ORIGINAL RESEARCH

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Effect of tylosin on an experimental *Salmonella* infection in pigs

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Summary

Objective: To evaluate the effect of tylosin on an experimental *Salmonella typhimurium* infection.

Method: Tylosin was added to the ration of pigs infected with *Salmonella* (INFMED pigs) at 100 g per ton and fed for a period of 56 days post-inoculation and compared to an infected, nonmedicated control group (INF). Fecal samples were taken at least weekly for 56 days from both INF and INFMED pigs. Representative *Salmonella* isolates from each timepoint and pig were tested for susceptibility to 12 different antimicrobial compounds. At the termination of the study, samples of liver, spleen, ileocecal lymph node, and colon contents were examined for the presence of *Salmonella*.

Results: There was no difference in the overall mean $\log_{10}$ counts of *Salmonella* between the INFMED and INF groups (P=.75), nor in the prevalence of positive fecal samples between the two groups (P=.66). Medication significantly reduced the mean duration of *Salmonella* shedding in INFMED pigs compared to INF pigs (P=.05). There was no difference between treatment groups in the number of *Salmonella* isolations from tissues taken at necropsy nor on the antimicrobial susceptibility of *Salmonella* isolates from the fecal or tissue samples.

Implications: Feeding tylosin to pigs experimentally inoculated with *S. typhimurium* had no effect on the quantity of *Salmonella* in the feces or on prevalence of *Salmonella* in the population of pigs tested. Treatment with tylosin did not change susceptibility to 12 antibiotics. However, treatment with tylosin did reduce the duration of *Salmonella* shedding in the feces. These findings suggest that tylosin did not reduce the competitive exclusion capacity of the normal intestinal bacteria.

Keywords: swine, *Salmonella*, tylosin

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Antibiotics and antimicrobial agents fed to pigs have been consistently associated with an improvement in feed efficiency and growth rate.[1] In order to receive United States regulatory approval for feed additives used as performance enhancers, the manufacturer of the product must investigate whether feeding the product will have an adverse microbiological effect on the animals or on humans. Feeding an antibiotic might:

- increase the number of *Salmonella* in the environment by increasing the quantity in the feces,
- elevate the prevalence of animals harboring the pathogen,
- extend the duration of shedding, or
- cause the acquisition of new resistance determinants.[2–4]

The rationale for using *Salmonella* as an indicator of gut effects is based on the possibility that performance-enhancing antibiotics could suppress susceptible intestinal Gram-positive microflora, thereby disrupting their competitive exclusion protection, and allow a relative increase in *Salmonella* concentrations.

Macrolide antibiotics are administered to livestock for both performance enhancement and therapy.[5] Tylosin is a macrolide antibiotic that has been approved for use in swine feeds in the United States at 10–40 g per ton to increase rate of weight gain and improve feed efficiency, or at 100 g per ton to control swine enteric diseases, such as porcine proliferative enteropathy.[6] Tylosin is active primarily against Gram-positive bacteria, but is also active against *Mycoplasma*, spirochetes, chlamydiae, and some noncoliform Gram-negative bacteria.[7]

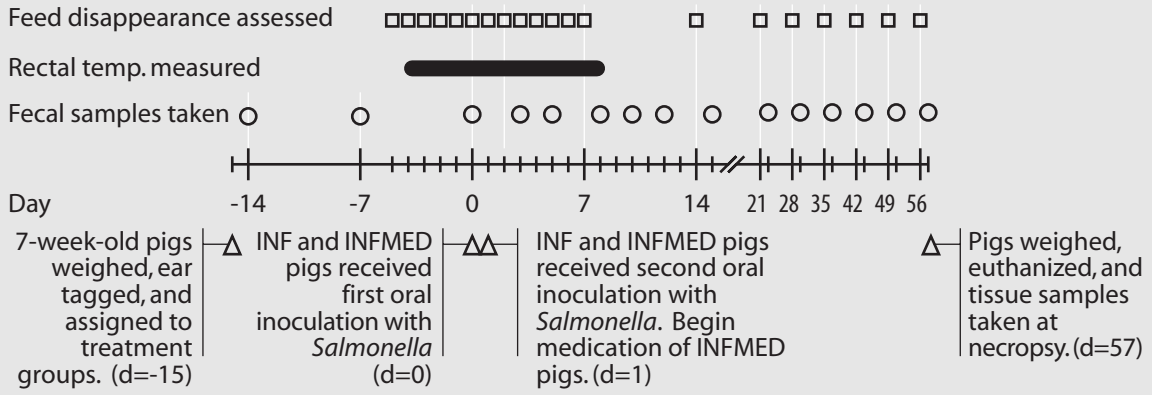
The purpose of this study was to determine the effect of feeding the highest approved concentration of tylosin—representing the greatest selection pressure for resistance emergence—on the quantity, prevalence, and duration of *Salmonella* shedding, as well as to determine the antimicrobial susceptibility of *Salmonella* after an experimental infection in pigs.

