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K-STATE Research and Extension

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Effects of Zinc Source and Level in Low ABC-4 Diets on Nursery Pig Growth Performance and Fecal Characteristics

Jonathan Riedmüller,[1] Jamil E. G. Faccin, Alessandra R. Monteiro,[2] Jürgen Zentek,[1] Joel M. DeRouchey, Jordan T. Gebhardt,[3] Robert D. Goodband, Jason C. Woodworth, and Mike D. Tokach

Summary

A total of 360 weanling barrows (DNA 200 × 400; initially 13.0 ± 0.07 lb) were used in a 38-d study to evaluate the effects of diets containing different levels of a novel Zn source (HiZox, Animine Precision Minerals, Annecy, France) and different levels of crude protein (CP) in low acid-binding capacity at pH 4 (ABC-4) diets on growth performance and fecal characteristics. Pigs were randomly assigned to pens (5 pigs per pen) and pens were assigned to 1 of 6 treatments with 12 pens per treatment. Diets were fed in 3 phases: phase 1 from d 0 to 10, phase 2 from d 11 to 24, and phase 3 from d 25 to 38. All diets were formulated to have low ABC-4. Treatment 1, the negative control (NC), was formulated to contain 150 ppm of Zn (HiZox) throughout the experiment (d 0 to 38). Treatment 2, the positive control (PC), was formulated to contain 3,000 ppm (phase 1) and 2,000 ppm (phase 2) of Zn (ZnO). Treatment 3 (low HiZox) contained 500 ppm (phase 1) and 300 ppm (phase 2) of Zn. Treatment 4 (low HiZox + low CP) was formulated similar to treatment 3 but contained lower CP (19.3% CP) than the NC, PC, low, and high HiZox treatments (21.3% CP). Treatment 5 (high HiZox) contained 800 ppm (phase 1) and 500 ppm (phase 2) of Zn. Treatment 6 (high HiZox + low CP) was formulated to be similar to treatment 5 but contained less CP (19.3% CP). In phase 3, all pigs were fed a common diet containing 150 ppm of Zn (HiZox) and 21.3% CP. For the experimental period (d 0 to 24), pigs fed high HiZox + low CP had poorer F/G ($P < 0.05$) than NC, PC, low HiZox, and high HiZox. In addition, ADG (quadratic, $P = 0.007$) and ADFI (quadratic, $P = 0.018$) increased as HiZox increased, and pigs fed diets with low CP were less feed efficient ($P = 0.043$) than those fed the same levels of HiZox but with high CP. Overall, pigs fed low CP diets had poorer F/G ($P = 0.041$) than pigs fed similar levels of HiZox with high CP. For fecal characteristics, pigs fed low CP had higher ($P = 0.008$) dry matter (DM) and an interaction between day and CP ($P = 0.040$) was detected for fecal scores with low CP diets improving stool consistency to a greater extent on d 10 than on d 23. In summary, increasing levels of HiZox improved performance of nursery pigs during phases 1 and 2, and pigs fed a regimen of 800 and 500 ppm of HiZox in the first

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2 phases in low ABC-4 diets had similar performance to pigs fed pharmacological levels of Zn from ZnO in the overall period. Finally, pigs fed low CP diets had improved fecal characteristics, but poorer F/G throughout the nursery period.

Introduction

The inclusion of pharmacological levels of Zn in nursery pig diets is widely known to reduce post-weaning diarrhea and improve growth performance. Zn also has many other beneficial effects such as improving gut health and gastrointestinal microbiota. However, many studies have shown that the use of high levels of ZnO might stimulate the promotion of resistance of antibiotics in the pig's gut microbiome. Also, pharmacological levels of Zn increases Zn concentrations in swine waste. ZnO is further known to have 21,863 meq/kg of ABC-4, one of the highest values among feed ingredients.[4] Thus, the use of high levels of Zn through ZnO in weanling pig diets has the potential to drastically increase the stomach pH, which might decrease the utilization of nutrients and increase the chance for pathogens to reach the intestine. Recently, new diet formulation approaches have reduced ABC-4 values to a range from 200 to 300 meq/kg as an alternative to low ZnO diets, without impairing pig performance.[4] Low CP diets are also known to reduce diarrhea, but the growth rate of pigs can be decreased. New feeding alternatives and new Zn sources have been studied to find ways to feed lower levels of Zn and still keep the positive effects on growth performance, fecal dry matter, and microbiota. Currently, there are limited studies exploring the effects of different sources and levels of Zn in low ABC-4 diets on nursery pigs. Different physicochemical characteristics of Zn sources may permit feeding low Zn concentrations if used in conjunction with low ABC-4 diets. This study aimed to explore the effects of different levels of a novel Zn source (HiZox) in low ABC-4 diets with low or high CP content.

