

The effect of water quality, acidification, and zinc source on growth performance of nursery and finishing pigs

by

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## Abstract

This thesis contains three chapters summarizing research: 1) evaluating the effect of zinc sources on growth performance and body weight variation of grow-finish pigs, 2) evaluating water characteristics and their effects on reducing water pH using citric acid, and 3) evaluating the effect of dietary ABC-4 formulation strategy, water source, and water acidification on the growth performance and fecal dry matter of nursery pigs. Chapter 1 utilized 3,159 pigs to determine the effect of zinc (Zn) source on growth, carcass characteristics, and within-pen body weight (BW) and hot carcass weight (HCW) variation in a commercial finishing research setting. Experimental diets contained 100 mg/kg of Zn from Zn hydroxychloride (ZnHyd), zinc sulfate (ZnSO<sub>4</sub>), or zinc oxide (ZnO). Overall, there were no differences observed for growth performance or carcass characteristics. Zinc source did not affect BW or HCW variation at first marketing or final marketing. Chapter 2 involved the collection of 45 water samples from swine production sites across six states that were analyzed to determine the effects of their chemical characteristics on pH reduction with citric acid. Analyses included hardness, pH, calcium (Ca), and magnesium (Mg). Based on the findings of this research, water hardness, Ca, Mg, and initial pH cannot fully predict the amount of citric acid required to reach a stable sample pH of 4.0. However, relationships were observed that can partially explain the variation in the amount of acid required. Chapter 3 utilized 987 nursery pigs to determine the effects of dietary ABC-4 formulation strategy, water acidification, and water source on growth performance, and fecal dry matter (DM) of weanling pigs. Treatments included diets formulated to either a low or high acid binding capacity-4 (ABC-4) value, with high ABC-4 diets containing pharmacological levels of ZnO and low ABC-4 diets containing only basal nutritional levels of Zn. Water was supplied from either rural or well sources, with water source being acidified or not. Ultimately,

formulating diets with ZnO at pharmacological levels improved ADG and ADFI compared to low ABC-4 diets without pharmacological levels of ZnO, with the main effects of water source and acidification having minor impacts. Fecal dry matter tended to be increased in rural water compared to pigs offered well water, but removals and mortality were not affected. In summary, these experiments provide greater understanding of the effect of zinc source when provided to growing-finishing pigs, the relationship between water quality parameters and the ability to change water pH, and the effect of feed and water acidification on growth performance of nursery pigs.

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# **Chapter 1 - Effects of different dietary zinc sources on growth performance and carcass characteristics of finishing pigs**

## **Abstract**

Zinc is a component of many digestive enzymes that make it important for protein, carbohydrate, and lipid metabolism. There are several inorganic and organic Zn sources available for commercial use in swine diets; however, efficacy of these sources varies and data is not consistent. Therefore, a total of 3,159 pigs ( $337 \times 1050$ , PIC; initially  $21.9 \pm 0.77$  kg) were used to determine the effect of Zn source on growth, carcass characteristics and within-pen body weight (BW) and hot carcass weight (HCW) variation in a commercial finishing research setting. Pigs were housed in mixed gender pens with 27 pigs per pen and 39 pens per treatment. Individual pig weights were taken at allotment, first marketing, and final marketing events to calculate within-pen body weight variation. Experimental diets contained 100 mg/kg of Zn from: 1) zinc hydroxychloride (ZnHyd; IntelliBond Z, Selko USA, Indianapolis, IN); 2) zinc sulfate ( $\text{ZnSO}_4$ ), or 3) zinc oxide (ZnO). Experimental diets were fed in meal form in 4 phases. The data from the study was divided into three phases with phase 1 fed from approximately 21.9 to 52.9 kg, phase 2 from 52.9 to 91.6 kg, and phase 3 from 91.6 to 137.3 kg. During phase 1, pigs fed diets containing ZnO had increased ( $P < 0.05$ ) average daily gain (ADG) and gain-to-feed (G:F) compared to those fed the diet containing ZnHyd. In phase 2, Zn source had no effect on ADG, average daily feed intake (ADFI), or G:F. During phase 3, pigs fed diets containing ZnHyd had increased ( $P < 0.05$ ) ADG and G:F compared to those fed ZnO. Overall, there were no differences observed for growth performance or carcass characteristics. Zinc source did not affect BW or HCW variation at first marketing or final marketing. In conclusion, the Zn sources

evaluated in this study, when added at levels commonly used in commercial production, had similar overall growth performance, carcass characteristics, and weight variation at marketing.

### **List of Abbreviations**

ADFI, average daily feed intake

ADG, average daily gain

BW, body weight

G:F, gain-to-feed ratio

CV, coefficient of variation

## **Introduction**

Zinc is an essential nutrient and the second most abundant trace element in the body (Shurson et al., 2022). It is a divalent cation and is found throughout animal tissues, with bone and muscle accounting for 30 and 60%, respectively, of the body's Zn content (Gaudré, 2015). Numerous biological functions including structural and functional integrity of over 2,000 transcription factors and almost every signaling and metabolic pathway are dependent on one or more enzymes that require Zn (Suttle, 2010; Shurson et al., 2022). Zinc is also found in the liver, intestines and kidney which all play a key role in Zn homeostasis (Gaudré, 2015). Decreased appetite, impaired growth, parakeratosis, and reproductive failure are the typical Zn deficiency signs (NRC, 2012).

The NRC (2012) requirement estimates for Zn are 50 to 60 mg/kg of the diet for finishing pigs from 25 to 135 kg BW. This requirement estimate was derived from data from Smith et al. (1962) and Miller (1970) who observed that adding 50 mg/kg of Zn alleviated deficiency symptoms and improved growth performance compared to pigs fed diets containing low levels of Zn. However, a recent industry survey of vitamin and mineral fortification in swine diets

concluded that nutritionists are commonly adding an average of 1.7 times the NRC (2012) requirement estimates of Zn in diets fed to finishing pigs (Faccin et al., 2023).

A variety of Zn sources are used with varying bioavailability. Zinc sulfate is considered 100% bioavailable, whereas ZnO is only considered 50 to 80% bioavailable (Kretzel et al., 2016). ZnO is the most commonly used Zn source followed by ZnSO<sub>4</sub> (Bonetti et al., 2021). As new Zn sources become available, they must be evaluated to compare their efficacy to the sources that are commonly used. Zinc hydroxychloride is an inorganic source of Zn that is produced through reactions of high purity metal, water, and hydrochloric acid (Leisure et al., 2014). This process results in hydroxychloride crystals that contain Zn which is covalently bound to hydroxyl groups and chloride (Cao et al., 2000). During the reaction that produces ZnHyd, covalent bonds are produced that are expected to reduce the reactivity with other components within the diets resulting in improvements in bioavailability (Cao et al., 2000; Shurson et al., 2022). However, there is little published data available that demonstrates the impact of ZnHyd compared to other Zn sources on finisher pig performance and that which exists does not demonstrate a consistent response. Therefore, the objective of this study was to compare the effects between ZnHyd, ZnSO<sub>4</sub>, and ZnO on growth performance, removals and mortality, carcass characteristics and BW and HCW coefficient of variation.

## **Materials and Methods**

### **General**

The Kansas State University Institutional Animal Care and Use Committee approved the protocol used in this experiment. The study was conducted at a commercial research-finishing site in southwest MN. The site contained four identical finishing barns that were naturally ventilated, double-curtain-sided, with completely slatted floors over a deep pit for manure

storage. A total of three barns with 39 pens within each barn were used. Each pen was 3.05 × 5.49 m providing approximately 0.62 m<sup>2</sup> per pig. Ad libitum access to feed and water was provided via a 5-hole stainless steel dry self-feeder and a bowl waterer. Daily feed additions to each pen were accomplished using a robotic feeding system (FeedPro; Feedlogic Corp., Wilmar, MN) able to record feed deliveries for individual pens.

### ***Animals and diets***

A total of 3,159 pigs (337 × 1050, PIC; initially 21.9 ± 0.77 kg) were initially used. Pigs were housed in mixed gender pens with 27 pigs per pen and 39 pens per treatment. There were a similar number of barrows and gilts in each pen. Pens were blocked by initial weight and randomly assigned to 1 of 3 treatments in a randomized complete block design. Treatments consisted of diets containing 100 mg/kg Zn from either ZnHyd (IntelliBond Z, Selko USA, Indianapolis, IN), ZnSO<sub>4</sub>, or ZnO. Treatment diets were prepared with 3 separate vitamin and trace mineral premixes, each containing the respective Zn source as the only source of supplemental Zn in the diet. The remainder of the premix contained the same sources and levels of other vitamins and trace minerals with Cu supplied by tribasic copper chloride (Intellibond C, Selko USA, Indianapolis, IN) to provide 150 mg/kg of Cu and manganese supplied by manganese hydroxychloride (Intellibond M, Selko USA, Indianapolis, IN) to provide 30 mg/kg of Mn. Vitamin-trace mineral premixes remained at a constant level across all four phases. All diets were manufactured at New Horizon Farms feed mill in Pipestone, MN, and were formulated to meet or exceed the NRC (2012) requirement estimates for respective weight ranges of growing-finishing pigs (Table 1). Representative samples for each phase and treatment were collected from the feeders and stored at -20 °C until analysis.

Zn concentration in the feed samples was analyzed at the Kansas State University Soils Laboratory using an ICP analyzer. Before submission for analysis, feed samples were ashed followed by a digestion in 4 M nitric acid at 90°C for 4 h. Diets were fed in meal form in 4 phases with phase 1 fed from approximately 21.9 to 50 kg, phase 2 from 50 to 75 kg, phase 3 from 75 to 100 kg and phase 4 from 100 kg to market.

Pens of pigs were weighed and feed disappearance measured approximately every 14 days to determine ADG, ADFI, and G:F. Data from the study was summarized in 3 periods around common weighing events from approximately 21.9 to 52.9 kg (period 1), 52.9 to 91.6 kg (period 2), and 91.6 to 137.3 kg (period 3). At allotment, first marketing, and final marketing events all pigs were individually weighed to determine within-pen body weight variation. At first marketing, the heaviest 6 pigs were selected from each pen, tattooed with a pen identification number, and transported to a USDA inspected packing plant (JBS Swift, Worthington, MN) for carcass data collection. At the final marketing event, approximately 23 d after the first, the remaining pigs in the pen were marketed following the same procedures. Carcass measurements collected for both marketing events included hot carcass weight (HCW), carcass yield, loin depth, and backfat depth and percentage carcass lean was calculated based on a proprietary equation from the plant. Carcass yield was calculated by dividing HCW by the final live weight recorded at the farm.

### ***Statistical analysis***

Data were analyzed as a completely randomized design for one-way ANOVA using the lmer function from the lme4 package in R (version 4.1.1 (2021-08-10), R Foundation for Statistical Computing, Vienna, Austria). Pen was considered the experimental unit and treatment was considered a fixed effect in the statistical model and weight block considered a random

effect for all analysis. Carcass data were analyzed using individual carcass observations and the statistical model included pen as a random intercept to account for subsampling. For analysis of loin depth, backfat depth, and percentage lean, HCW was used as a covariate. Individual weight data collected at allotment, the first and final marketing events was used to calculate within-pen coefficient of variation at each time point which was analyzed on a pen-basis like other growth performance responses. Removals and mortality were analyzed using a binomial distribution utilizing the glmer function in R, where the denominator was the initial pen inventory, and the numerator was total pigs removed or dead during experimental period. All results were considered significant at  $P \leq 0.05$  and marginally significant between  $P > 0.05$  and  $P \leq 0.10$ .

## **Results and Discussion**

There are a variety of Zn sources available that can be included in trace mineral premixes for use in swine diets. The most common sources are inorganic ZnO and ZnSO<sub>4</sub>. However, novel Zn sources are being developed that need to be investigated to determine their response compared to the Zn sources that are commonly used today. Zinc sulfate is considered 100% bioavailable, whereas ZnO is 50 to 80% bioavailable (Shurson et al., 2022). Zinc hydroxychloride is believed to be more bioavailable than ZnO due to the covalent bonds created during the reaction required to produce ZnHyd (Cao et al., 2000). In a meta-analysis, Van kuijk et al. (2019) suggested that hydroxychloride minerals improved finisher pig performance compared to other inorganic forms and they speculated this may be because of improved bioavailability. In the current experiment, diets were formulated to provide 100 mg/kg of Zn, which is similar to the levels fed in commercial production and did not consider potential differences in Zn source bioavailability.

In period 1, pigs fed diets containing ZnO had increased ( $P < 0.05$ ) ADG and G:F compared to those fed the diet containing ZnHyd (Table 2). No differences in ADFI were observed during this period. In period 2, Zn source had no effect on ADG, ADFI, or G:F ( $P > 0.10$ ). In contrast to our findings, Villagómez-Estrada et al. (2021) observed increased ADG and G:F at the end of the growing period when pigs were fed ZnSO<sub>4</sub> compared to ZnHyd regardless of Zn inclusion within the diet (20 or 80 mg/kg). During period 3, pigs fed diets including ZnHyd had increased ( $P < 0.05$ ) ADG and G:F compared to pigs fed diets containing ZnO; with those fed ZnSO<sub>4</sub> intermediate. During this period there were no differences in ADFI between the treatments ( $P > 0.10$ ). Similarly, Villagómez-Estrada et al. (2021) found that during the finishing period (77.5 to 93.5 kg) pigs fed ZnHyd tended to have increased ADG compared to pigs fed ZnSO<sub>4</sub>.

Overall in our experiment, Zn source had no affect ( $P > 0.10$ ) on ADG, ADFI, G:F, and removals or mortality. Similarly, Carpenter et al. (2016) and Cemin et al. (2019) reported no evidence for Zn source to influence overall growth performance when comparing ZnHyd or ZnSO<sub>4</sub>. Mendonca et al. (2021) compared growth performance when supplementing either ZnHyd or ZnO (grower: 80 mg/kg; finisher phase:70 mg/kg) with different Cu sources and levels (grower phase: 100 or 150 mg/kg; finisher phase: 90 or 150 mg/kg). From 27.7 to 55.9 kg, pigs fed diets supplemented with ZnHyd containing 100 or 150 mg/kg of Cu from Tri-basic Cu (hydroxychloride source) tended to have a greater ADG than pigs fed ZnO with 150 mg/kg CuSO<sub>4</sub>. Similarly, during the finishing period (74.5 to 101.0 kg), Mendonca et al. (2021) observed greater ADG and BW when supplementing Zn and Cu hydroxychloride sources compared to pigs fed ZnO co-supplemented with 150 mg/kg CuSO<sub>4</sub>.



