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Effects of Tylosin Administration Routes on the Development of Antimicrobial Resistance in Fecal Enterococci of Finishing Swine

Abstract

Antibiotics can be administered via various routes in pigs, which may influence antimicrobial resistance development. A total of 40 barrows and 40 gilts (Line 600 × 241; DNA, Columbus, NE; initially 207 ± 7.9 lb) were used in a 35-d trial to determine the effects of tylosin administration route on pig growth performance and development of antimicrobial resistance in fecal *Enterococcus* spp. isolates. Pens of pigs (1 pig/ pen, 20 pigs/treatment) were blocked by initial body weight (BW) and gender. Within blocks, pens were randomly allotted to 1 of 4 treatments. The antibiotic treatments followed US label directions and were: 1) no antibiotic (Control); 2) 110 mg tylosin per kg of feed for 21 d (Feed); 3) 8.82 mg tylosin per kg of BW through intramuscular injection twice daily for the first 3 d of each wk during the 3-wk treatment period (Injection); and 4) 66 mg of tylosin per liter of drinking water for the first 3 d of each wk during treatment period (Water). Treatments were offered during d 0 to 21, after which all pigs were fed a common diet with no antibiotic until d 35. Fecal samples were collected on d 0, 21, and 35. No evidence for route × gender interactions ($P > 0.55$) were observed for any growth responses. From d 0 to 21, control pigs and pigs fed medicated feed had greater ($P < 0.05$) average daily gain (ADG) than those that received injected tylosin, with the ADG of pigs receiving tylosin through the water intermediate. There was no evidence for different average daily feed intake (ADFI) among treatment groups. Pigs that received tylosin through injection or water had poorer ($P < 0.05$) feed efficiency (F/G) compared with control pigs, but there was no evidence for difference from pigs receiving tylosin through feed. Among the medicated pigs, total tylosin dose administered was the greatest through injection, second highest through feed, with the water medication route the lowest. No evidence for route × day interactions ($P > 0.23$) were observed for the development of bacterial resistance to any antibiotics. Enterococcal isolates collected from pigs receiving tylosin via feed or injection were more resistant ($P < 0.05$) to erythromycin and tylosin compared with control pigs and those that received tylosin through water. The estimated probability of antimicrobial resistance to these 2 antibiotics was greater on d 21 and 35 than d 0. In summary, tylosin injection resulted in poorer ADG and F/G of finishing pigs, likely due to stress associated with handling and injection. Tylosin administration through injection and feed resulted in greater probability of enterococcal resistance to erythromycin and tylosin compared with in-water treatment, which is likely a combined effect of administration route and dosage.

Keywords

administration route, antimicrobial resistance, fecal enterococci, finishing pig, growth performance, tylosin

Cover Page Footnote

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Authors

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K-STATE Research and Extension

Swine Day 2018

Effects of Tylosin Administration Routes on the Development of Antimicrobial Resistance in Fecal Enterococci of Finishing Swine[1]

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Summary

Antibiotics can be administered via various routes in pigs, which may influence antimicrobial resistance development. A total of 40 barrows and 40 gilts (Line 600 × 241; DNA, Columbus, NE; initially 207 ± 7.9 lb) were used in a 35-d trial to determine the effects of tylosin administration route on pig growth performance and development of antimicrobial resistance in fecal *Enterococcus* spp. isolates. Pens of pigs (1 pig/pen, 20 pigs/treatment) were blocked by initial body weight (BW) and gender. Within blocks, pens were randomly allotted to 1 of 4 treatments. The antibiotic treatments followed US label directions and were: 1) no antibiotic (Control); 2) 110 mg tylosin per kg of feed for 21 d (Feed); 3) 8.82 mg tylosin per kg of BW through intramuscular injection twice daily for the first 3 d of each wk during the 3-wk treatment period (Injection); and 4) 66 mg of tylosin per liter of drinking water for the first 3 d of each wk during treatment period (Water). Treatments were offered during d 0 to 21, after which all pigs were fed a common diet with no antibiotic until d 35. Fecal samples were collected on d 0, 21, and 35. No evidence for route × gender interactions ($P > 0.55$) were observed for any growth responses. From d 0 to 21, control pigs and pigs fed medicated feed had greater ($P < 0.05$) average daily gain (ADG) than those that received injected tylosin, with the ADG of pigs receiving tylosin through the water intermediate. There was no evidence for different average daily feed intake (ADFI) among treatment groups. Pigs that received tylosin through injection or water had poorer ($P < 0.05$) feed efficiency (F/G) compared with control pigs, but there was no evidence for difference from pigs receiving tylosin through feed. Among the medicated pigs, total tylosin dose administered was the greatest through injection, second highest through feed, with the water medication route the lowest. No evidence for route × day interactions ($P > 0.23$) were observed for the development of bacterial resistance to any antibiotics. Enterococcal isolates collected from pigs receiving tylosin via feed or injection were more resistant

[1]This study was supported in part by a grant from the National Pork Board (#16-053).

