USPCE and NPB Swine Research Experience & Educations (SREE) FINAL RESEARCH GRANT REPORT

Project Title: Evaluating dietary acidifiers as alternatives for conventional feed-based antibiotics in nursery pig diets
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Scientific Abstract: A total of 360 weanling pigs (DNA 200 x 400; initially 9.7 ± 0.23 kg BW) were used in a 21-d experiment with 6 pigs/pen and 10 replicate pens/treatment. Pigs were weighed and allotted to pens based on BW in a completely randomized block design to one of 6 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; 6) 400 g/ton Chlortetracycline. A common diet was fed in phase 1 and 2 with treatments fed in phase 3. Pigs and feeders were individually weighed on a weekly basis to calculate average daily gain **(ADG)**, average daily feed intake **(ADFI)** and feed efficiency **(G:F)**. Data were analyzed using the PROC 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 overall (d 0 to 21). Specifically, from d 0 to 7, pigs fed CTC had increased ($P = 0.001$) ADG compared to those fed Acidifier B, Acidifier C and Carbadox, while 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 to all other treatments. From d 7 to 14 and d 14 to 21, pigs fed the Carbadox diet had decreased ($P < 0.0001$) ADG compared to all other treatments. During the overall period (d 0 to 21), pigs fed diets containing Carbadox had reduced ADG and ADFI ($P < 0.0001$), while pigs fed CTC had improved ($P < 0.0001$) ADG compared to all other treatments. Additionally, blood parameters, fecal consistency and fecal microbial populations were analyzed. Dietary treatment significantly impacted ($P < 0.05$) concentrations of protein, globulin, phosphorus, alkaline phosphatase, and sorbitol dehydrogenase in the blood. Treatment also significantly impacted ($P = 0.0005$) fecal score but did not affect ($P = 0.59$) fecal microbial growth from d 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.

Introduction: The transition from a liquid milk diet to solid feed results in drastic changes in the intestinal morphology and digestive abilities of weanling pigs. Feed additives are commonly included to combat these challenges and work to maximize nutrient absorption and utilization. Antibiotics and pharmacological levels of zinc and copper are among the most historically utilized feed additives in the nursery due to their proven ability to assist with post-weaning diarrhea and improve growth performance (Jacela et al., 2009; Shelton et al., 2011; Coble et al., 2017). However, there is growing consumer and regulatory pressure for swine producers to reduce their reliance on these products due to concerns regarding potential antibiotic resistance in human medicine or negative environmental impacts (Bager et al., 2000; Jondreville et al., 2003). Thus, animal scientists are actively investigating biological alternatives for these conventionally used antimicrobials. Dietary acidifiers have garnered interest as a possible avenue given data that suggest that they can provide prophylactic effects similar to antibiotics (Kim et al., 2005). These organic and inorganic acids can be fed solely or in most cases, as blends found in commercial additives. They work to reduce gastric pH, increase nutrient digestibility and limit the growth of pathogenic bacteria (Jacela et al., 2009). While dietary acidifiers have been heavily researched, data is still variable with regards to their efficacy since there are so many external factors that can impact their success, such as inclusion level, type of acid used, other nutritional components of the diet, and the age/existing health status of the pigs. Additionally, there is little direct comparison of commercial acidifiers with economic application, which ultimately limits producers' ability to make relevant, science-based decisions to include them.

Objectives: The objectives of this research include:

1. Evaluate commercial dietary acidifiers as alternatives to conventional feed-based antimicrobials by
   a. Determining their impact on growth performance and economics
   b. Identify any changes in blood parameters as indicators of immune status
   c. Characterize any shifts in the fecal microbiota
2. Expose 20 undergraduate students to swine research and careers in the swine industry
   a. A total of 20 students were a part of this research through a course-based undergraduate research project during the Spring 2020 semester

