Evaluating Dietary Acidifiers as Alternatives to Conventional Feed-Based Antibiotics in Nursery Pig Diets

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Cover Page Footnote
Appreciation is expressed to the National Pork Board and U.S. Pork Center of Excellence for their financial contributions.

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Payton L. Dahmer and Cassandra K. Jones

Summary
A total of 360 weanling pigs (200 × 400, DNA; initially 21.4 ± 0.23 lb BW) were used in a 21-d experiment with 6 pigs/pen, 10 replicate pens/treatment, and 2 separate nursery rooms, each with 30 pens. Pigs were weighed and allotted to pens based on BW in a completely randomized block design to one of six treatment diets: 1) negative control (no organic acids or antibiotics) and the control with 2) 0.25% acidifier A; 3) 0.3% acidifier B; 4) 0.5% acidifier C); 5) 50 g/ton carbadox; and 6) 400 g/ton chlor-tetracycline (CTC). Upon weaning, a common diet with no antibiotics or additives was fed for 21 d (Phases 1 and 2; days −21 to 0), followed by a 21-d experimental period (Phase 3; days 0 to 21) where treatment diets were fed. Pigs and feeders were individually weighed on a weekly basis to calculate ADG, ADFI, and F/G. Data were analyzed using the GLIMMIX procedure of SAS (v. 9.4, SAS Inst., Cary, NC) with pen as the experimental unit, treatment as a fixed effect, and room as a random effect. Dietary treatment had a significant impact (P < 0.05) on ADG, ADFI, and G:F each week and for the overall experimental period (days 0 to 21). Specifically, from days 0 to 7, pigs fed CTC had increased (P = 0.001) ADG compared with those fed acidifier B, acidifier C, and carbadox, whereas pigs fed the negative control and acidifier A diets were intermediate. Additionally, pigs fed the CTC diet had improved (P = 0.0002) ADFI when compared with all other treatments. From days 7 to 14 and days 14 to 21, pigs fed the carbadox diet had decreased (P < 0.0001) ADG compared with all other treatments. During the overall period (days 0 to 21), pigs fed diets containing carbadox had reduced ADG and ADFI (P < 0.0001), whereas pigs fed CTC had improved (P < 0.0001) ADG compared with all other treatments. Additionally, fecal consistency, and fecal microbial populations were analyzed on a subset of pigs (n = 5 pigs/treatment). Treatment also significantly impacted (P = 0.0005) fecal score but did not affect (P = 0.59) fecal microbial growth from days 0 to 21. In summary, CTC continues to be a valuable additive to improve performance in the nursery. Further investigation surrounding the efficacy of dietary acidifiers as antibiotic alternatives is warranted given inconclusive evidence in this study.

1 Appreciation is expressed to the National Pork Board and U.S. Pork Center of Excellence for their financial contributions.
Introduction
The weaning transition is a time of great stress and drastic changes in the piglet’s digestive abilities. Alterations in diet composition, comingling, and exposure to pathogens collectively induce health challenges such as post-weaning diarrhea and cause performance reductions.

Historically, feed-based antibiotics and pharmacological levels of Zn and Cu have been used widely to control these incidences.\(^2\)\(^3\)\(^4\) However, there is consumer and regulatory pressure to limit their use given concerns over the development of antimicrobial-resistant bacteria in humans or negative environmental impacts. Animal scientists are searching for alternatives – such as dietary acidifiers. These compounds, classified as organic or inorganic acids, are thought to improve growth performance by reducing gastric pH and limiting the growth of harmful enteric pathogens. Typically, blends of different acids are included in the diet. Literature is generally supportive of acidifiers improving nursery pig growth performance, but there has been little direct comparison of commercially available acidifier blends and commonly used antimicrobials. Therefore, the objective of this experiment was to evaluate three dietary acidifiers and their impacts on nursery pig growth performance, fecal score, and fecal microbial populations when compared with two feed-based antibiotics.

