategies that effectively manage PWD, while maintaining growth performance and supporting environmental sustainability in swine production.

Materials and Methods

Experimental procedures were approved by the Kansas State University Institutional Animal Care and Use Committee (IACUC #4942). The study was conducted at the Kansas State University Swine Teaching and Research Center in Manhattan, Kansas.

Animals and diets

A total of 360 pigs (DNA 241 × 600; initially 12.0 ± 0.07 lb) were used in a 42-day growth trial. Pigs were weaned at approximately 20 d of age and stratified into three BW categories: light, medium, and heavy. The pigs were then randomly assigned to pens within the BW categories, and pens were allotted to one of six dietary treatments. There

[1] 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. J. Anim. Sci. 79(4):934-94. doi:10.2527/2001.79493x.

[2] Gloaguen, M., N. Le Floc'h, E. Corrent, Y. Primot, and J. van Milgen. 2014. The use of free amino acids allows formulating very low crude protein diets for piglets. J. Anim. Sci. 92(2):637-644. doi:10.25287/jas/2013-6514.

were five pigs per pen and 12 replicates per treatment. Each pen (4 × 4 ft) was equipped with a four-hole dry self-feeder and a nipple waterer to provide *ad libitum* access to feed and water. The treatments were in a 3 × 2 factorial arrangement with main effects of crude protein (low, medium, high) and Zn (110 or 3,000 mg/kg). Crude protein levels were low (20.5% in phase 1; 20% in phase 2; Table 1), medium (22% in phase 1; 21.5% in phase 2), and high (23.5% in phase 1; 23% in phase 2). All basal diets contained 110 mg/kg Zn from $ZnSO_4$, and ZnO was added to reach 3,000 mg/kg where applicable. Diets were fed in three phases: phase 1 (d 0 to 10), phase 2 (d 10 to 25), and a common diet was fed to all pigs during phase 3 (d 25 to 42). Diets were fed in pellet form in phase 1 and mash form in phases 2 and 3. All diets were manufactured at the Kansas State University O.H. Kruse Feed Technology Innovation Center. All pigs were weighed individually on d 0, 10, 17, and 25, and pens of pigs were weighed using a floor scale on d 35 and 42 to determine ADG. Feed disappearance was measured on each of these days to calculate ADFI and F/G. A fecal sample was collected from the same three middle BW pigs per pen on d 10 and 25 for analysis of fecal dry matter (DM). After collection, samples were dried at 131°F (55°C) in a forced-air oven, and the ratio of dried to wet fecal weight determined the percentage DM.

Statistical analysis

Data were analyzed as a generalized, randomized block design using the lm 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, the model utilized treatment and block as a fixed effect. For fecal DM, in addition to the previous model components, sampling day was included as a fixed effect, and the pen was used as a random effect to account for the subsampling associated with multiple individual pigs analyzed from each pen. Contrast coefficients were used to evaluate the linear and quadratic effects of increasing crude protein on performance and fecal DM. Treatment differences were considered significant at $P \leq 0.05$ and marginally significant at $0.05 < P \leq 0.10$.

Results and Discussion

During phase 1 (d 0 to 10), there was a quadratic CP × Zn interaction ($P = 0.035$; Table 2) where increasing CP in low-added Zn diets did not influence feed efficiency (quadratic, $P = 0.375$). However, when CP increased in diets containing 3,000 mg/kg added Zn, a quadratic response was observed ($P = 0.035$), with the medium CP treatment having poorer F/G compared to the low and high CP levels. There were no other CP × Zn interactions observed ($P > 0.10$).

During phase 1, increasing CP resulted in a linear decrease in ADG and ADFI ($P \leq 0.047$; Table 3), but there was no evidence of a difference in F/G ($P \geq 0.375$).

During phase 2 (d 10 to 25), increasing CP resulted in a tendency for a linear reduction in ADFI ($P = 0.063$), and F/G was lowest for the medium CP treatment (quadratic, $P = 0.005$). There was no evidence of a difference in ADG based on CP level. For the main effect of added Zn, a similar response was observed compared to phase 1, where pigs fed a diet containing pharmacological levels of Zn had increased ($P \leq 0.033$) ADG and ADFI compared to pigs fed a diet without pharmacological Zn, with no effect on F/G. For the experimental period (d 0 to 25), as the dietary CP level increased, there was a linear decrease in ($P = 0.011$) ADFI, a tendency (linear, $P = 0.100$) for reduced ADG with increasing CP level, and a tendency (quadratic, $P = 0.052$) for the medium

CP treatment to have the lowest F/G. Pigs fed pharmacological levels of Zn had greater ADG, ADFI, and improved F/G ($P \leq 0.002$).

