ORIGINAL RESEARCH

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Effect of oral tiamulin on the development of porcine proliferative enteropathy in a pure-culture challenge model

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Summary

Objective: To determine the effect of tiamulin-medicated feed in pigs infected with pure cultures of *Lawsonia intracellularis* by monitoring fecal shedding of *L. intracellularis* by polymerase chain reaction (PCR), antibody response, clinical effects, and lesion development.

Methods: Thirty-two pigs were allocated to two replications of four treatments, each replication with four pigs per treatment; 1) noninfected and nonmedicated (CONTROL); 2) infected and nonmedicated(I-NM); 3) infected and medicated with feed containing tiamulin hydrogen fumarate (thf) at 35 g per ton (I-35); and 4) infected and medicated with feed containing thf at 50 g per ton (I-50). These diets were fed for 35 days, starting 7 days prior to inoculation (day -7). Pigs were intragastrically inoculated with either a pure culture of *L. intracellularis* or a placebo. All pigs were monitored daily until necropsied 28 days post infection (PI). Fecal shedding of *L. intracellularis* by PCR, antibody response, clinical signs, growth performance, and the extent of gross and microscopic lesions specific for porcine proliferative enteropathy (PPE) were determined.

Results: Fecal shedding of *L. intracellularis* was first observed 7 days PI, which coincided with increased diarrhea and loss of condition in the I-NM group. Antibody specific for *L. intracellularis* was first detected at 21 days PI. Medication in feed prevented the development of gross lesions, significantly reduced the prevalence and severity of microscopic lesions, and significantly reduced fecal shedding and seroconversion to *L. intracellularis.*

Implications: Seronegative pigs may shed *L. intracellularis* in their feces early in the course of infection. Medication status may affect the results of antemortem diagnostic tests for *L. intracellularis* infection. Larger sample sizes may be required to increase the probability of detecting infection in medicated populations of pigs. Dietary inclusion of tiamulin was effective in preventing/controlling the development of PPE.

Keywords: porcine proliferative enteropathy, ileitis, *Lawsonia intracellularis*, pathogenesis, lesions, serology, fecal shedding, tiamulin, feed

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Porcine proliferative enteropathy (PPE, ileitis) is a common enteric disease of grow-finish swine which may result in poor growth rate, diarrhea, and stunting or may be manifested as sudden death or bloody diarrhea in late finishing pigs and replacement gilts.[1] The etiologic agent has recently been identified as *Lawsonia intracellularis*.[2,3]

Porcine proliferative enteropathy occurs in virtually all swine production systems, including modern multiple-site or "high–health" herds in which early weaning and segregated rearing are practiced. A recent serologic survey conducted by the National Animal Health Monitoring System (NAHMS) using its swine serum bank (representing 198 farms) found the prevalence of PPE in the United States swine herd to be 96%.[4] The within-herd prevalence rate was 28%. Diagnostic investigations of PPE-affected farms in other countries suggest that approximately 35% of growing pigs are affected.[5,6] Subclinical effects such as reduced rate and efficiency of growth have, however, been difficult to quantify. Direct financial losses due to decreased growth rates (9%–31%) and feed inefficiency (6%–25%) have been estimated to cost $3.28–$11.48 per affected pig in the UK based on the results of five pure culture challenge studies.[7] MacKinnon[8] observed weight gain reductions of up to 50% and reduction in feed efficiency of up to 30% in pigs naturally infected with PPE compared to normal pigs. Annual costs to the pig production industries of several countries have been estimated at $20 million (United States),[9] $3–$6.5 million (United Kingdom),[7] and $25AUS per sow (Australia).[10]

Historically, PPE has been diagnosed by postmortem examination and histopathology. Recently, polymerase chain reaction (PCR) tests have been developed to detect the organism's DNA in feces and tissues.[11] In addition, an indirect-fluorescent antibody (IFA) serological test has been developed to detect IgG antibodies specific for *L. intracellularis*.[12]

The first successful experimental reproduction of the disease was by intragastic administration of crude gut homogenate derived from affected pigs.[13] Recently, the inoculation of pigs with pure cultures of *L. intracellularis* has allowed successful reproduction of disease without the confounding effect of other potentially pathogenic microflora and tissue factors inherent in crude inoculum preparations.[2,14] In addition, pure culture inoculation permits a uniform infective dose, thereby avoiding potentially significant variation in the actual challenge

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that individual pigs receive when administered crude gut inoculum from one or more donor animals.