Materials and Methods
The Kansas State University Institutional Animal Care and Use Committee approved the protocol for this experiment. The study was conducted at the Kansas State University Swine Teaching and Research Center in Manhattan, KS.

Animals and diets
A total of 360 weanling pigs (200 × 400, DNA; initially 21.4 ± 0.23 lb BW; approximately 21 d old) were utilized in a 21-d experiment. Upon weaning, pigs were individually weighed, tagged, and allotted to pens according to BW in a completely randomized block design. Blocking was completed by utilizing two separate environmentally controlled nursery rooms, each with 30 pens. Each pen (5 × 5 ft) included a four-hole dry self-feeder and a nipple waterer to provide pigs \textit{ad libitum} access to feed and water. A total of six pigs were placed into each of the 60 pens (10 replicate pens per treatment) and randomly assigned to one of six dietary treatments: 1) negative control (no organic acids or antibiotics) and the control with 2) 0.25% acidifier A (KEM-GEST, Kemin Industries, Des Moines, IA); 3) 0.3% acidifier B (ACTIVATE DA, Novus International, Saint Charles, MO); 4) 0.50% acidifier C (OutPace, PMI Additives, Arden Hills, MN); 5) 50 g/ton carbadox (Mecadox 10, Phibro Animal Health, Teaneck, NJ); or 6) control + 400 g/ton chlortetracycline (CTC; Deracin 100, PharmGate Animal Health, Wilmington, NC). The acidifiers used represent a variety of commonly used

\(^2\) Jacela, J.Y., J.M. DeRouchey, M.D. Tokach, R.D. Goodband, J.L. Nelssen, D.G. Renter, and S.S. Dritz. 2009. Feed additives for swine: Fact sheets – acidifiers and antibiotics. J. Swine Health Prod. 17(5):270-275. doi: 10.4148/2378-5977.7071.
\(^3\) Shelton, N.W., M.D. Tokach, J.L. Nelssen, R.D. Goodband, S.S. Dritz, J.M. DeRouchey, and G.M. Hill. 2011. Effects of copper sulfate, tri-basic copper chloride, and zinc oxide on weanling pig performance. J. Anim. Sci. 89:2440-2451. doi: 10.2527/jas.2010-3432.
\(^4\) Coble, K.F., J.M. DeRouchey, M.D. Tokach, S.S. Dritz, R.D. Goodband, J.C. Woodworth, and J.L. Usry. 2017. The effects of copper source and concentration on growth performance, carcass characteristics, and pen cleanliness in finishing pigs. J. Anim. Sci. 95:4052-4059. doi: 10.2527/jas2017.1624.
acid blends, and all inclusion levels were based on the manufacturer’s recommendations. The CTC was included at therapeutic levels based on a veterinary feed directive, whereas carbadox was included at subtherapeutic levels in the diet.

Upon weaning, pigs were fed common Phase 1 and Phase 2 starter diets without antimicrobials or acidifiers for 21 d and then fed experimental Phase 3 diets for 21 d. The transition to Phase 3 diets was considered day 0 of the experiment. All diets were formulated to meet or exceed NRC (2012)\(^5\) nutrient requirements. Treatments consisted of a standard corn- and soybean meal-based diet, whereas addition of dietary acidifiers or medications was included by the substitution of corn. Diets were manufactured by Hubbard Feeds (Hubbard Feeds, Beloit, KS) and were fed in pellet form during the common feed period, and meal form during the experimental period (Table 1).

**Data collection**

All pigs were weighed individually on days 0 and 21, and pen weights were collected utilizing a floor scale on days 7 and 14. Feeders from each pen were individually weighed on days 0, 7, 14, and 21 to record feed disappearance. Average daily gain (ADG) and average daily feed intake (ADFI) were calculated on a weekly basis.

