Growth performance and antibiotic tolerance patterns of nursery and finishing pigs fed growth-promoting levels of antibiotics

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Abstract
The objective of this study was to evaluate the impact of growth-promoting levels of antibiotics in diets for nursery and finishing pigs on growth performance and antibiotic tolerance patterns. Gilts (n=200, initial body weight was 6.2±0.003 kg), were allotted based on body weight to one of four treatments in a 2×2 factorial randomized complete block design. Nursery treatments consisted of feeding no antibiotics (CON) or an antibiotic diet (ANTI) containing chlortetracycline (CTC; 55 mg/kg). At the end of the nursery phase, one-half of the pigs receiving CON were switched to a diet containing antibiotic (virginiamycin; VIR, 11 mg/kg) and one-half of the pigs receiving ANTI were switched to CON for the remainder of the trial. This created four treatments for the finishing phase, consisting of: control in nursery and finishing (CC), antibiotic in nursery, control in finishing (AC), antibiotic in nursery, antibiotic in finishing (CA), or antibiotics throughout (AT). The pigs were weighed at the diet changes during the nursery (weeks 1, 3, and 5) and finishing (weeks 7, 9, 13, 17, and 20) phases. Fecal samples were collected at all diet changes for isolation of fecal coliforms and Enterococcus and subsequently tested for tolerance to CTC and VIR.

After 1 week, CON pigs weighed less (7.09 vs. 7.28 kg) and had lower ADG (149 vs. 180 g/day) and ADFI (174 vs. 192 g/day) than ANTI pigs (P<0.05). No performance differences were observed during the remainder of the study. At the initiation of the study (week 0), the ability of coliforms to grow in the presence of CTC and VIR, respectively, were 68 and 73% and increased to 90 and 96% at week 19 (time effect, P<0.001). At week 17, tolerance of coliforms to CTC was greater for CA (98%) than AC (96%, time×treatment effect, P<0.004). Enterococcus tolerance to CTC at week 7 was lower for CC (55%) compared to AT (76%), AC (74%) and CA (83%, time×treatment effect, P<0.001). At week 9, Enterococcus tolerant to CTC and VIR, respectively, was lower for CC (15 and 18%) than AT (31 and 40%), AC (35 and 35%), and CA (44 and 43%, time×treatment effect, P<0.001). Antibiotic growth promoters had little impact on growth performance in clean, isolated facilities with high labor inputs. The tolerance of bacteria to antibiotics fluctuated over time and persisted regardless of the use of antibiotic growth promoters.

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1. Introduction

The use of antibiotics in swine feeds has come under serious scrutiny in the last few years due to concerns about their potential effects on antibiotic resistance in humans. This has led to a ban of growth-promoting antibiotics in Sweden in 1986, in Denmark in finishing pigs in 1995 and all pigs in 1999, and the decision by the EU to ban five antibiotics for growth promotion...
in 1999. These decisions were largely influenced by public and political opinions and the use of antibiotics for growth promotion in the U.S. may be influenced similarly. Particularly considering the export market, the demand for antibiotic-free pork (whether it is produced completely without antibiotics or without subtherapeutic levels of antibiotics) is expected to become more prevalent.

Considering data collected from 1978 to 1985, Zimmerman (1986) reported an average improvement in daily gain of 15% in nursery pigs and 3.6% in finishing pigs when antibiotics were included in the feed. Feed efficiency was improved by 6.5% in nursery pigs and 2.4% in finishing pigs due to antibiotics. Effects may be different depending on factors such as disease status of the herd and management. Cromwell (2001) summarized that the improvements in daily gain and feed efficiency may be two times greater in farm experiments compared to research station tests. In addition, mortality rate was reduced from 15.6% to 3.1% in field trials under high disease levels compared to a decrease from 4.3% to 2.0% in low disease conditions with the addition of antibiotics. Our previous research (See et al., 1997) showed an improvement of 1.8 and 2.8% in feed efficiency of finishing pigs in a clean environment when fed two different antibiotics.