Procedures

The Kansas State University Institutional Animal Care and Use Committee approved the protocol used in this experiment. The experiment was conducted at the Kansas State University Segregated Early Weaning Facility in Manhattan, KS. The facility offered two identically structured, fully enclosed nursery barns. The environment was controlled by mechanical ventilation. Each barn had 40 pens, of which 36 pens were used for the trial. Each pen measured 4 × 4 ft. A 4-hole stainless steel dry self-feeder (Thorp Equipment, Thorp, WI) and a bowl/nipple waterer in each pen provided feed and water *ad libitum*.

A total of 360 weanling barrows (DNA 200 × 400; initially 13.0 ± 0.07 lb) were used in a 38-d study to evaluate the effects of diets containing different levels of a novel Zn source (HiZox, Animine Precision Minerals, Annecy, France) and different levels of crude protein (CP) in low ABC-4 diets on growth performance and fecal score and dry matter (DM). Pigs were randomly assigned to pens (5 pigs per pen) and pens were assigned to 1 of 6 treatments with 12 pens per treatment. The experiment was divided into 3 feeding phases: phase 1 from d 0 to 10, phase 2 from d 11 to 24, and phase 3 from d 25 to 38. All diets were formulated using ingredients known to have low ABC-4. Treatment 1 served as the negative control (NC), which was formulated to contain

[4] Stas, E., Tokach, M., DeRouchey, J., Goodband, R., Woodworth, J., Gebhardt, J. Evaluation of the acid-binding capacity of ingredients and complete diets commonly used for weanling pigs, Transl Ani Sci, 6, 3 (2022). https://doi.org/10.1093/tas/txac104.

150 ppm of Zn (HiZox) throughout the experiment (d 0 to 38). Treatment 2 served as a positive control (PC) and was formulated to contain 3,000 ppm (phase 1) and 2,000 ppm (phase 2) of Zn (ZnO). Treatment 3 (low HiZox) contained 500 ppm (phase 1) and 300 ppm (phase 2) of Zn. Treatment 4 (low HiZox + low CP) was formulated to be similar to treatment 3, but contained lower CP (19.3% CP) than the NC, PC, low, and high HiZox treatments (21.3% CP). Treatment 5 (high HiZox) contained 800 ppm (phase 1) and 500 ppm (phase 2) of Zn. Finally, treatment 6 (high HiZox + low CP) was similar to treatment 5 but contained less CP (19.3% CP). In phase 3, all pigs in the treatments were fed a common diet containing 150 ppm of Zn (HiZox) and high CP. Phases 1 and 2 compositions are presented in Tables 1 and 2. A pelleted form of the diets was used in phase 1 and meal form was used in phases 2 and 3.

Individual pig weights and feed disappearance were measured on days: 0, 10, 18, 23, 31, and 38 to calculate ADFI, ADG, and F/G. Feces were collected from the same three pigs in each pen at the end of phases 1 and 2, respectively. Fecal samples were dried (131°F for 24 h) to determine DM. The collected samples were scored in advance using a 5-point scoring system by a single observer. Scores were assigned on following appearance: 1 = watery feces; 2 = unformed feces; 3 = soft formed feces; 4 = firm formed feces; and 5 = hard feces.