Fecal scoring was also conducted by two independent, trained scorers on days 0, 1, 2, 7, 14, and 21 to categorize the consistency of piglet feces per pen. A numerical scale from 1 to 5 was used: 1) being hard pellet-like feces, 2) a firm formed stool, 3) a soft moist stool that retains shape, 4) a soft un-formed stool, and 5) a watery liquid stool. Additionally, fecal samples were collected from 30 pigs (five pigs per treatment) on days 0 and 21 for analysis of enteric bacteria by the Iowa State University Veterinary Diagnostic Laboratory (Iowa State University, Ames, IA). Samples were plated without incubation or enrichment on selective media and incubated at 98.6° F for 24 h. Suspect colonies were serogrouped for final identification.

**Statistical analysis**

Data were analyzed as a completely randomized block design using the GLIMMIX procedure of SAS (v. 9.4, SAS Institute, Inc., Cary, NC), with pen as the experimental unit. Treatment was included as a fixed effect, and room was included as a random effect in the statistical model. All comparisons incorporated Tukey-Kramer multiple comparison adjustments. Preplanned pairwise contrasts were also utilized to compare medicated diets and none (CTC or carbadox vs. control) as well as organic acid diets and none (acidifier A, acidifier B, or acidifier C vs. control). Results were considered significant if \(P \leq 0.05\) and a trend if \(0.05 > P \leq 0.10\).

**Results and Discussion**

From days 0 to 7, pigs fed the diet containing CTC had improved \((P = 0.001)\) ADG compared with those fed diets with acidifier B, acidifier C, or carbadox, whereas pigs fed the control or acidifier A treatments were intermediate. Additionally, ADFI was greater \((P = 0.0002)\) for pigs fed the CTC diet when compared with those fed all other treatments. Feed efficiency was improved \((P = 0.007)\) for those pigs fed the CTC or acidifi-

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\(^5\) National Research Council. 2012. Nutrient Requirements of Swine: Eleventh Revised Edition. Washington, DC: The National Academies Press. https://doi.org/10.17226/13298.
fier A diets when compared with pigs fed carbadox. Overall, (days 0 to 21), ADG was the greatest ($P < 0.0001$) for pigs fed CTC when compared with all other treatments. Likewise, ADFI was increased ($P < 0.0001$) for pigs fed the CTC diet when compared with those fed the control, acidifier A, acidifier B, and carbadox diets, whereas those fed acidifier C were intermediate. Feed efficiency was decreased ($P < 0.0001$) for pigs fed the carbadox treatment when compared with those on all other diets. There was no evidence of differences ($P = 0.129$) in piglet BW on day 0 of the experiment; however, by day 7, pigs fed CTC were heavier ($P = 0.001$) compared with those fed the control, acidifier B, or carbadox treatments. Thus, by the end of the 21-d experiment, pigs fed CTC were the heaviest ($P < 0.0001$) and those fed carbadox were the lightest.

For the duration of the experiment, there was no evidence ($P = 0.11$) of a significant dietary treatment × day interaction with regard to fecal score. However, the main effect of treatment significantly impacted the fecal score ($P = 0.0005$), with a mean fecal score of 3.2 for pigs fed the negative control, acidifier A, acidifier B, acidifier C, and CTC. This indicates that pigs fed the carbadox treatment had a lower average fecal score throughout the experiment when compared with all other diets, suggesting that these pigs had firmer feces when compared with their contemporaries. Additionally, fecal score was also impacted by sampling day ($P < 0.0001$), with mean scores of 3.1, 3.1, 3.0, 3.2, 3.3, and 3.3 for days 0, 1, 2, 7, 14, and 21, respectively. No impact ($P = 0.59$) was observed by dietary treatment on nursery pig fecal microbial growth, with mean growth values of 3.37, 3.60, 3.47, 3.44, 3.23, and 3.38 reported for the negative control, acidifier A, B, C, carbadox, and CTC, respectively. However, the main effect of day ($P = 0.0016$) indicated that the growth of enteric bacteria was reduced from day 0 to day 21 (day 0 average growth = 3.6; day 21 average growth = 3.2).