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($P < 0.05$) to erythromycin and tylosin compared with control pigs and those that received tylosin through water. The estimated probability of antimicrobial resistance to these 2 antibiotics was greater on d 21 and 35 than d 0. In summary, tylosin injection resulted in poorer ADG and F/G of finishing pigs, likely due to stress associated with handling and injection. Tylosin administration through injection and feed resulted in greater probability of enterococcal resistance to erythromycin and tylosin compared with in-water treatment, which is likely a combined effect of administration route and dosage.

Introduction

Use of antimicrobial feed additives to promote growth has been a valuable tool for efficient swine production for the past six decades. However, the emergence of antimicrobial resistance constitutes a major public health concern. Therefore, in swine production systems, there is considerable interest and effort in identifying feeding and management practices that maintain and improve production efficiency without promoting the occurrence of antimicrobial resistance in bacteria.

Antibiotics are administered either in feed, in water, or parenterally. The oral route through either feed or water is by far the most common route of administration of antibiotics. Oral administration is more convenient when treating a large number of animals compared with individual pig treatment through the injectable route. Nevertheless, oral administration exposes gut bacteria directly to high concentrations of antibiotics and thus has been hypothesized to have a greater potential in promoting the emergence and amplification of antimicrobial resistance. However, to our knowledge, no study has been carried out to compare the impacts of oral administration through feed or water versus injectable antibiotic administration on the development of resistance in pigs. Therefore, the objective of this study was to determine the effects of tylosin administration route on the growth performance and the development of antimicrobial resistance in fecal enterococci of finishing pigs.

Procedures

The Kansas State University Institutional Animal Care and Use Committee approved the protocol used in this experiment. The study was conducted at the Kansas State University Swine Teaching and Research Center in Manhattan, KS. Pigs were housed in an environmentally controlled barn with a completely slatted concrete floor. Each pen (5 × 5 ft) was equipped with a single-hole stainless steel feeder and cup waterer for *ad libitum* access to feed and water. Each drinker was equipped with an individual water reservoir and water cup allowing for independent water treatment. Each 2 pens (1 barrow pen and 1 gilt pen sharing the same treatment) were segregated by solid pen dividers to minimize nasal contact and manure cross-contamination among pigs from different treatment groups.

A total of 40 barrows and 40 gilts (Line 600 × 241; DNA, Columbus, NE; initially 207 ± 7.9 lb) were individually housed and used in a 35-d trial. Pigs were individually weighed, blocked by initial BW, gender, and barn location, and assigned to pens 17 d prior to the start of the experiment. Early allotment was done in order to avoid pig movement across pens on d 0 and minimize cross-contamination for fecal sample collection. On d 0, experimental treatments were assigned to pens, which include:

1) pigs fed a corn-soybean meal-based diet (Table 1) with no antibiotic (Control); 2) pigs fed the control diet with 110 ppm of tylosin (100 g Tylan®100/ton of feed; Elanco Animal Health, Indianapolis, IN) per kg feed for 21 d (Feed); 3) pigs fed the control diet and received 8.82 mg injectable tylosin (1 mL Tylan®200/50 lb BW; Elanco Animal Health, Indianapolis, IN) per kg of BW twice daily for the first 3 d of each week during the 3-wk treatment period (Injection); and 4) pigs fed the control diet and received 66 mg of tylosin (Tylan®Soluble; Elanco Animal Health, Indianapolis, IN) per L of drinking water for the first 3 d of each week during the 3-wk treatment period (Water). Antibiotic treatments were terminated on d 21 and all pigs were fed a common diet from d 21 to 35.

Pigs were weighed and feed disappearance was recorded on d 0, 7, 14, 21, 28, and 35 to determine ADG, ADFI, and F/G. The water reservoir was weighed and refilled twice daily to determine daily water consumption for each pig. Previously, all pigs had received dietary chlortetracycline and tiamulin for approximately 14 d immediately after weaning; however, pigs were not treated by other antibiotics through feed or water from d 14 after weaning until the start of experimental treatments.

All diets were provided in meal form and were manufactured by the Kansas State University O.H. Kruse Feed Technology Innovation Center, Manhattan, KS. Complete diet samples were obtained and delivered to Kansas State University Swine Laboratory, Manhattan, KS, and stored at -4°F until analysis. Feed samples were analyzed for dry matter (DM), crude protein (CP), ether extract, calcium (Ca), and phosphorus (P) (Ward Laboratories, Inc., Kearney, NE).

Fecal samples were collected into individual Whirl-Pak bags (Nasco, Ft. Atkinson, WI) on d 0 (baseline), 21 (end of treatment period), and 35 (end of common period). Samples were transported on ice to the Molecular Epidemiology and Microbial Ecology laboratory at Kansas State University (Manhattan, KS) for bacterial isolation and antimicrobial susceptibility analysis.