Tiamulin (Denagard™) is a member of the diterpene class of antibiotics. Diterpene class antibiotics are reserved for use in food producing animals and are not used in human medicine. Tiamulin is lipophilic and therefore concentrates within cells and tissues and acts by inhibiting microbial protein synthesis. When given to pigs orally via feed or water, or parenterally by injection, tiamulin achieves high tissue concentrations in the enteric and respiratory tracts as well as in the tonsil. Tiamulin has good in vitro activity against Gram-positive bacteria, mycoplasmas, anaerobes, spirochetes (e.g., *Serpulina* spp.), and selective activity against Gram-negative pathogens, including *L. intracellularis*,[15,16] *Actinobacillus pleuropneumoniae*, *Haemophilus parasuis*, and *Pasteurella multocida*.

The purpose of this study was to evaluate the impact of dietary tiamulin on the development of clinical signs and lesions of PPE and to characterize the progression of *L. intracellularis* infection in both medicated and nonmedicated pigs using serial antemortem diagnostic techniques (PCR and serology). The effects of antimicrobial treatment on the results of these new antemortem diagnostic tests for PPE have not previously been published.

Materials and methods

Animals

Thirty-two healthy pigs of mixed breed and sex were purchased from a closed swine herd with no clinical or diagnostic history of PPE, *Salmonella*, or *Serpulina* infections. Adults and offspring from this herd were previously shown to be free from *L. intracellularis* infection by culture, histopathology, serological testing, and fecal and tissue PCR techniques. Offspring have been shown to be susceptible to *L. intracellularis* infection and were used previously to successfully reproduce PPE. The pigs were from five litters, weaned at 17–21 days of age, and ranged in weight from 23–30 lb (10–13 kg) when purchased at 6–7 weeks of age. No antibiotics had been administered to these pigs before purchase.

Treatments

Ten days before inoculation with *L. intracellularis* (day -10) (Figure 1), all pigs were identified by eartag, weighed, and randomly allocated to pens and blocked to replication by weight. The 16 pigs in each replicate were allocated to four pens (four to a pen), and each pen was randomly assigned to one of the following four treatments:

- noninfected, nonmedicated controls (CONTROL);
- infected, nonmedicated controls (I-NM);
- medicated with tiamulin hydrogen fumarate (thf) (Denagard®, Boehringer Ingelheim Vetmedica Inc.) in feed at 35 g per ton (I-35) and infected with *L. intracellularis*; or
- medicated with thf in feed at 50 g per ton (I-50) and infected with *L. intracellularis*.

(Denagard products are not currently approved for use against PPE.)

The noninfected pens were segregated at one end of the building due to biosecurity requirements. The assigned diets were fed for the entire study period, from day -7 to test end on day 28 (a total of 35 days). Inoculation with *L. intracellularis* occurred on day 0, which resulted in a 28-day PI observation period.