In summary, this study demonstrated that feeding CTC can benefit nursery pig health and growth performance. The addition of dietary acidifiers did not alter nursery pig growth performance when compared with a control. Continued investigation into optimal inclusion levels, the mode of action, and the economic benefits of utilizing dietary acidifiers in place of antibiotics is warranted.

*Brand names appearing in this publication are for product identification purposes only. No endorsement is intended, nor is criticism implied of similar products not mentioned. Persons using such products assume responsibility for their use in accordance with current label directions of the manufacturer.*
Table 1. Diet composition (as-fed basis)\(^1\)

| Ingredient, % | Control   | Acidifier A | Acidifier B | Acidifier C | Carbadox | CTC  |
|---------------|-----------|-------------|-------------|-------------|----------|------|
| Corn          | 65.69     | 65.34       | 65.44       | 65.09       | 64.90    | 65.39|
| Soybean meal, 46.5% CP | 30.20     | 30.20       | 30.20       | 30.20       | 30.20    | 30.20|
| Calcium carbonate | 1.00      | 1.00        | 1.00        | 1.00        | 0.66     | 1.00 |
| Limestone phosphate, 21% | 0.95      | 0.95        | 0.95        | 0.95        | 0.95     | 0.95 |
| Sodium chloride | 0.58      | 0.58        | 0.58        | 0.58        | 0.58     | 0.58 |
| L-Lys         | 0.55      | 0.55        | 0.55        | 0.55        | 0.55     | 0.55 |
| DL-Met        | 0.27      | 0.27        | 0.27        | 0.27        | 0.27     | 0.27 |
| L-Thr         | 0.07      | 0.07        | 0.07        | 0.07        | 0.07     | 0.07 |
| L-Val         | 0.14      | 0.14        | 0.14        | 0.14        | 0.14     | 0.14 |
| Trace mineral premix | 0.15      | 0.15        | 0.15        | 0.15        | 0.15     | 0.15 |
| Vitamin w/phytase\(^3\) | 0.25      | 0.25        | 0.25        | 0.25        | 0.25     | 0.25 |
| Experimental ingredient | N/A       | 0.25        | 0.30        | 0.50        | 1.00     | 0.20 |
| Total         | 100.00    | 100.00      | 100.00      | 100.00      | 100.00   | 100.00|

Calculated analysis

| Standardized ileal digestibility (SID) amino acids, % | Acidifier A | Acidifier B | Acidifier C | Carbadox | CTC  |
|-----------------------------------------------------|-------------|-------------|-------------|----------|------|
| Lys                                                 | 1.33        | 1.33        | 1.33        | 1.33     | 1.33 |
| Ile:Lys                                             | 51          | 51          | 51          | 51       | 51   |
| Leu:Lys                                             | 107         | 107         | 107         | 107      | 107  |
| Met:Lys                                             | 38          | 38          | 38          | 38       | 38   |
| Met and Cys:Lys                                     | 58          | 58          | 58          | 58       | 58   |
| Thr:Lys                                             | 63          | 63          | 63          | 63       | 63   |
| Trp:Lys                                             | 20          | 20          | 20          | 20       | 20   |
| Val:Lys                                             | 69          | 69          | 69          | 69       | 69   |
| Total Lys, %                                        | 1.47        | 1.47        | 1.47        | 1.47     | 1.47 |
| ME, kcal/kg                                         | 3,264       | 3,255       | 3,258       | 3,247    | 3,242 |
| NE, kcal/kg                                         | 2,320       | 2,313       | 2,315       | 2,306    | 2,302 |
| CP, %                                               | 20.2        | 20.2        | 20.2        | 20.2     | 20.2 |
| Ca, %                                               | 0.74        | 0.74        | 0.74        | 0.74     | 0.74 |
| P, %                                                | 0.58        | 0.59        | 0.58        | 0.57     | 0.57 |
| Available P, %                                      | 0.29        | 0.31        | 0.29        | 0.29     | 0.29 |

\(^1\) A total of 360 weanling pigs (200 × 400, DNA) were used in a three-phase nursery trial with 6 pigs per pen and 10 replicates per treatment. A common diet was fed from d -21 to 0 (Phases 1 and 2); Treatment diets were fed from d 0 to 21 (Phase 3).

