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**Non Ruminant Nutrition**

# Effect of added calcium carbonate without and with benzoic acid on weanling pig growth performance, fecal dry matter, and blood Ca and P concentrations

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## ABSTRACT

The objective of these studies was to determine the effects of increasing levels of calcium carbonate ($CaCO_3$) with and without benzoic acid on weanling pig growth performance, fecal dry matter (DM), and blood Ca and P concentrations. In experiment 1, 695 pigs (DNA Line 200 × 400, initially 5.9 ± 0.02 kg) were used in a 28 d study. Pigs were weaned at approximately 21 d of age and randomly assigned to pens and then pens were allotted to one of five dietary treatments. Treatment diets were fed from weaning (day 0) to day 14, with a common diet fed from days 14 to 28. Dietary treatments were formulated to provide 0%, 0.45%, 0.90%, 1.35%, and 1.80% added $CaCO_3$ at the expense of ground corn. From days 0 to 14 (treatment period), average daily gain (ADG) and G:F decreased (linear, $P \leq 0.01$) as $CaCO_3$ increased. From days 14 to 28 (common period) and for the overall experiment (days 0 to 28), there was no evidence of differences in growth performance between treatments. For fecal DM, there was a trend (quadratic, $P = 0.091$) where pigs fed with the highest $CaCO_3$ diets had the greatest fecal DM. Experiment 2 used 360 pigs (DNA Line 200 × 400, initially 6.2 ± 0.03 kg) in a 38 d study. Upon arrival to the nursery facility, pigs were randomly assigned to pens and then pens were allotted to one of six dietary treatments. Dietary treatments were fed in three phases with treatment diets fed from days 0 to 10 and days 10 to 24, and a common phase 3 diet fed from days 24 to 38. Dietary treatments were formulated to provide 0.45%, 0.90%, and 1.35% added $CaCO_3$ with or without 0.5% benzoic acid (VevoVitall, DSM Nutritional Products, Parsippany, NJ) added at the expense of ground corn. There was no evidence ($P > 0.05$) for any $CaCO_3$ by benzoic acid interactions. For the experimental period (days 0 to 24), there was a tendency for benzoic acid to increase ADG ($P = 0.056$), average daily feed intake (ADFI; $P = 0.071$), and gain-to-feed ratio (G:F; linear, $P = 0.014$) as $CaCO_3$ decreased. During the common period (days 24 to 38), pigs previously fed benzoic acid had increased ($P = 0.045$) ADG and marginally increased ($P = 0.091$) ADFI. For the overall study, pigs fed benzoic acid had increased ADG ($P = 0.011$) and ADFI ($P = 0.030$), marginally increased G:F ($P = 0.096$) and final body weight ($P = 0.059$). Serum Ca decreased (linear, $P < 0.001$) as $CaCO_3$ decreased in the diet. These data show that decreasing the $CaCO_3$ content in the nursery diet immediately after weaning may improve ADG and G:F. Dietary addition of benzoic acid may also provide beneficial effects on ADG and ADFI, regardless of dietary Ca level.

## LAY SUMMARY

The focus of this research was to determine the impacts of lowering the acid binding capacity (ABC-4) of the diet. The ABC-4 is the amount of acid required to lower the pH of a feed ingredient. Therefore, we investigated low calcium carbonate ($CaCO_3$) additions and the interaction between $CaCO_3$ and benzoic acid when provided in the diets of nursery pigs immediately after weaning. Two experiments were conducted where the first experiment showed that increasing $CaCO_3$ decreased average daily gain (ADG) and gain-to-feed (G:F). In the second experiment, there were no interactions between $CaCO_3$ level and benzoic acid inclusion. However, increasing $CaCO_3$ decreased gain-to-feed, and benzoic acid inclusion increased ADG and feed intake.

**Key words:** acid binding capacity, benzoic acid, calcium, calcium carbonate, growth, nursery pig

**Abbreviations:** ABC-4, acid binding capacity; ADFI, average daily feed intake; ADG, average daily gain; BW, body weight; Ca:P, calcium-to-phosphorus ratio; $CaCO_3$, calcium carbonate; CP, crude protein; DDGS, dried distillers grains with solubles; DM, dry matter; ESBM, enzymatically treated soybean meal; FYT, phytase unit; G:F, gain-to-feed; GIT, gastrointestinal tract; NE, net energy; SID, standardized ileal digestibility; STTD, standard total tract digestibility

## INTRODUCTION

Acid binding capacity (ABC-4) is a feed ingredient's ability to resist a change in gastrointestinal tract (GIT) pH. A high ABC-4 is associated with high pH when the ingredient is suspended within an aqueous solution. A high gastric pH has been observed to result in GIT challenges including increased intestinal bacteria such as *Escherichia coli* (Smith and Jones, 1963) whereas a low stomach pH improves protein digestion (Kil et al., 2011) and intestinal health (Li et al., 2008).

Although Ca is important to maximize bone mineralization (Lagos et al., 2019) and other physiological functions (Crenshaw, 2001), certain Ca containing ingredients have a high ABC-4. Calcium carbonate ($CaCO_3$) is one such ingredient with a relatively high ABC-4 (Jasaitis et al., 1987; Lawlor et al., 2005). Calcium carbonate has an ABC-4 of

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12,932 mEq/kg, whereas other ingredients such as corn, soybean meal, corn DDGS, and dicalcium phosphate have lower ABC-4 (111, 642, 96, and 3,098 mEq/kg, respectively; Lawlor et al., 2005). An improvement in growth performance has been observed with lower dietary ABC-4 (Lawlor et al., 2006).

To help lower gastric pH, dietary acidifiers have been extensively evaluated. Benzoic acid has been observed to decrease *E. coli* in the lower GIT (Guggenbuhl et al., 2007; Diao et al., 2021) and improve growth performance (Guggenbuhl et al., 2007; Chen et al., 2017; Bergstrom et al., 2020; Bradley et al., 2020). Benzoic acid has also been observed to lower the pH of the stomach and lower GIT organs of nursery pigs (Kluge et al., 2006; Halas et al., 2010). Our hypothesis was that short-term reduction in dietary $CaCO_3$ and the addition of benzoic acid would decrease dietary ABC-4 resulting in increased pig performance. Therefore, the objective of this study was to investigate the effects of increasing levels of $CaCO_3$ and the interactive effects of $CaCO_3$ level with or without benzoic acid on the growth performance, fecal dry matter (DM), and blood Ca and P concentrations of nursery pigs.