Materials and methods

Study design

Thirty-five 7-week-old Chester White-Hampshire-Duroc pigs were obtained from a local breeder, weighed, and identified by ear tag. The pigs, whose mean weight on the first day of the trial was 11 kg (25 lb) (Figure 1), were stratified on the basis of weight and sex and assigned to one of the following treatments:

Figure 1



Study timeline

- INFMED pigs (n = 10) inoculated with *Salmonella* by oral gavage on 2 consecutive days (days 0 and 1 of the trial) with 10 mL of the inoculum suspended in a buffered salt solution (hemaglutination or HA buffer), and subsequently fed tylosin-treated ration;
- INF pigs (n = 10) inoculated with *Salmonella* in the same manner as the INFMED pigs, and subsequently fed nonmedicated control ration; and
- CONTROL pigs (n = 15) that were not infected with *Salmonella* and received a nonmedicated control ration.

Each inoculated animal received a total of 5.2×10^{10} colony-forming units (cfu) in the first dose and 5.6×10^{10} cfu in the second dose, as determined by triplicate plate counts of the inoculum suspensions. Feed and water were withheld from the pigs from approximately 24 hours before the first inoculation until approximately 2 hours afterward. Control group pigs were then provided with ad libitum access to feed for the duration of the study. Feed and water were made available for 1 hour to the INF and INFMED pigs and then were withheld from approximately 20 hours before the second inoculation until approximately 1 hour after the second dose was administered.

Pigs were housed by treatment in 35 separate, contiguous rooms in three isolation buildings, with one pig per room. Within each building, pigs were placed so that a CONTROL-group pig was housed in the room between each INFMED and INF pig.

Biosecurity

Building sides were each handled as a unit by a single technician, who wore new disposable coveralls, plastic boots, and disposable gloves each time he entered a room. Traffic of other visitors and personnel was restricted and controlled. The animals were cared for in the same order, from one end of a building side to the other, throughout the study.

Salmonella strain

The *Salmonella* inoculation strain was a swine-origin *S. typhimurium* (298–1NA) that displayed a typical reaction on a triple sugar iron (TSI) agar slant and was agglutinated by group-B somatic typing serum. It was susceptible to amikacin, ampicillin, carbenicillin, cephalothin, chloramphenicol, gentamycin, kanamycin, triple sulfa, tetracycline, and trimethoprim/sulfamethoxazole. The strain was categorized as intermediate in susceptibility to streptomycin and resistant to nalidixic acid by disk diffusion antimicrobial susceptibility tests as described below. The tylosin minimum inhibitory concentration (MIC) was 1024 µg per mL.

Performance measures

Individual body weights were taken the day before pigs were placed in the isolation rooms and on the day the trial ended (Figure 1).

Rectal temperatures were taken daily from 4 days prior to the first inoculation to 7 days after the second inoculation. General health conditions were noted throughout the experiment.

Feed disappearance was determined by weighing back feed daily from 5 days prior to the first inoculation to 6 days after the second inoculation, and then weekly for the remainder of the study.

