Determination of Probiotic and/or Chlortetracycline Inclusion Effects on Nursery Pig Growth Performance

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Abstract
A total of 300 nursery pigs (DNA 200 × 400, Columbus, NE; initially 13.0 lb BW) were used in a 42-d study evaluating the effects of feeding chlortetracycline (CTC) in combination with probiotics on nursery pig performance. Probiotics are a class of antimicrobial alternatives designed to enhance growth performance and digestive tract health. Pigs were weaned at approximately 21 d of age and allotted to pens based on initial BW. Pigs were fed a common pelleted starter diet for 4 d and then weighed, and pens were allotted to 1 of 6 dietary treatments based on BW in a completely randomized design. The treatments were arranged in a 2 × 3 factorial with main effects of chlortetracycline (0 vs. CTC at 400 g/ton from d 0 to 42) and probiotic (0 vs. 1 lb/ton Bioplus 2B (Chr. Hansen USA, Inc., Milwaukee, WI)) vs. 1 lb/ton Poultry Star (Biomin America, Inc., San Antonio, TX). Experimental diets were fed in 2 phases (Phase 1: d 0 to 14 and Phase 2: d 14 to 42) and fed in meal form. On d 14 and 28, CTC was removed from the diet according to FDA regulations. For overall performance, there were no interactions (P > 0.05) between added probiotics and CTC. However, pigs fed CTC had improved (P < 0.001) ADG, ADFI, and overall BW compared with those fed diets without CTC. While adding Poultry Star to the diet increased (P < 0.05) BW and ADFI on d 14, there were no consistent benefits of feeding either probiotic alone or in combination with CTC.

Keywords
antibiotic, growth performance, nursery, probiotic

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Cover Page Footnote
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Summary
A total of 300 nursery pigs (DNA 200 × 400, Columbus, NE; initially 13.0 lb BW) were used in a 42-d study evaluating the effects of feeding chlortetracycline (CTC) in combination with probiotics on nursery pig performance. Probiotics are a class of antimicrobial alternatives designed to enhance growth performance and digestive tract health. Pigs were weaned at approximately 21 d of age and allotted to pens based on initial BW. Pigs were fed a common pelleted starter diet for 4 d and then weighed, and pens were allotted to 1 of 6 dietary treatments based on BW in a completely randomized design. The treatments were arranged in a 2 × 3 factorial with main effects of chlortetracycline (0 vs. CTC at 400 g/ton from d 0 to 42) and probiotic (0 vs. 1 lb/ton Bioplus 2B (Chr. Hansen USA, Inc., Milwaukee, WI) vs. 1 lb/ton Poultry Star (Biomin America, Inc., San Antonio, TX)). Experimental diets were fed in 2 phases (Phase 1: d 0 to 14 and Phase 2: d 14 to 42) and fed in meal form. On d 14 and 28, CTC was removed from the diet according to FDA regulations. For overall performance, there were no interactions ($P > 0.05$) between added probiotics and CTC. However, pigs fed CTC had improved ($P < 0.001$) ADG, ADFI, and overall BW compared with those fed diets without CTC. While adding Poultry Star to the diet increased ($P < 0.05$) BW and ADFI on d 14, there were no consistent benefits of feeding either probiotic alone or in combination with CTC.

Key words: antibiotic, growth performance, nursery, probiotic

Introduction
The use of antimicrobials in feed and their positive benefits on growth performance during the nursery stage of weaned pig production is firmly established. In the past, producers widely used antimicrobials throughout the nursery stage of swine production, even in the absence of a health challenge. With the changing perspectives on the use of feed antimicrobials, alternative technologies are being considered that can possibly replace the growth performance benefits of feed grade antimicrobials.

1 Appreciation is expressed to the National Pork Board for financial support.
2 Department of Diagnostic Medicine/Pathology, College of Veterinary Medicine,
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Probiotics are one such technology that enhances gut function for improved nutrient uptake. Therefore, the objective of this study was to compare the growth performance of nursery pigs fed diets containing antimicrobials and/or probiotics.