The objectives of the current experiment were to determine the effects of growth-promoting antibiotics on performance of nursery and finishing pigs obtained from a commercial source and to determine antibiotic tolerance patterns in response to antibiotics during the nursery and finishing stages.

2. Materials and methods

2.1. General procedures

All experimental procedures were approved for use by the North Carolina State University Institutional Animal Care and Use Committee. Female pigs selected from sow farms with known health status were purchased from a commercial swine production company in North Carolina and shipped to the Swine Evaluation Station in Clayton, NC. The herd had a prolonged history of PRRS virus infection and marginal production and had returned to normal levels of production before the initiation of this study. The sows from which the pigs were selected were not fed antibiotics, the pigs did not receive creep feed, and no antibiotic injections were given to pigs prior to the initiation of this study.

Minor cuts or scrapes were treated with iodine. Any pigs determined to need medication for treatment of minor cuts or scrapes had to wait 15 min before being weighed. Pigs were housed ve pigs per pen using 40 pens. A solid concrete wall separated pens receiving different dietary treatments during the phase. All pigs were weighed and feed intake was measured in 4-week intervals to coincide with the diet phase changes, which occurred as follows: the phase I diet was fed from 22.7 to 45.4 kg, phase II from 45.4 to 68.0 kg, phase III from 68.0 to 90.7 kg, and phase IV from 90.7 to 113.4 kg (Table 1).

Ultrasound measurements to determine backfat, and longissimus muscle area (LMA) were taken at the end of the trial. All diets were manufactured at an FDA licensed commercial feed mill using stringent sequencing and standard operating procedure, any time antibiotic containing diets were color coded using red iron oxide to ensure proper delivery and feeding. Control diet was then transported in bulk. Upon bulk delivery, approximately 50 kg of ground corn (10% of the mixer capacity). All diets were manufactured at an FDA licensed commercial feed mill using stringent sequencing and flushing procedures to avoid cross-contamination of antibiotic growth promoters between treatments. Thus, control diets were always manufactured first, followed by diets containing antibiotic. As a standard operating procedure, any time antibiotic containing diets were manufactured, the mixer, auger system, bagging unit, and bulk delivery system were flushed with approximately 300 kg of ground corn (10% of the mixer capacity). All nursery diets were bagged in 22.5 kg bags. Finisher diets were transported in bulk. Upon bulk delivery, approximately 50 kg of control diet was used to flush the bottom and auger system of the truck and discarded to minimize any potential cross-contamination of diets due to transport. Control diet was then augmented into the designated control feed bin first, followed by the antibiotic containing diet into its designated feed bin. All antibiotic containing diets were color coded using red iron oxide to ensure proper delivery and feeding.

This experiment was designed to monitor effects of feeding growth-promoting levels of antibiotics; therefore, every effort was made to limit medications that would affect the results. Minor cuts or scrapes were treated with iodine. Any pigs determined to need medication for treatment of

2.2. Performance

During the 5-week nursery phase, gilts were assigned to one of two dietary treatments: 1) negative control diet (CON) without antibiotic or 2) an antibiotic diet (ANTI) containing growth-promoting levels of chlortetracycline (CTC; 55 mg/kg; Aureomycin 90, Alpharma Inc., Fort Lee, NJ; Table 1). The antibiotic treatment was selected based on its widespread use as a growth-promotant in the swine industry as reported by the Animal and Plant Health Inspection Service, USDA (2002). Pigs were fed a three-phase dietary nursery program designed to be representative of diets used in the swine industry (1.6, 1.4, and 1.2% total lysine for the prestarter, starter I and starter II diets, respectively) and were provided in pelleted form. Diets were not supplemented with growth-promoting levels of Cu and Zn in order to provide the greatest opportunity to evaluate the effects of antibiotics. Pig performance (body weight, feed intake, daily gain, and gain-to-feed ratio) was determined at each diet change.