Statistical analysis

Data were analyzed as a completely randomized design. Pen was considered the experimental unit and barn was used as random effect. Treatments were used as the fixed effect. Data were analyzed in three different approaches: multiple comparisons (all 6 treatments); dose response of HiZox (NC, Low, and High HiZox); and the interactive effect of HiZox level and CP (high and low HiZox with high and low CP). For growth performance and fecal DM, data were analyzed using the GLIMMIX procedure of SAS (v. 9.4, SAS Institute, Inc., Cary, NC). When testing the HiZox dose response, linear and quadratic contrasts were used. Fecal dry matter and fecal score were analyzed as repeated measures representing multiple observations in each pen over time. For fecal score, data were analyzed as categorical outcomes using a multinomial response distribution. Data were fit using the GLIMMIX procedure and were summarized using the FREQ procedure and reported as the percentage of observations within each fecal score category by treatment and day. For all growth and fecal data, Tukey adjustment was used for multiple comparisons. All results were considered significant at $P \leq 0.05$ and marginally significant at $0.05 < P \leq 0.10$.

Results and Discussion

In Phase 1 (d 0 to 10), pigs fed high HiZox had improved ADG ($P < 0.05$), ADFI ($P < 0.05$), F/G ($P < 0.05$), and were heavier ($P < 0.05$) at d 10 compared to NC, with pigs fed other treatments intermediate (Table 3). Increasing HiZox increased ADG (quadratic, $P < 0.001$) and ADFI (quadratic, $P = 0.005$).

During phase 2 (d 11 to 24), pigs fed diets with low CP (treatments 4 and 6) had poorer F/G ($P = 0.043$) than pigs fed low and high HiZox with high CP (treatments 3 and 5). Pigs fed the NC, PC, and low HiZox diets had improved F/G ($P = 0.001$) compared with pigs fed high HiZox + low CP.

For the experimental period (d 0 to 24), ADG (quadratic, $P = 0.007$) and ADFI (quadratic, $P = 0.018$) improved as HiZox increased. Pigs fed high HiZox + low CP had poorer F/G ($P < 0.05$) than pigs fed the NC, PC, low HiZox, and high HiZox treatments. Pigs fed diets with low CP (treatments 4 and 6) were less feed efficient ($P = 0.043$) than those fed the same Zn levels but with high CP (treatment 3 and 5).

For the overall period (d 0 to 38), pigs fed diets with low CP (treatments 4 and 6) had poorer F/G ($P = 0.041$) than pigs fed the same HiZox levels but with high CP (treatments 3 and 5) pigs. Pigs fed high HiZox + low CP had poorer F/G than pigs fed high HiZox only ($P < 0.05$), with other treatments intermediate.

For fecal characteristics, pigs fed low CP diets had higher ($P = 0.008$) DM compared to pigs fed high CP (Table 4). There was an interaction detected between day and CP ($P = 0.040$) for fecal consistency where lowing the dietary CP increased fecal consistency to a greater extent on day 10 than on d 23 (Figure 1).

In conclusion, during the first 10 d after weaning, 800 ppm of HiZox in a low ABC-4 diet improved ADG, ADFI and F/G compared to lower levels of Zn and similar to pharmacological levels of ZnO. Lowering dietary CP was effective in increasing fecal DM, however, pigs were less feed efficient. This study did not show an interaction between Zn and CP concentrations. Therefore, the inclusion of HiZox at 800 and 500 ppm in low ABC-4 diets in phases 1 and 2, respectively, with a CP content of 21.3% might be a possible means to reduce the use of pharmacological levels of Zn from ZnO in nursery diets.

Table 1. Phase 1 diet composition (as-fed basis)