Fecal samples were stored at 39.2°F prior to processing. Approximately 1 g of feces from each sample was suspended in 9 mL of phosphate buffer saline. Fifty microliters of the fecal suspension were then spread plated onto M-*Enterococcus* agar plates for the selective isolation of *Enterococcus* spp. from each fecal sample. Unless otherwise specified, all the culture media was obtained from Difco (Becton, Dickinson and Company, Sparks, MD). M-*Enterococcus* plates were incubated at 107.6°F for 24 to 36 h. Two putative colonies (pin-point red, pink, or metallic red) were selected from each M-*Enterococcus* agar; each of these colonies was individually streaked onto a blood agar plate (Remel, Lenexa, KS) and incubated at 98.6°F for 24 h. Preliminary genus confirmation of each of the enterococcal isolates was done by esculin hydrolysis. The 2 confirmed *Enterococcus* isolates per original fecal sample were preserved using cryo-protect beads (Cryocare; Key Scientific Products, Round Rock, TX) and stored at −112°F for future use.[5]

[5]Amachawadi, R. G., N. W. Shelton, X. Shi, J. Vinasco, S. S. Dritz, M. D. Tokach, J. L. Nelssen, H. M. Scott, and T. G. Nagaraja. 2011. Selection of *tcrB* gene mediated copper resistant fecal enterococci in pigs fed diets supplemented with copper. Appl. Environ. Microbiol. 77:5597–5603. doi:10.1128/AEM.00364-11.

The microbroth dilution method as outlined by the Clinical and Laboratory Standards Institute (2013)[6] was used on one of each *Enterococcus* spp. bacterial isolate per original fecal sample to determine the minimal inhibitory concentrations using a Sensititre (TREK Diagnostic Systems, Oakwood Village, OH) micro-broth dilution procedure. *Enterococcus* spp., bacterial isolate preserved in cryo-protect beads was streaked onto a blood agar plate and incubated at 98.6°F for 24 h. Individual colonies were selected and suspended in demineralized water (TREK Diagnostic Systems) and turbidity was adjusted to 0.5 McFarland turbidity standards. Then, 10 µL of the bacterial inoculum was added to Mueller–Hinton broth and vortexed to mix. A Sensititre automated inoculation delivery system (TREK Diagnostic Systems) was used to dispense 100 µL of the broth into National Antimicrobial Resistance Monitoring System panel plates designed for Gram-positive (CMV3AGPF; TREK Diagnostic Systems; Table 2) bacteria. *Enterococcus faecalis* ATCC 29212 (American Type Culture Collection, Manassas, VA) strains were included as quality controls for *Enterococcus* susceptibility testing. Plates were incubated at 98.6°F for 18 h and then bacterial growth was assessed using Sensititre ARIS and Vizion systems (TREK Diagnostic Systems). Clinical and Laboratory Standards Institute guidelines were used to classify each bacterial isolate as resistant or nonresistant (intermediate and susceptible) according to the breakpoints established for each antimicrobial.[6]

All response criteria were analyzed using generalized linear mixed models. The linear predictors included the fixed effects of tylosin administration route (control, in-feed, injectable, and water), gender (gilt and barrow), and their interaction. The model also included the random effects of BW and location block and block × route interaction. The random effect was used to identify a pair of pens with 1 barrow pen and 1 gilt pen sharing the same treatment as the level of replication for tylosin administration route.

Growth performance responses as well as water and medication intake were measured at the pen level. Residual assumptions were checked using Studentized residuals. For antimicrobial resistance data, frequency tables of resistant and nonresistant isolates for each antibiotic were initially evaluated. For gentamicin, kanamycin, streptomycin, and vancomycin, none of the fecal isolates were categorized as resistant and thus no further statistical analyses were performed. For each remaining antibiotic, subcategory frequency tables were further evaluated for tylosin administration route, sampling day, and their interaction. These tables were used to identify potential extreme category problems during model fitting. Subcategories with all resistant or nonresistant isolates or frequencies close to these extremes can lead to model fitting problems due to zero variance components.

Antimicrobial resistance probability was analyzed using a model assuming a Bernoulli distribution response and a logit link function. Due to the presence of subcategory extremes, it was not possible to fit the 3-way interaction among administration route, sampling day, and gender for chloramphenicol, linezolid, nitrofurantoin, penicillin, quinupristin/dalfopristin, tigecycline, ciprofloxacin, daptomycin, erythromycin, lincomycin, tetracycline, and tylosin. In addition, 2-way interactions with administra-

[6]Clinical and Laboratory Standards Institute. 2013. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals. Approved Standard, 4th Ed. Document VET01-A4. CLSI, Wayne, PA.

tion route by sampling day were eliminated for linezolid, nitrofurantoin, penicillin, quinupristin/dalfopristin, and tigecycline, as well as any interaction involving gender for ciprofloxacin, daptomicin, erythromycin, lincomycin, tetracycline, and tylosin. Overdispersion was assessed using the maximum-likelihood-based fit statistic Pearson Chi-Square over degree of freedom. In all cases, final models used for inference showed no evidence for overdispersion.

Pairwise comparisons were conducted using a Tukey-Kramer or Bonferroni adjustment. Statistical models were fit using the GLIMMIX procedure of SAS (Version 9.4; SAS Inst. Inc., Cary, NC). In all cases, the final model used for inference was fit using residual (pseudo-) likelihood implemented with a Newton-Raphson optimization with ridging. Least square mean estimates of probability of resistance are presented, along with corresponding 95% confidence intervals. Results were considered significant at $P \leq 0.05$, and marginally significant with P-values > 0.05 and ≤ 0.10.