Materials & Methods: A total of 360 weanling pigs (DNA 200 × 400; initially 9.4 ± 0.23 kg BW; approximately 21 d old) were fed for 21 d at the Kansas State University Swine Teaching and Research Center (Manhattan, KS). Upon weaning, pigs were individually weighed 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 (1.52 × 1.52 m) that included stainless steel 4-hole dry self-feeders and nipple waterers to provide *ad libitum* access to feed and water. There were 6 pigs placed in each pen with 10 replicate pens assigned to one of 6 dietary treatments: 1) negative control (diet with no antibiotics or acidifiers); 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); and 6) 400 g/ton chlortetracycline (CTC) (Deracin® 100, Pharmgate Animal Health, Wilmington, NC). Pigs were fed common phase 1 and phase 2 starter diets with no antimicrobials for 21 d, then fed experimental diets for 21 d. All pigs were weighed individually on d 0 and d 21 and pen weights were collected utilizing a floor scale on d 7 and d 14. Feeders from each pen were individually weighed on d 0, 7, 14, and 21. Average daily gain (ADG) and average daily feed intake (ADFI) were then calculated on a weekly basis. Whole blood samples were collected from the same 30 pigs (5 pigs/treatment) on d 0 and 21 and submitted to the Kansas State University Veterinary Diagnostic Laboratory for complete blood panel, serum chemistry and hepatic profile analyses via spectrophotometry. Additionally, fecal samples from 30 pigs (5 pigs/treatment) were collected with sterile cotton-tipped collection swabs on d 0 and 21 for analysis of fecal microbial populations. These samples were analyzed by the Iowa State University Veterinary Diagnostic Laboratory. Finally, fecal scoring was also conducted by two independent, trained scorers on d 0, 1, 2, 7, 14, and 21 to categorize the consistency of piglet feces per pen utilizing a numeric scale from 1 to 5, with 1 being the hardest and 5 being the softest fecal consistency. Data were analyzed using the GLIMMIX procedure of SAS (SAS Institute, Inc., version 9.4, Cary, NC) with pen as the experimental unit. Treatment was included as a fixed effect and room (block) was included as a random effect in the statistical model. All comparisons incorporated Tukey-Kramer multiple comparison adjustments and pre-planned pairwise contrasts were used to compared medicated diets to the control as well as the acidified diets to the control. Results were considered significant if $P \leq 0.05$.

This experiment was conducted as part of the course-based research experience in the Department of Animal Sciences and Industry at Kansas State University. To complete the stated objectives above, 20 undergraduate students were responsible for assisting with data collection, maintaining independent weekly Excel data sheets with animal weights, and calculating mean fecal scores, ADG, ADFI, and G:F at the end of the experiment. Students also calculated economic impact of the dietary treatments, including diet cost, cost/lb of gain, and income over feed. Students were assisted with statistical analysis using SAS (v.9.4, Cary, NC) and developed independent data tables, research abstracts, and research posters, which were presented at the Department of Animal Sciences & Industry Undergraduate Research Forum in May 2020.

Student learning outcomes were assessed using an anonymous, online self-assessment survey that was completed within 2 weeks of students completing the undergraduate research experience. These experimental procedures (proposal #8609) were determined by the Kansas State University Research Compliance Office as exempt under the criteria set forth in the Federal Policy for the Protection of Human Subjects, 45 CFR 46.101(b)(1)(i). The survey asked

13 independent questions, with a 5-point Likert scale used for response options as described by Cavus and Uzunboylu (2009). Responses could include (1) strongly disagree, (2) disagree, (3) neither agree nor disagree, (4) agree, and (5) strongly agree. All 20 students in the course completed the survey and agreed to have their responses used for research purposes.

Results:

Growth Performance: For the overall experiment (d 0 to d 21), ADG was the greatest ($P < 0.0001$) for pigs fed CTC compared to all other treatment. Likewise, ADFI was increased ($P < 0.0001$) for pigs fed the CTC diet when compared to those fed the control, acidifier A, acidifier B and carbadox diets, while those fed acidifier C were intermediate. Feed efficiency was decreased ($P < 0.0001$) for pigs fed the carbadox treatment when compared to those on all other diets. By the end of the 21 d experiment pigs fed CTC were the heaviest ($P < 0.0001$) and those fed carbadox were the lightest (BW: 24.6 kg and 19.5 kg, respectively).

Economics: Feed costs were calculated for dollars per kg of feed, dollars per pig and dollars per kg of gain utilizing current ingredient prices. Income over feed (IOF) was also calculated by subtracting the feed cost per pig from a predicted revenue. The predicted revenue was a fixed amount, set at $0.25 per kg of gain, taking into consideration the current market price at the time of the experiment. This calculation was done on a per pen basis in order to have proper replication for statistical analysis. Economic data was included in the statistical model previously described. Given all treatment diets were formulated from the control, differences in the cost of each diet depend solely on the price of the additive included. Feed cost per pig was calculated: Feed Cost, $/pig = Feed Cost, $/kg of feed × (ADFI Overall × 21). Feed cost per kg of gain was also calculated: Feed Cost, $/kg of gain = Feed Cost, $/pig ÷ (ADG Overall × 21). Significant differences in feed cost, both per pig and per kg of gain were observed across treatments ($P < 0.0001$). While costs associated with feeding the diet containing CTC were statistically higher ($P < 0.0001$), IOF calculations determined that the margin of profit is potentially greater ($P < 0.0001$) by including CTC in the diet when compared to the other additives used in this study.

Blood Parameters: Blood data indicate that pigs fed CTC had lower total protein concentrations ($P = 0.01$) compared to those fed carbadox, while the remaining treatments were intermediate. Globulin levels were increased ($P < 0.0001$) in pigs fed the carbadox treatment compared to those fed CTC, acidifier A or the negative control. The pigs consuming carbadox also showed increased ($P = 0.04$) alkaline phosphatase concentrations compared to pigs fed acidifier A, while other dietary treatments were intermediate. Finally, pigs fed carbadox had significantly increased ($P = 0.02$) levels of sorbitol dehydrogenase when compared to pigs consuming all other treatments.