Carcass characteristics were not statistically affected by Zn source at the first or final marketing event, or overall. However, pigs fed ZnHyd had numerically greater HCW which is in line with other trials. Cemin et al. (2019) observed that pigs fed ZnHyd tended to have greater HCW and carcass yield compared to pigs that received ZnSO<sub>4</sub>. Similarly, Carpenter et al. (2016) compared ZnHyd and ZnSO<sub>4</sub> and observed that pigs fed diets with ZnHyd had increased HCW compared to pigs that received supplemental Zn as ZnSO<sub>4</sub>. Likewise, Villagómez-Estrada et al. (2021) observed greater carcass yields when pigs were fed ZnHyd compared to ZnSO<sub>4</sub>, however, no differences in HCW were observed. Mendonça et al. (2021) observed greater HCW for pigs fed ZnHyd compared to those who were fed diets containing supplemented Zn in the form of ZnO. A meta-analysis by Van Kuijk et al. (2019), where they compared added ZnHyd to inorganic Zn sources supplemented at 80 ppm, reported improved carcass lean for pigs fed ZnHyd compared to inorganic Zn sources.

Zinc source has been hypothesized to reduce BW and HCW coefficient of variation; however, the mode of action is not understood, nor been successfully confirmed. During this trial within pen pig BW or HCW coefficient of variation was not influenced by treatment at either the first or final marketing event (Tables 2 and 3). However, pigs fed the ZnHyd treatment had numerically the lowest CV for overall pigs marketed and the captured HCW. Attempts to reduce body weight variation are evaluated to assist producers when marketing pigs as packing plants specify that pigs be marketed within certain weight ranges to avoid discounts for pigs outside the desired weight ranges. Therefore, evaluating ways to reduce pig BW coefficient of variation is critical to reduce economic losses. Tolosa et al. (2021) completed a meta-analysis that evaluated the relationship between pig body weight and variation from birth to market. It was reported that the mean CV of the population has a decreasing quadratic relationship with pig BW. Coefficient

of variation of pig BW decreased as live weight increased, which matches results observed in this trial. The largest CV was observed at the time of allotment, and it decreased at the time of marketing for all treatments. Previous research by Cordoba et al. (2023) compared manganese hydroxychloride (MnHyd) and manganese sulfate (MnSO<sub>4</sub>) and observed numeric reductions in BW CV and HCW CV for pigs fed MnHyd compared to pigs fed MnSO<sub>4</sub> at marketing. Similarly, during this trial a numeric reduction in BW CV and HCW CV was observed with pigs fed the ZnHyd compared to the other Zn sources. While not statistical, the economic relevance of this is important for producer's consideration as 0.72% of the pigs fed ZnHyd were below the weight window of 104 kg whereas 2.33% and 2.40% were below the same threshold for the pigs fed ZnSO<sub>4</sub> and ZnO, respectively. Similarly, 95.38% of the pigs fed ZnHyd were above 113 kg, whereas 93.32% and 93.71% were above the same threshold for pigs fed ZnSO<sub>4</sub> and ZnO, respectively. Economically, these trends in more pigs within the marketing window when ZnHyd is fed are meaningful for producers and worthy of additional research to confirm the response.

## **Conclusion**

In summary, when comparing Zn added to finishing pig diets at commercial levels from ZnHyd, ZnSO<sub>4</sub>, or ZnO, Zn source had small effects depending on phase, but no overall effect on growth performance, removals or mortality, carcass characteristics, or BW and HCW coefficient of variation.

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**Table 1.1.** Composition of diets (as-fed basis)<sup>1</sup>

Ingredients, %	Phase			
	1	2	3	4
Corn	54.63	60.98	65.87	68.37
Soybean meal (47.7% CP)	17.34	11.19	6.67	4.22
DDGS <sup>2</sup>	25.00	25.00	25.00	25.00
Limestone	1.45	1.40	1.30	1.25
Monocalcium phosphate (21% P)	0.30	0.20	---	---
Salt	0.40	0.40	0.40	0.40
L-Lys HCl	0.48	0.45	0.43	0.43
DL- Met	0.02	---	---	---
Thr <sup>3</sup>	0.11	0.10	0.08	0.08
L-Trp	0.03	0.03	0.03	0.04
Vitamin trace mineral premix <sup>4</sup>	0.20	0.20	0.20	0.20
Phytase <sup>5</sup>	0.05	0.05	0.03	0.02
Total	100	100	100	100
Analyzed zinc concentration, mg/kg	112.4	111.5	109.8	125.0
SID amino acids <sup>6</sup> %				
Lys	1.08	0.91	0.78	0.72
Ile:Lys	60	60	60	60
Leu:Lys	155	168	183	190
Met:Lys	29	30	33	34
Met and Cys:Lys	56	59	64	66
Thr:Lys	62	63	64	65
Trp:Lys	18.4	18.1	18.0	18.3
Val:Lys	71	73	76	77
Lys:NE, g/Mcal	4.62	3.82	3.26	3.05
NE, kcal/kg	2,385	2,424	2,460	2,475
CP, %	20.4	17.9	16.1	15.2
Ca, %	0.65	0.60	0.51	0.49
STTD P <sup>7</sup> , %	0.43	0.39	0.33	0.31

<sup>1</sup> Phases 1, 2, 3, and 4 were fed from 22.7 to 50, 50 to 75, 75 to 100, and 100 kg to marketing, respectively.

<sup>2</sup> DDGS = corn dried distiller's grains with solubles.

<sup>3</sup> ThrPro (CJ America Bio, Downers Grove, IL)

<sup>4</sup> Treatments included per kg of feed: 100 mg of Zn from hydroxychloride (IntelliBond Z, Selko USA, Indianapolis, IN), ZnSO<sub>4</sub>, or ZnO, 110 mg Fe; 30 mg Mn from manganese hydroxychloride; 150 mg Cu from tribasic copper chloride; 0.30 mg I; 0.30 mg Se; 4,885 IU vitamin A; 1,217 IU vitamin D; 24 IU vitamin E; 2.4 mg vitamin K; 0.02 mg vitamin B12; 45.6 mg niacin; 15.2 mg pantothenic acid; and 4.6 mg riboflavin.

<sup>5</sup> Optiphos 2500 (Huvepharma Inc., Peachtree City, GA) provided 1,251 FTU/kg for phases 1 and 2 and 626 and 500 FTU/kg for phases 3 and 4 with an assumed release of 0.13%. STTD P release %; Phase 1 and 2; 0.14, Phase 3; 0.11, Phase 4; 0.10.

<sup>6</sup>SID = standardized ileal digestible.

<sup>7</sup>STTD P = standardized total tract digestible phosphorus.

**Table 1.2.** Effects of zinc source on growing and finishing pig performance<sup>1,2</sup>

Item <sup>3</sup>	Zn			SEM	P =
	Hydroxychloride <sup>4</sup>	ZnSO <sub>4</sub>	ZnO		
BW, kg					
Initial	21.9	21.8	21.9	0.77	0.919
End period 1	52.7	52.8	53.3	0.66	0.327
End period 2	91.5	91.1	92.1	0.70	0.111
First marketing event					
Marketed pigs	129.0	128.3	128.7	0.96	0.810
All pigs	119.1	117.9	118.8	0.84	0.239
Final marketing event	138.2	136.9	136.9	1.05	0.218
Average market weight <sup>5</sup>	136.1	134.8	134.7	0.91	0.120
Period 1					
ADG, kg	0.71 <sup>b</sup>	0.72 <sup>ab</sup>	0.74 <sup>a</sup>	0.009	0.024
ADFI, kg	1.51	1.49	1.50	0.022	0.427
G:F, g/kg	474 <sup>b</sup>	486 <sup>a</sup>	490 <sup>a</sup>	3.3	0.001
Period 2					
ADG, kg	0.91	0.90	0.91	0.013	0.194
ADFI, kg	2.41	2.38	2.43	0.033	0.106
G:F, g/kg	379	377	376	2.5	0.494
Period 3					
ADG, kg	1.02 <sup>a</sup>	1.00 <sup>ab</sup>	0.98 <sup>b</sup>	0.015	0.003
ADFI, kg	3.45	3.41	3.39	0.027	0.129
G:F, g/kg	296 <sup>a</sup>	294 <sup>ab</sup>	289 <sup>b</sup>	3.0	0.016
Overall					
ADG, kg	0.88	0.87	0.87	0.008	0.199
ADFI, kg	2.44	2.42	2.43	0.029	0.309
G:F, g/kg	361	361	360	1.9	0.596
Removals, %	7.6	6.1	5.4	0.94	0.100
Mortality, %	0.6	0.6	0.6	0.27	0.700
Total removals and mortalities, %	8.3	6.9	5.9	0.99	0.100
Coefficient of variation, % <sup>6</sup>					
Allotment	17.9	17.9	17.9	0.69	0.999
First marketing event <sup>7</sup>					
Marketed pigs <sup>8</sup>	5.4	5.2	5.7	0.34	0.538
Remaining pigs <sup>9</sup>	10.2	10.5	10.4	0.42	0.844
All pigs	11.6	12.0	11.8	0.44	0.793
Final marketing event	8.9	9.4	9.5	0.34	0.433
All marketed pigs <sup>10</sup>	8.7	8.8	9.1	0.24	0.533

<sup>1</sup>A total of 3,159 pigs (PIC 337 × 1050; initially 21.9 ± 0.77 kg) were used in a 134-d growth trial. with 27 pigs per pen and 39 replications per treatment.



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<sup>2</sup>Zinc was included in the diet at 100 ppm for each of the dietary treatments.

<sup>3</sup>BW = body weight; ADG = average daily gain; ADFI = average daily feed intake; G:F = gain-to-feed ratio.

<sup>4</sup>Intellibond Z (Zn hydroxychloride, Selko USA, Indianapolis, IN).

<sup>5</sup>Average marketing weight = (first marketing weight + final marketing weight)/total pigs marketed.

<sup>6</sup>Within pen Coefficient of Variation = (SD of individual weights / mean of individual weights) × 100.

<sup>7</sup>First marketing event was on day 119 for barn 1, day 112 for barn 2, and day 102 for barn 3.

<sup>8</sup>The 6 heaviest pigs were selected, individually weighed, tattooed with a pen identification number, and transported to a USDA inspected packing plant where carcass data was collected.

<sup>9</sup>The remaining pigs that were not removed during the first marketing event.

<sup>10</sup>All marketed pigs CV includes the CV for the marketed pigs at the first marketing and the CV of all pigs marketed at the final marketing event.

**Table 1.3.** Effect of zinc source on market pig carcass characteristics<sup>1</sup>

Item	Zn			SEM	P =
	Hydroxychloride <sup>2</sup>	ZnSO <sub>4</sub>	ZnO		
Count					
HCW first marketing	198	173	208	---	---
HCW final marketing	614	632	596	---	---
HCW Overall	812	805	804	---	---
HCW, kg <sup>3</sup>					
First marketing <sup>4</sup>	96.6	96.0	96.1	0.77	0.673
Final marketing	100.8	100.3	100.2	0.71	0.475
Overall	99.7	99.3	99.1	0.62	0.405
Carcass yield, %					
First marketing	74.7	74.7	74.7	0.27	0.986
Final marketing	72.9	73.2	73.3	0.19	0.185
Overall	73.5	73.4	73.6	0.19	0.504
Loin depth, mm <sup>5</sup>					
First marketing	63.3	62.1	63.1	0.51	0.797
Final marketing	67.3	67.0	67.4	0.41	0.662
Overall	66.4	66.0	66.4	0.76	0.490
Backfat depth, mm <sup>5</sup>					
First marketing	16.5	16.3	16.2	0.29	0.593
Final marketing	16.2	16.3	16.0	0.18	0.370
Overall	16.3	16.2	16.0	0.42	0.478
Lean, % <sup>5</sup>					
First marketing	56.4	56.5	56.6	0.20	0.772
Final marketing	57.1	57.0	57.2	0.13	0.334
Overall	56.9	56.9	57.1	0.31	0.510
Coefficient of variation, % <sup>6</sup>					
First marketing	6.1	5.8	6.4	0.40	0.547
Final marketing	8.9	9.1	9.3	0.31	0.665
All carcasses	8.8	8.9	9.0	0.27	0.885

<sup>1</sup>A total of 3,159 pigs (PIC 337 × 1050; initially 21.9 ± 0.77 kg) were initially utilized in a 134-d growth trial with 27 pigs per pen and 39 replications per treatment in 3 barns.

<sup>2</sup>Intellibond Z (Zn hydroxychloride, Selko USA, Indianapolis, IN)

<sup>3</sup>HCW = hot carcass weight.

<sup>4</sup>At the first marketing event the 6 heaviest pigs per pen were selected, individually weighed, tattooed with a pen identification number, and transported to a USDA inspected packing plant where carcass data was collected. At final marketing the remaining pigs in the pen followed the same procedure.

<sup>5</sup>Adjusted using HCW as a covariate.

<sup>6</sup>Coefficient of Variation = (SD of individual HCW / mean of individual HCW) × 100.

## **Chapter 2 - Evaluating water characteristics and their effects on reducing water pH using citric acid and Activate WD Max**

### **Abstract**

A total of forty-five water samples from swine production sites across six states were used to determine the amount of CitraSol (Northwest Livestock Distribution, Medina, MN), a citric acid product, required to reduce water pH to a common end point. Water samples were analyzed for pH, Ca, Mg, and hardness. Total hardness was calculated using Ca and Mg concentrations and expressed as mg of CaCO<sub>3</sub>/L. Water hardness ranged from 142 to 1,181 mg CaCO<sub>3</sub>/L with an average of 441.2 mg CaCO<sub>3</sub>/L. Initial pH ranged from 7.42 to 8.47 with an average of 7.91. In triplicate, CitraSol was added to 10 mL samples of each water source to reach a stable pH of 5.0 and 4.0 ± 0.05. An inverse relationship between water hardness and initial pH was observed (quadratic,  $P = 0.002$ ;  $R^2 = 0.22$ ). The amount of CitraSol required to reach a stable pH of 4.0 increased (quadratic,  $P < 0.001$ ) as hardness, Ca, and Mg increased ( $R^2 = 0.30$ , 0.27, 0.28, respectively). Surprisingly, high initial pH water required less (quadratic,  $P < 0.001$ ;  $R^2 = 0.31$ ) CitraSol to reach a pH of 4.0. We hypothesize this was partially due to the reduction in the amount of free Ca ions as the water becomes more alkaline in nature. A sub sample of the water samples was titrated using Activate WD Max (Novus International, Chesterfield, MO) to determine if the amount of acid required to reduce water pH to the same common end point was acid specific. A direct relationship between the amount of CitraSol and Activate WD Max (linear,  $P < 0.001$ ;  $R^2 = 0.87$ ) to reach a pH of 4.0 was observed, suggesting that data from one acid may allow prediction of the quantity required of another acid to reach the same target pH. Similarly, titrating to a pH of 5.0 can predict the amount of acid required to reach a pH of 4.0 (linear,  $P < 0.001$ ;  $R^2 = 0.99$ ). In conclusion, pH, Ca, Mg, and hardness cannot fully predict the

amount of acid required to reach a stable water pH of 4.0. However, relationships were observed that can partially explain the variation in the amount of acid required. This data suggests that acid titrations of individual water sources should be completed to determine the amount of acid required to reach a final pH of 4.0.