At the end of the nursery phase, one-half of the pigs receiving CON were switched to a diet containing antibiotic (virginiamycin; VIR, 11 mg/kg; Stafac 10, Phibro Animal Health, West Caldwell, NJ) and one-half of the pigs receiving ANTI were switched to CON for the remainder of the trial. This created four dietary treatments for the finishing phase, consisting of: control in nursery and finishing (CC), antibiotic in nursery, control in finishing (AC), control in nursery, antibiotic in finishing (CA), or antibiotics throughout (AT). Pigs were fed a four phase diet program during the finishing phase (1.05, 0.95, 0.85, and 0.75% total lysine for diet phase I to IV, respectively) which lasted 16 weeks. Finishing pigs weighed and feed intake was measured in 4-week intervals to coincide with the diet phase changes, which occurred as follows: the phase I diet was fed from 22.7 to 45.4 kg, phase II from 45.4 to 68.0 kg, phase III from 68.0 to 90.7 kg, and phase IV from 90.7 to 113.4 kg (Table 1). Ultrasound measurements to determine backfat, and longissimus muscle area (LMA) were taken at the end of the trial.

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This experiment was designed to monitor effects of feeding growth-promoting levels of antibiotics; therefore, every effort was made to limit medications that would affect the results. Minor cuts or scrapes were treated with iodine. Any pigs determined to need medication for treatment of
scours, cough, lameness, prolapse, or other reasons were removed from the study.

2.3. Collection of fecal samples

Fecal samples were taken from pigs in each pen at the diet changes during the nursery phase, 2 weeks after the nursery phase and at the diet changes during the finishing phase for the entire study period. Feces was collected by inserting a gloved finger into the rectum of the pig, placing the feces into a sterile Whirl-pak bag and storing on ice until being processed within 6 h of collection.

2.4. Isolation of E. coli and Enterococcus

One gram of homogenized feces was removed from the Whirl-pak bag, serially diluted in sterile water and streaked on MacConkey and m-Enterococcus agar (Baltimore Biologics Laboratory, Sparks, MD) plates for the isolation of E. coli and Enterococci, respectively. E. coli and Enterococci were chosen for evaluation because these are the most common indicators of selective pressure of antibiotic usage (Anderson et al., 2003). The plates were incubated at 37 °C for 24 h. A target number of forty-eight colonies of each bacterium representing each composite sample were selected and transferred to 96-well microplates. Colonies that appeared pink on the MacConkey’s agar were presumed to be E. coli and were transferred to 96-well microplates containing Colilert (Idexx, Westbrook, ME) for confirmation. After incubation for 24 h, those microwells that turned yellow and fluoresced under UV light confirmed the presence of E. coli. Bacterial isolates that grew into a deep red color on m-Enterococcus agar were presumed to be Enterococcus. The deep red colonies were transferred to 96-well microplates containing Enterococcosel broth (Becton Dickison, Sparks, MD). After incubation for 24 h at 37 °C, colonies that turned the broth black confirmed presence of Enterococcus. Bacterial colonies were then stored in the 96-well microplates at 4 °C until further analysis.

### Table 1

Composition of experimental diets fed throughout the study (as fed basis).

<table>
<thead>
<tr>
<th>Time (weeks)</th>
<th>Pre-starter</th>
<th>Starter 1</th>
<th>Starter 2</th>
<th>Grower 1</th>
<th>Grower 2</th>
<th>Finisher 1</th>
<th>Finisher 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–1</td>
<td>29.30</td>
<td>54.22</td>
<td>50.94</td>
<td>59.29</td>
<td>63.35</td>
<td>66.75</td>
<td>69.78</td>
</tr>
<tr>
<td>1–3</td>
<td>16.00</td>
<td>25.00</td>
<td>28.04</td>
<td>23.26</td>
<td>19.27</td>
<td>15.94</td>
<td>12.94</td>
</tr>
<tr>
<td>3–5</td>
<td>–</td>
<td>–</td>
<td>10.00</td>
<td>10.00</td>
<td>10.00</td>
<td>10.00</td>
<td>10.00</td>
</tr>
<tr>
<td>5–9</td>
<td>17.17</td>
<td>0.26</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>9–13</td>
<td>10.00</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>13–17</td>
<td>7.72</td>
<td>8.32</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>17–20</td>
<td>4.00</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