Feed

Prior to inoculation, all pigs were fed a complete corn-soybean ration containing 16% protein. After inoculation, the infected INF and CONTROL groups continued to be fed this ration. The INFMED group received the same base ration medicated with 100 g tylosin premix per ton of feed. Each batch of feed was tested for and determined to be free of the presence of *Salmonella* and/or contaminating concentrations of antibiotics and antimicrobials.

Fecal sampling periods

Fecal samples were obtained from each pig at 14 and 7 days prior to and on the days of inoculation. Samples were cultured to confirm the absence of indigenous *Salmonella*.

Fecal samples were taken for *Salmonella* quantitation or detection at 3, 5, 8, 10, 12, 15, 22, 29, 36, 43, 50, and 57 days of the study.

At the end of the 57-day study period, all surviving animals in each of the three groups were euthanized, and necropsy was performed by a veterinarian. Approximately 1-g samples of liver, spleen, ileocecal lymph node, and colon content were aseptically collected for *Salmonella* isolation by an enrichment procedure. Necropsy samples were also taken from one pig that died during the trial.

Bacteriological culture

Three-gram samples of pretreatment fecal and feed samples were cultured for extraneous *Salmonella* contamination, using a brilliant green broth (BGB) enrichment, and subsequently plated onto a BG agar, as previously described.[8]

Fecal samples (3 g) and necropsy samples (1 g) were cultured by the same enrichment procedure, followed by plating onto MacConkey agar supplemented with 50 µg per mL nalidixic acid, as previously described.[8]

A maximum of five *Salmonella*-suspect colonies per sample were taken from the quantitative count agar plates, or from positive enrichment plates when quantitative plates were negative or not used, and inoculated onto TSI slants. The slants were incubated overnight at 37°C and observed for TSI reactions typical of *Salmonella*. Isolates giving typical reactions were serologically typed with specific diagnostic serum to confirm the identity as group B *Salmonella*.

Confirmed *S. typhimurium* isolates were tested for antimicrobial susceptibility by the disk diffusion method.[9] Susceptibility was determined to the same group of antibiotics as those identified during the inoculation strain characterization. Owing to the high MIC of tylosin, and given that enteric Gram-negative bacteria are impermeable to macrolides, no further testing was warranted.

Statistical analysis

Because pigs were housed individually, the pig was the experimental unit in all statistical analysis. Duration of shedding was not submitted to repeated-measures analysis.

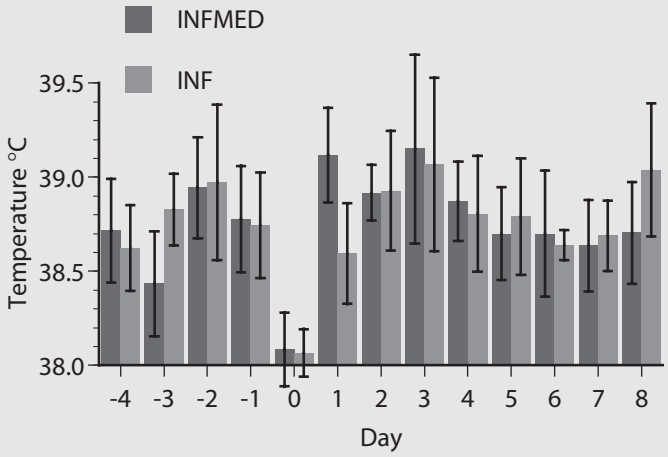
SAS computer software (SAS, Cary, North Carolina) was used to compare study groups and *Salmonella* counts using repeated-measures ANOVA. Analysis to compare duration of *Salmonella* shedding was based on the last-day counts ($\log_{10} > 0.7$), because all surviving animals were shedding at least qualitatively detectable amounts of *Salmonella* on the last sampling day. The effect of treatment on *Salmonella* prevalence was analyzed using a log-linear model (Grizzle-Starmer-Koch procedures).

Results

Feed assays

Feed tests demonstrated an acceptable homogeneity and stability of tylosin in the feed, the absence of other contaminating antibiotics, and all feed samples were negative for *Salmonella* contamination.