### **List of Abbreviations**

ABC-4 = acid binding capacity-4

Ca = calcium

CaCO<sub>3</sub> = calcium carbonate

IA = Iowa

KS = Kansas

Mg = magnesium

MN = Minnesota

MO = Missouri

OH = Ohio

TN = Tennessee

## **Introduction**

Water is a fundamental nutrient required for all living organisms and is essential for normal metabolic functions including body temperature regulation, waste excretion, and is necessary to maximize feed consumption (Romoser et al., 2022). Although necessary for survival, water research has received little attention, especially in livestock production (Patience, 2012). Research conducted with water often focuses on optimal water delivery and requirements rather than water quality (Lozinski et al., 2022). Water quality can be evaluated using three broad criteria including: physical, chemical, and microbiological characteristics, with most of the

attention placed on chemical and microbiological criteria. When evaluating chemical criteria, the focus is mainly on the concentration of minerals including Ca, Mg, sulfates, nitrates, and other minerals. These contaminants can be naturally found in well water and can vary based on well location, design, and depth (Patience, 2012). Although not demonstrated to be a health concern to the pig, elevated levels of hardness, or the additive concentrations of Ca and Mg salts in the water source can lead to scale build up in water delivery and treatment systems (Patience, 2013). The material inside water lines can lead to problems with water filters and nipple drinkers, thus reducing water flow rate (Patience, 2013). To clean water lines, acidifiers can be added as they are believed to help reduce microbial growth and loosen debris within the line. Furthermore, acidifiers can be added to water systems to reduce pH which is suggested to improve performance of newly weaned pigs (Gottlob et al., 2005; Xu et al. 2022).

Xu et al. (2022) observed benefits in growth performance of nursery pigs when adding acids to the drinking water, while Parker et al. (2007) observed benefits in broiler chicken health when supplementing organic acids in the water. However, Gentry et al. (2010) observed no differences in nursery pig mortality or ADG when including any of three different acidifiers to the water. There are many commercially available water acidifiers including organic, inorganic, and acid-blend products. Citric acid is a commonly used organic acid because it is relatively inexpensive and readily accessible. The commercial acidifier, Activate WD Max, is an organic acid blend that can be used as an alternative to citric acid. We hypothesized that certain chemical characteristics of water such as Ca and Mg concentrations might negatively influence the ability of acidifiers to reduce water pH. Therefore, the objective of this study was to determine if different water quality characteristics associated with different water sources

influenced the amount of acidifier that need to be added to reach a stable pH endpoint of 5.0 and 4.0.

## **Materials and Methods**

### **General**

A total of forty-five water samples were collected from commercial swine production facilities located in IA, KS, MN, MO, OH, or TN. A 25 mL subsample of each water sample was sent to the Kansas State University Soils Laboratory, Manhattan, KS, and analyzed to determine pH, Ca, and Mg (Table 1). Analyzed Ca and Mg concentrations were then used to calculate total hardness expressed in calcium carbonate equivalence using the equation described by Boyd (2015): Total Hardness, mg CaCO<sub>3</sub>/L = (Ca, mg/L × 2.479) + (Mg, mg/L × 4.118)

Using 10 mL samples of water per replicate, initial pH was recorded and increasing amounts of CitraSol (Northwest Livestock Distribution, Medina, MN), a citric acid product, was added to achieve a final pH of 5.0 and 4.0 ± 0.05. To analyze water pH during the acid titration process, a Mettler Toledo Seven Compact SS20 pH/Ion meter (Mettler Toledo, Columbus, Ohio) was used. During each titration, the water samples were placed in a beaker containing a metal stir bar and the beaker was located on a stir plate to continuously homogenize the water sample. The amount of CitraSol required to reach each pH was recorded for each replicate and the average was calculated for each sample for each pH level. Activate WD Max (Novus International, Chesterfield, MO; an organic acid blend including lactic acid, phosphoric acid and methionine hydroxy analogue) was titrated in a subsample of 13 water samples following the same procedure used with CitraSol in order to compare the quantity of a second acidifier required to reduce pH.

### ***Statistical analysis***

Data were analyzed using the `lm` and `ggplot` functions in R (Version 4.3.0 (2023-03-01), R Foundation for Statistical Computing, Vienna, Austria) to determine the linear and quadratic relationships between water measurements including starting pH, hardness, Ca, and Mg concentrations to the amount of CitraSol required to reach the target pH. The linear relationship between the amount of CitraSol vs Activate WD Max required to reach the same target pH was also analyzed. All results were considered significant at  $P \leq 0.05$ . Correlation coefficients ( $R^2$ ) were also determined.

## **Results and Discussion**

Patience (2012) described three broad criteria that are used when evaluating water quality. These include physical, microbiological, and chemical properties. Physical criteria include qualities, which are believed to be of minimal consideration in pig production, including color, turbidity, and odor. Most attention is placed on the microbiological properties including microbial growth and contamination; along with chemical properties including concentrations of Ca, Mg, sulfates, nitrates, and other minerals.

When evaluating the chemical properties, total hardness can be used to characterize water quality. Hardness is the measure of the sum of divalent cations including Ca and Mg salts in the water (Nyachoti et al., 2022), and is expressed as calcium carbonate ( $\text{CaCO}_3$ ) equivalence (Kober, 1993). Hardness, for water supply purposes, is often classified into four categories; soft (less than 50 mg/L), moderately hard (50 - 150 mg/L), hard (150 - 300 mg/L), and very hard ( $> 300$  mg/L; Boyd, 2015). Geographic location and water source (municipal vs well water) are critical variables that directly affect water hardness. In alkaline water, bicarbonate is the major

inorganic carbon species and preferentially binds to acids that have a low polarizability, such as  $\text{Ca}^{2+}$ , and  $\text{Mg}^{2+}$  (Pearson 1963; Pearson 1968), creating calcium and magnesium carbonate.

Water hardness represents the total concentration of dissolved components ( $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ ) in the solution, and reflects the sum of free ion species and concentrations of various soluble complexes that may form (Essington, 2015). For most free species and soluble complexes, analytical methods are not available to directly measure their concentrations and differentiate from each other. Therefore, only total concentrations of the mineral can be measured and the distribution of the element between free and complex species must be estimated (Essington, 2015). Assuming that cations present within water ( $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ , and others) form only one complex with each ligand (a molecule that can form a complex with a metal), a typical water source can contain over 100 soluble complexes and free species (Essington, 2015), making a water source extremely complex.

To understand the variation in acid binding material in the water supply from swine facilities, forty-five samples were collected across six states. Total hardness ranged from 142 to 1,181 mg  $\text{CaCO}_3/\text{L}$  with an average of 441.2 mg  $\text{CaCO}_3/\text{L}$  (Table 1). On average, the forty-five water samples would be classified as very hard (Boyd, 2015). Initial sample pH ranged from 7.42 to 8.47 with an average of 7.91. An inverse relationship between sample hardness and initial pH was observed (quadratic,  $P = 0.002$ ;  $R^2 = 0.22$ ; Table 2; Figure 1), where an increase in sample hardness resulted in a lower starting pH. We hypothesize this is partially due to greater concentrations of free Ca and Mg ions in the water at a lower pH. As the hydrolysis of free species of minerals occurs as water becomes more alkaline, the concentrations of free Ca and Mg that is accounted for when calculating hardness decreases. High initial sample pH was associated with a reduction (quadratic,  $P < 0.001$ ;  $R^2 = 0.31$ ; Figure 2) in the amount of CitraSol required to



reach a pH of 4.0. Although initially unexpected, Essington (2015), stated that close to 100% of the total K, Na, Cl, and NO<sub>3</sub> in alkaline water occur as free species. However, only 89% percent of the total Ca and 88% of the total SO<sub>4</sub> occur as free species; resulting in less Ca available to bind the added CitraSol. Conversely, Essington (2015) determined that free, uncomplexed species of Ca<sup>2+</sup>, Mg<sup>2+</sup>, K<sup>+</sup>, Na<sup>+</sup>, SO<sub>4</sub><sup>2-</sup>, Cl<sup>-</sup>, and NO<sub>3</sub><sup>-</sup> dominate acidic water solutions.

Additionally, acidic water contains approximately 100% of the total Ca, Mg, K, Na, Cl, and NO<sub>3</sub> and 84% of the total SO<sub>4</sub>, as the free species. Thus, more uncomplexed species present in lower pH water are available to bind to the added acidifier meaning that an increase in the amount of acidifier is required to lower pH in acidic water compared to alkaline water. Sample pH does not fully predict ( $R^2 = 0.31$ ) the variation in the amount of CitraSol required to reduce water pH to 4.0, we hypothesize that it is possible that metal citrate precipitates may be forming in water with lower starting pH, resulting in greater amount of acid required to lower water pH (Hettiarachchi, 2024).

A low correlation between the amount of CitraSol to obtain a stable pH of 4.0 and hardness, Ca, and Mg as single predictors was observed (Figures 3, 4, and 5), leading us to conclude that there are other variables that affect the ability of an acidifier to reduce water pH. Although species availability can be estimated by water pH, element species distribution is environment and element specific (Essington, 2015). The amount of CitraSol required to reach a sample pH of 4.0 increased (quadratic,  $P < 0.001$ ) as hardness, Ca, and Mg increased ( $R^2 = 0.30, 0.27, 0.28$ , respectively). This was as expected due to the acid binding characteristics of both Ca and Mg. A high correlation between Ca and Mg concentrations was observed (linear,  $P < 0.001$ ;  $R^2 = 0.80$ ; Figure 6) concluding that Ca and Mg concentrations are related and an increase in the concentration of one of these minerals is often associated with an increase of the other.

Adding CitraSol to reach a target pH of 5.0 can predict (linear,  $P < 0.001$ ;  $R^2 = 0.99$ ; Figure 7) the amount of acid required to reach a pH of 4.0. Therefore, the amount of CitraSol required to reach one pH endpoint can be used to predict the amount of CitraSol required to reach a lower pH endpoint.

When also using a second acidifier (Activate WD Max), it was determined that the amount of acid required to reduce water pH was specific to the acid used in the titration. A direct relationship between the amount of CitraSol and Activate WD Max (linear,  $P < 0.001$ ;  $R^2 = 0.87$ ) to reach a pH of 4.0 was observed, suggesting that the amount of acidifier required to reach a target pH from one acidifier can be used to predict the amount of a different acidifier needed to reach the same target pH endpoint.

## **Conclusion**

In conclusion, water pH, Ca, Mg, and hardness cannot fully predict the amount of CitraSol required to reach a stable pH of 4.0. Therefore, a titration of specific acidifiers in unique water sources is necessary to determine their amount required to reach a final target water pH.

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**Table 2.1.** Range of analyzed water characteristics<sup>1</sup>

Variable	Minimum	Average	Maximum
Sample pH	7.42	7.91	8.47
Calcium, mg/L	33.6	110.4	300.1
Magnesium, mg/L	8.5	40.2	105.0
Total hardness, mg CaCO <sub>3</sub> /L <sup>2</sup>	142.3	441.2	1181.3

<sup>1</sup>A total of 45 swine production-site water samples across six states were collected and analyzed.

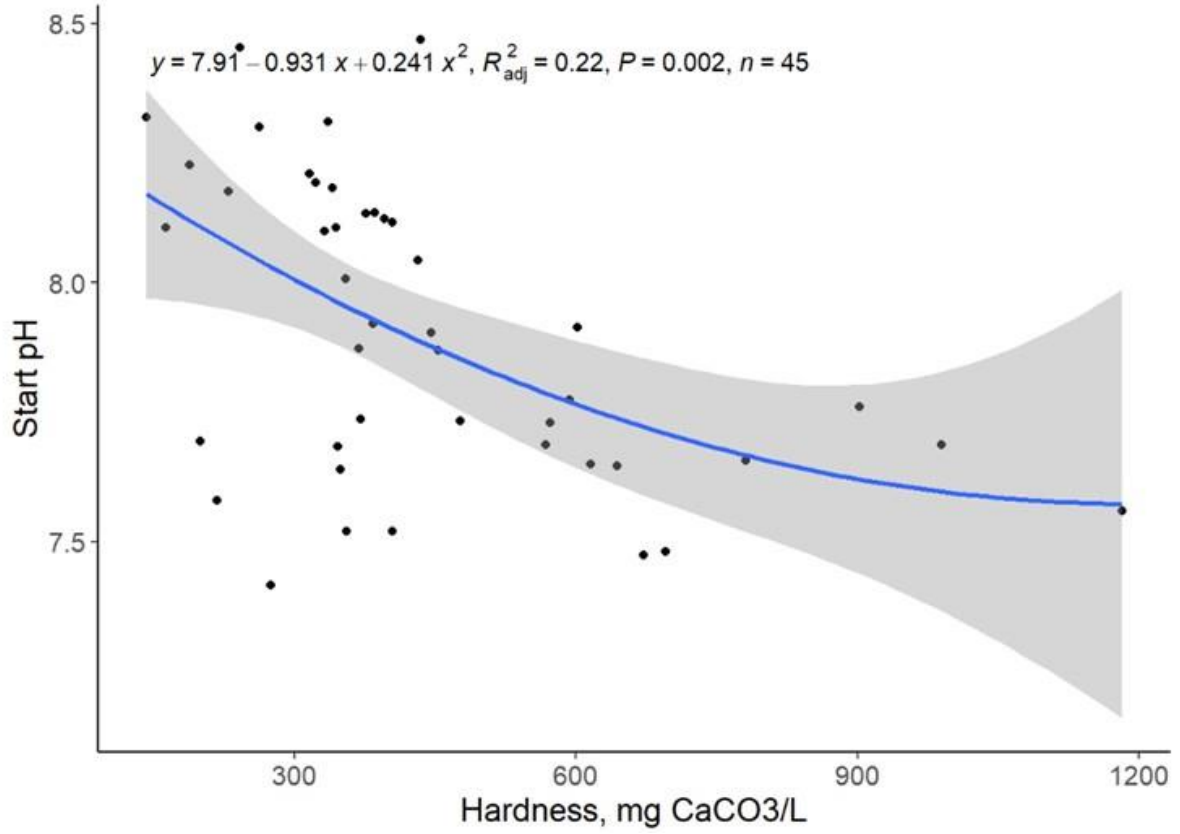
<sup>2</sup>Total hardness, mg CaCO<sub>3</sub>/L = (Ca, mg/L × 2.479) + (Mg, mg/L × 4.118)