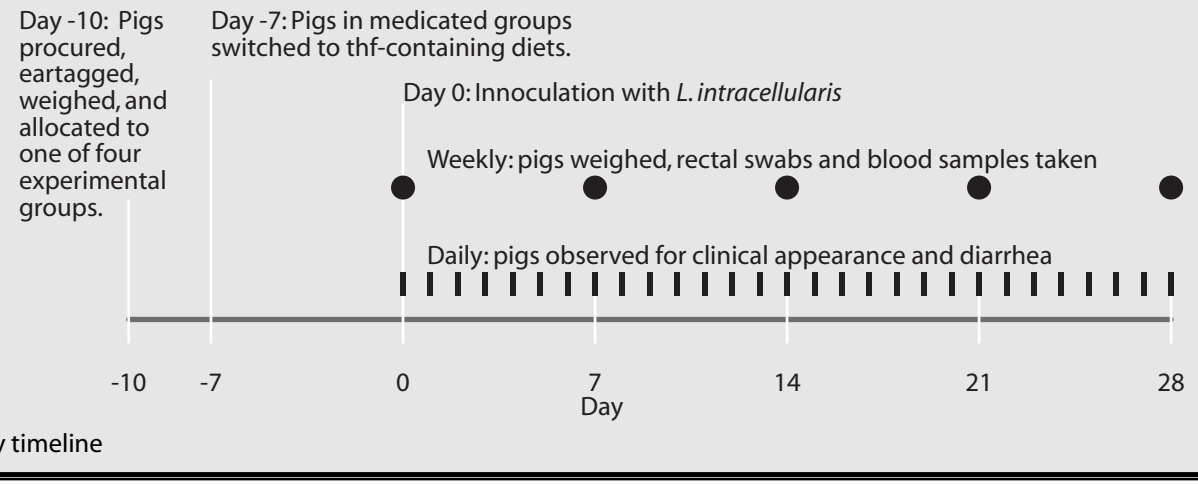
Water/Feed

Water and feed were available ad libitum for the duration of the study. A corn–soymeal-based diet was formulated to meet or exceed NRC requirements for growing pigs. The nonmedicated diet was fed to all pigs during the 3-day acclimation period (day -10 to day -7). On day -7, pigs in the I-35 and I-50 groups were switched to their respective thf-containing diets for the 35-day duration of the trial. The diet provided to the CONTROL and I-NM pigs was not medicated. The assigned diets were fed for the entire study period, from day -7 to test end on day 28.

Infection

Lawsonia intracellularis strain N343 is a recent field isolate from the ileum of a sow from a farm in Minnesota. The in vitro minimum inhibitory concentration (MIC)/susceptibility of this isolate for tiamulin was not determined. Low-passage cultures of strain N343 were grown using continuous cell culture techniques for 8–12 weeks, harvested, processed as previously described, and quantified using the Reed-

Figure 1



Study timeline

Meunsch method.[2] On day 0, pigs designated as "infected" were inoculated with $TCID_{50}$ $10^{6.2}$ organisms as a 20-mL inoculum by gastric intubation. Twenty mL of inoculum and 40 mL of air were drawn into a 60-mL syringe and administered to each pig, the air flushing all inoculum into the stomach. All noninfected pigs received tissue-culture media (placebo).

Facilities

The test facility was an isolated, totally confined, environmentally controlled single room with twenty 5 ft × 7 ft (1.5 m × 2.1 m) pens separated by solid partitions on woven wire flooring over a shallow pit. Ambient temperatures were held at approximately 85°F (29°C) for the first week and lowered incrementally each week to 75°F (24°C) during the final week. Heat lamps provided supplemental zonal heating. A single-sided adjustable stainless steel self-feeder and one automatic nipple waterer serviced each pen. The CONTROL pigs were segregated in one end of the building, separated from infected pens by several empty pens and a plastic sheet barrier. Other standard biosecurity measures (separate boots and clothes) were practiced to prevent inadvertent transmission of *L. intracellularis* by animal caretakers.

Measurements and observations

Each pig was weighed on day -7, day 0, day 7, day 14, day 21, and day 28. Feed disappearance was measured for the same weekly intervals. Rectal swabs were taken on day 0 (before infection), day 7, day 14, day 21 and day 28. Polymerase chain reaction was used to detect *L. intracellularis* from fecal swabs, and reported as positive or negative. Blood samples were collected from all pigs on day 0, day 7, day 14, day 21, and day 28 to detect IgG-specific antibodies for *L. intracellularis* using an IFA test. The investigators were blinded for this portion of the study.

Each pig was observed daily for diarrhea and clinical appearance and a composite pen score was assigned for diarrhea severity (scale: 0 = normal feces, 1 = mild [pasty feces], 2 = moderate [fluid], or 3 = severe [watery]), for the presence or absence of blood, and for clinical appearance (0 = normal, 1 = gaunt, 2 = moribund or dead).

On day 28, all remaining live pigs (31 of 32) were euthanized, necropsied, and sampled. Microscopic examination of the jejunum, ileum, and colon was used to determine the presence of lesions (hematoaxylin and eosin) and intracellular curved bacteria (Warthin-Starry silver stains) to score the degree of crypt cell hyperplasia (scale: 0 = normal morphology; 1 = mild, focal enterocyte hyperplasia; 2 = moderate, multifocal enterocyte hyperplasia; 3 = severe diffuse enterocyte hyperplasia) and quantitate inflammation (scale: 0 = normal architecture, 1 = focal suppurative cryptitis; 2 = moderate suppurative cryptitis and lymphoplasmacytic infiltrates into lamina propria; 3 = severe, diffuse cryptitis and marked lymphoplasmacytic infiltrates and Peyer's patches hyperplasia). The pathologist was not blinded for the clinical scoring or the gross pathological assessment portions of this study, but was blinded by the nature of the sample submission and processing procedure for the microscopic lesion assessment.