	Treatments					
Item	**NC[1]**	**PC[1]**	**Low HiZox**	**Low HiZox + Low CP**	**High HiZox**	**High HiZox + Low CP**
Ingredients, %						
Corn	50.07	49.64	50.02	55.45	49.98	55.41
Soybean meal, 46% CP[1]	16.04	16.07	16.04	9.83	16.04	9.83
Lactose	15.00	15.00	15.00	15.00	15.00	15.00
Corn oil	2.00	2.00	2.00	2.00	2.00	2.00
Fermented soybean meal[2]	9.75	9.75	9.75	9.75	9.75	9.75
Spray-dried bovine plasma	2.50	2.50	2.50	2.50	2.50	2.50
Calcium carbonate	0.50	0.50	0.50	0.52	0.50	0.52
Monocalcium P, 21% P	0.95	0.95	0.95	1.04	0.95	1.04
Salt	0.70	0.70	0.70	0.70	0.70	0.70
L-Lys-HCl	0.44	0.44	0.44	0.64	0.44	0.64
DL-Met	0.29	0.29	0.29	0.35	0.29	0.35
L-Thr	0.21	0.21	0.21	0.30	0.21	0.30
L-Trp	0.04	0.04	0.04	0.08	0.04	0.08
L-Val	0.08	0.08	0.08	0.19	0.08	0.19
L-Ile	---	---	---	0.11	---	0.11
L-His	---	---	---	0.08	---	0.08
Vitamin premix[3]	0.31	0.31	0.31	0.31	0.31	0.31
Trace mineral premix	0.11	0.11	0.11	0.11	0.11	0.11
Fumaric acid	0.50	0.50	0.50	0.50	0.50	0.50
Formic acid	0.50	0.50	0.50	0.50	0.50	0.50
ZnO	---	0.42	---	---	---	---
HiZox[4]	0.02	---	0.07	0.07	0.11	0.11

continue

Table 1. Phase 1 diet composition (as-fed basis)

	Treatments					
Item	**NC[1]**	**PC[1]**	**Low HiZox**	**Low HiZox + Low CP**	**High HiZox**	**High HiZox + Low CP**
Calculated analysis						
SID AA, %						
Lys	1.36	1.36	1.36	1.36	1.36	1.36
Ile:Lys	58	58	58	58	58	58
Leu:Lys	116	116	116	105	116	105
Met:Lys	41	41	41	43	41	43
Met and Cys:Lys	58	58	58	58	58	58
Trp:Lys	65	65	65	65	65	65
Thr:Lys	20.7	20.7	20.7	20.7	20.7	20.7
Val:Lys	70	70	70	70	70	70
His:Lys	36	36	36	36	36	36
NE, kcal/lb	1,032	1,027	1,031	1,050	1,031	1,050
Ca, %	0.50	0.50	0.50	0.50	0.50	0.50
STTD P, %	0.47	0.47	0.47	0.47	0.47	0.47
ABC-4, meq/kg[1]	200	292	200	172	200	172
Chemical analysis						
Zn, ppm	189	2,780	492	486	762	850
CP, %	21.3	21.7	22.3	19.9	21.7	19.9

[1] NC = negative control. PC = positive control. CP = crude protein. ABC-4 = acid-binding capacity at pH 4.
[2] MEPro (Prairie AquaTech, Brookings, SD).
[3] Ronozyme 2700 (DSM Nutritional Products, Inc, Parsippany, NJ) provided an assumed 0.13% release of STTD P with 567 FTU/lb in the final diet.
[4] HiZox (Animine Precision Minerals, Annecy, France).

Table 2. Phase 2 diet composition (as-fed basis)

	Treatments					
Item	**NC[1]**	**PC[1]**	**Low HiZox**	**Low HiZox + Low CP**	**High HiZox**	**High HiZox + Low CP**
Ingredients, %						
Corn	57.81	57.45	57.760	63.21	57.76	63.18
Soybean meal, 46% CP[1]	21.49	21.49	21.48	15.20	21.49	15.20
Lactose	7.50	7.50	7.50	7.50	7.50	7.50
Corn oil	1.00	1.00	1.00	1.00	1.00	1.00
Fermented soybean meal[2]	7.65	7.65	7.65	7.65	7.65	7.65
Calcium carbonate	0.50	0.50	0.50	0.50	0.50	0.50
Monocalcium P, 21% P	1.00	1.00	1.00	1.00	1.00	1.00
Salt	0.70	0.70	0.70	0.70	0.70	0.70
L-Lys-HCl	0.52	0.52	0.52	0.71	0.52	0.71
DL-Met	0.29	0.29	0.29	0.34	0.29	0.34
L-Thr	0.24	0.24	0.24	0.33	0.24	0.33
L-Trp	0.05	0.05	0.05	0.09	0.05	0.09
L-Val	0.12	0.12	0.12	0.22	0.12	0.22
L-Ile	---	---	---	0.11	---	0.11
L-His	---	---	---	0.09	---	0.09
Vitamin premix[3]	0.30	0.30	0.30	0.30	0.30	0.30
Trace mineral premix	0.11	0.11	0.11	0.11	0.11	0.11
Fumaric acid	0.35	0.35	0.35	0.35	0.35	0.35
Formic acid	0.35	0.35	0.35	0.35	0.35	0.35
ZnO	---	0.28	---	---	---	---
HiZox[4]	0.02	---	0.04	0.04	0.07	0.07