**Table 2.2.** Relationship between water characteristics and CitraSol<sup>1</sup> (mL) required to reach a stable pH of 4.0<sup>2</sup>

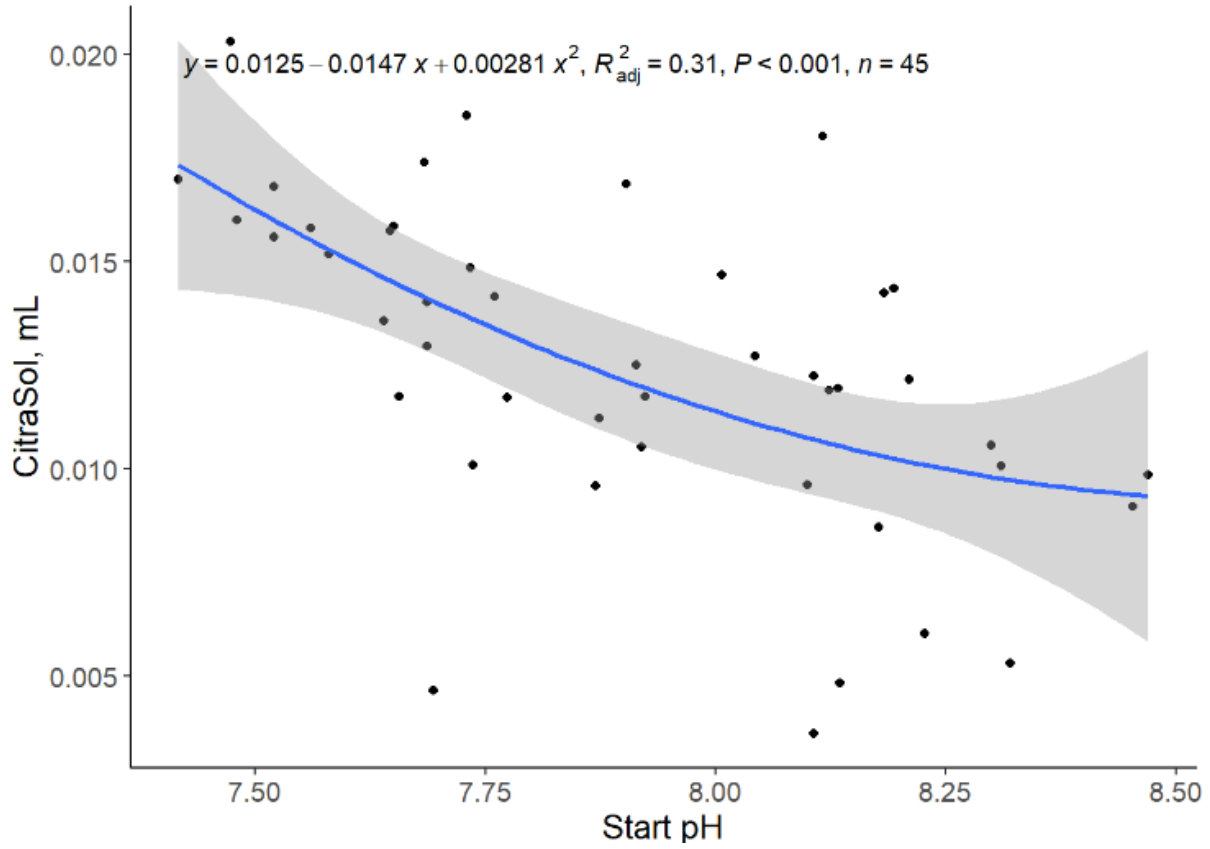
Relationship	Linear		Quadratic	
	<i>P</i> =	R <sup>2</sup>	<i>P</i> =	R <sup>2</sup>
Start pH and hardness	< 0.001	0.22	0.002	0.22
Acid required (mL) to reach pH of 4.0 relationship with:				
Hardness	0.001	0.19	< 0.001	0.30
Ca concentration	0.002	0.19	< 0.001	0.27
Mg concentration	0.002	0.18	< 0.001	0.28
Starting pH	< 0.001	0.31	< 0.001	0.31
Acid required (mL) to reach pH of 5.0	< 0.001	0.99	---	---

<sup>1</sup> CitraSol, Northwest Livestock Distribution, Medina, MN.

<sup>2</sup>A total of 45 swine production-site water samples across six states were collected and analyzed.