## MATERIALS AND METHODS

The Kansas State University Institutional Animal Care and Use Committee approved the protocols (4035) used in these experiments. The studies were conducted at the Kansas State University Segregated Early Weaning Facility in Manhattan, KS. The facility has two identical barns that are completely enclosed, environmentally controlled, and mechanically ventilated. Each pen contained a four-hole, dry self-feeder, and a cup waterer to provide ad libitum access to feed and water. Pens (1.22 × 1.22 m) had metal tri-bar floors and allowed approximately 0.30 $m^2$/pig.

### Animals and Diets

*Experiment 1.* A total of 695 castrated male pigs (DNA Line 200 × 400, Columbus, NE; initially 5.9 ± 0.02 kg) were used in two groups in a 28-d study with 5 pigs per pen and 27 or 28 replications (pens) per treatment. There were 350 and 345 pigs in group 1 and 2, respectively. Upon arrival to the nursery research facility, pigs were randomly assigned to pens and then pens were allotted to one of five dietary treatments. Treatment diets were fed from days 0 to 14 and a common phase 2 diet was fed from days 14 to 28. Dietary treatments were formulated to provide 0%, 0.45%, 0.90%, 1.35%, or 1.80% added $CaCO_3$ at the expense of ground corn (Table 1). The calculated dietary Ca levels were 0.49%, 0.66%, 0.84%, 1.01%, and 1.18%, which were below and above the total Ca requirement estimate of 0.85 and 0.80 for 5 to 7 and 7 to 11 kg pigs (NRC, 2012). Standardized total tract P concentration was maintained well above NRC (2012) requirement estimates to account for the high Ca levels decreasing P absorption (Wu et al., 2018). The corresponding dietary ABC-4 values of 424, 481, 539, 597, and 655 mEq/kg, respectively. Treatment diets were fed in meal form for group 1 and pellet form for group 2, with the common phase 2 diet fed in meal form in both groups. At the time of manufacturing for each group, a single base diet was manufactured at Hubbard Feeds in Beloit, KS, packaged in 22.7 kg bags and then transported to Manhattan, KS, where $CaCO_3$ and corn additions were mixed with the base diet to form experimental treatments. In group 2, diets were subsequently pelleted at the O.H. Kruse Feed Technology Innovation Center at Kansas State University, Manhattan, KS.

*Experiment 2.* A total of 360 castrated male pigs (DNA Line 200 × 400, Columbus, NE; initially 6.2 ± 0.03 kg) were used in a 38 d study with 5 pigs per pen and 12 replications (pens) per treatment. Upon arrival to the nursery research facility, pigs were randomly assigned to pens and then pens were allotted to 1 of 6 dietary treatments. Dietary treatments were formulated like experiment 1 (Tables 2 and 3) and fed in three phases with treatment diets fed from days 0 to 10 (phase 1) and days 10 to 24 (phase 2) with a common phase 3 diet fed from days 24 to 38. Dietary treatments were formulated to provide 0.45%, 0.90%, or 1.35% added $CaCO_3$ with or without 0.5% benzoic acid (VevoVitall, DSM Nutritional Products) added at the expense of ground corn. In phase 1, the calculated dietary Ca levels were 0.66%, 0.83%, and 1.01% with corresponding dietary ABC-4 values of 450, 508, and 566 mEq/kg, respectively, without benzoic acid included. In phase 2, the calculated dietary Ca levels were 0.54%, 0.72%, and 0.89% (387, 445, and 502 mEq/kg of ABC-4 without benzoic acid, respectively). The addition of benzoic acid decreased ABC-4 by approximately 30 mEq/kg when added to the phases 1 and 2 diets. Similar to experiment 1, standard total tract digestibility phosphorus (STTD P) was formulated to exceed requirement estimates so not to be limiting because of high Ca concentrations. A single base diet was manufactured at Hubbard Feeds and packaged in 22.7 kg bags and then transported to Manhattan, KS. Calcium carbonate, benzoic acid, and corn additions were then mixed at the O.H. Kruse Feed Technology Innovation Center at Kansas State University. All treatment diets in experiment 2 were fed in meal form.

### Data Collection

Pigs were weighed individually, and feed disappearance was measured for each pen on days 0, 14, and 28 in experiment 1, and on days 0, 10, 24, and 38 in experiment 2 to determine average daily gain (ADG), average daily feed intake (ADFI), and gain-to-feed ratio (G:F). On day 10 of experiment 1 and on day 7 of experiment 2, ~40 g of feces were pooled from 3 randomly selected pigs per pen and then dried at 55 °C for 48 h to determine fecal DM. On day 21 of experiment 2, blood was collected from the jugular region of 1 randomly selected pig per pen (72 pigs; 12 observations per treatment), centrifuged at 4 °C and 1800 × *g* for 30 min, and 1.0 mL serum collected.

### Diet Sampling and Chemical Analysis

In both experiments, complete diet samples of each treatment were taken with a grain probe from every other bag (phase 1) and every third bag (phase 2) upon completion of manufacturing. Samples were combined to obtain a homogenous sample of each diet and were stored at −20 °C until further analysis.

Six subsamples of each diet were collected by using a riffle splitter and ground with a food processor to create a homogeneous sample. After creating these homogenous samples, three subsamples were submitted to the K-State Research and Extension Soil Testing Laboratory in Manhattan, KS, for analysis of Ca (AOAC 985.01, 2006) and P (AOAC 985.01, 2006). In experiment 1, ABC-4 was determined as described by Lawlor et al. (2005). In experiment 2, 0.5 mL of serum