- **Ingredients**
  - Corn
  - Soybean meal, dehulled
  - Wheat middlings
  - Dried whey
  - Oat groats
  - Dried whey permeate
  - Poultry meal
  - Animal fat
  - Soy protein concentrate
  - Fish meal
  - Dicalcium phosphate
  - Calcium carbonate
  - Salt
  - L-lysine-HCl
  - DL-methionine
  - Se premix
  - Trace mineral premix
  - Vitamin premix
  - Starter vitamin
  - Choline chloride
  - L-tryptophan
  - L-threonine
  - Vitamin E premix
  - Aureomycin 90
  - Stafac
  - Ferric oxide

- **Laboratory analysis**
  - ME, Mcal/kg
  - Crude Protein, %
  - Calcium, %
  - Phosphorus, %
  - Lysine, %

1. Provided 0.3 ppm Se in the final diet.
2. Provided 20 ppm Cu, 0.75 ppm I, 70 ppm Fe, 50 ppm Mn, and 90 ppm Zn in the final diet.
3. Provided 1300 IU/kg vitamin A, 230 IU/kg vitamin D, 22.7 IU/kg vitamin E, 4.4 mg/kg menadione, 0.22 mg/kg biotin, 1.25 mg/kg folic acid, 43 mg/kg niacin, 215 mg/kg pantothenic acid, 0.1 mg/kg pyridoxine, 0.033 mg/kg vitamin B12 in the final diet.
4. Included in antibiotic containing diets only. Ferric oxide was used as a coloring agent to identify the antibiotic containing diets.
5. Provided 55 mg chlortetracycline/kg diet.
6. Provided 11 mg virginiamycin/kg diet.
7. Calculated values.
2.5. Antibiotic tolerance patterns

The procedures used in this study to test the ability of bacteria to grow in the presence of an antibiotic were adapted from Antibiotic Resistance Analysis (ARA) procedures described by Wiggins et al. (1999). Briefly, E. coli and Enterococci isolates were transferred using a stainless steel 48-prong replica plater (Sigma Chemical Co., St. Louis, MO) from the 96-well microplates and inoculated onto trypticase soy agar (Becton Dickinson, Sparks, MD) plates containing CTC at 60, 80, and 100 μg/mL, and VIR at 8, 16, and 32 μg/mL, for testing the tolerance of bacteria to grow on the culture media at all sample periods. In addition, isolates were inoculated onto a control plate containing no antibiotic. After inoculation, these plates were incubated for 24 h at 37 °C. An isolate was considered tolerant to a given concentration of antibiotic if growth of that isolate was comparable to the growth on the control plate. This method allowed for the generation of an antibiotic tolerance pattern of bacteria in swine feces which can be used for tracking of nonpoint pollution sources.

2.6. Statistical analyses

Performance data were analyzed as a randomized complete block design using the GLM procedures (SAS Ins., Cary, NC) with pen as the experimental unit. The model included block and treatment. Repeated measures in time were used for BW, ADG, ADFI, and G:F. Antibiotic tolerance data were analyzed as a complete randomized design using the MIXED procedures of SAS (1998). The model included treatment, level of antibiotic, time, and all appropriate interactions. Time was used as a repeated measure. Significance was declared at $P<0.05$ for all variables measured. A significant time × treatment interaction was observed and showed differences between treatments for total number of fecal coliforms, total number of Enterococcus, and percent of fecal coliforms that grew in the presence of VIR at the initial sampling period. Therefore, these values were used as covariates in further analyses.

3. Results

3.1. Growth performance

Analysis of the nursery diets for antibiotic concentrations indicated that the antibiotic containing prestarter diet contained a lower level of CTC (37.3 mg/kg) than targeted (55 mg/kg). In addition, the starter I and starter II CON diets were found to contain low levels of CTC (3.43 and 5.57 mg/kg, respectively), which was not expected. All other diets fed during the nursery phase contained the proper amount of antibiotic or no antibiotic. Extreme care was taken to avoid cross-contamination during diet manufacturing. Control diets were always manufactured first and all nursery diets were bagged, which should have eliminated any possible contamination. During the nursery phase, 3 pigs were removed from the CON treatment and 2 pigs were removed from the ANTI treatment due to medical treatment.