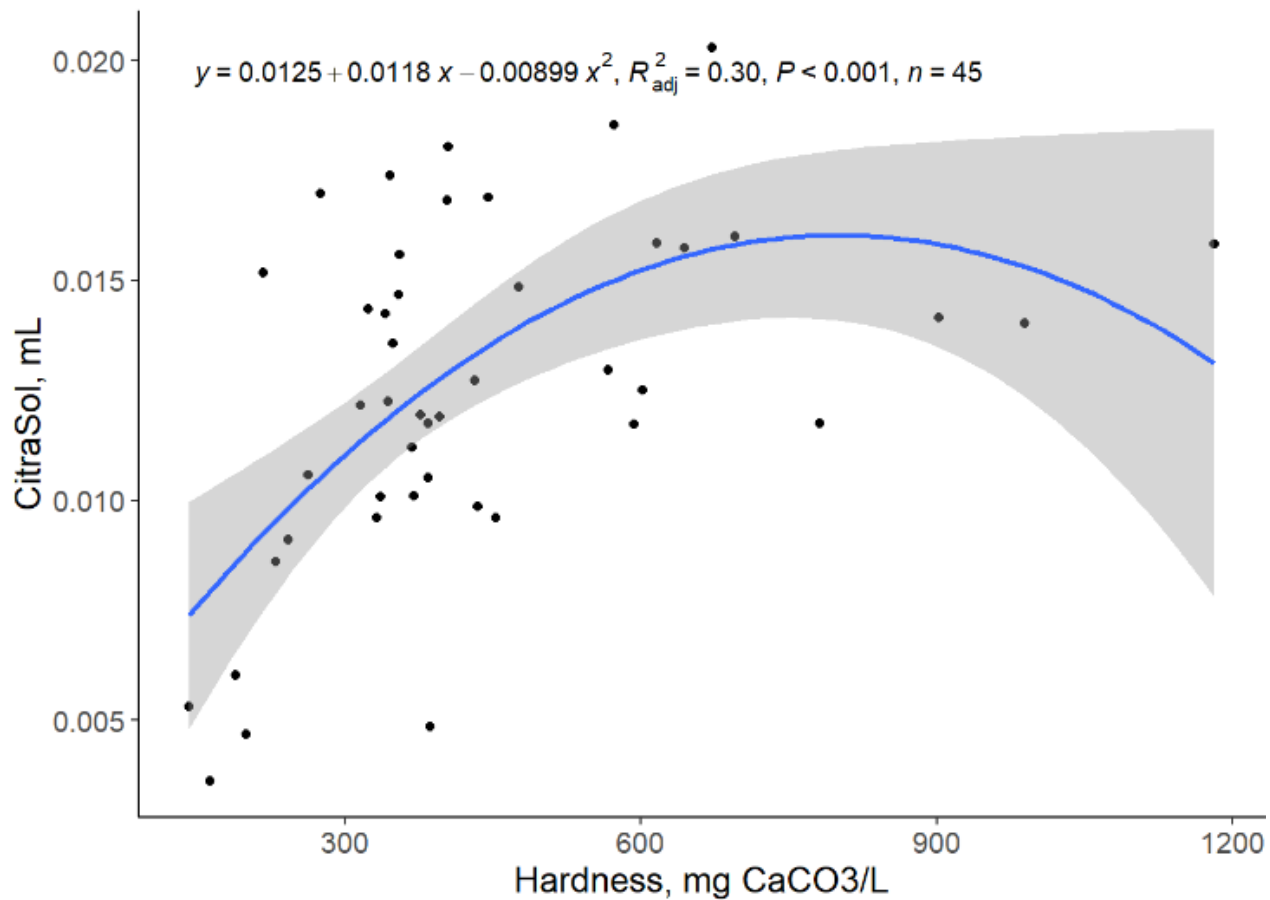


**Figure 2.1.** Relationship between starting water pH and hardness.

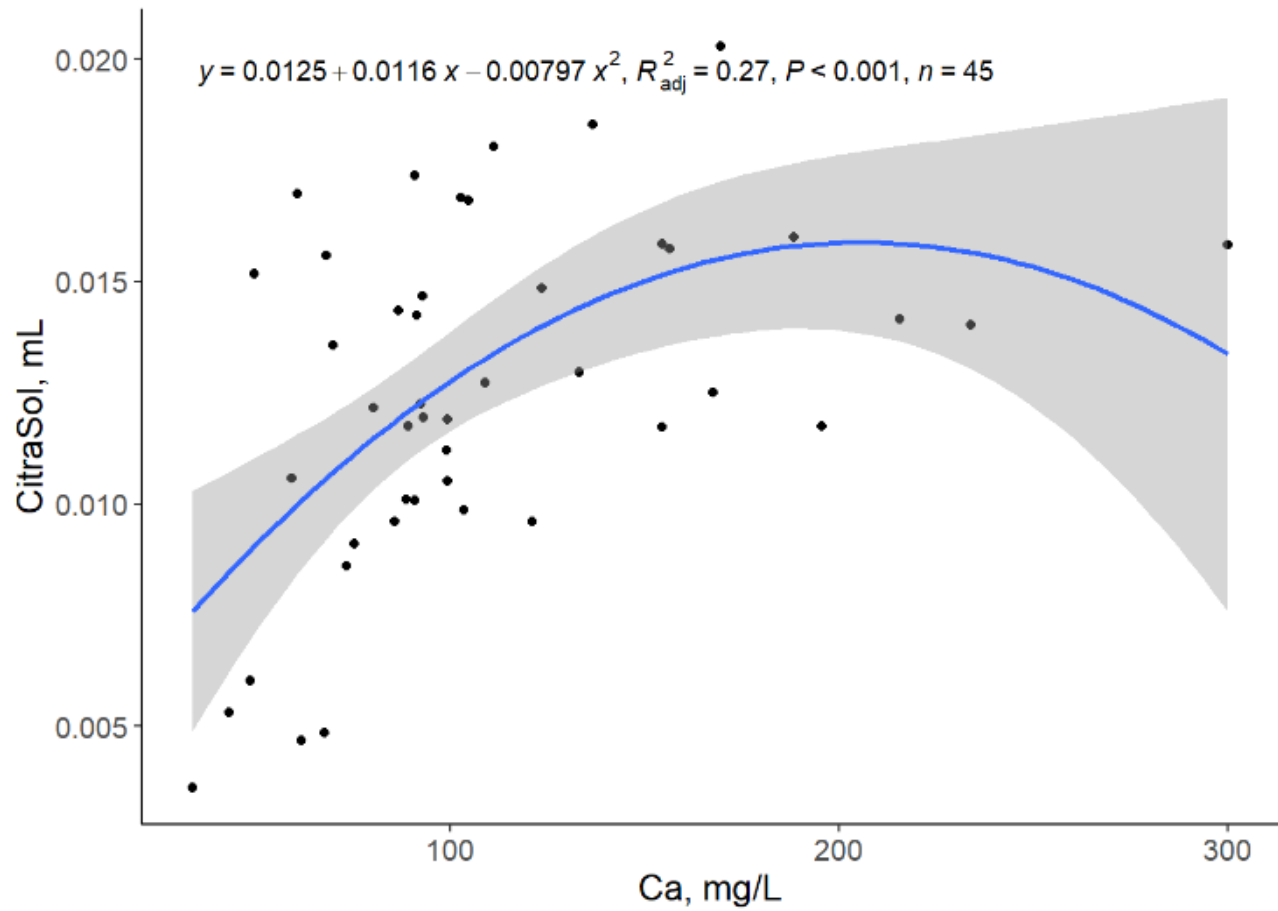


**Figure 2.2.** Relationship between mL of CtiraSol required to reach a pH of 4.0 and water starting pH.

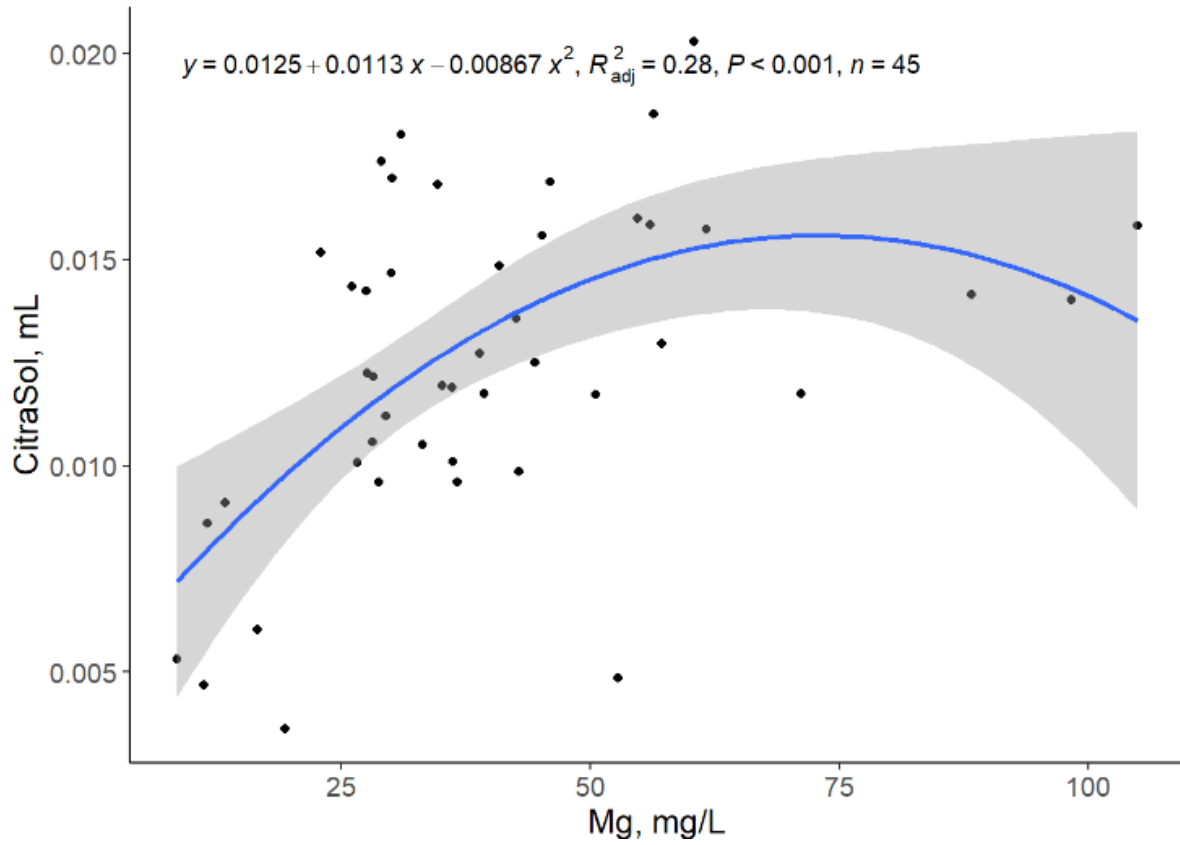




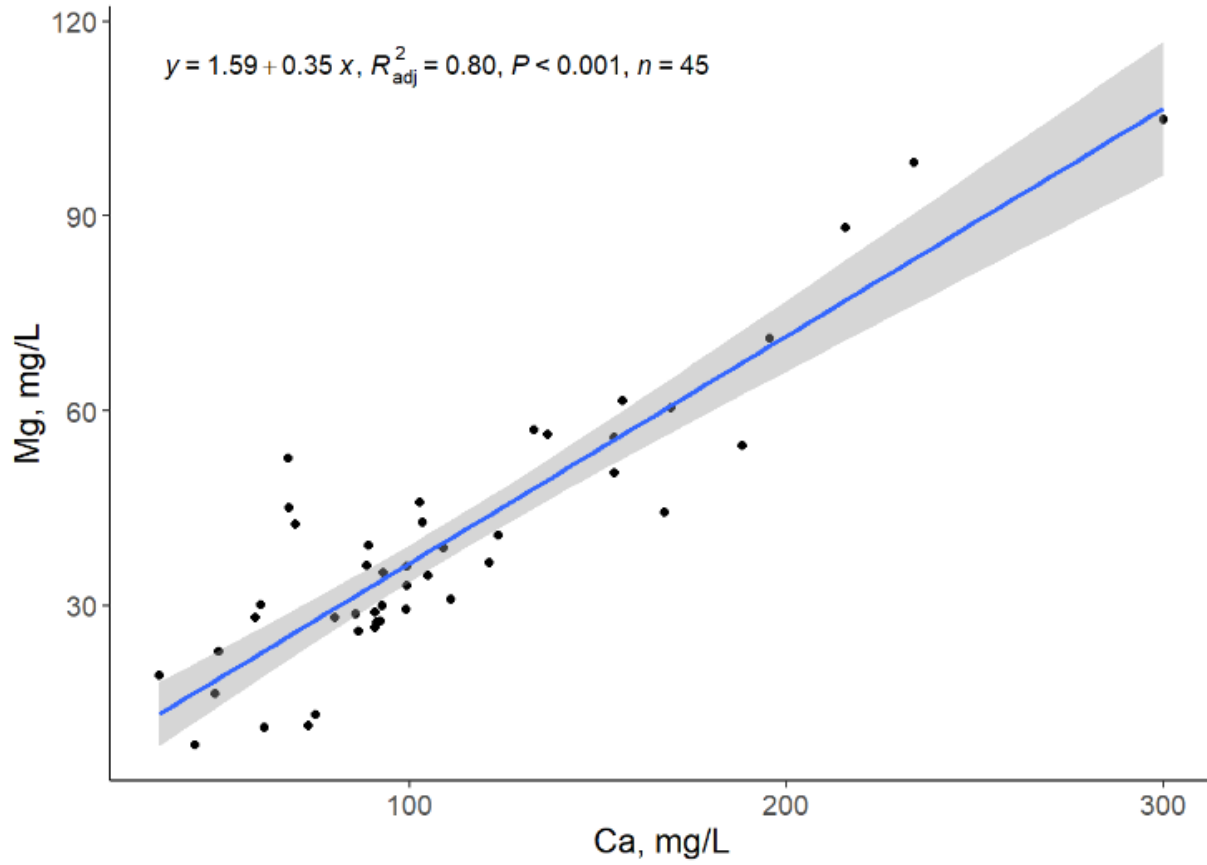
**Figure 2.3.** Relationship between mL of CitraSol required to reach a pH of 4.0 and water hardness.



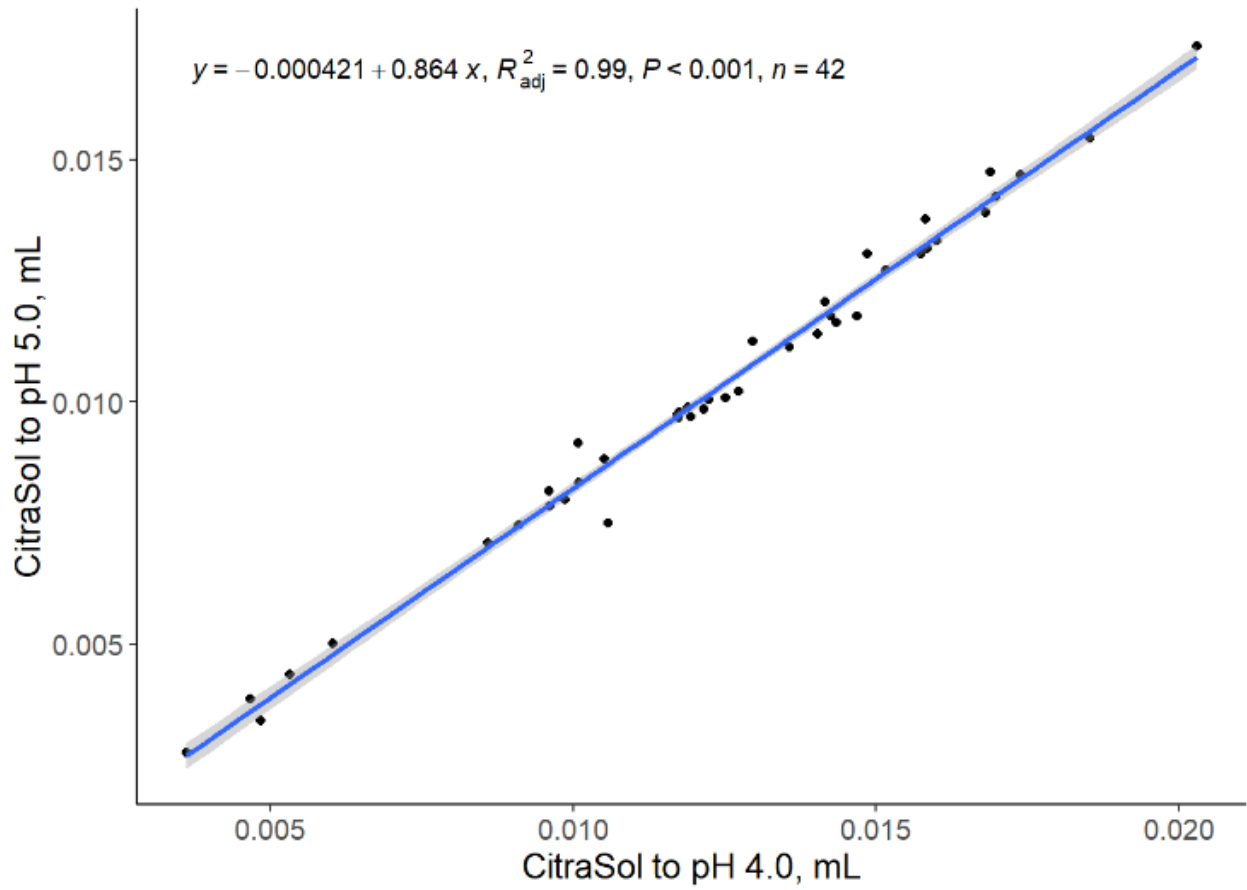
**Figure 2.4.** Relationship between mL of CitraSol required to reach a pH of 4.0 and water Ca concentration.



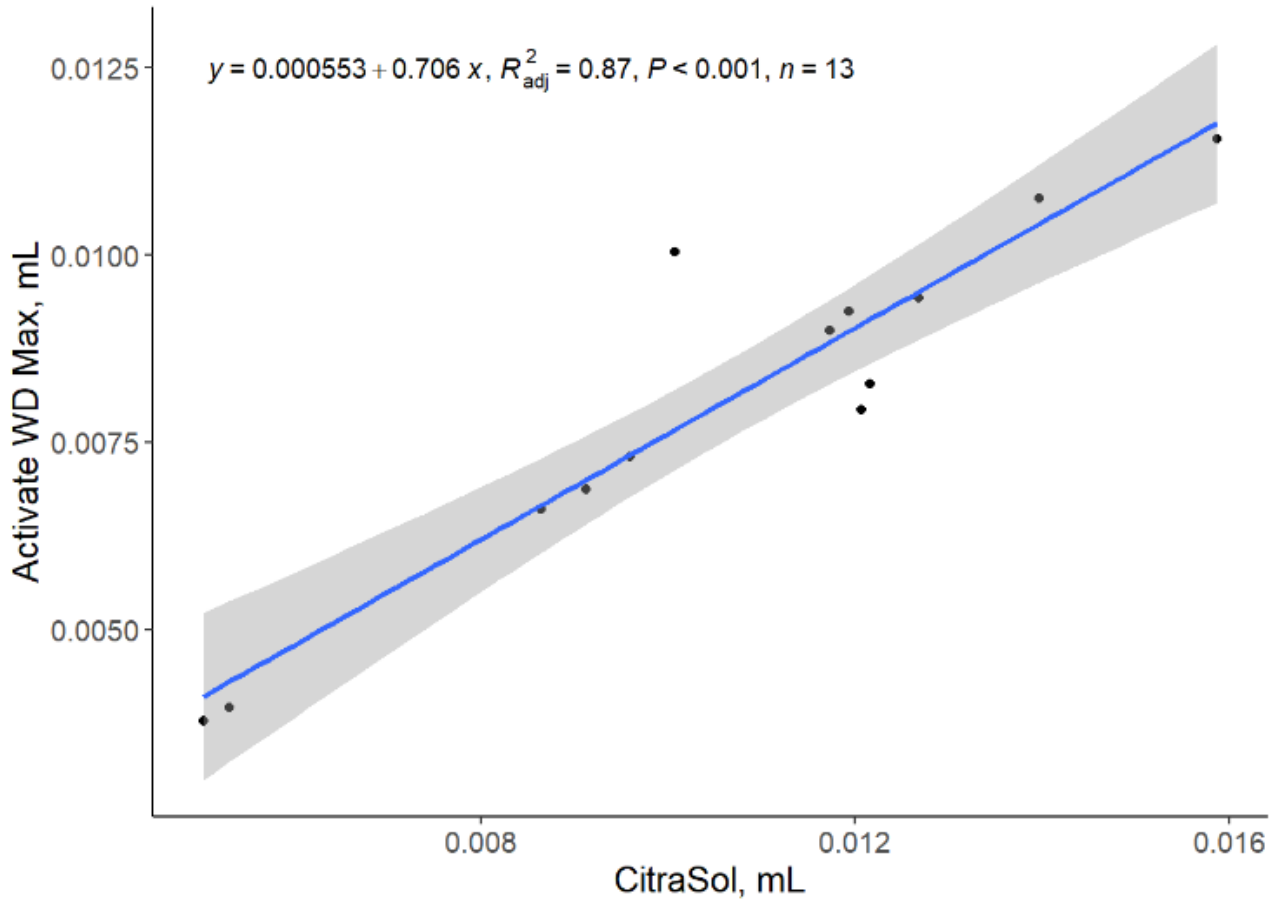
**Figure 2.5.** Relationship between mL of CitraSol required to reach a pH of 4.0 and water Mg concentration.



**Figure 2.6.** Relationship between water Mg and Ca concentrations.



**Figure 2.7.** Relationship between quantity of CitraSol required to reach a pH of 5.0 and 4.0 in water samples.



**Figure 2.8.** Relationship between amount of CitraSol and Activate WD Max required to each a pH of 4.0 in water samples.

# **Chapter 3 - Effect of dietary formulation strategy, water source, and water acidification on growth performance and fecal dry matter of nursery pigs**

## **Abstract**

Low ABC-4 diet formulation strategies have recently gained attention as a possible alternative to feeding growth promotional levels of ZnO to weaned pigs. It is unclear if the response to these diet formulation strategies is dependent on water source or water acidification. Therefore, the objective of this experiment was to determine the effect of dietary formulation strategy, water source, and water acidification on growth performance and fecal dry matter (DM) of weanling pigs. A total of 987 pigs [PIC 800 × (Fast LW × PIC L02), initially  $6.8 \pm 0.39$  kg] were used in a 33-day trial with treatments arranged in a  $2 \times 2 \times 2$  factorial design with main effects of dietary formulation strategy (low ABC-4 vs high ABC-4 with ZnO), water source (rural vs well), and water acidification (with or without). Experimental diets were fed in 2 phases followed by a common phase 3 diet. Diets were formulated to have a dietary ABC-4 value of either 199 or 427 meq/kg in phase 1, and 249 or 441 meq/kg in phase 2. The high ABC-4 diet formulation strategy contained pharmacological levels of Zn as ZnO (3,026 and 2,270 mg/kg Zn in phase 1 and 2, respectively), whereas the low ABC-4 diet had low levels of Zn (110 mg/kg) provided by the trace mineral premix. Water was supplied by either rural or well sources. PerpHect H20 (Acid Products Company Inc., Chicago, IL) was used to acidify both water sources to a targeted pH of 4.0. Overall, (d 0 to 33), a tendency for a 3-way interaction was observed for ADG ( $P = 0.081$ ) where pigs fed the high ABC-4 diet with ZnO had better performance than those fed the low ABC-4 formulation strategy when using acidified or non-

acidified well water or acidified rural water. However, ABC-4 formulation strategy did not result in a significant increase in ADG when pigs were provided non-acidified rural water. For overall ADFI, a 3-way interaction was observed ( $P = 0.034$ ) where pigs fed the high ABC-4 diet with ZnO had better ADFI than those fed the low ABC-4 formulation strategy regardless of water source or water acidification with the greatest magnitude of improvement in the acidified rural water. Pigs that received rural water tended ( $P = 0.072$ ) to have increased fecal DM compared to pigs that received well water. No differences were observed for removals and mortality. Formulating high ABC-4 diets with ZnO resulted in increased ( $P < 0.05$ ) ADG and ADFI compared to low ABC-4 diets without ZnO which we hypothesize was primarily due to the ZnO in the high ABC-4 diets as opposed to the ABC-4 level itself. In conclusion, formulating diets with pharmacological ZnO improved performance compared to low ABC-4 diet formulation strategies with marginal impacts on performance from water source or water acidification.