During the common phase (d 25 to 42), pigs previously fed the medium CP level had the lowest (quadratic, $P = 0.018$) ADG, and increasing the CP level worsened F/G (linear, $P = 0.045$; quadratic, $P = 0.051$). Pigs previously fed pharmacological Zn tended ($P = 0.052$) to have lower ADG, with no evidence of a difference for ADFI or F/G.

Overall (d 0 to 42), pigs fed the medium CP diets tended (quadratic, $P \leq 0.098$) to have the lowest ADG and ADFI. There was no evidence of a difference ($P \geq 0.254$) for F/G based on CP level. Pigs fed pharmacological levels of Zn in the experimental phase tended ($P = 0.066$) to have greater overall ADG, with no evidence of a difference ($P \geq 0.242$) for ADFI or F/G.

There was no evidence of a CP × Zn × day interaction ($P \geq 0.209$) or CP × day interaction ($P \geq 0.498$) for fecal DM. A Zn × day interaction was observed ($P = 0.015$) for fecal DM, where pharmacological Zn increased fecal DM on d 10 ($P < 0.001$) and d 25 ($P = 0.024$), but the magnitude of improvement was greater on d 10.

In summary, there was minimal evidence of interactions between dietary CP and Zn level. In phase 1, pigs had greater ADFI and ADG with low CP diets, which also tended to be observed in the overall trial period. Pharmacological Zn resulted in improvements in ADG, ADFI, and F/G during the experimental period. During the common period, pigs previously fed pharmacological Zn had lower ADG, which resulted in only a tendency for improved ADG for the overall trial period, with no overall difference in ADFI or F/G. Pharmacological levels of added Zn improved fecal DM, consistent with previous research, although no evidence of a difference in fecal DM was observed based on dietary CP level.

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

Item	Crude protein:	Phase 1 Low	Phase 1 Medium	Phase 1 High	Phase 2 Low	Phase 2 Medium	Phase 2 High	Phase 3
Ingredient, %								
Corn		45.82	41.73	37.78	60.30	56.21	52.28	67.81
Soybean meal, 47.7% CP		16.82	21.30	25.61	20.24	24.71	29.03	28.13
Whey powder		25.00	25.00	25.00	10.00	10.00	10.00	---
Soy protein concentrate[2]		7.50	7.50	7.50	5.00	5.00	5.00	---
Soybean oil		1.00	1.00	1.00	---	---	---	---
Calcium carbonate		0.45	0.46	0.48	0.69	0.71	0.73	0.73
Monocalcium phosphate, 21.5% P		0.90	0.85	0.80	1.11	1.05	0.99	1.05
Salt		0.58	0.58	0.58	0.65	0.65	0.65	0.60
L-Lys-HCl		0.36	0.22	0.08	0.45	0.31	0.17	0.55
DL-Met		0.21	0.17	0.13	0.21	0.17	0.13	0.22
L-Thr		0.17	0.10	0.04	0.20	0.14	0.08	0.24
L-Trp		0.05	0.03	---	0.05	0.03	---	0.06
L-Val		0.15	0.07	---	0.16	0.08	---	0.16
Trace mineral premix		0.15	0.15	0.15	0.15	0.15	0.15	0.15
Vitamin premix		0.25	0.25	0.25	0.25	0.25	0.25	0.25
Choline chloride, 60%		0.05	0.05	0.05	---	---	---	---
Phytase[3]		0.06	0.06	0.06	0.06	0.06	0.06	0.06
ZnO[4]		+/-	+/-	+/-	+/-	+/-	+/-	---
TiO_2[5]		0.50	0.50	0.50	0.50	0.50	0.50	---