Figure 2



Mean (±95% CI) temperature data from 10 pigs (exception: only 9 pigs from day 7–8) for the tylosin-medicated pigs due to a death). Pigs were inoculated on days 0 and 1.

Clinical data

Pig temperature

Initial pre-inoculation temperatures fluctuated around 38.3–38.9°C (101–102°F). Mean body temperature decreased to 38°C (~100.5°F) on Day 0 (first day of challenge). Body temperatures greater than 39.4°C (103°F) were observed in only eight of the INFMED- and INF-group pigs on days 1–5 of the study, at which time temperatures rapidly returned to pre-inoculation levels (Figure 2).

Pig weight

Pig weight gain did not differ significantly among study groups. There were no remarkable clinical findings other than the death of one INFMED pig, which was attributed to acute pneumonia, peritonitis, and enteritis.

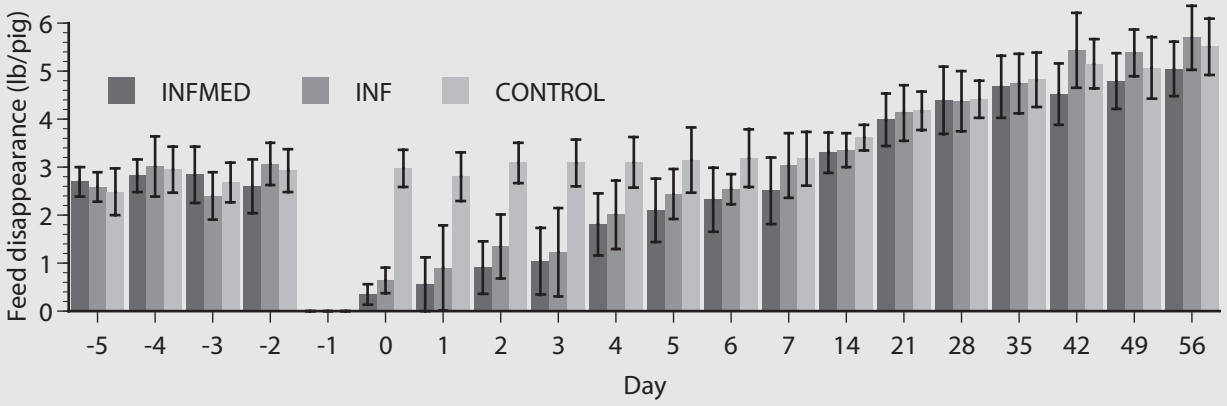
Feed disappearance

Feed disappearance of all pigs was reduced immediately after inoculation (Figure 3). Mean feed disappearance in the INFMED and INF pigs returned to approximately three-fourths of the pre-inoculation level by day 4 of the study. By day 8 of the study, feed disappearance for the infected pigs no longer differed significantly from that of the non-infected controls. Thus, the INFMED pigs consumed the same amount of feed as the INF pigs, with feed disappearance in both groups returning to that of the CONTROL group by day 8 of the study.

Salmonella isolations

No *Salmonella* were detected in any of the fecal samples taken prior to inoculation, including samples taken on the first day of inoculation (day 0). A nalidixic-acid-resistant *Salmonella* was isolated from a single CONTROL-group pig on day 57. All other CONTROL-group samples were negative for the inoculated *Salmonella* strain.

Figure 3



Mean (±95% CI) of feed consumption data from 10 pigs (9 pigs days 7–56) for the tylosin-medicated pigs; feed was withheld at day -1 and day 0 prior to inoculation. Pigs were inoculated on days 0 and 1.

Salmonella quantitation

Quantitative *Salmonella* $\log_{10}$ counts were obtained from the majority of the animals for the first four samplings (Figure 4). Some pigs then exhibited intermittently quantifiable fecal samples.

Initial average $\log_{10}$ counts were 5.20 for INFMED and 5.0 for INF pigs. Subsequently, $\log_{10}$ counts decreased over time, but remained over 1.0 in both infected groups throughout the observation period. The overall mean $\log_{10}$ counts (2.12 for INFMED group pigs and 2.14 for INF-group pigs) did not differ significantly (P =.75).