### **List of Abbreviations**

ABC, acid binding capacity

ABC-4, acid binding capacity-4

ADFI, average daily feed intake

ADG, average daily gain

BW, body weight

DM, dry matter

G:F, gain-to-feed ratio

### **Introduction**

Weaning is stressful and can lead to sub-optimal growth performance and gastrointestinal tract disturbances leading to post-weaning diarrhea (PWD). After weaning, secretion of HCl is



limited, resulting in increased gastric pH, creating the ideal environment for the proliferation of bacteria that cause PWD (Bonetti et al., 2021). To mitigate the reduction in growth rate and prevent PWD, pharmacological levels of Zn from ZnO have been widely used (Shelton et al., 2011; Hutchens et al., 2021; De Mille et al., 2022). However, due to recent environmental and antimicrobial resistance concerns, alternative strategies to pharmacological levels of Zn are needed. One potential alternative is formulating diets to low acid binding capacity-4 (ABC-4) levels. Poor acidification of the stomach can be partially attributed to the utilization of ingredients that are known to bind acid. Lawlor et al. (2005) and Stas et al. (2022) reported acid ABC-4 values of common nursery feed ingredients used in diet formulation. Stas et al. (2023) observed that formulating diets to low ABC-4 values improved pig growth performance and increased fecal dry matter (DM) compared to diets formulated to high ABC-4 values without added ZnO. Additionally, Warner et al. (2022) observed increased ADG and feed efficiency when calcium carbonate concentrations, a high ABC-4 ingredient, was reduced in the diet. Other research has focused on the growth promotional benefits of acidifying the water offered to pigs with some research showing benefits (Walsh et al., 2007; Xu et al., 2022) and others not observing a positive response (Nyachoti et al., 2005; Gentry et al., 2010). To our knowledge, there is no known research that demonstrates if the response to different ABC-4 diet formulation strategies is dependent on the acidity of the water that is also offered to the pig.

Factors including geographic location and water source directly impact the concentration of contaminants within the water source (Patience, 2012). Although not considered a health concern to pigs, Ca and Mg within the water source can cause buildup of scale in water lines, and nipple waterers (Patience, 2013). The additive concentration of free Ca and Mg ions is described as the hardness of the water and has been found to negatively impact the ability of acidifiers to

reduce water pH (Corso et al., 2024). It is well understood that well water sources have increased concentrations of minerals due to well location, design and depth (Patience, 2012) compared to municipal water sources that are supplied in rural areas and often referred to as rural water.

Benefits have been reported when utilizing low ABC-4 diet formulation strategies, however to our knowledge, no research has evaluated the effects of dietary ABC-4 formulation strategy in conjunction with varying water sources, and water acidification. We hypothesized that the impact of acidification in well water may be greater than in rural water and pigs fed diets formulated to low ABC-4 strategies will benefit less from the water acidification. Therefore, the objective of this experiment was to determine the interactive effects of dietary ABC-4 diet formulation strategy, water acidification, and water source on growth performance, and fecal dry matter in weanling pigs.

## **Materials and Methods**

### **General**

The Kansas State University Institutional Animal Care and Use Committee approved the protocol used in this experiment. At weaning, pigs were housed in a temperature-controlled nursery facility located in south-central Minnesota. A total of 48 pens were used with each pen (1.9 × 3.1 m) having plastic grated flooring, one cup waterer, and one 3-hole stainless steel self-feeder. Access to feed and water was provided *ab libitum*. Pigs were allowed approximately 0.27 m<sup>2</sup> per pig. The barn was equipped to utilize either rural or well water simultaneously, with 4 water lines available to use in each pen with two water lines for each source. Daily feed additions to each pen were accomplished using a robotic feeding system (FeedPro; Feedlogic Corp., Wilmar, MN) able to record feed deliveries for individual pens.

### *Animals and diets*

A total of 987 pigs [PIC 800 × (Fast LW × PIC L02), initially  $6.8 \pm 0.39$  kg, approximately 24 d of age] were used in a 33-d trial and were housed in mixed gender pens resulting in 6 pens per treatment. There was a similar number of barrows and gilts in each pen. Pens were blocked by initial weight and randomly assigned to 1 of 8 treatments in a randomized complete block design. Treatments were arranged as a  $2 \times 2 \times 2$  factorial with main effects of dietary ABC-4 formulation strategy (low or high with ZnO), water source (rural or well), and water acidification (with or without). Experimental diets were fed in 2 phases followed by a common phase 3 diet. Diet formulation strategy was achieved by utilizing specialty ingredients and acidifiers to reduce the dietary ABC-4 value in the low diet and using conventional ingredients plus pharmacological levels of ZnO in the high ABC-4 diet. Experimental diets were formulated to have a dietary ABC-4 value of either 199 or 427 meq/kg in phase 1, and 249 or 441 meq/kg in phase 2. The high ABC-4 diets in phase 1 and 2 contained 3,026 and 2,270 mg/kg Zn, respectively, with low ABC-4 diets containing 110 mg/kg of supplemental Zn from the trace mineral premix. Phase 3 was a common corn-soybean meal-based diet with an ABC-4 value of 426 meq/kg. Phase 1 and 2 diets were manufactured at New Vision feed mill in Worthington, MN and the Phase 3 diet was manufactured at the New Fashion Pork feed mill in Round Lake, MN. All diets were formulated to meet or exceed the NRC (2012) requirement estimates for respective weight ranges of nursery pigs (Table 1). Diets were fed in meal form in all phases with phase 1 fed from approximately 6.8 to 7.8 kg, phase 2 from 7.8 to 14.7 kg, and phase 3 from 14.7 to 19.2 kg.

PerpHect H20 (Acid Products Company Inc., Chicago, IL), an inorganic sulfuric acid product was used as the water acidifier and applied via stock solution utilizing a medicator and

delivered at a rate of 1:128 into the water source to reduce water pH to  $4.0 \pm 0.40$ . Initially, 9 oz acid/gal of water was added to the stock solution for the well water treatment, while 6 oz acid/gal of water was added to the stock solution for the rural water treatment to reach the target pH. Four pens per treatment were randomly selected and water pH was monitored throughout the trial and stock solution acid inclusion was adjusted to reach the targeted pH. Water samples from each water source were collected on d 0, 11 and 18 and sent to the Kansas State University Soils Laboratory, Manhattan, KS, to be analyzed for pH, Ca, and Mg concentrations utilizing the ICP-OES procedure (Table 4). To calculate total hardness the equation from Boyd (2015) was used.

Pigs were weighed on d 0, 11, 18, 26 and 33 of the trial and fecal samples were collected from 5 average weight pigs per pen on d 11 and 18 to determine fecal dry matter (DM) percentage. Fecal samples were individually placed in a sterile bag and stored at the Kansas State University Applied Swine Nutrition Laboratory in a  $-20^{\circ}\text{C}$  freezer until the time of analysis. After collection, fecal samples were dried at  $55^{\circ}\text{C}$  in a forced air oven and the ratio of dried to wet fecal weight determined the fecal percentage of DM.

### ***Statistical analysis***

Experimental data were analyzed using R Studio (version 4.1.1 (2021-08-10), R Foundation for Statistical Computing, Vienna, Austria) with pen serving as the experimental unit, treatment considered a fixed effect and weight block considered a random effect for all analysis. Main effects and all 2- and 3-way interactions of dietary ABC-4 formulation strategy, water source, and water acidification were evaluated. Fecal dry matter was analyzed over time as repeated measures. Removals and mortality were analyzed using a binomial distribution, where the denominator was the initial pen inventory, and the numerator was total pigs removed or dead

during trial. All results were considered significant at  $P \leq 0.05$  and marginally significant at  $0.05 \leq P \leq 0.10$ .

## **Results**

### **Water source analysis**

The well water samples had greater concentrations of both Ca and Mg compared to the rural water source at each of the collection time points (Table 2). However, rural water samples had increased pH compared to that of the well water samples. Due to the greater concentrations of both Ca and Mg, hardness of the well water was elevated compared to that of the rural water. Water analysis results remained consistent across all 3 collection time points.

### **Growth performance**

Three-way interactions were observed throughout the study (Table 3). For d 0 to 26 and overall ADFI, a 3-way interaction was observed ( $P < 0.05$ ) where pigs fed the high ABC-4 diet with ZnO had better performance than those fed the low ABC-4 formulation strategy regardless of water source or water acidification with the greatest magnitude of improvement in the acidified rural water. A 3-way interaction was observed ( $P = 0.004$ ) for d 26 BW where the lowest BW was observed for pigs fed diets formulated to the low ABC-4 strategy regardless of water source or acidification. However, for pigs fed diets formulated to the high ABC-4 strategy with ZnO, the best performance was when pigs were offered acidified rural water, with the other pigs fed that formulation strategy not different. A 3-way interaction was observed ( $P = 0.036$ ) for d 33 BW where the lowest BW was observed for pigs fed diets fed the low ABC-4 formulation strategy and well water as well as the low ABC-4 formulation strategy with acidified rural water and the highest BW was observed for pigs fed the diets formulated to the high ABC-4 strategy with ZnO with acidified rural water or non-acidified well water with other treatments intermediate. For the

experimental period from d 0 to 26, a tendency for a 3-way interaction was observed for ADG ( $P = 0.062$ ) where pigs fed the high ABC-4 diet with ZnO and offered acidified water had better performance than all other treatments that were also provided rural water. However, for pigs offered well water, those fed the high ABC-4 diet with ZnO had greater performance than the low ABC-4 formulation strategy when water was acidified, but with no water acidification, diet formulation strategy did not influence daily gain. Overall, (d 0 to 33), a tendency for a 3-way interaction was observed for ADG ( $P = 0.081$ ) where pigs fed the high ABC-4 diet with ZnO had better performance than those fed the low ABC-4 formulation strategy when using acidified or non-acidified well water or acidified rural water. However, ABC-4 formulation strategy did not result in a significant increase in ADG when pigs were provided non-acidified rural water although a similar pattern was observed compared to the before mentioned combinations of water source and water acidification.

During the experimental period (d 0 to 26), a water source by diet formulation strategy interaction was observed where pigs fed the high ABC-4 diet with ZnO had a greater ( $P < 0.05$ ) improvement in ADG and ADFI than those fed the low ABC-4 formulation strategy diets when they were offered rural water compared to well water. An ABC-4 formulation strategy by water acidification interaction was observed where water acidification did not impact ADG or ADFI of the pigs fed the low ABC-4 formulation strategy diets, whereas water acidification improved ADG and ADFI when pigs were fed the high ABC-4 plus ZnO diets. Additionally, feed efficiency was greater ( $P < 0.05$ ) for pigs fed the low ABC-4 diets and no water acidification compared to the high ABC-4 plus ZnO diets without water acidification with the other treatments intermediate. For main effects, high ABC-4 plus ZnO diets had better ( $P < 0.05$ ) ADG and ADFI

compared to low ABC-4 formulation strategy diets with no differences observed based on water source or water acidification.

During period 3 when pigs were fed a common diet (d 26 to 33), a tendency for a water source by water acidification interaction was observed ( $P = 0.099$ ) for G:F but the means did not separate. For main effects, high ABC-4 plus ZnO diets had better ( $P < 0.05$ ) ADG and ADFI compared to low ABC-4 formulation strategy diets with no differences observed based on water source or water acidification.

Overall (d 0 to 33), an ABC-4 formulation strategy by water acidification interaction was observed where water acidification did not impact ADG or ADFI of the pigs fed the low ABC-4 formulation strategy diets, whereas water acidification improved ADG and ADFI when pigs were fed the high ABC-4 plus ZnO diets. For main effects, high ABC-4 plus ZnO diets had better ( $P < 0.05$ ) ADG and ADFI compared to low ABC-4 formulation strategy diets with no differences observed based on water source or water acidification.

Dietary ABC-4 formulation strategy, water source, or water acidification had no impact on removals and mortality during the entire trial. There were no 2- or 3-way interactions ( $P > 0.10$ ) observed for fecal dry matter (DM). However, pigs provided rural water had marginally ( $P = 0.074$ ) greater fecal dry matter compared to pigs that were provided well water.

## **Discussion**

During the period after weaning, stomach acid secretion is low as the amount of lactic acid in the stomach is reduced compared to a suckling pig. Switching from a liquid to grain-based diet may increase gastric pH resulting in higher prevalence of PWD (Cranwell et al., 1968; Yen, 2001; Stas et al., 2022). To mitigate the reduction in growth rate and reduce diarrhea prevalence, Zn in the form of ZnO is commonly supplemented at pharmacological levels in nursery diets (Meyer et

al., 2002; Faccin et al., 2023). Due to recent environmental and antimicrobial resistance concerns, alternatives to ZnO supplementation in the nursery are being explored. Recently, strategies including reducing the acid-binding-capacity of the diet have been explored as a viable alternative to pharmacological levels of ZnO (Stas et al. 2023). Other strategies including water acidification may be an alternative to ZnO supplementation in the nursery, however further research is warranted to validate this potential strategy.

It is understood that large variations in water characteristics can occur between sources. As expected, well water samples collected had a lower pH than rural water samples and contained greater concentrations of acid binding contaminants including both Ca and Mg. Natural contamination of the well water is likely to occur due to well location, depth and design (Patience, 2012). Nyachoti et al. (2005), compared the effect water source (ground vs surface) may have on nursery pig performance and found that water source did not affect ( $P > 0.10$ ) pig performance. Additionally, Lozinski et al. (2022) compared water sources with varying water quality and observed no impact ( $P > 0.10$ ) on overall growth performance of nursery pigs. Similarly, during this trial water source had little impact on nursery pig performance. Although not fully unexpected, we speculate that the elevated levels of buffering material within the well water source may impact gut pH of the newly weaned pig, therefore impacting growth performance. Although we saw no differences in performance, pigs that received rural water tended ( $P = 0.074$ ) to have increased fecal DM compared to those that received the well water, which may reflect reductions in buffering material within the rural water. As anticipated, rural water sources had increased starting pH and reduced concentrations of Ca and Mg compared to that of the well water source. A recent study by Corso et al. (2024) analyzed water samples from



swine facilities to determine pH, Ca and Mg concentrations. They observed reductions in water pH as Ca and Mg concentration became greater, which aligns with the findings from this trial.

To reduce water pH there are many commercially available water acidifiers including organic, inorganic, and acid-blend products. The strength of acids varies based on chemical structure and will impact the efficiency to reduce water pH. PerpHect H<sub>2</sub>O is an inorganic sulfuric acid product that is used to reduce water pH and is a stronger acid compared to organic acids such as commonly used citric acid. Utilizing a stronger acid has the potential benefit of reducing pH at an expediated rate, however palatability and corrosive characteristics of some acids should be considered. As expected, during this trial more acid was required to reduce the pH of the well water compared to the rural water as greater concentrations of both Ca and Mg were observed in the well water samples.