Results and Discussion

Growth Performance

No evidence for route × gender interactions ($P > 0.55$) were observed for any of the growth responses (Table 3). During the treatment period (d 0 to 21), the main effect of administration route marginally contributed to explain ADG ($P = 0.100$), whereby the control pigs and pigs fed medicated feed had greater ($P < 0.05$) ADG than those receiving tylosin through injection, with the ADG of pigs receiving tylosin through water intermediate. For the main effect of gender, barrows grew marginally faster ($P = 0.091$) than gilts during the treatment period. Average daily feed intake was greater ($P = 0.031$) in barrows than in gilts but there was no evidence for any effect of tylosin administration route on ADFI. Feeding tylosin resulted in similar F/G as that of pigs receiving no antibiotic treatment. In contrast, administration of tylosin through injection or water worsened F/G ($P < 0.05$) compared with pigs from the control group. No evidence of gender effect was observed for F/G during the treatment period. During the post-treatment period (d 21 to 35), no evidence for any effects of administration route or gender were observed for any growth responses. Overall (d 0 to 35), growth performance was not influenced by the tylosin administration route; barrows had marginally greater ($P = 0.071$) ADFI than gilts but no evidence of different ADG or F/G were observed.

For average daily water intake, there was no evidence for any effects of tylosin administration route nor gender. Among the medicated pigs, total tylosin dose administered was the greatest through injection, second highest through feed, with the water medication route the lowest ($P < 0.01$).

Antimicrobial Resistance

Table 4 illustrates the estimated probability of antimicrobial resistance of enterococcal isolates, in response to tylosin administration route and sampling day, to antibiotics of critically importance to human medicine,[7] namely ciprofloxacin, daptomycin, erythromycin, gentamicin, kanamycin, linezolid, penicillin, streptomycin, tigecycline, tylosin,

[7]World Health Organization. 2012. Critically important antimicrobials for human medicine – 3rd rev. WHO Document Production Services, Geneva, Switzerland.

and vancomycin. No enterococcal isolates showed any resistance to gentamicin, kanamycin, streptomycin, and vancomycin for the duration of the study. For ciprofloxacin, there was no evidence for any interactive or main effects of tylosin administration route, gender, or sampling day on antimicrobial resistance in the study period. For daptomycin, only a main effect of sampling day was apparent on antimicrobial resistance ($P < 0.001$), whereby regardless of administration route or gender, the probability of resistance decreased during treatment period and increased thereafter (57, 27, and 46% on d 0, 21, and 35, respectively). There was no evidence of any tylosin administration route effect on antimicrobial resistance to daptomycin. For erythromycin, no evidence of route × sampling day interaction was apparent, but both main effects significantly ($P < 0.05$) contributed to explain antimicrobial resistance. Overall, the probability of antimicrobial resistance to erythromycin was marginally greater ($P < 0.10$) when gilts or barrows received tylosin via either feed or injection relative to water (76, 78, and 52%, respectively), with that of control pigs (57%) intermediate. Moreover, the probability of resistance to erythromycin increased from d 0 to d 21 and d 35 (50, 75, and 73%, respectively), regardless of tylosin administration route. For linezolid, penicillin, and tigecycline, there was no evidence for any effects of tylosin administration route, gender, and sampling day on antimicrobial resistance. For tylosin, the main effect of administration route marginally contributed to explain antimicrobial resistance ($P = 0.068$), whereby the probability of resistance to tylosin was greater ($P < 0.05$) in enterococcal isolates collected from pigs receiving tylosin via feed and injection (69 and 70%, respectively) compared with control pigs and those receiving oral tylosin through drinking water (50 and 50%, respectively). The probability of resistance to tylosin increased ($P < 0.01$) from d 0 to d 21 and d 35 (41, 70, and 68%, respectively).

Table 5 shows the estimated probability of antimicrobial resistance of enterococcal isolates to antibiotics considered highly important or important to human medicine, namely chloramphenicol, quinupristin/dalforistin, lincomycin, tetracycline, and nitrofurantoin (WHO, 2012). There was no evidence for any effects of tylosin administration route, gender, and sampling day on antimicrobial resistance to chloramphenicol, quinupristin/dalforistin, lincomycin, and tetracycline. For nitrofurantoin, only the main effect of sampling day significantly contributed to explain antimicrobial resistance ($P = 0.002$), whereby the probability of resistance to nitrofurantoin remained similar during treatment period and decreased ($P < 0.01$) thereafter (22, 27, and 2% on d 0, 21, and 35, respectively) regardless of gender or tylosin administration routes.