continued

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

Item	Crude protein:	Phase 1 Low	Phase 1 Medium	Phase 1 High	Phase 2 Low	Phase 2 Medium	Phase 2 High	Phase 3
Calculated analysis								
SID amino acids, %								
Lys		1.35	1.35	1.35	1.30	1.30	1.30	1.30
Ile:Lys		60	66	71	57	63	68	53
Leu:Lys		117	125	132	116	125	133	113
Met:Lys		37	35	34	37	36	34	37
Met & Cys:Lys		58	58	58	58	58	58	58
Thr:Lys		64	64	64	64	64	64	64
Trp:Lys		21.1	21.2	21.1	20	20	20	20
Val:Lys		74	74	74	73	73	73	70
His:Lys		34	38	41	35	39	42	35
SID Lys:CP		6.59	6.13	5.75	6.50	6.04	5.66	6.50
NE, kcal/lb		1,149	1,136	1,124	1,115	1,102	1,089	1,108
SID Lys:NE, g/Mcal		5.33	5.39	5.45	5.64	5.64	5.64	5.32
CP, %		20.5	22.0	23.5	20.0	21.5	23.0	20.0
STTD P, %		0.58	0.58	0.58	0.54	0.54	0.54	0.48
Ca:P		1.01	1.01	1.01	1.10	1.10	1.10	1.10

[1] Phase 1 diets were fed in pellet form, phases 2 and 3 were fed in meal form. All diets were manufactured at the O.H. Kruse Feed Technology Center in Manhattan, KS.
[2] Bunge SPC Pure Pro (Bunge, Chesterfield, MO).
[3] HiPhorius 2,400 (2,400 FYT/g; DSM-Firmenich, Parsippany, NJ) provided at 1,500 FYT/kg with an assumed release of 0.12% STTD P.
[4] All diets contained 110 mg/kg added zinc from $ZnSO_4$. Treatment diets for phases 1 and 2 with pharmacological levels of Zn had additional Zn provided in the form of ZnO added at 0.40% to provide a total of 3,000 mg/kg added Zn.
[5] Titanium dioxide (Brenntag Specialties, Inc., South Plainfield, NJ) was included at 0.50% in all diets as an indigestible marker for fecal Zn excretion.

Table 2. Interactive effects of dietary crude protein and added Zn on nursery pig growth performance and fecal DM[1]

Added Zn, mg/kg:	110			3,000				CP × Zn, *P* =	
Crude protein:	Low	Med	High	Low	Med	High	SEM	Linear	Quadratic
BW, lb									
d 0	12.0	12.0	12.0	12.0	12.0	12.0	0.07	0.965	0.775
d 10	15.7	15.5	15.2	17.0	16.5	16.7	1.86	0.792	0.239
d 25	29.9	30.1	29.0	31.7	31.2	31.2	3.18	0.651	0.234
d 42	54.6	53.9	52.7	55.6	54.0	55.0	4.55	0.404	0.223
Phase 1 (d 0 to 10)									
ADG, lb	0.37	0.35	0.33	0.49	0.45	0.46	0.019	0.669	0.328
ADFI, lb	0.46	0.42	0.41	0.52	0.51	0.48	0.018	0.870	0.399
F/G[2]	1.25	1.20	1.26	1.07	1.14	1.04	0.041	0.458	0.035
Phase 2 (d 10 to 25)									
ADG, lb	0.95	0.94	0.92	0.98	0.98	0.97	0.023	0.675	0.940
ADFI, lb	1.36	1.30	1.29	1.41	1.34	1.37	0.029	0.693	0.616
F/G	1.43	1.38	1.41	1.44	1.37	1.42	0.020	0.945	0.365
Experimental period (d 0 to 25)									
ADG, lb	0.72	0.70	0.68	0.78	0.77	0.76	0.016	0.563	0.751
ADFI, lb	1.00	0.95	0.94	1.05	1.01	1.01	0.020	0.665	0.914
F/G	1.39	1.34	1.38	1.34	1.31	1.32	0.018	0.622	0.625
Common phase (d 25 to 42)[3]									
ADG, lb	1.45	1.40	1.40	1.40	1.31	1.40	0.027	0.360	0.155
ADFI, lb	2.13	2.10	2.09	2.06	2.00	2.12	0.043	0.207	0.287
F/G	1.47	1.50	1.49	1.47	1.53	1.52	0.020	0.461	0.601
Overall (d 0 to 42)									
ADG, lb	1.01	0.98	0.97	1.03	0.99	1.02	0.016	0.324	0.338
ADFI, lb	1.46	1.41	1.40	1.45	1.41	1.46	0.024	0.247	0.621
F/G	1.44	1.43	1.45	1.41	1.43	1.43	0.014	0.744	0.597
Fecal dry matter, %[4]									
d 10	16.9	13.6	15.4	19.7	20.4	20.8	1.15	0.258	0.173
d 25	19.4	18.7	18.3	20.8	20.5	21.4	1.13	0.433	0.836