continued

Table 2. Phase 2 diet composition (as-fed basis)

	Treatments					
Item	**NC[1]**	**PC[1]**	**Low HiZox**	**Low HiZox + Low CP**	**High HiZox**	**High HiZox + Low CP**
Calculated analysis						
SID AA, %						
Lys	1.35	1.35	1.35	1.35	1.35	1.35
Ile:Lys	58	58	58	58	58	58
Leu:Lys	115	115	115	103	115	103
Met:Lys	42	42	42	44	42	44
Met and Cys:Lys	58	58	58	58	58	58
Trp:Lys	65	65	65	65	65	65
Thr:Lys	20.4	20.4	20.4	20.4	20.4	20.4
Val:Lys	70	70	70	70	70	70
His:Lys	36	36	36	36	36	36
NE, kcal/lb	1,134	1,130	1,134	1,150	1,133	1,151
Ca, %	0.52	0.52	0.52	0.52	0.52	0.52
STTD P, %	0.46	0.46	0.46	0.46	0.46	0.46
ABC-4, meq/kg[1]	244	305	244	212	244	212
Chemical analysis						
Zn, ppm	195	1,900	301	331	495	494
CP, %	22.2	20.8	22.6	19.9	22.2	20.0

[1] NC = negative control. PC = positive control. CP = crude protein. ABC-4 = acid-binding capacity at pH 4.
[2] MEPro (Prairie AquaTech, Brookings, SD).
[3] Ronozyme 2700 (DSM Nutritional Products, Inc, Parsippany, NJ) provided an assumed 0.13% release of STTD P with 567 FTU/lb in the final diet.
[4] HiZox (Animine Precision Minerals, Annecy, France).

Table 3. Effects of different Zn sources and levels, and CP in low ABC-4 diets on nursery pig performance[1]

	Treatments							HiZox level[2]		
								P =		
Item	NC	PC	Low HiZox	Low HiZox + Low CP	High HiZox	High HiZox + Low CP	SEM	Linear	Quadratic	Treatment Probability, *P* =
BW, lb										
d 0	13.0	13.0	13.0	13.0	13.0	13.0	0.072	0.930	0.952	0.999
d 10[3]	15.0^{b}	15.7^{ab}	15.7^{ab}	15.9^{ab}	16.5^{a}	15.9^{ab}	0.338	0.042	0.001	0.004
d 24	26.9	28.0	27.4	27.4	28.6	27.3	0.607	0.443	0.007	0.098
d 38	45.3	45.9	45.5	46.1	46.4	45.3	0.935	0.909	0.250	0.818
d 0 to 10 (Phase 1)										
ADG, lb[3]	0.20^{b}	0.27^{ab}	0.27^{ab}	0.29^{ab}	0.35^{a}	0.28^{ab}	0.034	0.042	<0.001	0.004
ADFI, lb	0.24^{b}	0.27^{ab}	0.27^{ab}	0.29^{ab}	0.31^{a}	0.29^{ab}	0.023	0.137	0.005	0.039
F/G	1.41^{b}	1.06^{ab}	1.07^{ab}	1.06^{ab}	0.91^{a}	1.03^{ab}	0.136	0.011	0.010	0.016
d 11 to 24 (Phase 2)										
ADG, lb	0.91	0.94	0.90	0.88	0.93	0.88	0.034	0.671	0.429	0.326
ADFI, lb[4]	1.09	1.13	1.09	1.12	1.15	1.16	0.040	0.848	0.077	0.334
F/G[5]	1.19^{a}	1.20^{a}	1.21^{a}	1.28^{ab}	1.24^{ab}	1.32^{b}	0.030	0.643	0.159	0.001
d 0 to 24 (Experimental period)										
ADG, lb	0.60	0.65	0.63	0.62	0.68	0.62	0.027	0.442	0.007	0.091
ADFI, lb[4]	0.72	0.76	0.73	0.76	0.79	0.78	0.030	0.684	0.018	0.156
F/G [3,5]	1.19^{a}	1.17^{a}	1.17^{a}	1.22^{ab}	1.16^{a}	1.26^{b}	0.021	0.333	0.284	< 0.001
d 25 to 38 (Phase 3)										
ADG, lb	1.20	1.18	1.18	1.22	1.22	1.25	0.039	0.622	0.541	0.537
ADFI, lb[4]	1.80	1.82	1.76	1.79	1.82	1.87	0.070	0.651	0.206	0.271
F/G	1.51	1.54	1.49	1.47	1.50	1.50	0.025	0.746	0.583	0.268
d 0 to 38 (overall)										
ADG, lb	0.80	0.83	0.83	0.83	0.85	0.83	0.025	0.428	0.087	0.585
ADFI, lb	1.08	1.11	1.10	1.13	1.12	1.13	0.029	0.548	0.287	0.533
F/G[5]	1.35^{ab}	1.35^{ab}	1.33^{ab}	1.36^{ab}	1.31^{a}	1.37^{b}	0.017	0.519	0.069	0.045