It has been reported in studies (NCR-89 Committee on Confinement Management of Swine, 1986;[8] Pilcher et al., 2015[9]) that feeding tylosin at a low dosage (44 or 22 ppm) promoted ADG and F/G of growing-finishing pigs. However, other studies (Lillie et al.,

[8]NCR-89 Committee on Confinement Management of Swine. 1986. Effect of space allowance and tylosin feeding on performance of growing-finishing pigs. J. Anim. Sci. 62:871–874.
[9]Pilcher, C. M., R. Arentson, and J. F. Patience. 2015. The interaction of fiber, supplied by distillers dried grains with solubles, with an antimicrobial and a nutrient partitioning agent on nitrogen balance, water utilization, and energy digestibility in finishing pigs. J. Anim. Sci. 93:1124–1132. doi:10.2527/jas2013-7309

1997;[10] Dritz et al., 2002;[11] Van Lunen et al., 2003[12]) have suggested a lack of growth-promoting response of tylosin when fed to finishing pigs. In the present study, we did not observe any evidence for a difference in growth performance among pigs fed tylosin-medicated feed and those with no antibiotic treatment. A potential reason for this observation is the excellent performance of control pigs; pigs in the present study were individually housed and had approximately 15% greater ADFI and 20% greater ADG than the normally group-housed pigs of similar weight range and raised on the same research site. In addition, the good hygienic conditions of a university research environment may have also contributed to the lack of growth response to feed antibiotic. Pigs from the injection group had poorer ADG and F/G than control pigs, which was likely a result of stress associated with the injection procedure. However, it remains unclear why pigs offered medicated water were less feed efficient than control pigs.

We initially hypothesized that oral administration exposes gut bacteria directly to high concentrations of antibiotics, thus creating a greater potential in promoting the development of antimicrobial resistance. However, results from the present study suggested greater promoting effects of injectable and in-feed tylosin on the development of enterococcal resistance to erythromycin and tylosin over the oral water administration routes. Two possible reasons can be speculated for this observation. The first is potential excretion of injected tylosin and its metabolites into the gastrointestinal tract of pigs that exerted selection pressure on bacteria to become resistant. Secretion from the liver into the gastrointestinal tract and urinary excretion of absorbed tylosin and the metabolite desmycosin has been reported (Worth, 1971;[13] Wal and Bories, 1973[14]). Secondly, the effects of administration route on the development of antimicrobial resistance may be dose-dependent. The treatment dose and procedure administered in each tested route followed the label instruction of corresponding tylosin product. Based on these dosages, pigs provided the oral water treatment received only 21 and 43% of the total tylosin doses administered to those on the injection and in-feed treatments, respectively (Table 3). Moreover, a recent review by Pyöräla et al.[15] has suggested that applying macrolide antibiotics, including tylosin, in feed or through injections creates long-acting concentrations of active substance in pigs, which may particularly contribute to the development of antimicrobial resistance. The slow absorption and release of tylosin in injected pigs and the uninterrupted tylosin administration through feed may have

[10]Lillie, R. J., L. T. Frobish, N. C. Steele, and G. Graber. 1977. Effect of dietary copper and tylosin and subsequent withdrawal on growth, hematology and tissue residues of growing-finishing pigs. J. Anim. Sci. 45:100–107.

[11]Dritz, S. S., M. D. Tokach, R. D. Goodband, and J. L. Nelssen. 2002. Effects of administration of antimicrobials in feed on growth rate and feed efficiency of pigs in multisite production systems. J. Am. Vet. Med. Assoc. 220:1690–1695.

[12]Van Lunen, T. A. 2003. Growth performance of pigs fed diets with and without tylosin phosphate supplementation and reared in a biosecure all-in all-out housing system. Can. Vet. J. 44:571–576.

[13]Worth, H. M. 1971. How do safety evaluations and residues studies assure wholesome pork. Symposium proceedings: Swine feed additives, producer and consumer. University of Kentucky, College of Agriculture, Lexington. p. 61.

[14]Wal, J. M. and Bories, G. F. 1973. Tritiation of tylosin and metabolic study in the rat. J. Antibiot. 26:687–691.

[15]Pyörälä, S., K. E. Baptiste, B. Catry, E. van Duijkere, C. Greko, M. A. Moreno, M. C. Pomba, M. Rantala, M. Ružauskas, P. Sanders, E. J. Threlfall, J. Torren-Endo, and K. Törneke. 2014. Macrolides and lincosamides in cattle and pigs: Use and development of antimicrobial resistance. Vet J. 200:230–239. https://doi.org/10.1016/j. tvjl.2014.02.028.

created continuous selection pressure on resistant bacteria in contrast to the lower dosage and intermittently administered tylosin treatment through water.

In addition, it is surprising that we did not observe any evidence for a route × day interaction for the development of resistance to tylosin and erythromycin. This suggested an increased resistance rate over time even in enterococcal isolates collected from pigs that received no tylosin treatment. It is possible that the resistant bacteria might have been transmitted from the tylosin-treated pigs to control pigs through fecal contamination even though isolation measures were provided among pens. Indirect physical contact of pigs via personnel movement across pens could also lead to contamination of resistant bacteria. However, we currently cannot explain the reason why the resistance of enterococcal isolates to daptomycin was decreased from baseline to treatment period and then increased back to baseline levels after a 2 wk wash-out period (Table 4).