Performance data during the nursery phase are summarized in Table 2. After 1 week, pigs fed no antibiotics (CON) weighed less (7.09 vs. 7.29 kg) and had lower ADG (149 vs. 180 g/day) and ADFI (174 vs. 192 g/day) and G:F was greater than pigs fed antibiotics (ANTI). No performance differences were found at weeks 3 and 5 of the nursery, or for the overall nursery period. Analysis of the antibiotic containing finishing diets indicated that the actual concentration of virginiamycin was slightly lower (7.48 mg/kg) than what was targeted (11 mg/kg). Analysis of control diets showed a small amount of virginiamycin in the phase I finishing diet (1.97 mg/kg), however virginiamycin was undetectable in the other control diets. In the finishing phase, pigs removed from the trial due to medical treatment were as follows: 3 from CC, 2 from AT, 1 from AC, and 0 from CA. During the finishing period, no differences were found in ADG, ADFI or G:F between any treatment groups (Table 3). Backfat and LMA measurements were not different between treatments.

3.2. Antibiotic tolerance patterns

While culturing fecal samples, isolates that were selected from MacConkey’s agar and considered to be E. coli did not always fluoresce under UV light after incubation in Colilert. We could not confirm that every isolate was, in fact, E. coli; therefore data are reported as fecal coliforms. There was no effect of dietary treatment ($P=0.093$) on total number of fecal coliforms isolated (data not shown). A time effect ($P<0.001$) showed that number of fecal coliforms decreased from weeks 0 to 3 and remained relatively consistent throughout week 19, with the exception of an increased number of isolates on week 7 (mean coliform counts ×10⁶ were 119.6, 55.3, 3.4, 6.8, 43.8, 2.9, 4.4, 3.4, and 8.9 for weeks 0, 1, 3, 5, 7, 9, 13, 17, and 19, respectively).

No interactive effects on CTC or VIR tolerance were observed between the level of antibiotic used in the culture medium and dietary treatment. Therefore, main effects are presented and discussed.

### Table 2

<table>
<thead>
<tr>
<th>Treatment</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
</tr>
<tr>
<td>Antibiotic</td>
<td></td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td></td>
</tr>
<tr>
<td>Initial</td>
<td>6.19</td>
</tr>
<tr>
<td>Week 1</td>
<td>7.09&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Week 3</td>
<td>12.4</td>
</tr>
<tr>
<td>Week 5</td>
<td>21.9</td>
</tr>
<tr>
<td>ADG (kg)</td>
<td></td>
</tr>
<tr>
<td>Weeks 0–1</td>
<td>0.15&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Weeks 1–3</td>
<td>0.38</td>
</tr>
<tr>
<td>Weeks 3–5</td>
<td>0.67</td>
</tr>
<tr>
<td>Weeks 0–5</td>
<td>0.40</td>
</tr>
<tr>
<td>ADFI (kg)</td>
<td></td>
</tr>
<tr>
<td>Weeks 0–1</td>
<td>0.17&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Weeks 1–3</td>
<td>0.48</td>
</tr>
<tr>
<td>Weeks 3–5</td>
<td>0.80</td>
</tr>
<tr>
<td>Weeks 0–5</td>
<td>0.48</td>
</tr>
<tr>
<td>Gain:feed</td>
<td></td>
</tr>
<tr>
<td>Weeks 0–1</td>
<td>0.85&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Weeks 1–3</td>
<td>0.79</td>
</tr>
<tr>
<td>Weeks 3–5</td>
<td>0.85</td>
</tr>
<tr>
<td>Weeks 0–5</td>
<td>0.83</td>
</tr>
</tbody>
</table>

<sup>a,b</sup> Means without a common superscript within a row are different ($P=0.05$).

<sup>c,d</sup> Means without a common superscript within a row are different ($P=0.07$).

<sup>1</sup> Values are means of 20 pens with 5 pigs per pen.