Duration of *Salmonella*

The duration of *Salmonella* shedding in INFMED-group pigs was significantly shorter than that of INF-group pigs (P =.05) (Table 1).

Prevalence of *Salmonella*

Salmonella prevalence did not differ significantly between INFMED- and INF-group pigs (P =.66) (Table 1).

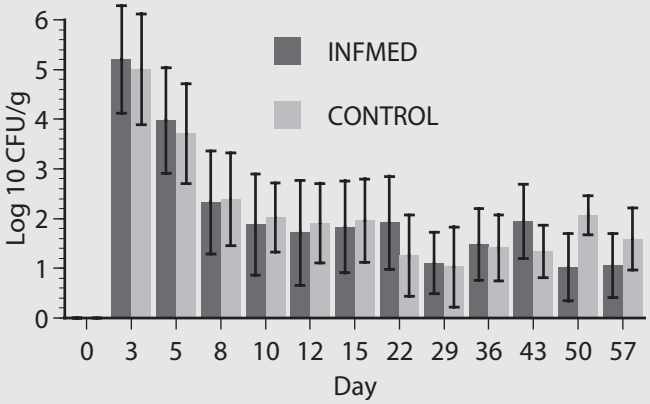
Salmonella isolation from tissues

All samples from the pig that died on test were positive. Numerically similar results were obtained from pigs in the INFMED and INF groups (Figure 5).

Antibiotic susceptibility of isolated *Salmonella*

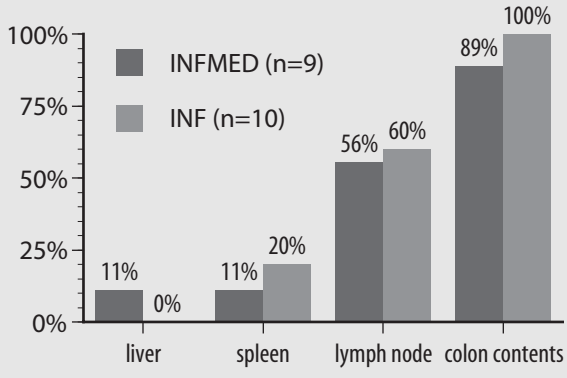
A total of 110 fecal samples from INFMED pigs yielded 107 isolates for susceptibility testing. INF pigs provided 110 isolates from 115 samples. The CONTROL group had only one isolate from 180 samples. Isolates from five necropsy samples from the pig that died during the study and from 33 enrichment-positive samples taken at necropsy at the end of the study were also tested. Results were not obtained for two isolates (pig No. 366 on day 22 and pig No. 361, colon content at necropsy) nor for two antibiotics of one isolate of pig No. 377 on day 22. Except for the expected resistance to nalidixic acid, none of the isolates developed resistance to any of the antimicrobial agents.

Figure 4



Mean (±95% CI) of quantitative *Salmonella* recovery data from 10 pigs (9 pigs days 7–8). Pigs were inoculated on days 0 and 1.

Figure 5



Salmonella isolation from specific tissues at necropsy (day 57; pigs were inoculated on days 0 and 1)