In summary, feeding tylosin did not promote the growth performance of finishing pigs; in contrast, tylosin injection harmed ADG and F/G. We hypothesize this is due to stress associated with the injection procedure. Tylosin administration through injection and feed resulted in more prevalent resistance to erythromycin and tylosin in fecal enteroccocal isolates compared with those collected from pigs that received no or oral tylosin through the water. We hypothesize that the total tylosin dosage could have affected the resistance response to administration route because the oral treatment through the water resulted in a lower dose administered than the injection and in-feed treatments.

Table 1. Diet composition (as-fed basis)

	Non-medicated	Medicated
Corn	85.95	85.90
Soybean meal	11.91	11.91
Monocalcium P (21% P)	0.40	0.40
Limestone	0.90	0.90
Salt	0.35	0.35
L-Lys-HCl	0.23	0.23
L-Thr	0.06	0.06
Trace mineral premix	0.10	0.10
Vitamin premix	0.08	0.08
Phytase[1]	0.02	0.02
Tylan 100[2]	---	0.05
Total	100.00	100.00
Calculated composition		
Standardized ileal digestible (SID) AA, %		
Lys	0.65	0.65
Ile:Lys	65	65
Leu:Lys	169	169
Met:Lys	31	31
Met and Cys:Lys	62	61
Thr:Lys	67	67
Trp:Lys	17	17
Val:Lys	77	77
Total Lys, %	0.74	0.74
Crude protein, %	13.02	13.02
Net energy, kcal/lb	1,159	1,158
Ca, %	0.45	0.45
P, %	0.39	0.39
STTD P with phytase,[3] %	0.28	0.28
Analyzed composition, %		
Dry matter	89.69	89.60
Crude protein	12.80	12.65
Fat	2.75	2.25
Calcium	0.52	0.47
Phosphorus	0.35	0.31

[1]Ronozyme Hiphos 2700 (DSM Nutritional Products, Inc., Parsippany, NJ), providing 184.3 phytase units (FTU)/lb and an estimated release of 0.10% available P.
[2]Elanco Animal Health (Indianapolis, IN).
[3]STTD = standardized total tract digestible.

Table 2. Resistance breakpoints and evaluated concentrations for antimicrobials of National Antimicrobial Resistance Monitoring System Gram-positive bacteria panel (CMV3AGPF; WHO, 2012)

Antimicrobial	WHO classification[1]	Concentration, μg/mL	Breakpoint, μg/mL[2]
Chloramphenicol	Highly important	2-32	≥32
Ciprofloxacin	Critically important	0.12-4	≥4
Daptomycin	Critically important	0.25-16	N/A[3]
Erythromycin	Critically important	0.25-8	≥8
Gentamicin	Critically important	128-1,024	>500
Kanamycin	Critically important	128-1,024	≥1,024
Lincomycin	Highly important	1-8	≥8
Linezolid	Critically important	0.5-8	≥8
Nitrofurantoin	Important	2-64	≥128
Penicillin	Critically important	0.25-16	≥16
Quinupristin/alfopristin	Highly important	0.5-32	≥4
Streptomycin	Critically important	512-2,048	>1000
Tetracycline	Highly important	1-32	≥16
Tigecycline	Critically important	0.015-0.5	N/A[4]
Tylosin tartrate	Critically important	0.25-32	≥32
Vancomycin	Critically important	0.25-32	≥32

[1]World Health Organization (WHO) categorization of antimicrobials according to importance for human medicine (WHO, 2012).

[2]Breakpoints established by Clinical and Laboratory Standards Institute (2013).

[3]N/A = not applicable. A susceptibility breakpoint of ≤ 4 μg/mL for daptomycin exists but no resistant breakpoint has been established. In this study, isolates with a minimal inhibitory concentration ≥ 8 μg/mL were categorized as resistant.

[4]A susceptibility breakpoint of ≤ 0.25 μg/mL for tigecycline exists but no resistant breakpoint has been established. In this study, isolates with a minimal inhibitory concentration ≥ 0.5 μg/mL were categorized as resistant.

Table 3. Effects of tylosin administration route and gender on growth performance of finishing pigs[1]

	Route[2]					Gender			*P* <		
	Control	Feed	Injection	Water	SEM	Barrow	Gilt	SEM	Route	Gender	Route × gender
Treatment (d 0 to 21)											
ADG, lb	2.77[a]	2.78[a]	2.54[b]	2.69[ab]	0.074	2.75	2.64	0.051	0.100	0.091	0.551
ADFI, lb	8.02	8.20	7.83	8.43	0.218	8.33	7.91	0.157	0.219	0.031	0.824
F/G	2.90[b]	2.97[ab]	3.10[a]	3.14[a]	0.062	3.04	3.01	0.038	0.058	0.618	0.702
Post-treatment (d 21 to 35)											
ADG, lb	2.64	2.68	2.55	2.59	0.071	2.62	2.61	0.053	0.569	0.882	0.991
ADFI, lb	8.24	8.13	7.78	8.09	0.177	8.16	7.96	0.153	0.178	0.304	0.903
F/G	3.13	3.05	3.09	3.15	0.068	3.14	3.08	0.058	0.697	0.429	0.916
Overall (d 0 to 35)											
ADG, lb	2.72	2.74	2.54	2.65	0.060	2.70	2.63	0.038	0.127	0.159	0.746
ADFI, lb	8.11	8.17	7.81	8.29	0.187	8.26	7.93	0.146	0.241	0.071	0.844
F/G	2.98	2.99	3.09	3.13	0.051	3.07	3.03	0.032	0.151	0.289	0.560
Water intake, L	6.14	6.45	6.87	6.06	0.287	6.56	6.20	0.241	0.179	0.310	0.566
Tylosin intake, g	-	8.61[b]	18.00[a]	3.69[c]	0.148	10.20	10.01	0.123	0.001	0.262	0.425