**Table 1.** Experiment 1 phases 1 and 2 diet composition (as fed basis)[1]

| | $CaCO_3$, % | | | | | |
|---|---|---|---|---|---|---|
| Item, % | 0 | 0.45 | 0.90 | 1.35 | 1.80 | Phase 2[3] |
| Ingredient | | | | | | |
| Corn[2] | 44.07 | 43.62 | 43.17 | 42.72 | 42.27 | 48.57 |
| Soybean meal, 46.5% CP | 17.65 | 17.65 | 17.65 | 17.65 | 17.65 | 23.73 |
| Spray-dried whey | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 | – |
| Whey permeate | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 |
| Corn DDGS | 5.00 | 5.00 | 5.00 | 5.00 | 5.00 | 7.50 |
| ESBM[4] | 5.00 | 5.00 | 5.00 | 5.00 | 5.00 | 5.00 |
| Menhaden fish meal | 2.50 | 2.50 | 2.50 | 2.50 | 2.50 | – |
| Spray-dried bovine plasma | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 | – |
| Choice white grease | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Monocalcium P, 21.5% P | 0.80 | 0.80 | 0.80 | 0.80 | 0.80 | 1.00 |
| $CaCO_3$ | – | 0.45 | 0.90 | 1.35 | 1.80 | 0.90 |
| Zinc oxide | 0.40 | 0.40 | 0.40 | 0.40 | 0.40 | 0.25 |
| Sodium chloride | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 | 0.50 |
| Vitamin premix with phytase[5] | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |
| Trace mineral premix | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 |
| L-Lys-HCL | 0.40 | 0.40 | 0.40 | 0.40 | 0.40 | 0.55 |
| DL-Met | 0.19 | 0.19 | 0.19 | 0.19 | 0.19 | 0.22 |
| L-Thr | 0.18 | 0.18 | 0.18 | 0.18 | 0.18 | 0.23 |
| L-Trp | 0.03 | 0.03 | 0.03 | 0.03 | 0.03 | 0.04 |
| L-Val | 0.09 | 0.09 | 0.09 | 0.09 | 0.09 | 0.13 |
| Total | 100 | 100 | 100 | 100 | 100 | 100 |
| Calculated analysis | | | | | | |
| SID amino acids, % | | | | | | |
| Lys | 1.35 | 1.35 | 1.35 | 1.35 | 1.35 | 1.35 |
| Ile:Lys | 56 | 56 | 56 | 56 | 56 | 55 |
| Leu:Lys | 118 | 118 | 118 | 118 | 117 | 116 |
| Met:Lys | 36 | 36 | 36 | 36 | 36 | 37 |
| Met & cys:Lys | 58 | 58 | 58 | 58 | 58 | 57 |
| Thr:Lys | 64 | 64 | 64 | 64 | 64 | 63 |
| Trp:Lys | 19.3 | 19.3 | 19.3 | 19.3 | 19.3 | 19.3 |
| Val:Lys | 70 | 70 | 70 | 70 | 70 | 70 |
| Total Lys, % | 1.51 | 1.51 | 1.51 | 1.52 | 1.52 | 1.51 |
| NE, kcal/kg | 2,558 | 2,545 | 2,534 | 2,520 | 2507 | 2,494 |
| SID Lys:NE, g/Mcal | 6.03 | 6.00 | 5.97 | 5.95 | 5.92 | 6.26 |
| CP, % | 21.5 | 21.5 | 21.4 | 21.4 | 21.4 | 21.4 |
| Ca, % | 0.49 | 0.66 | 0.84 | 1.01 | 1.18 | 0.72 |
| P, % | 0.68 | 0.68 | 0.68 | 0.68 | 0.68 | 0.63 |
| STTD P, % | 0.58 | 0.58 | 0.58 | 0.58 | 0.58 | 0.48 |
| Formulated Ca:P | 0.72 | 0.97 | 1.23 | 1.48 | 1.74 | 1.21 |
| Ca:STTD P | 0.84 | 1.13 | 1.45 | 1.74 | 2.03 | 1.50 |
| ABC-4[6] | 424 | 481 | 539 | 597 | 655 | 475 |
| Chemical analysis[7] | | | | | | |
| DM, % | 91.4 | 91.4 | 91.5 | 91.5 | 91.5 | – |
| CP,% | 19.9 | 19.8 | 19.7 | 20.8 | 20.2 | – |
| Ash, % | 6.1 | 6.5 | 7.0 | 7.5 | 7.9 | – |
| Ca, % | 0.61 | 0.80 | 0.99 | 1.15 | 1.37 | 0.89 |
| P, % | 0.75 | 0.75 | 0.77 | 0.70 | 0.71 | 0.60 |
| ABC-4, mEq/kg[8] | 318 | 347 | 376 | 368 | 409 | – |

[1]Phase 1 experimental diets were fed from 5.9 to 8 kg.
[2]Corn level altered with increasing $CaCO_3$ inclusions.
[3]A common diet was fed following the experimental period.
[4]HP 300, Hamlet Protein, Findlay, Ohio.
[5]Ronozyme HiPhos 2700 (DSM Nutritional Products) provided a 0.13 release of STTD P % for 750 FYT/kg inclusion in the diet.
[6]ABC-4 was calculated based on published ingredient values (Lawlor et al., 2005).
[7]Three representative samples were collected from each treatment diet and two representative samples were collected from the phase 2 diet, ground with a food processor, and submitted for analysis to the Kansas State University Soil Testing Laboratory for Ca and P analysis. ABC-4 analysis was performed as described by Lawlor et al. (2005).
[8]ABC-4 was measured and calculated as the amount of acid in milli-equivalents required to bring 1.0 kg of ground feed to a pH of 4.0.