Mesenteric lymph node, ileum, and colon from all pigs were submitted to the Iowa State University Veterinary Diagnostic Laboratory for isolation of *Salmonella*, pathogenic *Escherichia coli*, and *Serpulina* spp. Polymerase chain reaction on ileal and colonic mucosal scrapings and and indirect-fluorescent antibody labeling technique on histologic sections of ileum and colon were used to detect and confirm the presence of *L. intracellularis* at necropsy.

Statistical analysis

The individual pig was considered to be the experimental unit for statistical analysis of the categorical data that applied to individual animals including: prevalence and severity of gross and microscopic lesions, fecal PCR, IFA on ileal and colonic mucosal scrapings, and serology. The clinical appearance scores, diarrhea scores, average daily gain, feed intake, and feed conversion data were statistically evaluated using the pen as the experimental unit. The day 0 body weights and weight gain for day -7 to day 0 were tested as possible covariates. Only the feed intake variable needed day 0 bodyweight as a covariate. A Fisher's Exact test was used to analyze the pathological and clinical categorical data while ANOVA was used to analyze productivity data. If the overall F-test or Fisher's Exact test had a significance level $<.05$, then multiple comparisons were used to compare all pairs of treatments. If the overall F-test or Fisher's Exact test had a significance level $>.05$, then no further tests were used to compare the treatments.

Results

CONTROL pigs all remained negative for *L. intracellularis* infection by serology, PCR, and clinical and pathological assessment.

No clinical evidence of PPE was detected in either of the groups that received medication. There were no differences in effectiveness between the two medication inclusion rates tested for any parameter measured.

Bacteriology

All bacterial culture attempts were negative for pathogenic *E. coli*, *Salmonella* spp., and *Serpulina* spp., verifying the presence of an uncomplicated *L. intracellularis* infection.

Lesions

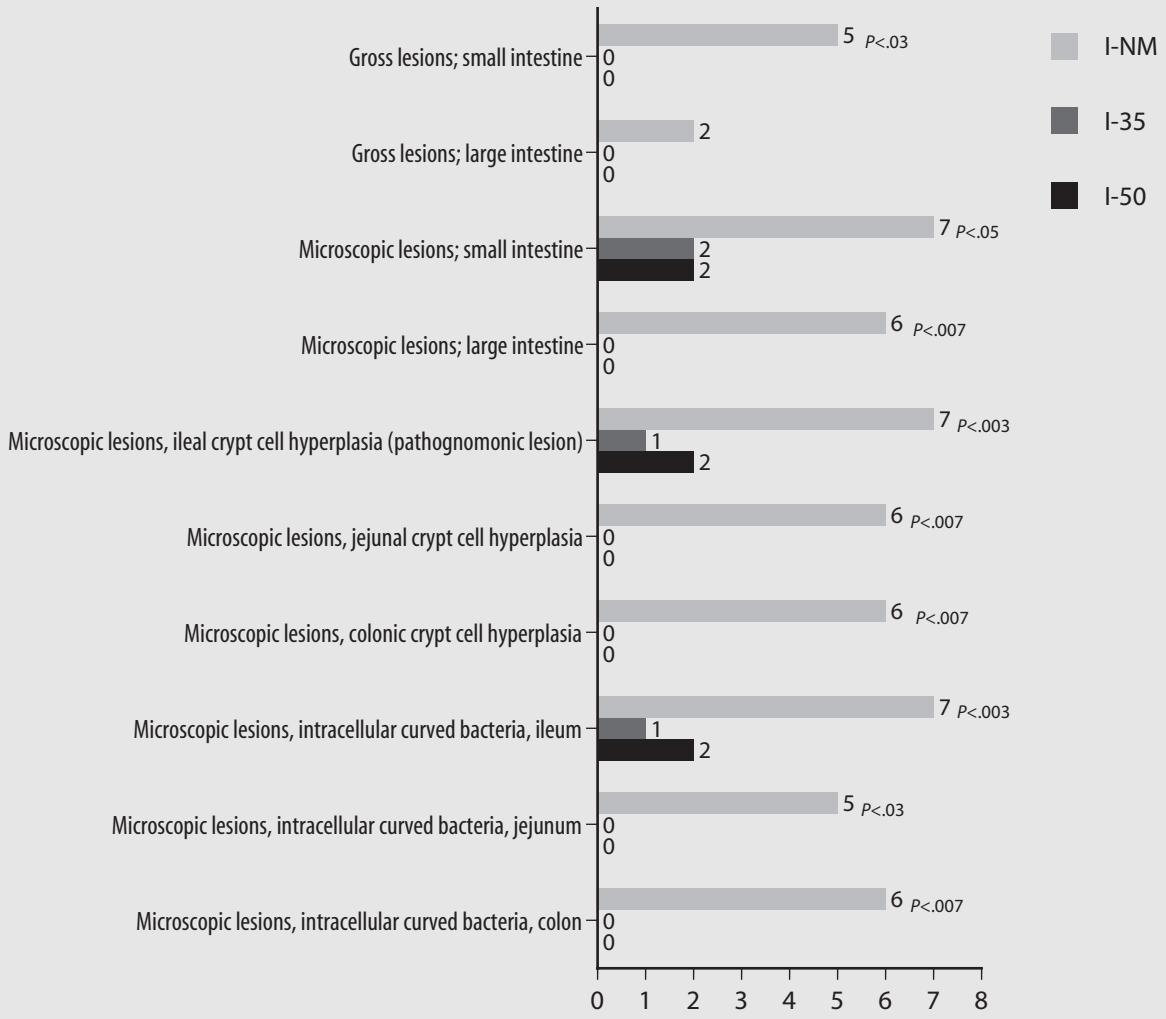
No gross lesions were observed in either small or large intestines of medicated pigs but were present in small intestines (six of eight) and large intestines (two of eight) of the I-NM pigs (Figure 2).