[1]There were a total of 40 barrows and 40 gilts (Line 600 × 241, DNA, Columbus, NE; initially 207 ± 7.9 lb) housed with 1 pig per pen and 10 replicate pens per treatment per gender.

[2]Control = pigs received no antibiotic; feed = pigs received 110 mg tylosin per kg feed for 21 d; injection = pigs received 8.82 mg tylosin per kg BW through intramuscular injection twice daily for the first 3 d of each wk during the 3-wk treatment period; water = 66 mg tylosin per liter of drinking water for the first 3 d of each wk during treatment period.

[abc]Means with different superscripts within a row differ ($P < 0.05$).

ADG = average daily gain. ADFI = average daily feed intake. F/G = feed efficiency.

Table 4. Effects of tylosin administration route and time on fecal enterococci resistant prevalence to critically important antimicrobials[1]

	Route[2]				Probability, $P <$		
	Control	Feed	Injection	Water	Route	Day	Route × day
Ciprofloxacin					0.318	0.904	0.986
Baseline (d 0)	10 [2, 33][3]	20 [8, 43]	20 [8, 43]	0 [.]			
Treatment (d 21)	10 [2, 33]	25 [11, 48]	20 [8, 43]	15 [5, 38]			
Post-treatment (d 35)	10 [2, 33]	25 [11, 48]	10 [2, 33]	15 [5, 38]			
Daptomycin					0.312	0.001	0.708
Baseline (d 0)	70 [47, 86]	55 [33, 75]	60 [38, 79]	40 [21, 62]			
Treatment (d 21)	40 [21, 62]	25 [11, 48]	25 [11, 48]	20 [8, 43]			
Post-treatment (d 35)	50 [29, 71]	40 [21, 62]	40 [21, 62]	55 [33, 75]			
Erythromycin					0.025	0.004	0.258
Baseline (d 0)	55 [33, 76]	65 [42, 83]	45 [24, 67]	35 [17, 58]			
Treatment (d 21)	50 [28, 71]	80 [57, 93]	95 [72, 99]	50 [28, 71]			
Post-treatment (d 35)	65 [42, 83]	80 [57, 93]	75 [51, 90]	70 [46, 87]			
Gentamicin					N/A[4]	N/A	N/A
Baseline (d 0)	0 [.]	0 [.]	0 [.]	0 [.]			
Treatment (d 21)	0 [.]	0 [.]	0 [.]	0 [.]			
Post-treatment (d 35)	0 [.]	0 [.]	0 [.]	0 [.]			
Kanamycin					N/A	N/A	N/A
Baseline (d 0)	0 [.]	0 [.]	0 [.]	0 [.]			
Treatment (d 21)	0 [.]	0 [.]	0 [.]	0 [.]			
Post-treatment (d 35)	0 [.]	0 [.]	0 [.]	0 [.]			
Linezolid					0.688	0.942	-
Baseline (d 0)	0 [.]	20 [8, 42]	10 [2, 35]	0 [.]			
Treatment (d 21)	20 [7, 47]	10 [2, 35]	0 [.]	0 [.]			
Post-treatment (d 35)	15 [5, 37]	10 [3, 32]	10 [3, 32]	0 [.]			
Penicillin					0.697	0.187	-
Baseline (d 0)	5 [0.7, 27]	10 [2, 33]	0 [.]	0 [.]			
Treatment (d 21)	0 [.]	5 [0.7, 27]	0 [.]	0 [.]			
Post-treatment (d 35)	10 [2, 33]	10 [2, 33]	10 [2, 33]	0 [.]			
Streptomycin					N/A	N/A	N/A
Baseline (d 0)	0 [.]	0 [.]	0 [.]	0 [.]			
Treatment (d 21)	0 [.]	0 [.]	0 [.]	0 [.]			
Post-treatment (d 35)	0 [.]	0 [.]	0 [.]	0 [.]			

continued

Table 4. Effects of tylosin administration route and time on fecal enterococci resistant prevalence to critically important antimicrobials[1]