**Table 2.** Experiment 2 phase 1 diet composition (as-fed basis)[1]

| Benzoic acid[2]: | Without | | | With | | |
|---|---|---|---|---|---|---|
| Ingredient, % CaCO$_3$, %: | 0.45 | 0.90 | 1.35 | 0.45 | 0.90 | 1.35 |
| Corn | 44.82 | 44.30 | 43.79 | 44.33 | 43.84 | 43.36 |
| Soybean meal, 46.5% CP | 17.47 | 17.55 | 17.60 | 17.47 | 17.50 | 17.54 |
| Spray-dried whey | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 |
| Whey permeate | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 |
| Corn DDGS | 5.00 | 5.00 | 5.00 | 5.00 | 5.00 | 5.00 |
| Fermented soybean meal[3] | 4.00 | 4.00 | 4.00 | 4.00 | 4.00 | 4.00 |
| Menhaden fish meal | 2.50 | 2.50 | 2.50 | 2.50 | 2.50 | 2.50 |
| Spray-dried bovine plasma | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 | 2.50 |
| Choice white grease | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Monocalcium P 21.5% P | 0.80 | 0.80 | 0.80 | 0.80 | 0.80 | 1.00 |
| CaCO$_3$ | 0.45 | 0.90 | 1.35 | 0.45 | 0.90 | 1.35 |
| Zinc oxide | 0.40 | 0.40 | 0.40 | 0.40 | 0.40 | 0.40 |
| Sodium chloride | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 |
| Vitamin premix with phytase[4] | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |
| Trace mineral premix | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 |
| L-Lys-HCl | 0.40 | 0.40 | 0.40 | 0.40 | 0.40 | 0.40 |
| DL-Met | 0.19 | 0.19 | 0.19 | 0.19 | 0.19 | 0.19 |
| L-Thr | 0.17 | 0.17 | 0.17 | 0.17 | 0.17 | 0.17 |
| L-Trp | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 |
| L-Val | 0.08 | 0.08 | 0.08 | 0.08 | 0.08 | 0.08 |
| Benzoic acid | – | – | – | 0.50 | 0.50 | 0.50 |
| Total | 100 | 100 | 100 | 100 | 100 | 100 |
| Calculated analysis | | | | | | |
| SID amino acids, % | | | | | | |
| Lys | 1.35 | 1.35 | 1.35 | 1.35 | 1.35 | 1.35 |
| Ile:Lys | 57 | 57 | 57 | 57 | 57 | 57 |
| Leu:Lys | 120 | 120 | 119 | 120 | 119 | 119 |
| Met:Lys | 36 | 36 | 36 | 36 | 36 | 36 |
| Met & cys:Lys | 59 | 59 | 59 | 59 | 59 | 59 |
| Thr:Lys | 64 | 64 | 64 | 64 | 64 | 64 |
| Trp:Lys | 19.2 | 19.2 | 19.2 | 19.2 | 19.2 | 19.2 |
| Val:Lys | 70 | 70 | 70 | 70 | 70 | 70 |
| Total Lys, % | 1.51 | 1.52 | 1.52 | 1.51 | 1.51 | 1.51 |
| NE, kcal/kg | 2,542 | 2,529 | 2,518 | 2,529 | 2,516 | 2,505 |
| SID Lys:NE g/Mcal | 5.87 | 5.91 | 5.94 | 5.90 | 5.93 | 5.96 |
| CP, % | 21.5 | 21.5 | 21.5 | 21.5 | 21.4 | 21.4 |
| Ca, % | 0.66 | 0.83 | 1.00 | 0.66 | 0.83 | 1.00 |
| P, % | 0.66 | 0.66 | 0.66 | 0.66 | 0.66 | 0.66 |
| STTD P, % | 0.58 | 0.58 | 0.58 | 0.58 | 0.58 | 0.58 |
| Formulated Ca:P | 0.99 | 1.26 | 1.52 | 1.00 | 1.26 | 1.53 |
| Ca:STTD P | 1.14 | 1.43 | 1.72 | 1.14 | 1.43 | 1.72 |
| ABC-4, mEq/kg[5] | 450 | 508 | 566 | 420 | 477 | 535 |
| Chemical analysis, %[6] | | | | | | |
| DM, % | 90.5 | 90.7 | 91.0 | 90.8 | 90.9 | 90.4 |
| CP, % | 20.1 | 20.3 | 19.8 | 20.6 | 20.7 | 20.6 |
| Ash, % | 6.8 | 6.9 | 6.9 | 6.6 | 6.9 | 7.1 |
| Ca | 0.77 | 0.90 | 1.04 | 0.74 | 0.89 | 1.05 |
| P | 0.68 | 0.65 | 0.63 | 0.63 | 0.65 | 0.58 |

[1]Phase 1 experimental diets were fed for 10 days.
[2]VevoVitall, DSM Nutritional Products.
[3]ME Pro, Prairie Aquatech, Brookings, SD.
[4]Ronozyme HiPhos 2700 (DSM Nutritional Products) provided a 0.12 release of STTD P % for 1250 FYT/kg inclusion in the diet.
[5](ABC-4 was calculated based on published or estimated ingredient values (Lawlor et al., 2005).
[6]Three representative samples were collected from each treatment diet, ground with a food processor, and submitted for analysis to the Kansas State University Soil Testing Laboratory.

**Table 3.** Experiment 2 phases 2 and 3 diet composition (as-fed basis)[1]

| Benzoic acid[2]: | Without | | | With | | | |
|---|---|---|---|---|---|---|---|
| Ingredient, % CaCO$_3$, %: | 0.45 | 0.90 | 1.35 | 0.45 | 0.90 | 1.35 | Phase 3[3] |
| Corn | 50.16 | 49.67 | 49.19 | 49.62 | 49.13 | 48.65 | 64.71 |
| Soybean meal, 46.5% CP | 23.75 | 23.79 | 23.82 | 23.79 | 23.83 | 23.86 | 31.30 |
| Whey permeate | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 | – |
| Corn DDGS | 7.50 | 7.50 | 7.50 | 7.50 | 7.50 | 7.50 | – |
| Fermented soybean meal[4] | 3.85 | 3.85 | 3.85 | 3.85 | 3.85 | 3.85 | – |
| Choice white grease | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | – |
| Monocalcium P, 21.5% P | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| CaCO$_3$ | 0.45 | 0.90 | 1.35 | 0.45 | 0.90 | 1.35 | 0.85 |
| Zinc oxide | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | – |
| Sodium chloride | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.60 |
| Vitamin premix with phytase[5] | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |
| Trace mineral premix | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 |
| L-Lys-HCL | 0.55 | 0.55 | 0.55 | 0.55 | 0.55 | 0.55 | 0.52 |
| DL-Met | 0.22 | 0.22 | 0.22 | 0.22 | 0.22 | 0.22 | 0.23 |
| L-Thr | 0.22 | 0.22 | 0.22 | 0.22 | 0.22 | 0.22 | 0.22 |
| L-Trp | 0.04 | 0.04 | 0.04 | 0.04 | 0.04 | 0.04 | – |
| L-Val | 0.12 | 0.12 | 0.12 | 0.12 | 0.12 | 0.12 | 0.06 |
| Benzoic acid | – | – | – | 0.50 | 0.50 | 0.50 | – |
| Total | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| Calculated analysis | | | | | | | |
| SID amino acids, % | | | | | | | |
| Lys | 1.35 | 1.35 | 1.35 | 1.35 | 1.35 | 1.35 | 1.35 |
| Ile:Lys | 56 | 56 | 56 | 56 | 56 | 56 | 55 |
| Leu:Lys | 118 | 118 | 118 | 118 | 118 | 118 | 114 |
| Met:Lys | 37 | 37 | 37 | 37 | 37 | 37 | 37 |
| Met & cys:Lys | 58 | 58 | 58 | 58 | 58 | 58 | 58 |
| Thr:Lys | 63 | 63 | 63 | 63 | 63 | 63 | 63 |
| Trp:Lys | 19.3 | 19.3 | 19.3 | 19.3 | 19.3 | 19.3 | 20.3 |
| Val:Lys | 70 | 70 | 70 | 70 | 70 | 70 | 69 |
| Total Lys, % | 1.51 | 1.51 | 1.51 | 1.51 | 1.51 | 1.51 | 1.49 |
| NE, kcal/kg | 2,503 | 2,489 | 2,476 | 2,487 | 2,476 | 2,463 | 2,423 |
| SID Lys:NE, g/Mcal | 6.14 | 6.17 | 6.20 | 6.17 | 6.21 | 6.24 | 5.67 |
| CP, % | 21.5 | 21.4 | 21.4 | 21.4 | 21.4 | 21.4 | 21.2 |
| Ca, % | 0.54 | 0.72 | 0.89 | 0.54 | 0.72 | 0.89 | 0.68 |
| P, % | 0.61 | 0.61 | 0.61 | 0.61 | 0.61 | 0.60 | 0.61 |
| STTD P, % | 0.51 | 0.51 | 0.51 | 0.51 | 0.51 | 0.51 | 0.47 |
| Formulated Ca:P | 0.89 | 1.18 | 1.47 | 0.89 | 1.18 | 1.47 | 1.13 |
| Ca:STTD P | 1.06 | 1.41 | 1.75 | 1.06 | 1.41 | 1.75 | 1.45 |
| ABC—4, mEq/kg[6] | 387 | 445 | 502 | 357 | 415 | 473 | 412 |
| Chemical analysis, %[7] | | | | | | | |
| DM | 89.9 | 90.2 | 90.4 | 90.0 | 89.9 | 90.2 | – |
| CP, % | 19.7 | 18.7 | 19.0 | 18.9 | 20.5 | 19.3 | – |
| Ash, % | 5.2 | 6.2 | 7.2 | 5.4 | 5.8 | 6.0 | – |
| Ca | 0.58 | 0.74 | 1.04 | 0.61 | 0.79 | 1.07 | – |
| P | 0.52 | 0.51 | 0.53 | 0.58 | 0.59 | 0.54 | – |