\(^2\) Diets included either 0.25% Acidifier A (KEM-GEST, Kemin Industries, Des Moines, IA); 0.3% Acidifier B (ACTIVATE DA, Novus International, Saint Charles, MO); 0.5% Acidifier C (OutPace, PMI Additives, Arden Hills, MN); 50 g/ton carbadox (Mecadox 10, Phibro Animal Health, Teaneck, NJ); or 400 g/ton CTC (Deracin 100, Pharmgate Animal Health, Wilmington, NC).

\(^3\) Premix provided per lb of premix: 110 g Fe from iron sulfate; 110 g Zn from zinc sulfate; 26.4 g Mn from manganese oxide; 11 g Cu from copper sulfate; 198 mg I from calcium iodate; and 198 mg Se from sodium selenite.
Table 2. Effects of dietary treatment on nursery pig growth performance

| Item:                  | Acidifier A | Acidifier B | Acidifier C | Carbadox | CTC     | SEM | Treatment Medicated vs. none | Acidifier vs. none |
|------------------------|-------------|-------------|-------------|----------|---------|-----|-----------------------------|--------------------|
| BW, lb                 |             |             |             |          |         |     |                             |                    |
| d 0                    | 20.7        | 21.4        | 21.2        | 21.8     | 20.9    | 22.3| 2.30                        | 0.129              |
| d 7                    | 27.1        | 28.2        | 27.6        | 28.2     | 27.1    | 30.2| 2.50                        | 0.001              |
| d 14                   | 35.7        | 37.5        | 36.8        | 37.7     | 34.0    | 40.6| 3.30                        | < 0.0001           |
| d 21                   | 48.5        | 49.4        | 49.4        | 50.0     | 43.0    | 54.2| 3.90                        | < 0.0001           |
| ADG, lb/d              | 0.99        | 1.04        | 0.95        | 0.90     | 0.86    | 1.15| 0.35                        | 0.001              |
| d 7 to 14              | 1.34        | 1.37        | 1.32        | 1.40     | 1.37    | 1.54| 0.22                        | 0.0002             |
| F/G                    | 1.35        | 1.32        | 1.39        | 1.56     | 1.79    | 1.34| 0.38                        | 0.007              |
| ADG, lb/d              | 1.21        | 1.30        | 1.32        | 1.37     | 0.99    | 1.48| 0.21                        | < 0.0001           |
| d 14 to 21             | 1.76        | 1.72        | 1.79        | 1.76     | 1.28    | 1.96| 0.20                        | < 0.0001           |
| ADFI, lb/d             | 1.79        | 1.85        | 1.76        | 1.96     | 1.65    | 2.09| 0.39                        | 0.002              |
| F/G                    | 1.48        | 1.42        | 1.33        | 1.43     | 1.67    | 1.41| 0.62                        | 0.050              |
| ADG, lb/d              | 1.32        | 1.34        | 1.34        | 1.34     | 1.04    | 1.52| 0.15                        | < 0.0001           |
| d 0 to 21              | 1.85        | 1.85        | 1.83        | 1.96     | 1.70    | 2.14| 0.33                        | < 0.0001           |
| ADFI, lb/d             | 1.40        | 1.38        | 1.37        | 1.46     | 1.63    | 1.41| 0.17                        | < 0.0001           |
| F/G                    | 0.13        | 0.13        | 0.13        | 0.15     | 0.13    | -   | -                           | -                  |
| Feed cost, $/lb feed³  | 2.31        | 2.38        | 2.36        | 2.58     | 2.38    | 2.79| 0.44                        | < 0.0001           |
| Feed cost, $/pig⁴      | 2.31        | 2.38        | 2.36        | 2.58     | 2.38    | 2.79| 0.44                        | < 0.0001           |
| Feed cost, $/lb gain⁵  | 2.31        | 2.38        | 2.36        | 2.58     | 2.38    | 2.79| 0.44                        | < 0.0001           |