**Methods**

This trial was conducted in collaboration with the Department of Diagnostic Medicine/Pathobiology, College of Veterinary Medicine, Kansas State University. The main objective of the study was to evaluate the impact of probiotics on the emergence and dissemination of antimicrobial resistance among bacteria present in the gut. Two probiotics were chosen for our animal experiments based on our preliminary results on antimicrobial resistance carriage in them. Poultry Star (Biomin America, Inc., San Antonio, TX) was chosen in this study because the product contained resistance genes for 3 classes of antimicrobials. BioPlus 2B (Chr. Hansen USA, Inc., Milwaukee, WI) was chosen in this study because of the absence of antimicrobial resistance genes. This report describes the growth performance of these same pigs; the impact of these 2 probiotic sources on antimicrobial resistance will be reported elsewhere.

The Kansas State University Institutional Animal Care and Use Committee approved the protocol for this experiment. The study was conducted at the K-State Segregated Early Weaning Facility in Manhattan, KS.

A total of 300 nursery pigs (DNA 200 × 400, Columbus, NE; initially 13.0 lb BW) were used in a 42-d study with 5 pigs per pen and 10 replications per treatment. Each pen (4 ft × 4 ft) had metal tri-bar flooring, one 4-hole self-feeder and a cup waterer to provide ad libitum access to feed and water. Pigs were weaned at approximately 21 d of age and allotted to pens based on initial BW. Pigs were fed a common starter diet for 4 days and then allotted to 1 of 6 dietary treatments based on BW in a completely randomized design.

The 6 dietary treatments were based on a corn-soybean meal diet and arranged in a 2 × 3 factorial with main effects of antimicrobial (0 vs. chlortetracycline (CTC) at 400 g/ton from d 0 to 42; Zoetis Services, LLC., Florham Park, NJ), and probiotic (0 vs. 1 lb/ton BioPlus 2B; Chr. Hansen USA, Inc., Milwaukee, WI or 1 lb/ton Poultry Star; Biomin America, Inc., San Antonio, TX). The treatment ingredients were substituted for an equivalent amount of corn in the respective diets to form the experimental treatments (Table 1). Experimental diets were fed in 2 phases (Phase 1: d 0 to 14 and Phase 2: d 14 to 42) and fed in meal form. On d 14 and 28, CTC was removed from the diet according to FDA regulations. Experimental diets containing CTC resumed feeding on d 15 and 29. Pigs and feeders were weighed every 7 d to determine ADG, ADFI, and F/G.

All experimental diets were fed in meal form and were prepared at the K-State O.H. Kruse Feed Technology Innovation Center, Manhattan, KS. The 4 d common starter diet was fed in pellet form. Multiple diet samples were collected at manufacturing, and pooled samples of each diet were submitted for analysis of DM, CP, Ca, and P (Ward Laboratories, Inc., Kearney, NE; Table 2).
Growth data were analyzed as a randomized complete block design using the PROC GLIMMIX procedure of SAS (SAS Institute, Inc., Cary, NC) with pen as the experimental unit. The main effects of CTC and probiotics as well as their interactions, were evaluated using preplanned CONTRAST statements. These contrast statements were arranged as a $2 \times 3$ factorial with the main effects of CTC and each of the probiotics. Differences between treatments were determined by using least squares means. A $P$-value $\leq 0.05$ was considered significant and $0.05 < P \leq 0.10$ was considered marginally significant.

Results and Discussion

From d 0 to 14, a CTC $\times$ Bioplus 2B interaction ($P = 0.002$) was observed for ADFI (Table 4). The interaction occurred because pigs fed diets containing the combination of CTC and BioPlus 2B had greater ADFI compared to pigs fed the control diet or the diet with only BioPlus 2B, while pigs fed CTC intermediate. Pigs fed diets containing CTC had improved ($P = 0.0001$) ADG and ADFI compared to those without.