Fecal Consistency and Fecal Microbial Populations: For the duration of the experiment, there was no evidence ($P = 0.11$) of a significant dietary treatment × day interaction with regards to fecal score. However, the main effect of treatment significantly impacted fecal score ($P = 0.0005$), with a mean fecal score of 3.2 for treatments 1, 2, 3, 4, and 6, This indicates that pigs fed the carbadox treatment had a lower average fecal score throughout the experiment when compared to all other diets, suggesting that these pigs had firmer feces when compared to 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 d 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 d 0 to d 21 (d 0 average growth = 3.6; d 21 average growth = 3.2).

Student Learning Outcomes: Student perception of their undergraduate research experience was highly positive. All students indicated that they ‘agreed’ or ‘strongly agreed’ with all assessed statements (Table 1).

Table 1. Student perceptions of undergraduate research experience[1].

Statement	Average Respon (n = 20)
The undergraduate research experience met my expectations.	4.7
I can apply the scientific method to answer research questions.	4.8
I am competent in quantitative skills, such as data analysis.	4.4
The undergraduate research experience improved my critical thinking skills.	4.8
I can apply critical thinking skills to distinguish fact during the interpretation of results.	4.9
I have experience with computers and software to analyze data and apply it to animal science.	4.5
I have an appreciation for ethical practices, such as the use of animals for research purposes.	5.0
I can work as a team.	4.9
I had adequate guidance from my undergraduate research mentor.	5.0
The undergraduate research experience made me want to conduct additional research in the futur	4.7
The undergraduate research experience helped me identify if research may be a future career path	4.4
I would recommend undergraduate research to a friend.	4.8

[1]Student survey responses to the question, "How accurate is this statement" using a 5-point Likert scale ranging from 1: strongly disagree to 5: strongly agree based on their completion of a conventional, participant-based undergraduate research experience vs. a course-based research experience.

Discussion: Previous research tells us that the addition of antibiotic agents to nursery diets can improve piglet health and performance (Cromwell et al., 2002; Zimmermann, 1986). Likewise, our study observed that the overall ADG of pigs fed diets containing chlortetracycline was greatest when compared to those fed a control or diets with commercial acidifiers. Data surrounding the efficacy of dietary acidifiers as antibiotic alternatives is extremely variable, and in published data where improvements in growth performance have been reported, there is no evidence of optimal inclusion levels. In many cases, no significant differences have been reported between acidifiers fed (Partanen et al., 2001; Roth and Kirchgessner, 1998). Similarly, the current study did not observe differences between the tested acidifiers. Literature suggests that acidifiers can work through a variety of mechanisms, including altering the microbiota of the digestive tract and modulating the immune response. We attempted to characterize any shifts in fecal microbial populations from the dietary treatments but saw no differences in the type or prevalence of fecal bacteria between products testes. Additionally, serum metabolites were analyzed to evaluate immune function. While dietary treatment significantly impacted some of the evaluated parameters, no criteria were outside of normal biological reference intervals, indicating no changes in immune response as a result of treatment. Finally, our study aimed to provide economic application to the data and determine any cost benefit to producers by including acidifiers in their diets. In this experiment, there was no economic incentive to be found my including the tested acidifiers and the inclusion of CTC resulted in the greatest potential economic return as demonstrated by our calculation of IOF costs. However, it is extremely hard for information from one study to be applied widely across the industry, as many additional factors can affect this outcome. In summary, feeding CTC improved nursery pig growth performance. The addition of dietary acidifiers did not alter nursery pig growth performance when compared to a control. Continued investigation into optimal inclusion levels, the mode of action and economic justification of utilizing dietary acidifiers in place of antibiotics is warranted.

Student Statement: Twenty students were exposed to undergraduate research using this unique mechanism. Of the students that were part of this project, the following students have been identified as having pursued swine-related career opportunities include:

- Jenna Bromm – begins her M.S. with the K-State Applied Swine Nutrition Team in January 2021
- Zach Buessing – begins his M.S. at the University of Illinois in June 2021
- Ivan Bueso-Interiano – Completed an internship with Carthage Veterinary Services in Summer 2020 and was recently accepted to the K-State McNair Scholars Program
- Kaylee Farmer – began her M.S. in meat science at K-State in August 2020
- Shyanne Osterhaus – began her D.V.M. at K-State in August 2020
- Linnea Rimmer – begins her M.S. in nonruminant nutrition at K-State in August 2021

- Megan Anguano, Erin Cocjin, Lauren Duncan, Anna Hixon, Kate Nelson, Gabriela Olivier, Scotney Reichenberger, Destiny Serrano-Quiroz, Colton Stucky, Sydney Tastad, Ryan Tipton, and Nadia Zange continue their undergraduate studies at K-State.