<sup>2</sup> Chlorotetracycline at 55 mg/kg of complete diet.
The time×treatment interaction is primarily explained by the observation that pigs fed CC had an increase in the amount of fecal coliforms that grew in the presence of VIR from week 5 to week 7, whereas all other treatments showed a decrease from week 5 to week 7, with the AT treatment decreasing to dramatically lower levels than all others.

There was no effect of dietary treatment on number of Enterococcus isolated from fecal samples. Number of Enterococcus decreased during the nursery phase and then remained relatively constant throughout the duration of the study (mean Enterococcus counts×10^5 were 29.5, 10.5, 1.0, 0.8, 1.3, 4.3, 1.4, and 0.7 for weeks 0, 1, 3, 5, 7, 9, 13, 17, and 19, respectively; time effect, P = 0.049). There was an expected effect of antibiotic level in the culture medium (P < 0.001 on percent of Enterococcus that grew in the presence of CTC and VIR. Increasing level of antibiotic decreased tolerance. Percent of Enterococcus tolerant to CTC and VIR also showed a significant effect of time (P < 0.001) and a time×treatment interaction (P < 0.001; Fig. 2A and B). The time×treatment interaction for percent of Enterococcus that grew in the presence of CTC and VIR is explained by the observation that pigs fed the CC treatment had the lowest amount of Enterococcus tolerant to CTC and VIR at weeks 5, 7, and 9 compared to all other treatments, whereas tolerance of Enterococcus to CTC and VIR increased dramatically from week 9 to week 13 for pigs fed CC to levels greater than pigs fed AT and CA.

### 4. Discussion

While we were able to observe differences in performance during the first week of the study, we were unable to detect differences in ADG, ADFI, and GF for the remainder of the nursery phase. Our results do not fully agree with previous research on growth performance of pigs fed antibiotics compared to those fed no antibiotics during the nursery phase (Cromwell, 2002). Roof and Mahan (1982) found that carbadox improved weaned pig growth and feed intake over 5 weeks, however, the effects were most prominent in the first 2 weeks. It has been reported that improvements in growth performance of pigs due to the use of antibiotic growth promoters is often greater in field trials as compared to research station trials (Cromwell, 2002) as well as in pigs with a high level of immune activity (Hardy, 2002). It should be noted that the growth rates of all pigs in the present study were very good, and this reflects a high health status of the pigs. In this instance, we would not expect antibiotic growth promoters to have a large effect on growth performance. We also found no impact of growth-promoting antibiotics on performance of gilts during the finishing phase. Moser et al. (1985) found that virginiamycin did not affect growth performance of finishing pigs. Weber et al. (2001) reported no carry-over effects on growth of finishing pigs when fed growth-promoting antibiotics in the nursery.

### Table 3

<table>
<thead>
<tr>
<th>Treatment</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC</td>
<td>21.3 22.5 20.6 21.6 0.75</td>
</tr>
<tr>
<td>CA</td>
<td>40.1 41.6 39.8 40.3 1.17</td>
</tr>
<tr>
<td>AC</td>
<td>67.1 70.2 66.3 67.5 1.47</td>
</tr>
<tr>
<td>AT</td>
<td>95.5 97.1 94.8 95.2 1.81</td>
</tr>
<tr>
<td>Week 0</td>
<td>119.6 120.7 118.9 118.5 1.81</td>
</tr>
</tbody>
</table>

1. Values are means of 10 pens with 5 pigs per pen. No differences were observed between treatments for any measure (P > 0.05).
2. Treatments were as follows: control diet without antibiotics in nursery and finishing (CC); antibiotic (chlortetracycline, 55 mg/kg) in nursery, control in finishing (AC); control in nursery, antibiotic (virginiamycin, 11 mg/kg) in finishing (CA); or antibiotics throughout (AT).