Means within a row that do not share a common superscript differ P > 0.05.

¹A total of 360 weanling pigs (6 pigs per pen, 10 pens/treatment) were fed a common diet during Phase 1 and Phase 2 with treatment diets fed during Phase 3.

²Diets included either 0.25% Acidifier A (KEM-GEST, Kemin Industries, Des Moines, IA); 0.3% Acidifier B (ACTIVATE DA, Novus International, Saint Charles, MO); 0.5% Acidifier C (OutPace, PMI Additives, Arden Hills, MN); 50 g/ton carbadox (Mecadox 10, Phibro Animal Health, Teaneck, NJ); or 400 g/ton CTC (Deracin 100, Pharmgate Animal Health, Wilmington, NC).

³Calculated using ingredient prices as of April 28, 2020.

⁴Feed cost, $/pig = feed cost per lb of feed × (ADFI overall × 21).

⁵Feed cost, $/lb of gain = feed cost per pig ÷ (ADG overall × 21).
Table 3. Impact of dietary treatment on nursery pig average fecal score and fecal microbial growth

| Item                        | Control | Acidifier A | Acidifier B | Acidifier C | Carbadox | CTC | SEM | Treatment | Day | Treatment × day |
|-----------------------------|---------|-------------|-------------|-------------|----------|-----|-----|-----------|-----|-----------------|
| Average fecal score<sup>2</sup> | 3.2<sup>a</sup> | 3.2<sup>a</sup> | 3.2<sup>a</sup> | 3.2<sup>a</sup> | 2.9<sup>b</sup> | 3.2<sup>a</sup> | 0.05072 | 0.0005 | < 0.0001 | 0.11 |
| Average microbial growth<sup>3</sup> | 3.37    | 3.60        | 3.47        | 3.44        | 3.23     | 3.38 | 0.144 | 0.59      | 0.002 | 0.47          |

<sup>1<sup>Means within the same row that do not share a common superscript differ *P* < 0.05. Values reported are least square means, representing the main effect of dietary treatment.

<sup>2<sup>Diets included either 0.25% Acidifier A (KEM-GEST, Kemin Industries, Des Moines, IA); 0.3% Acidifier B (ACTIVATE DA, Novus International, Saint Charles, MO); 0.5% Acidifier C (OutPace, PMI Additives, Arden Hills, MN); 50 g/ton carbadox (Mecadox 10, Phibro Animal Health, Teaneck, NJ); or 400 g/ton CTC (Deracin 100, Pharmgate Animal Health, Wilmington, NC).

<sup>3<sup>Fecal scores were collected on d 0, 1, 2, 7, 14, and 21 of the experiment by two trained, independent scorers using a numerical scale: 1 = hard, pellet-like feces; 2 = firm, formed stool; 3 = soft, moist stool that retains shape; 4 = soft, unformed stool; 5 = watery, liquid stool.

<sup>4<sup>Fecal samples from 30 pigs (5 pigs/treatment) were collected on d 0 and 21 via rectal swab and plated for analysis of enteric bacteria by the Iowa State University Veterinary Diagnostic Laboratory (Iowa State University, Ames, IA). Culture growth from d 0 to d 21 was reported using a numeric scale: 0 = No significant growth; 1 = Low; 2 = Few; 3 = Moderate; 4 = High.