[1] A total of 360 pigs were used in a 38-d trial. There were 5 pigs per pen and 12 replicate pens per treatment. NC = 150 ppm of Zn (HiZox). PC = 3,000 and 2,000 ppm of Zn (ZnO) for phases 1 and 2, respectively. Low HiZox = 500 and 300 ppm of Zn for phases 1 and 2, respectively. Low HiZox + Low CP = similar to previous with 2% lower CP (19.3%). High HiZox = 800 and 500 ppm of Zn for phases 1 and 2, respectively. High HiZox + Low CP = similar to previous with 2% lower CP (19.3%). At phase 3 (d 25 to 38) all pigs were fed a common diet with 150 ppm of Zn from HiZox and 21.3% CP.

[2] NC, Low HiZox, and High HiZox were included in the HiZox dose response analysis (only treatments with high CP were included).

[3] $P \leq 0.095$ for the interaction between HiZox and CP.

[4] $P \leq 0.087$ for the main effect of HiZox.

[5] $P \leq 0.043$ for the main effect of CP. Different letters (a and b) represent statistical difference ($P < 0.05$).

NC = negative control. PC = positive control. CP = crude protein. HiZox = a novel Zn source (Animine Precision Minerals, Annecy, France). ABC-4 = acid-binding capacity at pH 4.

Table 4. Fecal dry matter of pigs submitted to different Zn and CP levels at nursery[1]

	Treatment							HiZox level[2]		
								P =		
Fecal DM, %	NC	PC	Low HiZox	Low HiZox + Low CP	High HiZox	High HiZox + Low CP	SEM	Linear	Quadratic	Treatment *P* =
d 10	27.3	26.5	26.9	29.1	27.5	30.2	0.35	0.815	0.596	0.135
d 23	26.3	25.5	26.2	27.5	26.4	27.3	0.35	0.933	0.960	0.915

[1] A total of 360 pigs were used in a 38-d trial. There were 5 pigs per pen and 12 replicate pens per treatment. NC = 150 ppm of Zn (HiZox). PC = 3,000 and 2,000 ppm of Zn (ZnO) for phases 1 and 2, respectively. Low HiZox = 500 and 300 ppm of Zn for phases 1 and 2, respectively. Low HiZox + Low CP = similar to previous with 2% lower CP (19.3%). High HiZox = 800 and 500 ppm of Zn for phases 1 and 2, respectively. High HiZox + Low CP = similar to previous with 2% lower CP (19.3%). At phase 3 (25 to 38 d) all pigs were fed a common diet with 150 ppm of Zn from HiZox and 21.3% CP.
[2] NC, Low HiZox and High HiZox were included in the HiZox dose response analysis (only treatments with high CP were included).
HiZox level × day, *P* = 0.720; HiZox level, *P* = 0.596; Zn × CP × day, *P* = 0.770; Day, *P* = 0.005; Zn × CP, *P* = 0.944; Main effect of Zn, *P* = 0.488; Main effect of CP, *P* = 0.008.
NC = negative control. PC = positive control. CP = crude protein. HiZox = a novel Zn source (Animine Precision Minerals, Annecy, France).