	Route[2]				Probability, *P* <		
	Control	Feed	Injection	Water	Route	Day	Route × day
Tigecycline					0.279	0.832	-
Baseline (d 0)	85 [63, 95]	90 [68, 98]	95 [71, 99]	100 [.]			
Treatment (d 21)	90 [68, 98]	90 [68, 98]	100 [.]	95 [74, 99]			
Post-treatment (d 35)	90 [68, 98]	90 [68, 98]	100 [.]	85 [62, 95]			
Tylosin					0.068	0.001	0.233
Baseline (d 0)	45 [24, 68]	55 [32, 76]	30 [13, 54]	35 [17, 58]			
Treatment (d 21)	50 [28, 72]	75 [51, 90]	90 [67, 98]	50 [28, 72]			
Post-treatment (d 35)	55 [32, 76]	75 [51, 89]	75 [51, 89]	65 [41, 83]			
Vancomycin					N/A	N/A	N/A
Baseline (d 0)	0 [.]	0 [.]	0 [.]	0 [.]			
Treatment (d 21)	0 [.]	0 [.]	0 [.]	0 [.]			
Post-treatment (d 35)	0 [.]	0 [.]	0 [.]	0 [.]			

[1]Values represent the estimated probability of resistance among 20 enterococcal isolates per sampling day (d 0, 21, or 35); susceptibility was determined according to National Antimicrobial Resistance Monitoring System (CLSI, 2013, footnote 6 from main text) established breakpoints. One fecal sample was collected per pen per day and 1 enterococcal isolate per fecal sample was assessed. There was a total of 80 pigs (Line 600 × 241, DNA, Columbus, NE; initially 207 ± 7.9 lb) housed with 1 pig per pen and 10 replicate pens per treatment per gender.

[2]Control = pigs received no antibiotic; feed = pigs received 110 mg tylosin per kg feed for 21 d; injection = pigs received 8.82 mg tylosin per kg BW through intramuscular injection twice daily for the first 3 d of each wk during the 3-wk treatment period; water = 66 mg tylosin per liter of drinking water for the first 3 d of each wk during treatment period.

[3]Indicates 95% confidence interval.

[4]N/A represents statistics were not conducted because all enterococcal isolates were identified as susceptible.

Table 5. Effects of tylosin administration route and time on the fecal enterococci resistant prevalence to highly important and important antimicrobials[1]

	Route[2]				Probability, *P* <		
	Control	Feed	Injection	Water	Route	Day	Route × day
Chloramphenicol					0.331	0.234	0.935
Baseline (d 0)	19 [7, 44][3]	14 [4, 38]	3 [0.3, 26]	4 [0.4, 28]			
Treatment (d 21)	10 [2, 33]	9 [2, 32]	4 [0.4, 28]	5 [0.4, 28]			
Post-treatment (d 35)	19 [7, 44]	14 [4, 38]	19 [7, 44]	8 [2, 32]			
Lincomycin					0.996	0.555	0.340
Baseline (d 0)	95 [72, 99]	86 [61, 96]	76 [52, 90]	91 [67, 98]			
Treatment (d 21)	100 [.]	91 [67, 98]	95 [71, 99]	81 [56, 93]			
Post-treatment (d 35)	86 [62, 96]	95 [72, 99]	95 [72, 99]	95 [72, 99]			
Nitrofurantoin					0.331	0.002	-
Baseline (d 0)	20 [7, 43]	10 [2, 33]	35 [17, 58]	25 [10, 49]			
Treatment (d 21)	25 [10, 49]	30 [13, 54]	15 [5, 38]	40 [20, 63]			
Post-treatment (d 35)	0 [.]	0 [.]	0 [.]	10 [3, 31]			
Quinupristin/Dalfopristin					0.688	0.942	-
Baseline (d 0)	0 [.]	20 [8, 42]	10 [2, 35]	0 [.]			
Treatment (d 21)	20 [7, 47]	10 [2, 35]	0 [.]	0 [.]			
Post-treatment (d 35)	15 [5, 37]	10 [3, 32]	10 [3, 32]	0 [.]			
Tetracycline					0.753	0.104	0.747
Baseline (d 0)	80 [55, 93]	80 [55, 93]	75 [50, 90]	80 [55, 93]			
Treatment (d 21)	80 [55, 93]	90 [65, 98]	95 [70, 99]	80 [55, 93]			
Post-treatment (d 35)	90 [65, 98]	85 [60, 96]	95 [70, 99]	90 [65, 98]			

[1]Values represent the estimated probability of resistance among 20 enterococcal isolates per sampling day (d 0, 21, or 35); susceptibility was determined according to National Antimicrobial Resistance Monitoring System (CLSI, 2013, footnote 6 from main text) established breakpoints. One fecal sample was collected per pen per day and 1 enterococcal isolate per fecal sample was assessed. There was a total of 80 pigs (Line 600 × 241, DNA, Columbus, NE; initially 207 ± 7.9 lb) housed with 1 pig per pen and 10 replicate pens per treatment per gender.

[2]Control = pigs received no antibiotic; feed = pigs received 110 mg tylosin per kg feed for 21 d; injection = pigs received 8.82 mg tylosin per kg BW through intramuscular injection twice daily for the first 3 d of each wk during the 3-wk treatment period; water = 66 mg tylosin per liter of drinking water for the first 3 d of each wk during treatment period.

[3]Indicates 95% confidence interval.