From d 14 to 28, no interactions between CTC and either BioPlus 2B or Poultry Star were observed. Pigs fed diets with CTC had improved ($P = 0.0001$) ADG, ADFI, and BW compared to pigs not fed CTC. Also, pigs fed diets containing Poultry Star had a tendency for greater ($P = 0.052$) ADFI than those not fed Poultry Star.

From d 28 to 42, a CTC $\times$ Poultry Star interaction ($P = 0.050$) was observed for ADFI. The interaction occurred because pigs fed diets containing CTC only had greater ADFI compared to the control, while diets containing either Poultry Star or Poultry Star with CTC were intermediate. Furthermore, a tendency for a CTC $\times$ BioPlus 2B interaction ($P = 0.077$) was observed for F/G. The interaction occurred because pigs fed diets containing CTC in combination with BioPlus 2B had improved F/G comparative to pigs fed diets containing CTC or BioPlus 2B alone. Feeding CTC increased ($P = 0.045$) ADFI, with no impact on ADG or F/G.

For the overall study (d 0 to 42), no CTC by probiotic interactions were observed. Pigs fed diets containing CTC had greater ($P = 0.001$) ADG, ADFI, and overall BW compared to those not fed CTC.

In summary, feeding probiotics alone or in combination with CTC did not consistently improve nursery pig growth performance. This main effect of CTC on growth performance throughout the study was similar to previous research with an increase in growth rate driven by increased feed intake. In certain phases of the nursery, the addition of one of the probiotics (Poultry Star) with CTC had an additive effect on growth performance, but in later phases this benefit was not found. This warrants further research on whether in certain phases of nursery production it is beneficial to feed probiotics in combination with CTC to increase performance. In conclusion, this study further characterized the positive benefits of feeding CTC during the nursery phase on pig performance.
Table 1. Experimental diet composition (as-fed basis)¹

| Item                                | Phase 1 | Phase 2 |
|-------------------------------------|---------|---------|
| Ingredient, %                       |         |         |
| Corn                                | 55.75   | 62.50   |
| Soybean meal, 46.5% CP              | 25.35   | 33.40   |
| Dried whey                          | 10.00   | ---     |
| HP 300²                             | 5.00    | ---     |
| Limestone                           | 1.05    | 1.18    |
| Monocalcium phosphate, 21%          | 1.20    | 1.20    |
| Sodium chloride                     | 0.30    | 0.35    |
| L-Lys HCl                           | 0.45    | 0.45    |
| DL-Met                              | 0.20    | 0.20    |
| L-Thr                               | 0.20    | 0.20    |
| L-Trp                               | 0.03    | 0.03    |
| L-Val                               | 0.10    | 0.10    |
| CTC-50³                             | ---     | ---     |
| Bioplus 2B⁴                         | ---     | ---     |
| Poultry Star⁵                       | ---     | ---     |
| Phytase⁶                            | 0.02    | 0.02    |
| Trace mineral premix⁷                | 0.15    | 0.15    |
| Vitamin premix                      | 0.25    | 0.25    |
| Total                               | 100     | 100     |

Calculated analysis

Standardized ileal digestible (SID) amino acids, %

|               | Phase 1 | Phase 2 |
|---------------|---------|---------|
| Lys           | 1.35    | 1.35    |
| Me: Lys       | 36      | 36      |
| Met and Cys: Lys | 57  | 58      |
| Thr: Lys      | 65      | 64      |
| Trp: Lys      | 19.1    | 19.3    |
| Val: Lys      | 70      | 70      |
| Total Lys, %  | 1.49    | 1.50    |
| ME, kcal/lb   | 1,496   | 1,482   |
| CP, %         | 21.4    | 21.9    |
| Ca, %         | 0.75    | 0.75    |
| P, %          | 0.69    | 0.66    |
| Available P, %| 0.49    | 0.43    |