Samples collected at the beginning of the trial (week 0) showed that a large percentage of the coliform bacteria isolated was able to grow in the presence of CTC and VIR. Differences between treatments in baseline percentage of fecal coliforms that grew in the presence of VIR were observed, therefore, this percentage was used as a covariate in subsequent analysis of tolerance of coliforms to VIR. As expected, there was an effect (P < 0.05) of antibiotic level used in the culture medium on percent of fecal coliforms tolerant to CTC and VIR. As level of antibiotic increased, percent of fecal coliforms tolerant to the antibiotic decreased. Regardless of dietary treatment, percent of fecal coliforms that were able to grow in the presence of CTC was 73% at 60 μg/mL, 70.5% at 80 μg/mL, and 66% at 100 μg/mL. Similarly, the percent of fecal coliforms that grew in the presence of VIR was 79% at 8 μg/mL, 76% at 16 μg/mL, and 72% at 32 μg/mL. A significant effect of time (P < 0.001) and a treatment×time interaction (P < 0.05) was noted for percent of fecal coliforms tolerant to CTC (Fig. 1A) and VIR (Fig. 1B). The time effect indicates that percent of fecal coliforms exhibiting tolerance to CTC decreased after 1 week, and tolerance to both CTC and VIR gradually increased to the end of the nursery phase, at which point the antibiotic regimen was changed. Tolerance to both antibiotics decreased from week 5 to week 7, and then gradually increased to the end of the study for all treatments. The time×treatment interaction is primarily explained by the
Escherichia coli are the predominant isolate in the fecal microflora of animals, and have the ability to transfer antibiotic resistance genes to other species of bacteria (Anderson et al., 2003). In the current study, we observed that a high level of fecal coliforms isolated from nursery pigs were able to grow in the presence of CTC and VIR even at the beginning of the study. These pigs received no antibiotic treatments before weaning and came from a farrowing facility in which antibiotics were not fed to the sows. Langlois et al. (1988) found that 59% of fecal coliforms isolated from pigs not exposed to antibiotics for 13 months were resistant to tetracycline. After the first week in the nursery, percent of fecal coliforms able to grow in the presence of CTC and VIR decreased for all treatment groups.

While Enterococcus isolated from CC pigs showed the lowest tolerance to antibiotics, Enterococcus isolated from pigs fed CA and AC treatments exhibited the highest tolerance. This was unexpected, since CA pigs were fed no antibiotic growth promoters during the nursery period. Factors other than exposure to antibiotics can lead to increased shedding of antibiotic-resistant bacteria. Mathew et al. (2003) found that various environmental stressors such as cold stress, heat stress, overcrowding, and intermingling affected apramycin resistance in E. coli isolated from pigs. At the end of the nursery phase of the present study, the growth promoter used in the diet was switched from CTC to VIR. When measured 2 weeks after the switch (week 7), percent of fecal coliforms tolerant to CTC and VIR decreased for all treatment groups except CC. Research has shown that when the use of one antibiotic is discontinued, resistance to other antibiotics may also decline (Salyers, 2002). Fecal coliform tolerance to CTC and VIR gradually increased from week 7 to the end of the present study for all treatment groups. Similarly, Salyers (2002) reported that a change in antibiotic regimen may only decrease resistance to a low level, and when an organism is re-exposed to an antibiotic, resistance can increase quickly.