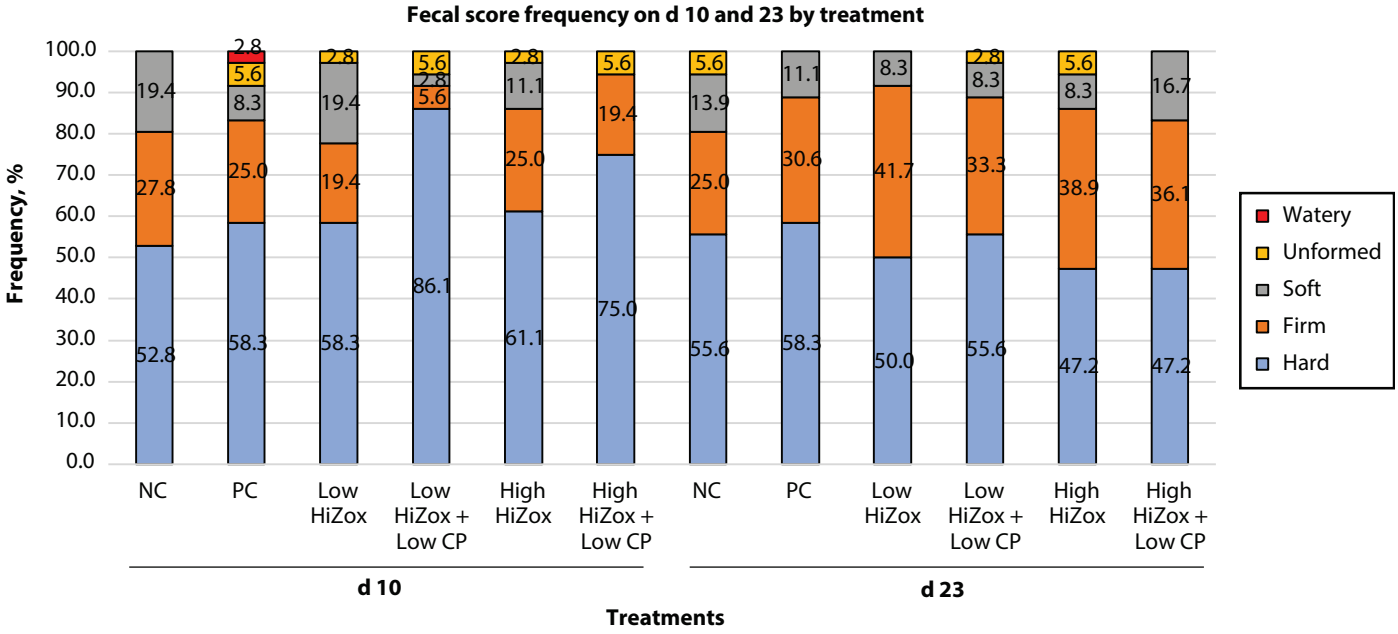

Figure 1. Fecal score frequency on d 10 and 23 by Zn source and Zn concentration and CP level. A total of 360 pigs were used with 5 pigs/pen. Each bar represents 36 observations. Fecal scores were assigned to each pig by a single observer. Fecal scores were assigned based on a 5-point scale: 1 = watery feces; 2 = unformed feces; 3 = soft formed feces; 4 = firm formed feces; and 5 = hard feces. Frequency was determined by the number of each fecal score over the total number of observations of each treatment. HiZox level × day, *P* = 0.378; HiZox level, *P* = 0.473; Zn × CP × day, *P* = 0.396; Zn × CP, *P* = 0.441; Zn × day, *P* = 0.853; main effect Zn, *P* = 0.449; CP × day, *P* = 0.040.