¹Phase 1 diets were fed from d 0 to 14 (~13.0 to ~19 lb BW) and Phase 2 diets from d 14 to 42 (~19 to 55 lb BW). A common starter diet was fed to all pigs for 4 days after weaning.
²Hamlet Protein, Inc., Findlay, OH.
³Zoetis Services, LLC., Florham Park, NJ.
⁴Chr. Hansen USA, Inc., Milwaukee, WI.
⁵Biomin America, Inc., San Antonio, TX.
⁶HiPhos 2700 (DSM Nutritional Products, Inc., Parsippany, NJ), providing 184.3 phytase units (FTU)/lb and an estimated release of 0.10% available P.
⁷Trace mineral premix containing 17 ppm Cu and 110 ppm Zn.
Table 2. Diet analysis, %\textsuperscript{1}

|               | CTC | Bioplus 2B | Poultry Star |
|---------------|-----|------------|--------------|
|               | -   | -          | -            |
|               | +   | +          | +            |
|               | -   | -          | -            |
| Phase 1 diets |     |            |              |
| DM            | 89.5| 89.5       | 90.1         |
| CP            | 21.1| 21.4       | 21.3         |
| Ca            | 0.85| 0.91       | 0.93         |
| P             | 0.74| 0.70       | 0.72         |
| Phase 2 diets |     |            |              |
| DM            | 88.0| 88.3       | 88.0         |
| CP            | 21.7| 20.7       | 21.5         |
| Ca            | 0.85| 0.99       | 0.96         |
| P             | 0.66| 0.69       | 0.67         |

\textsuperscript{1}Complete diet samples were obtained from each treatment during manufacturing and composited. Samples of diets were then submitted to Ward Laboratories, Inc. (Kearney, NE) for analysis.
Table 3. Effects of probiotic and/or antimicrobial on nursery pig performance