Results from using acidifiers in swine production vary, therefore, the impact water acidification has on nursery pig performance is unclear and may be due to variation in water source, water characteristics or acidifiers used. Xu et al. (2022) observed improvements in growth performance of nursery pigs when adding acids to the drinking water. Likewise, Walsh et al., (2007) observed improvements in ADG and ADFI for pigs that received an organic acid blend in the water source compared to pigs that did not. However, Gentry et al. (2010) observed no differences in ADG of nursery pigs when supplementing organic acids in the water. Similarly, Nyachoti et al., (2005), observed no differences ( $P > 0.10$ ) in performance when acidifying the water source. During our trial, feeding the high ABC-4 diet with ZnO and acidified rural water resulted in numerically the greatest ADG and ADFI during the experimental period. However, when comparing the performance of pigs when fed the low ABC-4 diet with or without water

acidification, there were no differences in performance. Additional research is needed to further investigate the interactive effects of water acidification and diet formulation strategy.

Although results on the impact water acidification has on nursery pig performance are inconsistent, research utilizing low ABC-4 diets has shown improvements in nursery pig performance. Stas et al. (2023) compared dietary ABC-4 levels (low and high) with and without ZnO and concluded that when ZnO is not present in the diet, low ABC-4 diets can improve growth performance, however when ZnO was present, no differences were observed. Throughout the current trial dietary ABC-4 formulation strategy was impactful on pig performance with the high ABC-4 formulation strategy that included ZnO improving growth performance compared to pigs fed the low ABC-4 diet without ZnO. We speculate that this response is likely driven by the inclusion of ZnO. Similarly, Hutchens et al. (2021) , observed an increase ( $P < 0.05$ ) in BW at d 42 for nursery pigs when fed pharmacological levels (3,000 mg/kg vs 110 mg/kg) of Zn from d 0 to 21 compared to pigs fed low levels of ZnO. Consequently, we suggest that the responses observed between low and high ABC-4 diet formulation strategies was driven by the inclusion of ZnO in the high ABC-4 diet as opposed to the ABC-4 level itself.

## **Conclusion**

In summary, the interactions between water acidification, water source, and dietary ABC-4 formulation strategy were impactful on overall growth performance. However, removals and mortality or fecal DM were not affected. Formulating diets to high ABC-4 values when including ZnO at pharmacological levels will improve ADG and ADFI compared to low ABC-4 diets without ZnO. This response was likely because of the presence of the ZnO in the high ABC-4 diets as opposed to the ABC-4 level itself. Therefore, supplementing pharmacological levels of Zn in the nursery remains the more effective option to maximize growth performance of

nursery pigs compared to formulating diets with low ABC-4 ingredients and no ZnO with water acidification or water source having minimal impact.

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**Table 3.1.** Diet composition (as-fed basis)<sup>1</sup>

ABC-4 formulation strategy <sup>2</sup> :	Phase 1		Phase 2		Phase 3	
	Low	High	Low	High	Common <sup>3</sup>	
Item	ZnO:	-	+	-	+	-
Ingredients, %						
Corn		53.61	48.25	47.89	49.20	65.68
Soybean meal		12.82	12.77	22.75	22.75	30.26
Corn DDGS		---	5.00	10.00	10.00	---
Whey powder		---	16.60	---	11.10	---
Whey permeate		15.00	---	10.00	---	---
Specialty soy protein concentrate <sup>4</sup>		10.25	---	4.05	---	---
Fermented soybean meal <sup>5</sup>		---	10.10	---	2.60	---
Spray-dried bovine plasma		2.50	2.50	---	---	---
Choice white grease		2.00	2.00	1.00	1.00	---
Limestone		0.28	0.28	0.43	0.46	0.68
Monocalcium phosphate		0.75	0.35	0.70	0.50	1.00
Salt		0.30	0.35	0.48	0.48	0.60
L-Lys-HCL		0.46	0.43	0.56	0.56	0.50
DL-Met		0.21	0.19	0.22	0.21	0.28
L-Thr		0.21	0.18	0.23	0.23	0.30
L-Trp		0.05	0.05	0.06	0.06	0.05
L-Val		0.10	0.08	0.11	0.13	0.20
Choline chloride		0.04	0.04	---	---	---
Phytase <sup>6</sup>		0.04	0.04	0.04	0.04	---
Fumaric acid		0.50	---	0.50	---	---
Formic acid		0.50	---	0.60	---	---
ZnO <sup>7</sup>		---	0.41	---	0.30	---
Vitamin premix		0.25	0.25	0.25	0.25	0.25
Trace mineral premix		0.15	0.15	0.15	0.15	0.15
Total		100	100	100	100	100
Calculated analysis						
SID amino acids, %						
Lys		1.35	1.35	1.35	1.35	1.25
Ile:Lys		55	57	56	56	55
Met:Lys		36	34	38	37	40
Met and Cys:Lys		58	58	58	58	58
Thr:Lys		64	64	64	64	66
Trp:Lys		21.1	21.5	20.2	20.2	20.0
Val:Lys		70	71	70	70	73
His:Lys		35	35	35	35	33
Total Lys, %		1.50	1.50	1.51	1.50	1.38
NE, kcal/kg		2,616	2,454	2,489	2,463	2,875



SID Lys:NE, g/Mcal	5.16	5.50	5.43	5.48	5.48
CP, % <sup>8</sup>	20.7	20.7	21.5	21.1	19.7
Ca, %	0.47	0.48	0.53	0.55	0.63
P, %	0.53	0.54	0.55	0.55	0.60
STTD P, %	0.45	0.45	0.45	0.45	0.47
Calculated ABC-4, meq/kg	199	427	249	441	426

<sup>1</sup>Phase 1 diets were fed from approximately 6.8 to 7.8 kg, phase 2 from 7.8 kg to 14.7 kg, and phase 3 from 14.7 kg to 19.2 kg.

<sup>2</sup>Low ABC-4 and high ABC-4 diets were formulated to 199 or 427 meq/kg in phase 1 and 249 or 441 meq/kg in phase 2.

<sup>3</sup>Common phase 3 diet contained phytase in the vitamin mineral premix providing 1,250 FTU/kg and an estimated release of 0.13% STTD P. L-Ile and Tribasic copper chloride were also supplemented at 0.03, and 0.03 % of the diet respectively.

<sup>4</sup> AX3 Digest; Proteka; Newport Beach, CA.

<sup>5</sup>Fermex 200; Purina Animal Nutrition; Shoreview, MN.

<sup>6</sup>Optiphos Plus 2500 G (Huevepharma; Sofia, Bulgaria); provided 876 FTU/kg in phases 1 and 2.

<sup>7</sup>High ABC-4 diets contained 3,026 mg/kg or 2,270 mg/kg of supplemental Zn from ZnO and the vitamin trace mineral premix, while the low ABC-4 diet contained Zn only from the vitamin trace mineral premix in phases 1 and 2 respectively.

<sup>8</sup>CP = crude protein

**Table 3.2.** Analyzed pH, Ca, and Mg concentrations of rural and well water sources<sup>1</sup>

	pH	Calcium, mg/L	Magnesium, mg/L	Total Hardness, mg CaCO <sub>3</sub> /L <sup>2</sup>
d 0				
Rural	8.01	116.7	36.0	437.4
Well	7.85	258.7	95.6	1034.8
d 11				
Rural	7.93	116.2	35.6	434.8
Well	7.75	262.2	95.9	1044.9
d 18				
Rural	7.88	115.0	35.2	430.0
Well	7.66	259.9	95.2	1036.3

<sup>1</sup>Total hardness, mg CaCO<sub>3</sub>/L = (Ca, mg/L × 2.479) + (Mg, mg/L × 4.118).

**Table 3.3.** Evaluating the interactive effects of dietary ABC-4 level, water source, and water acidification on growth performance of nursery pigs<sup>1</sup>

	Water Source								SEM	<i>P</i> = 3-way interaction
	Rural				Well					
	No acidifier		Added acidifier <sup>2</sup>		No acidifier		Added acidifier			
High ABC-4 <sup>3</sup>	Low ABC-4	High ABC-4	Low ABC-4	High ABC-4	Low ABC-4	High ABC-4	Low ABC-4			
Body weight, kg										
d 0	6.8	6.8	6.8	6.7	6.8	6.8	6.8	6.8	0.17	0.665
d 11	7.9	7.6	8.4	7.4	7.9	7.5	8.0	7.4	0.39	0.200
d 26	15.1 <sup>b</sup>	14.1 <sup>c</sup>	16.1 <sup>a</sup>	13.7 <sup>c</sup>	15.3 <sup>b</sup>	14.3 <sup>c</sup>	15.2 <sup>b</sup>	14.1 <sup>c</sup>	0.59	0.004
d 33	19.7 <sup>ab</sup>	18.7 <sup>bc</sup>	20.5 <sup>a</sup>	18.0 <sup>c</sup>	19.9 <sup>a</sup>	18.5 <sup>c</sup>	19.8 <sup>ab</sup>	18.4 <sup>c</sup>	0.73	0.036
Period 1 (d 0 to 11)										
ADG, g	94	64	121	43	72	50	98	46	20.0	0.476
ADFI, g	133	109	155	94	119	103	133	100	16.5	0.285
G:F, g/kg	670	543	753	366	553	402	712	382	94.9	0.635
Period 2 (d 11 to 26)										
ADG, g	457 <sup>bcd</sup>	423 <sup>de</sup>	503 <sup>a</sup>	414 <sup>e</sup>	480 <sup>ab</sup>	440 <sup>cde</sup>	466 <sup>bc</sup>	429 <sup>de</sup>	14.9	0.014
ADFI, g	633 <sup>b</sup>	554 <sup>c</sup>	686 <sup>a</sup>	536 <sup>c</sup>	657 <sup>ab</sup>	560 <sup>c</sup>	646 <sup>ab</sup>	564 <sup>c</sup>	22.4	0.006
G:F, g/kg	722	764	734	775	732	788	721	761	11.2	0.559
Experimental period (d 0 to 26)										
ADG, g	293 <sup>bc</sup>	262 <sup>d</sup>	331 <sup>a</sup>	248 <sup>d</sup>	294 <sup>bc</sup>	264 <sup>cd</sup>	301 <sup>b</sup>	254 <sup>d</sup>	13.5	0.062
ADFI, g	407 <sup>b</sup>	354 <sup>c</sup>	447 <sup>a</sup>	336 <sup>c</sup>	411 <sup>b</sup>	353 <sup>c</sup>	415 <sup>ab</sup>	352 <sup>c</sup>	15.2	0.015
G:F, g/kg	718	739	740	734	715	748	723	721	10.5	0.735
Period 3 (d 26 to 33)										
ADG, g	658	654	623	614	644	603	649	615	29.5	0.854
ADFI, g	948	909	954	902	959	895	951	899	27.1	0.639
G:F, g/kg	695	717	653	681	671	674	682	685	22.6	0.927
d 0 to 33										

ADG, g	366 <sup>abc</sup>	341 <sup>cd</sup>	390 <sup>a</sup>	322 <sup>d</sup>	363 <sup>bc</sup>	333 <sup>d</sup>	371 <sup>ab</sup>	326 <sup>d</sup>	13.3	0.081
ADFI, g	515 <sup>b</sup>	466 <sup>c</sup>	549 <sup>a</sup>	451 <sup>c</sup>	520 <sup>ab</sup>	463 <sup>c</sup>	523 <sup>ab</sup>	460 <sup>c</sup>	15.3	0.034
G:F, g/kg	709	730	710	713	698	720	708	708	10.5	0.869
Removals, %	5.2	6.1	6.8	6.8	10.0	7.6	6.9	9.9	2.95	0.400
Mortality, %	0.7	0.0	0.7	0.7	0.7	0.7	0.7	0.7	0.77	1.000
Total Removals, %	6.1	6.3	7.7	7.8	11.0	8.6	7.8	10.9	3.04	0.500
Fecal DM, % <sup>4</sup>										
d 11	19.8	20.8	18.9	19.8	20.1	18.7	17.5	18.6	1.00	0.978
d 18	19.9	20.5	19.3	19.3	19.1	18.1	19.1	19.8	---	---

<sup>a-e</sup>Means within row with different superscripts differ ( $P < 0.05$ ).

<sup>1</sup>A total of 987 pigs [PIC 800 × (Fast LW × PIC L02), initially  $6.8 \pm 0.39$  kg] were used in a 33-day trial with 6 replicates per treatment.

<sup>2</sup>PerpHect H20 (Acid Products Company Inc., Chicago, IL) was added to the water source via a medicator at a rate of 1:128 to reach a target pH of  $4.0 \pm 0.40$ .

<sup>3</sup>High ABC-4 diets were formulated to 427 and 441 meq/kg; low ABC-4 diets were formulated to 199 and 249 meq/kg for phases 1 and 2 respectively. High ABC-4 diets included 3,026 and 2,270 mg /kg of Zn in phases 1 and 2, while the low ABC-4 diet contained 110 mg/kg Zn provided from the trace mineral premix.

<sup>4</sup>The P-values presented in the data table show the effect of water source × dietary ABC-4 formulation strategy × water acidification × day; source,  $P = 0.075$ ; ABC-4 formulation strategy,  $P = 0.616$ ; acidification,  $P = 0.271$ .

**Table 3.4.** Interactive effect of water source and water acidification on nursery pig performance<sup>1</sup>

	Source				SEM	<i>P</i> = Source × Acid
	Rural		Well			
	No acidifier	Added acidifier <sup>2</sup>	No acidifier	Added acidifier		
Body weight, kg						
d 0	6.8	6.8	6.8	6.8	0.17	0.900
d 11	7.8	7.9	7.7	7.7	0.38	0.477
d 26	14.6	14.9	17.8	14.7	0.57	0.068
d 33	19.2	19.2	19.2	19.1	0.71	0.752
Period 1 (d 0 to 11)						
ADG, g	79	82	61	72	19.2	0.486
ADFI, g	121	125	111	116	15.9	0.850
G:F, g/kg	607	560	478	547	84.9	0.179
Period 2 (d 11 to 26)						
ADG, g	440	459	460	447	13.9	0.006
ADFI, g	594	611	609	605	21.1	0.168
G:F, g/kg	743	754	760	741	8.9	0.031
Experimental period (d 0 to 26)						
ADG, g	277	289	279	277	12.7	0.147
ADFI, g	380	392	382	383	14.4	0.338
G:F, g/kg	728	737	731	722	8.7	0.147
Period 3 (d 26 to 33)						
ADG, g	656	618	624	632	25.3	0.137
ADFI, g	928	928	927	925	24.1	0.961
G:F, g/kg	706	667	672	683	17.1	0.099
d 0 to 33						
ADG, g	353	356	348	348	12.7	0.741
ADFI, g	491	500	491	492	14.5	0.359
G:F, g/kg	719	711	709	708	8.1	0.604

Removals, %	5.7	6.8	8.7	8.3	2.11	0.600
Mortality, %	0.0	0.7	0.7	0.7	0.57	1.000
Total Removals, %	6.2	7.7	9.7	9.2	2.16	0.500
Fecal DM, % <sup>3</sup>						
d11	20.3	19.4	19.4	18.1	0.71	0.529
d18	20.2	19.3	18.6	19.5	0.71	---

<sup>1</sup>A total of 987 pigs [PIC 800 × (Fast LW × PIC L02), initially  $6.8 \pm 0.39$  kg] were used in a 33-day trial with 6 replicates per treatment.