[1]Phase 2 experimental diets were fed from days 10 to 24.
[2]VevoVitall, DSM Nutritional Products.
[3]Phase 3 common diet was fed from days 24 to 38.
[4]ME Pro, Prairie Aquatech.
[5]Ronozyme 2700 (DSM Nutritional Products) provided a 0.13 release of STTD P % for 1250 FYT/kg inclusion in the diet.
[6]ABC-4 was calculated based on published or estimated ingredient values (Lawlor et al., 2005).
[7]Three representative samples were collected from each treatment diet, ground with a food processor, and submitted for analysis to the Kansas State University Soil Testing Laboratory.

was submitted to the Kansas State University Veterinary Diagnostics Laboratory for analysis of Ca and P (Cobas c 501, Roche Diagnostics).

## Statistical Analysis

In experiment 1, data were analyzed as a randomized complete block design with pen serving as the experimental unit. Treatment was included in the statistical model as a fixed effect, and block was incorporated in the model as a random intercept to account for initial pen average body weight, barn, and group (groups 1 and 2). In experiment 2, data were analyzed as a completely randomized design with pen serving as the experimental unit. Treatment was included in the statistical model as a fixed effect, and barn was incorporated in the model as a random intercept to account for pigs being housed in two identical nursery barns. Data were analyzed using R Studio (Version 3.5.2, R Core Team. Vienna, Austria). Contrasts were used to test for linear and quadratic responses to $CaCO_3$ (experiments 1 and 2) and for the main effects of $CaCO_3$, benzoic acid, and their interaction (experiment 2). Model assumptions including normality were evaluated using visual assessment of studentized residual plots and appeared to be reasonably met. Differences between treatments were considered significant at $P \leq 0.05$ and marginally significant at $0.05 < P \leq 0.10$.

# RESULTS

The analyzed ABC-4 values of the experimental diets in experiment 1 were lower than calculated (Table 1) however increased with increasing $CaCO_3$ in the diet as expected. Crude protein content was relatively similar across dietary treatments as expected and ash content increased with increasing $CaCO_3$ concentrations (Tables 1, 2, and 3).

## Experiment 1

There was no evidence for treatment × group interactions, so data from both groups were combined. From days 0 to 14 (treatment period), ADG (linear, $P = 0.010$) and day 14 BW (linear, $P = 0.006$) decreased as $CaCO_3$ increased (Table 4). Likewise, G:F decreased (linear, $P < 0.001$) as $CaCO_3$ increased with no evidence for difference in ADFI observed. For fecal DM collected on day 10, there was a trend (quadratic, $P = 0.091$) where pigs fed the highest $CaCO_3$ had the greatest DM where other treatments were relatively similar.

From days 14 to 28 (common period) and for the overall experiment (days 0 to 28), there was no evidence for differences in growth performance between treatments.

## Experiment 2

For all response criteria, there was no evidence for $CaCO_3$ × benzoic acid interactions observed (Table 5). From days 0 to 10, pigs fed benzoic acid tended to have increased ($P = 0.092$) ADG and had increased ($P = 0.042$) ADFI (Table 6). From days 10 to 24, pigs fed decreasing $CaCO_3$ had improved (linear, $P = 0.022$) G:F but ADG, ADFI, and day 24 BW were not influenced by dietary treatment. For the experimental period (days 0 to 24), there was a tendency observed for benzoic acid to improve ($P = 0.056$) ADG and ($P = 0.071$) ADFI, and an improvement (linear, $P = 0.014$) was observed in G:F as $CaCO_3$ decreased in the diet.

During the common period (days 24 to 38), pigs previously fed benzoic acid had increased ($P = 0.045$) ADG and

**Table 4.** Effects of increasing $CaCO_3$ on weanling pig growth performance, experiment 1[1]

| | CaCO3, %[2] | | | | | | *P* = | |
|---|---|---|---|---|---|---|---|---|
| Item | 0.00 | 0.45 | 0.90 | 1.35 | 1.80 | SEM | Linear | Quadratic |
| BW, kg | | | | | | | | |
| Day 0 | 5.9 | 5.9 | 5.9 | 5.9 | 5.9 | 0.02 | 0.846 | 0.498 |
| Day 14 | 8.0 | 8.0 | 7.8 | 7.7 | 7.7 | 0.11 | 0.006 | 0.641 |
| Day 28 | 14.7 | 14.9 | 14.8 | 14.5 | 14.6 | 0.23 | 0.397 | 0.612 |
| Experimental period (days 0 to 14) | | | | | | | | |
| ADG, g | 149 | 147 | 136 | 129 | 133 | 7.2 | 0.010 | 0.443 |
| ADFI, g | 171 | 170 | 170 | 164 | 171 | 7.4 | 0.676 | 0.627 |
| G:F, g/kg | 864 | 854 | 805 | 783 | 776 | 18.8 | < 0.001 | 0.585 |
| Common period (days 14 to 28)[3] | | | | | | | | |
| ADG, g | 477 | 489 | 498 | 485 | 494 | 14.9 | 0.331 | 0.470 |
| ADFI, g | 659 | 682 | 678 | 660 | 669 | 15.4 | 0.798 | 0.424 |
| G:F, g/kg | 723 | 725 | 730 | 734 | 735 | 10.4 | 0.182 | 0.914 |
| Overall (days 0 to 28) | | | | | | | | |
| ADG, g | 312 | 314 | 312 | 304 | 311 | 9.7 | 0.569 | 0.954 |
| ADFI, g | 413 | 417 | 418 | 408 | 416 | 11.1 | 0.918 | 0.920 |
| G:F, g/kg | 753 | 751 | 745 | 744 | 745 | 7.1 | 0.246 | 0.736 |
| Fecal DM, %[4] | | | | | | | | |
| Day 10 | 22.5 | 22.1 | 21.9 | 21.5 | 24.4 | 1.1 | 0.303 | 0.091 |

[1]A total of 695 weanling barrow pigs (DNA 200 × 400; initially 5.9 ± 0.02 kg) approximately 21 d of age were used in two 28-d experiments with 5 pigs per pen and 27 or 28 pens per treatment.
[2]Analyzed Ca of the treatment diets were 0.61%, 0.80%, 0.99%, 1.15%, and 1.37%, respectively.
[3]Analyzed Ca of the common phase 2 diet was 0.89%.
[4]Feces from three pigs from each pen were pooled, weighed, and dried to measure fecal DM.