Table 1

Prevalence and duration of *Salmonella* shedding

Pig no.	Post-challenge sampling day 3	5	8	10	12	15	22	29	36	43	50	57	Days positive	Shedding duration (Days)
INFMED group														
354	1	1	1	1	1	0	0	0	0	0	0	0	41.7%	11
358	1	1	–	–	–	–	–	–	–	–	–	–	–	–
365	1	1	0	0	0	0	0	0	1	1	1	0	41.7%	49
366	1	1	1	1	–	–	1	–	1	1	–	1	66.7%	56
375	1	1	1	0	1	1	1	0	1	1	0	0	66.7%	42
377	1	1	1	1	0	1	1	0	0	1	1	0	66.7%	49
379	1	1	1	1	0	1	1	1	0	1	1	1	83.3%	56
392	1	1	0	0	0	0	0	0	0	0	0	0	16.7%	4
395	1	1	1	1	1	1	1	0	1	1	0	0	75.0%	42
398	1	1	1	0	1	1	0	1	0	0	0	0	50.0%	28
Pigs positive	100.0%	100.0%	77.8%	55.6%	44.4%	55.6%	55.6%	22.2%	44.4%	66.7%	33.3%	22.2%	56.5%	
Pigs positive														
INF group														
355	1	1	0	0	0	0	0	0	0	0	1	0	25.0%	49
357	1	1	1	1	1	1	1	0	0	1	1	0	75.0%	49
360	1	1	1	1	0	0	0	1	1	1	1	0	66.7%	49
361	1	1	1	1	1	1	1	0	1	1	1	1	91.7%	56
370	1	1	1	0	0	0	0	0	0	1	0	1	41.7%	56
389	1	1	1	1	1	1	1	0	1	1	1	1	91.7%	56
390	1	1	1	1	1	1	1	1	1	1	1	1	100.0%	56
391	1	1	1	1	1	1	0	0	0	0	1	1	66.7%	56
393	1	1	0	1	0	0	0	0	0	0	1	1	41.7%	56
400	1	1	0	1	1	1	0	0	0	0	1	0	50.0%	49
Pigs positive	100.0%	100.0%	70.0%	80.0%	60.0%	60.0%	40.0%	20.0%	40.0%	60.0%	90.0%	60.0%	65.0%	

1 *Salmonella* detected
0 *Salmonella* not detected
– No sample available

Discussion

Persistent colonization was successfully established in all infected pigs after they were administered a high dose of *Salmonella*. Detecting *Salmonella* in only one of 15 CONTROL pigs on the last day of sampling confirmed the sensitivity of the test system to detect contamination resulting from failed biosecurity measures or from a "silent" carrier pig. Thus, it is unlikely that cross-contamination or carrier status among the experimental groups confounded our results.

The proportion of culture-positive INF pigs during this study is consistent with results reported for other induced-model infections.[10,11] Other researchers have investigated *Salmonella* colonization and persistence within tissues, mortality, and pig temperatures using induced *S. typhimurium* model infections in swine.[10–12] Wood, et al.,[11,12] reported minimal recovery of *Salmonella* from the liver and spleen, with 45% positive ileocolic lymph nodes and 71% recovery from the cecum, 3% mortality,[11] and body temperatures that spiked immediately after inoculation and returned to baseline by day 4.[11,12] The consistency of our results to these frequencies of recovery, mortality, and temperature patterns substantiates the validity of the experimental model.

The results of the present study on tylosin are similar to those reported for other feed additive antibiotics evaluated in *Salmonella* infection models. Evangelisti, et al.,[10] found only one instance of an oxytetracycline-resistant isolate when tested against eight antibiotics during their evaluation of the effect of 150 g per ton oxytetracycline on *S. typhimurium* in swine. Furthermore, Evangelisti, et al.,[10] observed no difference in the quantity of *Salmonella* shedding, prevalence, or

duration of infection between treated and nontreated pigs. Chlortetracycline at 55 mg per kg did not increase or prolong shedding of a tetracycline-susceptible *S. typhimurium* in a model infection,[8] whereas virginiamycin fed at 55 mg per kg tended to numerically increase and prolong shedding, although the differences were not significant. In another study that investigated the effect of feeding a combination of aureomycin (100 g per ton), sulfamethazine (100 g per ton), and penicillin (50 g per ton) on pigs orally inoculated with *S. choleraesuis*, medicated pigs had reduced mortality and clinical illness associated with increased weight gain and feed efficiency compared to the nonmedicated group.[13] Moreover, fewer *Salmonella* were isolated from medicated pigs than from nonmedicated pigs; thus, prevalence and duration of shedding were reduced in the presence of the antibiotic.

Implications

- The use of tylosin at the highest approved dose (100 g per ton) did not cause increased *Salmonella* excretion and reduced the duration of shedding.
- The use of tylosin in the presence of *Salmonella* did not appear to disrupt the competitive exclusion capacity of the normal gut microflora.
- The use of tylosin did not select for *Salmonella* with new resistance phenotypes.

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