<sup>2</sup>PerpHect H20 (Acid Products Company Inc., Chicago, IL) was added to the water source via a medicator at a rate of 1:128 to reach a target pH of  $4.0 \pm 0.40$ .

<sup>3</sup>The P-values presented in the data table show the effect of water source × water acidification × day; source,  $P = 0.075$ ; ABC-4 formulation strategy,  $P = 0.616$ ; acidification,  $P = 0.271$ .

**Table 3.5.** Interactive effect of water source and dietary ABC-4 on nursery pig performance<sup>1</sup>

	Water Source				SEM	<i>P</i> = Source × ABC- 4 formulation
	Rural		Well			
	High ABC- 4 <sup>2</sup>	Low ABC- 4	High ABC- 4	Low ABC- 4		
Body weight, kg						
d 0	6.8	6.8	6.8	6.8	0.17	0.749
d 11	8.1	7.5	7.9	7.5	0.38	0.308
d 26	15.6 <sup>a</sup>	13.9 <sup>b</sup>	15.3 <sup>a</sup>	14.2 <sup>b</sup>	0.57	0.015
d 33	20.1	18.4	19.8	18.5	0.71	0.283
Period 1 (d 0 to 11)						
ADG, g	108	54	85	48	19.2	0.136
ADFI, g	144 <sup>a</sup>	102 <sup>c</sup>	126 <sup>b</sup>	101 <sup>c</sup>	15.9	0.066
G:F, g/kg	712	455	632	392	84.9	0.840
Period 2 (d 11 to 26)						
ADG, g	480 <sup>a</sup>	419 <sup>b</sup>	473 <sup>a</sup>	435 <sup>b</sup>	13.9	0.040
ADFI, g	659 <sup>a</sup>	545 <sup>b</sup>	652 <sup>a</sup>	562 <sup>b</sup>	21.1	0.092
G:F, g/kg	728	769	726	775	8.9	0.624
Experimental period (d 0 to 26)						
ADG, g	312 <sup>a</sup>	255 <sup>b</sup>	297 <sup>a</sup>	259 <sup>b</sup>	12.7	0.043
ADFI, g	427 <sup>a</sup>	345 <sup>b</sup>	413 <sup>a</sup>	352 <sup>b</sup>	14.4	0.048
G:F, g/kg	729	737	719	734	8.7	0.503
Period 3 (d 26 to 33)						
ADG, g	641	634	646	609	25.3	0.320
ADFI, g	951	905	955	897	24.1	0.607
G:F, g/kg	674	699	676	679	17.1	0.471
d 0 to 33						
ADG, g	378	331	367	330	12.7	0.250
ADFI, g	532	459	522	462	14.5	0.172
G:F, g/kg	709	721	730	714	8.1	0.927

Removals, %	6.0	6.5	8.3	8.7	2.10	0.900
Mortality, %	7.1	0.0	7.3	7.2	0.57	1.000
Total Removals, %	6.9	7.0	9.3	9.7	2.14	0.900
Fecal DM, % <sup>3</sup>						
d11	19.4	20.3	18.8	18.7	0.71	0.448
d18	19.6	19.9	19.1	19.0	0.71	---

<sup>a-c</sup> Means within row with different superscripts differ ( $P < 0.05$ ).

<sup>1</sup>A total of 987 pigs [PIC 800 × (Fast LW × PIC L02), initially  $6.8 \pm 0.39$  kg] were used in a 33-day trial with 6 replicates per treatment.

<sup>2</sup>High ABC-4 diets were formulated to 427 and 441 meq/kg; low ABC-4 diets were formulated to 199 and 249 meq/kg for phases 1 and 2 respectively. High ABC-4 diets included 3,026 and 2,270 mg /kg of Zn in phases 1 and 2, while the low ABC-4 diet contained 110 mg/kg Zn provided from the trace mineral premix.

<sup>3</sup>The P-values presented in the data table show the effect of water source × dietary ABC-4 formulation strategy × day; source,  $P = 0.075$ ; ABC-4 formulation strategy,  $P = 0.616$ ; acidification,  $P = 0.271$ .



**Table 3.6.** Interactive effect of dietary ABC-4 and water acidification on nursery pig performance<sup>1</sup>

	Diet ABC-4 Level <sup>2</sup> :		Low		SEM	<i>P</i> = Acid × ABC-4 formulation	
	High	Low	No	Yes			
	Acidification <sup>3</sup> :	No	Yes	No	Yes		
Body weight, kg							
d 0		6.8	6.8	6.8	6.8	0.17	0.765
d 11		7.9 <sup>b</sup>	8.2 <sup>a</sup>	7.6 <sup>c</sup>	7.4 <sup>c</sup>	0.38	0.001
d 26		15.2 <sup>b</sup>	15.7 <sup>a</sup>	14.2 <sup>c</sup>	13.9 <sup>c</sup>	0.57	0.004
d 33		19.8 <sup>a</sup>	20.1 <sup>a</sup>	8.6 <sup>b</sup>	18.2 <sup>b</sup>	0.71	0.044
Period 1 (d 0 to 11)							
ADG, g		83 <sup>b</sup>	110 <sup>a</sup>	57 <sup>c</sup>	45 <sup>c</sup>	19.2	0.001
ADFI, g		126 <sup>b</sup>	144 <sup>a</sup>	106 <sup>c</sup>	97 <sup>c</sup>	15.9	0.005
G:F, g/kg		612 <sup>ab</sup>	733 <sup>a</sup>	473 <sup>bc</sup>	374 <sup>c</sup>	84.9	0.014
Period 2 (d 11 to 26)							
ADG, g		468 <sup>a</sup>	484 <sup>a</sup>	432 <sup>b</sup>	421 <sup>b</sup>	13.9	0.023
ADFI, g		645 <sup>a</sup>	666 <sup>a</sup>	557 <sup>b</sup>	550 <sup>b</sup>	21.1	0.058
G:F, g/kg		727	727	776	768	8.9	0.534
Experimental period (d 0 to 26)							
ADG, g		293 <sup>b</sup>	316 <sup>a</sup>	263 <sup>c</sup>	251 <sup>c</sup>	12.7	0.001
ADFI, g		409 <sup>b</sup>	431 <sup>a</sup>	354 <sup>c</sup>	344 <sup>c</sup>	14.4	0.003
G:F, g/kg		716 <sup>b</sup>	732 <sup>ab</sup>	743 <sup>a</sup>	728 <sup>ab</sup>	8.7	0.012
Period 3 (d 26 to 33)							
ADG, g		651	636	629	614	25.3	0.983
ADFI, g		953	952	902	901	24.1	0.994
G:F, g/kg		683	668	696	683	17.1	0.940
d 0 to 33							
ADG, g		365 <sup>b</sup>	380 <sup>a</sup>	337 <sup>c</sup>	324 <sup>c</sup>	12.7	0.001
ADFI, g		518 <sup>b</sup>	536 <sup>a</sup>	465 <sup>c</sup>	456 <sup>c</sup>	14.5	0.008
G:F, g/kg		704	709	725	710	8.1	0.144
Removals, %		7.3	6.8	8.2	8.2	2.03	0.600
Mortality, %		0.7	0.7	0.0	0.7	0.57	1.000
Total Removals, %		8.3	7.8	7.3	9.2	2.09	0.500
Fecal DM, % <sup>4</sup>							
d11		19.9	18.2	19.8	19.2	0.71	0.391
d18		19.5	19.2	19.3	19.6	0.71	---

<sup>a-c</sup>Means within row with different superscripts differ (*P* < 0.50).

<sup>1</sup>A total of 987 pigs [PIC 800 × (Fast LW × PIC L02), initially 6.8 ± 0.39 kg] were used in a 33-day trial with 6 replicates per treatment.

<sup>2</sup>High ABC-4 diets were formulated to 427 and 441 meq/kg; low ABC-4 diets were formulated to 199 and 249 meq/kg for phases 1 and 2 respectively. High ABC-4 diets included 3,026 and 2,270 mg

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/kg of Zn in phases 1 and 2, while the low ABC-4 diet contained 110 mg/kg Zn provided from the trace mineral premix.

<sup>3</sup> PerpHect H20 (Acid Products Company Inc., Chicago, IL) was added to the water source via a medicator at a rate of 1:128 to reach a target pH of  $4.0 \pm 0.40$ .

<sup>4</sup>The P-values presented in the data table show the effect of dietary ABC-4 formulation strategy  $\times$  water acidification  $\times$  day; source,  $P = 0.075$ ; ABC-4 formulation strategy,  $P = 0.616$ ; acidification,  $P = 0.271$ .

**Table 3.7.** Main effect of dietary acid binding capacity-4 (ABC-4), water source, and water acidification on nursery pig performance and fecal dry matter (DM)<sup>1</sup>

	ABC-4 <sup>2</sup>		SEM	P =	Water source		SEM	P =	Water Acidification <sup>3</sup>		SEM	P =
	High	Low			Rural	Well			No	Yes		
Body weight, kg												
d 0	6.8	6.8	0.16	0.716	6.8	6.8	0.16	0.594	6.8	6.8	0.16	0.960
d 11	8.0 <sup>a</sup>	7.5 <sup>b</sup>	0.38	<0.001	7.8	7.7	0.38	0.100	7.8	7.7	0.38	0.239
d 26	15.4 <sup>a</sup>	14.1 <sup>b</sup>	0.57	<0.001	14.8	14.7	0.57	0.834	14.8	14.7	0.57	0.420
d 33	19.9 <sup>a</sup>	18.4 <sup>b</sup>	0.70	<0.001	19.2	19.1	0.70	0.596	19.2	19.2	0.70	0.954
Period 1 (d 0 to 11)												
ADG, g	96 <sup>a</sup>	51 <sup>b</sup>	18.8	<0.001	81 <sup>a</sup>	67 <sup>b</sup>	18.8	0.016	70	77	18.8	0.221
ADFI, g	135 <sup>a</sup>	101 <sup>b</sup>	15.5	<0.001	123 <sup>a</sup>	114 <sup>b</sup>	15.5	0.049	116	121	15.5	0.313
G:F, g/kg	672 <sup>a</sup>	424 <sup>b</sup>	79.4	<0.001	583	512	79.4	0.103	542	553	79.4	0.793
Period 2 (d 11 to 26)												
ADG, g	476 <sup>a</sup>	427 <sup>b</sup>	13.3	<0.001	449	454	13.3	0.423	450	453	13.3	0.609
ADFI, g	656 <sup>a</sup>	554 <sup>b</sup>	20.5	<0.001	602	607	20.5	0.506	601	608	20.5	0.348
G:F, g/kg	727 <sup>b</sup>	772 <sup>a</sup>	7.4	<0.001	749	751	7.4	0.786	751	748	7.4	0.577
Experimental period (d 0 to 26)												
ADG, g	305 <sup>a</sup>	257 <sup>b</sup>	12.3	<0.001	283	278	12.3	0.264	278	283	12.3	0.277
ADFI, g	420 <sup>a</sup>	349 <sup>b</sup>	13.9	<0.001	286	383	13.9	0.515	381	388	13.9	0.225
G:F, g/kg	724	735	7.7	0.062	733	727	7.7	0.306	730	730	7.7	0.991
Period 3 (d 26 to 33)												
ADG, g	643	622	22.9	0.156	637	628	22.9	0.539	640	625	22.9	0.340
ADFI, g	953 <sup>a</sup>	901 <sup>b</sup>	22.4	<0.001	928	926	22.4	0.854	927	926	22.4	0.939
G:F, g/kg	675	689	13.5	0.352	687	678	13.5	0.560	689	675	13.5	0.349
d 0 to 33												
ADG, g	373 <sup>a</sup>	330 <sup>b</sup>	12.4	<0.001	355	348	12.4	0.110	351	352	12.4	0.733
ADFI, g	527 <sup>a</sup>	460 <sup>b</sup>	14.1	<0.001	496	492	14.1	0.426	491	496	14.1	0.319
G:F, g/kg	706	718	6.6	0.103	715	709	6.6	0.311	714	710	6.6	0.491
Removals, %	7.0	7.5	1.54	0.800	6.2	8.5	1.67	0.200	7.1	7.5	1.54	0.800

Mortality, %	0.7	0.0	1.92	1.000	0.0	0.7	1.91	1.000	0.0	0.7	1.92	1.000
Total Removals, %	8.0	8.2	1.54	0.900	6.9	9.5	1.68	0.100	7.8	8.5	1.56	0.700
Fecal DM, % <sup>4</sup>												
d 11	19.1	19.5	0.50	0.754	19.8	18.7	0.50	0.712	19.9	18.7	0.50	0.255
d 18	9.3	19.4	0.50	---	19.8	19.0	0.50	---	19.4	19.4	0.50	---

<sup>a,b</sup>Means within row with different superscripts differ ( $P < 0.050$ ).

<sup>1</sup>A total of 987 pigs [PIC 800 × (Fast LW × PIC L02), initially  $6.8 \pm 0.39$  kg] were used in a 33-day trial with 6 replicates per treatment.

<sup>2</sup>High ABC-4 diets were formulated to 427 and 441 meq/kg; low ABC-4 diets were formulated to 199 and 249 meq/kg for phases 1 and 2 respectively. High ABC-4 diets included 3,026 and 2,270 mg /kg of Zn in phases 1 and 2, while the low ABC-4 diet contained 110 mg/kg Zn provided from the trace mineral premix.

<sup>3</sup>Acidifier was added to the water source via a medicator at a rate of 1:128 to reach a target pH of  $4.0 \pm 0.40$ .

<sup>4</sup>The  $P$ -values represented in the data table show the effect of the main effect × day; source,  $P = 0.075$ ; ABC-4 formulation strategy,  $P = 0.616$ ; acidification,  $P = 0.271$ .