**Table 5.** Interactive effects of $CaCO_3$ with or without benzoic acid on nursery pig growth performance, fecal DM and blood calcium and phosphorus concentration, experiment 2[1]

| Benzoic acid[2]: | Without | | | With | | | | *P* = | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | $CaCO_3$ × benzoic acid | |
| Item CaCO$_3$, %: | 0.45 | 0.90 | 1.35 | 0.45 | 0.90 | 1.35 | SEM | Linear | Quadratic |
| BW, kg | | | | | | | | | |
| Day 0 | 6.2 | 6.2 | 6.2 | 6.2 | 6.2 | 6.2 | 0.03 | 0.948 | 0.636 |
| Day 10 | 7.5 | 7.4 | 7.5 | 7.5 | 7.6 | 7.6 | 0.12 | 0.451 | 0.534 |
| Day 24 | 13.2 | 13.1 | 12.9 | 13.3 | 13.2 | 13.4 | 0.31 | 0.535 | 0.784 |
| Day 38 | 21.0 | 20.8 | 20.8 | 21.5 | 21.3 | 21.5 | 0.46 | 0.847 | 0.871 |
| Phase 1 period (days 0 to 10) | | | | | | | | | |
| ADG, g | 130 | 117 | 128 | 132 | 140 | 146 | 13.9 | 0.451 | 0.471 |
| ADFI, g | 137 | 132 | 144 | 147 | 146 | 160 | 10.4 | 0.715 | 0.878 |
| G:F, g/kg | 940 | 846 | 855 | 891 | 954 | 913 | 57.4 | 0.241 | 0.188 |
| Phase 2 period (days 10 to 24) | | | | | | | | | |
| ADG, g | 401 | 399 | 385 | 416 | 402 | 410 | 25.1 | 0.677 | 0.446 |
| ADFI, g | 526 | 530 | 517 | 538 | 526 | 563 | 23.4 | 0.292 | 0.259 |
| G:F, g/kg | 762 | 753 | 743 | 773 | 763 | 729 | 20.4 | 0.359 | 0.657 |
| Experimental period (days 0 to 24) | | | | | | | | | |
| ADG, g | 287 | 274 | 276 | 297 | 291 | 295 | 12.4 | 0.655 | 0.936 |
| ADFI, g | 362 | 355 | 359 | 375 | 365 | 387 | 13.2 | 0.516 | 0.602 |
| G:F, g/kg | 791 | 771 | 768 | 793 | 797 | 763 | 11.2 | 0.751 | 0.129 |
| Common period (days 24 to 38) | | | | | | | | | |
| ADG, g | 553 | 554 | 562 | 584 | 580 | 581 | 15.9 | 0.693 | 0.966 |
| ADFI, g | 829 | 829 | 827 | 851 | 840 | 859 | 16.7 | 0.766 | 0.561 |
| G:F, g/kg | 666 | 669 | 679 | 686 | 689 | 676 | 10.4 | 0.301 | 0.513 |
| Overall (days 0 to 38) | | | | | | | | | |
| ADG, g | 384 | 373 | 379 | 403 | 396 | 396 | 11.7 | 0.904 | 0.736 |
| ADFI, g | 531 | 522 | 527 | 550 | 539 | 554 | 13.1 | 0.736 | 0.775 |
| G:F, g/kg | 721 | 714 | 718 | 732 | 736 | 715 | 7.6 | 0.281 | 0.160 |
| Fecal DM, %[3] | | | | | | | | | |
| Day 7 | 27.5 | 30.4 | 29.8 | 28.1 | 27.3 | 28.3 | 1.31 | 0.303 | 0.159 |
| Serum[4] | | | | | | | | | |
| Ca, mg/Dl | 10.7 | 11.2 | 11.6 | 11.0 | 11.3 | 11.6 | 0.21 | 0.355 | 0.942 |
| P, mg/dL | 11.0 | 10.6 | 11.0 | 10.9 | 11.3 | 10.8 | 0.32 | 0.897 | 0.156 |

[1]A total of 360 weanling barrows (DNA 200 × 400, initially 6.2 ± 0.03 kg) approximately 21 d of age were used in a 38-d experiment with 5 pigs per pen and 12 pens per treatment.
[2]DSM Nutritional Products.
[3]Feces from three pigs from each pen were pooled, weighed, and dried to measure fecal DM.
[4]Blood was collected from 1 pig per pen on day 21 and submitted to the Kansas State University Veterinary Diagnostic Lab for Ca and P analysis.

marginally increased ($P = 0.091$) ADFI. For the overall study, pigs fed with benzoic acid had increased ADG ($P = 0.011$) and ADFI ($P = 0.030$) and marginally improved G:F ($P = 0.096$) and final BW ($P = 0.059$); however, no overall impact of $CaCO_3$ level was observed.

For fecal DM on day 7, there was no evidence for differences among treatments. For serum analysis on day 21, serum Ca increased (linear, $P < 0.001$) as the level of $CaCO_3$ in the diet increased, while no difference in serum P was observed.