Microscopic lesions were more prevalent in I-NM pigs (seven of eight had lesions in their small intestines and six of eight in large intestines) when compared to medicated pigs (four of 16 with lesions in their small intestines and none of 16 with lesions in their large intestines) (Figure 2).

Serology

Lawsonia intracellularis seroconversion was first detected at day 21 (Figure 3). By day 28, the frequency of seropositivity was significantly higher in pigs in the I-NM group (P=.03) compared with pigs in the other groups.

Figure 2



Prevalence of gross and microscopic PPE lesions at necropsy

Fecal samples

Fecal swabs positive by PCR first appeared in two of eight I-NM pigs on day 7 (Figure 4). By day 21, more fecal samples from the I-NM pigs tended to be positive by PCR (P =.08) than those from the other groups. By day 28, significantly more fecal samples from I-NM pigs were positive for *L. intracellularis* by PCR than in the other groups (P=.03).

Health scores

Diarrhea was sporadic in the I-NM group and only occasionally was blood observed. Daily diarrhea scores tended to be higher in the I-NM group pigs compared with the other three groups by as early day 6–7 (P=.20) which coincides with the initial detection of *L. intracellularis* being shed in the feces of pigs in the I-NM group on day 7. The differences in daily diarrhea scores continued to diverge between the I-NM pigs and the other three groups, with the scores reaching a statistically significant difference by day 24–day 28 (P =.03). Clinical appearance scores of the I-NM group were significantly higher than those of the other groups by day 5 (P=.007). Mean daily appearance and diarrhea scores as well as the percent of pig days with clinical appearance or diarrhea scores > 0 were significantly higher for the I-NM group versus the medicated groups for the 28-day post-infection period (Figure 5).