[1] A total of 360 pigs (DNA 240 × 600; initially 12.0 ± 0.07 lb) were used in a 42-d growth study with five pigs per pen and 12 pens per treatment.
[2] Quadratic effect of CP in 110 ppm Zn diets, $P = 0.375$; quadratic effect of CP in 3,000 ppm Zn diets, $P = 0.035$.
[3] All experimental groups were fed the same common diet containing 110 mg/kg added Zn.
[4] The same three middle BW pigs per pen were sampled on days 10 and 24. Linear and quadratic CP × Zn × day, $P \geq 0.209$.

Table 3. Main effects of dietary crude protein and added Zn on nursery pig growth performance and fecal DM[1]

	Crude protein				*P* =		Added Zn, mg/kg			*P* =
Item	Low	Med	High	SEM	Linear	Quadratic	110	3,000	SEM	ZnO
BW, lb										
d 0	12.0	12.0	12.0	0.05	0.849	0.988	12.0	12.0	0.04	0.722
d 10	16.3	16.0	15.9	0.14	0.055	0.417	15.5	16.7	0.12	< 0.001
d 25	30.8	30.7	30.1	0.31	0.110	0.559	29.7	31.4	0.26	< 0.001
d 42	55.1	54.0	53.9	0.53	0.110	0.432	53.8	54.9	0.43	0.078
Phase 1 (d 0 to 10)										
ADG, lb	0.43	0.40	0.39	0.013	0.047	0.496	0.35	0.47	0.011	< 0.001
ADFI, lb	0.49	0.47	0.45	0.013	0.009	0.969	0.43	0.51	0.010	< 0.001
F/G	1.16	1.18	1.16	0.029	0.875	0.375	1.25	1.09	0.024	< 0.001
Phase 2 (d 10 to 25)										
ADG, lb	0.96	0.96	0.94	0.017	0.367	0.644	0.94	0.98	0.014	0.033
ADFI, lb	1.38	1.32	1.33	0.020	0.063	0.171	1.32	1.37	0.017	0.026
F/G	1.43	1.36	1.41	0.014	0.246	0.005	1.41	1.41	0.012	0.930
Experimental period (d 0 to 25)										
ADG, lb	0.75	0.74	0.72	0.012	0.100	0.975	0.70	0.77	0.010	< 0.001
ADFI, lb	1.03	0.98	0.97	0.014	0.011	0.214	0.96	1.02	0.011	< 0.001
F/G	1.37	1.33	1.35	0.013	0.363	0.052	1.37	1.33	0.010	0.002
Common phase (d 25 to 42)[2]										
ADG, lb	1.43	1.36	1.40	0.019	0.286	0.018	1.42	1.37	0.016	0.052
ADFI, lb	2.10	2.05	2.11	0.031	0.784	0.217	2.11	2.06	0.025	0.210
F/G	1.46	1.52	1.51	0.014	0.045	0.051	1.49	1.50	0.012	0.253
Overall (d 0 to 42)										
ADG, lb	1.02	0.98	1.00	0.012	0.094	0.084	0.99	1.01	0.010	0.066
ADFI, lb	1.46	1.41	1.43	0.017	0.300	0.098	1.42	1.44	0.014	0.346
F/G	1.42	1.43	1.44	0.010	0.254	0.751	1.44	1.42	0.010	0.242
Fecal dry matter, %[3]										
d 10	18.3	17.0	18.1	0.81	0.825	0.222	15.3	20.3	0.66	< 0.001
d 25	20.1	19.6	19.9	0.81	0.828	0.704	18.8	20.9	0.66	0.024

[1] A total of 360 pigs (DNA 240 × 600; initially 12.0 ± 0.07 lb) were used in a 42-d growth study with five pigs per pen and 12 pens per treatment.

[2] All experimental groups were fed the same common diet containing 110 mg/kg added Zn.

[3] The same three middle BW pigs per pen were sampled on days 10 and 24. Linear and quadratic CP × day, $P \geq 0.498$; Zn × day, $P = 0.015$.