## DISCUSSION

Calcium (Ca) is one of the most abundant macrominerals required by the pig and 0.8% of the animals' body is Ca (Hendriks and Moughan, 1993). Of that 0.8%, 96% to 99% is present in bone tissue in the form of hydroxyapatite. Calcium is therefore essential for bone formation and mineralization. In the present study, a reduction of calcium carbonate was used to decrease the ABC-4 of the diet and as a consequence, the total dietary Ca percentage decreased. However, the analyzed Ca values were higher than the calculated values and revealed that the pigs fed the diets at or below 0.45% $CaCO_3$ in both experiments and the 0.90% $CaCO_3$ in experiment 2 were below the NRC (2012) requirement estimates of 0.85% and 0.80% for 5 to 7 kg and 7 to 11 kg, respectively. However, if providing a 0.10% Ca release value for phytase inclusion, only the diet with no $CaCO_3$ used in experiment 1 was Ca deficient. It can be assumed that difference in analyzed Ca is due to a discrepancy in a Ca concentration of one or more ingredients used in the experiments and/or analytical error. As well, analyzed ABC-4 values were

**Table 6.** Main effects of $CaCO_3$ and benzoic acid on nursery pig growth performance, fecal DM and blood calcium and phosphorus concentrations, experiment 2[1]

| | $CaCO_3$, %: | | | | P = | | Benzoic acid[2]: | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Item | 0.45 | 0.90 | 1.35 | SEM | Linear | Quadratic | Without | With | SEM | *P* = |
| BW, kg | | | | | | | | | | |
| Day 0 | 6.2 | 6.2 | 6.2 | 0.03 | 0.793 | 0.283 | 6.2 | 6.2 | 0.03 | 0.257 |
| Day 10 | 7.5 | 7.5 | 7.6 | 0.10 | 0.598 | 0.403 | 7.4 | 7.6 | 0.10 | 0.156 |
| Day 24 | 13.3 | 13.1 | 13.2 | 0.26 | 0.620 | 0.665 | 13.1 | 13.3 | 0.23 | 0.294 |
| Day 38 | 21.2 | 21.1 | 21.2 | 0.38 | 0.821 | 0.678 | 20.9 | 21.4 | 0.35 | 0.059 |
| Phase 1 period (days 0 to 10) | | | | | | | | | | |
| ADG, g | 131 | 129 | 137 | 12.0 | 0.550 | 0.529 | 125 | 139 | 11.2 | 0.092 |
| ADFI, g | 142 | 139 | 152 | 8.8 | 0.228 | 0.245 | 138 | 151 | 8.2 | 0.042 |
| G:F, g/kg | 915 | 900 | 884 | 47.9 | 0.485 | 0.990 | 880 | 919 | 44.3 | 0.293 |
| Phase 2 period (days 10 to 24) | | | | | | | | | | |
| ADG, g | 408 | 400 | 397 | 23.4 | 0.397 | 0.832 | 395 | 409 | 22.8 | 0.188 |
| ADFI, g | 532 | 528 | 540 | 20.4 | 0.595 | 0.566 | 524 | 542 | 19.4 | 0.174 |
| G:F, g/kg | 768 | 758 | 736 | 18.1 | 0.022 | 0.611 | 752 | 755 | 17.2 | 0.799 |
| Experimental period (days 0 to 24) | | | | | | | | | | |
| ADG, g | 292 | 282 | 286 | 10.3 | 0.519 | 0.451 | 279 | 295 | 9.4 | 0.056 |
| ADFI, g | 368 | 360 | 373 | 10.5 | 0.690 | 0.288 | 358 | 376 | 9.4 | 0.071 |
| G:F, g/kg | 792 | 784 | 766 | 8.5 | 0.014 | 0.595 | 777 | 784 | 7.3 | 0.374 |
| Common period (days 24 to 38) | | | | | | | | | | |
| ADG, g | 569 | 567 | 572 | 11.7 | 0.846 | 0.839 | 557 | 582 | 10.0 | 0.045 |
| ADFI, g | 840 | 835 | 843 | 12.6 | 0.849 | 0.607 | 828 | 850 | 10.9 | 0.091 |
| G:F, g/kg | 676 | 679 | 678 | 7.3 | 0.883 | 0.851 | 672 | 684 | 6.0 | 0.159 |
| Overall (days 0 to 38) | | | | | | | | | | |
| ADG, g | 393 | 384 | 387 | 9.5 | 0.531 | 0.485 | 378 | 398 | 8.7 | 0.011 |
| ADFI, g | 541 | 530 | 541 | 10.3 | 0.982 | 0.288 | 527 | 548 | 9.1 | 0.030 |
| G:F, g/kg | 726 | 725 | 716 | 5.8 | 0.163 | 0.550 | 718 | 727 | 5.1 | 0.096 |
| Fecal DM, %[3] | | | | | | | | | | |
| Day 7 | 27.8 | 28.8 | 29.0 | 1.09 | 0.249 | 0.657 | 29.2 | 27.9 | 1.00 | 0.126 |
| Serum [4] | | | | | | | | | | |
| Ca, mg/dL | 10.8 | 11.3 | 11.6 | 0.15 | < 0.01 | 0.808 | 11.2 | 11.3 | 0.13 | 0.470 |
| P, mg/dL | 10.9 | 11.0 | 10.9 | 0.23 | 0.959 | 0.941 | 10.9 | 11.0 | 0.19 | 0.591 |

[1]A total of 360 weanling barrows (DNA 200 × 400, initially 6.2 ± 0.03 kg) approximately 21 d of age were used in a 38-d experiment with 5 pigs per pen and 24 pens per $CaCO_3$ treatment and 36 pens per benzoic acid treatment. There was no $CaCO_3$ × benzoic acid interactions observed ($P > 0.10$).
[2]DSM Nutritional Products.
[3]Feces from three pigs from each pen were pooled, weighed, and dried to measure fecal DM.
[4]Blood was collected from 1 pig per pen on day 21 and submitted to the Kansas State University Veterinary Diagnostic Lab for Ca and P analysis.

lower than the calculated values and could be attributed to variation in the ingredient values compared to those provided from Lawlor et al. (2005).

In the present study the Ca:P ratio increased as the concentration of $CaCO_3$ increased. The Ca:P ratio appears to be more critical to growth performance and bone mineralization at low P levels (Wu et al., 2018). Qian et al. (1996) observed decreased ADG when Ca:P increased from 1.2:1 to 2:1. This response was greater when diets were fed with deficient P levels. To avoid this concern, dietary STTD P in the present experiments were at least 0.58%, which is much greater than NRC (2012) requirement estimates of 0.45% and 0.40% for pigs weighing 5 to 7 and 7 to 11 kg, respectively. Wu et al. (2018) observed interactive effects of dietary Ca and P in the first 24 d of the nursery period. They observed increasing Ca by increasing $CaCO_3$ significantly reduced G:F in diets fed at the NRC (2012) requirement estimate (0.45% STTD P) for 5 to 7 kg pigs, but no difference compared to the diets that were fed above the requirement estimate (0.56% STTD P). These data altogether show that pigs fed with adequate dietary P can maintain performance when fed with a wide range of Ca levels. Thus, improvements in performance when nursery pigs were fed lower Ca levels may be a result of reduced diet ABC-4.