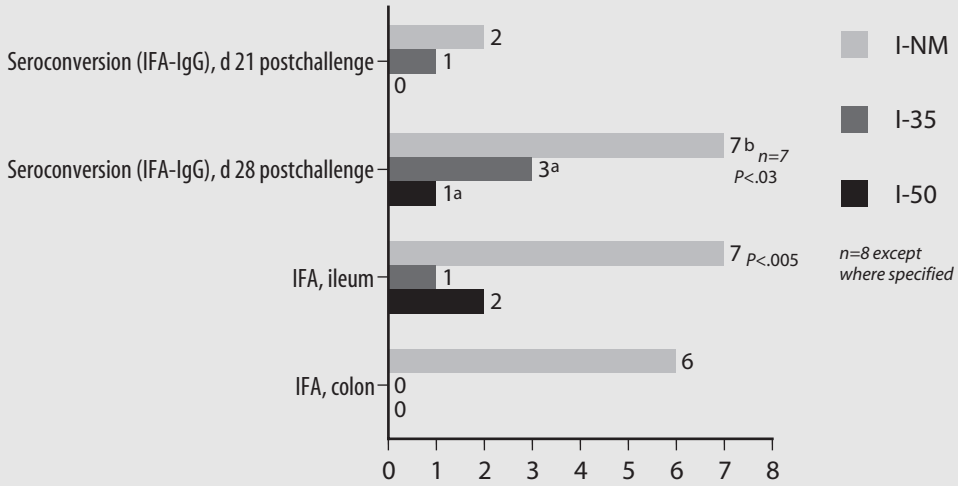
Growth performance

Of the growth performance parameters measured, only F:G differed significantly between the I-NM pigs and the tiamulin-treated groups, and only for the first 2 weeks after inoculation (data not shown; P<.04). No other growth parameters significantly differed among the groups at any time during the trial.

Discussion

The negative bacterial cultures we observed in this study verify that the experimental inoculation resulted in an uncomplicated *L. intracellularis* infection. Indirect-fluorescent antibody labeling technique on histologic lesions specifically confirmed the presence of *L. intracel-*

Figure 3



Seroconversion to *L. intracellularis* over study period and IFA results on intestinal sections at slaughter

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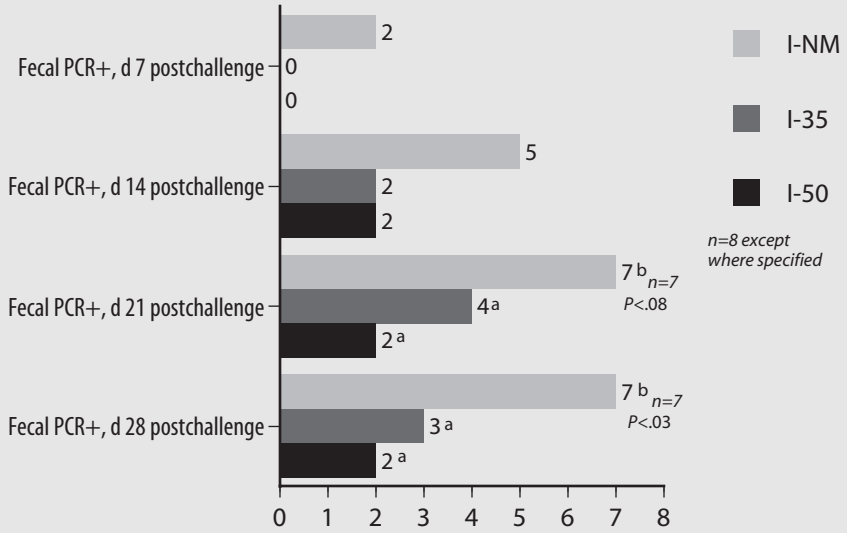
Figure 4



Fecal shedding of *L. intracellularis* over study period

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lularis within lesions.

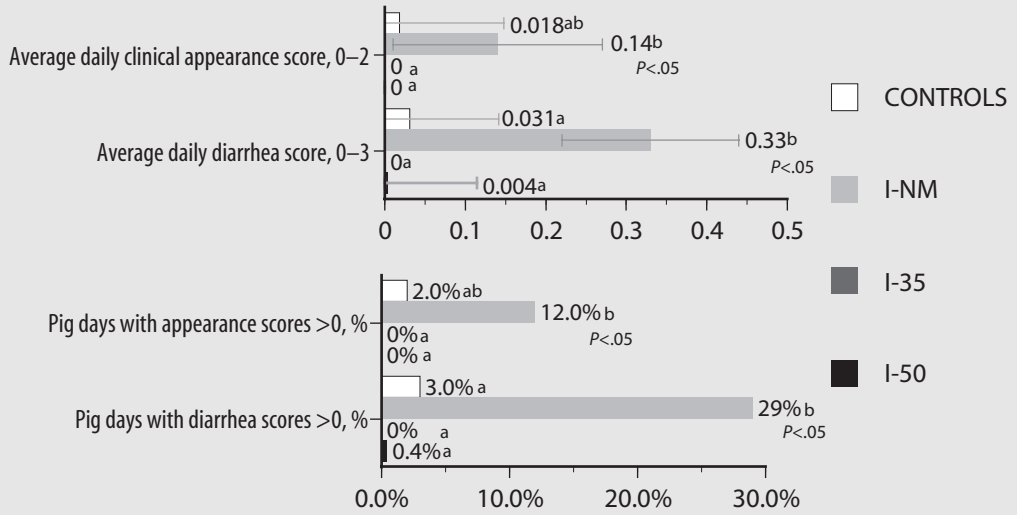
Dietary inclusion of tiamulin completely prevented the development of PPE gross lesions and significantly reduced the prevalence and severity of PPE microscopic lesions when compared to pigs in the I-NM group. The difference in the prevalence of lesions we observed between the small and large intestines of the I-NM pigs is typical of observations made in the field by practitioners and diagnosticians.