Fig. 1. Percentage of fecal coliforms that were able to grow on culture media containing chlortetracycline (CTC; A) and virginiamycin (VIR; B). Fecal coliforms were isolated from pigs fed control diets without antibiotics in the nursery and finishing phase (CC, diamond with dotted line), control diets in the nursery and antibiotic (virginiamycin at 11 mg/kg) in the finishing phase (CA, squares with long dash), antibiotic in the nursery phase (chlortetracycline at 55 mg/kg) and control in the finishing phase (AC, triangles with short dash), or antibiotics in the nursery and finishing phase (AT, circle with solid line). The arrow signifies the change in antibiotics from CTC to VIR at week 5. A: time effect (P < 0.001), time × treatment interaction (P = 0.009), PSEM = 6.45. B: time effect (P < 0.001), time × treatment interaction (P < 0.05), PSEM = 2.35. a,bDifferent superscripts indicate means are different within time period (P < 0.05).
data collection and animal management throughout the study. Additionally, pigs were housed such that contact between pigs receiving different treatment diets was not possible. Langlois et al. (1988) reported that 40.5% of fecal coliforms isolated from pigs after 126 months with no antibiotic exposure were resistant to tetracycline. The use of medication in pigs over several years results in increased amounts of resistant bacteria which can be passed from one generation to the next, and resistant bacteria that become stabilized in the gastrointestinal tract, may eventually dominate the intestinal flora (Langlois et al., 1988). We cannot discount, however, that the small amounts of antibiotics detected in the starter I and starter II CON diets (3.43 and 5.57 mg/kg CTC, respectively) as well as in the phase I CON finishing diet (1.97 mg/kg) fed to CC pigs may have impacted the antibiotic tolerance patterns that were observed for this treatment group. All diets were mixed at a commercial feed mill using strict quality control measures that should have eliminated the possibility of cross-contamination between diets. Specifically, control diets were always manufactured first and handled in bags for the nursery diets and bulk for the finishing diets. Following the manufacturing of the antibiotic containing diets, the mixer and feed handling equipment was flushed with ground corn (10% of the mixer capacity) to clean the system. Similarly, control feed was used to flush the feed delivery system of the bulk feed truck and discarded prior to unloading the control feed first in specifically designated feed bins, followed by the antibiotic containing feed. Further, antibiotic containing feed was color coded to ensure that the proper feed was always presented to the appropriate pens of pigs. It is possible that even the small amount of antibiotic detected in some of the feeding phases was enough to allow resistant bacteria to develop and proliferate in the gut of pigs.

The commensal bacteria, Enterococci, are also able to serve as a reservoir of antibiotic resistance genes (Jackson et al., 2004). Similar to fecal coliforms, a large percentage of Enterococcus isolated from pigs at the beginning of the study exhibited the ability to grow in the presence of CTC and VIR, although pigs in the nursery were only fed CTC. Tolerance of Enterococcus to both antibiotics remained high throughout the nursery until week 5, at which time pigs

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**Fig. 2.** Percentage of Enterococcus that were able to grow on culture media containing chlortetracycline (CTC; A) and virginiamycin (VIR; B). Enterococcus were isolated from pigs fed control diets without antibiotics in the nursery and finishing phase (CC, diamond with dotted line), control diets in the nursery and antibiotic (virginiamycin at 11 mg/kg) in the finishing phase (CA, squares with long dash), antibiotic in the nursery phase (chlortetracycline at 55 mg/kg) and control in the finishing phase (AC, triangles with short dash), or antibiotics in the nursery and finishing phase (AT, circle with solid line). The arrow signifies the change in antibiotics from CTC to VIR at week 5. A: time effect (P<0.001), time × treatment interaction (P<0.001), PSEM = 5.72. B: time effect (P<0.001), time × treatment interaction (P<0.05), PSEM = 5.77. a,b Different superscripts indicate means are different within time period (P<0.05).
receiving the AT treatment had a larger percentage of Enterococcus able to grow in the presence of antibiotics than pigs receiving the CC treatment. Aarestrup et al. (2001) reported Enterococcus resistance to VIR was between 40–60% for pigs fed VIR during a three year time period. In the current study, when CTC was removed and VIR was included as the growth-promoting antibiotic, Enterococcus tolerance to both CTC and VIR decreased for all treatment groups from weeks 5 to 9. Enterococcus able to grow in the presence of both CTC and VIR then increased from week 9 to the end of the study. Enterococcus faecalis is intrinsically resistant to VIR (Aarestrup et al., 2001), and our culture media could not select between different species of Enterococcus. Therefore, it is possible that the decrease in resistance from week 5 to week 9 was due to a decrease in Enterococcus species susceptible to VIR, and by week 9, E. faecalis may have proliferated in the gut of the pigs and caused the noticeable increase in resistance.

5. Conclusion

The results of this study showed that the use of antibiotic growth promoters in clean, well-managed facilities did not improve growth performance of growing and finishing pigs. However, we observed increased ADG and ADFI of pigs receiving growth-promoting antibiotics in the first week after weaning, a time of high stress for pigs. Understanding difference scenarios in which antibiotics improve growth will help determine their usefulness in swine production. The results further indicate that tolerance of indicator bacteria to antibiotics develop and persist over time regardless of the use of antibiotic growth promoters.

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