The use of ABC-4 has been evaluated more extensively in European nursery diets (Blank et al., 1999; Mroz et al., 2000; Lawlor et al., 2006) compared to US-based formulations. Lawlor et al. (2005) conducted acid/base titrations to develop ABC-4 ingredient values that were used in diet formulation in the present study. With the purpose of evaluating ABC-4 diet values on growth performance, Lawlor et al. (2006) fed different Ca and P levels with and without acidifiers. They observed that low Ca (0.28%) and P (0.51%) concentrations for the first 7 d postweaning improved ADFI and tended to improve feed conversion through the first 14 d. The improvement in feed conversion they found agrees with the present experiments. The change in Ca investigated by Lawlor et al. (2006) was completed by removing limestone flour (1.2%) and dicalcium phosphate (1.0%), ultimately lowering ABC-4 values from 315 to 207 mEq/kg. In a separate experiment, Lawlor et al. (2006) observed no growth response to diet Ca level when feeding limestone flour from 0.03% to 1.19% (340 to 500 mEq/kg ABC-4) of the diet while maintaining constant total P. Finally, Lawlor et al. (2006) also examined keeping limestone flour and dicalcium phosphate constant and included 2.0% formic acid, which lowered the ABC-4 from 315 to 180 mEq/kg. They reported a mineral level × acid interaction with increased ADFI when pigs were fed formic acid with adequate (NRC, 1998) Ca and P in the first 7 d, but no difference when formic acid was fed with low Ca and P levels. This interaction is in contrast with the present study, where no interaction was found between acid and Ca level, which could be attributed to the type or level of acidifier used or other ingredient combinations that made up the experimental diets.

Given the NRC (2012) requirement estimates of 0.85% and 0.80% Ca for pigs weighing 5 to 7 kg and 7 to 11 kg, respectively, the pigs in the present studies fed the low $CaCO_3$ levels were deficient in Ca on a calculated basis (≤ 0.66% and 0.72% in experiments 1 and 2, respectively). However, diet Ca values from chemical analysis revealed that only the 0% added $CaCO_3$ diets were below while the 0.45% were only slightly below the NRC (2012) requirement estimates. Also, all diets contained added phytase which is shown to increase Ca availability. These calculations were not considered in the present studies, and therefore, even the diet without $CaCO_3$ would most likely been at or very close to the pigs Ca requirement.

The concern of feeding below the pig's Ca requirement for an extended period is that bone mineralization would likely be limited. This is supported by Lagos et al. (2021) who fed weaned pigs' diets with 50% (NRC, 2012) estimated Ca and P levels for 16 d. These authors reported decreased bone mineralization at day 15 with no differences in the growth performance. While we did not measure bone mineralization directly, in experiment 2 there were statistical differences for serum Ca with serum Ca percentage decreasing as the level of dietary $CaCO_3$ decreased. However, the serum Ca levels for all treatments were within normal biological ranges 9 to 13 mg/dL (Puls, 1994). The response to serum Ca being reflective of diet Ca concentration agrees with previous studies (Nielsen et al., 1971; Mahan, 1982; Hall et al., 1991; Lagos et al., 2019). We hypothesize that the short-term reduction in Ca concentrations by removing $CaCO_3$ might only marginally affect bone mineralization, but the benefits of low $CaCO_3$ on growth performance by either decreasing ABC-4, or a response to less mineral in the diet on net energy (NE) concentration would out-weigh this potential transitory effect on bone mineralization. However, further research on the impacts of bone mineral content should be assessed to confirm our hypothesis.

The use of acidifiers in nursery diets and the proposed mechanism of action has been extensively researched and reviewed (Kim et al., 2005; Kil et al., 2011; Suiryanrayna and Ramana, 2015). Acidifiers added to feeds are used to decrease stomach and lower GIT pH, improve nutrient digestibility, and decrease pathogen proliferation. A low stomach pH is required for adequate protein digestion because pepsinogen is more rapidly cleaved to produce pepsin at a pH 2.5 to 3.0 (Herriott, 1938). Pepsin is the primary proteolytic enzyme of the stomach. Although it was not the objective of this study to measure gastric pH, numerical changes in stomach pH with different acidifiers has been researched (Kil et al., 2011) where it was reported that statistical differences of stomach pH are variable due to other dietary ingredient additions with varying ABC-4 estimates. Certain ingredients with high ABC-4 may interact with acidifiers to lessen their response. Although we did not see an interaction between calcium carbonate and benzoic acid in the present study, an interaction with other ingredients or a combination of ingredients that have relatively high ABC-4 values may exist, but this would require further investigation.

In the present study, it was hypothesized that an acidifier would further decrease the ABC-4 of the diet and potentially provide additional benefit regardless of $CaCO_3$ level in the diet. Therefore, benzoic acid was used due to previous research evaluating early nursery diets where improvements in growth performance and nutrient utilization have been shown (Guggenbuhl et al., 2007; Chen et al., 2017; Diao et al., 2021). Guggenbuhl et al. (2007) compared feeding 0.50% benzoic acid to a control diet and observed increased total digestibility of nitrogen, energy, and apparent ileal digestibility of Lys and Thr. Although it was not measured, the improvement of ADG and ADFI when benzoic acid was fed in the present study might be attributed to an increase in nutrient digestibility. The inclusion of benzoic acid in the present study decreased the calculated ABC-4 by 30 mEq/kg. It is unclear whether the magnitude was sufficient to illicit a statistical growth response that could be attributed to lower ABC-4.

In our diet formulation, $CaCO_3$ and benzoic acid were added at the expense of ground corn, and as a result the dietary energy decreased in these diets. Decreasing the dietary NE by 1% has been shown to decrease G:F by 1% (Nitikanchana et al., 2015). It was therefore expected in the present study that we could observe reductions in G:F as $CaCO_3$ increased in the diet. However, in experiment 1 increasing $CaCO_3$ from 0% to 1.80% reduced diet NE by 2%, while we observed a 10% reduction in G:F when the experimental diets were fed. Similarly, in experiment 2, increasing $CaCO_3$ reduced NE by 0.9% and 1% in phases 1 and 2, respectively, and yet we observed a 3% reduction in G:F when treatment diets were fed. From these observations, we can conclude that the change in G:F may not be solely attributed to dietary NE.

In summary, increasing $CaCO_3$ in the early nursery diets with adequate dietary P decreased BW, ADG, and G:F. This response is potentially due to the increase in the ABC-4 of the diet or change in NE concentration. The use of benzoic acid showed an improvement in ADG and ADFI and marginal improvement in G:F, and the response was independent of the dietary $CaCO_3$. While not unexpected, serum Ca level decreased with reduced diet Ca levels. Additional research needs to be conducted to determine if the results were a direct result of changes in ABC-4 from altering the diet $CaCO_3$ concentration or other mechanisms.

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