Because our experimental design did not include noninfected, medicated pigs, we were unable to determine what noninfected pigs would have gained if they had been given the advantage of an antibiotic as a growth promotant. Due to the difficulty of separating "growth promotant" from "therapeutic" effects, comparison of our productivity results between the medicated and nonmedicated groups should be interpreted with caution, even though recognized confounding infections have been ruled out. However, the productivity data could be considered specific for the effects of *L. intracellularis* infection when comparing the CONTROL group to the I-NM group. The magnitude of the numerical reductions in productivity measures we observed between the CONTROL and the I-NM groups are consistent with those reported from other pure culture infections.[7]

The few significant differences we observed in productivity between the medicated and I-NM groups were probably due to the relatively small number of replications (n=2). Consistent trends in the data suggest that infection resulted in decreased productivity and that medication appeared to protect against these detrimental effects. Further research with more pens of pigs is necessary to confirm these observations.

In this study, medication was provided prior to infection to evaluate the effectiveness of dietary medication in preventing/controlling PPE in a

Figure 5



Appearance and diarrhea observations

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population of pigs with significant subsequent *L. intracellularis* exposure. In food animal/population medicine, medication is typically initiated during the period(s) of risk identified from experience with previous batches of pigs in a particular production system, or when the first animals in the population are recognized to be diseased. Therefore, most animals in the field are medicated prior to or shortly after exposure to prevent and control the biologic and economic effects of disease on the population. This study demonstrates that dietary tiamulin effectively prevents and controls the impact of a subsequent *L. intracellularis* challenge on a population of pigs.

Previous researchers have observed that pigs reared in segregated early-weaning systems can be seropositive for *L. intracellularis* at weaning; however, this is likely due to maternal antibody.[17] In other field observations of naturally occuring disease, pigs were observed to be negative for *L. intracellularis* by PCR and IFA assay serology until the late nursery or the grow/finish phase,[18] well past the time when medicated feed is typically first provided. In addition, PPE is primarily a disease of grow/finish rather than nursery pigs.[1]

Tiamulin has been reported to possess good in vitro activity against *L. intracellularis*[15,16] and to be effective in treating, controlling, or preventing PPE in two separate pure-culture *L. intracellularis* challenge models,[14,19] a field study with the naturally occurring disease,[20] and in clinical use by swine practitioners.[21–23] This study confirms the ability of tiamulin to control the clinical and pathological effects of PPE and demonstrates the potential impact of effective medication on the results of new antemortem diagnostic tests. Medication may interfere with expected fecal shedding of and seroconversion to *L. intracellularis*, which may confound interpretation of these tests in medicated populations. Accurate identification of the timing of *L. intracellularis* infection may facilitate the design of more effective treatment, control, and possibly eradication strategies.

Implications

- *Lawsonia intracellularis* fecal shedding may occur in seronegative pigs early in the course of infection, which emphasizes the need for appropriate diagnostic test selection and interpretation.
- Medication status may affect the results of antemortem diagnostic tests for *L. intracellularis* infection.
- Medication may decrease the frequency of detectable *L. intracellularis* fecal shedding and seroconversion, necessitating larger sample sizes to increase the probability of detecting infection in medicated populations of pigs.
- Tiamulin is effective in the prevention/control of *L. intracellularis* infection/PPE.

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