|                  | CTC | BioPlus 2B | Poultry Star | Probability, P < |
|------------------|-----|------------|--------------|------------------|
|                  |     |            |              | CTC | BioPlus 2B | Poultry Star | CTC × BioPlus 2B | CTC × Poultry Star |
|                  |     |            |              | SEM |       |          |                |                  |
| d 0 to 14        |     |            |              |     |       |          |                |                  |
| ADG, lb          | 0.35<sup>a</sup> | 0.43<sup>b</sup> | 0.36<sup>a</sup> | 0.47<sup>a</sup> | 0.41<sup>bc</sup> | 0.47<sup>a</sup> | 0.023 | 0.001 | 0.356 | 0.108 | 0.505 | 0.976 |
| ADFI, lb         | 0.50<sup>b</sup> | 0.57<sup>ab</sup> | 0.52<sup>b</sup> | 0.61<sup>a</sup> | 0.56<sup>ab</sup> | 0.61<sup>a</sup> | 0.022 | 0.001 | 0.124 | 0.018 | 0.002 | 0.938 |
| F/G              | 1.46 | 1.30 | 1.48 | 1.30 | 1.36 | 1.31 | 0.165 | 0.001 | 0.788 | 0.362 | 0.861 | 0.252 |
| d 14 to 28       |     |            |              |     |       |          |                |                  |
| ADG, lb          | 1.00<sup>b</sup> | 1.12<sup>ab</sup> | 0.94<sup>c</sup> | 1.15<sup>a</sup> | 1.01<sup>bc</sup> | 1.18<sup>a</sup> | 0.044 | 0.001 | 0.795 | 0.242 | 0.310 | 0.868 |
| ADFI, lb         | 1.45<sup>bc</sup> | 1.70<sup>a</sup> | 1.40<sup>c</sup> | 1.74<sup>a</sup> | 1.54<sup>b</sup> | 1.77<sup>a</sup> | 0.043 | 0.001 | 0.935 | 0.052 | 0.239 | 0.810 |
| F/G              | 1.47 | 1.53 | 1.53 | 1.53 | 1.54 | 1.51 | 0.048 | 0.592 | 0.612 | 0.865 | 0.437 | 0.565 |
| d 28 to 42       |     |            |              |     |       |          |                |                  |
| ADG, lb          | 1.50<sup>bc</sup> | 1.55<sup>ab</sup> | 1.44<sup>bc</sup> | 1.58<sup>a</sup> | 1.54<sup>bc</sup> | 1.49<sup>ab</sup> | 0.044 | 0.227 | 0.788 | 0.860 | 0.361 | 0.195 |
| ADFI, lb         | 2.36<sup>bc</sup> | 2.55<sup>a</sup> | 2.32<sup>bc</sup> | 2.47<sup>bc</sup> | 2.49<sup>bc</sup> | 2.44<sup>ab</sup> | 0.058 | 0.045 | 0.350 | 0.872 | 0.738 | 0.050 |
| F/G              | 1.58 | 1.65 | 1.62 | 1.57 | 1.61 | 1.65 | 0.033 | 0.521 | 0.582 | 0.617 | 0.077 | 0.570 |
| d 0 to 42        |     |            |              |     |       |          |                |                  |
| ADG, lb          | 0.94<sup>bc</sup> | 1.03<sup>a</sup> | 0.90<sup>c</sup> | 1.06<sup>a</sup> | 0.98<sup>ab</sup> | 1.04<sup>bc</sup> | 0.029 | 0.001 | 0.808 | 0.340 | 0.214 | 0.545 |
| ADFI, lb         | 1.42<sup>bc</sup> | 1.60<sup>a</sup> | 1.38<sup>c</sup> | 1.60<sup>a</sup> | 1.52<sup>ab</sup> | 1.61<sup>a</sup> | 0.036 | 0.001 | 0.573 | 0.173 | 0.531 | 0.215 |
| F/G              | 1.52 | 1.56 | 1.56 | 1.51 | 1.55 | 1.54 | 0.029 | 0.820 | 0.869 | 0.797 | 0.171 | 0.519 |
| BW, lb           |     |            |              |     |       |          |                |                  |
| d 0              | 13.0 | 13.0 | 13.0 | 13.0 | 12.9 | 13.1 | 0.118 | 0.093 | 0.896 | 0.613 | 0.837 | 0.143 |
| d 14             | 18.1<sup>c</sup> | 19.1<sup>bc</sup> | 18.0<sup>c</sup> | 19.6<sup>bc</sup> | 18.7<sup>bc</sup> | 19.6<sup>c</sup> | 0.345 | 0.001 | 0.388 | 0.043 | 0.354 | 0.914 |
| d 28             | 32.0<sup>c</sup> | 34.7<sup>bc</sup> | 31.3<sup>c</sup> | 35.7<sup>a</sup> | 32.8<sup>bc</sup> | 36.1<sup>a</sup> | 0.748 | 0.001 | 0.832 | 0.135 | 0.265 | 0.706 |
| d 42             | 53.3<sup>bc</sup> | 56.4<sup>ab</sup> | 52.1<sup>c</sup> | 57.6<sup>a</sup> | 54.6<sup>abc</sup> | 56.9<sup>a</sup> | 1.152 | 0.001 | 0.988 | 0.438 | 0.289 | 0.728 |

<sup>1</sup><sup>ab</sup> Means within the same row with different superscripts differ (P ≤ 0.05).

<sup>1</sup>A total of 300 pigs (DNA 200 × 400) were used in a 42-d study with 5 pigs per pen and 10 replications per treatment. On d 14 and 28, antibiotics were removed from the diet according to FDA regulations. Experimental diets containing antibiotics resumed feeding on d 15 and 29.<n
<sup>2</sup>CTC-50 (Zoetis Services, LLC., Florham Park, NJ) added at 0.40% of the diet.<n
<sup>3</sup>Bioplus 2B (Chr. Hansen USA, Inc., Milwaukee, WI) added at 0.05% of the diet.<n
<sup>4</sup>Poultry Star (Biomin America, Inc., San Antonio, TX) added at 0.05% of the diet.